TRANSCRIPTION: FACTORS, REGULATION AND DIFFERENTIATION

Organizers: David Baltimore and Rodrigo Bravo

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Plenary Sessions *Page*

Poster Sessions

Structure of Regulatory Proteins

B 001 SPECIFICITY AND STABILITY OF LEUCINE ZIPPER INTERACTIONS.

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The leucine zipper motif was originally proposed by McKnight's group as a hypothetical dimerization motif in a new class of DNA binding proteins (Landschultz et al., Science 240: 1759 [1988]). Biochemical, spectroscopic, genetic, and X-ray crystallographic studies show that the leucine zipper of the yeast transcriptional activator, GCN4, folds as a two-stranded, parallel coiled coil (O'Shea et al., Science 243: 538 [1989]; Oas et al., Biochemistry 29: 2891 [1990]; Hu et al., Science 250: 1400 [1990]; Goodman & Kim, Biochemistry 30: 11615 [1991]; O'Shea et al., Science 254: 539 [1991]).

The isolated leucine zipper regions from the nuclear oncogene products, Fos and Jun, are sufficient to mediate specific heterodimer formation (O'Shea et al., Science 245: 646 [1989]). This provides a simple model system for studying the specificity of protein-protein interactions: two α-helices that prefer to interact with each other rather than with themselves. It is found that destabilization of the Fos homodimer by acidic residues provides a thermodynamic driving force favoring heterodimers, and that eight amino acid residues from Fos and from Jun are sufficient to direct preferential heterodimer formation (O'Shea et al., Cell 68: 699 [1992]).

B 002 SYSTEMATIC STRUCTURAL ANALYSIS OF PROTEIN-DNA INTERACTIONS, Carl O. Pabo, Massachusetts Institute of Technology, Howard Hughes Medical Institute, Cambridge, MA 02139.

Methods are being developed for the systematic structural analysis of protein-DNA interactions. We begin by using a simple geometric approach to characterize the spatial relationship between every amino acid and every base in a protein-DNA complex. This method of "parsing" the structure allows a systematic comparison of contacts in different complexes. These comparisons should enhance our understanding of the evolutionary and structural relationships between different families of DNA-binding proteins, and should help provide a solid basis for the design of novel DNA-binding proteins.

B 003 STEREOCHEMICAL PRINCIPLES OF DNA TARGET SELECTION BY THE STEROID/NUCLEAR RECEPTORS, Paul B. Sigler, Yale University, Department of Molecular Biophysics and Biochemistry, and The Howard Hughes Medical Institute, New Haven, Ct., 06510.

Transcriptional regulatory proteins interact with one another, and with the basal transcription apparatus, to produce a near continuum of responses. These molecular contacts are often put into action once the protein binds to the DNA regulatory element. The steroid/nuclear receptor family is a well studied system that exhibits such DNA-dependent specific protein-protein interactions including those responsible for DNA target selection.

The DNA targets of the steroid/nuclear receptor family are distinguished by: (1) the base sequence of the 6-basepair 'half-sites'; and, (2) the orientation of the half-sites as well as the number of basepairs between them. We have studied the crystal structure of the glucocorticoid receptor's DNA-binding domain (GR-DBD) in complexes with a variety of DNA targets. We have also studied DNA complexes of mutational variants of the GR-DBD designed to recognize alternative half-site sequences and alternative half-site arrangements. These studies reveal; (1) the stereochemistry of half-site recognition, and (2) structural features influenced by DNA-binding that select for targets with appropriate orientation and spacing of half-sites.

Transcription-I

B 004 CHARACTERIZATION OF THE TFIID COMPLEX AND TRANSCRIPTIONAL CO-ACTIVATORS, Timothy Hoey, Brian Dynlacht, Robert Weinzierl, Siegfried Ruppert, Edith Wang, and Robert Tjian, University of California , Berkeley CA 94720

The general transcription factor TFIID is a multiprotein complex composed of the the TATA binding protein (TBP) and several associated factors (TAFs). There are seven major TAFs, ranging in size from 30 to 200 kD, in the Drosophila TFIID complex. There are several lines of evidence indicating that the TAFs are essential for mediating regulated transcription by upstream activators. Purified TBP, lacking TAFs, is able to direct basal but not activated transcription. In contrast, immunopurified TFIID complex is able to mediate transcription activation in a reconstituted in vitro reaction. The TAFs can be separated from TBP by treatment with denaturing agents. Transcription experiments reconstituted with purified TAFS indicate that the co-activator activity is contributed by the TAFs. In order to understand the molecular mechanisms involved in transcriptional regulation we are further characterizing the TAF proteins and their biochemical activities. We have cloned and sequenced several of the genes encoding the Drosophila or human TAFs, and found that TAF proteins from these two species are highly homologous. We have monoclonal antibodies specific for these proteins and are currently characterizing the protein-protein interactions mediated by TAFs. Preliminary experiments suggest that some of the TAFs function as co-activators by serving as "adaptors" that are sites of protein-protein interaction between the activation domains of regulatory transcription factors and the general initiation factors.

B 005 ROLE OF CHROMATIN STRUCTURE IN THE REGULATION OF TRANSCRIPTION BY RNA POLYMERASE II, Rohinton T. Kamakaka, Glenn E. Croston, Leslie A. Kerrigan, Suman M. Paranjape, Michael D. Bulger, Catherine P. George, Curtis M. Tyree, Sharon L. Wampler, and James T. Kadonaga, Department of Biology, University of California, San Diego.

We have been carrying out a biochemical analysis of transcription by RNA polymerase II by using purified sequence-specific factors, fractionated and partially purified basal transcription factors, and chromatin templates. A general strategy that has been employed in these studies is to recreate in vitro transcriptional effects that are observed in vivo, and then to dissect the molecular mechanisms by which these phenomena occur. The basal transcriptional apparatus comprises RNA polymerase II as well as several auxiliary factors, which are commonly referred to as the basal or general factors. Several of the basal factors, including the TATA-box binding polypeptide (TBP) of TFIID, TFIIA, TFIIB, TFIIE, and TFIIF, have been purified and cloned from various organisms, but the exact number of factors that are required for basal transcription has not yet been elucidated. Recent work suggests that the mechanism of basal transcription varies at different promoters. In addition, it appears that there may be another class of factors, which have been named coactivators, mediators, or adapters, that are required for activation of transcription by the promoter- and enhancer-binding factors. The first portion of the talk will cover our recent work on basal transcription by RNA polymerase II in Drosophila. The remainder of the talk will describe studies involving the reconstitution and transcriptional analysis of chromatin templates. In this area, we have been investigating the ability of promoter- and enhancer-binding factors to counteract chromatin-mediated repression of transcription. In principle, promoter- and enhancer-binding factors may activate transcription by either or both of the following two mechanisms. First, the sequence-specific factors may facilitate the inherent transcription reaction -- we refer to this as "true activation." Alternatively, the promoter- and enhancer-binding factors may counteract a general repression of basal transcription by a nonspecific DNA binding entity (*i.e.*, chromatin) -- we have designated this effect as "antirepression." By using transcriptionally repressed templates as either histone H1-DNA complexes or H1-containing chromatin, we have found that some sequence-specific transcription factors function only as antirepressors, whereas other factors are able to act as both true activators and antirepressors. With the chromatin templates, we have been able to reconstitute threshold phenomena as well as long distance activation of transcription. We have also examined the mechanistic basis of transcriptional antirepression by the hybrid transcriptional activator GAL4-VP16 by using the fractionated and partially purified basal transcription factors with the transcriptionally repressed templates. In these studies, we have found that binding of GAL4-VP16 to the template was not sufficient for antirepression. It appears that an additional factor, which we have somewhat awkwardly designated as a co-antirepressor, is necessary for GAL4-VP16-mediated antirepression. Thus, transcriptional antirepression appears to be a process with a complexity beyond that of a simple effect involving transcription factor-mediated removal of histones from the DNA template.

B 006 YEAST RNA POLYMERASE II TRANSCRIPTION: STRUCTURE, MECHANISM AND REGULATION, Roger D. Kornberg, Daniel I. Chasman, Seth A. Darst, Peter David, Aled M. Edwards, William J. Feaver, Peter M. Flanagan, Opher Gileadi, N. Lynn Henry, Raymond J. Kelleher III, Yang Li, Yahli Lorch, Gavin Meredith, Claudia Poglitsch, Michael H. Sayre, and Herbert Tschochner, Department of Cell Biology, Stanford Medical Center, Stanford CA 94305.

A set of five purified general initiation factors and RNA polymerase II constitute a nearly fully defined transcription system from *Saccharomyces cerevisiae*. (Current information points to the possible existence of a sixth factor.) Three of the general initiation factors, termed **b**, **d**, and **e**, have been identified with mammalian factors δ/BTF2/TFIIH, TFIID, and TFIIB, respectively, and gene cloning will soon allow identification of the remaining factors, **a** and **g**. Direct binding to RNA polymerase II has been demonstrated for factors **b**, **e**, and **g**. Factor **b** is both a kinase, whose preferred substrate is the CTD of RNA polymerase II, and also a DNA-dependent ATPase. Studies concerning the requirement for factor **b** in transcription with polymerase lacking a CTD will be described. The CTD-less enzyme is fully active in reconstituted transcription but inert in a crude transcription system, due an inhibitor(s) whose characterization is underway.

Transcription with purified general initiation factors and RNA polymerase II is unresponsive to activator proteins, such as GAL4 derivatives. Various inhibitory proteins have been purified that abolish transcription, and whose effects are reversed by activator proteins, in reactions that also require the previously described mediator of activation and additional factors. Further resolution of the mediator and studies of its mode of action in the context of inhibitors and activators are in progress.

Structural analyses of the yeast RNA polymerase II transcription apparatus include the following: X-ray structure determination of TFIID at approximately 3 Å resolution; structure determination of RNA polymerase II by a combination of electron microscope crystallography and X-ray analysis of crystals diffracting to at least 4.1 Å resolution; and electron microscope crystallography of complexes of RNA polymerase II with general initiation factors and anti-epitope antibodies.

Transcription-II

B 007 PHEROMONE-RESPONSIVE TRANSCRIPTION IN YEAST, Yi-lu O. Yuan, Ilana L. Stroke, and Stanley Fields, Department of Microbiology, State University of New York at Stony Brook, Stony Brook, N.Y. 11794.

Saccharomyces cerevisiae **a** and α cells each transcribe a set of cell-type-specific genes: **a**-specific genes are expressed only in **a** cells and are inducible by α-factor, and α-specific genes are expressed only in α cells and are inducible by **a**-factor. This cell-type-specific and signal-responsive transcription is regulated both by products encoded at the *MAT* locus and by the pheromone response pathway. The transcription factor STE12 is part of this response pathway and is required for expression of both **a**- and α-specific genes. STE12 binds *in vitro* to the pheromone response element (PRE), the DNA sequence present in the control region of **a**-specific genes that is responsible for pheromone induction. In response to treatment of **a** cells with α-factor, STE12 rapidly is hyperphosphorylated, and this modification correlates with its ability to mediate induced transcription.

In the case of α-specific genes, there is no evidence that STE12 binds directly to these genes, nor does the DNA element that is responsible for α-specific and **a**-factor-inducible transcription closely resemble the PRE. Instead, the *MATα*-encoded protein α1 and the *MCM1* product bind to this DNA element. To test the hypothesis that STE12 is involved in the activation of α-specific genes by interacting with the α1 protein, we have cloned both *STE12* and *MATα1* functional homologs from the related yeast, *Kluyveromyces lactis*. Comparison of the *K. lactis* STE12 protein sequence with its *S. cerevisiae* homolog indicates that the DNA-binding domains are highly conserved (78% identity) and these domains bind to the PRE with similar affinity *in vitro*. However, the remainder of the proteins, required for uninduced transcription and pheromone-induced transcription, is very divergent (10-20% identity) with the exception of three short stretches of 9-15 amino acids. The *K. lactis* gene on a low copy plasmid complements approximately 10-fold less efficiently for mating in α *ste12* cells than in **a** *ste12* cells, and it similarly mediates **a**-specific transcription better than α-specific transcription.

Comparison of the predicted *K. lactis* α1 protein with the *S. cerevisiae* α1 protein indicates that the *K. lactis* protein is considerably larger, with the two α1 homologs 30% identical in the region of overlap. Mating of an α *ste12* strain carrying the *K. lactis STE12* gene could be enhanced more than 1000-fold if the strain also carried the *K. lactis MATα1* gene. Based on hybrid proteins containing portions of STE12 from either *K. lactis* or *S. cerevisiae*, this enhancement by the *K. lactis* α1 requires the STE12 sequences involved in mediating transcriptional activation. These results provide evidence for a genetic interaction between STE12 and α1, suggesting that the two proteins may also physically interact. They suggest that STE12 may operate via two different mechanisms to mediate pheromone-responsive transcription in yeast: by direct DNA-binding and by protein-protein interaction with the DNA-bound α1 protein.

Cell Cycle-I (Joint)

B 008 CELL CYCLE PHOSPHORYLATION, Tony Hunter, Jeroen den Hertog, Rick Lindberg, Jill Meisenhelder, David Middlemas, John Pines, Byron Sebastian, Sharon Tracy, and Peter van der Geer, The Salk Institute, La Jolla, California 92037

Receptor protein-tyrosine kinases (PTKs) transduce signals across the plasma membrane in response to extracellular ligands. Many receptor PTKs act to trigger cells to enter the cell cycle from the G0 state. CSF-1 is a growth factor for myeloid precursors, and the CSF-1 receptor is a PTK in the PDGF receptor PTK family. We have identified Tyr697, 706, and 721 (the site responsible for the binding of PI-3 kinase) in the CSF-1 PTK kinase insert as autophosphorylation sites, and analyzed their role in CSF-1-mediated immediate early gene induction, growth stimulation, and morphological responses by mutagenesis. Eck is a receptor-like PTK , which is mainly expressed in epithelial cells, and which is a member of a family of receptor PTKs, (Eph, Elk, Eek, Cek4/Mek4, Sek, Cek5/Nuk). We have identified a potential ligand for Eck in conditioned media of transformed cells. The TrkB receptor PTK is closely related to but distinct from the Trk and TrkC receptor PTKs. *trkB* is primarily expressed in brain as a 140 kDa glycoprotein and two smaller ~90 kDa C-terminally truncated proteins. TrkB PTK activity is stimulated by NT-3 and BDNF. NT-3/BDNF stimulates phosphorylation of TrkB on two Tyr, and phosphorylation of PLCγ1, and its association with activated TrkB. In collaboration with Hakan Persson (Karolinska Institutet) we have found that kindling-induced hippocampal seizures cause rapid but transient inductions of *trkB* mRNAs and TrkB proteins in rat hippocampus, without any effect on the levels of *trk* and *trkC* RNAs. Concomitantly BDNF mRNA is induced in the same hippocampal areas, suggesting that paracrine or autocrine stimulation of TrkB is involved in repair of neuronal damage. To study the role of protein-tyrosine phosphatases (PTPs) in the cell cycle we have cloned the mouse homologue of the PTPα receptor-like PTP. PTPα is phosphorylated constitutively in NIH3T3 cells, predominantly on two Ser, which we have identified as Ser180 and Ser204. TPA treatment of NIH3T3 cells stimulates phosphorylation at both Ser, and recombinant PTPα is phosphorylated at Ser180 and Ser204 by purified protein kinase C. Phosphorylation of PTPα at Ser180 and Ser204 increases its PTP activity in vitro. We have identified the SH3/SH2 domain-containing protein Nck as a novel substrate for the PDGF and EGF receptor PTKs, and the vSrc oncoprotein.

Progression through the somatic cell cycle is governed by a series of cyclin/cdk complexes that are formed and activated at different times during the cycle. The G2/M transition is controlled by cyclin A/cdc2 and cyclin B1/cdc2 complexes, that are activated by action of the cdc25 phosphatase on cdc2. We have proved that cdc25B can act as a dual specificity protein phosphatase, which can dephosphorylate both P.Thr14 and P.Tyr15 in cyclin B1-associated cdc2. Cyclin B1 is accumulates in the cytoplasm until the start of prophase, and is then translocated into the nucleus before nuclear lamina breakdown. In prophase cyclin B1 localizes to the mitotic asters, and in metaphase cyclin B1 localizes to the spindle poles and microtubules. In contrast to cyclin B, cyclin A is predominantly a nuclear protein, and is associated with cdk2 rather than cdc2. We have analyzed cyclin A/B1 chimeras to determine what dictates the localization of the two cyclins and the association with different cdks. Unexpectedly we have found that cyclin A/cdk2 complexes are stimulated by cdc25B treatment, implying that this complex is negatively regulated by phosphorylation, and we have identified Thr14 and Tyr15 in cdk2 as the regulatory phosphorylation sites. We have also found that cyclin E/cdk2 complexes are also activated by cdc25B. This implies that different cdc25's may play a role in regulating transitions in the cell cycle other than G2/M.

B 009 GROWTH FACTOR-REGULATED MAMMALIAN G1 (D-TYPE) CYCLINS AND THEIR CYCLIN-DEPENDENT KINASES. Charles J. Sherr,[1,2] Martine F. Roussel,[2] David Strom,[2] Jun-ya Kato,[2] and Hitoshi Matsushime,[1,2] Howard Hughes Medical Institute and Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN 38105.

Colony-stimulating factor-1 (CSF-1) is required throughout the G1 phase of the macrophage cell cycle in order for cells to enter S phase, after which they can complete the S, G2, and M phases in its absence. Macrophages deprived of CSF-1 accumulate in early G1 and progress synchronously through the cell cycle after readdition of the growth factor, but withdrawal of CSF-1 at any time during G1 results in their inability to enter S phase. Different D-type cyclins (originally designated *CYL* genes) are expressed in G1 as part of the delayed early response to growth factor stimulation. The three murine cyclin D genes are much more related to their human counterparts than to each other, suggesting nonredundancy of their functions. In CSF-1-starved macrophages restimulated to enter the cell cycle, cyclin D1 synthesis is maximally induced in early G1 and persists as long as CSF-1 is present; much lower levels of cyclin D2 accumulate maximally at the G1/S transition, whereas cyclin D3 is not expressed in these cells. Different patterns of cyclin D expression are observed in other proliferating cell types, depending on their lineage.

The cyclin D1 encoded polypeptide, $p36^{D1}$, forms complexes during G1 with a novel cyclin-dependent, serine/threonine kinase ($p34^{cdk4}$) distinct from the known cdk's, $p34^{cdc2}$ (*cdk1*), $p33^{cdk2}$, and $p36^{cdk3}$. When CSF-1-deprived macrophages are stimulated to enter the cell cycle, $p34^{cdk4}/p36^{D1}$ complexes accumulate during G1 and then decrease during S phase, in concert with a rise and fall in *cdk4* mRNA levels. By late G1, the majority of $p34^{cdk4}$ is found in complexes, with free $p36^{D1}$ present in excess. Thus, expression of cyclin D1 in macrophages is growth factor-regulated and nonperiodic, whereas *cdk4* gene expression appears to be cell cycle-regulated and, possibly, rate limiting for complex formation. Cyclins D2 and D3 form complexes with $p34^{cdk4}$ in interleukin-2-dependent T cells. We do not exclude the possibility that D-type cyclins might also interact with other *cdk* partners.

In collaboration with Mark E. Ewen and David M. Livingston (Dana Farber Cancer Institute, Boston, MA), we found that D-type cyclins can form stable complexes *in vitro* with the retinoblastoma gene product, pRb. Binding of pRb to $p35^{D2}$ or $p34^{D3}$ requires amino acid sequences within the pRb "pocket" that are necessary for its interactions with DNA tumor virus transforming proteins (T antigen, E1a, and E7), as well as the intact pRb C-terminus. Because cyclins D2 and D3 preferentially associate *in vitro* with pRb, whereas cyclins D1 and D3 are the most efficient in forming *in vitro* complexes with $p34^{cdk4}$, stable ternary complexes containing pRb, cyclin D3 and $p34^{cdk4}$ can be readily assembled from recombinant proteins. Although $p34^{cdk4}$ alone lacks kinase activity, such complexes exhibit a serine/threonine kinase activity that phosphorylates pRb, but not exogenously added, soluble histone H1. Neither of two biologically inactive pRb mutants (Cys-706 to Phe or a C-terminal truncation mutant) assembled into complexes with cyclin D3/$p34^{cdk4}$ or underwent phosphorylation *in vitro*. In contrast, a kinase-defective $p34^{cdk4}$ mutant (containing Met for Lys at its ATP binding site) formed ternary complexes with cyclin D3 and pRb but was unable to catalyze pRb phosphorylation. Cotransfection of pRb together with cyclins D2 or D3 into Rb-negative SAOS-2 osteosarcoma cells induced pRb hyperphosphorylation, suggesting that D-type cyclin-regulated kinase(s) might, at least in part, regulate G1 exit through this mechanism.

B 010 Mos PROTO-ONCOGENE AND CELL CYCLE REGULATION. George F. Vande Woude, Renping Zhou, Ira Daar, Nelson Yew, Wayne Matten, Kenji Fukasawa, B.K. Sathyanarayana, and J. Ronald Rubin. ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702.

The mos proto-oncogene product, $p39^{mos}$, is required for meiotic maturation in vertebrate oocytes and is an active component of cytostatic factor (CSF), an activity in unfertilized amphibian eggs believed to be responsible for their arrest at metaphase II. CSF arrests egg development at M-phase and is believed to function by stabilizing maturation promoting factor (MPF). Thus, $p39^{mos}$ functions downstream in the signal pathway during M-phase, and at a major cell cycle control point. Its activity is indirectly responsible for the stabilization of MPF at metaphase II, but we have also shown that Mos is necessary and sufficient to initiate G2/M transition. This link between proto-oncogene function and M-phase cell cycle regulation could be responsible for certain phenotypes of transformed cells. Thus, we have shown that the ras oncoprotein can also substitute for Mos in meiotic maturation and has authentic CSF activity.

We have found that $p39^{mos}$ is associated with and phosphorylates tubulin in vitro. Our analyses have also shown that β-tubulin is preferentially associated with and phosphorylated by $p39^{mos}$ from either transformed cells or unfertilized eggs. In transformed cells, $p39^{mos}$ co-localizes with microtubules including those of the metaphase spindle pole and early telophase mid-body and asters. Moreover, we have determined that $p39^{mos}$ binds to the tubulin heterodimer with a Kd in the nanomolar range, and have identified regions on $p39^{mos}$ which associate with tubulin that correspond to the putative substrate binding region in a 3D model of the Mos kinase derived from the cAMP-dependent protein kinase (cAPK) crystal structure. We speculate that Mos may contribute to the formation of the spindle pole as well as the spindle and thereby contribute, as CSF, to metaphase arrest. Constitutive expression of $p39^{mos}$ in somatic cells is sufficient for morphological transformation, but only cells expressing low levels of Mos can grow as transformed cells. We postulate that this amount of product is not sufficient to cause mitotic arrest but is sufficient to impart M-phase phenotypes during interphase. Altered cell morphology and loss of contact inhibition during interphase could be due to cytoskeletal changes that normally occur during M-phase. Overexpression of Mos in somatic cells leads to growth arrest, inappropriate chromosome condensation and karyokinesis in the absence of cytokinesis. The latter process is perhaps related to the regulatory role of Mos in nuclear division during meiosis. It can also provide an explanation for chromosomal instability in tumor cells.

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Cell Cycle-II (Joint)

B 011 KINASES AND PHOSPHATASES THAT REGULATE THE CELL CYCLE IN DROSOPHILA. David M. Glover, Cancer Research Campaign Laboratories, Cell Cycle Genetics Group, Department of Anatomy and Physiology, Medical Sciences Institute, The University, Dundee DD1 4HN, Scotland, UK.

The entry into mitosis is universally regulated by the $p34^{cdc2}$ kinase, which is itself activated by the tyrosine phosphatase cdc25 or its homologues. Drosophila has two cdc25 homologues, encoded by the genes string and twine, which we have isolated by their ability to rescue a fission yeast ts mutant. Whereas string appears to control entry into mitosis in somatic tissue, twine is expressed exclusively in the germ-line. Meiosis does not occur in homozygous twine males which produce cysts containing 16 rather than 64 spermatids, and in homozygous twine females, it occurs prematurely.

Both cdc2 and cdc25 homologues appear to be regulated by their phosphorylation state. Both serine-threonine protein phosphatases PP1 and PP2A have been implicated in this control. Drosophila mutants in the gene for the major isoform of PP1 and 87B show an elevated mitotic index and have cells with overcondensed chromosomes, and collapsed or multipolar spindles. Mutations in the gene for the 55 kDa regulatory subunit of PP2A at 85F, on the other hand, show two specific types of anaphase defect. Most frequently we observe intact lagging chromatids that have undergone separation from their sisters, but which remain at the position formerly occupied by the metaphase plate. This is suggestive of a specific anaphase function. The second class of abnormal anaphase figures show stretched chromosomes in which chromatids remain attached, but not at their centromeric regions, so as to bridge the mitotic poles, possibly reflecting a defect earlier in S-phase.

Relatively little is known of the phosphatases that oppose kinases other than cdc2 required for the mitotic cycle. aurora, a Drosophila gene essential for centrosome separation, encodes a 40 kDa serine-threonine protein kinase. Mutation in aurora results in monopolar spindles in larval neuroblasts. Whereas the function of aurora seems to be required early in the mitotic cycle, mutation in polo leads to a variety of abnormal late events in mitosis. These include bipolar spindles in which one pole can be unusually broad; and monopolar spindles, as well as non-disjunction in meiosis. polo encodes a protein kinase that is the Drosophila homologue of that encoded by the budding yeast gene cdc5. Assays for polo kinase activity in the mitotic cycles of syncytial embryos shows it to have cyclical activity peaking at late anaphase-telophase.

B 012 BIOCHEMICAL ANALYSIS OF SIGNALS COMING TO AND EMITTED BY RB AND OTHER CELL CYCLE REGULATING POCKET PROTEINS. D. M. Livingston, T. Chittenden, J. DeCaprio, R. Eckner, M. E. Ewen, P. Farnham*, Y. Li*, E. Flemington, W. Kaelin, W. Krek, N. Modjtahedi, C. Sherr**, and S. Shirodkar. Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA; *McArdle Laboratory, University of Wisconsin, Madison WI; and, **St. Jude Hospital and Research Institute, Memphis, TN.

RB, p107, and p300 are all specific nuclear binding targets of E1A and SV40 T antigen, both DNA tumor viral transforming proteins. Binding by the latter to these proteins is believed to contribute measurably to their transforming function. Indeed, there is abundant evidence that each of these proteins can operate in one or more aspects of cell cycle control. In the cases of RB and p107, cell cycle regulation is provided, at least in part, by each of these proteins modulating, in a timely manner, the action of at least one transcription factor, E2F-1, which, itself, is believed to promote exit from G0/G1 and passage through S. E2F-1 has now been cloned and aspects of its structure suggest possible ways in which RB and p107 might influence its action.

In its un(der)phosphorylated state, RB is believed to contribute to a block to exit from G0/G1 which can be overcome, at least in part, by specific RB phosphorylation beginning in the second half of G1. Recently, we have accumulated evidence suggesting a possible role of one or two D-type G1 cyclin-cdk kinase complexes in the process of releasing cells from a G1 block erected by un(der)phosphorylated RB. Specific interactions between RB and each of these two cyclins were detected in vitro. In addition, we have accumulated indirect evidence pointing to analogous interactions in vivo. The data imply that one or more D cyclin-cdk complexes interact, directly or indirectly, with unphosphorylated RB in vivo and that at least one outcome of these encounters is passage out of G1.

Rel/NF-kB Family

B 013 ACTIVITY AND EXPRESSION OF RELB, Rodrigo Bravo, Daniel Carrasco, Pawel Dobrzanski, and Rolf-Peter Ryseck, Bristol-Myers Squibb Pharmaceutical Research Institute, Department of Molecular Biology, P.O. Box 4000, Princeton, N.J. 08543-4000.

RelB is a 558 amino acids protein containing a region of 300 amino acids, the Rel homology domain (RHD), which present a high similarity to c-Rel and other members of the Rel/NF-kB family. In addition to the RHD and to a non-conserved C-terminal region present in the Rel proteins, mouse RelB contains a unique N-terminus of 102 amino acids. In contrast to other members of the Rel/NF-kB family, RelB does not form stable homodimers and therefore is not able to bind efficiently to NF-kB sites. However, it forms heterodimers with p50B(p49)- and p50-NF-kB that do bind to different NF-kB binding sites with a similar or higher affinity to that shown by p50-NF-kB homodimers. These heterodimers are significantly more active in enhancing the transcription of a kB-dependent promoter *in vivo* than p50- or p50B-NF-kB homodimers. This data indicates that RelB, like p65, provides the heterodimer with an activating domain. To analyze the RelB regions which contribute to this transactivation, different deletion mutants of RelB and RelB/p50 chimeras were generated, and their activating potential was tested by cotransfection into F9 cells. The results demonstrate that for full activation, RelB requires both the N-terminal 100 amino acids preceding the RHD and the last 180 C-terminal amino acids. We have also demonstrated that the leucine zipper-like structure present in the N-terminus of RelB contributes to the transcriptional activity.

IkBα can associate with RelB/p50-NF-kB heterodimers and inhibit their DNA binding activity. To determine the region of RelB interacting with IkBα RelB deletion mutants and hybrid molecules between RelB and p50-NF-kB have been constructed. The results of these experiments will be discussed.

In order to better understand the biological role of RelB, we have studied by *in situ* hybridization the pattern of *relB* expression during mouse development and compared it with other members of the *rel* family.

The results show that members of the *rel* family genes have different temporal and spatial patterns of expression, in particular in lymphoid tissues. In 13 day embryos, p50- and p65-NF-kB are detected in many different tissues with the highest levels in the thymus. In contrast, c-*rel* and *relB* gene expression is mainly restricted to the thymus and is only significantly detected in the late stages of development.

In adult thymus, c-*rel*, *relB* and p50B-NF-kB mRNAs are detected in the medullar region, while p50- and p65-NF-kB are found mainly in the cortical region of this organ.

In adult spleen, all members studied are primarily expressed in the white pulp. c-*rel*, in particular, is highly expressed in germinal centers and in the marginal zone. In contrast, *relB* transcripts are mainly detected in the periarterial lymphatic sheath. These observations suggest that the members of the *rel* family have a differential role during mouse embryogenesis and in particular during lymphoid development.

B 014 THE REL/NF-κB FAMILY OF TRANSCRIPTION FACTORS, Nancy R. Rice[1], Frédérique Logeat[2], Mary Lee MacKichan[2], Odile Le Bail[2] and Alain Israel[2], [1]Frederick Cancer Research and Development Center, Frederick MD 21702-1201 and [2]Institut Pasteur, 75015 Paris, FRANCE.

The NF-κB transcription factor is a key regulator involved in the control of the expression of a large number of genes whose products participate in the immune response (MHC class I, Immunoglobulin κ light chain, Interferon-ß, IL6, IL2 receptor, TNF-α, GM-CSF, T-cell receptor-ß chain). It is an heterodimer of two subunits (p50 and p65) which is constitutively nuclear only in B cells. In other tissues, and in particular in T cells, it is kept as an inactive complex in the cytoplasm through interaction with an inhibitor, IκB. Following a series of seemingly unrelated external stimuli (IL1, IL6, TNF-α, PMA, LPS in pre-B cells, anti-CD3 and anti-CD28 in T cells), IκB undergoes a change in its phosphorylation state and dissociates from NF-κB which is then translocated into the nucleus where it binds to its target κB sites. The cloning of p50 revealed that this subunit is synthesized as a 105 kD cytoplasmic precursor (p105) which is unable to bind DNA. The N-terminal part, which corresponds to p50, is highly homologous to the c-rel protooncogene product and to the Drosophila *dorsal* morphogen. C-rel also binds to κB sites and can form heterodimers with p50 or p65.

The C-terminal part of p105 contains 7 repeats similar to the ones found in a cytoplasmic protein called ankyrin (where they are involved in the attachment to the cytoskeleton and to integral membrane proteins) and also in the 3 IκB-like molecules cloned so far. This suggests that this C-terminal part might behave as an IκB-like molecule. We will present data showing that two of the ankyrin repeats of p105 are responsible for its cellular localisation and unability to bind DNA. In addition to this "cis-acting" IκB-like activity, p105 also exhibits a "trans" activity : in the cytoplasm of various cell lines it is found associated with c-rel or p65 and is responsible for their cytoplasmic retention. This mechanism presents a major difference with the classical IκB system : while IκB dissociates from its target following a (de)phosphorylation event, the cytoplasmic complexes containing p105 yield active nuclear p50 containing dimers following processing of p105 by a cytoplasmic protease activity. We have undertaken the purification of this activity.

Transcription Factors and Differentiation-I

B 015 NOVEL REGULATORY ROLE FOR 3' UNTRANSLATED REGIONS IN DIFFERENTIATON, Helen M. Blau, Farzan Rastinejad, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305.

Differentiation of skeletal muscle entails the activation and continued expression of a battery of genes for tissue-specific function. To date, efforts to identify regulators of myogenesis have yielded DNA-binding transcription factors. In order to explore novel regulatory pathways in myogenesis, we have used a genetic complementation approach. A differentiation defective myoblast mutant (NMU2) was isolated that expresses MyoD, but lacks regulators necessary to activate α-cardiac actin and myogenin promoters. NMU2 cells with stably transfected muscle-specific promoter constructs were transfected with a cDNA expression library. Four cDNAs activated the promoter constructs as well as the endogenous myogenin gene. Three activating cDNAs were identified as muscle structural genes. The activity mapped to the 3' untranslated region (3'UTR) of the cDNAs. These data suggest that a trans activity by 3'UTRs mediates a feedback loop for promoting and stabilizing the differentiated state of muscle.

B 016 **TRANSREGULATION OF HOMEODOMAIN PROTEIN FUNCTION BY DCoH.** Gerald R. Crabtree, Weidong Wang, Linda Hansen and Dirk Mendel. Stanford University School of Medicine, Stanford, CA 94305.

Studies of homeodomain proteins in a variety of species have lead to the conclusion that the function of a homeodomain protein is dictated largely by the homeodomain itself. However, genetic studies of fusion genes between *Ante* and *Ubx* in *Drosophila* indicate that regions outside the homeodomain can modulate the function of the homeodomain protein in a tissue-specific manner. These modifying effects are thought to arise from the interactions with uncharacterized protein, termed transregulators. In the course of studying the mammalian homeodomain protein HNF-1 (LFB-1), we and others found that this transcription factor was unable to transactivate its cotransfected target genes in certain cell lines but fully competent in others. This lead us to look for a protein that could transregulate the function of HNF-1. We found that an 11kDa protein copurified with HNF-1. The amino acid sequence of this protein was found to be unique and over 94% conserved Transfections of DCoH transactivates HNF-1α-directed transcription by over 200-fold, thereby explaining the tissue-selective action of *HNF-1*. *In liver cell extracts, DCoH* is present in both the cytosol and nuclei and while nearly 100% of HNF-1α is complexed to DCoH, most DCoH is not between Xenopus, mice, rats and man. When overexpressed with HNF-1, it stabilizes dimers of HNF-1 (hence the name Dimerization Cofactor for HNF-1) and forms a tetrameric structure containing 2 DCoH molecules and 2 HNF-1 molecules. *In vitro*, either HNF-1α or β can participate in the tetrameric complex, associated with HNF-1 implying the existence of other DCoH-associated proteins. Since DCoH does not contain an activation domain that can be detected in a Gal 4 fusion protein, the basis for its ability to provide transactivation is uncertain. Recently, DCoH was found to be identical to a dehydratase that removes water from 4α-carbinolamine, a cofactor for phenylalanine hydroxylase. At present, we are not certain if the enzymatic activity of DCoH could be involved in transcription or if it is simply a bifunctional protein.

B 017 **SERUM RESPONSE FACTOR-HOMEODOMAIN COMPLEXES AND THE SPECIFICITY OF NUCLEAR SIGNAL TRANSDUCTION.** Michael Gilman, Cyrille Alexandre, Ricardo Attar, Dorre Grueneberg, Gary Lee[1], Sridaran Natesan, Ann Ryan[1], Henry Sadowski, and Ken Simon[1], Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, and [1]Graduate Program in Molecular and Cellular Biology, SUNY Stony Brook, Stony Brook, NY 11794.

Cell growth and differentiation is coordinated by extracellular signals. Cells and their progenitors encounter many such signals during development, and they must be able to distinguish among them and respond in an appropriate fashion. Moreover, the same signal is often used by different cells as an inductive signal for very different patterns of gene expression and subsequent development. And, more generally, the intracellular signal transduction pathways triggered by different signals often overlap, suggesting that highly specific signal-receptor interactions become reduced to more generic intracellular signals after transduction across the membrane. In such cases, there is little or no intrinsic information in the signal that specifies the cellular response. Instead, it is the cell's developmental history or identity that determines its response to a signal. How is signal transduction information interpreted by the apparatus that determines cell identity? Our recent data suggest that this may be accomplished by the formation of physical complexes between homeodomain proteins and proteins of the MADS box family. Homeodomain proteins have well-established roles in assigning cell identity in animals and lower eukaryotes. Serum response factor (SRF), a member of the MADS box family, is a major target for transcriptional activation of the c-fos gene by growth and differentiation factors. In an effort to identify human proteins that interact with SRF, we designed a genetic screen in yeast for genes encoding proteins that interact with the related yeast MADS box protein MCM1 to activate a cell-type-specific pheromone-inducible gene (1). We repeatedly isolated a cDNA encoding a novel protein of the homeodomain family, which we have named Phox1. In several different in vitro and in vivo assays, we have determined that Phox1 indeed interacts with SRF as well as MCM1, and the principal effect of this interaction is an enhancement of the kinetics of binding of SRF to its binding site in the c-fos gene. We have shown that this activity is shared with certain Drosophila homeodomain proteins with closely related structures, and that the activity is independent of the DNA binding activity of the homeodomain itself. Thus, the ability to interact with SRF represents a second independent activity of the homeodomain. We propose that one way in which homeodomain proteins may assign cell identity is by interacting with SRF and related proteins to specify the transcriptional response to inductive signals. We are now studying the DNA-binding specificity and structural organization of SRF-homeodomain complexes. In addition, we have begun to ask whether SRF binding sites are associated with known targets of homeodomain action in Drosophila.

1. Grueneberg, D.A., Natesan, S., Alexandre, C., and Gilman, M.Z. Science 257, 1089 (1992).

Phosphorylation of Transcription Factors-I (Joint)

B 018 **MYC IS INVOLVED IN A NETWORK OF PROTEIN INTERACTIONS.** D. Ayer[1], E.M. Blackwood[1,2], M.W. King[3], L. Kretzner[1], K. Tietje[1], and R.N. Eisenman[1]. [1]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle; [2]Department of Pathology, University of Washington School of Medicine, Seattle; [3]Center for Medical Education, Indiana State University, Terre Haute.

The proteins encoded by the *myc* family of proto-oncogenes are members of a larger group of proteins distinguished by the presence of a basic region followed by putative helix-loop-helix and zipper domains (bHLH-Zip proteins). This region is known to mediate DNA binding and protein-protein interactions. Myc family proteins self-associate and bind DNA poorly but readily form sequence-specific DNA binding complexes with Max, another bHLH-Zip protein. Max is a highly conserved protein that can form homodimers but preferentially heterodimerizes with Myc. *In vitro*, Max homodimers bind the CACGTG E-box consensus sequence less tightly than Myc:Max homodimers. In avian and mammalian cells alternative splicing generates two Max proteins (Max and Max9) which *differ by a nine residue insertion N-terminal to the basic region.* Max proteins in *Xenopus laevis* differ from human Max by the absence of a 24 residue region and from each other by a 27 residue insertion, both in the region C-terminal of the HLH-Zip. In gel retardation assays both human Max9 homodimers and Max9:Myc heterodimers display a significantly slower off-rate for binding to CACGTG than the corresponding complexes with Max.

In vivo, Max proteins have been found to be highly stable and expressed at essentially equivalent levels in resting and proliferating cells. Since Myc is so highly regulated and its short half-life is not affected by heterodimer formation we presume that *in vivo* the ratio of Max homodimers to Myc:Max heterodimers is continuously dependent on the rate of synthesis of Myc.

Since Max is present at times when Myc is not expressed and is generally in excess when Myc is expressed we were led to search for other proteins that might interact with Max. Using Max as a probe we screened a λgt11 expression library and identified a novel bHLH-Zip protein: Mad. Mad homodimerizes poorly but binds Max *in vitro* to form a sequence-specific DNA binding complex with properties very similar to those of Myc:Max. Both Myc:Max and Mad:Max heterocomplexes are favored over Max homodimers and have similar binding specificity and apparent stability. Furthermore, unlike Max, the DNA binding activity of the heterodimers is unaffected by CKII phosphorylation. Mad does not associate with Myc nor *with other* bHLH-Zip proteins tested.

To determine the potential role of these proteins in gene expression we carried out *in vivo* transactivation assays using a reporter gene linked to promoter-proximal CACGTG binding sites. These experiments demonstrate that Myc:Max complexes activate, while Max homodimers and Mad:Max complexes repress, transcription. Our findings suggest that Max lies at the center of a network of interacting proteins in which the relative levels of Max's dimerization partners can generate opposing transcriptional functions. Since Myc's function appears to be related to proliferation and differentiation, we believe this network may regulate genes that control these aspects of cell behavior.

B 019 SIGNAL TRANSDUCTION BY TYROSINE PHOSPHORYLATION OF TRANSCRIPTION FACTORS: A DIRECTOR EFFECTOR MODEL Xin-Yuan Fu and Guang-Rong Sun, Department of Biochemistry, Mount Sinai School of Medicine, New York, NY 10029

The primary transcription factor induced by interferon-α (termed ISGF3, interferon Stimulated gene factor 3) is a complex of four (113, 91/84 and 48 kD) proteins. The 113 and 91/84 kD proteins are present in the cytoplasm before interferon treatment and translocated to the nucleus in response to interferon-α. Sequence comparison has shown that the 113 and 91/84 kD proteins have 42% sequence identities but are derived from different members of a new gene family.

It has been shown that the 113 and 91/ 84 kD proteins of ISGF3 contain conserved SH2 and SH3 (*src* homology regions 2 and 3) domains and are immediately phosphorylated in the cytoplasm by *an interferon-α-induced* protein tyrosine kinase. Phosphorylated 113 and 91/84 kD proteins can be immunoprecipitated by specific anti-phospho-tyrosine antibodies as well as antibodies against proteins of ISGF3. A phospho-amino acid analysis of [^{32}P]-labeled 113 and 91 kD proteins has shown predominant tyrosine phosphorylation of both proteins only after interferon-α treatment. Moreover, a tyrosine kinase activity associated with the ISGF3 complex is detected in an *in vitro* kinase assay.

Two kinase inhibitors, staurosporine and genistein, inhibit this interferon-α-induced tyrosine phosphorylation of the 113 and 91/84 kD proteins both *in vivo* and *in vitro*. The tyrosine phosphorylated 113 and 91/84 kD proteins can form active ISGF3 complexes with the purified 48 kD protein *in vitro* to bind an interferon stimulated regulatory element (ISRE). Phosphatase treatment of these 113 and 91/84 kD proteins results in inhibition of this ISGF3 complex formation *in vitro*. These observations indicate that interferon-a-induced tyrosine phosphorylation is necessary for activation of the transcription factor ISGF3 and expression of induced genes. A "DIRECT EFFECTOR" model has been proposed on the mechanism of signal transduction induced by interferon.

We are in progress of further identifying the tyrosine kinase involved in this direct signaling, and dissecting functional domains of ISGF3 proteins which may interact with the tentative kinase.

B 020 POSITIVE AND NEGATIVE REGULATION OF AP-1 AND OTHER TRANSCRIPTION FACTORS BY PROTEIN PHOSPHORYLATION CASCADES, Michael Karin, Tod Smeal, Bernard Binetruy, Tiliang Deng and Anning Lin, Department of Pharmacology, School of Medicine, University of California, San Diego, La Jolla, CA 92093-0636.

AP-1 is a transcriptional activator composed of homo- and heterodimeric Jun and Jun/Fos complexes. It is involved in the activation of various target genes, such as: collagenase, stromelysin, IL2 and TGFß1, by tumor promoters, growth factors and cytokines. In addition, AP-1 activity is elevated in response to expression of transforming oncogenes including *H-ras*, *v-src*, and *v-raf* and is required for cell proliferation. AP-1 activity is subject to complex regulation both transcriptionally and post-transcriptionally. Transcriptional control determines which of the *jun* and *fos* genes is expressed at any given time in any given cell type. Therefore, transcriptional control determines the amount and composition of the AP-1 complex. Transcription of the *jun* and *fos* genes is subject to both positive and negative autoregulation and is highly inducible in response to various stimuli including those associated with cell proliferation. AP-1 activity is also regulated at the post-transcriptional level. Both cJun and cFos are phosphoproteins that are subject to regulated phosphorylation. In the case of cJun, phosphorylation of sites near the DNA-binding domain inhibits its DNA-binding activity while dephosphorylation reverses this inhibition. Phosphorylation of cJun on sites located within its activation domain increases its ability to activate transcription without affecting its DNA binding activity. The signalling pathways that modulate the phosphorylation of these sites and their regulation will be discussed.

B 021 THE ACTIVATION OF THE HSP70 PROMOTER BY E1A COINCIDES WITH A RELEASE OF TBP FROM AN INTERACTION WITH THE INHIBITORY FACTOR *Drl*, Virginia B. Kraus[1], Juan A. Inostroza[2], Danny Reinberg[2], Elizabeth Moran[3], and Joseph R. Nevins[1], [1]Section of Genetics, Howard Hughes Medical Institute, Duke University, Durham, N. C. [2]Robert Wood Johnson Medical School, UMDNJ, Piscataway, N. J.; [3]Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.

Recent studies have shown that the adenovirus $E1A_{12S}$ protein (the oncogenic E1A product) can *trans*-activate transcription by releasing the E2F transcription factor from inhibitory complexes with proteins such as the retinoblastoma gene product. However, E2F cannot be the only target for $E1A_{12S}$ activation since several cellular promoters have been found to be activated by the $E1A_{12S}$ protein despite the fact that they lack E2F sites. Indeed, we show that activation of the hsp70 promoter by the $E1A_{12S}$ product requires the TATAA sequence. Moreover, whereas activation of E2 transcription via E2F requires the CR1 and CR2 domains of E1A, activation of the hsp70 promoter requires the N terminal domain of the E1A protein and does not require the CR2 sequences. We conclude that the targeting of distinct transcription factors, leading to *trans*-activation of transcription of multiple promoters, involves distinct domains of the E1A proteins that are also required for oncogenic activity. Given the fact that the TATAA element appears to be the target for the activation of the hsp70 promoter, we have addressed the possibility that interactions with the TATAA-binding protein TBP might be altered by E1A. Recent experiments have identified a factor termed *Drl* that interacts with and inhibits the transcriptional activity of TBP. We find that the $E1A_{12S}$ protein can disrupt the interaction of *Drl* with TBP, allowing TBP to interact with TFIIA. This disruption is dependent on the N terminal E1A sequences that are also essential for trans-activation of the hsp70 promoter. It would thus appear that the activation of hsp70 through the TATAA element may be mechanistically similar to the activation of the E2 promoter via E2F, in each case involving a release of the responsible transcription factor from an inactive complex.

Phosphorylation of Transcription Factors-II (Joint)

B 022 IDENTIFICATION OF SIGNALS FOR PHOSPHORYLATION OF RNA POLYMERASE AND TRANSCRIPTION FACTORS, William S. Dynan, Arik Dvir, and Scott R. Peterson. Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215.

We report here the purification and characterization of a template-associated protein kinase that phosphorylates the C-terminal domain (CTD) of the largest subunit of RNAP II. Prior biochemical studies have shown that the CTD is phosphorylated at about the same time that transcription begins, suggesting that phosphorylation might provide a signal that allows entry into the elongation phase of the reaction. Separately, genetic studies have shown that the CTD is involved in the response to certain DNA-binding transcriptional activator proteins. Taken together, these observations raise the intriguing possibility that some activator proteins might work by stimulating a CTD kinase, which would then transduce an activation signal by phosphorylating the RNA polymerase. The properties of the kinase that we have isolated suggest that it may participate in such a signal transduction process.

We developed a specific assay for the template-associated kinase and used this assay to purify the kinase to apparent homogeneity from human cells. The purified kinase phosphorylates the CTD of native RNA polymerase in a reaction that is dependent on DNA and the general transcription factors TFIID (TBP), TFIIB, and TFIIF. The kinase has two components. One is catalytic, and appears to be composed of a single 350 kDa polypeptide. The other is a DNA-binding regulatory component that functions to recruit the catalytic component to the DNA. This regulatory component has been identified as Ku autoantigen, based on the molecular weights of its component polypeptides, its DNA binding properties, and its reactivity with anti-Ku monoclonal antibodies. Ku autoantigen interacts with DNA by a characteristic bind-and-slide mechanism. Purified Ku autoantigen has previously been shown to function as a transcriptional activator protein, although specific DNA sequences are not required, and the mechanism by which Ku acts has therefore been uncertain. We suggest Ku protein tethers the kinase catalytic subunit to DNA, and that the ability of Ku protein to slide along DNA provides a mechanism for bringing the kinase to the transcription complex.

Separate experiments show that, under certain circumstances, the transcriptional activator protein, GAL4-VP16, can replace Ku protein as the kinase regulatory component. GAL4-VP16 stimulates the ability of the purified kinase catalytic component to phosphorylate a CTD fusion protein in vitro. Both the GAL4 and VP16 portions of the activator protein appear to be involved in the interaction with the kinase. The VP16 portion of the protein is itself phosphorylated in the reaction.

Stimulation of CTD phosphorylation is a novel and potentially general mechanism of action for transcriptional activator proteins.

B 023 THE REGULATION OF TRANSCRIPTION DURING THE CELL CYCLE, Nathaniel Heintz, Franca LaBella, Rosanna Martinelli and Neil Segil. Howard Hughes Medical Institute, The Rockefeller University, New York, New York.

Transcriptional induction of histone gene expression during the S phase of the mammalian cell cycle involves coordinate activation of a set of transcription factors which interact with subtype specific consensus sequences within the histone gene promoters. In the case of histone H2b genes, the transcription factor Oct1 binds to the S phase regulatory sequences and mediates cell cycle regulation. Recent investigations have established that Oct1 undergoes a complex program of phosphorylation during the cell cycle which correlates with changes in its functional activity. Both in vivo and in vitro studies have established that mitotic hyperphosphorylation of Oct1 at ser385 within the homeodomain results in inhibition of DNA binding, and that this residue is specifically phosphorylated by PKA in vitro. These results demonstrate that Oct1 activity is modulated by phosphorylation during the cell cycle, and suggest that characterization of additional posttranslational modifications to Oct1 that occur during the cell cycle may shed light on transcriptional regulation by the POU domain proteins.

To assess whether other transcription factors which modulate histone gene transcription during the cell cycle are regulated by mechanisms similar to those controlling Oct1 activity, we have extended these studies to a second histone gene regulatory factor. H1TF2 is a novel, heterodimeric CCAAT binding protein which participates in histone H1 cell cycle control. Analysis of H1TF2 phosphorylation in vivo has established that it is also modified late in the cell cycle, and that its phosphorylation during mitosis also results in loss of DNA binding activity. Thus, two functionally related but entirely distinct transcription factors involved in temporal regulation of transcription during the cell cycle appear to be coordinately regulated by common molecular mechanisms. These results provide a biochemical framework for further work on the coordinate regulation of transcription during the mammalian cell cycle, and suggest that phosphorylation may be a key regulatory step in modulating the activities of these factors and their close relatives under other biological circumstances.

B 024 IRF-1 AND IRF-2 ; A LINK BETWEEN THE INTERFERON SYSTEM AND CELL GROWTH CONTROL. Tadatsugu Taniguchi, Hisashi Harada, Nobuyuki Tanaka, Motoo Kitagawa, Takashi Fujita*, Nobumasa Watanabe, Jun Sakakibara, Hitomi Yamamoto, Takatoshi Kawakami.

Cytokines regulate cell growth in a positive or negative manner by inducing their respective target genes. Interferons(IFNs) represent a family of cytokines with many biological activities including anti-proliferative activity. In the process of analyzing the mechanism of the IFN-β gene expression, we have identified specific DNA motifs in the promoter region of the gene. The motifs include the ubiquitous NFκB site, and Oct-1 site, and the other is more specific to the IFN genes characterized particularly by the presence of the hexamer AAGTGA motif. We have found specific nuclear factors interacting with this motif, IRF-1 and IRF-2 molecules. The functional role of IRF-1 and IRF-2 is getting clearer. There is ample evidence on the involvement of these nuclear factors in the IFN-β gene induction. In fact, evidence has been provided that, following viral induction, IRF-1 is synthesized and phosphorylated to act on the IFN-β promoter. We detect in the nuclear extract of NDV-infected mouse L929 cells a factor complex which involves IRF-1 and shown a higher affinity to the IFN-β promoter than IRF-1 or IRF-2(monomer). Our experimental data suggest that the complex is heterodimer which consists of IRF-1 and IRF-2, and it interacts with the promoter involving a larger DNA sequence element than either IRF-1 or IRF-2.

In the context of the regulation of cell growth by IFNs, it is interesting that the IRF-1 gene is IFN-inducible *per se*. Hence it may be one of the target genes for the anti-proliferative function of IFNs. Recently, we have proveded evedence that restained cell growth depend upon the balance between these two competitive factors. In fact, the IRF-1/IRF-2 expression ratio oscillates during the cell cycle, and perturbation of this ratio results in profoundly altered growth properties of NIH3T3 cells. These findings suggest a novel mechanism in the regulation of cell growth and imply the involvement of the two mutually-antagonistic transcription factors in oncogenesis.

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Transcription Factors and Differentiation-II

B 025 FROM GRADIENTS TO ZONES: MODES OF SPATIAL GENE REGULATION IN THE *DROSOPHILA* EMBRYO, Herbert Jäckle, Abteilung Molekulare Entwicklungsbiologie, Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany.

Segmentation during *Drosophila* embryogenesis is controlled by a cascade of zygotic gene activities that derive from polarly localized maternal factors which provide positional information along the entire longitudinal axis of the egg. *The zygotic target genes* can be subdivided into three distinct classes depending on the mutant phenotype and the spatial patterns of wildtype gene expression: Gap genes are expressed in broad contiguous domains that fail to develop in the corresponding gap mutant embryos; pair-rule genes are expressed in a repetitive banded pattern corresponding in position to alternating segment equivalents that fail to develop in pair-rule mutant embryos; segment polarity genes are expressed in the equivalents of anterior or posterior portions of each segment which are absent in the corresponding mutant larvae.

The cascade of zygotic segmentation genes is initiated by maternal gene products forming anterior-posterior and posterior-anterior gradients in the early embryo. After local activation in response to the maternal gene products, the gap gene expression patterns are spatially controlled by mutual interaction among the gap genes themselves. The gap-gene protein products, mostly zinc finger-type proteins, form broad and overlapping protein gradients along the longitudinal axis of the embryo. These local protein gradients contain the information for the control of zones of expression of the other gap genes which in turn provide spatial cues for generating the periodic pattern of subordinate pair-rule and homeotic gene expression.

Our results show that most of the genetic interactions within the segmentation gene cascade depend on direct DNA-protein interactions which are modulated through homo- and heterodimer formation of the gap-gene proteins that cause regulatory switches such as turning an activator into a repressor. The implication of such interactions on pattern formation in the early embryo will be discussed.

B 026 ANTAGONISM BETWEEN c-jun AND Jun-D IN THE CONTROL OF FIBROBLAST GROWTH, Curtis Pfarr[1], Fatima Mechta[1], Giannis Spyrou[2], Dominique Lallemand[1], Serge Carillo[3], Marc Piechaczyk[3] and Moshe Yaniv[1], [1] Institut Pasteur, Paris, [2] Karolinska Institutet, Stockholm, [3] U.S.T.L., Montpellier

The Jun family of transcription factors mediate mitogenic and other signalling events in the nucleus by altering the expression of specific genes. Two members of this family, c-Jun and Jun-B, are able to transform cells either by themselves, or in some cell systems, in combination with other oncogene products. The third member of the Jun family, Jun-D, is unique in being unable to transform cells. Furthermore, Jun-D exhibits a distinct pattern of expression in different tissues and in cultured cells compared to either c-Jun or Jun-B. To further characterize these biological differences we have used quantitative immunoblotting and fluorescence microscopy to examine the endogenous levels and localization Jun-D and c-Jun in NIH-3T3 cells under different growth conditions. Contact inhibition or serum-starvation of cells leads to accumulation of Jun-D in the nuclei while the level of c-Jun decreases. When quiescent cells are stimulated with fresh serum, the nuclear-localized Jun-D is rapidly degraded while the level of c-Jun rises steadily. Following a short lag period Jun-D is synthesized de novo. In addition, cells constitutively expressing Jun-D grow slower than normal and accumulate in the G_0/G_1 phases of the cell cycle By contrast, cells constitutive for c-Jun expression accumulate in the S, G_2, and M phases but exhibit a normal or slighty faster growth rate. These data suggest that Jun-D functions as a negative regulator of fibroblast growth and may be important for proper cell-cycle progression. Our study has shown that two members of the Jun family are regulated in an opposing manner during cell growth transitions and may function antagonistically. By possessing both negatively and positively acting transcription factors in the same family, each with similar dimerization potential and DNA binding specificity, the cell may exert much finer control over its regulatory network. In addition, since aberrant function of many key regulatory proteins lead to transformation (in particular c-Jun and Jun-B) the existence of family members with antagonistic function may provide some level of protection against the runaway cell division characterizing oncogenesis. Thus, the Jun proteins may be the first example of a family of transcription factors encoded by both oncogenes (c-jun and jun-B) and an anti-oncogene (jun-D).

Signal Transduction and Disease (Joint)

B 027 FUNCTION OF THE RETINOBLASTOMA PROTEIN Wen-Hwa Lee Center for Molecular Medicine/ Institute of Biotechnology, University of Texas Health Science Center, San Antonio, Texas , 78245

A class of cellular genes in which loss-of-function mutations are tumorigenic has been proposed. The RB gene (RB) appears to operate in exactly this fashion. Consistent with its ubiquitous expression pattern, RB gene inactivation is not only limited to retinoblastomas. Many other cancers such as osteosarcoma, breast carcinoma, small cell lung carcinoma, bladder carcinoma and prostate carcinoma also contain inactivated RB genes. We have introduced, via retroviral-mediated gene transfer, a cloned RB gene into retinoblastoma, osteosarcoma, prostate carcinoma, bladder carcinoima and breast carcinoma cells that have inactivated endogenous RB genes. Expression of the exogenous RB gene consistently suppressed their tumorigenecity in nude mice. These results indicate that the RB gene is a general suppressor gene of multiple type of cancer cells.

To understand the biological function of RB, we have established mice model to address the role of RB during the developmental process. Our results suggest that mice without normal RB protein expression are embryonic lethal probably due to the ectopic mitosis following cell death in neuronal and hematopoetic systems. With only one copy of RB gene, mice appear to be phenotypically normal with brian tumor at the later age of about 10 months. However, overexpression of RB protein through its own promoter resulted in dwarfism of mice. The degree of dwarfism was inversely proportional to the amount of transgenic RB protein expressed. Delay of developmental stage was observed in mice with highest transgenic RB protein expressed (about 2 folds to its endogenous RB protein). These results indicated that the RB gene clearly plays an important role during the developmental process.

To further understand the biochemical function of RB, RB protein was expressed in E. coli or in insect cells and purified to a homogeneity. Microinjection of this protein into cells at different stages inhibits G1 progression of cell cycle, suggesting that the protein may be functional at the stage of G0 to early G1. The pure RB protein has an intrinsic property of forming multimer which was regulated by phosphorylation. This study provided a novel concept how RB regulating other proteins in a coordinate manner. Indeed, many endogenous cellular proteins exist that bind to the RB and thereby may mediate its function. Characterization of this group of proteins should shed light on what is the role of RB in the cell

Structure of Regulatory Proteins

B 100 CRYSTALS OF TBP DIFFRACTING TO 1.7Å, Daniel I. Chasman, Kevin M. Flaherty*, David B. McKay*, and Roger D. Kornberg*. Center for Cancer Research, MIT, Cambridge, MA 02139 and *Department of Cell Biology, Stanford University School of Medicine, Stanford.CA 94305.

It is a characteristic of transcription in eucaryotic cells that a promoter sequence associated with the transcribed DNA is recognized specifically by a complex of proteins or initiation factors which are in turn, recognized by RNA polymerase. In transcription by RNA polymerase II, TBP (TATA binding protein) stands at the center of these initiation complexes by binding both to the sequence "TATA" in the promoter DNA and to the other initiation factors. In transcription by RNA polymerases I and III, TBP is believed to serve similar functions although its recognition sequences at polymerase I and III promoters are less well characterized than at polymerase II promoters. We have purified large quantities of TBP from a recombinant strain of E. coli and have been able to form TBP crystals suitable for diffraction studies. Unit cell parameters and space group for our crystals have been determined by precession photography. The crystals are highly ordered diffracting x-rays of wavelength 1.08Å at the Stanford Synchrotron Radiation Laboratory (SSRL) to a maximum resolution of 1.7Å.

B 101 MULTIPLE AGGREGATION STATES OF HELIX-LOOP-HELIX PEPTIDES AS A CONTROL ELEMENT FOR GENE EXPRESSION, Robert Fairman, Rita K. Beran-Steed, Spencer J. Anthony-Cahill, James D. Lear, Walter F. Stafford, Tracy M. Handel, Peter J. Domaille, William F. DeGrado, Pamela A. Benfield, and Stephen L. Brenner. The DuPont Merck Pharmaceutical Co., Experimental Station, P.O.Box 80328, Wilmington, DE 19880.

The helix-loop-helix (HLH) motif is a structural domain required for protein-protein interactions and defines a class of gene regulatory DNA-binding proteins known to be important in cell development. We are studying the structural determinants for the interaction between the HLH domain from Id (a negative regulator of gene expression) and the HLH and DNA-binding domains from MyoD and E47 (site-specific DNA-binding regulators of gene expression). Electrophoretic mobility shift assays show that the HLH domain from Id differentially inhibits the DNA-binding activities of MyoD and E47. Sedimentation equilibrium and circular dichroism experiments have established the importance of a tetrameric state for the mechanism of inhibition. Mathematical analysis of the linked equilibria show that heterotetramers of MyoD and Id have sufficient free energy to disrupt the tight association between heterodimers of MyoD/E47 and DNA. We are currently determining the tertiary structure of E47 as a model of an HLH dimer using 3D and 4D heteronuclear NMR experiments to understand the underlying mechanism of specificity for both protein-protein and protein-DNA interactions.

B 102 BIOPHYSICAL AND CRYSTALLOGRAPHIC STUDIES ON USF, A MYC-RELATED BASIC/HELIX-LOOP-HELIX TRANSCRIPTION FACTOR, A.R. Ferré-D'Amaré, P. Pognonec, R.G. Roeder and S.K. Burley, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399

USF is a human transcription factor composed of an N-terminal activation domain and three C-terminal motifs, the basic (B) region, the helix-loop-helix (HLH) and the leucine-zipper (Z), which together define the so-called Myc family of DNA-binding proteins. The function of the four motifs was investigated biophysically utilizing highly purified proteins. The activation domain gives rise to extensive, nonspecific aggregation of the full-size protein, which nevertheless retains full DNA-binding activity. Deletion of this N-terminal region yields a BHLHZ protein that binds two molecules of DNA saimultaneously as a homotetramer; this was demonstrated using bimolecular ligation experiments and photon correlation spectroscopy. The dependence of tetramer formation on the leucine zipper was established by removal of the leucine zipper to yield a construct consisting of only B and HLH which functions as a monovalent homodimer. The interaction between DNA and full-size USF, BHLHZ, and BHLH was also examined by circular dichroism spectroscopy. These studies showed that the USF basic region undergoes a coil-to-helix folding transition on specific binding to DNA, thereby making it analogous to the basic regions of basic/leucine-zipper proteins. Co-crystals of a truncated form of USF bound to a synthetic oligonucleotide containing its specific recognition sequence have been obtained, and structure determination is underway. Recent results will be discussed.

B 103 STRUCTURAL STUDIES OF THE MOTA PROTEIN FROM BACTERIOPHAGE T4, Michael S. Finnin, David W. Hoffman, Stephanie J. Porter and Stephen W. White, Department of Microbiology, Duke University Medical Center, Durham, NC 27710.

Transcription of T4 genes is a temporally regulated process that can be divided into three distinct periods-early middle and late. Transcription from middle mode promoters has been shown *in vivo* and *in vitro* to be dependent on the viral protein MotA (modulator of transcription). Cells infected with *motA*$^-$ phage show reduced levels of MotA-dependent proteins and corresponding messenger RNA. MotA is thought to act as an accessory factor for RNA polymerase by binding to a -35 sequence on the middle mode promoter, (A/T)(A/T)TGCTT(T/C)A, called the "mot box".

The protein itself contains 211 amino acids with a molecular weight of 24kD. Protease cleavage experiments reveal that MotA contains two domains of roughly equal size. The N and C-terminal domains contain 95 and 96 amino acids respectively with a 20 amino acid hydrophilic, flexible region connecting them. Both domains were separately cloned and expressed in *E. coli*. A preliminary NMR analysis indicates that the C-terminal domain contains a five-stranded β-sheet with two alpha helical segments. NMR and circular dichroism show that the N-terminal domain has a high degree of alpha helical character. The N-terminal domain has been crystalized and crystals diffract to better than 2.5Å. The space group is $P3_{1(2)}21$ and the domain is present as a dimer in the asymmetric unit. This supports the idea that, like several other transcription factors, MotA functions as a dimer.

B 104 CRYSTALLOGRAPHIC STUDIES OF COMPLEXES OF THE TFIID TATA-BOX BINDING PROTEIN WITH DNA,
Joseph L. Kim, Dimitar B. Nikolov, and Stephen K. Burley, Laboratories of Molecular Biophysics, and Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10021

The TATA-box binding protein (TBP) is required for transcription by all three eukaryotic RNA polymerases. In class II transcription, TBP comprises the DNA binding component of the general transcription factor TFIID, a large, multiprotein complex consisting of TBP and a number of associated factors (TAFs). This macromolecular assembly binds to the TATA element via contacts with the minor groove, and thereby directs accretion of other class II initiation factors and RNA polymerase II to form the preinitiation complex. Cocrystals of TBP with synthetic DNA duplexes containing two different TATA box sequences have been obtained and are currently under study. Progress in the structure determination will be presented.

B 105 BIOPHYSICAL STUDIES ON THE STRUCTURE OF THE HEAT SHOCK TRANSCRIPTION FACTOR IN *Drosophila*
S.-J. Kim[1], J. Wisniewski[1], M. Wisniewska[1], M. S. Lewis[2] and Carl Wu[1], [1]LB, NCI, [2]BEIP, NCRR, NIH, Bethesda, MD 20892.

The heat shock transcription factor (HSF) mediates the transcriptional activation of heat shock genes by binding to cognate elements with high affinity and specificity. High affinity binding of HSF is dependent on the formation of a HSF trimer composed of identical subunits. We have produced the active form of full-length HSF protein in a baculovirus expression system, and purified the protein to ~95% homogeneity using conventional chromatography. Analytical ultracentrifugation was used to evaluate the state of HSF aggregation at pH 6.3 and pH 8.0. At both pH values, the baculovirus HSF appears to exist in a reversible monomer-trimer equilibrium, with the presence of some non-reversible higher order aggregates estimated to be larger than 16-mers. We failed to observe any species in either the dimeric or hexameric states of HSF. We have also over-expressed in bacteria a 130 amino acid peptide carrying the DNA-binding domain of HSF, and purified this domain to ~99% homogeneity by conventional chromatography. The peptide was shown to possess DNA binding activity when analyzed by DNase I footprinting and fluorescence spectroscopy. The hydrodynamic properties of the HSF peptide were studied by analytical centrifugation. The purified peptide exists as a monomer in solution, with a frictional ratio f/f_0 of ~1.1 and an axial ratio of ~2.5 (for a prolate ellipsoid), suggesting a relatively compact structure. Further details of the interaction of HSF peptide with the heat shock element were revealed by analysis of the fluorescence of tryptophan and tyrosine residues in the presence or absence of DNA, and by the pattern of proteolytic digestion using trypsin as a structural probe.

B 106 CRYSTAL STRUCTURE OF THE TFIID TATA-BOX BINDING PROTEIN: A CENTRAL TRANSCRIPTION INITIATION FACTOR,
Dimitar B. Nikolov, Alexander Gasch, Alexander Hoffmann, Masami Horikoshi, Nam-Hai Chua, Robert G. Roeder and Stephen K. Burley, Laboratories of The Rockefeller Uniiversity, 1230 York Avenue, New York, NY 10021-6399

The three-dimensional structure of a central eukaryotic transcription initiation factor component, the TATA-box binding protein (TBP or TFIIDτ), has been determined by X-ray crystallography at 2.6Å resolution. The highly symmetric α/β structure represents a novel three-dimentional fold and a new DNA-binding motif. Structural details and their biological implications will be presented.

B 107 MOLECULAR MODELING OF THE DNA RECOGNITION SITES OF A ZINC FINGER PROTEIN, Robert H. Whitson, Mark Sherman & Keiichi Itakura, City of Hope, Duarte, CA 91010

In order to examine its DNA sequence recognition, we have created a molecular model of EH-2, a recently cloned protein with four cys_2his_2 zinc fingers. As a structural framework, we used the zinc fingers of Zif 268, which, like EH-2, is a member of the GLI/Krüppel family. The structure of Zif 268, co-crystallized with its target DNA has been resolved to 2.1 Å (N.P. Pavelich and C.O. Pabo, *Science 252*:809, 1991). Based on this structure, R. Klevitt has proposed a model which predicts the target DNA squences of zinc finger proteins from their amino acid sequences (*Science 252*:1367 & 1393, 1991). This model assumes that three amino acids in each finger make base-specific contacts with the DNA. The recogniton sequence predicted for EH-2 (nnGnGGnAnAAn) matches well with its known target sequences. Also, methylation of the predicted G residues interferes with the binding of *in vitro*-transcribed EH-2. Using the crystal coordinates of Zif 268 and the Homology® computer program, we constructed a molecular model of three of the fingers of EH-2 and a target DNA sequence. The model was then energy-minimized using the Discovery® program. The energy-minimized structure showed hydrogen bonds between six amino acids of EH-2 and the phosphate backbone of the DNA. These bonds stabilize the DNA-protein complex, but do not influence sequence-specificity. Two amino acids in the EH-2 model, an arg and a thr, made H-bonds with bases (guanine and thymine, respectively) in the major groove of the DNA. The arg occupies a position in the zinc finger which is predicted to determine base specificity, but the thr does not . Nonetheless, the thymidine residue to which the thr binds is conserved in all of the EH-2 target sequences. In the energy-minimized structure, four amino acids which have side chains capable of making H-bonds with DNA bases lie in positions predicted to specify base sequence. These amino acids do not form H-bonds with the DNA in the deduced structure, however. These results suggest how the models may be further refined.

Transcription I

B 108 **TRANSCRIPTIONAL ACTIVATION BY SV40 LARGE T ANTIGEN: INTERACTIONS WITH MULTIPLE COMPONENTS OF THE TRANSCRIPTION COMPLEX.** James C. Alwine*, Janice M. Zabolotny*, Jia Hao Xiao[1], Irwin Davidson[1], and Maryann C. Gruda*
*Department of Microbiology, School of Medicine,,560 Clinical Research Building, 422 Curie Blvd., University of Pennsylvania, Philadelphia, PA 19104-6142 and [1]Laboratoire de Génétigue Moléculaire des Eucaryotes du CNRS, INSERM, Institut de Chimie Biologigue, Faculté de Médecine Strasbourg Cédex, France

SV40 large T antigen is a potent transcriptional activator of both viral and cellular promoters. Within the SV40 late promoter a specific upstream element necessary for T antigen transcriptional activation is the binding site for transcription enhancing factor I (TEF-I). The promoter structure necessary for T antigen mediated transcriptional activation appears to be simple. For example, a promoter consisting of upstream TEF-I binding sites (or other factor binding sites) and a downstream TATA or initiator element is efficiently activated. Since it has been demonstrated that transcriptional activation by T antigen does not require direct binding to the DNA then the most direct effect T antigen could have on these simple promoters would be through protein-protein interactions with either upstream bound transcription factors, the basal transcription complex or both. To determine whether such interactions occur, full length T antigen, or segments of it, were fused to the glutathione binding site (GST fusions) or to the Gal4 1-147 DNA binding domain (Gal4 fusions). Using the GST fusions it was found that TEF-1 and the TATA binding protein (TBP) bound different regions of T antigen. A GST fusion containing amino acids 5-172 (region T1) efficiently bound the TATA binding protein (TBP). TEF-I bound neither the region T1 nor a region between amino acids 168 to 373 (region T2); however, it bound efficiently to the combined region (T5) containing amino acids 5-383. The Gal4 fusions demonstrated that no region of T antigen could activate a promoter containing Gal4 binding sites, suggesting that T antigen does not contain an activation domain of the type defined by this assay. However, the Gal4 fusion proteins maintained their ability to activate promoters known to be activated by wild type T antigen. The fusion with Region T1, which binds only TBP, modestly activated the SV40 late promoter and the simple TEF-1/TATA promoter. Region T5, which binds both TBP and TEF-1, activated each of these promoters to levels equivalent to that of WT T antigen. The correlation between the binding of both TEF-I and TBP and the ability to achieve wild type levels of activation suggests that T antigen mediates transcriptional activation through direct interactions with multiple factors in the transcription complexes.

B 109 CELL TYPE SPECIFIC SUBSETS OF NUCLEAR FACTOR I (NFI) IN TRANSCRIPTIONAL CONTROL OF HUMAN PAPILLOMAVIRUS-16

Doris Apt, Yichun Liu, Terence Chong and Hans-Ulrich Bernard, Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511.

The transcription of Human Papillomavirus-16 (HPV-16) depends on an epithelial specific enhancer. Enhancer activation occurs through 7 binding sites for NFI, 3 for API, 1 for glucocorticoid/progesteron receptor and 3 for TEF-1. None of these factors is epithelial specific, suggesting that cell type specific differences are associated with apparently ubiquitous factors. We propose that one of the factors responsible for functional differences of the viral enhancer in different cell lines is NFI. Point mutations on fragments of the enhancer that retained epithelial-specific transcription verify the functional contribution of NFI to enhancer activation. A characteristic pattern of NFI binding proteins is found in epithelial cells, where the enhancer is active. In contrast, fibroblasts and lymphoid cell lines, which show a different profile of NFI proteins, do not support enhancer activation. These cells seem to miss a certain epithelial type NFI. In gain-of-function experiments, cotransfection of HeLa type NFI/CTF-1 activates the HPV-16 enhancer in cells which naturally do not contain endogenous NFI. In contrast we found that exogenous NFI/CTF-1 which forms heterodimers with endogenous forms of NFI in fibroblasts fails to activate the HPV-16 enhancer. We propose that heterodimer formation of NFI with different proteins in different cell types may lead to cell type specific function of the viral enhancer. Our research aims toward the cloning and identification of different NFI proteins from different cell lines, variations of cofactors for NFI and cooperative interactions of NFI with heterologous transcription factors.

B 110 OCT-2 FACTOR FACILITATES PREINITIATION COMPLEX ASSEMBLY AND IS CONTINUOUSLY REQUIRED AT THE PROMOTER FOR MULTIPLE ROUNDS OF TRANSCRIPTION. David N. Arnosti*, Alejandro Merino+, Danny Reinberg+, and Walter Schaffner*, *Institute of Molecular Biology II, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland, and +Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854

Octamer factor 2 (Oct-2, OTF-2) is a tissue-specific "upstream" promoter factor that binds to the octamer motif (ATGCAAAT) implicated in control of immunoglobulin gene transcription in B-lymphocytes. We have studied the role of Oct-2 in the process of transcription initiation in vitro using both nuclear extracts and purified basal transcription factors. Oct-2 specifically stimulates transcription from octamer-containing promoters in both systems. Thus, Oct-2 is a "true activator", rather than merely an "antirepressor" counteracting the effect of histones. In order-of-addition experiments, Oct-2 is required early, together with TFIID, to allow formation of a preinitiation complex. Oct-2 cannot functionally interact with cloned TATA binding protein (TBP) but rather requires "coactivators" found in the TFIID fraction. In single-round transcription experiments, early competition for Oct-2 by an octamer oligonucleotide is deleterious, but no effect is seen after assembly of a complete preinitiation complex. However, for multiple rounds of transcription, Oct-2 is continuously required at the promoter; this result argues against a "hit-and-run" mechanism whereby the activator becomes dispensible after organizing a TFIID-promoter complex, as has been proposed for the ATF activator. In agreement with our previous studies in vivo, the N-terminal glutamine-rich activation domain of Oct-2 is required for full activity in vitro, indicating that this domain directly interacts with basal transcription factors.

B 111 IDENTIFICATION OF "*CUT* REPEATS" AS NOVEL DNA BINDING DOMAINS OF THE HUMAN CCAAT DISPLACEMENT PROTEIN (CDP). Barbara Aufiero, Ellis J. Neufeld, Patricia M-J. Lievens, Stuart H. Orkin. Harvard Medical School, Department of Hematology, Boston, Mass. 02115.

CDP is a nuclear protein of 180-190 kD. Our laboratory has identified CDP as a putative repressor of the myeloid-specific gp91-phox gene, which encodes a component of the NADPH oxidase. The deduced CDP polypeptide reveals a novel, triplicated motif, the *cut* repeat, remarkably similar (80-90% conserved) to three domains (60-65 amino acids) first identified in Drosophila *cut*, a homeodomain protein important for cell-fate decisions in fly development. These motifs are unique to CDP and *cut*.

We engineered the *cut* domains from CDP (designated CDP1, CDP2, CDP3) into the bacterial expression vector pGEX2TK for expression of each as fusion proteins with glutathione S transferase (GST). Standard gel shift assays were used to test the ability of the GST-*cut* repeat fusion proteins to bind to the CDP target site in the gp91-phox promoter. Our results demonstrate that CDP2 and CDP3, but not CDP1, are able to bind the gp91-phox probe. CDP2 and CDP3 exhibit distinct binding specificities; a four nucleotide substitution in the gp91-phox probe abolishes CDP3 but not CDP2 binding activity. The CDP homeodomain expressed as a GST-fusion protein was also able to bind.

Further delineation of CDP/*cut* repeat DNA binding specificity has been achieved by using PCR-assisted DNA-target site selection. Sequence-specific DNA binding sites have been obtained for CDP2 and CDP3. CDP2 appears more promiscuous in DNA target sites. A kinetic analysis to determine relative binding affinities of CDP2 and CDP3 will be presented. These findings relate CDP to a group of proteins with multiple DNA binding domains. Close conservation of these novel DNA binding domains from Drosophila *cut* to CDP suggests that they are likely to play an important biological role in the regulation of gene expression.

B 112 **PROMOTER STRUCTURE OF THE *ACANTHAMOEBA* TFIID GENE**, Erik Bateman, Jie Min Wong and Feng Liu, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405

We have characterized, using *in vitro* transcription, the promoter of the *Acanthamoeba* TFIID gene. Deletion analysis of the promoter region shows that the minimal promoter required for efficient expression *in vitro* is located between -97 and +4 relative to the transcription start site. Three regions within the promoter are important for transcription *in vitro*; sequences between -97 and -35, the TATAAA box and the initiation region. The initiation region is dispensable but appears to position the transcription start site relative to the TATAAA box. The TATAAA box is absolutely required for transcription initiation whereas the upstream region stimulates transcription approximately five-fold. Mutants lacking the normal transcription start site, direct transcription from new positions that span ~11 bases with respect to the TATAAA box. Because this reflects initiation at rotationally distinct sites, we infer that the polymerase is not subject to strong constraints on its positioning or that it can form multiple, distinct complexes.

The upstream portion of the promoter was examined by identifying and isolating a factor which binds to sequences between ~-97 and -70. We have partly purified the protein(s) and are in the process of identifying precisely its binding site and cloning its gene from an *Acanthamoeba* expression library.

B 113 IDENTIFICATION OF A FACTOR THAT DIRECTS TBP BINDING TO A NON CONSENSUS TATA BOX, Pamela A. Benfield and Mark T Mitchell, Cardiovascular Molecular Biology Division, DuPont Merck Pharmaceuticals, Experimental Station, Wilmington, DE 19880-0328.

The brain creatine kinase (*ckb*) promoter contains a non-consensus TATA sequence (TTAA) that directs transcription *in vivo*. Immediately upstream lies a perfect consensus TATA sequence (TATA) that is not normally used *in vivo* but which is functional in *in vitro* transcription assays. Recombinant TBP binds poorly to each of these two sequences. We have investigated the ability of activities derived from HeLa nuclear extracts to direct TBP binding to the *ckb* promoter. HeLa nuclear extracts that have been subjected to phosphocellulose chromatography have been analyzed. An activity found in fraction A (possibly related to TFIIA) directs TBP binding to TATA but not to TTAA. Two activities appear to be present in fraction C that augment TBP binding. These activities can be further separated by CM Sepharose column chromatography. One of these activities directs TBP binding to TATA whereas the second directs binding to TTAA.

The complexes formed on TATA can be further super-shifted in gel retardation assays by recombinant TFIIB. However, the complex formed over TTAA appears unable to bind TFIIB as judged by this assay. TBP binding to TTAA in the presence of the factor found in HeLa phosphocellulose fraction C is sensitive to magnesium possibly accounting for the magnesium sensitivity of transcription driven by this promoter *in vitro*. We propose that TBP can complex with a family of factors that direct its binding to different core promoters. These factors may have tissue-specificity and contribute to the tissue-specific expression patterns of *ckb* and other core promoters.

B 114 **SRY PROTEIN RECOGNIZES KINKS IN DNA**, Marco E. Bianchi, Simona Ferrari, Vincent R. Harley* and Andrea Pontiggia**, Dip. di Genetica e di Biologia dei Microrganismi, Università di Milano, via Celoria 26, 20133 Milano, Italy; *Dept of Genetics, University of Cambridge, Downing St., Cambridge CB2 3EH, UK; **Istituto Scientifico H San Raffaele, via Olgettina 60, 20132 Milano, Italy

HMG boxes are DNA binding domains present in chromatin proteins, general transcription factors for nucleolar and mitochondrial RNA polymerases, and gene- and tissue-specific transcriptional regulators. The HMG boxes of HMG1, an abundant component of chromatin, interact specifically with four-way junctions, a DNA structure which has the shape of an X and contains angles of about 60° and 120° between its arms. We have shown that also the HMG box of SRY, the protein that determines the expression of male-specific genes in humans, recognizes four-way junction DNAs irrespective of their sequence. In addition, when SRY binds to linear duplex DNA containing its specific target AACAAAG, it produces a sharp bend of about 85°. Therefore, the interaction between HMG boxes and DNA appears to be predominantly structure-specific. The production or the recognition of a kink in DNA can serve several distinct functions, such as the repair of DNA lesions, the folding of DNA segments with bound transcriptional factors into productive complexes, or the wrapping of DNA in chromatin.

B 115 **GUANYLYLATION AND ADENYLYLATION OF THE α REGULATORY PROTEINS OF HERPES SIMPLEX VIRUS 1 REQUIRES A VIRAL COMPONENT, John A. Blaho, Clayton Mitchell, and Bernard Roizman, The Marjorie B. Kovler Viral Oncology Laboratories, The University of Chicago, Chicago, IL 60637**

Herpes simplex virus 1 genes form several groups whose expression is coordinately regulated in a cascade fashion. The first group, the α genes, do not require **de novo** viral protein synthesis and are induced by a structural component of the virion, the α trans-inducing protein (VP16). Expression of the later groups, β and γ, requires functional α proteins some of which have been shown to regulate gene expression at the transcriptional level (e.g. infected cell protein 4 or ICP4) or post transcriptionally (e.g. ICP27). We report the following: (a) ICP4, the major viral regulatory protein incorporated label from α^{32}P-GTP and α^{32}P-ATP. The labeling was favored at temperatures from 15°C to 27°C and by the presence of okadaic acid, a potent phosphatase inhibitor. (b) The conditions for labeling ICP4 with α^{32}P-GTP and α^{32}P-ATP and the stability of the label were different from those of ICP4 labeled with γ^{32}P-ATP. In particular, efficient labeling required the presence of a high molar excess of ATP or GTP. (c) Analyses of ICP4 labeled with ^{3}H-GTP and ^{3}H-ATP confirmed the hypothesis that ICP4 is nucleotidylylated. (c) Extensive analyses of other α proteins, particularly ICP0 (a promiscuous transactivator), ICP22, a protein which regulates late gene expression, and ICP27 (a protein which determines the nucleotide sequence content of mRNA) were shown to accept label from α^{32}P-ATP and α^{32}P-GTP. In this instance, only a fraction of the label is due to nucleotidylylation of the proteins. (d) Experiments with temperature sensitive viral mutants and metabolic inhibitors indicate that a viral gene product is required for these modifications. Preliminary studies indicate that the viral component involved in this modification is synthesized later in infection and is either a β or a γ protein.

References:

Blaho, J., and Roizman, B. J. Virol. **65**:3759-3769, 1991.

B 116 USING A RADIOLABELED BASIC-HELIX-LOOP-HELIX PROTEIN PROBE TO STUDY PROTEIN-PROTEIN INTERACTIONS, Michael A. Blanar and William J. Rutter, Hormone Research Institute and Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0534

Regulation of eukaryotic transcription is controlled by a complex series of interactions amongst various protein transcription factors. These interactions are coordinated at the target gene by specific protein binding sites in the DNA. The Basic-Helix-Loop-Helix (bHLH) family of DNA binding proteins is one example of a dimeric transcription factor whose DNA recognition element, CANNTG, is shared by many developmentally important and cell-specific genes. The HLH domain of this class of proteins has been demonstrated to be an important component required for HLH-specific dimer formation and for subsequent interactions with DNA. As has been demonstrated recently for the bHLH proteins E47 and MyoD, HLH proteins may form heterodimers that may exhibit characteristics different from either protein alone. The possibility of altered activity dependent on the formation of specific HLH heterodimers, may allow for enormous flexibility and exquisite control of gene regulation.

Utilizing the bHLH region of the ubiquitously expressed bHLH gene, E2A (which encodes, for example, the E12 and E47 proteins), we have generated a bHLH protein domain that can be radiolabeled with ^{32}P in vitro. As we have described previously [*Science* **256**:1014], a fusion polypeptide was expressed in bacteria in which a 17 amino acid extension (containing recognition sites for a monoclonal antibody, a specific endopeptidase, and a protein kinase) was appended onto the amino terminus of the protein of interest; in this case, the E2A bHLH domain. This protein is able to bind specifically to various bHLH recognition elements in an electrophoretic DNA-binding assay. In subunit exchange experiments, the E2A bHLH protein could form both homodimers and heterodimers with other bHLH proteins.

When radiolabeled E2A bHLH protein was used as a probe of a λgt11 expression library, we obtained cDNA clones encoding the HLH dimerization domains of both previously identified and novel HLH-containing proteins. Preliminary analysis of the distribution of the novel cDNAs suggests that their distribution may be cell-type-restricted. We are pursuing the tissue distribution of these factors by RNase-protection and in situ hybridization studies. Because HLH proteins are likely to be involved in cell-restricted gene expression in, for example, the developing lymphoid system, neural system, endocrine pancreas, and cardiac muscle, it is likely that the interaction of these novel proteins in vivo will have functional consequences in the regulation of gene expression.

B 117 INTERFERON-γ SENSITIVE RETINOBLASTOMA PROTEIN-DNA COMPLEXES, George Blanck, DeWayne Ussery, and Yanmei Lu, Department of Biochemistry and Molecular Biology, University of South Florida College of Medicine, Tampa, FL 33612

Two short, direct repeat motifs located at an intragenic HLA class II gene hypersensitive site resemble known promoter motifs and form multiple complexes with the retinoblastoma gene product (pRb) and with pRb related proteins, as determined by Electrophoretic Mobility Shift Assays (EMSAs), using extracts from various cell-lines. None of the pRb or pRb related complexes are formed if the EMSA extracts are preincubated with anti-pRb monoclonal antibodies. In contrast to previous motifs known to complex with pRb, the class II gene motifs *require* pRb or a pRb related protein to form a DNA-protein complex. The pRb complexes cannot be formed with extract from a retinoblastoma cell-line, but this extract can be used to form complexes consisting of pRb related proteins. Finally, the pRb complexes can be formed using extract from Human Papilloma Virus E7 producing HeLa cells, but not when using extract from HeLa cells treated with IFN-γ. IFN-γ does not affect the complexes with HeLa, pRb related proteins. Since the class II motif/pRb complexes are not affected by IFN-γ in cell lines not containing the E7 protein, which is known to destabilize pRb complexes, we are currently investigating the possibility that oncoproteins act in concert with IFN-γ to prevent pRb complex formation. We are also investigating the role of pRb in the IFN-γ response of the HLA class II genes.

B 118 DIFFERENTIAL INTERACTION OF α AND β THYROID HORMONE RECEPTOR WITH DIRECT REPEAT AND INVERTED PALINDROME TRE, Fausto Bogazzi, Beatrice Desvergne and Vera Nikodem, Genetics and Biochemistry Branch, National Institutes of Health, Bethesda , MD 20892

We have used two naturally occurring thyroid response elements (TRE) as models to study the characteristics of binding and transactivation by two isoforms of thyroid hormone receptor (TR), α and β. Single point mutations were directed to each base comprising malic enzyme (ME) and myelin basic protein (MBP) TRE. The mutants were then tested in transfections to attest their capacity to direct a T3 induction mediated by α or β TR. Mutations in the direct repeat (DR) of ME-TRE allowed the functional characterization of two binding sites of 6-7 nucleotides, in which mutations altered αas well as β transactivation. A spacer of three bases between the two binding sites was similarly observed for the two receptor isoforms. When the inverted palindrome (Inv Pal) of MBP-TRE was used in cotransfection with βTR, the mutations of 15 out of 16 bases affected the transactivation. Using αTR the affecting mutations were 9 out of 16 and principally located in the 3' half-site with a spacer of 5 bases. The widespread requirement of nucleotides for the binding of βTR to MBP-TRE was also confirmed using the methylation interference assay. DR and Inv Pal shared a similar pattern of methylated bases that prevent the formation of αTR/DNA binding complex (GG and C in 3' and GG or CC in 5' binding sites).We also observed a more efficient transactivation of Inv Pal by βTR with respect to αTR (201 T3 fold induction vs 34). The efficency of transactivation was similar (α: 35 and β: 28) for the two receptors when the DR was used. In conclusion our data -better characterizes the structural organization of ME and MBP-TRE -evidences a different relevance of the same nucleotides in term of transactivation and binding depending on the presence of TR isoforms -show a preferential transactivation of Inv Pal by βTR.

B 119 ISOLATION AND CHARACTERIZATION OF TRANS-ACTING FACTORS INVOLVED IN THE TRANSCRIPTIONAL REGULATION OF VIMENTIN, Alma M. Bracete, Richard J. Garzon and Zendra E. Zehner, Department of Biochemistry and Molecular Biophysics and the Massey Cancer Center, Medical College of Virginia, Richmond, Va. 23298.

The regulation of the chicken vimentin gene is a complex process involving both positive and negative regulatory sequences located in the 5'-flanking region of the gene. A 40 bp cis-acting element (-608 to -567) at least is partially responsible for the transcriptional repression of the gene during myogenesis and in cell types that do not express vimentin. This silencer element binds a protein factor (95 KDa), as determined by gel mobility shift assays, southwestern blots and footprint analysis. On the other hand, at early stages during development, the down-regulation exerted by the silencer element is overcome by a 75 bp antisilencer sequence located further upstream (-1144 to -1404) that appears to bind a 140 KDa protein. Interestingly, the antisilencer element is not an enhancer; it activates transcription only when silencer sequences are present. In order to elucidate the mechanism of action of the silencer and antisilencer in the developmental regulation of vimentin, their respective protein factors are purified by DNA affinity chromatography. In preliminary studies, ammonium sulfate enriched nuclear extracts from HeLa cells were prepared by standard procedures. The pellet obtained was loaded onto a Sephacryl S-300 gel filtration column (2.5 X 40 cm). The elution was monitored by A280 and fractions were assayed by footprint analysis. The elution profile of the silencer and antisilencer binding activities closely resembles that of other transcription factors such as AP-1 and SP-1 (Ve\Vo = 1.3-1.4). The affinity-purified factors' binding characteristics of the silencer and antisilencer elements are presented.

B 120 MCM1 POINT MUTANTS DEFICIENT IN α-SPECIFIC GENE EXPRESSION, Laurakay Bruhn and George F. Sprague, Jr., Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

MCM1 plays a central role in regulating transcription of cell type-specific genes in the yeast, *Saccharomyces cerevisiae*. MCM1 can bind to a palindromic DNA sequence, the P-box, and bring about transcription activation. MCM1's ability to activate transcription is modulated by its ability to interact with several coregulatory proteins including α1, α2, and STE12. Deletion analysis has demonstrated that the first third of MCM1, which includes an 80-residue segment homologous to mammalian SRF, is sufficient for DNA binding, transcription activation, and interaction with coregulators. To begin to understand the mechanisms by which MCM1 interacts with coregulators, we isolated MCM1 point mutants specifically deficient for interaction with α1. MCM1 and α1 act together at the upstream regions of α-specific genes in order to bring about their activation. These upstream regions contain a composite sequence element comprised of a degenerate P-box and an adjacent 10 bp sequence called the Q-box. α1 and MCM1 together bind to these QP-boxes although neither protein binds well alone. We have mutagenized the N-terminal third of MCM1 using PCR and have isolated point mutants which are deficient in ability to activate α-specific genes but are capable of activation from palindromic P-boxes *in vivo*. We expect mutants with this phenotype to be deficient for interaction with α1 or STE12. Preliminary *in vitro* DNA binding studies, involving a subset of the mutants, have identified a mutant that binds with normal affinity to palindromic P-boxes but binds poorly with α1 to QP-boxes suggesting that it is specifically deficient for interaction with α1. Analysis of the full set of mutants should reveal the MCM1 residues crucial for interaction with α1. In addition, we plan to assess the ability of the mutants to interact with other coregulators like STE12 and α2.

B 121 **ANALYSIS OF LONG-DISTANCE ACTIVATION OF RNA POLYMERASE II TRANSCRIPTION IN VITRO,** **Michael Bulger and James T. Kadonaga,** Department of Biology, University of California, San Diego, La Jolla, CA 92093

Biochemical studies of transcription of eukaryotic genes by RNA polymerase II have been hampered by the inability to demonstrate a number of phenomena known to occur in vivo, such as long-range activation of transcription by sequence-specific factors. Such studies, however, have typically been carried out on naked DNA templates, whereas chromatin most likely represents a better model of the state of the template in vivo. Previous work in our lab has shown that long-range activation of transcription can be achieved in vitro by using reconstituted chromatin templates. We are presently examining this phenomenon with a well-defined series of plasmid constructs. The effect of variation of distance and position of activator binding sites with respect to a gene will be shown.

B 122 DETECTION OF THE BINDING OF THE CELLULAR EGR-1 PROTEIN TO ITS RECOGNITION SEQUENCE, Xinmin Cao, Graeme R. Guy and Y. H. Tan, Institute of Molecular and Cell Biology, National University of Singapore, 10 Kent Ridge Crescent, Singapore, 0511

A 9 base pair sequence GCGGGGGCG has been identified as a high affinity binding site for the immediate-early gene Egr-1 using a recommbinant Egr-1 protein. In this study, we try to detect the binding of the native cellular Egr-1 protein to its recognition site in mobility shift DNA-binding assay. Using a 26 base pair oligonucleotide containing the Egr-1 binding site and flanking sequences in the Egr-1 promoter region as a probe, two specific binding complexes have been detected from nuclear extracts of various cell lines. The lower complex was undetectable in the untreated cells, but was enhanced by treatment of cells with serum, TPA, as well as okadaic acid. The time course of the binding was correlated to the production of Egr-1 protein induced by these agents and the binding was destroyed by incubation with the antibody against the Egr-1 fusion protein. The upper complex was formed constitutively in both treated and untreated cells and competed out by an excess of the oligonucleotide containing Sp-1 site , but not by Ap1 or NF kappa B sites. Antibody against Sp1 destroyed the binding. The formations of both complexes were inhibited by zinc chelators EGTA and 1,10-phenothroline. The regulation of transcription by Egr-1 and Sp1 through this overlapping sequence is under investigation.

B 123 HUMAN β-GLOBIN LOCUS CONTROL REGION (LCR): ANALYSIS OF THE 5' HS 2 SITE IN TRANSGENIC MICE, John J. Caterina, Carl A. Pinkert, Richard R. Behringer and Tim M. Townes, Department of Biochemistry, University of Alabama at Birmingham, Birmingham, AL 35294

A region of DNA located far upstream of the human β-globin locus is critically involved in the regulation of the β-globin gene family. This region, which consists of 5 developmentally stable, DNase I hypersenitive sites, is designated the β-globin Locus Control Region or LCR. LCR sequences have 2 important functions. First, they organize the entire β-globin locus into an "open" or DNase I sensitive domain and, secondly, they serve as a powerful enhancer of ε-, γ- and β-globin gene transcription. Specific sequences involved in these functions have recently been identified and cDNA clones that encode LCR binding factors have been isolated. Definition of the activities of these proteins will provide important insights into the mechanisms of LCR action.

B 124 RAP30 AND RAP74 HAVE SEPARATE FUNCTIONS IN THE INITIATION AND ELONGATION OF RNA CHAINS, Chun-hsiang Chang, Corwin F. Kostrub and Zachary F. Burton, Department of Biochemistry, Michigan State University, E. Lansing, MI 48824

RAP30 and RAP74 comprise subunits of the transcription factor called variously RAP30/74, TFIIF, βγ or FC. This factor is required for accurate transcription by RNA polymerase II, in addition to the other general initiation factors. Using recombinant RAP30 and RAP74, the functions of these subunits have been tested separately during the initiation and elongation phases of transcription. RAP30 is required for initiation of transcription, but RAP74 is dispensible for all initiation processes. RAP74, however, is essential for early elongation and may be required for promoter escape by polymerase. RAP30 is required to form a sarkosyl-resistant complex at 0.25% sarkosyl, showing that RAP30 is an initiation factor. RAP74, however, can be added to the transcription complex after sarkosyl, showing that this factor is dispensible for initiation functions. The same result is obtained using a pulse-chase protocol in which accurately initiated RNA is labeled during a short pulse, followed by a chase with excess unlabeled nucleoside triphosphates. RAP30 is required in order to label the transcript during the pulse, but RAP74 is not. Therefore, RAP30 is an initiation factor. RAP74 must be added during the chase, however, in order to obtain a run-off transcript. The following conclusions can be drawn from these experiments: 1) RAP74 is not required for RNA polymerase II to initiate phosphodiester bond formation from a promoter; 2) RAP74 is not required for ATP hydrolysis in initiation; and 3) RAP74 is required for early elongation of the transcript. We are currently doing experiments to determine the size of the nascent RNA synthesized in the absence of RAP74, to see how close to the promoter polymerase has stalled.

B 125 A DISTAL HEAT SHOCK ELEMENT PROMOTES THE RAPID RESPONSE TO HEAT SHOCK OF THE HSP26 GENE IN YEAST, *Junjie Chen and David S. Pederson*, Department of Microbiology &Molecular Genetics, University of Vermont School of Medicine, Burlington, VT05405

Induction of heat shock genes in response to heat and other stresses is mediated by Heat Shock Factor (HSF). Our recent footprinting experiments (in preparation) demonstrate that HSF binds constitutively to two heat shock elements (HSE's) in the promoter of the HSP26 gene in the yeast Saccharomyces cerevisiae. Here, we have investigated the role in transcription of the distal-most HSE (HSE-p), previously reported to not be involved in regulating expression of the HSP26 gene. Site-directed mutagenesis of HSE-p significantly reduces the rate of induction of transcription by heat shock and moderately reduced the maximum amount of HSP26 mRNA accumulated during heat shock. Binding of HSF to the mutated HSE cannot be detected by genomic footprinting, suggesting that reduced transcription is due to the failure of HSF to bind to the mutated HSE sequence. A synthetic promoter containing several HSE's induces transcription faster than does a synthetic promoter containing a single HSE. However, as with the native HSP26 gene, increasing the number of HSE's increased only moderately the amount of mRNA accumulated during heat shock. These results suggest that long range cooperative interactions among HSF molecules may stabilize their binding to DNA, and that as few as tow HSF:HSE complexes are sufficient to saturate HSF's target in the basal transcription apparatus.

B 126 TRANSCRIPTIONAL ACTIVATORS AND PREINITIATION COMPLEX ASSEMBLY, Bob K. Choy, Michael R. Green, Program in Molecular Medicine, University of Massachusetts Medical Center, Worcester, MA 01605

Accurate transcription by RNA polymerase II (RNAPII) involves a complex ordered assembly of general transcription factors on the promoter to form a preinitiation complex. The level of transcription can be modulated by transcriptional activators which appears to work by increasing the extent and/or the rate of preinitiation complex assembly. Previous experiments have used transcription from immobilized DNA templates to infer that acidic activators recruit TFIIB. In addition, the acidic activators were found to interact directly with the general transcription factor TFIIB. We have developed a gel filtration system to isolate preinitiation complexes, or stably DNA associated subcomplexes, from unbound factors. General transcription factors stably associated with DNA are detected by immunoblotting. We find that the activator increases the amount of TFIIB, but not TFIID, that is stably associated with the DNA template. Furthermore, factors that enter the complex following TFIIB (*eg.* $TFIIF^{30}$, RNAPII) also are found to be recruited by the activator. This corroborates the findings that TFIIB is the first factor whose binding is enhanced by acidic activators. We have also found that glutamine-rich and proline-rich activators also increased TFIIB assembly to a TFIID-DNA complex. However, the recruitment of TFIIB is not sufficient to explain how activators work. For example, cloned TATA-box binding protein (TBP) cannot support activation with acidic activators, yet it is able to support TFIIB recruitment. From this and other studies, the physical basis of TFIIB recruitment appears to be cooperative binding of TFIIB to promoter-bound TBP and the activator. However, factors after TFIIB are not recruited by the activator without TAFs. Furthermore, a single-bound activator recruits TFIIB as effectively as multiple-bound activators; however, multiple-bound activators are required for recruitment of factors entering after TFIIB, and transcriptional stimulation. Based on these results, we propose that activators must affect (directly or indirectly) multiple steps in the assembly pathway to stimulate transcription.

B 127 REGULATION OF THE EGFR GENE IN BREAST CANCER CELLS: CHROMATIN STRUCTURE ANALYSIS REVEALS THE INVOLVEMENT OF INTRON 1 SEQUENCES, Susan A. Chrysogelos, Lombardi Cancer Center and the Department of Biochemistry, Georgetown University, Washington, DC 20007

The progression of human breast cancer from hormone-responsiveness to a more aggressive, estrogen-independent state is often characterized by the loss of estrogen receptor (ER) and the acquisition of high levels of epidermal growth factor receptor (EGFR). Despite the tendency for an inverse relationship with ER, EGFR is a strong prognostic indicator independent of ER status. That is, coexpression of EGFR with ER overrides the benefits of ER expression and predicts for poor survival. Indeed, EGFR has been shown to be a better predictor for failure on endocrine therapy than ER status, strongly supporting the hypothesis that up-regulation of EGFR is a critical step in the progression to estrogen independence. To identify regions of the EGFR gene which are functionally implicated in its regulation, the DNase I sensitivity of the EGFR gene was analyzed in several human breast cancer cell lines with a wide range of EGFR levels. These studies indicate that there are structural differences in the promoter, first exon, and intron 1 of the EGFR gene which correlate with its expression. Specifically, a site around the exon 1/intron 1 boundary disappears in high expressors, while a group of sites in intron 1 appears in these cell lines. Additionally, a region in the promoter shows changes in both the level of sensitivity and the extent of the area which is susceptible. The appearance of new sites in intron 1 associated with an increase in expression indicates that these regions of the gene are probably involved in the binding of positive regulatory factors, while the loss of a site at the exon 1/intron 1 boundary as the gene is up-regulated suggests the action of a repressor which may block transcriptional elongation. Fine mapping of these regions by native genomic blotting has revealed the presence of multiple protein binding sites in both the promoter and first intron of the EGFR gene. Experiments are in progress to define the regulatory functions of the sequence elements which comprise these sites through transient transfection assays using CAT expression vectors in human breast cancer cell lines expressing high and low levels of EGFR.

B 128 IDENTIFICATION AND CHARACTERIZATION OF ESTROGEN RECEPTOR-ASSOCIATED PROTEINS

Carolyn J. Church and Geoffrey L. Greene; Dept. of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637

The human estrogen receptor (hER) is a member of the super-family of steroid hormone receptors that are activated by a ligand and subsequently bind specifically to DNA elements in the region of responsive genes, resulting in transcriptional activation or repression, depending on the target gene and tissue. Although much has been learned regarding the structure and composition of functional domains for the receptors, little information is available concerning the role of cellular accessory proteins required to mediate receptor function. We have used immuno-, steroid-, and site-specific DNA-affinity chromatography to purify proteins intimately associated with hER in extracts from MCF-7 human breast cancer cells and CHO cells stably transfected with hER. Analysis of eluted proteins by SDS-PAGE reveals a similar pattern by each technique, albeit with some differences. As expected the 66 kDa hER was observed in each case. In addition, a 70 kDa band, identified by Western blot as a member of the heat shock protein 70 family, was also observed. Another species that copurified with hER was a 55 kDa protein, identified by N-terminal sequencing as protein disulfide isomerase. Two additional bands at 40-45 kDa, which were observed only when hER was bound to DNA, await identification. Current efforts are focused on evaluating the effect of agonists and antagonists on hER interaction with the accessory proteins by treatment of cells in culture with these agents and subsequent immuno- or DNA-affinity chromatography. The structural domains involved in the interaction between hER and the associated proteins will be mapped by two methods: 1) hER antibodies directed to different domains of the receptor will be used to immunoprecipitate the complex and 2) mouse ER deletion mutants will be used to isolate the complex by the chromatographic approaches described above. These studies will yield valuable insight into the contribution of these accessory factors to hER function and, ultimately, an improved understanding of the molecular mechanism of hER mediated gene regulation.

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B 129 COOPERATION OF USF AND FOS-RELATED PROTEINS FOR COMPLEX FORMATION AND TRANSCRIPTION STIMULATION, Jan J.Cornelis[1],Serge Plaza[2], Christiane Dinsart[1] and Jean Rommelaere[1], [1]Angewandte Tumor Virologie, Deutsches Krebsforschungszentrum, Heidelberg, Germany, and [2]Unité d'Oncologie Moléculaire, Institut Pasteur de Lille, Lille, France.

The upstream promoter region of the non-structural gene of the virus minute-virus-of-mice (MVM) carries in its distal part a sequence with the E-box CACATG which binds a complex that contains the transcription factor USF/MLTF/UEF which is characterized by a basic DNA binding region and as dimerization motifs a helix-loop-helix domain and a leucine repeat. Electrophoretic mobility-shift assays and cross-linking of binding proteins to the target DNA followed by immunological identification of binding proteins revealed that a Fos protein forms part of the complex. Competitive electrophoretic mobility shift assays showed that both Fos and USF cooperate for binding of the complex to the E box. In contrast, no Fos protein was involved in the protein complex that binds to the prototype core USF-binding motif. Disruption of the binding site led to a reduction of the transcriptional activity of the resident promoter as measured by reporter gene assays. Our results point to the possibility that the transcriptional cooperation is mediated by a protein complex containing USF, Fos and possibly another component recognizing a low-affinity USF-binding site.

B 130 IDENTIFICATION OF A REGION IN THE HORMONE BINDING DOMAIN OF BOTH THE ANDROGEN AND GLUCOCORTICOID RECEPTORS THAT IS INVOLVED IN TRANSCRIPTIONAL ACTIVATION: CONSTRUCTION OF A SUPER-SENSITIVE RECEPTOR Mark Danielsen, Shimin Zhang, and Xiayuan Liang, Department of Biochemistry and Molecular Biology, Georgetown University Medical School, 3900 Reservoir Rd., N.W., Washington, DC 20007.

In order to map the regions of the hormone binding domain of steroid receptors that are involved in hormone binding specificity, we have constructed a series of hybrid proteins that consist of the glucocorticoid receptor (GR) with up to 15 amino acids of the androgen receptor (AR) systematically replacing the equivalent GR sequence. One of these constructs, GA11, has 10 amino acids of the AR replacing the corresponding region of the GR. This hybrid protein, unlike wild type GR, is now induced by androgens. Surprisingly, this induction is not due to a change in hormone binding specificity of the hybrid, rather the hybrid responds to 50 to 100 fold less hormone than wild type GR. Even more surprisingly, this super-sensitive receptor binds glucocorticoids with near normal hormone binding constants. Taken together these data suggest that the hybrid GA11 maps a region of both the GR and AR that interacts with the transcriptional machinery.

B 131 DISSECTION OF THE FIRST ZINC FINGER DOMAIN OF EVI-1 SHOWS THAT NOT ALL FINGERS BIND DNA, Ruud Delwel, Brent Kreider and James N. Ihle, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101 and Department of Hematology, Erasmus University, Rotterdam, The Netherlands

The Evi-1 gene has been implicated in the etiology of acute myelogenous leukemia in mice and in man. The gene encodes a zinc finger (ZF), DNA-binding protein, that contains an amino-terminal first domain of seven fingers and a carboxyl domain with three fingers. To study the DNA binding abilities of the first domain, a glutathione-S-transferase (GST) fusion protein was generated. Using an oligonucleotide containing a central 35 base random sequence (35R) and PCR approaches a consensus binding sequence of GA(T/C)AAGA(T/C)AAGATAA was defined. To determine which fingers were responsible for DNA binding, GST-fusion constructs of fingers 1-3, 2-4, 3-5, 4-6 and 5-7 were made. Only ZF 4-6 and 5-7 bound the consensus sequence. Binding and PCR amplification studies using an oligonucleotide containing a core of 15 random bases, demonstrated that only ZFs 4-6 and 5-7 bound DNA and yielded a consensus of GA(T/C)AAGATAA. To further evaluate the contribution of ZFs 1-3, GST-fusion proteins containing ZF 2-7, 3-7, 4-7 were compared with a construct containing ZF 1-7. Binding and amplification experiments with the 35R demonstrated that ZFs 1-7 were required for selection of the full consensus sequence, with progressively less ZFs, selection of the 15 bp core changed to selection of a 10 bp core and finally a 5 bp core. The various GST-fusion proteins were also used in gel shift assays with oligonucleotides of GACAAGATAA(CT), GATAAGATAA(TT), GATAAGACAA (TC), GACAAGACAA(CC). Constructs containing ZFs 1-7 bound the oligonucleotides in the order CT>CC>TT=TC However, all other constructs bound the four olionucleotides comparably. The results demonstrate that the first domain has a DNA binding consensus of 15 nucleotides with a repetitive element composed of GA(T/C)AA. DNA binding is predominantly through ZFs 4-7 although the full first domain is required for optimal binding and confers an element of sequence specificity that distinguishes GACAA from GATAA in the first core recognition element. The significance of the ability of Evi-1 to bind to GATA containing elements will be discussed.

B 132 HOMEODOMAIN DNA SEQUENCE RECOGNITION AND COOPERATIVITY, Claude Desplan, Nathalie Dostatni, Thomas Lecuit, Guojun Sheng and David Wilson, Howard Hughes Medical Institute, The Rockefeller University, New York City 10021.

The extensive homeodomain (HD) family of transcription factors mediates a large number of specific developmental regulatory functions. However, the various putative HD binding sites which have been defined are composed of only five to six base pairs and exhibit little selectivity in distinguishing between divergent HDs. This lack of specificity is consistent with the small size of the HD and with the fact that it has been shown to bind DNA as a monomer. Because few natural HD binding sites have been identified and very little is known about the DNA binding specificity differences between HDs, we employed a new technique ("SELEX") for isolating, from random synthetic DNA libraries, optimal DNA binding sites for different HDs.

We applied this *in vitro* selection to the HD from the product of the *Drosophila* segmentation gene *paired (prd)*, related to the mouse *pax-3* gene. Surprisingly, we find that the HD is by itself capable of binding as a cooperative dimer to a palindromic sequence, each half of the palindrome resembling the previously defined HD consensus sequence TAATTG. This implies that some of the previously identified HD binding sites constitute half-sites. We have also found that the ninth amino acid position of the recongition helix modulates the dimeric nature of the Prd HD. Depending on the identity of this residue, the cooperative binding can either be abolished, or induced to occur with an altered spacing between monomers. Furthermore, this residue specifies the preferred base pairs at the center of the palindrome, which is consistent with the previously defined role of this position to interact with the bases immediately downstream of the TAAT core recognition sequence for monomeric HDs.

The ability of dimeric homeoproteins to recognize long, and therefore rare, palindromic sequences may increase their specificity to that required *in vivo*. We present examples of other HDs from the Prd class and from divergent classes (such as the Hox class) which cooperatively bind to palindromic DNA sequences. Interestingly, some HDs can bind cooperatively as heterodimers, thus multiplying the number of potential target sequences recognized by homeoproteins.

B 133 SPECIES SPECIFIC INTERACTION OF THE GLUTAMINE - RICH ACTIVATION DOMAINS OF Sp1 WITH THE TATA-BOX BINDING PROTEIN, TBP. Andrew Emili, Jack Greenblatt and C. James Ingles. Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada, M5G 1L6

Several sequence-specific activators of RNA polymerase II mediated transcription in eukaryotic cells have been shown to interact directly with the TATA-box binding protein (TBP), a subunit of the general initiation factor TFIID. Our laboratories previously demonstrated a specific interaction of TBP with the potent acidic activation domain of VP16, which can activate transcription in the cells of such divergent species as yeasts, insects, and mammals. Unlike VP16, however, the GC-box binding factor Sp1 activates RNA polymerase II directed transcription in mammalian and insect cells, but not in yeast. By using techniques of affinity chromatography, we have now assessed the ability of the activation domains of Sp1 to interact with the TBPs of distinct species.

The glutamine-rich activation domains A and B of Sp1 were expressed in *E.coli* as fusions to GST. As with purified GST-VP16, we found that GST-Sp1(domain A) coupled to a column matrix could bind directly to human TBP. GST-Sp1(domainA) also interacted specifically with *Drosophila* TBP, but failed to bind to yeast TBP. Domain A of Sp1 also bound to an N-terminal truncated form of human TBP, suggesting that Sp1 contacts species-specific residues within the otherwise conserved C-terminal region of TBP. Similar results were obtained using domain B of Sp1 as the affinity column ligand. The ability of the Sp1 activation domains to interact only with TBP derived from species in which Sp1 can activate transcription suggests that this contact plays a role in Sp1-mediated transactivation.

B 134 CHARACTERIZATION OF YEAST RNA POLYMERASE II TRANSCRIPTION FACTOR b AND CTD KINASE, William John Feaver, Opher Gileadi and Roger D. Kornberg, Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305

Yeast RNA polymerase II initiation factor b was purified from whole cell extracts based on its ability to restore transcription activity to nuclear extracts inactivated by mild heat treatment. Factor b was found to copurify with three polypeptides of 85, 73 and 50 kilodaltons and with a protein kinase activity that phosphorylates the COOH-terminal repeat domain (CTD) of the largest polymerase subunit. The gene encoding the 73 kilodalton subunit has been cloned and was found to be essential for cell growth. The deduced protein sequence exhibited no similarity to known protein kinases. However, the sequence was found to be similar to that of the 62 kilodalton subunit of Hela transcription factor BTF2, suggesting that this factor is the human counterpart of yeast factor b. Affinity purified polyclonal antibodies raised against the 73 kilodalton subunit were found to inhibit both transcription and CTD kinase activities. Photoaffinity labeling with 3' - O - (4 - benzoyl)benzoyl-adenosine 5'-triphosphate (ATP) identified an ATP-binding site in the 85 kilodalton polypeptide suggesting that this subunit may contain the catalytic domain of the kinase.

B 135 INTERFERONγ RAPIDLY INDUCES IN HUMAN MONOCYTES A NOVEL DNA-BINDING FACTOR THAT RECOGNIZES THE GAMMA RESPONSE REGION WITHIN THE PROMOTER OF THE GENE FOR THE HIGH AFFINITY Fcγ RECEPTOR, David S. Finbloom, Kevin C. Wilson, Gerald M.Feldman, Division of Cytokine Biology, Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892

Interferonγ (IFNγ), a cytokine secreted from stimulated T cells, is a potent activator of monocytes and macrophages and, therefore, is a critical component for both host defense and inflammation. IFNγ transcriptionally activates several early response genes in monocytes which are important for the ultimate phenotype of the activated macrophage. One of these genes is the high affinity Fc receptor for IgG, (FcγRI). Recently Pearse et al (Proc. Natl. Acad. Sci. 88:11305, 1991) defined within the promoter region of the FcγRI gene an element, the gamma response region (GRR), which was necessary for IFNγ induced enhancement of FcγRI. We describe the induction by IFNγ of a novel DNA binding factor, FcRFγ, (FcγRI DNA binding factor, IFNγ induced), that specifically recognizes the GRR element. Electrophoretic mobility shift assays (EMSA) demonstrated the presence of FcRFγ in human monocytes within one minute after exposure to IFNγ. On EMSA, FcRFγ consisted of two complexes termed $FcRF\gamma_1$ and $FcRF\gamma_2$. Preliminary purification and UV-crosslinking studies suggest that $FcRF\gamma_2$ is a 40-50 kDa protein. $FcRF\gamma_1$ may be either a dimer of $FcRF\gamma_2$ or one molecule of $FcRF\gamma_2$ complexed with another protein of about equal size. The nuclear concentration of FcRFγ rapidly increased, peaked at 15 min, and then fell after 1-2 hr. Dose response studies revealed: 1) as little as 0.05 ng/ml IFNγ induced FcRFγ, 2) maximum activation occurred at 1 ng/ml; and 3) steady state levels of FcγRI mRNA closely paralleled that of FcRFγ. Since FcRFγ was activated in cells normally not expressing FcγRI RNA, other regulatory mechanisms must control FcγRI restricted tissue expression. Activation of FcRFγ by IFNγ was inhibited by pretreatment with 500 nM staurosporin and 25 μM phenyl arsine oxide. These data suggest that a kinase and possibly a phosphatase activity are required for IFNγ induced signalling of FcRFγ in monocytes.

B 136 DESIGN OF HIGH AFFINITY DNA BINDING MYC ANALOGUES: CRITICAL SIDE CHAIN AND CONFORMATIONAL REQUIREMENTS, David E. Fisher and Phillip A. Sharp, Center for Cancer Research, MIT, Cambridge, MA 02139 and Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

The Myc oncoproteins belong to the b-HLH-ZIP family and recognize the palindromic core sequence CACGTG. This family of proteins forms homo- or heterodimers via interactions in helix-loop-helix and leucine zipper domains and contacts DNA via an adjacent "basic domain" of approximately 20 amino acids. Using gel filtration and sedimentation analyses, these proteins have been shown to form tetramers in solution but bind DNA as dimers. This DNA contact has been analyzed in detail to shed light on biochemical features of this recognition process. Methylation interference studies demonstrated that binding occurs within the major groove of DNA. To determine critical amino acids for DNA recognition, the basic domain sequences of CACGTG-binding proteins were analyzed for homologies. Conserved positions were detected every 3-4 residues. This spacing is reminiscent of consecutive turns of alpha-helical peptide and suggested that the basic domain utilizes one face of an alpha-helix to contact DNA. Using the protein TFEB as a homodimeric model, circular dichroism was performed in the absence and presence of target DNA. Alpha-helical content was shown to increase significantly upon addition of DNA, suggesting that the basic domain is disordered in the absence of DNA, but alpha-helical in its presence. Exhaustive "alanine scanning" mutagenesis was undertaken in the basic domain. Four conserved amino acids were shown to be critical as demonstrated by obliteration of DNA binding by their mutation. Three other residues strongly stabilized the interaction. Based on their positions, the presence of a DNA "anchoring" subdomain has been postulated. DNA bending was also analyzed, electrophoretically, for 5 proteins in this family. All were found to produce minor groove oriented bends of 74^{o}-82^{o}. Utilizing the structural information from these studies, artificial Myc basic domain analogues were designed. Basic region positions predicted not to contact DNA were systematically mutated to alanine. Six wild-type residues ultimately remained within a polyalanine backbone. Binding affinities as much as >30 fold higher than c-Myc's basic region were obtained. Stronger DNA binding may arise from enhanced alpha-helicity of polyalanine. This design strategy may be applicable to other helix-dependant biomolecular interactions. High affinity Myc family analogues may be useful in the design and study of protein:DNA interactions and biological effects of Myc induced oncogenesis.

B 137 TRANSCRIPTIONAL REPRESSION BY THE THYROID HORMONE RECEPTOR *IN VITRO*.
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The thyroid hormone receptor (TR) belongs to the steroid/nuclear receptor superfamily of ligand inducible transcription factors. Numerous studies have demonstrated that in the absence of thyroid hormone, the unliganded TR acts as a repressor of transcription on genes bearing TR-response elements (TRE's). In order to examine the molecular mechanism of this repression, we expressed the human TRα1 cDNA in both *E. coli* and Sf9 cells (using baculovirus) and tested whether hTRα1 would repress transcription from a series of heterologous promoters containing TRE's. Using an *in vitro* transcription asssay, hTRα1 repressed transcription: 3-fold from a human immunodeficiency virus-1 (HIV-1) promoter; 6-fold from an adenovirus major late (AdML) promoter; and 13-fold from a herpes thymidine kinase (TK) promoter. Interestingly, hTRα1 repressed transcription from AdML and HIV-1 promoter constructs containing only minimal promoter sequences (the TATA element and the initiation region). These data suggest that unliganded TR inhibits transcription by either direct or indirect interactions with one or more of the general transcription factors. We are currently investigating which specific target factors and steps in preinitiation complex assembly are negatively affected by the TR.

B 138 CLONING OF PROTEINS THAT INTERACT WITH THE TRANSCRIPTIONAL ACTIVATION REGION OF THE ADENOVIRUS E1a PROTEIN, Christian C. Fritz and Michael R. Green, Program in Molecular Medicine, University of Massachusetts Medical Center, 373 Plantation St. , Worcester, Massachusetts 01605

The Adenovirus E1a protein contains a potent transcriptional activation domain. Several lines of evidence suggest that E1a's activation region interacts with a unique cellular target, that is not used by, for example, acidic activation regions.
To isolate such an 'adaptor' protein we have used a gentic screen in yeast (Fields and Song 1989) to identify mammalian proteins that specifically bind to the transcriptional activation region of E1a. A human c-DNA library was constructed as a fusion to the potent activation region of the herpes simplex virus VP16 protein. This library was transformed into a yeast strain expressing a GAL4-E1a fusion protein. c-DNA sequences that encode proteins that bind to E1a are predicted to activate transcription through a GAL4-E1a : cDNA-VP16 activator complex.

In a screen of 10^{6} yeast colonies 500 primary positives were obtained. From these primary candidates, two overlapping c-DNAs were isolated whose protein products bind specifically to the E1a portion of the GAL4-E1a fusion protein. Several mutant E1a proteins were analyzed for binding to these candidates in the yeast assay. With one notable exeption, mutants in the cysteine rich region of the activation domain that are defective for transactivation do not bind the protein encoded by these two c-DNAs. When these partial c-DNAs are expressed in an antisense orientation in mammalian cells, one clone increases transcriptional activation by GAL4-E1a. Since this effect does not seem to be limited to activators carrying an E1a activation domain, we are currently investigating whether this protein could be a general negative factor. We are also testing whether this protein which is present in nuclear extracts, appears associated with TBP in the TFIID complex. Comparison of nucleic acid sequences from these clones with a current version of the Genbank database indicates that this gene has not previously been characterized.

B 139 **BIOCHEMICAL ANALYSIS OF DISTINCT MECHANISMS OF BASAL TRANSCRIPTION FROM DIFFERENT PROMOTERS,** Catherine P. George and James T. Kadonaga, Department of Biology, University of California, San Diego, La Jolla, CA 92093

RNA polymerase II transcription requires a host of general transcription factors, in addition to RNA polymerase II, for the accurate initiation of basal level transcription. These general factors have been purified to various degrees and many have been cloned. The precise role of each factor in the initiation of transcription is still in question. Recent data has indicated that there is more than one mechanism by which pre-initiation complexes assemble on a promoter. We have found that different basal promoters exhibit distinct properties. Class II promoters differ in their requirement for each general factor. Additionally, the ability to be squelched by either excess TFIIB or excess TBP, and the ability to be transcribed by pol II and a minimal set of purified recombinant factors are properties that vary from promoter to promoter. We have constructed a series of minimal and hybrid promoters to identify and to characterize the sequences that confer these properties to each promoter. Intriguing results will be presented.

B 140 POLYMERASE PROCESSIVITY ON THE DROSOPHILA hsp70 GENE, Charles Giardina and John T. Lis,
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The uninduced hsp70 gene in Drosophila is associated with a paused RNA polymerase II molecule that has an associated transcription bubble in the region of +14 through +42 (as detected in vivo by the single-stranded DNA probing reagent, $KMnO_4$). Upon heat-shock induction, transcription of the hsp70 gene increases over 100-fold. Interestingly, DNA melting at this pause site is unchanged after induction, indicating that the polymerase continues to pass through a pausing step. Presumably, the rate of both paused polymerase release and pause site filling are increased equally - about 100-fold - upon heat shock induction. Heat-shock induction also generates single-stranded regions over the transcriptional start site, as well as at sites downstream of the pause site. These additional regions of melting could represent polymerases loading on to the gene and leaving the pause site.

It is believed that the purine nucleotide analog DRB causes the formation of less-processive, terminator-prone RNA polymerase II elongation complexes. We were interested in determining the effect of DRB on the release of the paused polymerase on the hsp70 gene. It was found that DRB does not affect the level of polymerase at the pause site in uninduced cells (as determined by in vivo UV crosslinking and $KMnO_4$ probing). In induced cells, polymerases are sufficiently processive in the presence of DRB to transcribe through the pause site, but terminate transcription before reaching the 3' end of the gene. Interestingly, DRB reduces the degree of DNA melting over the transcriptional start site. We propose that the melted start site complex inhibited by DRB is a station at which the polymerase is modified. This modification is not critical for the elongation of polymerase through the pause site, but is critical for transcription through to the end of the gene.

B 141 INTERACTIONS BETWEEN DISTAL ENHANCER AND SEPARATE AND DISTINCT TISSUE SPECIFIC PROMOTER ELEMENTS DETERMINE APO-LIPOPROTEIN AI GENE EXPRESSION IN LIVER AND INTESTINE, Geoffrey S. Ginsburg and Sotirios K. Karathanasis, Department of Cardiology, Beth Israel and Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115

The genes coding for apolipoproteins AI (apoAI), CIII (apoCIII) and AIV (apoAIV) are closely linked and tandemly organized within a less than 15 kilobase (kb) DNA segment in the genomes of various mammalian and avian species. In mammals all three of these genes are expressed predominantly in the liver and the intestine. To determine whether expression of the apoAI gene in liver and intestine is controlled by the same or distinct transcriptional mechanisms we transfected various *human apoAI* gene plasmid constructs into human hepatoma (HepG2) and human colon carcinoma (Caco-2) cells and studied their expression. The results showed that separate and distinct apoAI promoter elements determine expression of the apoAI gene in HepG2 and Caco-2 cells. A DNA element located approximately 5-kb 3' to the apoAI gene and which spans portions of the coding regions in the apoCIII and apoAIV genes including their intergenic region (apoCIII/apoAIV element) dramatically enhanced transcription of the apoAI gene in both HepG2 and Caco-2 cells but only when combined with the corresponding promoter elements. Cotransfection experiments revealed that the hepatocyte nuclear factor 4 (HNF4) transactivates apoAI gene expression in Caco-2 but not in HepG2 cells and further potentiates transcriptional enhancement by the apoCIII/apoAIV element in Caco-2 cells. These results suggest that the decision to transcribe the apoAI gene in liver or intestine is controlled by separate and distinct tissue specific apoAI promoter elements and that the level of transcription is further enhanced by the apoCIII/apoAIV element. Furthermore, these results suggest that HNF4 facilitates functional interaction(s) between the apoAI promoter and the apoCIII/apoAIV element in intestinal cells.

B 142 A Y-BOX CONSENSUS SEQUENCE IS REQUIRED FOR BASAL EXPRESSION OF HUMAN MULTIDRUG RESISTANCE (*mdr*1) GENE
Merrill E. Goldsmith, Mary Jane Madden, Charles S. Morrow, and Kenneth H. Cowan, Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Resistance to many natural product antineoplastic drugs is caused by overexpression of the multidrug resistance (*mdr*1) gene which encodes a drug efflux pump. The mechanism responsible for this overexpression in patient tumors is not understood. Basal transcription of the human *mdr*1 promoter was studied by chloramphenicol acetyl transferase (CAT) reporter fusion gene analysis in two parental and doxorubicin-resistant human tumor cell lines. Previous work from our laboratory demonstrated that deletion of *mdr*1 promoter sequences from -4740 to -136 relative to the start of transcription (at +1) had little effect on *mdr*1 promoter activity. In our current experiments deletion of *mdr*1 DNA sequences from -456 to -88 caused a modest increase in expression. Deletion of nucleotide sequences from -88 to -69, however, resulted in a 5-10 fold reduction in mdrCAT expression. Further deletion of sequences between -69 to -38 essentially eliminated mdrCAT activity. The region between -69 to -38 contains several putative GC boxes or Sp1 recognition sequences. DNase I footprint analysis demonstrated that the region from -84 to -69 was protected from nuclease digestion. The sequence between -81 and -72 is perfectly homologous with the 10bp Y-box consensus sequence found in the promoters of all major histocompatibility complex class-II (MHC II) genes. The Y-box sequence in MHC II genes is required for accurate and efficient transcription and contains the sequence CCAAT in the reverse orientation (Dorn *et al.* (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84, 6249-6253). Single or double point mutations in the reverse CCAAT sequence of the Y-box consensus reduced expression of mdrCAT vectors 5-10 fold. A double point mutation in this sequence eliminated nucleoprotein binding in an electrophoretic mobility shift assay. Several distinct families of proteins which bind to the Y-box consensus sequences in other genes have been identified. Our results suggest that proteins which bind to the putative Y-box consensus sequence are critical for basal transcriptional regulation of the human *mdr*1 gene.

B 143 INTERACTION OF THE PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR WITH OTHER NUCLEAR PROTEINS

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The peroxisome proliferator activated receptor expressed in the baculovirus system binds to a DNA-target element (PPARE) in the promoter of the acyl-CoA oxidase gene which is constituted by a direct repeat of the hexameric motif AGG(A/T)CA spaced by one nucleotide (Tugwood et al. (1992) *EMBO J.* 11, 433-439). We purified PPAR to >90% homogeneity from baculovirus infected insect cells by Ni^{2+}-chelate chromatography after tagging the amino terminus of the receptor with 6 histidine residues. Purified PPAR does no longer bind to its DNA-recognition motif but DNA-binding can be reconstituted by addition of extracts from non-infected Spodoptera frugiperda cells (Sf-9).

In analogy to the heteromerization of other nuclear receptors with the retinoid X receptor (RXR), rRXRα can substitute the PPAR complementing activity in Sf-9 insect cells. Thus, *in vitro* translated rRXRα supplements binding of PPAR to a PPARE. Furthermore, the expression of RXR in CHO cells is a prerequisite for the response to peroxisome proliferators of a reporter gene under the control of a PPARE upstream of the Herpes Simplex thymidine kinase promoter.

Additionally to rRXRα, an activity present in nuclear extracts from COS cells appears to enhance binding of PPAR to its responsive element. Different electrophoretic mobilities of PPAR containing protein-DNA complexes formed with Sf-9 cell extracts, COS cell extracts or rRXRα suggest that PPAR can interact with several distinct nuclear factors. The implications of heteromerization of PPAR with other nuclear factors for the response to activators of PPAR such as WY 14,643 or fatty acids will be discussed.

B 144 DYNAMIC INTERPLAY BETWEEN THE GLUCOCORTICOID RECEPTOR AND A LIVER-SPECIFIC FACTOR DURING TRANSCRIPTIONAL ACTIVATION OF THE RAT TYROSINE AMINOTRANSFERASE GENE

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The tyrosine aminotransferase (TAT) gene is expressed specifically in liver cells where its transcription is increased by glucocorticoid hormones. Two remote glucocorticoid responsive units (GRUs) interact cooperatively to promote full induction of the TAT gene (1). These two GRUs function in a cell-type specific way (2, 3). They are constituted of multiple contiguous and overlapping binding sites for three trans-acting factors: the glucocorticoid receptor (GR), C/EBP (and the C/EBP-like family) and a liver-specific factor: HNF5 (3). One of the GR-binding site is also an HNF5-binding site and these two factors cannot interact simultaneously with that site (4).

In vivo footprinting analysis of the two GRUs reveal that: 1) the interaction of the GR with DNA does not last long enough to be detectable, 2) before maximal GR-mediated activation of transcription is achieved (10 min.), one of the GR-binding site is occupied by HNF5 and not by the GR, 3) the interaction of HNF5 with its target sites appears to require beforehand an alteration of the chromatin structure which is mediated by the activated GR over only one of the two GRUs (4).

Based on these observations, we propose a hit-and-run mechanism for GR-mediated activation of transcription of the TAT gene which involves GR-dependant alteration of chromatin structure.

1. Grange *et al* (1989) Nucl. Acid Res. **17**, 8695-8709.
2. Grange *et al* (1989) Exptl. Cell Res. **180**, 220-233.
3. Grange *et al* (1991) Nucl. Acid Res. **19**, 131-139.
4. Rigaud *et al* (1991) Cell **67**, 977-986.

B 145 A MEMBER OF THE NF1 FAMILY BINDS TO DISTINCT CIS-ACTING ELEMENTS FROM THE PROMOTER AND 5'-FLANKING REGION OF THE HUMAN CRBP1 GENE. Sylvain L. Guérin, Winnie Eskild*, Jacques Simard, and Vidar Hansson*, Molecular Endocrinology Laboratory, Laval University Medical Center, Québec, Canada, G1V 4G2 and *Institute of Molecular Biochemistry, University of Oslow, 0317 Oslow 3, Norway.

The gene encoding human cellular retinol-binding protein 1 (CRBP1) is expressed in a number of tissues and particularly high levels are found in liver, epididymis and testis. To better understand how this gene is controlled, we have studied the nuclear proteins which bind to the promoter and 5'-flanking region of the CRBP1 gene and the effects of such interaction on gene expression in several cell types. Within the first 500 base pairs of the 5'-flanking and promotor region, DNaseI footprinting identified 7 specific sequences which interacted with nuclear proteins from liver and prostate. Two of these sequences (designated as Fp1 and Fp5) were highly homologous, sharing the core sequence "GGCCAAC", which is homologous to the half site of the concensus sequence for the NF1 binding site. Our results from competition experiments in both gel mobility shift and DNaseI footprinting indicated that a commom protein interacted with both elements. Furthermore, immunological and biochemical data indicated that this protein belongs to the NF1 family of transcription factors. Transient transfections of several cell types were used to study the ability of the Fp1 and Fp5 sequences to control gene expression. Only Fp1 possess the ability to induce reporter gene expression, when inserted upstream of the heterologous basal promotor from the mouse p12 gene. Hence, despite the close sequence homology, the common core sequence and a similar ability to bind nuclear proteins in vitro, Fp1 and Fp5 appear to function differently in vivo.

B 146 ARABIDOPSIS RNA POLYMERASE II SUBUNIT GENES. Tom J. Guilfoyle, Tim Ulmasov, Rob Larkin, Xiang Yang Shi, and Yi Li, Department of Biochemistry, University of Missouri, Columbia, MO 65211

We have cloned full length Arabidopsis cDNAs and genes for the three largest subunits (205, 135, and 35 kDa) and a small subunit (19.6 kDa) which are found in plant RNA polymerase II, but not in I or III. The three largest Arabidopsis RNA polymerase II subunits are related to bacterial RNA polymerase β', β, and α subunits, respectively. The two larger subunit cDNAs were obtained by screening an Arabidopsis cDNA library with yeast probes and the smaller subunit cDNAs were obtained by subunit antibody screening of an Arabidopsis cDNA expression library. Each gene, with the possible exception of the α homologue, is present as a single copy in the Arabidopsis genome. We are testing whether the plant subunits can complement yeast cells defective in specific subunit genes. We are expressing the cloned cDNAs in *E. coli* and in *in vitro* transcription/translation systems to study subunit interactions. The promoters of the genes have been fused to the *E. coli uidA* reporter gene which encodes β-glucuronidase (GUS), and the expression patterns of the genes are being analyzed in transgenic tobacco plants.

B 147 **DMS-II IS REQUIRED AND SUFFICIENT FOR THE RNA POLYMERASE II RNA CLEAVAGE ACTIVITY** Hongliang Guo and David H. Price, Department of Biochemistry, University of Iowa, Iowa City, IA 52242

RNA polymerase II can remove nucleotides from the growing point of nascent RNA in the presence of the elongation factor, S-II, which was initially shown to suppress pausing and stimulate elongation. We examined the *Drosophila* factor, DmS-II, using a dC-tailed template transcription system and showed that purified, bacterially expressed, DmS-II was able to induce the polymerase to back-up. The backup activity required an intact transcription elongation complex and was Mg^{2+} dependent. The RNA cleavage activity was sensitive to α-amanitin. The shortened transcripts remained in elongation complexes and could be chased into longer transcripts. Delaying the addition of nucleotides to the reaction containing DmS-II (allowing backup) did not effect the ability of the factor to suppress pausing or stimulate elongation in the presence of nucleotides. The DmS-II mediated nuclease activity of RNA polymerase II was not dependent on the RNA being in heteroduplex. Kinetic experiments indicated that the DmS-II mediated backup is very fast. More than 80% of the short released products are dinucleotides with some mononucleotides also being released. The RNA cleavage is an obligatory step during suppression of pausing by DmS-II. Other details of the action of DmS-II are currently being examined.

B 148 DIFFERENTIAL ACTIVATION OF TRANSCRIPTION BY GLUCOCORTICOID AND PROGESTERONE RECEPTORS ON CHROMATIN TEMPLATES. Gordon L. Hager, Trevor Archer and Catherine Smith, Lab of Molecular Virology, National Cancer Institute, Bethesda, MD 20892.

The mouse mammary tumor virus (MMTV) promoter adopts a phased array of six nucleosomes when introduced into cells. The transcription factor NF1 is excluded from its binding site in the promoter by this nucleoprotein structure. In contrast, on transiently introduced templates, NF1 is bound constitutively. Thus, induction by glucocorticoids is a bimodal process involving receptor-dependent remodelling of chromatin and direct receptor-mediated recruitment of additional transcription factors.

We have addressed the ability of different receptors to mediate chromatin remodelling, as opposed to transactivation via protein-protein interactions in transient transfections. Using a B-galactosidase-based fluorescence-activated cell sorting procedure, we obtained a cell population greatly enriched in transfected cells. We report that in this population transiently-expressed progesterone receptor can activate transiently-transfected MMTV templates, but not corresponding chromatin templates. Therefore, in contrast with the endogenous glucocorticoid receptor, newly expressed progesterone receptor does not productively interact with the chromosomal MMTV promoter in these cells. These results indicate that nucleoprotein structure can mediate differential interaction of transcription factors with the in vivo template.

B 149 **The Identification of a Truncated Form of HNF1-β Which Potentially Functions as a Repressor of Transactivation by HNF1-β**

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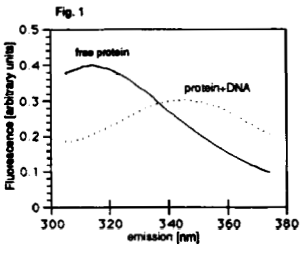
HNF-1α and 1β (also known as LF-B1 and LF-B3) are distantly related to the homeodomain protein family. HNF-1α and 1β are unique among mammalian homeoproteins in that they dimerize, via their amino termini, before binding to their target DNA sequence. We have recently cloned a cDNA for a naturally occurring truncated form of HNF1-β, which encodes the amino terminal part of the protein, but lacks the putative DNA binding homeodomain and carboxy-transactivation domain. This gives rise to the possibility that a dominant-negative heterodimer of HNF1 proteins could be formed. Western analysis shows that the truncated form of HNF1-β exists at a high concentration in liver. The liver contains nearly equal amounts of full-length HNF1-α and HNF1-β protein, but in gel mobility shift assays virtually all of the DNA binding activity is due to HNF1-α. We are currently testing the hypothesis that the truncated form of HNF1-β preferentially dimerizes with full length HNF1-β, creating a non-DNA binding dimer, similar to the MyoD/Id interaction.

B 150 PURIFICATION AND CHARACTERIZATION OF YEAST RNA POLYMERASE II GENERAL INITIATION FACTOR g, N. Lynn Henry, Michael H. Sayre, and Roger D. Kornberg, Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305

RNA polymerase II general initiation factor g was purified to near homogeneity from yeast whole cell extract on the basis of its function in a reconstituted transcription system. Polypeptides of 30, 54, and 105 kDa co-purified with transcriptional activity, forming a complex with a mass of 300 kDa as judged by gel filtration, but only 100 kDa based on sedimentation in glycerol gradients. After separation of the three polypeptides under denaturing conditions, transcription activity could be reconstituted; the 54 and 105 kDa subunits were both essential, while the 30 kDa subunit was slightly stimulatory. The factor was required for initiation at all promoters tested, including those from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and adenovirus. Factor g can stably associate with RNA polymerase II, as shown by co-sedimentation in a glycerol gradient.

B 151 THE BINDING OF YEAST TBP TO DNA: ANALYSIS OF KINETIC AND THERMODYNAMIC PROPERTIES WITH FLUORESCENCE TECHNIQUES. Stefan Hermann and Diane K. Hawley; Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, OR 97403



Yeast Tata Binding Protein (yTBP) is a 27 kilodalton protein that binds to DNA in a sequence specific manner. yTBP has a single tryptophan, located in its N-terminus, that emits fluorescent light when excited at 275 nm. The emission maximum is at 315 nm. Upon binding to DNA the fluorescence is quenched and the emission maximum shifts to 345 nm (see fig. 1). We have used these signals to follow the kinetics of binding of yTBP to various DNA sequences. From the temperature dependance of the kinetic parameters we were able to estimate activation energies. Using titration techniques, we have determined the binding constants for low affinity binding sites.

B 152 FUNCTIONAL ANALYSES OF ATF-2 STRUCTURE,

James P. Hoeffler[1], David J. Kroll[1], Gary L. Johnson[2], Lynn Heasley[1], Joseph Avruch[3], John M. Kyriakis[3], and Hany Abdel-Hafiz[1]. [1]University of Colorado HSC, Denver, CO 80262, [2]National Jewish Hospital, Denver, CO 80262, [3]Mass General Hospital East, Charlestown, MA 02129.

Dimerization of leucine zipper-containing proteins has characteristically been associated with the formation of a coiled coil structure between two compatible leucine zipper motifs. In the present study we demonstrate the association of the leucine zipper of CREB with a zinc finger motif of ATF-2. The association of the CREB leucine zipper with the ATF-2 zinc finger is stabilized if the ATF-2 leucine zipper is intact, implying that the preferred interactive structure of ATF-2 juxtaposes the amino-terminal zinc finger motif of this protein with the carboxy-terminal leucine zipper of this same protein. Furthermore, we demonstrate that the association of the CREB leucine zipper with the ATF-2 zinc finger *in vitro* blocks the association of the adenoviral E1a protein with ATF-2. Similarly, overexpression of full-length CREB, or a truncated version of this protein corresponding to the carboxy-terminal 74 amino acids which make up the DNA-binding and dimerization domains, can block the ATF-2 mediated transcriptional stimulation by E1a *in vivo*. We have expressed full-length and truncated peptides corresponding to various regions of the ATF-2 protein in bacteria and the baculovirus insect cell system in order to investigate the precise mechanisms and structural requirements for productive transcriptional coupling by this factor. Analyses of these recombinant ATF-2 products demonstrated that bacterially expressed truncated (350-505), but not full-length ATF-2, was able to bind a consensus CRE-containing oligonucleotide, suggesting the N-terminal moiety may serve as a negative regulator of DNA-binding activity. In contrast, the full-length ATF-2 protein expressed utilizing a recombinant baculovirus was fully competent to bind DNA. Protein phosphatase 2A reversed the DNA-binding activity by dephosphorylating the ATF-2 polypeptide. MAP kinase catalyzed the phosphorylation and stimulated the DNA-binding activity of bacterially expressed full-length ATF-2. Phosphopeptide mapping of phosphorylated ATF-2 proteins identified a single peptide in the N-terminal moiety of ATF-2 phosphorylated by p42 or p54 MAP kinase (MAPK). Therefore, we propose that phosphorylation of this regulatory site is sufficient to induce an allosteric structural change in the ATF-2 protein, which allows dimerization and subsequent DNA-binding.

B 153 ANALYSIS OF TATA-BINDING PROTEIN AND ASSOCIATED FACTORS

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Using a panel of monoclonal antibodies specific for the TATA-binding protein (TBP) we have examined the composition of TBP-containing complexes in human cell lines of various origin. Immunoprecipitation experiments with [35S]-methionine and [32P]-phosphate labeled lysates have resulted in the identification of at least 10 putative TBP-associated factors (TAFs). These proteins remained associated with TBP under both mild and stringent assay conditions, indicating that the interaction of these TAFs with TBP is relatively stable. The metabolic half-life of TBP was found to be more than 10 hours in HeLa cells, implying that TBP itself is a stable protein.

Surprisingly, no major differences in TBP-TAF complexes could be detected when lysates from several cell lines were compared. Immunoprecipitation and immunoblotting analysis showed that the mobility of TBP in denaturing protein gels differed significantly between individual cell lines. We are currently investigating the nature of these differences in TBP mobility.

B 154 CLASSIFICATION OF TATA BOX SEQUENCES,

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The DNA sequence of the TATA box is highly conserved, and mutations in the TATA box alter the level of transcription both *in vivo* and *in vitro*. In addition, studies from several laboratories have indicated that certain activator proteins require particular TATA box sequences to activate transcription. To understand the relationship between the sequence of the TATA box and the mechanism of preinitiation complex assembly, we are studying the effect of TATA box mutations on both transcription initiation *in vitro* and the binding of the general transcription factor TBP. We have generated a number of basepair mutations in the TATA box of the Adenovirus Major Late promoter, which contains a consensus TATA box sequence. We have characterized these altered sequences for their transcriptional activity, affinity for TBP, and for the stability of the resulting TBP:TATA complexes. These assays have allowed us to place different TATA box sequences into several classes. The implications of our findings to the role of TBP in transcription initiation and to mechanisms of activation will be discussed.

B 155 FUNCTIONAL SELECTION AND CHARACTERIZATION OF BINDING SITES FOR THE E. COLI *TRP* REPRESSOR,

Barry K. Hurlburt and Piotr J. Czernik, Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205

trp repressor controls transcription initiation in four operons involved in tryptophan biosynthesis: *trp*, *aroH*, *trpR*, and *mtr*. The dimeric repressor, when bound by two molecules of L-tryptophan, binds symmetrical operator sequences within the promoters of the regulated operons. The structure of the crystalline *trp* repressor/operator complex lead to a controversial proposal: "indirect readout", the conformation of the operator resulting from the constituent bases, was responsible for specificity of binding. We applied the functional selection/PCR amplification strategy (variations of this technique have been termed: CASTing, SELEX, SAAB, etc) to examine the DNA-determinants of specificity for the *trp* repressor/operator interaction without bias based on known operator sequences. Purified *trp* repressor was coupled to Sepharose CL6B and the resultant affinity matrix was used to select binding-competent DNAs from a randomized 30-bp template (complexity $\approx 1 \times 10^{16}$). DNAs with tryptophan-dependent, high-affinity binding were eluted by competition with the tryptophan analog β-indole acrylic acid and amplified by PCR. Following 7 cycles of selection and amplification, the percent-bound DNA had increased 500-fold and reached saturation. The selected DNAs were cloned, sequenced and used in binding-site mapping and quantitative binding affinity measurements. The consensus sequence from the selected DNAs defined only a half-site. This result supports previous biochemical and genetic studies indicating that the two halves of *trp* repressor bind DNA independently. Evolution of a binding-competent half sites could have occurred independently.

B 156 ANALYSIS OF THE REGULATORY REGION OF THE MURINE GENES FOR THE β SUBUNITS OF THE GM-CSF, IL-3 and IL-5 RECEPTORS, Takashi Iwamoto, Daniel M Gorman, and Atsushi Miyajima, DNAX Research Institute, Palo Alto, CA94304

The high affinity receptors for hematopoietic growth factors, IL-3, IL-5, and GM-CSF are composed of α and β subunits. Each high affinity receptor has a cytokine-specific α subunit which binds its ligand with low affinity by itself. There are two homologous β subunits (AIC2A and AIC2B) in the murine receptors. AIC2A is the specific β subunit for the IL-3 receptor and AIC2B is shared by the three receptors. These receptor subunits are expressed mainly in hematopoietic cells. To study the cell type specific expression of these genes we have characterized the chromosomal genes for the two mouse β subunits, AIC2A and AIC2B. Both genes were mapped on murine chromosome 15 and consist of 14 exons and span about 28 kb each. Sequence analysis revealed that they are 95% identical up to 700 bp from the transcription initiation sites. Interestingly, potential recognition sequences for hematopoietic transcription factors including GATA-1 and PU.1 are present in the 5' flanking region and a stretch of 20 bp including the initiation site is homologous to the corresponding region of the erythropoietin receptor and the IL-7 receptor genes. Using the luciferase gene as a reporter gene we found promoter activity in the 5' flanking sequences. We are currently analyzing these sequences to find regulatory elements responsible for transcription.

B 157 REGULATION OF VON WILLEBRAND FACTOR GENE EXPRESSION INVOLVES GATA ELEMENT. Nadia Jahroudi and Dennis C. Lynch. Dana-Farber Cancer Institute and Departments of Medicine and Pathology, Harvard Medical School, Boston, MA 02115

von Willebrand factor is a large heavily glycosylated protein which is expressed exclusively in endothelial cells and megakaryocytes. We have studied the mechanism of transcriptional regulation of von Willebrand factor (vWf) gene and identified the cis-acting elements which regulate its highly restrictive cell type specific expression. Plasmids containing fragments of vWf 5' region fused to the human growth hormone structural gene were transiently transfected into bovine aortic endothelial(BAE) cells, which express their endogenous vWf gene and cells such as HeLa and bovine smooth muscle cells which do not. Results of these experiments indicated that DNA element(s) located within the 90 base pairs immediately upstream of the vWf transcription start site can activate the expression of the fused growth hormone gene. This promoter activity was observed when constructs were transfected into all cell types used. Furthermore element(s) located between 300 and 500 base pairs upstream of the vWf transcription start site have a strong inhibitory effect on the observed promoter activity. The effect of the negative element was abolished only in BAE cells when the entire first exon was included in the construction of the expression vector. Thus a fragment of vWf gene spanning 500 bp of 5' flanking region and the entire 246 bp of the first exon appears to regulate the cell type specific expression of the vWf gene. Sequence analysis of the first exon indicated that there is a SP1 binding site, a GATA element, and an octamer binding site located within the terminal 100 bp of the first exon. To investigate the role of these elements in regulation of vWf gene expression, they were subjected to point mutation. Transfection of BAE cells with a plasmid containing the 500 bp 5' flanking region and the first exon containing the mutated GATA site was shown to abolish the expression of this plasmid,while the mutation of either SP1 or octamer binding site individually or in combination had no effect on promoter activity. Gel mobility assay also indicated the formation of a specific complex by the wild typeDNA fragment and the BAE nuclear extract which was different from the complex formed with the fragment containing mutated GATA site. These results suggest that cell type specific regulation of vWf gene is controlled by a complex mechanism which involves a negative regulatory element(s), a core promoter and positive regulatory elements such as the GATA site located downstream of the transcription initiation site.

B 158 **Lipid Modification of FBR (Gag-Fos) Controls Transactivation of Alpha (III) Collagen,** Robert M. Jotte, Nobuyuki Kamata, and Jeffrey T. Holt, Department of Cell Biology, Vanderbilt University,Nashville, TN 37232

Post-translational modification of transcription factors mediates many of the processes involved in cellular growth and differentiation. Previously, we have shown that myristylation of FBR results in decreased transactivation of the TRE/AP-1 binding site (MCB 11:765) and decreased transrepression of the c-fos promoter relative to Fos (MCB 12:876). Here we show that FBR transactivates the alpha (III) collagen promoter. Transient transfections in HeLa cells and 3T3-L1 fibroblasts demonstrate a 25-40 fold induction of the alpha (III) collagen promoter-CAT reporter by FBR which occurs at the level of CAT mRNA. Analysis of FBR/c-Fos chimeras indicates that induction by FBR is dependent upon the N-terminally myristylated glycine in the gag sequence. Detailed 5'deletions and internal deletions of the collagen III promoter in HeLa cells map FBR transactivation to a region within 3 bp of a potential SV40 core C/EBP consensus binding sequence. C/EBP transiently co-transfected in HeLa cells represses the ability of FBR to transactivate the alpha (III) promoter-CAT reporter to a level below basal activity. Gel shift analysis with this SV40 core sequence demonstrates specific binding to C/EBP. However, myristylated FBR inhibits C/EBP binding, while MR (non-myristylated FBR) and c-Fos do not. DNAse I footprinting with HeLa nuclear extracts demonstrates binding in a region overlapping the SV40 core sequence. This footprinted region is crucial to collagen III gene regulation, since the collagen III promoter deleted of -67 to -93 (which lacks FBR transactivation in HeLa cells) is activated in differentiated 3T3-L1 cells which normally down-regulate collagen III expression. These results indicate that FBR's ability to transactivate alpa (III) collagen is attributable to a cotranslational modification due to retroviral transduction. This v-fos specific induction maps to a C/EBP binding region implicated in both negative and positive regulation of alpha (III) collagen gene expression.

B 159 **MFT1: AN ACE1-RELATED PUTATIVE TRANSCRIPTION FACTOR REQUIRED FOR GROWTH UNDER STRESS CONDITIONS**

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We have identified a gene, *MFT1*, which encodes a protein of 417 amino acids with significant sequence similarity to the transcription factor ACE1. ACE1 is a copper-dependent transcription activator of the yeast metallothionein gene, *CUP1*. MFT1 and ACE1 share 53% amino acid identity at their N-terminal domains. This domain is required for DNA- and copper-binding of ACE1. Two amino acids of ACE1 known to be essential for DNA-binding are present in MFT1 at identical positions. The arrangements of the cysteine residues in MFT1 and ACE1 are similar and suggest that MFT1 is also a metal-binding protein.

Loss-of-function mutations in *MFT1* lead to a slow growth of *mft1* mutants, indicating that MFT1 is important even under normal growth conditions. Moreover, *mft1* mutants are hypersensitive against a variety of stress conditions including heavy metal exposure (Cd^{2+}, Pb^{2+}, Zn^{2+}) and oxidative stress (H_2O_2) and they are respiratory deficient. Interestingly, *mft1* mutants are also heat-shock sensitive. They are inviable at 37°C.

We constructed a MFT1-β-galactosidase fusion protein that can rescue normal growth of *mft1* mutants even under stress conditions. This fusion protein localizes to the nucleus, supporting the notion that MFT1 acts as an transcription factor. The target genes are not known at present but they may include known stress-inducible genes.

B 160 THE EBNA2-RELATED RESISTANCE TOWARDS IFNα IN BL CELLS EFFECTS INDUCTION OF IFN-INDUCED GENES BUT NOT THE ACTIVATION OF ISGF-3, Kayoko Kanda, Thomas Decker, Pierre Åman,Margret Wahlström, Bengt Kallin and Alexander von Gabain. Department of Bacteriology, Karolinska Institute Box 60400 S10401 Stockholm,Sweden.

The initial studies with interferons (IFN) concerned their antiviral effect which they induce in target cells. Later studies have disclosed that several lytic viruses have been found to counteract the antiviral action of IFN. A lack of response towards IFN has been attributed to an impaired induction of IFN induced genes (ISGs) in the case of IFN resistant variants of the Daudi cell line and in connection with hepatitisB virus and adenovirus infection. In the case of adenovirus and hepatitisB virus-related IFN resistance, adenovirus E1A and hepatitis virus terminal protein mediate the transcriptional block of ISGs to be due to inhibition of ISGF3 activation. The present result suggest that the insensible effect to IFN is involved in expressing Epstein-Barr virus nuclear antigen 2 (EBNA2) in human lymphoma cells. We studied the expression of ISGs in pairs of cell lines, differing in the expression of EBNA2. In EBNA2 expressing cells, the induction of five ISGs by α-IFN was strongly reduced or in some cases eliminated. CAT reporter gene constructs containing different IFN response elements, ISREs, were transfected into EBNA2 negative and EBNA2 positive cells. Induction of CAT activity by IFNα was suppressed in EBNA2 positive cells. However gel shift assays of EBNA2 positive and EBNA2 negative cells exhibited a nearly identical pattern of ISRE-binding proteins. Most important activation of the ISGF3 was not inhibited in IFN resistant cells expressing EBNA2. The mechanism of the EBNA2 related IFN resistance seems to be distinct from both the resistance mediated by hepatitis virus and adenovirus products as well as from the IFN resistance found IFN resistant in BL cell variants.

References:

Åman,P. and A. von Gabain. (1990) EMBO J., 9, 147-152.

Kanda,K., Decker, T., Åman, P.,Wahlström, M., von Gabain, A. and B. Kallin. (1992) Mol.Cell.Biol., in press.

B 161 **Transcriptional regulation of the rat liver sodium-dependent bile acid transporter gene** Saul Karpen, M. Ananthanarayanan, and Frederick J. Suchy. Department of Pediatric Gastoenterology/Hepatology, Yale University School of Medicine, New Haven, CT 06510

Recently, the cDNA encoding the rat liver sodium-dependent bile acid transporter gene, [*Nbtcp* ;whose product transports bile acids across the sinusoidal surface of the hepatocyte] has been cloned [Hagenbuch *et al* PNAS **88** 10629-10633, 1991]. Analysis of the expression of the *Nbtcp* gene would provide insight into the transcriptional regulation of this integral component of the enterohepatic circulation of bile. We have screened a rat genomic library with the *Nbtcp* cDNA, and isolated three overlapping lambda clones that appear to fully encompass the *Nbtcp* gene. The span is approximately 16 kilobases [kb], and the clones also include 9 kb of 5' upstream, and 5 kb of 3' flanking sequences. Southern blots indicate it to be a single copy gene. Primer extension analysis places the mRNA transcription start site at 10 nucleotides [nt] upstream of the published cDNA sequences. A 1.3 kb fragment spanning from an upstream BglII site to cDNA nt +42 was subcloned into the unique HindIII site of the basic luciferase expression vector pSVoAL Δ5' [de Wet *et al* Mol & Cell Biol **7**, 725-737, 1987]. Four hours after plating primary rat hepatocyte cultures, this recombinant plasmid [p1.3*Nbtcp*] was transfected in serum-free media via lipofection overnight. The cells were harvested 24 hours later. Contro transfections with pSVoALΔ5' and pSV2ALAΔ5' [containing the SV40 promoter-enhancer] were also performed. p1.3*Nbtcp* directed luciferase activity at approximately 10% of the level of pSV2ALAΔ5', and hence appears to contain a functional promoter. Preliminary studies suggest that physiologic concentrations of cholic acid upregulates p1.3*Nbtcp* activity. Sequence analysis of the 5' upstream region reveals homologies to several known transcription factor recognition sequences, including a TATA box at nt -28, and C/EBP, CRE, and GRE binding sites. This assay system will be used to explore the effects of various hormones and bile acids, as well as promoter sequence deletion and mutation experiments, on *Nbtcp* gene promoter activity.

B 162 *SIN1* INTERACTS WITH A PROTEIN COMPLEX THAT BINDS THE REGULATORY REGION OF THE YEAST *HO* GENE. Don Katcoff, Eyal Yona, Orly Dgany, Hadas Friedman and Yael Cohen, Dept. of Life Sciences, Bar Ilan University, Ramat Gan 52900, Israel

Recently, evidence has been mounting suggesting that a number of chromatin components previously thought to primarily or exclusively have structural function, also have a regulatory role in eukaryotic transcription. Notably, in yeast, histone H4 N-terminal sequence has been shown to be required for promoter activation of certain genes in vivo, and mutations in histone H3 *(SIN2)* or in *SIN1* (which has some sequence similarity to HMG1) are able to suppress *swi1, swi2,* and *swi3* mutations, restoring transcription to *HO* as well as a number of other genes. Furthermore, a functional interaction has been shown between the C-terminal domain of RNA polymerase II and the SIN1 protein. We are seeking to answer the question of how SIN1 molecules that are known to bind DNA non-specifically and that are found throughout the chromatin in large numbers, are able to specifically affect the transcription of a relatively small set of genes.

In the work carried out in our laboratory, we have identified a novel protein that specifically binds the *HO* regulatory region on the one hand, and on the other somehow appears to contact the SIN1 protein. The protein that we have identified binds a small defined DNA sequence in the *HO* promoter in-vitro, and is detected in cell extracts made from wild type cells. However, it is undetectable in extracts made from *sin1-2* mutants. We have shown that the DNA binding activity does not contain SIN1, since extracts from *sin1Δ* strains retain the activity. Mixing of wild type with *sin1-2* extracts has shown that one or more components in extracts made from *sin1-2* strains is able to prevent the DNA binding activity in wild-type extracts. *SIN1* and *sin1-2* have been cloned and overexpressed in bacteria as fusion proteins. Sin1-2 fusion protein added to an extract used in a gel retardation assay can dissociate the DNA/protein complex while a similar SIN1 fusion protein has no effect on the complex at similar concentrations. These data imply that the sin1-2 protein can interact directly with the DNA/protein complex. Sequencing of *sin1-2* has revealed that *sin1-2* is a point mutation very close to the C-terminus which substitutes a lysine for a glutamic acid. Interestingly, protein/DNA complex made from *sin1Δ* extracts is far more resistant to dissociation by *sin1-2* extract than the complex made from wild type extracts. Several models of SIN1 function are presented to explain these and published results.

B 163 TRANSCRIPTION FACTORS REGULATING THE EXPRESSION OF NA,K-ATPASE ALPHA 1 SUBUNIT GENE, Kawakami Kiyoshi, Suzuki Yuriko, Hirayama Yasutaka, Masuda Kazuyuki, Watanabe Yuko and Nagano Kei, Department of Biology, Jichi Medical School, Minamikawachi, Tochigi, 329-04 Japan

Na,K-ATPase is the enzyme responsible for maintaining Na^+ and K^+ gradients across the cell membrane. It is composed of two subunits named alpha and beta. Three isoforms have been identified both for alpha and beta subunits. The alpha 1 subunit gene (ATP1A1) is expressed virtually in all animal tissues. We have identified cis-acting elements of the gene which are common to several cell lines of various tissue origin[1]. The elements include Sp1 binding consensus sequenece and the newly identified ARE (ATP1A1 regulatory element). The binding factors to the ARE are cell type specific. That is, C1 and C2 complexes were identified in B103 (rat neuroblastoma cell line) nuclear extract, while C1, C2 and C3 complexes were identified in MDCK (canine kidney cell line) or HeLa nuclear extract by gel retardation assay using ARE sequence as the probe. The core sequence of the ARE is found in several other genes involved in cellular energy metabolism such as malate dehydrogenase and pyruvate kinase, suggesting the sequence to be a common regulatory element responsive to the state of cellular energy metabolism. We have identified two cDNA clones which specifically bind to the ARE by South Western cloning with a probe of ARE sequence. One of them was identical to HEB (Hu et al., MCB12 1031-1042, 1992). The other clone is now under characterization. The binding region of the factors coded by the cDNAs were analyzed with use of GST-fusion protein. The binding region of the factors coded by these clones were distinct from those of gel retardation complexes. The preliminary purification of the binding factors revealed that the factor for C3 complex binds to WGA column, wherease factors for C1 and C2 did not.

[1]Suzuki, Y. et al., MCB12 in press, 1992

B 164 **CLONING OF A *DROSOPHILA* HOMOLOG OF RAP74, A GENERAL RNA POLYMERASE II TRANSCRIPTION FACTOR**

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Factor 5 is a *Drosophila* RNA polymerase II initiation factor which also affects the elongation phase of transcription. To further our understanding of how factor 5 functions during initiation and elongation, we have used a RAP74 cDNA to isolate a full-length cDNA that putatively encodes the large subunit of *Drosophila* factor 5 (factor 5a). A *Drosophila* K_c cell cDNA library was probed at low stringency with a cDNA encoding RAP74, the large subunit of the human counterpart of factor 5. A 1100 bp cDNA with extensive sequence similarity to the amino terminus of RAP74 was isolated. The K_c cell cDNA was used to screen a *Drosophila* embryonic cDNA library and a 1929 bp cDNA with similarity to the entire RAP74 sequence was isolated. The deduced amino acid sequence of the *Drosophila* clone was 43% identical and 65% similar to the sequence of human RAP74. Based on this similarity, we propose that this cDNA encodes the large subunit of *Drosophila* factor 5 (factor 5a). Production of factor 5a using bacterial expression systems or an *in vitro* transcription/translation system yields a protein product of approximately 85 kD, consistent with the molecular mass of the large subunit of purified factor 5. Sequence comparisons of the *Drosophila* and human proteins and the position of introns deduced from the sequence of *Drosophila* genomic DNA suggest that the proteins contain highly conserved N-terminal and C-terminal domains separated by a central region rich that is highly charged (51% D+E+K+R) but less well conserved. Evidence is presented which suggests that at least one copy of the gene for factor 5a in K_c cells has been disrupted by the insertion of a 412 transposable element. In contrast, the factor 5a gene in *Drosophila* embryos or adult flies does not seem to be disrupted by a 412 element.

B 165 ROLE OF DNA BENDING IN TRANSCRIPTION ACTIVATION AND REGULATORY SPECIFICITY AMONG FOS AND JUN FAMILY PROTEINS, T.K. Kerppola and T. Curran, Department of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, NJ 07110

We have previously demonstrated that Fos and Jun induce opposite orientations of DNA bending upon binding to the AP-1 site (Cell, 66: 317, Science, 254: 1210). To investigate the functional role of DNA bending in transcription activation, we have mapped the domains in Fos and Jun that control DNA bending. The region that determines DNA bend orientation was localized to the basic DNA contact surface. A 33 amino acid peptide encompassing the basic region was sufficient to induce a bend in the same orientation as the full length protein. The regions that determine the DNA bend angle were localized to proline-rich domains in both Fos and Jun. These regions coincide with the transcription activation domains of these proteins. In Jun, the amino-terminal repressor region reduced the DNA bend angle. Deletions across this region displayed a perfect correlation between DNA bend angle and transcription activation. Thus, DNA bending is likely to be integral to transcription activation by Fos and Jun.

The AP-1 site is recognized by a large number of bZIP family proteins. To investigate if DNA bending could confer functional specificity to members of this family, we determined the DNA bends induced by twelve bZIP family proteins at several variants of the AP-1 site. Each protein induced a DNA bend of distinct orientation and magnitude. The sequence of the recognition site had different effects on the DNA bend angle depending on the binding protein. Therefore, DNA bending can provide specificity to proteins binding to the AP-1 site. First, different complexes can induce distinct DNA bends when binding to the same site. Second, a complex binding to different sites can have diverse effects on DNA structure.

The structural differences between Fos and Jun correlate with functional differences between these proteins. Several regulatory interactions between Fos/Jun and the glucocorticoid receptor (GR) have been reported. We have analyzed the functional interactions between Fos, Jun and GR *in vitro* using purified proteins. The GR DNA binding domain inhibited DNA binding and transcription activation by Fos-Jun heterodimers, but not Jun homodimers, implicating Fos as the primary target of GR inhibition. Fos and Jun are targets of multiple kinases that phosphorylate different sites in the proteins. Mutation of the phosphorylation sites in Jun had different effects on DNA binding and transcription activation by Fos-Jun heterodimers and Jun homodimers.

B 166 TRANSCRIPTIONAL ANALYSIS OF YEAST TFIID POINT MUTANTS BY ACIDIC ACTIVATORS IN YEAST AND HUMAN IN VITRO SYSTEMS.

Tae Kook Kim[1], Shigeru Hashimoto[1], Peter M. Flanagan[2], Raymond J. Kelleher III[2], Roger D. Kornberg[2], Masami Horikoshi[1,3] & Robert G. Roeder[1], [1]Lab. of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021. [2]Dept.of Cell Biology, Stanford University of Medicine, Stanford, CA 94305. [3]Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tyoko 113, JAPAN.

The TATA-binding factor TFIID plays a key role in eukaryotic transcription initiation. Consistent with its central role in preinitiation complex assembly , TFIID and the derived TATA-binding subunit (TFIIDτ) have been shown to be targets for various regulators including acidic activators. Here, functional in vivo and in vitro analyses were carried out to identify residues in yeast TFIID which are important for transcriptional activation by acidic activators. Three individual point mutations (L114K, L189K and K211L) abolished transcriptional activation by acidic activators (GAL4-VP16/AH) while maintaining basal transcription in a yeast-derived TFIID-dependent in vitro transcription system. With these defective TFIID point mutants, we also analyzed direct interactions with the acidic activation domain of VP16 and the effects on growth of yeast cells. Transcriptional activities of these activation-defective point mutants were also determined in a TFIID-deficient heat-treated nuclear extract from human (HeLa) cells. TFIID mutants L189K and K211L, but not L114K, mediate normal acidic activator responses in the HeLa system. These results suggest that species-specific interactions within TFIID are involved in the function of acidic activators. This species-specificity may reflect differences in the ability of yeast TFIID to interact with cofactors that may be required for transcriptional activation by acidic activators. The inability of mutant L114K to function in either yeast or human systems raises the possibility either that the altered site is involved in direct interactions with acidic activation domains in both organisms or that an evolutionary conserved cofactor function is involved.

B 167 COMPARITIVE STUDY OF THE PROMOTER REGIONS OF THE HUMAN AND RAT SPERMATID TRANSITION PROTEIN 1 GENES TO IDENTIFY DNA-PROTEIN BINDING SITES. Malathi K. Kistler, E.A. Shipwash and W.S. Kistler, Department of Chemistry, University of S. Carolina, Columbia, SC 29210.

During spermatogenesis the nuclear proteins undergo replacement in a stage specific manner. Transition proteins (Tp) are basic proteins that replace histones but are themselves eventually displaced by protamines in the maturing sperm. We have obtained genomic clones for rat and human Tp1, and comparitive alignment of the upstream DNA sequences of these shows remarkable similarities within a 100 base pairs upstream of the transcriptional start sites. Examination of this 100 bp region for potential transcription factor binding sites revealed a cyclic AMP response element (CRE) , a CACC / Sp1box and a CAAT motif. The rat Tp1 gene displays a perfect CRE element (TGACGTCA), while human Tp1 has two nucleotides deviating from the consensus sequence (TGACagCA). Gel mobility shift experiments were conducted using HeLa nuclear protein extract and DNA fragments from rat and human Tp1 upstream regions. A set of retarded bands that were competed only with cold CRE oligo nucleotide were obtained for both human and rat. Another shifted band that was competed with a synthetic 21-mer containing a Sp1 binding site was also obtained with the rat and the human genes. DNAseI footprinting experiments gave protected regions corresponding to the CRE and Sp1 binding sites. These experiments with HeLa cell extracts showed that there are potential binding sites for CRE and Sp1. Gel mobility shift experiments with rat testis nuclear protein extracts and the CRE regions of rat and human TP1 revealed an extra band that is a candidate for a tissue-specific variant of theCRE binding protein.

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B 168 CHARACTERIZATION OF THE ETS-DOMAIN GENE, PU.1, Michael J. Klemsz**, Richard A. Maki and Robert Hromas*, Depts. of **Microbiology and Immunology and *Biochemistry and Molecular Biology and the Walther Oncology Center, Indiana University School of Medicine, Indianapolis, IN., 46202, and La Jolla Cancer Research Foundation, La Jolla, CA., 92037.

The trans-activating protein PU.1 is a member of a growing family of transcription factors related to the *ets* oncogene. The region of identity between these proteins, termed the ETS-domain, encodes for a novel DNA binding motif. We have studied PU.1 and compared it to ets-2 and Fli-1 to understand how ETS-domain proteins bind to DNA and function as transcription factors. Using degenerate oligonucleotides, we have defined consensus DNA binding sites for PU.1 and ets-2. The two proteins are 40% identical in the ETS-domain. Their consensus DNA binding sites are the same at 9 of 12 bases. This difference, however, prevents either protein from binding to the others consensus site. To study the binding motif, we have made amino acid substitutions in the ETS-domain of PU.1. These data show that the conserved amino acids of this motif are required for DNA binding, and defines the ETS-domain as a new DNA binding structure. These three related genes were used in co-transfection studies to determine their ability to trans-activate from appropriate DNA binding sites. Additionally, we have made deletions in the PU.1 and Fli-1 proteins to begin to define their activation domains.

B 169 **CHARACTERIZATION OF p21^{SNF}, A NOVEL bZIP PROTEIN COMPONENT OF TAX-RESPONSE COMPLEX-1 (TRC-1) IN HTLV-TRANSFORMED T-CELLS,** Daniel P. Kolk, Wolfgang M. Klump, Tracy L. Martin, Ian B. Murton, Wolfgang H. Fischer, and William Wachsman, Research Service, San Diego VAMC, The Salk Institute, and Division of Hematology-Oncology, UCSD School of Medicine, La Jolla, CA 92093-0677

The human T-cell leukemia virus (HTLV) encoded Tax is required for efficient viral replication and acts in-trans to regulate expression of specific genes involved in transformed T cells. It appears to act indirectly on several cis-acting sequences, including the cyclic-AMP response element (CRE) and NF-κB sites. Others have identified factors that bind to Tax-response elements, but none that are expressed specifically in HTLV-transformed T cells. We have identified a factor, termed Tax-response complex-1 (TRC-1), that specifically binds the CRE-like 21-bp repeats in the HTLV LTR. TRC-1 is detected only in productively infected HTLV-transformed T cells. Its presence is independent of Tax, however, indirect functional data suggest that Tax is required for transactivation of CRE sequences that interact specifically with TRC-1. We purified TRC-1 by affinity chromatography and determined that it is composed of 4 polypeptides of 21 (p21^{SNF} [small nuclear factor]), 40, 41, and 43 (p40/43) kD. UV cross-linking and reconstitution studies indicated that both p21 and p40/43 are necessary to form TRC-1. Western blot analysis revealed that p40/43 is differentially phosphorylated JunB. Using probes derived from partial amino sequencing of p21^{SNF}, we obtained cDNA and genomic clones of this factor. Analysis indicated that it is a novel member of the basic region/leucine zipper (bZIP) transcription factor family. Northern blot and RNase protection assays showed that *snf* and *jun*B mRNA are constitutively expressed in HTLV-transformed T cells, but are not detectable in the non-infected CEM or Jurkat human T-cell lines. Comparative analysis of *snf* cDNA and genomic clones suggest that it is alternatively spliced in the HTLV-1-transformed SLB-I T-cell line. We are currently investigating whether Tax regulates expression of p21^{SNF} and how Tax regulates the action of TRC-1 on the HTLV LTR and other Tax-responsive genes in T cells.

B 170 START-SITE SELECTION BY RNA POLYMERASE II Richard Kollmar and Peggy J. Farnham, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

Two types of positioning elements control site-specific initiation by RNA polymerase II: A "selector", such as the TATA box, directs the enzyme to the correct region in the promoter; the "initiator" then determines the exact start site within that region. We have compared the sequences of known initiator elements to the eucaryotic start-site consensus and have analyzed published data about their function; we find that all initiator elements belong to the same family.

The minimal functional promoter of the Syrian hamster CAD gene between –81 and +26 contains two Sp1 binding sites and a close match to the start-site consensus, but no consensus TATA box. We are characterizing the positioning elements of the CAD promoter to answer these two questions:

1. Can an Sp1 binding site function as a selector?
2. What sequences define the CAD initiator?

B 171 THE PU.1 ACTIVATION DOMAIN BINDS RB AND TFIID *IN VITRO*: RB SHOWS SEQUENCE SIMILARITY TO TFIID AND TFIIB. Tony Kouzarides, Christian Hagemeier, Andrew Bannister and Alistair Cook, Wellcome/CRC Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QR, U.K.

We have used an assay we term "GST pull-down" to analyse protein-protein interactions between regulatory transcription factors and the TATA-binding factor, TFIID or the Retinoblastoma tumour suppressor protein, RB. In this assay, *in vitro* translated, radiolabelled transcription factors are incubated with GST-TFIID or GST-RB. Following "pull-down" of the GST-fusion with glutathione agarose beads the amount of co-precipitated transcription factor is established. Using this assay we find that five factors, Myc, MyoD, C/EBP, Fos and PU-1 can interact with TFIID and RB whereas two other factors, Sp1 and CTF, cannot bind to either TFIID or RB.

We have characterized further the interaction of TFIID and RB with the PU-1 protein (a lymphoid specific transcription factor). We find a 75 amino acid activation domain of PU-1 contacts the C-terminal, basic region of TFIID and the pocket domain of RB.

These results prompted us to examine the RB and TFIID sequences for primary sequence conservation. This analysis revealed that the RB pocket domain has two separate regions which show homology to TFIID and a second general transcription factor, TFIIB. The TFIID similarity overlaps domain A, whereas the TFIIB similarity overlaps domain B of the RB pocket. These results suggest that RB may mimic functions performed by TFIID and TFIIB.

B 172 ELEMENTS IN THE WHEY ACIDIC PROTEIN GENE NECESSARY FOR POSITION INDEPENDENT EXPRESSION IN TRANSGENIC MICE

Michael John Krnacik and Jeffrey M. Rosen, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030

It is clear that the expression of a gene in a given system, requires not only the appropriate cis and trans-acting elements, but also that the gene in question be situated within a region of the genome capable of transcription, commonly termed "open" or "active" chromatin. Two types of elements have been discovered which seem to have the ability to create active chromatin. They are locus control regions (LCRs) and matrix attachment regions (MARs). When included in exogenous genes, both types of regions can confer integration site-independent expression to the gene. They may or may not enhance the absolute level of expression. Using a functional assay in transgenic mice, we have identified a region necessary for position independent expression of rat milk protein gene encoding whey acidic protein (WAP). This region is more highly conserved than the WAP coding region and is novel in that it is located within the 3'UTR and it has no homology with previously identified LCRs or MARs.

A genomic rWAP transgene (+2020) comprised of 949bp of 5' flanking sequences, intragenic sequences, and extending 70 base pairs into the 3' flanking DNA, was expressed at endogenous levels in a position-independent manner in independent lines of mice. Internal deletions within the +2020 3'UTR or replacement of the rWAP 3'UTR and flanking region by analogous sequences from the SV40 late region resulted in position dependent expression (1). This result has lead us to ask 1) Is the rWAP 3'UTR sufficient for position independent expression of a heterologous transgene? and 2) What is the mechanism of the rWAP 3'UTR influence? In an attempt to answer the first question, we have are characterizing lines of mice carrying a ß-casein promoter/CAT transgene with either the +2020 rWAP 3'end (CCW) or the analogous ß-casein 3'end (CCC). Preliminary results show 4/4 CCW lines express CAT mRNA while only 1/4 CCC lines express. To address the second question, we have begun by using electrophoretic mobility shift assays (EMSAs) to look for specific interactions between the rWAP UTR and nuclear factors *in vitro*. Initial results indicate at least two distinct nuclear factors associate specifically with this region. Finally, we have established an *in vivo* nuclear matrix association assay which we will employ in an attempt to correlate nuclear matrix association with the influence of the rWAP 3'UTR.

1.Dale, T.C., M.J. Krnacik, C. Schmidhauser, C.L-Q. Yang, M.J. Bissell, and J.M. Rosen. (1992) High Level Expression of the Rat Whey Acidic Protein Gene is Mediated by Elements in the Promoter and the 3' Untranslated Region. Mol. Cell. Biol. 12, pp 905-14.

B 173 DUAL FUNCTION OF THE NUCLEOLAR TRANSCRIPTION FACTOR UBF: TRANSACTIVATOR AND ANTIREPRESSOR

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We have established a reconstituted transcription system from mouse cells which contains four partially purified fractions (i.e. RNA polymerase I, TIF-IA, TIF-IB, and TIF-IC) to analyze the mode of UBF action. UBF, the factor which interacts both with the proximal and distal element of the rDNA promoter and with the 140 bp enhancer repeats, is a doublet of 97 and 94 kD polypeptides which originate from alternative splicing. UBF is a phosphoprotein and the degree of phosphorylation is affected by the growth rate of the cells. UBF is phosphorylated both *in vitro* and *in vivo* by casein kinase II. Multiple serine residues in the carboxy-terminal hyperacidic tail of UBF are the targets of phosphorylation.

We have cloned the two forms of murine UBF and show that only the 97kD polypeptide (UBF1) is able to transactivate rDNA transcription. The shorter form (UBF2) is transcriptionally inactive, although it binds with the same affinity as UBF1 to the gene promoter and enhancer. Consistent with their different trans-activating properties, the ratio of UBF1 to UBF2 as well as their corresponding mRNAs changes in response to cell growth and differentiation.

Using co-immunoprecipitation assays, Far-Western blots and protein-affinity chromatography with matrix-bound RNA polymerase I, we demonstrate that UBF interacts specifically with RNA polymerase I. This interaction between UBF and polymerase I appears to have been conserved during evolution since mammalian UBF binds also to highly purified RNA polymerase I from yeast. Specific interaction between UBF and RNA polymerase I involves the 34.5 kD subunit of the yeast enzyme and the 65 kD subunit of the mouse enzyme.

Furthermore, data will be presented showing that an important part of the transactivating function of UBF is to overcome the inhibitory action of a negative-acting DNA binding protein which competes with TIF-IB for binding to the promoter and thus prevents the assembly of preinitiation complexes. This inhibitory factor has been purified to homogeneity and shown to consist of two polypeptides of 75 and 95kD. The complex interplay of positively and negatively acting factors provides a versatile mechanism which enables the cell to adapt the rate of rDNA transcription to a variety of extracellular signals.

B 174 CLONING OF A cDNA ENCODING THE 220 kDa SUBUNIT OF THE HUMAN TRANSCRIPTION FACTOR IIIC

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Transcription Factor IIIC (TFIIIC) is an activity required for the assembly of a pre-initiation complex on 5S RNA, tRNA and adenovirus VA RNA genes. The most purified form of human TFIIIC, which is still able to bind to the internal control region of the VA RNA gene, contains five polypeptides ranging in molecular weight from 220 to 63 kDa (Kovelman and Roeder, in the press). The major subunit (TFIIICα, 220 kDa) can be directly cross-linked to the DNA binding site. As a first step to elucidate in further detail the quaternary structure of TFIIIC, as well as the mechanism of transcription activation and DNA recognition, we have cloned a cDNA encoding TFIIICα. Antibodies raised against the recombinant protein can specifically inhibit RNA polymerase III transcription, and the transcriptional activity can be rescued by adding back partially purified TFIIIC. The results of immunoprecipitation and supershift experiments will also be discussed. The availability of the TFIIICα cDNA clone will allow us to investigate possible modifications of TFIIIC, as well as interactions with other subunits of TFIIIC, and with other polIII factors. Moreover, the expression of an epitope tagged TFIIICα in human cells could constitute a powerful tool for purification and further characterization of natural TFIIIC and associated factors.

B 175 A SINGLE AMINO ACID EXCHANGE TRANSFERS VP16-INDUCED POSITIVE CONTROL FROM THE OCT-1 TO THE OCT-2 HOMEODOMAIN.

Jiann-Shiun Lai[1,2], Michele A. Cleary[1,3], and Winship Herr[1], [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; [2]Genetics Program, and [3]Department of Molecular Microbiology, State University of New York at Stony Brook, Stony Brook, NY 11794

The selective association of the herpesvirus transactivator VP16 with the human Oct-1 homeodomain is a model for differential positive transcriptional control by homeodomains. VP16 discriminates between the closely related homeodomains of Oct-1 and Oct-2 by distinguishing among their seven amino acid differences; these differences lie on the surface that is thought to be accessible when the homeodomain is bound to DNA. Only two of these seven differences are recognized by VP16, one in each of the first two α helices of the tri-α-helical homeodomain. The major determinant for selective association with VP16 in vitro and VP16-induced positive control in vivo is a single glutamic acid residue at position 22 in the first α helix of the Oct-1 homeodomain, but the acidic properties of this residue are not critical for association with VP16 in vitro or in vivo, because it can be replaced by glutamine with little or no deleterious effect. Mere replacement of the single corresponding alanine residue in the Oct-2 homeodomain with the key glutamic acid residue is sufficient to confer on the Oct-2 homeodomain the ability to associate with VP16 in vitro and respond to VP16-induced positive control in vivo. Thus, the specificity of homeodomain positive control can be conferred by a single amino acid difference.

B 176 A NOVEL HUMAN INSULINOMA-ASSOCIATED cDNA, IA-1, ENCODES A PROTEIN WITH "ZINC-FINGER" DNA-BINDING MOTIFS, Michael S. Lan, Yasuhiro Goto, Mark G. De Silva, Antonio Toscani, Bellur S. Prabhakar and Abner Louis Notkins, Laboratory of Oral Medicine, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892

A subtraction library was constructed from human insulinoma (beta cell tumor) and glucagonoma (alpha cell tumor) cDNA phagemid libraries. Differential screening of 153 clones with end-labeled mRNAs from insulinoma, glucagonoma and HeLa cells resulted in the isolation of a novel cDNA clone designated IA-1. This cDNA clone has a 2838 bp sequence consisting of an open reading frame of 1530 nucleotides which translates into a protein of 510 amino acids with a pI value of 9.1 and a molecular mass of 52,923 daltons. At the 3'-untranslated region there are seven ATTTA sequences between two polyadenylation signals (AATAAA). The IA-1 protein can be divided into two domains based upon the features of its amino acid sequence. The N-terminal domain of the deduced protein sequence (1-250 a.a.) has four classical pro-hormone dibasic conversion sites and an amidation signal sequence, Pro-Gly-Lys-Arg. The C-terminal domain (251-510 a.a.) contains five putative "zinc-finger" DNA-binding motifs of the form X_3-Cys-X_{2-4}-Cys-X_{12}-His-X_{3-4}-His-X_4 which has been described as a consensus sequence for members of the Cys_2-His_2 DNA-binding protein class. Northern blot analysis revealed IA-1 mRNA in five of five human insulinoma and three of three murine insulinoma cell lines. Expression of this gene was undetectable in normal tissues. Additional tissue studies revealed that the message is expressed in several tumor cell lines of neuroendocrine origin including pheochromocytoma, medullary thyroid carcinoma, insulinoma, pituitary tumor and small cell lung carcinoma. The restricted tissue distribution and unique sequence motifs suggest that this novel cDNA clone may encode a protein associated with the transformation of neuroendocrine cells.

B 177 CLONING OF A NOVEL TRANSCRIPTION FACTOR THAT BINDS TO THE INTERFERON RESPONSE SEQUENCE OF THE 202 GENE IN MURINE LEUKEMIA CELLS.T S. Landolfo, M. Gariglio, M. Gaboli, Z. Dembic*, R.G. Clerc*. University of Turin, Medical School, Italy; *Hoffmann La Roche, Basel, Switzerland.

The 5' flanking region of the IFN inducible gene 202 contains a 42bp IFN-stimulatable response element (ISRE), called GA box, which has been demonstrated to be necessary and sufficient to make the expression of a gene responsive to IFN. In bandshift assays this GA box yielded with nuclear extraxts obtained from IFN-α/β treated L1210 cells a DNA-protein complex which was maximally induced 30' after IFN treatment. A lambda gt11 cDNA expression library was constructed from poly A+RNA prepared from L1210 cells treated with IFN α/β for 30'. By screening this library (7 x 10^5 recombinant phages) with the GA box as a probe we have isolated one single positive clone that bind the GA box but not other unrelated regulatory sequences. The protein encoded by the 4181bp full length cDNA is 708 Aa in length and has an estimated Mr of 78 kd. Upon cotransfection in NIH3T3 cells, GAbf-1 could transactivate a reporter plasmid in which the GA trimer was linked to the minimal for promoter and the CAT gene. The full length cDNA of GAbf-1 (has been fully sequenced and) showed no homology to any of the previous cloned transcription factors that bind to the ISREs of the IFN-inducible genes and therefore represents a member of a different gene family.

B 178 PURIFICATION OF TATA-BINDING PROTEINS FROM PLANTS, Rob Larkin and Tom Guilfoyle, Department of Biochemistry, University of Missouri, Columbia, MO 65211

We have isolated and sequenced soybean cDNA clones encoding a TATA-binding protein (TBP) of about 22 kDa. We have expressed the N-terminal half of a soybean TBP cDNA in *E. coli* and raised polyclonal antibodies to the expressed TBP. These antibodies recognize TBPs in both monocotyledonous and dicotyledonous plants. We have fractionated wheat germ and other plant WCEs by Polymin P precipitation, phosphocellulose chromatography, and heparin Affigel chromatography and used the TBP antibody to determine how plant TBPs fractionate. The bulk of the TBPs from ungerminated wheat germ and soybean embryos does not precipitate with Polymin P and is found in the Polymin P supernatant. When these Polymin P supernatants are fractionated on phosphocellulose (PC100, PC300, PC600, and PC1000 fractions), nearly all of the TBPs are recovered in the PC1000 fraction, although minor amounts are also found in the PC300 and PC600 fractions. Based on gel filtration chromatography, we estimate that the bulk of the TBPs in ungerminated embryos have a native molecular mass of about 22 kDa and, like yeast, are not associated with TAFs. Upon germination, the TBPs show some alteration in their fractionation patterns. We are attempting to determine if germination results in the association of TAFs with the TBPs.

B 179 Reconstitution of Chromatin with Core Histones Purified from Yeast Strains Carrying Mutations in the N-termini of Histones H3 and H4.

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The objective of these experiments is to better characterize the role of chromatin in RNA polymerase II transcriptional regulation. The approach is to use both biochemical and genetic analyses. The best organism for this two pronged attack is yeast. There exists a large background of genetic studies in yeast on the role of chromatin in transcriptional regulation. These studies form the foundation of the experiments described here. Michael Grunstein and his colleagues have shown through genetic analyses that positively charged residues in the N-terminal tails of histone H3 and H4 have an important role in the activation and repression of transcription by RNA polymerase II [Durrin *et al.* (1991)*Cell*, **65**, 1023-1031; Johnson *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6286-6290]. Mutations in the N-termini of histone H3 and H4 may mimic the hyperacetylation of the basic residues in these domains, which has correlated with actively transcribed chromatin regions, by removing the positive charges. The effect of mutations in the N-termini of histone H3 and H4 on the stability of nucleosomes has been investigated. In addition, the average number of supercoils formed in the DNA template per nucleosome formed during reconstitution with wild type and mutant core histones has been measured. Finally, the relative ability of nucleosomes reconstituted with wild type or mutant core histones to repress RNA polymerase II transcription in vitro has been determined.

B 180 MOLECULAR ANALYSIS OF TISSUE INHIBITOR OF METALLOPROTEINASE (TIMP-1) PROMOTER, Elaine T. Lea, T.K. Chen, and Mark D. Johnson, Departments of Cellular, Molecular and Structural Biology, and Pathology, Northwestern University, Chicago IL 60611

Levels of proteolytic activity by matrix metalloproteinases (ie. interstitial and type IV collagenases) are altered in tumor cells, resulting in enhanced invasive and metastatic capabilities. Thus, TIMP-1, a high affinity inhibitor of matrix metalloproteinases, may help control extracellular matrix stability and degradation. TIMP-1 has been previously shown to be transcriptionally induced by various agents including serum, EGF, PDGF, phorbol esters and TGF-beta. Analysis of a TIMP-1 promoter/ chloramphenicol acetyltransferase (CAT) construct containing 3.9kb of mouse genomic sequence 5′ to the start site confers responsiveness to serum, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), TGF-beta and okadaic acid (OA), in both transient transfections and stable expression assays. Under conditions resulting in the induction of endogenous TIMP gene expression, relative CAT activities are induced as follows: 20% serum (1.5 fold), 100 ng/ml TPA (3.7 fold), 5 ng/ml TGF-beta (1.4 fold) and 100 ng/ml OA (1.5 fold). In addition, corresponding inductions of CAT mRNA are seen. Sequence analysis shows regions of homology to AP-1, AP-2, PEA-3 and NF-1 response elements within 150 bp of the start site. The AP-1 and PEA-3 elements are also found in the promoters of stromelysin and interstitial collagenase, suggesting that the binding of transcription complexes to these sequences may mediate the responses of metalloproteinases and TIMP-1 to mitogens. Understanding this balance will help define the role of TIMP-1 in tissue and wound repair as well as in tumor invasion and metastasis. (Supported by NIH grant CA 49916)

B 181 PURIFICATION OF THE PUTATIVE SOS REPRESSOR FROM *BACILLUS SUBTILIS*, Charles M. Lovett, Jr., Kerry Cho, Thomas O'Gara, and Joanna Lowell, Department of Chemistry, Williams College, Williamstown, MA 01267

Inducible DNA repair in the bacterium *Bacillus subtilis* is similar to the well characterized SOS system of *Escherichia coli*. Exposure of *B. subtilis* cells to a variey of treatments that damage DNA results in enhanced DNA repair capacity, enhanced mutagenesis, and inhibition of cell division. Corresponding to these induced physiological responses, several damage-inducible (*din*) or SOS genes are transcriptionally activated by a mechanism that requires the RecA protein, activated by an SOS inducing signal. We report here the purification of a protein from *B. subtilis* crude extracts that binds specifically to DNA sites upstream of four distinct *din* genes: *dinA*, *dinB*, *dinC*, and *recA*. We show by mutational analysis and competition experiments that the protein binds to the consensus sequence 5'-GAACN$_4$GTTC-3' located within the promoter regions of these four *din* genes. Gel filtration chromatography resolves the DNA binding activity into two distinct peaks corresponding to 15-kDa and 30-kDa proteins, which we attribute to a monomer-dimer equilibrium. The specific DNA binding activity of this protein decreases significantly in RecA$^+$ cells following treatment with UV or mitomycinC; the rate of this decrease correlates with the induction kinetics of *din* promoters and RecA protein. *B. subtilis recA* mutants, deficient in SOS induction, show no change in the level of the DNA binding activity following DNA damaging treatments; however, addition of purified *B. subtilis* RecA, activated in vitro by the addition single-stranded DNA and dATP, abolishes the DNA binding activity in crude extracts from *recA* mutants. Taken together these results suggest that the protein is a *B. subtilis* SOS gene repressor which is inactivated following DNA damage by a mechanism requiring a functional RecA protein. Surprisingly, activated RecA does not abolish the binding activity of the purified repressor; instead, RecA increases the protein's apparent affinity for the putative SOS operators. This suggests that RecA interacts with the repressor but that, unlike *E. coli*, additional factor(s) are involved in the regulation of the *B. subtilis* SOS system.

B 182 TRANSCRIPTIONAL ACTIVATION OF THE GENE ENCODING RAT PEROXISOMAL HYDRATASE-DEHYDROGENASE BY HETEROLOGOUS PEROXISOME PROLIFERATOR ACTIVATED RECEPTORS, S. Marcus, B. Zhang, K. Miyata, S. Subramani*, R. Rachubinski, J. Capone, Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5 and *Department of Biology, UCSD, La Jolla, CA, 92093

A peroxisome proliferator responsive element (PPRE) has been localized to a region between -2965 and -2924 upstream of the transcription start site of the rat hydratase-dehydrogenase gene (*HD*). Transient transfection studies in rat hepatoma cells showed that this element was necessary and sufficient to confer ciprofibrate responsiveness to a heterologous promoter, irrespective of its orientation or position. Gel retardation analysis showed that rat liver nuclear factors could interact with this element in a sequence-specific manner. The minimal PPRE contains 3 imperfect, directly repeated nuclear receptor half-site motifs TGACCT (Fig.), suggesting that the binding activity in rat extracts is due to a peroxisome proliferator activated receptor (PPAR). Co-transfection of unresponsive COS-1 cells with cDNAs encoding either mouse PPAR or *Xenopus* α-PPAR, and a reporter construct containing the *HD* PPRE, led to a 2- to 4- fold induction of reporter activity in the presence of the peroxisome proliferator ciprofibrate. Gel retardation analysis using extracts of cells expressing cDNAs encoding α, β, or γ *Xenopus* PPARs or mouse PPAR, showed that these receptors interact specifically with the PPRE. Mutations in the PPRE that abolished *in vitro* binding to the PPARs also resulted in a loss of ciprofibrate responsiveness *in vivo*. *In vitro* transcribed and translated PPARs could interact with the PPRE only in the presence of a COS-1 cell nuclear extract, indicating that an additional cellular factor is required for PPAR/PPRE interaction. These results indicate that heterologous PPARs can activate the rat *HD* gene and further suggest that PPAR binding to the PPRE is necessary, but not sufficient, to mediate ciprofibrate responsiveness.

-2956 -2924
5′-CCTCTCCTTTGACCTATTGAACTATTACCTACA

B 183 HOMEODOMAIN/LEUCINE ZIPPER PROTEINS IN PLANTS.

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To test the hypothesis that plants, like other eukaryotes, use homeobox- containing genes to control developmental processes, we set out to isolate such genes from plants. We screened cDNA libraries from carrot and *Arabidopsis thaliana* using a degenerate pool of oligonucleotides matching the most conserved part of *Antennapedia*. One clone was isolated from each species. The clone from *A. thaliana* was then used as a probe in a second screen to isolate additional clones from this plant. At present, six homeobox- containing clones have been sequenced.

The homeodomains of all these clones show at most 30-40% overall sequence identity to any homeodomain from the animal kingdom, but contain all four invariant and six out of eight highly conserved amino acid residues found in other homedomains. More remarkably, all proteins contain a putative leucine zipper next to the carboxy- terminus of the homeodomain. The distance between the leucine zipper and the homeodomain is identical in all proteins, indicating that it is critical for proper functioning of these proteins. Although there is no obvious sequence- similarity between these proteins outside the homeodomain/ leucine zipper, all of them have short stretches rich in prolines, acidic residues or residues with hydroxyl groups, which may potentially function as activating domains in transcriptional regulation.

B 184 CHARACTERISATION OF THE TATA-BINDING PROTEIN HOMOLOGUE IN THE EXTREMELY A+T-RICH HUMAN MALARIAL PARASITE, PLASMODIUM FALCIPARUM, Michael B. McAndrew, Paul F.G. Sims and John E. Hyde, Dept. of Biochemistry and Applied Molecular Biology, University of Manchester Institute of Science and Technology (UMIST), Manchester, UK

The TATA-box, present in many RNA Pol II promoters, is recognised by the TATA-binding protein (TBP or TFIID). This, with associated factors, is required for correct initiation of both TATA-containing and TATA-less promoters. TBP is also involved in transcription of RNA pol I and pol III promoters. TBP contains a highly conserved 180 residue C-terminal domain. This encodes a repeated region exhibiting DNA-binding activity, separated by a basic region involved in protein-protein interactions. The N-terminus of TBP is species-specific and mediates interactions with transcription factors such as Sp1 in higher eukaryotes, although its function in lower eukaryotes is unclear.

The genome of the malaria parasite P. falciparum is 81% A+T-rich with many intergenic regions exceeding 90% A+T. Thus, sequences resembling the consensus TATA-box are very common, often appearing several times in the 100bp upstream of genes. We wondered if the malarial TBP homologue recognised TATA sequences, and if so, how it differentiated between genuine and fortuitous occurrences.

We have characterised the gene encoding the plasmodial homologue of TBP. This gene exists as a single copy on chromosome 4 and encodes a protein of 228 aa with a Mr of 26kDa. The C-terminus is overall poorly conserved in comparison to others, exhibiting only 38 and 42% homology to the human and yeast domains. However, residues identified as being functionally important are conserved, as is the overall molecular architecture. DNA binding studies using recombinant plasmodial TBP are currently in progress.

B 185 TRANSCRIPTION FACTOR Sp1 BINDS AN EGF RESPONSE ELEMENT IN THE HUMAN GASTRIN PROMOTER. Juanita L. Merchant, Dale Shumaker, Diane Abraczinkas. Dept. of Internal Medicine, Gastrointestinal Division, University of Michigan, Ann Arbor, MI 48109-0682

Gastrin is the most potent gastric specific growth factor known and is the most potent hormonal stimulus of gastric acid secretion. It is synthesized by the antral G cell from which it is normally released during digestion in response to secretagogues. We have shown previously that EGF stimulates gastrin-reporter constructs and that this response is conferred by a 16 bp GC-rich DNA element (PNAS 86: 3036, 1989). This *g*astrin EGF Response Element or *g*ERE (-^{68}GGGGCGGGGTGGGGGG-^{53}) binds to nuclear protein in DNaseI footprinting and gel shift assays and does not appear to be related to other regulatory proteins that recognize GC-rich DNA elements . In particular, Sp1 did not bind to the *g*ERE element despite the presence of a high affinity Sp1 binding site in the 5' half-site of the gERE (Mol. Cell. Biol. 11: 2686, 1991). We now report that Sp1 does indeed bind to the EGF response element from the human gastrin promoter but only in the presence of zinc ion. Increasing concentrations of zinc added to the gel shift assay induces the appearance of several complexes. The major slower migrating complex binding to *g*ERE is supershifted with Sp1 antibody, comigrates with affinity-purified Sp1 and is competed specifically with the hMTIIa Sp1 binding site. In the absence of zinc, only the faster migrating complex is observed as previously described. This faster migrating complex is not recognized by Sp1 antibody. We therefore conclude that at least one of the slower migrating complexes is actually Sp1 or an Sp1-like protein. The faster migrating complex (*g*ERE binding protein or *g*ERP) comigrates with the 43 Kd protein marker on Southwestern blots. Moreover, when crude MKN-45 nuclear protein resolving adjacent to the 43 Kd marker is excised and eluted, the shifted complex formed by the eluted protein comigrates with the *g*ERP complex on gel shift assays. Although these results suggest that Sp1 may participate in EGF regulation of the gastrin promoter, EGF did not consistently induce the 8.2 kb Sp1 mRNA detected on Northern blots. Instead, regulation of Sp1 binding to *g*ERE may be a function of intranuclear zinc concentrations present after EGF induction rather than synthesis of new protein. In summary, the EGF response element of the human gastrin promoter binds at least two DNA binding proteins: Sp1 and the 43 Kd *g*ERP. Moreover, EGF regulation of the gastrin promoter appears to be the result of a complex interplay of DNA binding proteins whose binding rather than synthesis are differentially regulated.

B 186 FUNCTIONAL AND STRUCTURAL ANALYSIS OF MAMMALIAN TFIID COMPLEXES : SUBUNIT COMPOSITION OF B-TFIID, Rachel E. Meyers[1], H. Th. Marc Timmers[2], Phillip Sharp[1,1]Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge. [2]Laboratory of Physiological Chemistry, University of Utrecht, The Netherlands

The basal transcription factor TFIID, an essential component of the RNA Polymerase II transcription reaction, interacts with the TATA element of promoters. The DNA binding component of human TFIID has been cloned and antisera specific for this 38 kD component show that this protein fractionates into at least two distinct complexes of 300 kD (B-TFIID) and >700 kD (D-TFIID); the majority of the protein being present in the B-TFIID complex. Both complexes stimulate the basal transcription reaction with comparable efficiency. However, in contrast to the previously characterized D-TFIID, transcriptional initiation complexes formed with the B-TFIID complex do not respond to upstream factors containing acidic or glutamine-rich activation domains.

These functional and physical differences are likely to be caused by differences in the protein composition of the TFIID complexes. Using conventional and immunoaffinity chromatography, the B-TFIID complex was shown to consist of the 38kD protein and a 170kD protein of unknown identity. The 170 kD protein appears to be a unique component of the B-TFIID complex and is not contained within the larger D-TFIID complex. We are currently investigating the functions of the B-TFIID complex, and pursuing the purification and characterization of the 170 kD protein.

B 187 *IN VITRO* STUDIES ON THE HSP26 GENE PROMOTER : TRANSCRIPTION FACTORS MEET NUCLEOSOME. Cathy Mitchelmore, Gayl Wall and Peter Becker, European Molecular Biology Laboratory, Meyerhofstr. 1, 6900-Heidelberg, Germany.

Chromatin has been shown to play an important role in the transcriptional regulation of many genes. In the hsp26 gene promoter, a nucleosome is positioned *in vivo* (Thomas and Elgin, EMBO J. 7, 2191-2201, 1988) such that distal Heat Shock Elements (HSE 6 and 7) may be brought into juxtaposition with promoter-proximal elements. Although these distal elements are critical for heat shock induction *in vivo*, our data shows that promoter mutations abolishing Heat Shock Factor binding at these sites are fully active *in vitro* using heat-shocked *Drosophila* transcription extracts. Under these conditions, HSE 1 and 2 retain their functional importance. Using recombinant or purified transcription factors and a variety of nucleosome reconstitution procedures, we are trying to approach the *in vivo* situation. We show that the hsp26 promoter sequence, in the context of 5 kb of genomic DNA, directs strong rotational positioning of nucleosomes *in vitro*, but is not sufficient to specify the translational position observed *in vivo*. We are currently studying the ability of HSF and the GAGA Factor, which both have binding sites on either side of the *in vivo* nucleosome position, to determine the active conformation of the promoter in chromatin. With this bottom-up approach we hope to better understand the role of chromatin in transcriptional regulation of the hsp26 promoter.

B 188 PURIFICATION AND CHARACTERIZATION OF HUMAN TFIIIA, Beth Moorefield and Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021

Human TFIIIA has been purified from HeLa S100 using a combination of classical and affinity chromatographic methods. A single polypeptide of 42 kD copurifies with both 5S gene specific DNA binding and transcription activities and has been positively identified as TFIIIA by its ability to direct 5S gene transcription following renaturation from SDS PAGE gels. In addition, Western blot analysis reveals that this polypeptide is recognized specifically both by polyclonal antisera raised against Xenopus laevis TFIIIA, and by a monoclonal antibody generated to the Xenopus protein. These data suggest that human TFIIIA isolated from a somatic cell line is structurally related to TFIIIA derived from Xenopus oocytes. In addition, direct peptide sequencing of the human protein reveals that mammalian TFIIIA is more highly conserved with respect to the amphibian protein than might be expected from previously published reports. We have used the cross reacting monoclonal antibody to isolate a putative TFIIIA clone from a human placental cDNA library. Characterization of the properties of both the human protein and the cDNA will be presented.

B 189 **ACETYLATION OF HISTONE H4 IN *DROSOPHILA* HEAT SHOCK GENES,** **Rebecca J.L. Munks, Laura P. O'Neill, Jayne S. Lavender and Bryan M. Turner, Anatomy Department, University of Birmingham Medical School, Birmingham, U.K**

Antibodies specific for H4 molecules acetylated at each one of the four lysine residues acetylated in vivo (5, 8, 12, and 16) are being used to investigate the role of H4 acetylation in chromatin function in *Drosophila*. Immunolabelling of polytene chromosomes from larval salivary glands has shown that H4 molecules acetylated at each one of these sites have distinct patterns of distribution through the genome (Turner *et al.*, Cell 69, 375-384). Surprisingly, actively transcribed (puffed) regions do not label particularly strongly with any of these antisera. Thus, H4 acetylated at lys 5, 8, or 12 (lys 16 is rarely acetylated in autosomes) is distributed at only moderate levels through the entire heat shock puff at 87C (3 hsp70 genes) and is absent from the central region of the adjacent puff at 87A (2 hsp70 genes). To examine the distribution of acetylated H4 along hsp70 genes at higher resolution, chromatin fragments (up to six nucleosomes long) have been prepared from cultured Kc cells before and after heat shock and immunoprecipitated with antibodies to acetylated H4. DNA from antibody-bound and unbound fractions is analysed by slot blotting and the protein by Western blotting, to confirm enrichment of acetylated H4 in the bound fractions. Chromatin precipitated with an antibody (K12) to H4 acetylated at lys 8 is enriched about 3-5 fold in hsp70 genes both before and after heat shock. A greater enrichment is seen for the 5' regulatory region, which contains the TATA box and the Heat Shock Elements. Hybridisation of the same slot blots with the coding region of the H4 gene (whose transcription is not affected by heat shock, but is cell cycle dependent) shows no such enrichment either before or after heat shock. These results show that H4 in one or more nucleosomes along the hsp70 gene is acetylated at lys 8 and that the level of acetylation does not change on induction. Experiments in progress will determine whether this is true also for acetylation at other lysines. **Supported by grants from U.K Cancer Research Campaign.**

B 190 SEROTONIN$_{1A}$ RECEPTOR GENE STRUCTURE AND TRANSCRIPTIONAL REGULATION, Bita Nakhai, Annabel Bolos, David Goldman and David A. Nielsen, Section of Molecular Genetics, LN, NIAAA, Bethesda, MD 20892

Serotonergic activity is regulated, in part, by binding of serotonin to the serotonin (5-hydroxytryptamine, 5-HT$_{1A}$) receptor. Binding of the 5-HT$_{1A}$ receptor by 8-OH-DPAT reduces aggression in male rats. Furthermore, serotonin has been shown to be involved in a variety of physiological and behavioral functions such as intolerance to delay and control of temperature and sleep. To define the mechanisms controlling 5-HT$_{1A}$ receptor gene expression, we isolated several overlapping genomic clones from a human genomic library and are constructing gene fusions.

To determine the transcription start site(s), cDNA clones were isolated and sequenced from a human brain stem cDNA library. To confirm these start site(s), we are performing primer extension analysis of mRNA isolated from brain stem. The regulation of 5-HT$_{1A}$ receptor expression is being studied in various tissue culture cell lines that express this gene. The effects of various drugs on 5-HT$_{1A}$ receptor mRNA content in these cells are being investigated to elucidate factors controlling its expression. Various deletions of the 5-HT$_{1A}$ receptor gene promoter have been fused to the luciferase reporter gene. These will be transfected into several tissue culture cell lines to assay for 5-HT$_{1A}$ receptor gene expression. Regions necessary for tissue specific expression and regulation by the various drugs are to be identified through the use of various deletion constructs.

B 191 REGULATION OF c-FOS TRANSCRIPTION BY DNA BENDING MEDIATED BY p62^{DBF}/YY1. Sridaran Natesan and Michael Gilman, Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, New York 11724.

Regulation of c-fos transcription in response to a variety of extracellular signals is mediated by the serum response element (SRE). Previous work from our laboratory identified and characterized a 62 kd SRE binding protein termed Direct Binding Factor (DBF). DBF binds directly to the 5' side of the SRE in an asymmetrical fashion, in contrast to the symmetrical binding of another major SRE-binding protein, SRF. Recent data from Gualberto et al. (Mol. Cell. Biol. 12, 4209) suggested that the DNA-binding properties of DBF resemble those of YY1, a ubiquitously expressed zinc finger protein that functions as a transcriptional repressor or activator depending on the promoter context. We have confirmed using specific YY1 antibodies and affinity-purified DBF that these proteins are identical. We have also identified two additional YY1 binding sites in the c-fos promoter, located at approximately -246 and -55. Our initial analyses of DBF binding activity on various SRE mutants suggested that DBF may be a DNA bending protein. Circular permuation analyses using all three sites in the c-fos promoter showed that both YY1 and DBF bend the DNA in all three sites with the bend center located at the 5' side of the site. Phasing analyses using the SRE confirmed the DNA bending activity by YY1. To test whether DNA bending by YY1/DBF has any role in the regulation of c-fos transcription, we have introduced point mutations that abolish YY1 binding or reverse the orientation of the -55 binding site in a fos-CAT promoter fusion. Transient transfection assays using these constructs show that YY1 binding represses transcription in one orientation and activates in the other. We suggest that this unusual orientation-dependence of YY1 activity reflects its ability to bend DNA, either facilitating or interfering with contact between upstream factors and the basic transcription machinery.

B 192 MURINE HELIX-LOOP-HELIX TRANSCRIPTIONAL ACTIVATOR PROTEINS BINDING TO THE E-BOX MOTIF OF THE Akv MURINE LEUKEMIA VIRUS ENHANCER.
Anders Lade Nielsen, Niels Pallisgaard, Peder Lisby Nørby, Finn Skou Pedersen and Poul Jørgensen. Department of Molecular Biology, Aarhus University, DK-8000 Århus C (Denmark)
From NIH3T3 fibroblast cells, we have recently identified a cDNA sequence that codes for a protein, termed ALF1, with affinity for the E-box sequence of Akv murine leukemia virus. (MCB 12 page 3449 (1992)). The cDNA sequence of ALF1 suggests that we have recovered a gene similar, but not identical, to the gene for the transcription factor A1, the murine analog of the human transcription factor E47. Thus ALF1 codes for a new member of the basic-helix-loop-helix protein family. Two splice variants of ALF1 cDNA have been found, differing by a 72 bp insertion, coding for putative proteins of 682 aa and 706 aa, respectively. The two ALF1 mRNAs are expressed at varying levels in mouse tissues. *In vitro* DNA binding assays, using prokaryotically expressed ALF1 proteins, demonstrated specific binding of the ALF1 proteins to the Akv murine leukemia virus E-box motif ACAGATGG. The consensus binding sequence was determined as (A/C)CAG(G/C)TG(C/G/A/T) with the most frequently found bases underlined. Expression in NIH3T3 fibroblasts of ALF1 protein stimulated expression from an intact Akv enhancer-promoter region, as well as from a minimal promoter, indicating the existence of a transcriptional activator domain in ALF1.

B 193 MOUSE HOMOLOGUES TO *XENOPUS* DNA BINDING PROTEINS INTERACT WITH THE TESTIS-SPECIFIC MOUSE PROTAMINE 2 PROMOTER, B.S. Nikolajczyk[1], M.T. Murray[2] and N.B. Hecht[1], [1]Dept. Biology, Tufts Univ., Medford, MA 02155 [2]Center for Molecular Biology, Wayne State Univ., Detroit, MI 48202.

Y box binding proteins have been implicated in transcriptional control of both HLA class II and hsp 70 genes. The mouse protamine 2 (mP2) gene promoter, defined by transgenic mouse studies, contains a 9 of 12 consensus binding sequence for Y box proteins. The objective of this study is to determine if mouse germ-cell specific Y box binding proteins homologous to the *Xenopus* Y box binding proteins p54/p56 interact with the testis-specific mP2 promoter.

Western blot analysis using anti-p54/p56 antibody identified two immunologically similar proteins at 48 and 52 kDa in transcriptionally active testis nuclear extracts (TNX) but not in brain or HeLa nuclear extract. Both proteins remained soluble after heat treatment, a process utilized for purification of *Xenopus* p54/p56. DNA binding activity of purified p54/p56 and TNX was tested using a 62bp mP2 promoter fragment that contained the consensus binding sequence for Y box binding proteins. Both the denatured DNA fragment (ssDNA) and the isolated sense strand formed one major and two minor complexes with purified p54/p56. These complexes were competed with either double-stranded (ds) or ssDNA, but non-specific DNAs did not compete. Supershifts further confirmed the identity of the ssDNA-p54/p56 complex. dsDNA also bound purified p54/p56, but the anti-sense strand alone did not form specific complexes. Both dsDNA and ssDNA formed multiple specific complexes with TNX. The isolated sense strand formed a single complex with TNX, while the anti-sense strand did not specifically interact with TNX components. Furthermore, heat treated TNX formed a single complex with ssDNA. In conclusion, TNX contains proteins that are immunologically and functionally related to the Y box binding proteins p54/p56, and the Y box of the mP2 promoter specifically interacts with TNX proteins. Supported by NRSA to BSN and HD-28832 to NBH.

B 194 MOLECULAR CLONING AND CHARACTERIZATION OF AN IL-3 PROMOTER BINDING FACTOR. S. Nimer, M. Wolin, K. Kwan, C. Hong, M. Kornuc. UCLA School of Medicine, Los Angeles, CA 90024
To understand the transcriptional regulation of IL-3 expression in T-cells we and others have mapped regulatory elements within the 5' flanking region of the IL-3 promoter. We have correlated the presence of regulatory sequences with the binding of nuclear proteins from T cells. A region, located between bp -165 and -128 (alternately called footprint A, NF-IL3A, or the oct-like/CRE-like sequences), binds T-cell nuclear proteins in DNase I footprinting, electrophoretic mobility shift assays, and methylation interference assays. To characterize the DNA binding proteins that bind to this region (and regulate IL-3 expression) we screened a PHA-stimulated human T-cell cDNA library with radiolabelled concatamerized double-stranded synthetic oligonucleotides containing these IL-3 sequences. We obtained a 1.9 kb full-length cDNA clone that encodes a ~60 kD member of the bZIP family of transcription factors. This factor, which we call NF-IL3A, is similar to if not identical to a transcription factor recently cloned from human placenta, E4BP4. Northern blot analysis of poly A+ RNA demonstrates that this protein is expressed in both resting and activated T cells and PCR analysis of a variety of human hematopoietic cell lines suggests that the gene is ubiquitously expressed in the hematopoietic system. The NF-IL3A gene appears to be a single copy gene on Southern blot analysis. *In vitro* transcription and translation of this protein has just been completed to delineate its precise DNA binding characteristics and characterize its physiologic importance in regulating IL-3 gene expression.

B 195 THE HIV-1 TAT BINDING PROTEIN IS A TRANSCRIPTIONAL ACTIVATOR BELONGING TO A NEW FAMILY OF EVOLUTIONARILY CONSERVED GENES, Bella Ohana[1], Paul A. Moore[1], Steven M. Ruben[1], Christopher D. Southgate[2], Michael R. Green[2], and Craig A. Rosen[1], [1]Department of Gene Regulation, Roche Institute of Molecular Biology, 340 Kingsland St, Nutley, NJ 07110, [2]Program in Molecular Medicine, University of Massachusetts Medical Center, 373 Plantation Street, Suite 309, Worcester, MA 01605

The HIV Tat binding protein, TBP-1 was previously described and identified in our laboratory, through it's interaction with the HIV trans-activator protein, Tat. The observation that TBP-1 interacts with Tat and is localized to the nucleus, suggested the potential involvement of TBP-1 in transcriptional activity. Here we show through protein fusion experiments, that TBP-1, although unable to bind DNA, is a strong transcriptional activator when brought into proximity of several promoter elements. Transcriptional activity depends upon the integrity of at least two highly conserved domains; one resembling a nucleotide binding motif and the other motif common to proteins with helicase activity. Our studies further reveal that TBP-1 represents one member of a large, highly conserved, gene family that encode proteins demonstrating strong amino acid conservation across species. In addition, we have also identified a second family member, that although 77% similar to TBP-1, does not activate transcription from the promoters examined. This finding, together with the observation that TBP-1 does not activate each promoter examined, suggests that this gene family may encode promoter-specific transcriptional activators. Furthermore, in common with other transcriptional activator proteins, TBP-1 has the ability to form homodimers as well as heterodimerise with at least one related family member. The dimerization domain reside outside of the highly conserved region, and resembles a leucine zipper structure.

B 196 MAPPING THE RNA PRODUCT BINDING SITE ON T7 RNA POLYMERASE BY TARGETED MUTAGENESIS

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During transcription DNA directed RNA polymerases go through abortive cycling, a phase characterized by the synthesis and release of transcripts ranging from 2 to 9 bases in length. Once the RNA chain reaches a length of ~9 bases it becomes stably associated with the polymerase and the polymerase enters processive transcription. Muller *et al.*, (**Biochemistry** 27, 1988) obtained evidence that for bacteriophage T7 RNAP this transition is triggered by the binding of the RNA to a site located within the 20 kD N-terminal domain of the enzyme. Patra *et al.*, (**J. Mol. Bio.** 224, 1992) obtained evidence from mutagenic studies that at least part of this RNA product binding site lies within the first 50 N-terminal residues of T7 RNAP. We have saturated the region between T7 RNAP S12 and E48 (inclusive) with point mutations and characterized the abortive cycling behavior of these mutant enzymes. We find that mutations within two regions--E15-N22 and L37-G47--decrease the polymerase's ability to escape abortive cycling. Enzymes with mutations in these regions show increased levels of abortive transcripts, especially of 7-9mer products (corresponding to the RNA length at which the polymerase escapes abortive cycling). Gel-retardation assays reveal that these mutant enzymes display decreased affinity for single-stranded nucleic acids, a feature diagnostic of decreased affinity for the nascent RNA. In the crystal structure of T7 RNAP, E15-N22 and L37-G47 map to two α-helices which form one side of the DNA-binding cleft: these regions are appropriately positioned for binding to a 7-9 base RNA molecule which has its 3' end located in the polymerase's catalytic pocket. These experiments define part of the RNA binding site on the polymerase to which the RNA must bind for the enzyme to escape abortive cycling.

B 197 YEAST RNA POLYMERASE II: FUNCTIONAL REDUNDANCY OF CARBOXY-TERMINAL DOMAIN OF THE LARGEST SUBUNIT IN IN VITRO TRANSCRIPTION. Meera Patturajan, A. Antony and G. Ramananda Rao, Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012, India.

Eukaryotic RNA polymerase II contains two distinct structural domains: 1) a catalytic core consisting of subunits that are homologous to those present in other multisubunit RNA polymerases and 2) a unique carboxy-terminus of large subunit comprising tandem repeats of seven amino acids (YSPTSPS). The evolutionary conservation of the carboxy-terminal domain (CTD) in RNA polymerase II from animals, plants and protists suggests that it plays a fundamental role in transcription. The CTD is very sensitive to proteolytic cleavage, upon which it yields a mixture containing intact RNA polymerase II (consisting of IIO & IIA) and polymerase lacking CTD (IIB).

In Candida utilis CBS 4511, a fodder yeast, purification of RNA polymerase II using standard protocol had resulted in different forms of RNA polymerase II. Western blot analysis using polyclonal antibodies raised against bovine RNA polymerase II had revealed that these antibodies cross-reacted with all the forms. Partial digestion of RNA polymease II by chymotrypsin yielded RNA polymerase IIB wherein the resulting large subunit is 180 KD. The protease treatment removes the CTD of large subunit without detectably degrading other subunits of the enzyme. A time course of chymotrypsin treatment was done and analysis of subunits after SDS-PAGE indicated that the conversion of subunits IIo and IIa to a faster migrating species IIb is nearly complete by 20 min of incubation at room temperature. Aliquots drawn at different time intervals after commencing digestion were used to assay the transcription using calf thymus DNA. It was observed that partial removal of CTD had no significant effect on transcription. But on complete conversion of subunit IIa (215 KD) to subunit IIb (180 KD), there was 20% decrease in RNA polymerase activity. The apparent dispensability of some portion of CTD without significant loss of transcriptional activity, suggests that it may be functionally redundant (for transcription).

Subunits of RNA polymerase II have been reported to be phosphorylated. Hence it was of interest to examine whether CTD of large subunit of RNA polymerase II from C. utilis is phosphorylated and if so its effects on transcription. C. utilis cells were grown in the presence of ^{32}Pi and ^{32}P-labeled RNA polymerase II was immunoprecipitated using polyclonal antibodies. SDS-PAGE and autoradiography revealed that a subunit > 200 KD was phosphorylated and no phosphorylation was detected in polymerase IIb subunit. These results indicate that CTD of large subunit is phosphorylated. Phosphorylation has been implicated in regulation of CTD interaction with specific promoter-transcription factor complexes.

B 198 MULTISTATE RECEPTOR BINDING AT THE NON-CONSENSUS SITES OF A DELAYED SECONDARY CLUCOCORTICOID RESPONSE ELEMENT (sGRE), Farhang Payvar,* Per-Erik Stromstedt,+ and Guy C.-K. Chan,* The *Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, MO 63104, and the +Department of Medical Nutrition, Karolinska Institute, Huddinge University Hospital F60, Novum, S-141 86 Huddinge, Sweden.

The rat α_{2u}-globulin (RUG) sGREs memorize a hormonal pulse and clock the delayed transcriptional activation of an sGRE-linked promoter, Hess and Payvar (1992) *J. Biol. Chem.* **267,** 3490. These sGREs bind the purified glucocorticoid receptors via nonconsensus binding sites involving GRE-like hexanucleotide half-sites arranged as distinctive sequence motifs, Chan *et al.* (1991) *J. Biol. Chem.* **266,** 22634.

We examined the binding of the purified intact receptor and a truncated receptor (X556; Freedman *et al.*, 1988, *Nature* **334,** 556) to the RUG sGREs containing mutations within and outside of the hexanucleotide half-sites. The results show that the hexanucleotides specify a hierarchy of cooperative receptor binding; each of four hexanucleotides of two tandem receptor footprint sites bind the receptor inefficiently, but together they increase, by two orders of magnitude, the stable assembly of receptor multimers. Our data predicts that the interaction between receptor homodimers bound at each half-site is crucial for cooperative interactions within and between tandem footprint sites. Surprisingly, the hexanucleotides are not essential for binding of the monomeric receptors. In contrast, the sGRE sequence outside of the hexanucleotide half-sites specify binding of receptor monomers and stabilize multimer assembly. We will discuss the speculative notion that the sGREs bound cooperatively by multiple receptor homodimers or by monomeric receptors provide, respectively, windows of time lag and inductive phase.

B 199 *Abstract Withdrawn*

B 200 INTERACTION OF EC CELL SPECIFIC PROTEINS WITH TRANSCRIPTION FACTOR OCT-4.

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Oct-4 is a transcription factor expressed in the pluripotent progenitor cells of the early mouse embryo (1). Previously, we have shown that Oct-4 requires additional factors - termed coactivators or bridging factors - to exert its transcriptional activity from a distance (2).

To identify and characterize ES and EC cell specific factors that specifically interact with Oct-4 several *in vivo* and *in vitro* assays have been used. In a *functional squelching assay, overexpression* of an Oct-4 DNA binding mutant in P19 EC cells results in a strong decrease of transcriptional activation mediated by endogenous Oct-4. With improved binding conditions three additional Oct-4 containing DNA complexes are detectable in gel retardation assays, one of which appears to be EC cell specific. Finally, radiolabeled Oct-4 protein was used in a "far western like" approach to detect interacting proteins from EC and 3T3 cells and again, at least one protein appears to be specifically present in EC cells.

To identify the surface(s) required for protein-protein interaction, deletion mutants of Oct-4 are tested in the squelching assay. The region(s) determined will be further examined in the "far western" - like assay to see if the functional activity and the presence of one of the proteins correlate. Using this region as a probe an EC cell expression library will be screened in order to clone the respective factor. The identified cDNAs will then be tested in the *in vivo* assay which was originally used to determine the functional interaction of Oct-4 and E1A (2).

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(2) Schöler, H.R., Ciesiolka, T., and Gruss, P. *Cell* **66**, 291-304 (1991)

B 201 **CHARACTERIZATION OF A DNA-BINDING DOMAIN COMMON TO AN ERYTHROID-SPECIFIC TRANSCRIPTION FACTOR AND *ASPERGILLUS*.**

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The product of the *are*A gene of *Aspergillus nidulans* is a positive-acting regulatory protein containing a single, putative, DNA-binding zinc finger (1). This structure shows remarkable similarity to two zinc-fingers found in the mammalian regulatory protein, GATA-1 (GF-1, NF-1, Eryf-1) of human, mouse (2) and chicken erythroid cells. Both *are*A and GATA-1 can be described as "global" regulatory proteins in their respective organisms; *are*A activity involved in the control of nitrogen metabolism and GATA-1 in the control of erythroid-specific gene expression. We have developed a Gel-Shift assay using crude whole-cell extracts of *Aspergillus* and have used these to screen a wide range of *are*A mutants for DNA-binding activity. In addition we have overexpressed GATA-1 and a number of chimeric derivatives of GATA-1 and *are*A in *Aspergillus*, the effects of which have been studied by growth testing and Gel-Shift analysis.

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B 202 A CONSERVED DNA STRUCTURAL CONTROL ELEMENT MODULATES TRANSCRIPTION OF A MAMMALIAN GENE, Andrew J. Pierce, Robert C. Jambou, David E. Jensen and Jane Clifford Azizkhan, UNC Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599-7295, USA

The mammalian dihydrofolate reductase (DHFR) gene promoters contain several conserved sequence elements which have been shown to bind protein through *in vitro* footprinting assays, and yet there are other conserved DNA sequences that do not footprint. We report here that mutation of one of these conserved non-footprinting regions increases transcription from this promoter both in an *in vitro* transcription assay and *in vivo* as shown by a transient transfection assay. This sequence lies in a region sensitive to alterations in spacing between binding sites for the transcription factors SP1 and E2F. We show that this conserved region is flanked by sites hypersensitive to cleavage by methidiumpropyl-EDTA-Fe(II). Furthermore, multimers of a double-stranded oligonucleotide comprised of the region delineated by the hypersensitive sites display faster migration through polyacrylamide than control DNA. The difference in mobility is not the result of bending, nor does the primary sequence contain features which would predict altered mobility. We propose that this "Structural Control Element" is rigid and down-regulates transcription by inhibiting interactions between proteins binding adjacent to this region.

B 203 IN VITRO "COMPLEX" FORMATION BETWEEN THE VIRAL TRANSACTIVATOR ICP4 AND THE TATA BINDING PROTEIN (TBP).

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To understand how viral transactivators, stimulate RNA synthesis will require detailed information on how these proteins interact with the cellular proteins of the transcription complex. We have investigated the protein-protein interactions between the HSV-transactivator ICP4 and the human TATA-box binding protein component of TFIID (TBP). Radioactive TBP synthesized *in vitro* was immune precipitated in the presence of partially purified ICP4 by antisera directed against ICP4. In a complimentary experiment ICP4 radiolabeled *in vitro* was immunoprecipitated by an antiserum directed against a TBP fusion protein expressed in *E. coli*. The region of TBP involved in the protein-protein interaction resides within the carboxy-terminal half of the molecule since truncated TBP failed to coprecipitate with ICP4 using anti-ICP4 serum. The region of ICP4 previously defined as the DNA-binding domain (Codons 262 to 490) is involved in the interactions with TBP since a protien extract from bacteria expressing only this domain substituted for intact ICP4 in immunoprecipitation assays. To extend these observations, plasmids containing segments of the ICP4 gene [IE-3] have been constructed. The fusion proteins encoded by these plasmids will be expressed in *E. coli*. The function of analogous plasmids has been tested in cultured mammalian cells. Promoters for HSV genes show considerable variation in TATA-box structure and the ability to bind ICP4. A protein complex composed of TFIID and ICP4 can potentially make multiple contacts with DNA and bind to promoters with different affinities. Differential binding of this complex provides a mechanism for modulating gene expression.

B 204 RECOGNITION OF THE OCT-1 POU-HOMEODOMAIN SURFACE BY αTIF, Joel L. Pomerantz, Thomas M. Kristie, and Phillip A. Sharp, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Homeodomain proteins are transcription factors which are critical determinants in the processes of development and differentiation in a wide variety of organisms. The mechanisms by which these proteins exert extremely specific regulatory actions have remained largely undefined, since most characterized homeodomain proteins display similar DNA-binding target site specificities. The contribution of selective protein-protein interactions to the functional specificity of homeodomain proteins is modeled by the preferential assembly of the Oct-1 protein, over the closely related Oct-2, into a multiprotein complex on the Herpes Simplex Virus α/immediate early enhancer. This complex (C1) is composed of Oct-1, the viral αTIF protein(VP16), and an additional cellular component, the C1 factor. The specific recognition of Oct-1 in C1 complex assembly depends on determinants contained within the protein's POU-homeodomain. In order to map these determinants, variants of the Oct-1 POU-homeodomain were generated by site-directed mutagenesis, in which residues predicted to form the exposed surface of the domain-DNA complex were altered. Mutants with single amino acid substitutions on the surface of either helix 1 or 2 of the Oct-1 POU-homeodomain had diminished abilities to form the C1 complex. Each of the mutants deficient for C1 complex formation failed to interact cooperatively with the αTIF protein, suggesting that αTIF is principally responsible for recognition of Oct-1 in C1 complex assembly. The preferential recognition of Oct-1 over Oct-2 can be attributed to a single amino acid difference between the two proteins on the surface of helix 1.

B 205 **REGULATION OF THE HUMAN HEAT SHOCK RESPONSE,** Sridhar K. Rabindran, Raymond I. Haroun, Carl Wu, Laboratory of Biochemistry, Bg 37, Rm 4C-09, National Cancer Institute, NIH, Bethesda, MD 20892.

Human cells respond to heat and other forms of stress by the rapid synthesis of heat shock proteins. This heat shock response is mediated at the transcriptional level by the heat shock factor (HSF) which binds to heat shock elements (HSEs) in the promoter of heat shock genes. The activation of HSF for DNA binding is accomplished by a change in the oligomeric state from a monomer which is unable to bind to DNA to a trimer which binds to the HSEs with high affinity. To understand the mechanisms by which HSF might sense the stress signal in vertebrate cells, we cloned the heat shock factor in humans. Using degenerate primers deduced from regions of homology present between the yeast and *Drosophila* factors we amplified a DNA fragment from human cDNA using the polymerase chain reaction which was then used to obtain the full-length gene from a HeLa cell library. *HSF1* expressed in bacterial cells and in rabbit reticulocyte lysates in the absence of heat shock is active as a DNA binding transcription factor. However, expression of the protein in human 293 cells by transfection results in heat shock dependent activation suggesting that the intrinsic activity of the factor is under negative regulation in human cells. To examine the regions of HSF1 required for heat shock dependent regulation, wild-type and mutant forms of the protein were introduced into 293 cells by transfection. Using site-directed and nested-deletion mutagenesis, two domains within the carboxy-terminal region were identified that appear to act independently; one spans a region of homology between the two related, but distinct, human HSF proteins, while the other contains a leucine zipper motif which is conserved between insect and vertebrate factors. Human HSF1, like its counterparts in yeast and *Drosophila*, is a multi-zipper protein with the amino terminal zippers being involved in trimerization required for high affinity binding to DNA. Our results suggest that under normal conditions the carboxy-terminal zipper suppresses the aggregation of the amino terminal zipper elements possibly through heat shock sensitive coiled-coil interactions.

B 206 THE GAL4 C_6 ZINC CLUSTER DOES NOT CONFER DNA-BINDING SPECIFICITY *IN VITRO*, Richard J. Reece and Mark Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Ave, Cambridge, MA 02138.

GAL4 is a well characterized yeast transcriptional activator. Its amino terminal end contains a C_6 metal binding domain, that interacts with DNA, and a coiled-coil dimerization element. The carboxy terminus of GAL4 is an acidic activating region. GAL4 has been shown to bind to a 17 base-pair DNA sequence ($CGGN_{11}CCG$) found upstream of its target genes. The crystal structure of GAL4 with DNA indicates that specific interaction, between protein and DNA, occurs at the CGG triplets at each end of the binding site. The inner 11 bases only provide non-specific interactions. PUT3 is an activator of the proline utilization genes that bears a number of similarities to GAL4. PUT3 contains a putative C_6 metal binding domain and binds to a similar DNA sequence ($CGGN_{10}CCG$). We have localized the DNA-binding activity of PUT3 to residues 31-100 of the intact protein. These residues are also sufficient to provide a dimerization function - presumably *via* a coiled-coil structure as is the case in GAL4. We have also constructed, over-produced and purified various chimeric proteins of GAL4 and PUT3 in an attempt to define the elements within each protein that determine DNA-binding specificity. We have found that a molecule bearing the GAL4 zinc cluster and the PUT3 dimerization element binds to DNA with the specificity of PUT3. Similarly, a molecule bearing the PUT3 zinc cluster and the GAL4 dimerization element binds DNA identically to GAL4. Therefore, the metal binding domain of either protein contributes very little to its DNA-binding specificity. The implications of these findings to other fungal C_6 zinc cluster proteins will be discussed.

B 207 **PHYSICAL AND FUNCTIONAL INTERACTIONS OF TFII-I WITH THE GENERAL TRANSCRIPTION MACHINERY AND REGULATORY PROTEINS**

Ananda L. Roy, Ernest Martinez, Cindy Carruthers, Thomas Guthjar, Bernhard Kirschbaum and Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021

TFII-I has been shown to be a basal transcription factor which can substitute for TFIIA in transcription assays reconstituted with the Adenovirus ML core promoter and other basal initiation factors. Moreover the core promoter activity of TFII-I is correlated with the ability of TFII-I to bind specifically to the initiator (Inr) element. Further studies with the TATA- and Inr-containing ML core promoter have demonstrated that TFII-I and TFIIA can form distinct preinitiation complexes in the presence of other general factors. These observations indicate alternate pathways for preinitiation complex assembly and transcription initiation: one that is TFIIA and Inr independent and another that is dependent upon both TFII-I and Inr. In addition, TFII-I also was demonstrated to interact cooperatively with TFIIDτ (the TATA binding subunit of TFIID) in the formation of promoter complexes on a TATA-less promoter, thus providing a means to recruit TFIID on TATA-less promoters which nonetheless require TFIID for activation.

Apart from its core promoter function, TFII-I also has been shown to interact with USF and c-Myc, both members of the b-HLH class of regulators. Importantly, these interactions are not restricted just to the classical E-box site known to be recognized by such regulators, but can be observed at an Inr element as well. The physical and functional interactions through the Inr may have important ramifications as they show the involvement of regulatory proteins in transcription initiation via direct interactions with the general transcription machinery.

B 208 TATA BOX BINDING PROTEIN IS LIMITING FOR ESTROGEN RESPONSE

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The TATA box binding protein (TBP), a component of TFIID, is one of the candidate targets for transcription activation by the estrogen receptor. We tested whether estrogen response is limited by the availability of TBP, and additionally, whether titration of TBP causes the "squelching" observed when estrogen receptors are overexpressed in cells. To test this hypothesis, we transiently overexpressed vectors for the estrogen receptor and for TBP in HeLa and CHO cells, along with a reporter gene which contained an ERE at varying distances from the herpes virus thymidine kinase (tk) TATA-box.
We found that TBP overexpression substantially potentiated the estrogen response (2-8 fold), yet this effect was seen only when the ERE was combined with a simple TATA box, and not with the complete tk promoter, that contained SP-1 and NF-1 sites. In addition, TBP overexpression did not relieve the squelching seen with overexpression of estrogen receptors. Estrogen response potentiation by TBP in both CHO and HeLa cells was dose dependent in the range of 0.3-10 µgs of TBP plasmid. *In vitro* protein-protein binding assay demonstrated that TBP binds to glutathione-estrogen receptor agarose beads, but not to control beads.
We conclude that TBP interacts with the estrogen receptor, and is limiting for estrogen response when the ERE is combined with a simple TATA box but not with a full tk promoter, suggesting that this construct recruit TBP less efficiently when compared to more complex promoters. Furthermore, the squelching observed when estrogen receptors are overexpressed is not explained by the limiting amount of TBP.

B 209 α-NAPTHOFLAVONE-INDUCED CYP1A1 GENE EXPRESSION AND CYTOSOLIC ARYL HYDROCARBON RECEPTOR TRANSFORMATION,

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α-Napthoflavone (αNF) is a weak aryl hydrocarbon (Ah) receptor agonist and inhibits the induction of CYP1A1 gene expression of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). It has been suggested that the Ah receptor antagonist activity was due to the formation of αNF-cytosolic Ah receptor complexes which fail to undergo transformation. This hypothesis was consistent with data obtained in this and other studies using αNF concentrations from 10-1000 nM. However, 10 µM αNF was a strong Ah receptor agonist. Incubations of rat hepatic cytosol with 10 µM αNF caused transformation of the Ah receptor as determined in a gel retardation assay using a ^{32}P-labeled oligonucleotide containing a single dioxin responsive element (DRE). Incubation of rat hepatoma (H-4-II E) cells with 10 µM αNF not only resulted in the induction of CYP1A1 mRNA levels but it also increased cloramphenicol acetyl transferase (CAT) activity from a DRE-containing CAT reporter plasmid. Moreover, the rates of dissociation of the transformed cytosolic Ah receptor liganded with either TCDD or αNF from the DRE were similar. These data confirm that αNF is an Ah receptor agonist and exhibits partial antagonist activity via competition for receptor binding sites.

B 210 CLONING, EXPRESSION AND FUNCTIONAL ANALYSIS OF THREE PLANT HEAT STRESS TRANSCRIPTION FACTOR GENES; K.-D. Scharf, E. Treuter, S. Rose and L. Nover, Institute of Plant Biochemistry, O-4050 Halle

Induced binding of the tomato heat stress transcription factor(s) (HSF) is observed after treatment with heat or chemical stressors. Using differential screening of a λgt11 expression library with a functional HSE oligonucleotide we isolated three clones coding for tomato HSFs. They are characterized by a highly conserved 93 amino acid HSE-binding domain at the N-terminus characteristic of all eukaryotic HSFs. The C-terminal parts of the factors are not conserved except a pattern of heptad hydrophobic repeats of the leucine-zipper type. Surprisingly, two of the HSF are heat stress-induced themselves, suggesting a cascade of HSFs, which may influence quantitative and qualitative aspects of the hs response (1, 2). Functional analysis of the HSF clones was done (i) with the proteins overexpressed in E. coli and (ii) by transient expression in tobacco mesophyll protoplasts using hs promoter x gus reporter constructs. Depending on the position of inserted HSEs activator and repressor functions of different HSF constructs can be monitored. Analysis of C-terminal deletions identify acidic sequence elements with a central tryptophane residue to be important for activity control of all three HSFs.
(Scharf et al., Treuter et al. submitted for publication).

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B 211 PHYSICAL ASSOCIATION OF PIT-1 AND THE T_3R WITH EACH OTHER AND WITH SELECTED COMPONENTS OF THE BASAL TRANSCRIPTION MACHINERY,

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The pituitary-specific transcription of the rat growth hormone (rGH) gene promoter is regulated by a wide variety of transcription factors most of which are also present in cells that do not express GH. in vivo complementation experiments showed that two of these factors, the pituitary-specific transcription factor, Pit-1, and the thyroid hormone receptor (T_3R) synergistically activate the rGH promoter suggesting one model for explaining the integration of tissue-general information into tissue-specific expression patterns. A number of domains within Pit-1 were essential both to the activity of Pit-1 by itself and to the synergistic response with the T_3R. Interestingly, the mutation of one particular domain dramatically inactivated the synergistic activation of the rGH promoter with the T_3R without affecting the low-level activation observed with Pit-1 expression alone. Thus, at least some transcription factor functions may be synergism-specific. Assuming that the synergistic activation of transcription may require some direct communication of the synergistic factors either with themselves, with components of the basal transcription machinery or with novel factors, in vitro binding assays were developed. Far-western blots identified ca. six proteins that interact stably with labelled Pit-1. in vitro precipitation assays were also performed to test whether any factors known to be required for rGH promoter activity can interact. Pit-1 and the T_3R can physically associate with each other and with the same components of the basal transcription machinery (with some minor variations). Mutational studies identified domains required for these interactions.

B 212 **ROLE OF PHOSPHORYLATION OF THE RNA POLYMERASE II CTD IN BASAL TRANSCRIPTION**, Hiroaki Serizawa, Joan W. Conaway, Ronald C. Conaway, Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104

We previously purified RNA polymerase II initiation factor δ from rat liver and showed that it possesses both DNA-dependent ATPase activity and a protein kinase activity that phosphorylates the RNA polymerase II CTD. The δ-kinase phosphorylates the CTD on serine residues, in a reaction that can be strongly stimulated by DNA, but is not significantly affected by the presence of the TATA factor or other general initiation factors. A variety of evidence argues that DNA activates phosphorylation of polymerase by interacting with the CTD, making it more accessible to the kinase. In further studies the relationship between ATPase and kinase activities has been investigated by comparing their nucleotide specificities, DNA effector requirements, and sensitivities to a number of inhibitors. Results of these experiments argue that ATPase and kinase are distinct catalytic activities. Of particular interest are the findings (i) that CTD kinase, but not DNA-dependent ATPase, is strongly inhibited by H-8, a compound shown previously to inhibit a variety of protein kinases by competing with nucleoside triphosphates for active site binding, (ii) that DNA-dependent ATPase, but not CTD kinase, is inhibited by the non-hydrolyzable ATP analog ATPγS, and (iii) that promoter-specific transcription, reconstituted with highly purified RNA polymerase II and basal initiation factors, is inhibited by ATPγS but not by concentrations of H-8 that prevent conversion of the IIA form of RNA polymerase II to the highly phosphorylated IIO form of the enzyme. Taken together, these results argue that conversion of the IIA form of polymerase to the highly phosphorylated IIO form is not an obligatory step in basal transcription, but leave open the possibility that δ's associated DNA-dependent ATPase participates in ATP activation of the preinitiation complex.

B 213 THE IDENTIFICATION OF AMINO ACIDS INVOLVED IN DNA BINDING AND BINDING SITE RECOGNITION BY THE SERUM RESPONSE FACTOR (SRF), Peter E. Shaw, Friedrich von Hesler and Andrew D. Sharrocks. Spemann Laboratories, Max-Planck-Institut für Immunbiologie, Postfach 1169, 7800 Freiburg, Germany.

The Serum Response Factor (SRF) binds as a homodimer to a palindromic sequence in the *c-fos* Serum Response Element (SRE). A second protein, $p62^{TCF}$, binds in conjunction with SRF to form a ternary complex and it is through this complex that mitogenic stimulation of *c-fos* transcription is thought to take place.

A ninety amino acid domain of SRF, $core^{SRF}$, is able to dimerise, bind to the SRE and recruit $p62^{TCF}$. Analysis of $core^{SRF}$ mutants generated by site-directed mutagenesis has allowed the identification of residues essential for DNA binding and dimerisation. The results support the prediction of structural motifs involved in DNA binding.

The related SRF protein RSRFC4 harbours a region with sequence (and presumably structural) similarity to much of $core^{SRF}$. It binds to sequences resembling but distinct from SRF binding sites. The binding specificity of $core^{SRF}$ has been converted to that of RSRFC4. This $core^{SRF}$ derivative supports ternary complex formation at sites divergent from *bona fide* SREs. The required changes imply that SRF and RSRFC4 bind to their cognate sites in different ways.

B 214 **LOCALIZATION OF THE cAMP RESPONSIVE REGIONS OF THE HUMAN GROWTH HORMONE AND CHORIONIC SOMATOMAMMOTROPIN PROMOTERS**, Allan R. Shepard and Norman L. Eberhardt, Departments of Medicine and Biochemistry/Molecular Biology, Mayo Clinic, Rochester, MN 55905.

The human growth hormone (hGH) and chorionic somatomammotropin (hCS) genes are ~95% identical and yet are exclusively expressed in pituitary and placenta, respectively, and are differentially regulated by triiodothyronine (T3). These differences are explained in part by subtle structural differences between the two genes. We have examined 500 bp of the 5'-flanking region of the hGH and hCS genes for responsiveness to the cAMP elevating agent forskolin (F). The hCS and hGH promoters were coupled to the chloramphenicol acetyltransferase (CAT) reporter gene and transiently transfected into rat anterior pituitary tumor cells (GC). F treatment (48 hr) stimulated hGHp.CAT expression 3.6-fold, a value significantly greater ($p<0.01$) than that for hCSp.CAT (2.6-fold), suggesting that the hGH promoter contains additional cAMP responsive element(s). Site-specific mutations of the hCS and hGH promoter GHF-1, Sp1, AP-2 and T3 receptor (TR) binding sites did not affect cAMP responses, indicating that these factors do not contribute to cAMP responsiveness in GC cells. Mutations in the hCS -70/-40 T3 response element did abolish T3/cAMP-mediated synergism. A CGTCA motif with homology to the canonical CREB binding site (TGACGTCA), is present at -101/-97 in the hCS and hGH promoters and at -188/-184 in the hGH promoter. Elimination of the hCS -101/-97 sequence by either substitution or deletion mutations significantly reduced hCSp.CAT cAMP responsiveness by ~33% ($p<0.05$). The data indicate that the CGTCA motif partially mediates cAMP regulation of the hCS promoter. The presence of an additional CGTCA motif in the hGH promoter may account for the differential cAMP regulation of the hGH and hCS promoters.

B 215 A SIGLE AMINO ACID CHANGE IN THE HELIX 1 DOMAIN OF THE HELIX-LOOP-HELIX TRANSCRIPTION FACTOR, E12, CHANGES ITS OLIGOMERIZATION SPECIFICITY, Masaki Shirakata and Bruce M. Paterson, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

E12 is a transcription factor encoded in a E2A gene that also produces E47 and E2-5 transcripts by alternative splicing. Many types of cells express E2A proteins during development which are involved in the regulation of the differentiation process through the formation of functional complexes with cell type specific factors. The DNA-binding and dimerization domain of E12 is a basic-helix-loop-helix (bHLH) motif. The motif enables E12 protein to form a oligomer with another bHLH protein such as the myogenic factor, MyoD, and bind to the specific DNA sequence, CANNTG, referred to as an E-box. The oligomerization of bHLH proteins appears to have some specificity. E12 forms a dimer with MyoD or with other myogenic bHLH factors, but does not form a homodimer. E47 can make a stable homo-oligomer or a heterodimer with MyoD. Our mutation analysis in the HLH region of E12 demonstrates that a single amino acid replacement from Asp to Arg in the helix 1 domain dramatically changes the oligomerization specificity. The mutant E12 can form a homo-oligomer as well as a heterodimer with MyoD. The mutant E12 is equivalent to E47 in its ability to homodimerize. A previous study identified the inhibitory domain for homo-oligomer formation within the acidic region amino terminal to the basic region. The mutation releases the inhibitory effect of the acidic region and consequently converts the dimerization specificity of E12 to that of E47. The molecular basis of the above observation is not yet clear, but it suggests that a modification or a slight change induced in the conformation could regulate the oligomerization specificity of E12.

B 216 COFRACTIONATION OF HUMAN TBP WITH THE POLYMERASE III FACTOR TFIIIB.

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The TATA-binding protein, TBP, has been revealed as a general polymerase cofactor necessary for transcription by all three nuclear polymerases. In TATA-less promoters, TBP recruitment presumably reflects association with other proteins. Indeed, TBP is detected in multiprotein complexes within cell extracts. The pol II D-TFIID complex isolated from a high salt fraction (PC D) of HeLa or Drosophila extracts contains TBP and several associated factors (TAFs). A second TBP complex capable of supporting basal pol II transcription, B-TFIID, has been purified from a low salt fraction (PC B). The identification of TBP and three distinct TAFs in SL1, an essential pol I factor, suggested that TAFs may function in directing polymerase choice. Do the three polymerases utilize distinct TBP-TAF complexes: SL1 and D-TFIID for pol I and pol II respectively, and a third, unidentified complex for pol III?

Two activities, TFIIIB and TFIIIC, found in the HeLa PC B (0.35M) and PC C (0.6M) fractions respectively, are defined as necessary and sufficient, with pol III, for in vitro transcription of tRNA genes. Given the requirement for TBP in pol III transcription, and the observation that 75% of human TBP is fractionated into PC B, we addressed whether this fraction contained a TBP-TAF complex capable of supporting pol III transcription.

Three lines of evidence suggest that TFIIIB is a TBP-containing complex. First, that immunodepletion of TBP from the HeLa PC B fraction inactivates reconstituted pol III transcription of tRNA and 5S templates. Secondly, that TBP cofractionates with TFIIIB activity over four chromatographic steps. Finally, that these TFIIIB fractions are capable of supplying TBP in the form necessary for pol III initiation, and cannot be substituted for by other TBP complexes, or TBP alone.

B 217 HUMAN CYTOMEGALOVIRUS MAJOR IMMEDIATE EARLY 2 PROTEIN INTERACTS DIRECTLY WITH THE BASIC INTER-REPEAT OF TBP VIA A PROTEIN DOMAIN NECESSARY FOR HETEROLOGOUS PROMOTER TRANSACTIVATION,

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The human cytomegalovirus (HCMV) immediate early (IE) 1 and 2 proteins, which arise from differential splice products of the HCMV major IE region, are independently able to transactivate heterologous cellular and viral promoters. Also, IE1 acts as a positive autoregulator whereas IE2 negatively autoregulates.

We have recently shown that, unlike IE1 which acts TATA independently and does not interact directly with TATA binding protein (TBP), IE2 acts in a TATA dependent manner and interacts directly with the C-terminal conserved domain of TBP.

We present data to show that, like Adenovirus E1A, IE2 interacts with the basic inter-repeat of TBP but other sequences in the N-terminal repeat of TBP stabilise this interaction.

The region of IE2 responsible for interaction with TBP maps to a protein domain necessary for IE2-mediated transactivation of heterologous promoters in transient co-transfection assays.

Surprisingly, deletions of the first 290 amino acids of IE2 result in a substantial increase in TBP binding. This suggests that IE2 may also contain a domain which inhibits TBP interaction.

B 218 CLONING OF THE 110 KD SUBUNIT OF TFIIIC

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TFIIIC is a transcription factor required for the Pol III mediated transcription of 5S, VA, tRNA, and 7SL genes. HeLa cells have two forms of TFIIIC (active and inactive) which differ in their mobility in a gel shift assay. The active form has 5 subunits: 220 kD, 110 kD, 102 kD, 90 kD, and 63 kD. The inactive form appears to be composed of the same 5 subunits, except for the apparent replacement of the 110 kD subunit with a 95 kD subunit. In order to determine if the difference between the active and the inactive form lies in the 110 kD subunit, we have cloned a cDNA encoding the 110 kD subunit. The structure of the encoded protein will be discussed. Regions of the 110 kD subunit are being expressed and used to produce antibody to determine if the 110 and the 92 kD subunits are immunologically related and to investigate previous suggestions that the active and inactive form may reflect differences in phosphorylation or possible proteolysis. Epitope tagging and subsequent expression in mammalian cells are being used as a method to ease the purification of TFIIIC for future studies.

B 219 ACTIVATION OF HEAT SHOCK FACTOR 2 (HSF2) IN HEMIN-TREATED HUMAN ERYTHROLEUKEMIA CELLS, Lea Sistonen, Kevin D. Sarge, Benette Phillips, Klara Abravaya and Richard I. Morimoto, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208

Hemin treatment of human erythroleukemia K562 cells induces transcription of embryonic and fetal globin genes as well as the stress genes hsp70, hsp90 and grp78/BiP. As in heat-shocked cells, hemin-induced transcription of hsp70 is mediated by activation of heat shock transcription factor (HSF) through binding to the heat shock element (HSE). However, the kinetics and magnitude of hsp70 transcription differs greatly between heat-shocked and hemin-treated K562 cells. In contrast to a rapid and transient activation of HSF upon heat shock, HSF can be maintained in an activated state for an extended period of time in hemin-treated cells. Both hemin and heat shock treatments result in equivalent levels of HSF:HSE complexes as analyzed *in vitro* by gel mobility shift assay, yet transcription of the hsp70 gene is stimulated much less by hemin-induced HSF than by heat shock-induced HSF. Since two HSF genes, HSF1 and HSF2, have been cloned from human and mouse, we wished to determine whether HSF activated by hemin was identical to HSF activated by heat. By using specific antibodies raised against HSF1 and HSF2, we show that the HSE-binding activity in hemin-treated cells consists predominantly by HSF2 and in heat-shocked cells of HSF1. Genomic footprinting experiments reveal that HSF1 and HSF2 bind to the HSE of the human hsp70 promoter in a similar, yet not identical manner. Chemical cross-linking studies indicate that HSF2 undergoes a similar oligomerization during hemin treatment as has been shown for HSF1 upon heat shock. Yet, unlike HSF1, which exhibits heat-induced phosphorylation, HSF2 displays no hemin-induced covalent modification. Our results of differential activation of HSF1 and HSF2 in the same cell type support the hypothesis that these factors might mediate transient and sustained responses and/or responses to different stimuli in higher eukaryotes. Whether activation of HSF2 is directly involved in the differentiation process remains to be determined.

B 220 VDBP, A LIVER ENRICHED TRANSCRIPTION FACTOR IS INVOLVED IN REGULATION OF THE CHICKEN APO-VLDL II GENE, Marten P. Smidt, Jan Wijnholds and Geert AB, Laboratory of Biochemistry, University of Groningen, Nyenborgh 4, 9747 AG Groningen, The Netherlands.

The chicken Very Low Density Apolipoprotein II subunit is specificially expressed in the liver in an estrogen dependent way. The 300 bp 5' flanking region of the apo VLDL II gene contains two EREs (1) and a number of other binding sites for known transcription factors. One of the EREs overlaps with an element D which resembles the rat albumin gene Element D (2). A trimer of the chicken element D was used as a recognition site probe for cloning the VLDL gene D-box binding protein, VDBP (3). The VDBP construct was expressed in E. coli by using the expression system developed by Studier et. al. (4).
In vitro DNase I footprinting revealed in addition to element D several binding sites for the protein in the apo-VLDL II promotor region. The protected segment of element D overlaps one of the EREs. The interference or interplay of the VDBP with the ER will be investigated in futher detail by *in vitro* DNase I footprinting with recombinant chicken estrogen receptor (CER)(5) and transfection experiments. In Western blots, probed with antibodies made against the VDBP protein, it appeared that in liver two proteins are expressed with molecular weights of about 45 kD. Also possible degradation products were observed. Analysis of the tissue distribution of VDBP in chicken is in progress.

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B 221 NUCLEAR FACTOR I INDUCED DNA BENDING Vuk Stambolic[1] and Richard M. Gronostajski[1,2], [1]Department of Medical Biophysics, University of Toronto, Toronto, ONT M4X 1K9. [2]Cleveland Clinic Foundation, Dept. of Cancer Biology, 9500 Euclid Ave., Cleveland, Oh. 44195

Nuclear Factor I (NFI) comprises a family of site-specific DNA binding proteins that function in the regulation of transcription of a variety of cellular genes and in the replication of viral DNA. DNA mutational analysis has characterized the optimal sequence for NFI binding as TTGGC(N)$_5$GCCAA. NFI binds to these sequences as a dimer. NFI family members share a high degree of homology within their N-terminal 185 amino acids. It has been shown that the first 220 amino acids of the N-terminal domain of NFI are sufficient for DNA site-specific binding, stimulation of Adenovirus DNA replication and dimerization.

To assess NFI induced DNA bending, the circular permutation assay of Wu and Crothers was used. Seven NFI binding sites, differing in sequence within the spacer region and non-conserved residues were analyzed for DNA bending by a truncated form of NFI consisting of the 220 N-terminal amino acids of NFI-C (NFI-C220). The analysis has revealed that NFI-C220 binding to any of the investigated binding sites induces a bend in the DNA helix of approximately 50°. The centre of the NFI-C220 induced DNA bends is placed at, or very near to, the center of the binding site. Phasing analysis is currently underway to determine the direction of NFI-C220 induced DNA bends.

B 222 TFIIA INDUCES A CONFORMATIONAL CHANGE IN THE TATA BINDING PROTEIN (TBP), D. Barry Starr and Diane K. Hawley, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The role of TFIIA in *in vitro* transcription has been controversial for many years. Here we show that TFIIA has a profound effect upon the conformation of TBP bound at the TATA box. We use dimethyl sulfate (DMS) to probe the accessibility of the major and minor groove surfaces of the TATA box in complexes containing TBP, TFIIA, and TFIIB. We found that the minor groove contacts were protected from DMS methylation in all complexes probed. In contrast, the major groove was protected in complexes containing TBP or both TBP and TFIIB, but not in complexes containing TFIIA. We have recently shown that TBP does not require major groove contacts within the TATA box in order to bind. Preliminary evidence using similar techniques suggests that the major groove of the TATA box is important for *in vitro* transcription. Taken together, these results suggest that the function of TFIIA is to induce a conformational change in TBP such that the major groove of the TATA box becomes solvent accessible. Reconciliation of these results with recent findings from other laboratories regarding the function of TFIIA will be discussed.

B 223 COOPERATIVE DNA-BINDING INTERACTIONS BETWEEN A ZINC FINGER PROTEIN AND A HOMEODOMAIN PROTEIN AND THEIR ROLE IN GENE REGULATION, David J. Stillman, Robert M. Brazas, and Paul R. Dohrmann, Department of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City, UT 84132

We have previously identified two parallel pathways of transcriptional regulation in yeast (Genes and Dev. 6:93). SWI5 and ACE2 are yeast transcription factors with essentially identical zinc finger DNA-binding domains. However, SWI5 and ACE2 regulate different genes *in vivo*, HO and CTS1, despite having identical DNA-binding domains. SWI5 and ACE2 show additional parallels: Transcription of both genes and subcellular localization of the SWI5 and ACE2 proteins is regulated in the cell cycle. Additionally, HO and CTS1 show identical patterns of cell cycle regulated transcription.

We have now demonstrated that SWI5 and ACE2 recognize the same DNA sequences *in vitro* at the HO and CTS1 promoters. This raises the following question: How do SWI5 and ACE2 differentially regulate gene expression *in vivo* when they bind to the same DNA sequences *in vitro*? We will present evidence that additional DNA binding proteins are needed for specific promoter recognition.

The SWI5 protein has been expressed in E. coli and purified to homogeneity. The purified protein forms a "low affinity" complex *in vitro* with the HO promoter, since binding is competed by low concentrations of non-specific DNA. This result was surprising since genetic evidence demonstrated that SWI5 functions at the HO promoter via this site *in vivo*. A "SWI5 Stimulatory Factor" (SSF) was identified by the ability to promote "high affinity" binding of SWI5 in the presence of a large excess of non-specific carrier DNA. This assay has been used as an assay for purification of SSF. Final purification of SSF, an 83 kD protein, was achieved by a novel procedure, Cooperative Interaction Based DNA Affinity Chromatography ("CIBDAC").

The purified SSF protein was sequenced, leading to the determination that SSF is a homeodomain protein. The SSF protein has been expressed in E. coli, and the purified SSF protein has been used *in vitro* studies. Like SWI5, SSF binds to the HO promoter with low affinity. SWI5 and SSF show cooperativity in DNA-binding *in vitro*. No interaction between SWI5 and SSF is seen in the absence of DNA.

Yeast strains with null mutations in the gene encoding SSF have been constructed, and the results of *in vivo* studies on the the role of SSF on regulation of HO and CTS1 will be presented.

B 224 REGULATION OF RNA POLYMERASE I ACTIVITY IN *Acanthamoeba castellanii*, Christopher Terpening, Victor Duarte, and Marvin Paule, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523

Acanthamoeba castellanii is a small free-living amoeba found in soil and water which undergoes cellular differentiation into dormant cysts upon starvation. Concomitant with cyst formation, ribosomal RNA transcription is turned off in *Acanthamoeba*. This loss of transcriptional activity is mimicked in an *in vitro* assay on a specific template. This assay requires two protein components, 1) a fraction containing the transcription factors TIF-IB and UBF, and 2) a fraction containing the RNA polymerase I (pol I). Previous work has demonstrated that while the TIF-containing fraction is fully functional whether isolated from growing trophozoite cells or from cysts, the pol I is only active on the specific template when isolated from trophozoite cells. Thus, either a subunit(s) of the polymerase itself must be modified or the pol I fraction must contain a regulatory factor. A change in the mobility of the 39 kDa subunit of pol I on a Western blot occurs over the encystment time course. The apparent modification correlates with the loss of transcriptional activity. Numerous attempts to identify this modification have been made. New data suggest that encystment of the cells in the presence of sodium butyrate blocks the mobility shift in the Western, implying that the modification may be an acetylation. Transcriptional effects of sodium butyrate are being tested. A putative regulatory factor has been reported to be associated with, but separable from, the mouse pol I. However, no such factor is separated from *Acanthamoeba* pol I when purified in an identical manner. In order to more clearly ascertain whether or not a separable initiation factor is required for regulation, an assay using a mutant rRNA promoter linked to agarose beads is being developed. This bead-linked assay can also be used to determine whether the elongating pol I is capable of reinitiation at a distinct promoter following run-off.

B 225 TRANSCRIPTIONAL ACTIVATION OF mRNA PROMOTERS BY THE OCTAMER MOTIF IN NON-LYMPHOID CELLS. William Thomann and Winship Herr, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

The octamer motif, ATTTGCAT, is found in a variety of cellular and viral enhancers and promoters and binds the POU-homeodomain proteins Oct-1 and Oct-2. The octamer motif activates transcription of the U2 snRNA gene but not for the β-globin mRNA gene in non-B cells unless the B cell activator Oct-2 is present. This and other results establish Oct-1 as a preferential activator of snRNA transcription. There is, however, one mRNA promoter whose activation is octamer motif dependent in the absence of Oct-2, the histone H2B promoter.

To investigate this octamer motif dependence further, we used the histone H2B gene promoter, the non-responsive β-globin mRNA promoter, and a B-cell specific promoter, the immunoglobulin kappa light chain gene promoter, as test promoters in non-B cells. The histone H2B promoter is unique among octamer-containing promoters in that the octamer motif is close to the transcriptional start site. By deleting all promoter sequences 5' to the octamer motif in the H2B promoter, all of the remaining correctly initiated transcription is octamer motif dependent. Using point mutagenesis and transient transfection assays in the human embryonal kidney cell line, 293, we determined that there are many parameters required for octamer motif dependent mRNA transcription in non-B cells, and that changing any of these parameters results in a lower level of transcription. These parameters include; the affinity of the octamer motif; the position of the octamer motif in the promoter; the orientation of the octamer motif; and the 5' flanking sequences of the octamer motif. These results show the ubiquitous activity of the octamer motif for mRNA activation to be critically dependent on the precise promoter context.

Additionally, preliminary results show that the TATA box and surrounding sequences are important; when the histone H2B TATA box and surrounding sequences are replaced by corresponding sequences from the immunoglobulin kappa light chain gene promoter, transcriptional activity is lost. This result suggests that octamer motif dependent mRNA transcription is also dependent on the sequence of the TATA box.

B 226 ANALYSIS OF HUMAN AND MOUSE TATA-BINDING PROTEINS, USING MONOCLONAL ANTIBODIES

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Recombinant human TATA-binding protein (TBP) was used to generate monoclonal antibodies (MAbs) by standard hybridoma methods. Three hybridomas were isolated that produce MAbs that react strongly with human TBP in ELISA and immunoblot assays. Reaction of these MAbs with different peptides from proteolyzed TBP suggested that at least two epitopes were represented. Despite the strong reactivity of the MAbs in the ELISA, none of these MAbs could immunoprecipitate native TFIID or purified TBP effectively. However, when used in a gel shift assay, at least one of the MAbs supershifted the TBP-DNA complex, suggesting that the epitope for this MAb becomes accessible after binding to DNA. In immunoblot assays, none of the MAbs reacted with yeast TBP or with any protein in extracts prepared from *Xenopus* oocytes or silkworm silk glands; however, all of the MAbs reacted strongly with only one protein in mouse nuclear extracts. This mouse protein had a faster mobility on SDS-polyacrylamide gels (corresponding to approximately 30 kDa) than HeLa cell TBP, perhaps reflecting the differences in the number of the glutamine residues in the TBP molecules. The ability of the MAbs to inhibit the activity of native TFIID in HeLa and mouse nuclear extracts was examined.

B 227 THE BACTERIAL TRANSCRIPTION FACTOR OxyR RECOGNIZES DNA THROUGH A DEGENERATE CODE, Michel B. Toledano and Gisela Storz, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, NIH, Bldg. 18T, Room 101. Bethesda, MD 20892

Treatment of bacterial cells with low doses of H_2O_2 results in the induction of at least 30 proteins and resistance to killing by higher doses of H_2O_2. The expression of 9 of the H_2O_2-inducible proteins is controlled by the regulator OxyR. OxyR is homologous to the "LysR" family of bacterial regulators. As with other members of this family, OxyR is both a negative regulator of its own expression and a positive regulator of the H_2O_2-inducible proteins. OxyR binds to the promoters it regulates with high specificity and affinity, but unexpectedly, the binding sites show very little sequence similarity. This has led to the suggestion that OxyR might recognize DNA according to a degenerate code. In addition, the OxyR footprint patterns on each promoter sequence are different suggesting that OxyR may adopt different conformations at different binding sites. To gain insights into the mode of OxyR DNA recognition, we selected OxyR binding-sites out of a pool of random oligonucleotides. The analysis of 60 specific binding sites showed that the OxyR protein does have a degenerate DNA recognition code. The specific OxyR-DNA contacts were further examined by hydroxyl radical footprinting experiments. A model for how OxyR interacts with DNA will be presented.

B 228 INTERACTION OF MEMBERS OF THE STEROID / THYROID HORMONE SUPERFAMILIY WITH BASAL TRANSCRIPTION FACTORS, Ming-Jer Tsai, Aria Baniahmad, Nancy H. Ing, Ilho Ha, Danny Reinberg, Sophia Y. Tsai, and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030, and Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854

The mechanism of transcriptional activation by steroid hormone receptors is largely unknown. The basal transcription factor TFIID has been implicated as a target for transactivation. Recombinant TFIID has been shown to directly interact with the acidic activation domain of the herpesvirus VP16 and the adenovirus E1a proteins. Recent work shows that the binding of TFIIB seems to be the rate limiting step of transcriptional activation. Furthermore, recombinant human TFIIB also interacts directly with the VP16 activation domain suggesting that both TFIIB and TFIID basal transcription factors may be targets of transcriptional activation. We present evidence that the C-terminal domain of the human thyroid hormone receptor β functions as both an active silencer and a hormone dependent activator on different minimal promoters containing only a TATA-box. This suggests that the transcriptional machinery is not only the target for transcriptional activation but also for transcriptional silencing. In addition we show that the progesterone and the thyroid hormone receptor as well as other members of the steroid\thyroid hormone superfamily do interact with basal transcription factors. Protein-protein interaction experiments with recombinant TFIIB demonstrate specific interactions with steroid hormone receptors in solution. These facts implay that TFIIB is one of the targets for transcriptional modulation. Future studies will define the regions important for such interactions.

B 229 INTERACTION OF TRANSCRIPTION FACTORS WITH CHROMATIN USING AN IN VITRO NUCLEOSOME ASSEMBLY SYSTEM

Toshio Tsukiyama[1], Peter B. Becker[1,2] and Carl Wu[1] [1]Laboratory of Biochemistry, NCI, NIH, Bethesda, MD 20892, [2]Gene Expression Program, EMBL, Meyerhofstrasse 1, D-6900 Heidelberg, Germany.

We have developed a cell-free system derived from preblastoderm *Drosophila* embryos for the efficient assembly of cloned DNA into chromatin. The chromatin assembly system utilizes endogenous core histones and assembly factors, and yields long arrays of regularly spaced nucleosomes with a repeat length of 180 bp. We have used the assembly system and the heat shock gene 70 as a model for the analysis of transcription factor interactions with chromatin. Previous work from this and other laboratories has indicated binding of several transcription factors within hsp70 promoter sequences. Specifically, the TATA-binding factor TBP, the GAGA factor, and an arrested RNA polymerase molecule appear to interact with the hps70 promoter even in the absence of a heat shock stimulus. Binding of the heat shock transcription factor HSF is observed upon heat shock, and the bound HSF in thought to act by releasing the arrested polymerase. We have overexpressed TBP, GAGA factor, and HSF , and have purified these factors to near homogeneity. The purified transcription factors were introduced individually or in combination, before, during, and after the process of chromatin assembly, followed by micrococcal nuclease and DNase I digestion to map the position of nucleosomes and transcription factors on the DNA. The ability of the factors to create a nucleosome free region over the hsp70 promoter is under investigation.

B 230 COMPARISON OF NATIVE TFIID AND RECOMBINANT TBP IN BASAL TRANSCRIPTION, Curtis M. Tyree and James T. Kadonaga, Department of Biology, University of California, San Diego, La Jolla, CA 92093.

We have fractionated an in vitro transcription system from *Drosophila* embryo extracts containing the fractions TFIIB, TFIIE/F, TFIID, and RNA polymerase II. This system will transcribe a wide variety of promoters both possessing and lacking TATA boxes. We expressed the *Drosophila* TATA box-binding protein (TBP) in *E. coli* and substituted it for the TFIID fraction in reconstituted transcription assays. On different promoters, the TFIID fraction did not show a large difference in activity, but the TBP possessed a marked promoter specificity. The TFIID was further fractionated to yield a subcomponent that contained no TBP protein, but would complement TBP on certain promoters.
In addition, recombinant TFIIB, TFIIE, and TFIIF will substitute for their native *Drosophila* fractions in reconstituted transcription assays. This demonstrates that the TFIID fraction is a source of factors that may contribute to promoter specificity of basal transcription. Further work on this subject will be presented.

B 231 DNA-BINDING AND HETERODIMERIZATION PROPERTIES OF *DROSOPHILA* AND *BOMBYX* HOMOLOGUES OF NUCLEAR HORMONE RECEPTORS, George Tzertzinis, Anastasia M. Khoury, and Fotis C. Kafatos, Harvard University, Dept. of Cellular and Developmental Biology, 16 Divinity Ave., Cambridge MA, 02138, USA.

The *D.melanogaster* CF1 protein, a putative transcriptional regulator of chorion gene *s15*, is a member of the RXR subfamily of nuclear hormone receptors, and recognizes a region of the *s15* promoter with palindromic sequences similar to hormone response elements (HRE's) (Shea et al., Genes and Dev.,1990,4 , 1128). Genetic studies have shown that this RXR homologue is encoded by the ultraspiracle (*usp*) locus of the X chromosome and is required for pattern formation both maternally and zygotically (Oro et al., 1990, Nature, 347, 298).

Using PCR with degenerate primers based on CF1/*usp*, a homologous cDNA (BmCF1) was cloned from the silkmoth *Bombyx mori* . *B.mori* chorion genes have been shown to be succesfully regulated in transgenic flies. The zinc finger DNA-binding domain of BmCF1 has 96% identity in amino acid sequence to DmCF1 (*Drosophila melanogaster* CF1). The ligand domain is ~ 56% identical to DmCF1 and it also contains a set of leucine residues that have been proposed to participate in protein-protein interactions. The amino terminal domain which has been implicated in transcriptional activation in other receptors is very different in the *Drosophila* and *Bombyx* proteins. Both DmCF1 and BmCF1 have been expressed in bacteria and the proteins produced have been used to compare binding specificities towards the s15 binding site. The *s15* imperfect palindrome is TAGGTCACGTAAATGTCCAG. Full length protein apparently does not bind DNA whereas truncation of the ligand/ dimerization domain leads to high affinity specific binding. Using PCR-mediated binding site selection the optimal binding site for DmCF1 was determined to be the sequence GGGGTCAC which has a two base difference from the wild type *s15* site. The optimal binding site increases the binding affinity of DmCF1 by approximately 5-fold over the wild type DNA sequence. Both *Drosophila* and *Bombyx* proteins heterodimerize with mammalian hormone receptors and the heterodimers bind with high affinity to the *s15* binding site. This leads to the hypothesis that *in vivo* they act as heterodimers with as yet unidentified partners for modulation of gene expression. These partners should be identifiable by exploiting protein-protein interaction cloning methodologies. We are currently testing the transcriptional function of DmCF1 and BmCF1 in *Drosophila* tissue culture cells as well as in transformed flies that carry the *usp* mutation .

B 232 ACTIVATION OF THE c-fos SERUM RESPONSE ELEMENT (SRE) AND THE cfosAP-1 BINDING SEQUENCE (FAP) BY THE ACTIVATED Ha-ras PROTEIN IN A MANNER DEPENDENT OF PROTEIN KINASE C. Florian Überall, Sonja Kampfer, Wolfgang Doppler, Alexander Kekulé♣ and Hans H. Grunicke. Institute of Medical Chemistry and Biochemistry, University of Innsbruck, Fritz Preglstr. 3, A-6020 Innsbruck, Austria, ♣Max-Planck-Institute of Biochemistry, Am Klopferspitz 18a, D-8033 Martinsried, FRG.

Stable transfection of a MMTV-LTR-driven Ha-ras construct and the transient transfection of an EJras expression vector to the mouse mammary epithelial cell line HC11 activated the c-fos gene enhancer linked to the chloramphenicol acetyl transferase reporter gene (CAT) in a manner dependent of Protein Kinase C (PKC). Activation of c-fos expression was determined by measuring CAT-activity of a c-fosCAT construct in which the CAT gene is expressed under the control of the endogenous human c-fos promoter or in constructs spanning isolated sequences of the c-fos promoter (DSE, DSE/FAP) under the control of the thymidine kinase promoter of herpes simplex virus.

The serum response element (SRE) rather than the c-fosAP-1 binding sequence (FAP) was found to be responsible for the Ha-ras-induced activation of c-fos.

Constitutive expression of EJras in HC11 cells resulted in a translocation of PKC ε from the cytosolic fraction to plasma membrane. Phorbol ester (TPA)-induced expression of the c-fos enhancer was employed as an additional marker for intracellular PKC activity. Prolonged treatment of transfected HC11 cells with TPA caused downregulation of PKC ε. In PKC-depleted cells the expression of Ha-ras did not stimulate the DSE-TK-CAT or DSE-FAP-TK-CAT gene enhancer. The same inhibitory effect of the Ha-ras-mediated transactivation of cfosCAT-reporter plasmids was obtained if the PKC-inhibitors BM41440 (a thioether-alkyllysophospholipid) or hexadecylphosphocholine were used. These results suggest that the serum response element is responsible for the Ha-ras-induced activation of the endogenous c-fos gene enhancer and that the signal pathway from Ha-ras to the nucleus may involve an active PKC ε - isoenzyme.

B 233 MEDIATION OF TRANSCRIPTION STIMULATION BY USF AND Sp1 THROUGH TFIID-INITIATOR INTERACTIONS, Michael W. Van Dyke and Jo C. Wang, Department of Tumor Biology, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

The general transcription factor IID has been shown to interact with DNA sequences downstream of the start site of transcription on a subset of class II promoters (Nakajima et al., 1988, *Mol. Cell. Biol.*, 8:4028-4040) and that this interaction can be enhanced by the proximal binding of stimulatory transcription factors such as ATF and GAL4-AH (Horikoshi et al., 1988, *Cell* 54:665-669; *ibid.*, 1033-1042). Recently we have shown that this downstream interaction by TFIID is directed by sequences within the initiator element and that this interaction is required for maximal transcription efficiency in vitro. We have now investigated the role of this interaction in mediating transcription stimulation by transcription factors USF and Sp1. For both of these stimulatory factors, no qualitative or quantitative changes in the downstream promoter binding of TFIID were observed when either of these proteins were bound upstream of the TATA element. Both factors stimulated transcription to an equivalent degree, whether or not a strong initiator element was present in the promoter construct. This apparent additive and not multiplicative increase in transcription would suggest that upstream stimulatory factors such as USF and Sp1 increase transcription rate by a mechanism different from that used by TFIID-initiator binding.

B 234 CCAAT ELEMENT BINDING PROTEIN, NF-Y, INTERACTS WITH XEBP AND C/EBP AND MAY SERVE AS A CENTRAL NUCLEATING FACTOR IN PROTEIN COMPLEX FORMATION. B. Vilen, Y. Itoh-Lindstrom, J. Badley Clarke, Bernd Stein and J. P.-Y Ting. LCCC, UNC-CH, Chapel Hill, NC 27599.

This study will shed light on the mechanism by which a large number of tissue-specific promoters can be activated by a seemingly finite number of transcriptional activators. We show evidence that a CCAAT element binding protein, NF-Y, participates in direct protein-protein interactions with two unrelated DNA binding proteins suggesting that promiscuity in these interactions may play a crucial role in creating unique combinations of factors specific in gene activation.

The major histocompatibility complex class II genes contain two proximal promoter elements, X and Y, which are separated by a conserved spacer of two helical turns of DNA. Promoter analysis of the DRA gene has shown that the Y element, which contains an inverted CCAAT sequence and binds the CCAAT element binding protein NF-Y, must be stero-aligned with the X element for promoter function.[1,2] This requirement for helical periodicity is maintained when as many as 80bp separate the CCAAT element and the X element. Direct protein-protein interactions between NF-Y and X element binding proteins (XEBP) are now demonstrated by the binding of XEBP to solid-phase immobilized, bacterially expressed NF-Y. Immunoprecipitation studies are underway to confirm this interaction.

Previously it has been reported that the CCAAT element of the albumin promoter must be stereo-aligned with an upstream C/EBP binding site for synergistic activation of the albumin gene. To address whether NF-Y can interact with other promoter proximal binding factors, we performed immunoprecipitation and coprecipitation studies. Results indicate that NF-Y can indeed be immunoprecipitated with C/EBP$_\beta$. This indicates that NF-Y can participate in direct protein-protein interactions with at least two promoter proximal binding factors. Since many genes contain CCAAT elements, interactions at this site may represent a central nucleating point in protein complex formation on the proximal promoter.

[1]Vilen et al MCB 11 2406-2415. [2]Vilen et al JBC in press.

B 235 EMERGENCE OF NEW TRANSCRIPTION REGULATORY FUNCTIONS DURING EVOLUTION ; THE DROSOPHILA *SRY*β AND δ PROTEINS.

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Within the *Cys2/His2* finger protein multigene family, the drosophila *sry* β and δ proteins present an interesting problem in the diversification of structure and function. 4EMS induced lethal *sry* δ mutations of different strength have been characterized in detail (Genetics 131, 905-916, 1992), showing that *sry* δ may be involved in regulation of genes required for viability (with a sex-bias) and genes required for gonadal development. These four mutations correspond to single amino-acid replacements in either the third (out of six) zinc finger or a NH2-terminal domain proposed to be involved in specific protein-protein interactions required for binding of the protein *sry* δ at defined chromosomal sites (MCB 12, 724-733, 1992). None of the *sry* δ mutations is rescued by an extra copy of the *sry* β gene. Data will be presented on *sry* δ target genes and *in vitro* DNA recognition properties of the *sry* β, *sry* δ (wild type and mutant) and chimaeric *sry* β/δ proteins.

B 236 **THE ROLE OF TFIIB IN THE INITIATION OF RNA POLYMERASE II TRANSCRIPTION,** **Sharon L. Wampler, Lucy M. Lira, and James T. Kadonaga,** Department of Biology, University of California, San Diego, La Jolla, CA 92093

RNA polymerase II alone is unable to recognize a class II promoter and initiate specific transcription. Accurate transcription initiation requires, in addition to RNA polymerase, several other proteins referred to as the general transcription factors. One of these factors, TFIIB, has been purified and cloned from several sources including human, *Xenopus*, *Drosophila*, rat liver and yeast. We have cloned TFIIB from *Drosophila* (dTFIIB) and conducted a functional analysis of the recombinant protein. Recent evidence suggests that TFIIB plays an important role in gene activation. To further understand the role of this factor in both basal and activated transcription, we have constructed a series of deletions and point mutations in dTFIIB. Using *in vitro* transcription and gel shift analysis, we are performing experiments to delineate important functional domains in the dTFIIB polypeptide. Exciting results will be presented.

B 237 **Cooperation between *adenovirus* E1A and TBP proteins in E1B basal promoter activation**

Nobuo Horikoshi, Ullrich Graeven & Roberto Weinmann. The Wistar Institute, Philadelphia, PA 19104.

Direct interactions have been shown to occur between the adenovirus early E1A oncoprotein and the human TATA binding protein (TBP) 1,2. These interactions can be demonstrated in mammalian cells and in vitro with recombinant TBP or native TBP complexes. We localized the regions of interaction between E1A and TBP to conserved domain 3 containing the Zn finger of E1A, and the basic sequences between aminoacids 217 and 250 of TBP. Functional assays using an adenovirus E1B basal promoter construct demonstrate that E1A and TBP cooperate in the transactivation of this viral promoter. The unique TBP aminoterminal region (1-101) required for SP1 mediated ativation 12 is dispensable for E1A mediated activation of a basal promoter. A second TBP element in the basic region, required for basal transcription and DNA binding of the yeast TBP, is not required for cooperative E1A mediated transactivation, suggesting that this mutant TBP may be positioned in the transcriptional complex by interactions involving accesory proteins but not the DNA.

Supported by Deutsche Forschungsgemeinschaft Gr 984/2-1

B 238 **A PROMOTER-LINKED REGION REQUIRED FOR STIMULATION OF α-FETOPROTEIN TRANSCRIPTION BY DISTANT ENHANCERS,** Ping Wen, Nancy Crawford and Joseph Locker, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

The α-fetoprotein (AFP) gene is expressed at high level in fetal liver but repressed about 10,000 fold after birth. This tissue-specific and developmental stage-specific expression is controlled primarily at the level of transcription, governed by the 6 kb 5'-flanking sequences. We previously demonstrated that this region in rat contains three enhancers at -6.0, -4.2, and -2.5 kb, and a promoter extending to -201 bp. (The promoter alone can weakly stimulate transcription). The three distant enhancers act through the AFP promoter additively in HepG2 cells. In the present study, we identified by deletion analysis a promoter-linked segment (-179 to -155) required for interaction with distant enhancers but dispensable when enhancers were moved close to the promoter. This promoter-coupling element (PCE) was required for the interactions with each individual AFP enhancer, and appears to be able to accommodate three enhancers simultaneously. When coupled to the distant enhancers, the -155 promoter was stimulated about 4-fold less than the -179 promoter. Footprints with HepG2 extracts revealed protein binding at two sites in the region of the PCE. The identity of these DNA binding proteins is under investigation. The data indicate a positive transcriptional control mechanism by which the distant enhancers stimulate the AFP promoter through a specific promoter-linked element. Similar mechanisms may be involved in the stimulation of promoters by distant enhancers in other genes. The PCE could be one of the targets for the postnatal repression of AFP gene expression. (Supported by NIH Grant CA 43909).

B 239 **ACTIVATION TEMPERATURE OF HUMAN HEAT SHOCK FACTOR (HSF1) IS REPROGRAMMED IN DROSOPHILA CELL ENVIRONMENT.** Jan Wisniewski, Joachim Clos, Sridhar Rabindran and Carl Wu, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

The transcriptional regulation of eukaryotic heat shock genes is mediated by the activator protein called heat shock factor (HSF) whose activity is modulated at two separate levels: DNA binding and transcriptional activation. Induction of DNA binding activity is believed to be accomplished by a stress-induced trimerization of preexisting, inactive HSF monomer. While this general model of regulation appears to be shared amongst a wide range of eukaryotic species, there is significant variation in the temperature at which HSF binding activity (and its oligomerization) is induced. In Drosophila cells, HSF is activated above 32°C, while in human cells it is inactive at that temperature but undergoes activation above 42°C. In order to examine the regulation of HSF activation, we expressed human HSF1 and Drosophila HSF both in Drosophila Schneider 2 cells and human 293 cells. HSFs expressed in homologous cells were found to be regulated exactly as their endogenous counterparts. However, the results of heterologous expression were different. Surprisingly, in Drosophila cells the induction temperature of human HSF1 was reprogrammed to 32°C, while Drosophila HSF was fully active in human cells even when the culture was incubated at room temperature. Our results indicate that the temperature response of HSF protein is highly dependent on cellular environment, and suggest that HSF is unlikely to be directly sensitive to the absolute environmental temperature.

B 240 Members of the Thyroid/Steroid Receptor Superfamily That Regulate Apolipoprotein A1 Gene Expression, Norman C.W. Wong and Frances E. Carr, Dept. of Medicine Univ. of Calgary, Calgary, AB, CANADA, and Kyle Metabolic Unit, Walter Reed Army Medical Center, Washington, DC, 20307.

Apolipoprotein A1 (Apo A1) is the major protein constituent of the serum lipoprotein particle, HDL. This particle mediates reverse cholesterol transport (RCT), a process where by excess cholesterol from peripheral tissues is shuttled to the liver for disposal. An increase in Apo A1 will augment RCT and reduce the consequences of hypercholesterolemia. In an attempt to increase Apo A1 we have been studying this gene in rat liver and uncovered three cis-regulatory elements; A, B, and C [-232 to -187, -186 to -146, and -141 to -102, respectively]. Previous studies showed that Apo A1 mRNA is low in the liver of hypothyroid rats and is induced 20-fold following exposure to L-triiodothyronine (T3). To determine whether proximal sequences contained within the promoter mediated effects of T3, we constructed a template, Apo474-CAT containing the -474 to -7 sequence fused to the CAT gene. CAT activity increased 3-fold in human hepatoma cells, HuH-7 co-transfected with Apo474-CAT and human T3-receptor α (TRα). We speculated that site A mediated the effects of T3 because it contained a motif that matched closely with the T3-response element (TRE). Deletion of the A-site from Apo474-CAT not only abolished induction by T3 but the construct that lacked site A decreased 4-fold in response to T3. These observations suggest that the -474 to -7 contained both positive and negative TREs. Next we noted a striking similarity between the sequences in sites A and C. This lead us to speculate that similar proteins may interact with the two sites. Since ARP-1 and RXRα as well as TRα have been shown to interact with site A, we wondered whether these and other members of the same superfamily may bind to site C. Therefore, we chose 3 additional family members; HNF-4, COUP-TFII, and ARP-1 because their recognition sites were similar to sequences in site C. Both HNF-4 and ARP-1 but not COUP-TFII interacted with site C. Whereas, HNF-4 increased promoter activity 6- to 7-fold, ARP-1 repressed CAT activity by 2- to 4-fold. Both HNF-4 and ARP-1 expressed in COS cells bound to site C at the same motif. Based on the opposing effects of HNF-4/ARP-1 and their known tissue distribution, we postulated that balanced interactions of these two factors with site C regulate tissue specific expression of rat Apo A1. In summary, we have shown that the stimulatory effects of T3 on Apo A1 is mediated by TRα binding to site A of Apo A1 DNA. Two additional factors, HNF-4/ARP-1 from the same superfamily interact with site C to regulate tissue specific expression of the rat Apo A1.

B 241 STRUCTURAL AND FUNCTIONAL RELATIONSHIPS BETWEEN EUKARYOTIC NUCLEAR RNA POLYMERASES, Nancy A. Woychik, Keith McKune and Richard A. Young*, Department of Gene Regulation, Roche Institute of Molecular Biology, Nutley, NJ 07110 and *Whitehead Institute for Biomedical Research, Cambridge, MA 02142

The characterization of RNA polymerase subunit genes has revealed that some subunits are shared by the three nuclear enzymes, some are homologous, and some are unique to RNA polymerases I, II, or III. SDS-polyacrylamide gel electrophoresis of purified RNA polymerase II using an improved gel system revealed that the RPB9 subunit closely migrates with a previously unidentified subunit, RPB11. The gene which encodes the RPB11 subunit was isolated and shown to be present in single copy and localized directly upstream of the gene encoding topoisomerase I, *TOPI*, on chromosome XV. The sequence of the gene predicts an RPB11 subunit of 120 amino acids (13,600 daltons), only two amino acids shorter than the RPB9 polypeptide. *RPB11* was found to be an essential gene which encodes a protein closely related to an essential subunit shared by RNA polymerases I and III, AC19. Thus, at least nine of the twelve known RNA polymerase II subunits are related or identical to the subunits of RNA polymerases I and III, indicating that the three RNA polymerases may have significant structural and functional similarities. RPB11 contains a 19 amino acid segment found in three other yeast RNA polymerase subunits and the bacterial RNA polymerase subunit α. Some mutations that affect RNA polymerase assembly map within this segment, suggesting that this region may play a role in subunit interactions.

B 242 CCAAT BOX BINDING PROTEINS FACILITATE *IN VIVO* RECRUITMENT OF UPSTREAM DNA BINDING TRANSCRIPTION FACTORS. Kenneth L. Wright, Terry L. Moore and Jenny P.-Y. Ting, Lineberger Comprehensive Cancer Center, Uni. of North Carolina, Chapel Hill, NC 27599.

Studies of protein-protein interaction and promoter assembly have been limited by the necessity of using isolated proteins and DNA fragments in an *in vitro* system. We have now developed a system to address these questions in the more physiologically relevant chromatin environment of the intact cell. We have developed a panel of transfected cell lines that maintain stable, chromatin integrated wild type and mutant promoter constructs. These lines were analyzed by *in vivo* genomic footprinting which dissected the importance of specific DNA binding events on the recruitment of additional DNA binding factors and promoter assembly in an intact cell. Our previous *in vivo* genomic footprint studies of the major histocompatibility complex class II gene promoter, DRA identified four protein/DNA interactions including a CCAAT box element which binds to the CCAAT box binding factors NF-YA/B *in vitro*[1]. Functional analysis of the CCAAT box has shown it to be required for both basal and interferon-γ (IFN-γ) induced expression. *In vivo* analysis of the endogenous DRA promoter in an IFN-γ inducible glioblastoma cell line displayed interactions at the CCAAT box and an up-regulation of interaction at the X box upon IFN-γ induction. We now show that the *in vivo* protein/DNA interactions and IFN-γ induction on a stably integrated wild type DRA promoter are identical to those of the endogenous gene. Analysis of cell lines maintaining a mutated CCAAT box-DRA promoter exhibited a loss of the protein/DNA interactions at the CCAAT box and no transcriptional activity. More interestingly, the *in vivo* binding activity at the upstream X box was also significantly impaired. In contrast, mutation of two additional sites affected only the mutated interaction but had no effect on CCAAT box binding. This is the first report of an analysis capable of directly revealing the *in vivo* affect of individual DNA binding events on transcription factor-promoter assembly. These results implicate protein binding at the CCAAT box as a primary event in the recruitment of additional DNA binding proteins.

[1]Wright & Ting, PNAS 89:7601, 1992.

B 243 TRANSCRIPTIONAL AUTOREGULATION BY WILD TYPE P53 VIA AN NF-kB MOTIF, Hongyun Wu, Abdul Deffie, Valerie Reinke and Guillermina Lozano, Department of Molecular Genetics, University of Texas M.D.Anderson Cancer Center, Houston, Texas 77030

p53 is the most frequent target inactivated in various tumors. In transformation assays, addition of a p53 expression plasmid decreases the number of foci. Overexpression of wild-type p53 is incompatible with continued cell growth. Being defined as an important tumor suppressor gene, the mechanism by which p53 suppresses tumor formation is still unknown. We first noted that p53 could function as a transcriptional activator via a heterologous DNA binding motif. The correlation between the transcriptional regulation of various genes by p53 and its tumor suppression capability suggests that p53 controls cell growth by regulating key genes in the process. Finding the targets of p53 transactivation will be critical to our understanding of p53 in the cascade of events leading to tumor suppression. We studied the ability of p53 to regulate its promoter. Our results showed that wild-type p53 can up-regulate its expression in various cell lines. Deletion analysis of the promoter has minimized the region responsible for autoregulation to within 45 base pairs. Methylation interference was then performed to define the nucleotides involved in protein-DNA interaction. The nucleotides protected in this assay were identical to those protected by NF-kB. Site-directed mutation analysis and concatemerization of this site in front of a heterogenous promoter further suggested that the NF-kB motif is responsible for p53 autoregulation. Further experiments involving EMSA with antibody supershifts and transfection/transcription experiments will help us to define the protein(s) specifically involved in p53 autoregulation. The identification of these proteins will be important in the understanding of p53 in tumor suppression.

B 244 THE CARBOXY-TERMINAL DOMAIN OF THE RNA POLYMERASE II LARGEST SUBUNIT CAN FUNCTION AS AN ACTIVATOR OF TRANSCRIPTION, Hua Xiao,[1,2] John T. Lis[2] and James D. Friesen,[1] Department of Genetics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario M5G 1X8, Canada,[1] Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853, USA[2]

It is widely proposed that upstream activator proteins stimulate transcription by making direct contact with the general transcription machinery. In this study, we tested whether a domain of a protein that can make direct contact with the general transcription machinery could stimulate transcription. One candidate that we tested was the carboxy-terminal domain (CTD) of the RNA polymerase II largest subunit. It was recently shown that the heptapeptide repeat which constitutes the CTD interacts with the TATA-box-binding subunit (TBP) of the general transcription factor TFIID. We constructed hybrid proteins that contain the CTD of the yeast or Drosophila RNA polymerase II fused to the DNA-binding domain of the upstream activator protein GAL4, and tested the ability of the hybrid proteins to stimulate transcription in yeast. Our results showed that the GAL4-CTD fusion proteins stimulate transcription by over 1000-fold from a GAL1-lacZ reporter gene. This result prompted us to search for sequence similarities between the CTD and known transcriptional activation domains. We found that the proline-rich transcriptional activation domain of the CCAAT-box-binding factor CTF/NF1 contains a sequence that is identical to the heptapeptide repeats of the CTD. Deletion of a region including this CTD-like sequence from the proline-rich activation domain dramatically reduced its transcriptional activity, and insertion of the CTD heptapeptide repeats from RNA polymerase II into this deletion derivative restored the transcriptional activity. These results suggest that RNA polymerase II and at least one class of proline-rich activator proteins possess a common structural and functional component that can interact with the general transcription factor TFIID. We propose that an activator protein such as the GAL4-CTD can function at both pre-initiation and post-initiation steps to increase transcription.

B 245 DISTINCT ROLES FOR THE TWO cGATA-1 FINGER DOMAINS IN DNA-BINDING AND TRANSCRIPTIONAL ACTIVATION, Heng-Yin Yang and Todd Evans, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260

We have generated and analyzed by functional assays mutations of the chicken erythoid transcription factor GATA-1. The cGATA-1 protein contains two related finger domains highly conserved across species and characteristic of the family of GATA-binding factors. We find that mutations in the C-terminal finger or adjacent basic region abolish sequence-specific DNA-binding, confirming that this region constitutes a novel DNA-binding domain sufficient to recognize the consensus WGATAR motif. At least three separate regions outside of this finger II domain contribute in a cooperative manner to the trans-activation potential of the protein. As expected from the previous results analyzing the mouse homolog, we find that the N-terminal finger plays a role in DNA-binding by affecting the stability of the DNA/protein complex. In addition, we find mutations of finger I altered subtly in DNA-binding function which greatly diminish trans-activation. Our results support the notion that the GATA-1 protein must be positioned precisely on the GATA cis-element to enable the activation of target genes.

Transcription II

B 300 CHARACTERIZATION OF cis-ACTING ELEMENTS REGULATING TRANSCRIPTION OF THE DF3 BREAST CARCINOMA-ASSOCIATED ANTIGEN. Abe,M. and Kufe,D.W. Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

DF3 antigen, a polymorphic glycoprotein (300-500 Kd) containing 20 amino acid repeats is aberrantly overexpressed in the cytosol of human breast cancer cells. Expression of this antigen is regulated at the transcriptional level. DF3 genomic DNA was cloned from human MCF-7 breast carcinoma cells to study the mechanisms responsible for control of the DF3 gene. A region 1656 bp upstream to the DF3 transcription initiation site was ligated to the CAT reporter gene. Transient expression assays using a series of deleted constructs demonstrated that the region from position -618 to +31 contains the regulatory sequences necessary for DF3 transcription. Further analysis of internal deletions within this region and heterologous promoter constructs indicated the involvement of cis-acting elements in the fragment from positions -598 to -485. Deletions at either end of this region were associated with decreased or complete loss of trancriptional activity. These results suggested that the DF3 transcription is regulated by at least two distinct elements. Gel retardation, DNaseI footprinting and Southewestern studies have identified a protein (45 Kd) that recognizes sequenes at positions -505 to -485 (GGGAAGTGGTGGGGGGAGGGA). These results suggest that DF3 gene transcription is regulated by a factor that recognizes a novel consensus sequence.

B 301 THE HUMAN HEAT SHOCK PROTEIN HSP70 INTERACTS WITH THE HEAT SHOCK FACTOR (HSF), AND PREVENTS THE ACTIVATION OF HSF *IN VITRO*. Klara Abravaya, Michael P. Myers, Shawn P. Murphy, and Richard I. Morimoto. Department of Biochemistry, Molecular and Cellular Biology, Northwestern University, Evanston, IL 60208

All organisms respond to physiological stress conditions such as elevated temperature, by inducing the synthesis of a group of proteins called stress or heat shock proteins (hsps). The transcriptional induction of heat shock genes is mediated by the activation of a transcription factor, heat shock factor (HSF). A homeostatic mechanism where heat shock proteins negatively regulate their own expression has been proposed. It has been postulated that this regulation could be achieved by interaction of hsps with the protein that regulates their expression, HSF. We sought to determine whether the human heat shock protein, hsp70 interacts with HSF and whether hsp70 modulates HSF activity in human cells. By the use of monoclonal antibody specific for hsp70 (C92), in a gel mobility shift assay, we demonstrated that *in vivo* activated HSF is associated with hsp70 and that this interaction is detected as the levels of hsp70 increase in the cell. (Abravaya et al., 1992 Genes & Dev. 6:1153). Consistent with the property of 70kD hsps (their interaction with protein substrates is disrupted by ATP), HSF/hsp70 interaction is disrupted upon addition of ATP, but not upon addition of non-hydrolyzable analog AMP(PCP). Recombinant wild type hsp70 is able to associate with *in vivo* activated HSF, while an N-terminal deletion mutant which retains the peptide binding domain and a C-terminal deletion mutant retaining ATP- binding domain fail to interact with HSF. Complexes between HSF and hsp70 can also be formed using recombinant purified hsp70 and HSF. Association of hsp70 with HSF does not impair the DNA binding ability of activated HSF. A potential regulatory role of hsp70 in HSF activation is suggested using an *in vitro* system for HSF activation. HSF can be converted from a non-DNA binding form to a DNA binding form by treating cytoplasmic extracts by heat or non-ionic detergent NP40 (Larson et al., 1988 Nature 335:372; Mosser et al., 1990 PNAS 87:3748). We found that hsp70 blocks the conversion of HSF from an inactive form to an active form in this system. This inhibitory effect of hsp70 was relieved by ATP but not by AMP(PCP). We thus suggest that hsp70 may have a regulatory function in the activation of HSF.

B 302 EXPRESSION OF *GADD153* AND OTHER MEMBERS OF THE C/EBP FAMILY IN RESPONSE TO ACUTE STRESS .
Colette M.J.ap Rhys, Sara Carlson, Sherrie Reichenbaugh, Jennifer D. Luethy and Nikki J. Holbrook. Laboratory of Molecular Genetics, National Institute on Aging, Baltimore, MD 21224.
The *gadd153* gene encodes a protein related to the C/EBP family of transcription factors. *In vitro*, the formation of *gadd153* heterodimers with members of the C/EBP family has been shown to block their ability to bind to DNA and act as transcriptional activators. The *gadd153* gene has been shown to be responsive to a wide variety of acute stresses that result in arrest of cell growth or damage to DNA. In order to determine whether other members of the C/EBP family would respond similarly, cultured cells were stressed by inducing DNA damage by MMS or by UV light, and growth arrested by nutrient depletion or by the addition of PGA_2. The mRNA levels of C/EBPα were unchanged by these treatments. On the other hand, mRNA levels of *gadd153*, C/EBPβ and C/EBPδ were significantly elevated in response to all of these treatments. Since the expression of C/EBPβ and C/EBPδ increase significantly during the acute phase response, *in vivo* studies were undertaken in order to determine whether *gadd153* expression increases and, if so, to define the temporal nature of its expression in relation to C/EBPβ and C/EBPδ. LPS-induced acute inflammation in the rat resulted in a rapid increase in the expression of C/EBPβ and C/EBPδ . Maximal expresson occurred between 2 to 4 hours and declined thereafter. In contrast, *gadd153* showed no change in expression before 4 hours after LPS treatment but was highly induced by 8 hours. Examination of the *gadd153* promoter region revealed a putative C/EBP binding site. Cotransfection of a *gadd153* promoter-CAT construction with C/EBP expression vectors demonstrated that the *gadd153* promoter can be transactivated by C/EBPα, C/EBP/β and C/EBPδ in HeLa cells and to a much greater extent in HepG2 cells. A deletion of the *gadd153* promoter-CAT construction that removes the putative C/EBP site proved nonresponsive. Since *gadd153* expression overlaps with the expression of C/EBP/β and C/EBPδ under the described conditions, it is likely that they contribute to the activation of *gadd153* following stress.

B 303 CLONING OF A NOVEL c-FOS SRE-BINDING PROTEIN Ricardo Attar and Michael Gilman, Cold Spring Harbor Laboratory, P.O Box 100, Cold Spring Harbor, NY 11724
Transcription of the c-*fos*-proto-oncogene is induced within minutes after activation of several intracellular signal transduction pathways and rapidly repressed soon thereafter. Many of these signals act through a small regulatory element that flanks the c-*fos* gene, the serum response element (SRE). The SRE is a binding site for at least three cellular DNA-binding proteins. To achieve a better understanding of the mechanism by which the SRE regulates c-*fos* transcription, we have screened a HeLa cell cDNA expression library for phage expressing proteins that specifically bound an SRE oligonucleotide. We isolated a phage that encoded a lacZ fusion protein that specifically bound wild-type SRE oligonucleotides but not a mutant site that is inactive in vivo. The fusion protein binds specifically to the SRE in both a Southwestern blot and a mobility-shift assay. Analysis of the full length DNA sequence of this clone revealed that it was a previously unidentified gene that belongs to the family of zinc finger-containing proteins related to the Drosophila *Krüppel* gene. The clone contains seven tandem repeats that match the zinc-finger consensus for this gene family. We call this protein SRE-ZBP (serum response element-zinc finger binding protein). DMS-interference assays show that SRE-ZBP interact with the 3' region of the SRE. This gene is expressed in a variety of human cell lines at a very low level. Expression in HeLa cells is induced by serum and cycloheximide, suggesting that the SRE-ZBP belongs to the immediate early gene family. Antibodies raised against SRE-ZBP do not recognize any previously characterized SRE-binding proteins. Immunofluorescence assays on Cos cells transfected with the SRE-ZBP cDNA show that this protein is nuclear. Preliminary results suggest that the cellular SRE-ZBP protein has an apparent M_r of 70 KD. The presence of Zn fingers in this clone strongly suggests that it is a DNA-binding protein and probably a transcription factor.

B 304 REGULATORY ELEMENTS CONTROLLING THE EXPRESSION OF THE FIBRONECTIN RECEPTOR α4 SUBUNIT IN CORNEAL EPITHELIAL CELLS, Jean-François Audet, Christian Salesse and Sylvain L. Guérin, CHUL Research Center, Québec, (Canada), G1V 4G2

Extracellular matrix is a complex cross-linked structure of proteins and polysaccharides which organize the geometry of normal tissue. Some components of this matrix are involved in cell adhesion, migration and proliferation. One of these is fibronectin, a 440 kd glycoprotein that is recognized by the cellular α4/β1 and α5/β1 cellular fibronectin receptors, two members of the integrin superfamily. These receptors in combination with fibronectin seem to exert critical functions in the mechanism of reepithelialisation during wound healing. In fact, histochemical studies performed on rabbit cornea provided the evidence that high levels of fibronectin are detected during the reepithelialisation process and then returns to normal levels after healing. Immunological studies carried out using antisera raised against the fibronectin receptor clearly indicated that its concentration also increases during that same healing process. In order to define what are the cis-acting regulatory elements controlling the expression of the α4 subunit gene (a component of the FNR) during the reepithelialisation process, CAT reporter constructs containing various lengths of the promoter and 5'-flanking region of this gene were transiently transfected into primary cultures of rabbit corneal hepithelial cells. These results revealed that negative regulatory elements located in the 5'-flanking region of the α4 gene were clearly repressing the level of CAT gene expression when compared to the level driven by the basal α4 promoter. In addition, crude and heparin-sepharose enriched nuclear extracts prepared from either non-confluent (which simulate the healing process) or confluent cultures of hepithelial cells were used for both gel mobility shift assays and DNaseI footprinting experiments. A number of distinct DNA/protein complexes were identified and correlated with the results from transient transfection analyses. Here we present a detailed conclusion of the observations obtained from these studies.

B 305FUNCTIONAL CHARACTERIZATION OF THE TRANSCRIPTION FACTOR hUCRBP.
Kevin G. Becker, Paul Jedlicka and Keiko Ozato.
Laboratory of Molecular Growth Regulation,
National Institute of Child Health and Human Development,
National Institutes of Health, Bethesda, MD 20892.
We have cloned the transcription factor HUCRBP (YY1, NF-E1,δ), a C_2-H_2 type zinc finger protein which may be involved in the regulation of many viral and cellular genes, including the human LINE-1 element. hUCRBP accounts for much of the cellular UCR binding activity seen in EMSA gels. This was determined by (a) complete removal of binding activity by anti-hUCRBP antibody by (b) UV crosslinking of nuclear extracts with the UCR core element and by (c) western blot analysis. The protein sequence predicts that there are nine potential serine/threonine phosphorylation sites in hUCRBP. Treatment of nuclear extracts with potato acid phosphatase removes all binding to the UCR core element, while the phosphatase inhibitor 4-nitro-phenyl phosphate blocks this loss of activity. We have also carried out functional analysis of hUCRBP using transient co-transfection assays and show that hUCRBP can act as both a positive and negative regulator of transcription, and that this dual activity may depend upon the level of hUCRBP expressed.

B 306 TRANSCRIPTION OF A PROGESTERONE RESPONSIVE GENE IS SPECIFICALLY INHIBITED BY A TRIPLEX FORMING OLIGONUCLEOTIDE, Johanna M. Beekman[1], Nancy, H. Ing[1], Donald J. Kessler[2], Michael E. Hogan[2], Bert W. O'Malley[1] and Ming-Jer Tsai[1], Department of Cell Biology[1], Center for Biotechnology[2], Baylor College of Medicine, Houston, TX 77030

Antagonists of steroid hormone receptors are gaining medical popularity for reproductive control and cancer treatment. Oligonucleotides provide novel reagents for inhibition of gene expression because of their high affinity binding to specific nucleotide sequences. Single-stranded DNA oligonucleotides bind to the major groove of a double-stranded target DNA to form a triple-helix or "triplex" in a sequence specific manner. When triplex-forming oligonucleotides (TFOs) are targeted to a vital promoter region of a test gene, they have been shown to selectively repress transcription in a dose-dependent manner. We describe a 38 base, single-stranded DNA that forms a triple helix on progesterone response elements of a target gene. This triplex-forming oligonucleotide, containing natural deoxyguanosine and deoxythymidine, binds with a Kd = 100 nM at 37°C at physiological pH. Bandshift experiments show that the TFO blocks binding of progesterone receptors to the target DNA. Furthermore, it completely inhibited progesterone receptor-dependent transcription in vitro. This demonstration of specific gene inhibition by triplex formation joins growing evidence for therapeutical potential of oligonucleotides.

B 307 IDENTIFICATION OF A GROWTH HORMONE RESPONSE ELEMENT IN THE SOMATOSTATIN PROMOTER Nils Billestrup, Elisabeth D. Petersen, Johnny A. Hansen and Jens H. Nielsen, Hagedorn Research Laboratory, DK-2820 Gentofte, Denmark

Growth Hormone (GH) stimulates the proliferation of pancreatic islet cells as well as the transcription of the insulin and somatostatin genes. In order to test whether GH stimulates somatostatin gene transcription directly we have analyzed the ability of GH to stimulate expression of somatostatin promoter/CAT constructs. By transient transfection of the islet tumor cell line RIN 5-AH with various somatostatin promoter/CAT constructs it was observed that GH could stimulate expression 4-5 fold using constructs containing as little as 48 bp upstream of the transcription initiation site. The effect was dose-dependent with a half-maximal effect observed at 0.5 nM and maximal effect at 5 nM of GH. No effect of GH on expression of CAT fusion genes containing the RSV or α-chorionic gonadotropin promoters was observed. When the sequence from -44 to -33 of the somatostatin promoter was introduced upstream of a MMTV LTR minimal promoter/CAT construct either as a monomer or as a 3-mer, GH stimulation of CAT activity was observed. This indicates that a GH response element is present from -44 to -33 of the somatostatin promoter. This region of the somatostatin promoter contains a characteristic CAGAGAGAGA sequence which is also present in the promoter of other GH regulated genes. Gel shift assays using the -44 to -33 sequence as a probe and a nuclear extract from RIN 5-AH cells revealed the presence of a specific DNA/protein complex which was specific for the GAGA sequence. The presence of this complex was not dependent on whether the nuclear extract was prepared from control or GH stimulated cells. Using biotinylated DNA fragments containing the GAGA sequence and nuclear extracts from ^{35}S-Methionine and ^{35}S-Cystine labelled cells a protein with a Mr of 56 000 could be identified. These results indicate that GH directly stimulates transcription of the somatostatin gene by activating a transcription factor which can recognize the GH response element.

B 308 ATFa, MEMBERS OF THE ATF FAMILY, MEDIATE E1a-INDUCED TRANSACTIVATION BY DIRECTLY INTERACTING WITH E1a, Bruno Chatton, José L. Bocco, Jean Gœtz and Claude Kédinger, L.G.M.E. (CNRS), Unité 184 (INSERM) 11, rue Humann, 67000 Strasbourg, France.

The 289-aminoacid adenovirus E1a protein is both a potent activator of viral gene transcription and an oncoprotein. Three regions, designated regions 1, 2, and 3, highly conserved among E1a proteins from different serotypes are responsible for the various activities of E1a. Regions 1 and 2 are required for cellular transformation, whereas region 3 is necessary and sufficient for transcriptional activation. E1a is not a sequence-specific DNA-binding protein and may associate to promoter DNA by interacting with cellular transcription factors already bound to DNA. Several of the adenovirus early promoters, transcriptionally activated by the viral E1a protein, contain binding sites for members of the ATF family of transcription factors. These sites correspond to sequence motifs which have also been identified as cAMP-responsive elements (CRE) within the promoter of many cAMP-inducible genes.

We have isolated from a HeLa cell cDNA library three related clones encoding proteins (ATFa1, a2, a3) with specific ATF/CRE DNA-binding activities. Using cotransfection experiments, we show that these proteins mediate the transcriptional activation induced by E1a, and that the zinc-binding elements both present in the E1a region 3 and the ATFa proteins are essential for this effect. Using reciprocal co-immunoprecipitation and "far-western" experiments, we demonstrate that the E1a products directly contact the ATFa proteins and that the respective zinc-binding elements are partly involved in this interaction. The simultaneous alteration of both N-terminal and C-terminal domains of ATFa abolishes E1a binding, while either mutation alone fails to do so. Further deletional analysis of ATFa has revealed the existence of a negative regulatory domain located within the Leu-zipper region of ATFa. The nature and function of this element will be discussed.

B 309 CONSERVED REGION 1 OF THE ADENOVIRUS E1A GENE ENCODES A POTENT TRANSCRIPTIONAL ACTIVATOR FUNCTION, Maria Bondesson, Mattias Mannervik, Catharina Svensson and Göran Akusjärvi, Department of Microbial Genetics, Karolinska Institute, 104 01 Stockholm, Sweden

The adenovirus E1A 289 amino acid protein contains an efficient transcriptional activation domain, designated conserved region (CR) 3. Previous studies have shown that CR3 is a strong activator when fused to the DNA binding domain of Gal4 (Gal4 DBD) and assayed on a promoter containing Gal4 binding sites (Lillie & Green (1989) Nature, 338, 39-44). However, we show here that the amino terminal end of E1A (amino acids 23-188, lacking CR3) is as efficient as CR3 in activating transcription, when fused to the Gal4 DBD or the E2 DNA binding domain of bovine papilloma virus. Deletion of CR2 or a truncation of the protein at amino acid 91 created an activator that was stronger than CR3. In contrast, a deletion of CR1 abolished the activation potential completely. Collectively, these results show that CR1 encodes a very potent transcriptional activator function. CR1 has previously been shown to be important for the immortalization/transformation activity of E1A and has furthermore been shown to be involved in the repression of enhancer driven transcription.

Experiments where the activating capacity of the Gal4 /E1A fusion proteins was measured in the presence of an excess of free E1A proteins (not bound to DNA) showed that Gal4-CR1 mediated transcription was efficiently competed by an excess of wtE1A or E1A-12S proteins, but not by a CR1 mutant. Gal4 CR3 mediated transcription was competed by wtE1A or the CR1 mutant, but not by the E1A-12S protein. These results indicate that CR1 and CR3 mediate transcriptional activation through distinct factors which independently interact with the basal transcriptional machinery. In addition, CR1 and CR3 were able to activate transcription synergistically (around 10 times). The synergistic activation was also apparent when CR1 and CR3 were localized on heterodimeric molecules (one polypeptide chain encoding the CR1 activator and the other the CR3 activator), held together by the Gal4 dimerization domains.

B 310 ICSBP REQUIRES AN ACCESSORY MOLECULE FOR *HIGH* AFFINITY DNA BINDING AND MAY COMPETE WITH ISGF3, Chiara Bovolenta, Paul H. Driggers, Michael S. Marks, Jeffrey A. Medin, Alex D. Politis, Stephanie N. Vogel, John E. Coligan and Keiko Ozato, LMGR, NICHD, NIH, Bethesda MD, 20892.

ICSBP is a transcription factor involved in IFN-mediated gene induction. It belongs to the IRF family and is induced by IFN-γ. ICSBP has some homology with ISGF3γ. ISGF3γ is a subunit of the ISGF3 complex believed to act as an inducer of IFN regulated genes. By western blot analysis we show that ICSBP protein (48 kDa) is exclusively localized in the nucleus of immune cells. We have studied how ICSBP binds to the interferon stimulated response element (ISRE) derived from IFN inducible genes.
We noted that neither recombinant ICSBP (rICSBP) produced in a baculovirus vector (or in a bacterial vector) nor in vitro translated ICSBP binds to ISRE at high affinity. However we now show that rICSBP can bind at high affinity when mixed with IFN treated cell extracts. The newly formed ICSBP complex was identify in gel mobility shift assays, was specific for ISRE and was "supershifted" by anti-ICSBP antibody. The ability of ICSBP to bind to ISRE was dependent on treatment of cells with IFN and cell type specific. These results suggest that ICSBP, like ISGF3γ, requires a cytoplasmic accessory molecule to bind to ISRE. We present evidence that binding of ICSBP to the ISRE interferes with the binding of ISGF3. The ISGF3-ISRE complex is formed with IFNα treated cell extracts in gel shift assay. However when rICSBP was added, the intensity of the ISGF3 band was reduced in a concentration dependent manner. The reduction was specific for ICSBP since control proteins showed no reduction. Possible competition between ICSBP and ISGF3γ for an accessory molecule will be discussed.

B 311 SUPPRESSION OF MUTATIONS WITHIN THE GLUTAMINE-RICH REGIONS OF CREB BY A *GAL4* ACIDIC ACTIVATION DOMAIN, Paul Brindle, Steve Linke. Marc Montminy, The Salk Institute, La Jolla, CA 92037.
The cyclic AMP response element binding protein (CREB) activates transcription when phosphorylated at serine 133 by the cAMP-dependent protein kinase (PKA). A repressor of CRE-dependent transcription, termed CREM (N. S. Foulkes *et al.* Cell 64:739,1991), is nearly identical to CREB except that it lacks portions of two glutamine-rich regions that flank CREB's ser 133, termed Q1 (N-terminal of ser 133), and Q2 (C-terminal of ser 133). By comparison to CREM and by deletion mutagenesis of CREB's Q1 region (Gonzalez *et al.* MCB 11:1306, 1991), Q1 and Q2 have been postulated to be activation domains analogous to glutamine-rich activators found in SP1, for example. To test if Q2 was required for CREB function, we performed deletion mutagenesis studies and found that numerous deletions within Q2 decrease CREB activity. One model for how phosphorylation of ser 133 increases CREB activity is that phosphorylation causes an allosteric change that increases the activity of the Q1 and Q2 regions. This model suggests that the ability of CREB to function would be dependent on specific activation domains (Q1 and Q2), and their positions relative to ser 133. To test this hypothesis we exchanged the Q1 and Q2 regions with the *GAL4* acidic activation domain (aa 768-881). Remarkably, the *GAL4* acidic activator could suppress the effect of Q1 and Q2 deletion mutations, with the GAL4/CREB fusion proteins retaining the ability to be activated by PKA. In addition, the GAL4/CREB fusions had more activity than would be predicted from adding the activities of each activator tested separately, suggesting that multiple activation domains can act synergistically when fused in the same molecule. These results have several implications: 1) the Q1 and Q2 regions are not inseparable parts of a CREB activation domain in that they are replaceable and PKA inducibility is retained; 2) it is unlikely that phosphorylation of ser 133 activates CREB by causing a structural change in Q1 and Q2; 3) CREB is made up of modular activation domains that act synergistically with one another to activate transcription.

B 312 ALU SEQUENCES ARE INVOLVED IN THE CELL-SPECIFIC TRANSCRIPTIONAL REGULATION OF THE γ CHAIN OF Fc AND T CELL RECEPTORS, Anna T. Brini, Gai M. Lee and Jean-Pierre Kinet, Molecular Allergy and Immunology Section, National Institute for Allergy and Infectious Diseases, National Institutes of Health, 12441 Parklawn Drive, Rockville, MD 20852
The FcεRI γ chains are expressed in a variety of hematopoietic cells where they play a critical role in signal transduction. They are part of the high affinity IgE receptor in mast cells, basophils, Langerhans cells, and possibly other cells, part of the low affinity receptor for IgG (FcγRIIIA or CD16) in NK cells and macrophages, and part of the T cell antigen receptor in subsets of T cells. Here we report the transcriptional regulation of the γ chain gene by analyzing the 2.5 kb sequence upstream of the transcription start site. This sequence contains a promoter confering tissue specificity. It is functional only in hematopietic cells, regardless of whether they express the FcεRI γ chain transcripts. We have identified two adjacent regulatory elements, both of which contain Alu sequences. The first (-445/-365) is the positive element active in both basophils and T cells. The second (-365/-295) binds to nuclear factors different in basophils and T cells and acts as a negative element in basophils and as a positive one in T cells. Thus, Alu sequences found in the 5' region of the γ chain gene play a role in its cell-specific regulation.

B 313 TRANSCRIPTION INITIATION AT THE MURINE *REP-3* PROMOTER, Andrew B. Buermeyer and Peggy J. Farnham, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

The proximal promoter of the murine *Rep-3* gene has six binding sites for the transcription factor Sp1, but no close match to a consensus TATA box. We are studying how the preinitiation complex forms at this promoter. Promoter sequences extending from -49 to +14, relative to the start site at +1, are sufficient for accurate initiation of transcription. Two proteins bind to this minimal *Rep-3* promoter. The activator protein Sp1 binds to one site at -41. We have found that the second protein is the transcription factor YY1. YY1 in crude nuclear extracts or as a recombinant protein purified from bacteria bound to sequences from -21 to -13. This is approximately where a consensus TATA box is found in many class II promoters. At this position, YY1 could aid or block the interaction of TFIID with a nonconsensus TATA box in the *Rep-3* promoter. Both YY1 and Sp1 have been implicated in start site selection by RNA polymerase II. We are now investigating how these transcription factors influence the formation of preinitiation complexes at the proximal *Rep-3* promoter.

B 314 FUNCTIONAL ANALYSIS OF THE CAP SITE-PROXIMAL HUMAN INTERLEUKIN-1β POMOTER,
Jon A. Buras, Brian G. Monks, Bridget A. Martell and Matthew J. Fenton, Department of Medicine, Boston University Medical Center, Boston, MA 02118

Activated monocytes and macrophages express several potent proinflammatory cytokines, including interleukin 1β (IL-1β). Regulation of this gene appears to be mediated by both upstream and cap site-proximal (CSP) sequence elements. Interaction between these elements constitutes the molecular basis for inducible expression of the IL-1β gene. We have identified two novel nuclear proteins, termed NFβA and NFβC, which bind to specific sequences within the IL-1β CSP promoter, and a novel upstream factor, termed NFβB. NFβA binds to a highly conserved sequence adjacent to the TATA box of the IL-1β gene, whereas NFβC binds to a DNA sequence which spans the cap site of the IL-1β gene. Functional analyses show that NFβA is required for expression from the IL-1β CSP promoter and that NFβA is also involved in transcriptional transactivation or transrepression by the cytomegalovirus IE1 gene product in a cell type-specific manner. Studies using transfected RAW 264.7 and COS cells show that NFβA can activate transcription from a heterologous promoter in a distance and orientation independent manner. In these cells, transactivation mediated by NFβA is also dose-dependent, with increasing expression correlating with the placement of 1, 4, and 6 copies of the NFβA binding site upstream of the herpes virus thymidine kinase promoter. Fuctional comparison of the IL-1β CSP promoter and a thymidine kinase promoter containing a single added copy of the NFβA site in transfected RAW 264.7 and COS cells reveals the presence of a repressive element within the IL-1β promoter fragment. Our data suggest that NFβC is responsible for the reduced level of expression from the IL-1β CSP promoter. Interestingly, the DNA-binding activity of NFβC appears to require association with a distinct factor, termed NFβB, that binds to an unrelated sequence 300 bp further upstream. Competition experiments show that NFβC and NFβB can specifically interact, possibly through a shared subunit that is required for the DNA binding activity of NFβC. We propose that NFβB constitutes a subunit of NFβC that does not directly contact DNA, in addition to its ability to bind to DNA at a distinct site in the IL-1β promoter. The interaction between factors binding at both proximal and distal promoter elements suggests that the interplay of these factors may be important for coordinate regulation of IL-1β gene expression.

B 315 A mammalian sequence-specific DNA binding protein contains a protein domain similar to bacterial DNA ligases. Peter Burbelo, Atsushi Utani and Yoshihiko Yamada. Laboratory of Developmental Biology, National Institute of Dental Research, N.I.H., Bethesda, MD 20892.

A bidirectional promoter consisting of an 130 bp fragment has been shown to separate the transcriptional unit of both the α1 and α2 collagen IV genes. Within this bidirectional promoter are two CCTCCCCTT rich regions, which are also found in the promoters of a number of other matrix genes including laminin and fibronectin. Using Southwestern screening of a 14-day mouse embryo cDNA expression library with the CCTCCCCTT-containing oligonucleotide (CIV), a 1.4 kb partial cDNA encoding a DNA binding protein (SW) was obtained. Cloning of the full length cDNA for SW revealed it coded for a large basic protein (Mr=130 kd). Sequence analysis revealed that SW contained three notable protein motifs: (1) a 110 amino acid domain with 49% homology to *Thermus thermophilus* bacterial DNA ligase, (2) an acidic region commonly found in many transcription factors and (3) a nucleotide binding site found in many cell cycle control genes regulating DNA replication. Northern analysis revealed a high level of SW mRNA expression in the testis, spleen, thymus, and in the kidney. Gel shift assays of recombinant SW fusion protein showed sequence-specific DNA binding to the CIV sequence and not to other unrelated sequences. The 110 amino acid "ligase-like" domain of SW was found to be required for DNA binding. These results suggest that the SW may be a sequence-specific DNA binding protein involved in DNA replication and/or

B 316 cC/EBP, A CHICKEN TRANSCRIPTION FACTOR OF THE LEUCINE-ZIPPER C/EBP FAMILY, Cor F. Calkhoven, Geert AB and Jan Wijnholds, Department of Biochemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands.

The chicken apoVLDL II gene encoding the Very-Low-Density-apo-Lipoprotein II subunit is specifically expressed in liver and is strictly estrogen-dependent. The 300 bp 5' flanking sequence contains two EREs as well as recognition sequences for the ubiquitous factor COUP-TF and the liver-enriched factors LF-A1, C/EBP and DBP (1). The question is whether and how these factors interact in regulating transcription of the apoVLDL II gene. We have focussed on a binding site (element D) immediately flanking one of the EREs that is strikingly similar to element D of rodent albumin genes. From competition footprinting it appeares that binding *in vitro* to site D interferes with the occupation of the ERE. To study possible interactions between D-binding proteins and the estrogen receptor we have cloned cC/EBP (chicken CCAAT/Enhancer binding protein) using a rat C/EBPα probe (2). Comparative sequence analysis shows that the derived 324-amino acid chicken C/EBP sequence is highly similar (68,5 %) to the 358-residue C/EBPα sequence of rat (3). The C-terminal moieties constituting the basic, DNA-binding and leucine zipper, dimerisation domains are virtually identical (94 %). The N-terminal moieties are partially conserved (59 %); three highly conserved regions, designated I, II and III, can be distinguished and may correspond to individual functional domains. Our conserved region I coincides with the N-terminal *trans*-acting sequence defined in rat C/EBPα. Regions II and III map in a region of which the function is not clear. The conserved regions revealed in our investigations may help to further define the functional domains. We produced large quantities recombinant C/EBP in *E. coli*. Binding studies with recombinant cC/EBP are in progress.

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B 317 IDENTIFICATION OF A NEGATIVE REGULATORY ELEMENT IN THE PROMOTER OF THE MOUSE ALCOHOL DEHYDROGENASE GENE, *Adh-1*.
Lucinda G. Carr, Zhonghua Lin and Howard J. Edenberg, Departments of Medicine, Pharmacology, and Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202-5121

Mouse alcohol dehydrogenase (Adh-A2) catalyzes the rate-limiting step in ethanol metabolism in mouse liver. The mouse Adh-A2- encoding gene, *Adh-1*, is expressed in a tissue-specific and stage-specific manner. To analyze the transcriptional activity of *Adh-1* promoter regions, transfection studies were performed in which various 5'-flanking *Adh-1* sequences fused to the luciferase gene were introduced into H4IIE-C3 hepatoma cells. Initial studies reveal that there are positive regulatory elements between -229 to +53 and a negative regulatory element between nt -323 and -229. To identify the sequences implicated in supression of *Adh-1* gene expression, we performed gel retardation and footprint assays on the -323 to -229 promoter fragment to determine the regions of the promoter being bound by nuclear proteins from mouse liver and the hepatoma cell line, H4IIE-C3. DNaseI footprint assays revealed that the region between -324 and -297 was bound by a nuclear protein. Methylation interference assays were conducted to more precisely localize the protein-binding site within the nt -324 to -297 bp region. Methylation of two G's in the coding strand at nt -314 and - 304 and one G in the non-coding strand at nt -307 block formation of the protein-DNA complex. Thus, close protein-DNA contacts for this negative regulating site are within the sequence GAAGTTTCAGG, which may represent a novel regulatory sequence.
Supported by PHS AA07611, 08553, and 06460.

B 318 A DIRECT-REPEAT SEQUENCE IS REQUIRED FOR UP-REGULATION OF THE HUMAN BiP GENE BY BREFELDIN A, TUNICAMYCIN, AND CALCIUM IONOPHORE A23187, Chuck C.-K. Chao, Wai-Ching Yam and Nian-Kan Sun, Tumor Biology Laboratory, Department of Biochemistry, Chang Gung Medical College, Taoyuan, Taiwan 33332, Republic of China.

In this study, we report the transcriptional induction of polypeptide-binding protein (BiP), an endoplasmic reticulum-resident protein, by agents which blocks protein transport from the endoplasmic reticulum to the Golgi apparatus. The transcriptional rate of the BiP gene in mammalian cells was coordinately induced by calcium ionophore A23187 (18-20 fold), azetidine (8-15 fold), brefeldin A (15-20 fold), castanospermine (2.5-8 fold), 2-deoxyglucose (10-15 fold) and tunicamycin (4-5 fold). Analyses of the transfection with a PCR-cloned human BiP promoter indicate that HeLa cells utilized a transcriptional mechanism to increase the expression of BiP in resonse to A23187 and tunicamycin; whereas, utilized a post-transcriptional mechanism in response to azetidine, brefeldin, castanospermine and 2-deoxyglucose. Studies in NIH3T3 cells indicate a similar mechanism for the BiP induction by these agents except brefeldin A in which a transcriptional mechanism play a major role. We further demonstrated that in mammalian cells a region of the human BiP promoter responsive to A23187, brefeldin and tunicamycin was within a 69-bp region between -107 and -39, containing two direct-repeat sequences including CCAAT elements. Using a DNA mobility shift assay, a nuclear factor which recognizes the BiP promoter sequence was induced in both cell lines by A23187, azetidine and brefeldin A. The results indicate the regulation of BiP expression in mammalian cells at both the transcriptional and post-transcriptional level. The data also suggest that the nuclear factor may play a role in mediating transcriptional activation of the gene.

B 319 THE RB PROTEIN BEHAVES AS A TRANSCRIPTIONAL COACTIVATOR IN THE PRESENCE OF THE ADENOVIRUS E4 17 kD PROTEIN, IN UNDIFFERENTIATED CELLS, José L. Bocco, Bernard Reimund, Bruno Chatton and Claude Kédinger, L.G.M.E. (CNRS), Unité 184 (INSERM), 11 rue Humann, 67000 Strasbourg, France

A series of independent observations have recently demonstrated the reversible interaction of the retinoblastoma susceptibility gene product (Rb) with the cellular transcription factor E2F, in human or mouse cells. The E2F factor was initially identified in studies of the transcriptional control of the adenovirus E2a early promoter which contains two adjacent binding sites for this factor.

Transcriptional activation of the E2a promoter by E1a correlates with the ability of both early E1a proteins (289R and 243R) to sequester Rb and thereby release E2F from inactive complexes with this protein. In addition to its responsiveness to the E1a proteins, the E2a promoter is efficiently stimulated by a product of the viral E4 transcription unit, a 17 kD polypeptide encoded by the open reading frame 6/7 (E4-ORF6/7) of this early unit. The E4-dependent activation results from specific interactions between the E2F and E4-ORF6/7 proteins leading to the formation of complexes which bind cooperatively and stably to the two neighbouring E2F binding sites in the E2a promoter.

We have previously shown that in undifferentiated F9 cells (F9EC), the E2a promotrer is refractory to E2F-mediated activation by E1a. Using both band-shift and transfection experiments, we now show that in F9EC cells, the E4-ORF6/7 product recruites the Rb protein into a stable multi-protein complex with E2F and that in these cells, as opposed to differentiated cells, Rb is actively involved in the transcriptional stimulation of the E2a promoter by E4, in the absence of E1a activity.

Our results suggest that, depending on the cell state, Rb may behave either as a transcriptional activator (F9EC cells) or a transcriptional inhibitor (differentiated F9 cells). They also suggest that the adenovirus has evolved a dual strategy for the activation of its early genes, using E1a in differentiated cells, and the E4 product in undifferentiated cells where it circumvents the E1a functional defect.

B 320 SELECTIVE POSITIVE CONTROL OF A HOMEODOMAIN PROTEIN: VP16 RECRUITS OCT-1 BUT NOT OCT-2 TO AN OCT-1 RESPONSIVE SITE, Michele A. Cleary, Seth Stern, Masafumi Tanaka, and Winship Herr, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

The ubiquitous human POU-homeodomain protein Oct-1 and related B cell Oct-2 protein recognize the same regulatory site, the octamer motif, yet participate in different programs of transcription. The possession of different activation domains flanking their similar DNA-binding domains provides one mechanism for achieving differential activation. Another mechanism is demonstrated by the ability of Oct-1 to activate transcription from a site that bears little octamer similarity and that is responsive to neither Oct-1 nor Oct-2 alone. This activation is dependent on the potent herpesvirus transactivator VP16, which forms a multiprotein--DNA complex with Oct--1, but not Oct-2, on an immediate early gene promoter element called the TAATGARAT motif. In vivo, a form of the TAATGARAT motif that lacks an overlapping octamer motif is transcriptionally silent in Oct-1 and Oct-2-expressing cells, but can be activated by coexpression of VP16, presumably through its interactions with Oct-1. In vitro data support this observation by showing a recruitment of Oct-1 by VP16 to this site. An analogous recruitment of Oct-2 by VP16 does not occur.

Previously, the responsiveness of Oct-2 to VP16 could not be assayed in vivo due to interference by VP16 interaction with endogenous Oct-1. We have found that VP16 alone does not activate transcription well in mouse NIH 3T3 fibroblasts, probably because of differences in the human and mouse Oct-1 homeodomains as supported by in vitro mobility retardation analyses. After transient expression, however, human Oct-1, but not Oct-2, can activate transcription in these cells in response to VP16. This assay system shows that the Oct-1 DNA-binding domain is sufficient for VP16-induced positive control on the TAATGARAT immediate early gene promoter element and that the critical determinant for VP16-responsiveness is the Oct-1 homeodomain because substitution with the Oct-2 homeodomain is sufficient to disrupt activation. Thus through selective DNA-binding domain interaction and recruitment to a new regulatory site, VP16 expands the repertoire of sites responsive to the ubiquitous activator Oct-1, without affecting activation by its close relative Oct-2.

B 321 OLIGOMERISATION PROPERTIES AND DIRECT CALCIUM/CALMODULIN INHIBITION OF BASIC-HELIX-LOOP-HELIX E PROTEINS. B. Corneliussen, Y. Waltersson, C. Grundström, B. Hallberg, A. Thornell and T. Grundström. Department of Applied Cell and Molecular Biology, University of Umeå, S-90187 Umeå, Sweden.

The SL3-3 Enhancer Factor 2-1 (SEF2-1) gene encodes basic-Helix-Loop-Helix (bHLH) E proteins. We show that the gene spans more than 135 kb and is composed of at least 22 exons. Characterisation of many distinct cDNAs demonstrates a complex regulation by differential splicing. Two major types of encoded proteins with distinct N-terminals, denoted SEF2-1A and SEF2-1B (also named ITF2), were identified. Furthermore, the use of two alternative splice sites determines the presence of four extra amino acids in the region preceding the bHLH domain.

We have used a coupled *in vitro* transcription/translation system to study functional properties of SEF2-1 encoded proteins and E12/E47 proteins encoded by the related E2A gene. We show that the region preceding the bHLH domain of the proteins affects the stability of the DNA binding oligomers, and that homo-oligomers of SEF2-1 proteins lacking the 4 amino acids, SEF2-1-, and E12 are less stable than homo-oligomers of SEF2-1+ proteins and E47.

We also demonstrate that calcium loaded calmodulin directly interacts with both SEF2-1 and E12/E47 proteins and thereby specifically inhibits their DNA binding. This interaction is strictly calcium dependent and occurs through the highly conserved bHLH domains. These results imply a direct link between calcium regulation and bHLH transcriptional control.

B 322 DELINEATION OF A SMALL REGION WITHIN THE MAJOR TRANSACTIVATION DOMAIN OF THE GLUCOCORTICOID RECEPTOR THAT MEDIATES TRANSCRIPTIONAL TRANSACTIVATION, Karin Dahlman-Wright, Anthony Wright, Tova Almlöf and Jan-Åke Gustafsson, Centre for Biotechnology, Karolinska Institute, S-141 57 Huddinge, Sweden.

The glucocorticoid receptor is a soluble receptor protein that is activated by binding of steroid hormone and which subsequently binds to specific DNA elements and transactivates transcription. Previous deletion analysis of the human glucocorticoid receptor localised the major transcriptional transactivation activity of the receptor to a region of 185 amino acid residues in the amino terminus of the receptor protein. This region was named Tau 1 (Hollenberg S.M. and Evans R.M., 1988, Cell 55, 899-906).

To delineate the smallest active region within the Tau 1 domain we have tested the the transactivation activity of fusion proteins containing intact or deleted Tau 1 domains fused to the DNA-binding domain of the glucocorticoid receptor in yeast cells. Data showing deletions of the N- and C-termini of Tau 1 as well as internal deletions will be presented. These indicate sequences important for the transactivation activity of Tau 1. The activity of small fragments containing these sequences will also be presented.

B 323 REGULATION OF TRANSCRIPTION FROM THE β- AND γ-ACTIN PROXIMAL PROMOTERS. Sandra L. Danilition, Catherine Y. Taylor, and Neil G. Miyamoto, The Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, 500 Sherbourne Street, Toronto, Ontario, M4X1K9 CANADA

500 bp of 5' flanking sequences of the human β- and γ-actin promoters are sufficient for activation of transcription in transiently transfected HeLa cells. Sequence analysis of the promoters has revealed two common regulatory motifs located upstream of the TATA box (-29): a CArG box that interacts with serum response factor (SRF), and a CCAAT box that binds nuclear factor Y (NF-Y). In the β-actin promoter the CCAAT and CArG boxes are located at -91 and -62, respectively. Point mutation of either motif resulted in loss of both protein binding in vitro and transcriptional activity in vivo. In the γ-actin promoter the CCAAT and CArG boxes are located 30 bps further upstream from the TATA box at -122 and -92, respectively. Point mutation of either motif resulted in loss of protein binding in vitro; however, only mutation of the γ-actin CArG box resulted in significant loss of transcriptional activity in vivo.

In addition to the CCAAT and CArG boxes, the γ-actin proximal promoter contains a CACGTG sequence (E-box) at -143. This E-box specifically interacts in vitro with upstream stimulatory factor (USF). Interestingly, point mutation of the CACGTG motif such that DNA-protein interaction in vitro was disrupted showed no profound effect on transcriptional activity in vivo.

To further understand the role of SRF and NF-Y in regulating β- and γ-actin transcription, we investigated the formation of multi-protein complexes in vitro. It is well established that SRF interacts with the c-fos CArG box as a multi-protein complex, and we have identified similar complexes on the actin CArG boxes. Using the γ-actin CArG box as probe a complex containing SRF and a protein antigenically related to $p62^{TCF}$/ELK-1 was detected. We have also begun to analyze the specific DNA bends induced by SRF and NF-Y in vitro. Each of these factors individually bend the β-actin promoter DNA by more than 90°. Current studies are to determine more precisely the location and angle of the DNA bends induced by SRF and NF-Y, and to examine the DNA structure of the higher order protein-DNA complexes containing NF-Y, SRF, and/or $p62^{TCF}$/ELK-1.

B 324 IDENTIFICATION AND CHARACTERIZATION OF GABP-β-RELATED cDNAs. Fabienne Charles de la Brousse*, and Steven L. McKnight*, Department of Embryology, Carnegie Institution of Washington, Baltimore, Md 21210. * Tularik, Inc., South San Francisco, CA 94110.

GA Binding Protein-β (GABP-β), an ankyrin repeat containing polypeptide, forms a complex with GABP-α, an ETS-related DNA binding protein, that recognizes purine-rich target sequences in the regulatory region of Herpes Simplex Virus immediate early genes (LaMarco and McKnight, 1989; LaMarco et al., 1991; Thompson et al., 1991). We have used a standard molecular approach to identify and clone cDNAs of GABP-β-related proteins. We constructed a mouse DNA genomic library and screened it with probes representing the ankyrin repeats or the 3' dimerization region of GABP-β. Southern analysis of the library clones revealed one genomic clone that hybridized only weakly to the ankyrin repeats probe. This novel GABP-β-related fragment shows 80% sequence similarity with the region spanning the ankyrin repeats of GABP-β. Subsequent Northern blot analysis identified a RNA species of approximately 9kb that is present in most tissues examined. Since Northern analyses with the GABP-β cDNA had previously identified 2.7kb and 1.5kb mRNAs (LaMarco et al., 1991), these results suggest the presence of a novel GABP-β-like polypeptide in the mouse. Sequence analysis and characterization of putative GABP-β-related cDNAs is in progress. Cloning of full-length GABP-β-like cDNAs clones will permit studies that address the association of this novel polypeptide with ETS-related proteins such as GABP-α and the importance of this association with respect to transcription activation.

B 325 **MULTIPLE REGULATORY ELEMENTS MEDIATE INTERLEUKIN-6 GENE ACTIVATION BY PROSTAGLANDINS AND cAMP.** Ulrich Dendorfer and Towia A. Libermann, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

IL-6 expression is physiologically restricted to conditions associated with tissue injury and inflammatory reactions, and is aberrantly activated in a variety of autoimmune and malignant diseases. To elucidate the molecular mechanisms leading to constitutive activation of the IL-6 gene in autoimmune and malignant diseases, we have started to systematically investigate the regulation of the IL-6 gene in response to a variety of exogenous stimuli. Several extracellular mediators contribute to IL-6 induction via different signal transduction pathways, among which prostaglandins and their second messenger cAMP are particularly relevant.

We previously demonstrated that an apparent NF-κB binding site found upstream of the IL-6 gene is an indispensable component of the IL-6 promoter region for induction of IL-6 gene expression by LPS, double-stranded RNA, phytohemagglutinin, phorbol 12-myristate 13-acetate (PMA) and TNF-α in monocytes. In order to identify prostaglandin and cAMP–responsive *cis*–acting elements in the IL-6 promoter, we have generated point mutations at several potential transcription factor binding sites. A transient transfection assay involving IL-6 promoter/chloramphenicol acetyltransferase (CAT) reporter gene constructs and the murine monocytic cell line Pu5-1.8 was used to quantitate promoter function. Whereas a 1.2 kb fragment of the wildtype human IL-6 promoter is virtually inactive in unstimulated cells, addition of prostaglandins E1 or E2 (PGE), the prostaglandin analogue misoprostol, or dibutyryl cAMP leads to a dramatic increase in IL-6 promoter activity. Stimulation with both cAMP and LPS shows a marked synergistic effect. A significant reduction in inducibility by cAMP and PGE1 is observed for mutations within the multiple response element (MRE) and the AP-1, NF-IL6, and NF-κB binding sites. Mutations of additional putative regulatory elements have marginal effects. Mutations in the NF-κB binding site completely abolish LPS–induced promoter activity, but affect cAMP responsiveness to a much lesser extent. In contrast, the MRE mutations have stronger effects on cAMP signalling as compared to LPS stimulation.

We conclude that cAMP and prostaglandins act via multiple regulatory elements to induce IL-6 expression.

B 326 **A ZINC-DEPENDENT FACTOR IS REQUIRED FOR TRANSCRIPTION FROM BOTH HUMAN *c-myc* PROMOTERS, P1 AND P2, AND MEDIATES THEIR RELATIVE ACTIVITIES,** Edward DesJardins[1,2] and Nissim Hay[1,3], [1]The Ben May Institute and Departments of [2]Molecular Genetics and Cell Biology, and [3]Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637.

Expression of the nuclear proto-oncogene c-*myc* is implicated in the regulation of cell proliferation/differentiation and in the genesis of a variety of tumors. Normal regulation of c-Myc expression is found to occur through a number of mechanisms, including transcriptional initiation. Previous studies have delineated elements in the 5' upstream region and in the first exon of the human c-*myc* gene which are responsible for accurate and elevated transcription from both the P1 and P2 promoters. Our study characterizes one of these elements upstream of the P1 promoter. This element, which has the consensus sequence CCCTCCCC (the "CT element"), appears in an array of 5 tandem copies 101 base pairs upstream of the P1 promoter. We demonstrate that the integrity of all 5 of these elements is required to initiate transcription from P1 and for maximal activity of P2. Additionally, we show that these elements are able to function in an orientation and position independent manner, and can be functionally replaced by a heterologous enhancer element (MSV enhancer). A single copy of this same element also appears in an inverted orientation 51 base pairs upstream of the P2 promoter. We show that this single element is required for transcription from P2, and has an inhibitory effect on transcription from P1. Previous studies have shown that the five tandem elements upstream of P1 and the single element upstream of P2 are localized within DNase I hypersensitive sites which are prominent only when c-*myc* is actively transcribed. We have characterized a trans-acting factor, which we designate CTF (for "CT factor"), which binds these DNA elements located upstream of both P1 and P2 in a sequence-specific manner and requires zinc for its DNA binding activity. Methylation interference assays show identical guanine residues are required for DNA binding to the elements upstream of both P1 and P2. We show by UV DNA/protein crosslinking that this protein has an approximate MW of 105 kD.

B 327 INCREASED EXPRESSION OF THE LEISHMANIA TOR GENE ARISES FROM ITS SEPARATION FROM A SILENCER DURING DNA AMPLIFICATION, Siegfried Detke, Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, North Dakota, 58202.

Leishmania were made 100 to 500 times more resistant to toxic nucleosides by gradual adaptation to increasing levels of inosine dialdehyde or the adenosine analog tubercidin. Both of the resistant cells exhibited cross resistance to each of these compounds as well as to other toxic purine nucleoside analogs. These cells also exhibited a greatly reduced capability of accumulating exogenous adenosine, guanosine, thymidine and guanine. This decreased ability to accumulate nucleosides and at least one nucleobase appeared to be due to reduced activity of at least three distinct purine transporters as the differences between purine metabolizing enzymes were not sufficiently different to account for the decreased accumulation capability.

These effects arose from the amplification of a 55 kilobase region of DNA yielding an extrachromosomal DNA which was not detected in wild type cells or revertants which lost the resistance to toxic nucleosides. The resistant cells contained 2 to 4 times as much DNA homologous to the extrachromosomal DNA as did wild type cells and there appeared to be only a single copy of the TOR gene (i.e., Toxic nucleoside resistance gene) on each of these extrachromosomal DNAs.

The TOR gene from wild type Leishmania also conferred resistance to toxic nucleosides upon transformed Leishmania but only if it was separated from a regulatory element located approximately 20 kilobase away. This region of DNA was not present in the extrachromosomal DNA described above but would inactivate the TOR gene from the extrachromosomal DNA if cloned cis to this gene. The repression of the TOR gene by this regulatory element was position and orientation independent, properties characteristic of a silencer. It appears that resistance occurred not through a large increase in copy number of the target gene as reported in other systems but by the separation of a resistance conferring gene from a negative regulatory element which acts as a silencer.

B 328 EXPRESSION OF THE HUMAN ALCOHOL DEHYDROGENASE GENES *ADH1*, *ADH2* AND *ADH3*. Howard J. Edenberg, Celeste J. Brown, Lu Zhang. Dept. of Biochemistry & Molecular Biology, Indiana Univ. School of Medicine, Indianapolis, IN 46202-5122 USA

Humans have three closely related alcohol dehydrogenase genes, *ADH1*, *ADH2* and *ADH3*, that are all expressed at high levels in adult liver. These genes differ in their pattern of expression in other tissues. We are examining the transcriptional regulation of these genes in order to understand the subtle differences that lead to their differential expression.

The regions extending about 350 bp upstream from the transcriptional start site contain many sequences that bind nuclear proteins. We have mapped binding sites for the known transcriptional regulators C/EBPα, C/EBPβ, C/EBPδ, USF/MLTF, Sp1, CTF/NF-I, and HNF-1α. Comparisons among *ADH1*, *ADH2* and *ADH3* show differences in the affinities for some factors, which could affect the expression of these genes. There are interesting differences in the positions at which a CTF/NF-I-like protein binds to the three promoters. Within the region from -100 to -146 bp, all three genes show a footprint that can be eliminated by competition with a consensus NF-I oligo. The position of the footprint differs, however, despite great overall similarity in the sequences: *ADH3* shows a large footprint covering the left half of this region, *ADH1* shows a smaller footprint partially overlapping that of *ADH3*, and *ADH2* shows a small footprint at the right end that does not overlap either of the others. We believe the differences in binding are in part due to competition with other factors that bind nearby. This CTF/NF-I site acts to reduce transcription in CAT assays, in contrast to the stimulation seen in the promoters originally studied.

This and similar subtle differences among the three ADH genes should provide important information on the evolution of tissue-specificity.

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B 329 EFFECT OF CATIONS ON LOW AND HIGH AFFINITY DNA BINDING BY THE "ZINC FINGER" PROTEIN EGR-1, Steven A. Edwards, Department of Biochemistry. Meharry Medical College, Nashville, TN 37208

Egr-1 is a transcriptional activating protein containing three Cys-His zinc finger domains. Murine egr-1 was expressed in baculovirus infected insect cells. Cell extracts were tested for their ability to bind specifically to an Egr-1 target oligonucleotide. Binding was specific with a high affinity, Kd ~ 1 nM. DNA binding was blocked by an antiserum to the protein. High affinity DNA binding was completely inhibited by 1,10 phenanthrolene at 100 uM. Inhibition with EDTA or EGTA required high concentrations (>10 mM). Extracts were dialyzed vs EDTA and water, then the effect of various cations on DNA binding activity was measured. A rank order of the stimulatory effect found is as follows: Fe(III) > Co(II) > Ca(II) > Zn(II) > Mg(II) > Mn(II). Cd(II) inhibited DNA binding in both dialyzed and undialyzed extracts. Scatchard analysis of the effect of Ca(II) indicated that "high affinity" DNA binding was not affected, but that the amount of lower affinity, but specific, DNA binding by egr-1 extracts was substantially increased. The results will be discussed in terms of a two site model for DNA binding by egr-1.

B 330 **COUPLING OF TRANSCRIPTION AND DNA REPAIR IN OPEN CHROMATIN DOMAINS,** Klaus Erixon, Department of Medical Radiobiology, Karolinska Institute, S-10401 Stockholm.

An identical UV-dose response was found for the inactivation of transcription and for initiation of excision repair in normal human fibroblasts. It is concluded that the size of the targets/domains for repair and transcription are identical. Inhibition of RNA polymerase II transcription by the nucleoside analog 5,6-dichloro-1-ß-D-ribofuranosylbenzimidazol (DRB) also inhibits UV-dependent repair by reducing the number of domains that are incised. Thus transcription and repair seem to be initiated by a common event in potentially active chromatin domains. Both processes are blocked by the presence of a repair-induced strand break. The results are consistent with a requirement for torsional strain in DNA-domains and/or an association with the nuclear matrix for the initiation of both transcription and repair.

The kinetics of UV-induced DNA repair was followed by monitoring the single-strand breaks formed by incision at DNA lesions and by direct measurements of cyclobutyl-pyrimidine-dimers (CPD) by a lesion-specific endonuclease. The rate of CPD removal was measured both in the genome overall and in accessible regions of chromatin. The rate of incorporation of labelled nucleosides during repair was also determined.

At a dose of 2 J/m^2 (254 nm UV-light), 50% of the lesions measured by these assays have been removed in about 30 minutes. The kinetics indicate that all assays are looking at the same rate limiting event, an event that is involved in the preparation of chromatin domains for recognition and incision. These cells initially recognize and repair DNA lesions in a certain fraction of their genome by an efficient pathway which involves a DNA polymerase sensitive to aphidicolin and cytosinarabinoside. This preferential repair is localized in a part of the genome that is in an "open" chromatin conformation, such that the lesions are accessible to the exogenously added repair endonuclease in gently permeabelized cells.

It is suggested that the activation of a chromatin domain for incision depends on a conformational change also involved in transcription. About equal numbers of CPDs and non-CPDs are being repaired by this preferential pathway. About 200.000 repair-domains are present in these cells. The domains have a calculated average size of about 15 kbp.

B 331 **CLONING, EXPRESSION AND FUNCTIONAL ANALYSIS OF THREE POTATO B-ZIP PROTEINS THAT BIND TO THE MANNOPINE SYNTHASE BIDIRECTIONAL PROMOTER.** Dorothee Feltkamp, Robert Masterson, Jeff Schell and Sabine Rosahl. Max-Planck-Institut für Züchtungsforschung, Carl von Linné-Weg 10, D-5000 KÖLN 30, Federal Republic of Germany

We dissected the dual 1'-2' mas promoter from *Agrobacterium tumefaciens* into three parts. Gel retardation assays with potato nuclear extracts using these three fragments as a probe showed binding to the 1' and the central part of the mas promoter. By South-Western screening of a potato root expression library and cross-hybridization with PCR products we were able to isolate three cDNA clones, designated MASBF1,2 and 3 with high homology to each other. All three proteins contain a basic domain followed by a leucine zipper motif common to the bZip familiy of transcription factors. Sequence analysis revealed high homology to the tobacco TGA1a factor that binds specifically to the TGACG-motif in the as-1 element of the 35S promoter. This motif, also known as part of the ocs element, is present both in the 1' and 2' part of the mas promoter. After overexpression in *E. coli* all three proteins showed specific but differential binding to the 1' and 2' fragment of the dissected 1'2' mas promoter which could be competed by oligonucleotides corresponding to the ocs elements of this promoter as well as by the as-1 element. One half site of the 1' ocs element which showed strongest binding was multimerized and fused to the 35SΔ-60GUS construct as well as the 2' ocs element. Data will be presented on protoplast transformation with these constructs as well as cotransformation with constructs expressing one of the MASBFs.

B 332 REGULATION OF T CELL LYMPHOKINE GENE TRANSCRIPTION BY THE ACCESSORY MOLECULE CD28. James D. Fraser[1], and Arthur Weiss[1,2] Departments of Medicine and of Microbiology and Immunology[1] and the Howard Hughes Medical Institute[2], University of California at San Francisco, San Francisco, California 94143

T cell activation results in the production of multiple lymphokines. Efficient lymphokine gene expression appears to require both T cell antigen receptor (TCR) signal transduction and an uncharacterized second or costimulatory signal. CD28 is a T cell differentiation antigen that can generate intracellular signals that synergize with those of the TCR to increase T cell activation and interleukin 2 (IL-2) gene expression. In these studies we have examined the effect of CD28 signal transduction on granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 3 (IL-3), and interferon γ (IFN-γ) promoter activity. Stimulation of CD28 in the presence of TCR-like signals increases the activity of the GM-CSF, IL-3, and IFN-γ promoters by 3 to 6 fold. As previously demonstrated for the IL-2 promoter, the IL-3 and GM-CSF promoters contain distinct elements of similar sequence which specifically bind a CD28-induced nuclear complex (CD28RC). Mutation of the CD28 response elements in the IL-3 and GM-CSF promoters abrogates the CD28-induced activity without affecting phorbol ester and calcium ionophore-induced activity. UV cross-linking indicates that the CD28RC contains polypeptides of approximately 35,36 and 44 kD. These studies indicate that the TCR and CD28 regulated signal transduction pathways coordinately regulate the transcription of several lymphokines and that the influence of CD28 signals on transcription is mediated by a common complex.

B 333 A NOVEL 7 NUCLEOTIDE MOTIF LOCATED IN 3' UNTRANSLATED SEQUENCES OF THE IMMEDIATE-EARLY GENE SET MEDIATES PDGF INDUCTION OF THE JE GENE, Rolf R. Freter, Jean-Claude Irminger, Julie A. Porter, Susan D. Jones, and Charles D. Stiles, Department of Microbiology and Molecular Genetics, Division of Clinical Oncology, and Division of Cellular and Molecular Biology, Harvard Medical School and the Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115

A cohort of the serum and growth factor regulated "immediate-early gene set" is induced with slower kinetics than c-fos. Two of the first immediate-early genes characterized as such, c-myc and JE, are contained within this subset. Cis-acting genomic elements mediating induction of the slower responding subset of immediate-early genes have never been characterized. We have characterized two widely separated genomic elements which are together essential for induction of the murine JE gene by platelet-derived growth factor (PDGF), serum, Interleukin-1, and double-stranded RNA. One of these elements is novel in several regards. It is a 7-mer, TTTTGTA, found in the proximal 3' sequences downstream of the JE stop codon. The 3' element is position dependent and orientation independent. It does not function in polyadenylation, splicing, or destabilization of the JE transcript. Copies of the 7-mer or its inverse are found at comparable 3' sites in 25 immediate-early genes that encode transcription factors or cytokines. Given its general occurence, the 7-mer may be a required cis-acting control element mediating induction of the immediate-early gene set.

B 334 THE ACTIVITY OF THE LATE PROMOTER OF HUMAN PAPILLOMAVIRUS TYPE 8 IS REGULATED BY A CELLULAR REPRESSOR AND THE VIRAL TRANSACTIVATOR E2,
Pawel G. Fuchs, Michael May, Karin Grassmann and Herbert Pfister, Institut für Klinische und Molekulare Virologie, Friedrich-Alexander-Universität, D-8520 Erlangen, Germany

Two negative regulatory elements have been identified in the non-coding region (NCR) of the highly oncogenic, epidermodysplasia verruciformis-associated human papillomavirus 8 (HPV8). A 38 bp sequence (SIL) located in the 5'-part of the NCR upstream to the late promoter P_{7535} down-regulated expression from the homologous as well as the heterologous *tk*-gene promoter, independently of its orientation relative to the test promoter. It showed reduced activity when cloned downstream of the transcription units. Quantitative RNase protection analysis confirmed that the SIL-sequence acts as a silencer of transcription. SIL shows homology to a 29 bp motif (M29) in the 3' -part of the HPV8-NCR that displayed the same negative regulatory *properties as* SIL in functional tests with homo- and heterologous promoters. Gel retardation tests suggested that SIL and M29 specifically interact with the same nuclear factor. *In vivo* competition experiments and mutational analysis proved that binding of this protein is necessary for the negative activity of the SIL-element. DNaseI footprint within SIL overlaps partially with the binding site of the viral *trans*-activator protein E2. It could be shown that the expression of E2 overrides the inhibitory effect of SIL without interfering with the binding of the silencer-specific cellular factor. The bifunctional nature of SIL due to its interaction with the cellular repressor and the viral *trans*-activator offers an interesting possibility to regulate the switch from early to late transcription in the HPV life cycle. The protein binding to SIL and M29 may constitute a part of the cellular surveillance mechanism controlling the viral infection.

B 335 TRANSCRIPTIONAL ACTIVATION BY THE TAX PROTEIN OF HUMAN T CELL LEUKEMIA VIRUS TYPE 1 (HTLV-1)
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The *trans*-activator TAX of human T-cell leukemia virus type 1 (HTLV-1) activates multiple enhancers and stimulates transcription of the viral and some cellular genes but does not bind directly to the enhancer sequences.

We have previously shown that a fusion protein of TAX with the DNA binding domain of yeast GAL4 (GAL4-TAX) can activate transcription dependent on the GAL4-binding site in the reporter plasmid (J.Virology, **65**, 4525-4528). Furthermore, this activation with GAL4-TAX was interfered with by the free form of TAX . These results suggest that TAX associates with DNA through interaction with DNA binding protein(s) and also interacts with other transcription factor(s) to elicit the activation. To analyze the interaction with these proteins, we introduced a series of deletion mutations into the *tax* coding sequence and examined their activation capacities in fused form with GAL4 and also in their free form. Most mutant TAX proteins lost activities both in fused- and in free-form, however, a mutant lacking three amino acids at amino-terminus(Δ3-TAX) retained the activity as a GAL4 fusion protein but not in a free form. In addition, free form of Δ3-TAX could interfere with the transcriptional activation by GAL4-TAX. These observations indicate that the amino-terminal domain is involved in the interaction with DNA binding protein. As a candidate for such DNA binding protein, we demonstrated that CREB and CREM proteins make complex with TAX but not with Δ3-TAX.

B 336 THE Sμ-Cμ INTPON OF THE IMMUNOGLOBULIN HEAVY-CHAIN LOCUS IS A POSITION-DEPENDENT REPRESSOR OF TRANSCRIPTION IN ACTIVATED B CELLS,
Andrei Gartel, Leslie Morgan and Amy Kenter, Department of Microbiology and Immunology, College of Medicine, University of Illinois at Chicago, Chicago, IL 60612

The Sμ-Cμ intron of the IgH locus is conserved in evolution but its biological function is unknown. Switch recombination focuses on the repetitive sequences within Sμ region in mitogen activated normal B cells. In myelomas and lymphomas these breakpoints most frequently occur at the 5' end and upstream of the Sμ DNA while the Sμ-Cμ intron appears remarkably protected from recombination. We have considered the possibility that this intron contains regulatory elements which inhibit recombination from occuring downstream of the Sμ region. Since there may be overlap in the nucleoprotein complexes which drive transcription and recombination we decided to determine the transcriptional potential of this fragment. The Sμ-Cμ intron was cloned at different positions relative to the promoter in the TKCAT vector and the resulting constructions were transiently transfected into mitogen stimulated splenic B cells. By measuring CAT activities we found that this element acts as orientation-independent repressor of basal level of transcription when positioned upstream of the TK promoter. However, this fragment was inactive when placed in other positions in the plasmid. Furthermore, this fragment represses CAT activity in B cell line (J558) and does not affect CAT expression in T cell line (EL4) and nonlymphoid cell lines (HeLa, Hep G2). This suggests that Sμ-Cμ intron may be a B cell specific position-dependent repressor.

B 337 IDENTIFICATION AND CHARACTERIZATION OF *CIS*-ACTING SITES THAT MODULATE *GCR1*-DEPENDENT TRANSCRIPTIONAL ACTIVATION OF THE YEAST ENOLASE GENE *ENO2*,
Claire Mazow Gelfman, Catherine E. Willett, and Michael J. Holland, Department of Biological Chemistry, School of Medicine, University of California, Davis, California, 95616.

The yeast *GCR1* gene encodes a positive activator of glycolytic gene expression. Transcription of the yeast enolase genes *ENO1* and *ENO2* is reduced 50-fold in strains carrying a *gcr1* null mutation. To identify *cis*-acting sequences that modulate *GCR1*-dependent activation of transcription, a series of overlapping sequences from the 5' flanking region of the yeast enolase gene *ENO2* were ligated upstream from the core promoter regions of the *ENO2* gene as well as a yeast *CYC1*/LacZ gene fusion and tested for their ability to activate transcription in strains carrying the wild-type *GCR1* gene or a *gcr1* null mutant allele. A 60 base pair *ENO2* sequence was identified that conferred high level, *GCR1*-dependent, transcriptional activation on both promoters. A binding site for RAP1 was identified near the 3' end of the 60 base pair sequence. GCR1 protein was overexpressed in yeast and fractionated by MonoS chromatography. Using partially purified GCR1 protein, a binding site for GCR1 protein was identified adjacent to the RAP1 binding site by gel mobility supershift assay using an antibody directed against a GCR1 peptide. To access the role of RAP1 and GCR1 in activation of transcription, the 60 base pair sequence was divided into a 30 base pair sequence that bound RAP1 and GCR1 *in vitro* and a 30 base pair sequence that did not contain RAP1 or GCR1 binding sites. The 30 base pair sequence containing the RAP1 and GCR1 binding sites failed to activate transcription from the *ENO2* or *CYC1* core promoters whereas the 30 base pair sequence which lacked RAP1 or GCR1 binding sites conferred moderate levels of *GCR1*-independent transcriptional activation on both promoters. These observations suggest that the GCR1 protein activates transcription indirectly by staging binding and/or activation by a factor which binds immediately upstream from the GCR1 and RAP1 binding sites. We are currently testing the individual contributions of RAP1 and GCR1 binding within the 60 base pair *GCR1*-dependent element by testing the activity of site-directed base pair substitution mutations in each binding site.

B 338 ETS1 AND SP1 ACT SYNERGISTICALLY TO ACTIVATE TRANSCRIPTION OF THE ETS-RESPONSIVE REGION OF THE HLTV1 LTR, Jacques Ghysdael, Remy Bosselut, Rose-Aimée Bailly and Anne Gegonne, Institut Curie, Section Biologie, 91405 Orsay, France

Ets1 is the prototype of a family of transcriptional activators whose activity depends upon their binding to specific DNA sequences characterized by an invariant GGA core sequence (for review, see Ghysdael and Yaniv, Curr. Opin. Cell. Biol. 3, 484-492). We have previously demonstrated that transcriptional activation by Ets1 of the LTR of human T cell lymphotropic virus type 1 is strictly dependent upon the binding of Ets1 to two sites, ERE-A and ERE-B, localized in a 44 bp-long Ets-responsive region (ERR1) (Bosselut et al., EMBO J. (1990), 9, 3137-3144 ; Gitlin *et al.*, J. Virology (1991), 65, 5513-5523).

We report here that the activity of ERR1 as an efficient Ets1 response element in HeLa cells also depends upon the integrity of an Sp1 binding site localized immediately upstream of ERE-A. The response to Ets1 of an element restricted to the SP1/ERE-A binding sites is also strictly dependent upon both the Ets1 and Sp1 binding sites. Reconstitution experiments in *D. melanogaster* Schneider cells show that Ets1 and Sp1 act synergistically to activate transcription from either the ERR1 or the SP1/ERE-A elements and that synergy requires the binding of both Sp1 and Ets1 to their cognate sites. *In vitro*, Sp1 and Ets1 are shown to bind cooperatively to the SP1/ERE-A element, suggesting that synergy between these factors involves their cooperative binding to DNA. SP1/ERE-A elements are found in the enhancer/promoter region of several cellular genes, indicating that synergy between Ets1 and Sp1 is likely not to be restricted to the ERR1 region of the HLTV1 LTR. These results further strengthen the notion that Ets1 as well as other members of the Ets family usually function as components of larger transcription complexes to regulate the activity of a variety of viral and cellular genes.

B 339 MULTIPLE PROMOTERS, ALTERNATE SPLICING AND FACTORS INFLUENCING TRANSCRIPTIONAL REGULATION OF THE HUMAN PARATHYROID HORMONE-RELATED PROTEIN GENE, Matthew T. Gillespie, Jane A. Glatz, Takeshi Kiriyama, Larry J. Suva*, Seiji Fukumoto, Joan, K. Heath, Jane M. Moseley and T. John Martin, St. Vincent's Institute of Medical Research, Melbourne, Australia, *Department of Bone Biology and Osteoporosis Research, Merck, Sharp and Dohme Research Laboratories, West Point, PA, USA 19486

The parathyroid hormone-related protein (PTHrP) gene is an unusually complex transcriptional unit comprised of nine exons under the control of three promoter elements (5' to exons I, III and IV) which direct alternate 5' exon usage. In addition, alternative 3' splicing events results in protein products of 139, 141 or 173 amino acids in length. However little is known in relation to the regulation of the alternate splicing and factors which influence *PTHrP* gene expression. We have identified that estrogen, EGF and TGFβ1 upregulate *PTHrP* steady state mRNA and protein levels, whilst dexamethasone down-regulates PTHrP expression by 70 %. None of these agents appear to alter PTHrP mRNA stability, the half-life of which is 1-2 hr: each of the 3' untranslated sequences of PTHrP mRNA contains multiple copies of the instability motif AUUUA.

With the aim of identifying the genomic sequences responsible for transcriptional control, 5' flanking sequences of the human *PTHrP* gene were fused to a bacterial chloramphenicol acetyltransferase gene and these promoter/expression constructs were transfected into a variety of cell lines including, BEN, COLO 16, CV-1 and the keratinocyte line HaCaT. The promoter activity of one construct, pSMR38 (encompassing the region -1100 to -20 bp from the initiating ATG), was upregulated by estrogen, EGF and TGFβ1 treatments. The stimulatory effects (two- to threefold) of estrogen (10^{-9}M), EGF (10^{-8}M) and TGFβ1 (10^{-8}M) were equivalent to, or lower than, the enhanced production of PTHrP mRNA and protein in responsive cell lines. This region includes potential AP-1 and NF-1 binding sites, the latter which is known to be the TGFβ1-responsive element in the α(2) type 1 collagen gene, and these may be the responsive sequences by which EGF and TGFβ1 activate the expression of the *PTHrP* gene.

B 340 SEQUENCE DISCRIMINATION BY ALTERNATIVELY SPLICED ISOFORMS OF A DNA BINDING C_2H_2 ZINC FINGER DOMAIN Joseph A. Gogos, Jingmin Jin, Tien Hsu and Fotis C. Kafatos Department of Cellular and Developmental Biology, Harvard University, Cambridge MA 02138

The major form of the Drosophila putative chorion transcription factor, CF2, contains four zinc fingers of the C2H2 type in the C-terminal DNA binding domain. However transcripts of this gene are subject to three alternative modes of splicing, which are under strict developmental control and result in three isoforms that differ in the number of zinc fingers. One testis-specific isoform includes a frame-shifted segment of drastically different amino acid sequence in the C-terminal finger domain.

The other two developmentally regulated isoforms differ by an extra zinc finger motif inserted within the DNA binding domain. Detailed binding/selection studies with oligonucleotides of high and low degeneracy have determined the respective preferred DNA binding sites (GTA.TAT.ATA and GTA.TAT.TAT.ATA), which are distinguished by an internal duplication of TAT in the site recognized by the isoform with the extra finger. The dinstict binding properties of the two isoforms are reflected in recognition of distinct target promoter sequences: the isoform with the extra finger binds to the CF2 gene promoter suggesting autoregulation; the four finger isoform binds to the s15 chorion gene promoter leading, at low concentrations, to transcriptional activation as assayed in Drosophila Schneider cell line system. Thus, because of alternative splicing, the single CF2 gene generates in vivo multiple distinct DNA-binding proteins, which could act as developmental switches, regulating different gene sets in different tissues and developmental periods. Efforts are underway to clone some of these genes using immunoprecipitation protocols that enrich for chromatin fragments containing CF2 binding sites.

The results are consistent with a basic pattern of modular interactions between zinc fingers and trinucleotides, as in the Zif 268 complex with GC-rich DNA, and show for the first time how these modular finger/trinucleotide interactions can be used, in conjunction with alternative splicing, to change the binding specificity and increase the spectrum of sites recognized by a zinc finger DNA binding domain. They also suggest new rules for recognition of AT-rich DNA sites by zinc finger proteins; molecular modelling and extensive mutational analysis are underway to test these rules. We are particularly interested in the rules governing base pair selection by Glutamines and Lysines as well as in the effect that the length of the H-C link has in determining the DNA binding specificity of zinc finger domains.

B 341 REDOX REGULATION OF TRANSCRIPTION FACTOR ACTIVITY DURING T-LYMPHOCYTE ACTIVATION Sherilyn D. Goldstone and Nicholas H. Hunt, Department of Pathology, University of Sydney, NSW 2006, Australia.

The redox status of T lymphocytes is intimately associated with their proliferative capacity. We have previously demonstrated, by assessing intracellular oxidation of dichlorofluorescin to dichlorofluorescein, the occurrence of a burst of reactive oxygen species formation commencing 30-60 minutes after mitogenic stimulation of T lymphocytes. We also have shown that certain antioxidant compounds such as radical scavengers, iron chelators and aminothiols not only abrogate this oxidative response, but also inhibit T cell proliferation and result in a G1 phase cell cycle arrest.

Recent reports have suggested a role for redox mechanisms in the regulation of the activity of certain transcription factors. Given the requirement for both intracellular oxidative events and altered gene expression during the early stages of the proliferative response, we have investigated the effect of antioxidant treatment on the DNA binding activity of transcription factors AP-1 and NF-κB in mitogenically activated T lymphocytes. Inhibition of the DNA binding activity of both AP-1 and NF-κB by cysteamine, an aminothiol compound, was detectable by 1 hour post-stimulation, and continued for at least 8 hours post-stimulation. This aminothiol-induced inhibition of proliferation was found to correlate with inhibition of DNA binding activity of both of these factors, thus suggesting a link between the obligatory oxidative event and the altered gene expression which ultimately results in DNA synthesis and cell division.

B 342 INTERACTION OF SERUM RESPONSE FACTOR WITH HOMEODOMAIN PROTEINS. Dorre Grueneberg, Ken Simon, and Michael Gilman, Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, NY, 11724.

We describe an interaction between a new homeodomain protein and the serum response factor that suggests a model for how homeodomain proteins may specify the transcriptional response to inductive signals. Homeodomain proteins establish cell identity and the Serum Response Factor (SRF) activates genes in response to growth factors. We used expression cloning in yeast to isolate a human homeodomain protein that interacts with the yeast transcription factor, MCM1. MCM1 and SRF are MADS box proteins that are closely related in structure and function. The homeodomain protein, which we call Phox1, is 70% identical to the homeodomain of the Drosophila *paired* protein. We have determined that Phox1 enhances SRF binding to the SRE *in vitro*. The mechanism by which Phox1 enhances SRF activity is by accelerating the rate of exchange of SRF with its binding site, the SRE. The ability of Phox1 to enhance SRF binding resides within the homeodomain of Phox1 and is independent of DNA-binding activity of Phox1. In our original experiments, the mobility of the SRF/SRE complex did not change in the presence of Phox1, suggesting that Phox1 was not present in the complex. More recently, however, we have found conditions *in vitro* where we are able to detect stable ternary complexes containing Phox1, SRF and the SRE. The ternary complexes are dependent on the DNA-binding activity of Phox1 and residues in helix 2 of the homeodomain, which may be important for contacting SRF. Ternary complex formation *in vitro* correlates with the ability of Phox1 and SRF to activate a reporter gene *in vivo*.

B 343 TRANSCRIPTIONAL REGULATION OF GENES DIFFERENTIALLY EXPRESSED BETWEEN TUMORIGENIC AND NON TUMORIGENIC CLONES OF A HUMAN COLON CARCINOMA CELL LINE. Magali Gunther[1], Nicole Fossar[1], Madeleine Laithier[1], Thierry Frébourg[1], David A. Kulesh[2], Robert G. Oshima[2] and Olivier Brison[1], [1]Laboratoire d'Oncologie moléculaire, URA 1158 CNRS, Institut Gustave Roussy, 94805 Villejuif, France and [2]Cancer Research Center, La Jolla Cancer Research Foundation, La Jolla, CA 92037, USA.

Tumorigenic in nude mice (T) and non tumorigenic (N) subclones have been isolated from the SW613-S human colon carcinoma cell line. Several genes (TGF-α, IGF-2, ferritin-H,...) were shown to be overexpressed in T cells as compared to N cells as a consequence of an increased transcription rate (1,2,3). Among them the cytokeratin K18 gene was chosen as a model to study the mechanisms of this transcriptional regulation. Constructions made with its promoter fused to the CAT gene showed a higher level of expression in T than in N cells. By succesive deletions, a segment of 101 bp was shown to be sufficient for this increase. This region contains the TATA box and a GC-rich box which binds several factors as evidenced by band shift analyses. Deletions or point mutations in this box abolished binding and destroyed the activity of the promoter. One of the shifted bands was more abundant with N cell than with T cell extracts but mutations which specifically prevented the binding of this factor did not alter the differential activity of the promoter. The complete SV40 early promoter (72 bp repeats, GC-rich boxes and TATA box) as well as a chimeric promoter constructed with the enhancer and GC-rich upstream elements of the SV40 promoter and the TATA box of the K18 promoter were equally active both in N and T cells. Deletion of the 72 bp repeats in these two promoters confered on them a differential actvity. These results indicate that factors which bind to GC-rich regions are implicated in the differential expression of the K18 gene between N and T cells of the SW613-S line.

1) Lavialle *et al.* (1989) *Anticancer Res.* **9**, 1265-1280.
2) Modjtahedi *et al.* (1992) *Exp. Cell Res.* **201**, 74-82.
3) Modjtahedi *et al.* (1992) *Int. J. Cancer* 52, 1-8.

B 344 TWO DISTINCT PATHWAYS CAN INACTIVATE THE NF-κB ELEMENT IN THE E-SELECTIN PROMOTER, R. Hooft van Huijsduijnen, G. M. Smith, J. Whelan, R. Pescini, P. Ghersa and J.F. DeLamarter, GLAXO Institute for Molecular Biology, Chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland.

E-selectin (previously called ELAM-1) is transiently and tissue-specifically expressed on endothelial cells upon stimulation with cytokines. We show here that the E-selectin promoter in endothelial cells is under-methylated in comparison with non-expressing HeLa cells. When plasmid constructs with reporter genes driven by the E-selectin promoter were methylated *in vitro*, these constructs could no longer be transcribed in transient transfection assays or in *in vitro* transcription systems. We show that the NF-κB site in the promoter is a likely target for this repression. We have also studied another case of E-selectin transcription downmodulation which occurs 24 h post IL-1 stimulation. We can reproduce this downmodulation by introducing E-selectin promoter-driven constructs into cells before and after IL-1 induction of the cultures. This repression of transcription can also be ascribed to an inactivation of the NF-κB element, despite the high levels of DNA-binding NF-κB found late following induction. Thus, the NF-κB-site in the E-selectin promoter appears to be inactivated by promoter methylation and by a different mechanism that involves NF-κB when it is active in DNA binding.

B 345 **REGULATION OF THE CHICKEN OVALBUMIN GENE BY A SILENCER ELEMENT.** Sarah A. E. Haecker and Michel M. Sanders, Department of Biochemistry, University of Minnesota, Minneapolis, MN 55455

Most eucaryotic genes are controlled by a complex array of *cis*-acting regulatory domains. Two such domains were identified in the chicken ovalbumin (Ov) gene, a steroid-dependent regulatory element (SDRE, -900 to -780) and a negative regulatory element (NRE, -308 to -132). The SDRE is essential for the regulation of the Ov gene by steroid hormones, while the NRE appears to actively repress the gene in the absence of steroids. Deletional mutagenesis of the NRE identified three negative domains (-308 to -256, -239 to -220, and -174 to -132) that act independently to repress transcription. The internal domain, -239 to -220, shares sequence homology (TCTCTCCNA) with negative elements in several other genes and thus was investigated in more detail. To determine whether this negative domain functions as a silencer, an oligomer to this sequence was ligated in one and multiple copies, in both orientations, and in two positions relative to the CAT reporter plasmid driven by either the Ov or thymidine kinase (TK) promoters. These plasmids were transfected into primary oviduct cell cultures, and transcriptional activity of the promoters was determined by CAT assay. This negative domain was able to repress both the Ov and TK promoters about 3-fold in an orientation- and position-independent manner, indicating that this sequence acts mechanistically as a silencer. Multiple copies did not enhance the extent of repression. Gel mobility shift assays revealed that the amount of protein that binds to this sequence is unaffected by treatment of chickens with estrogen. Although the silencer binding protein is a phosphoprotein, treatment with phosphatase does not alter the amount of binding. Because this DNA binding protein is present in all tissues examined and because this sequence is present in the negative domains of several genes, this silencer element is a candidate for a relatively ubiquitous repressor sequence.

B 346 REDOX REGULATION OF *gadd153* and *grp78* STEADY STATE mRNA BY DITHIOTHREITOL

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Previously we have demonstrated that mRNA encoded by the growth arrest and DNA damage inducible (*gadd*) gene is elevated by dithiothreitol (DTT), a strong reducing agent which also increases expression of the glucose regulated gene *grp78*. The purpose of this work was to examine mechanisms by which alterations in redox status of intact cells modulate expression of *gadd153* and *grp78*. Northern analysis of poly(A)$^+$ RNA from renal epithelial LLC-PK$_1$ cells revealed that DTT and trans-4,5-dihydroxy-1,2-dithiane (DTTox) were strong inducers of *gadd153* and *grp78*. Interestingly, DTT but not DTTox increased chloramphenicol acetyl transferase (CAT) expression in LLC-PK$_1$ cells stably transfected with a gadd153-CAT construct. Increased CAT expression was not blocked by addition of catalase or superoxide dismutase indicating that formation of oxygen radicals during oxidation of DTT did not mediate the induction. DTT and DTTox displayed different dose response and time course curves for *gadd153* mRNA induction. In addition, induction of *gadd153* by DTT and DTTox could also be differentiated on the basis of sensitivity to cycloheximide and cytidine, a precursor of pyrimidine biosynthesis known to block induction of *grp78* by glucose deprivation. These results suggest that modulation of cellular thiol redox status in either the oxidized or reduced direction elevates *gadd153* and *grp78* mRNA, but by distinct mechanisms.

B 347 THE ACIDIC ACTIVATION DOMAINS OF VP16 AND THE EPSTEIN-BARR VIRUS R TRANSACTIVATOR ARE COMPOSED OF FUNCTIONAL SUBDOMAINS THAT CAN SYNERGIZE,

J. Marie Hardwick and Loretta Tse, Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD, 21287

The R transactivator (Rta) encoded by Epstein-Barr virus (EBV) is a potent activator of transcription via enhancer sequences located upstream of EBV genes. One of the primary targets of Rta is the enhancer within the viral lytic origin of replication. Via this enhancer, Rta regulates the expression of the EBV gene BHRF1 which has sequence homology to the bcl-2 oncogene. We have identified two domains of Rta which are important for transactivation. Domain 1 (amino acids 416-519) had weak activation activity when linked to GAL4. Deletion of domain 1 from Rta stimulated activity in Vero cells but nearly abolished activity in B cells. Domain 2 (amino acids 520-605) is a potent acidic activation domain. Deletion of this domain from Rta abolished activity in all cell types tested. Competition experiments indicated that the acidic activation domains of VP16 and Rta utilize overlapping but not identical sets of cellular transcription factors because neither could compete the other activator as efficiently as it competed for its own activity. Rta contains three overlapping sets of a pattern of hydrophobic residues shared with two activation domains present in VP16. These three subregions of the Rta domain 2 were subcloned onto GAL4 and found to function independently. When only one Rta subdomain was linked to GAL4, it exhibited synergistic activation on a target with five GAL4 binding sites compared to a target with only a single site. Similar results were obtained with VP16 subdomains. In contrast, the intact Rta domain 2 did not synergize on multiple sites, suggesting that the Rta subdomains were already synergizing with each other when bound to a single site. Unlike Rta which is an extremely potent activator when bound to a single site, the intact VP16 activation domain was a modest activator on a target with a single binding site but synergized on a target with multiple binding sites. These results suggest the possibility that activation domains may be composed of subdomains with different functions.

B 348 TWO DISTINCT TRANSCRIPTION FACTORS HAVE OVERLAPPING BINDING SITES IN THE 5'-FLANKING SEQUENCE OF THE MOUSE P12 GENE. Mario Harvey, Suzanne Robidoux and Sylvain L. Guérin, Unité d'Endocrinologie Moléculaire, Centre de recherche du CHUL, Québec, Canada G1V 4G2.

The product of the mouse p12 gene has been identified as a member of the Kazal family of secretory protease inhibitors. Its expression is restricted to the ventral prostate, the coagulating gland and the seminal vesicle. Although its precise function has not yet been precisely defined, it is likely that the p12 gene product is involved in the enzymatic regulation of the acrosome reaction at fertilization or in the neutralization of acrosin when the enzyme is prematurely activated. Few years ago, we initiated the characterization of the cis-acting regulatory elements controlling p12 gene expression at the molecular level. More than 15 distinct nuclear proteins were found to bind either the promoter or the 5'-flanking region of the p12 gene. Although a significant effort has been directed to the identification and characterization of the transcription factors binding to the basal promoter of the p12 gene, little is known about the proteins involved in prostate-specific control of p12 gene transcription. Using DNAseI as well as gel mobility shift assays, we present the evidence that two distinct proteins from different tissues (one from the liver and the other from the prostate) have overlapping binding sites in a region from the p12 5'-flanking sequence located between position -505 to -549 relative to the mRNA start site. Deletion of this particular region resulted in a significant reduction of CAT gene expression when transiently transfected in rat liver Hepa7.6 cells but an increased activity was observed in mouse liver CL-2 cells. A detailed analysis of the target sequence bound by the liver protein indicate that it possess a significant homology with the DNA target sequence recognized by a number of liver-specific transcription factor (NF-kB, LF-A1, HNF-1) as well as with the VRE element from the interferon β (βIFN) gene. On the other hand, analysis of the prostate-specific binding site revealed no homology with the target sequence for any other known transcription factor characterized so far.

B 349 INDUCTION OF INDOLEAMINE 2,3-DIOXYGENASE GENE EXPRESSION BY INTERFERON-γ: IDENTIFICATION OF AN INTERFERON-γ-INDUCED DNA-BINDING FACTOR, Hamdy H. Hassanain and Sohan L. Gupta, Hipple Cancer Research Center, 4100 S. Kettering Blvd., Dayton, OH 45439

Interferon (IFN)-γ induces the expression of a number of cellular genes leading to induced synthesis of many gene products which mediate the biological effects of IFN-γ. This includes the induction of indoleamine 2,3-dioxygenase (IDO) enzyme, which degrades tryptophan into N-formylkynurenine. The IDO gene represents an example of cellular genes which are induced strongly by IFN-γ but very poorly by IFN-α_2. The induction of IDO has been implicated in the antiproliferative and antiparasitic activities of IFN-γ. Earlier studies carried out with a genomic clone of the IDO gene have identified a 155 base pair (bp) region upstream of the IDO gene which was able to confer inducibility to a heterologous gene (chloramphenicol acetyltransferase, or CAT, gene linked to herpes thymidine kinase, or TK, promoter) by IFN-γ. This region contained a sequence element highly homologous to the ISRE (interferon stimulated response element) sequence found in all IFN-α_2 inducible genes. Thus, despite the presence of an ISRE homolog, IDO gene responds very poorly to IFN-α_2. Synthetic oligonucleotides spanning this 155 bp region were tested for their capacity to confer IFN-γ-inducibility to CAT gene. These experiments narrowed down the IFN-γ responsive sequence to a 39 bp fragment. This fragment contained the sequence homologous to the ISRE sequence. Binding to the IFN-α_2-inducible transcription factor ISGF3 was examined in order to investigate the lack of response to IFN-α_2. Gel mobility shift experiments were carried out to identify any IFN-γ-inducible DNA binding factor(s) which bind specifically to the IFN-γ responsive sequence upstream of the IDO gene. A specific IFN-γ-induced DNA-binding factor was detected with characteristics different from the IFN-α_2-regulated factor ISGF3. The appearance of this IFN-γ-induced factor was abolished if the cells were treated with IFN-γ in the presence of cycloheximide, indicating a need for new protein synthesis. This is in agreement with our earlier results which showed that the transcriptional activation of the IDO gene by IFN-γ required new protein(s) synthesis.

B 350 **CHARACTERIZATION OF PEA3, A NOVEL ETS TRANSCRIPTION ACTIVATOR,** John A. Hassell, Jihou Xin, Paul Desjardins and Sharen Bowman, Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario, Canada L8S 4K1

The *ets* gene family comprises over a dozen individual members that have been conserved between diverse species. Membership in the family is defined by the occurrence of an 85 amino acid stretch termed the ETS domain, which constitutes a sequence-specific DNA binding domain. Ets proteins act as transcriptional regulators which activate or repress transcription. Recently we identified a new murine member of this family termed PEA3. PEA3 binds to a motif known as the PEA3 element initially identified in the polyomavirus enhancer. This motif also occurs in the promoters of many cellular genes including those of growth factors and their receptors, proto-oncogenes and proteases. We are interested in learning how PEA3 acts to regulate transcription. To achieve this objective we have defined the optimal DNA binding site for PEA3 and begun to map its functional domains. We used a variation of the SAAB assay to define a consensus DNA binding site for PEA3. Whereas this sequence is very similar to the binding site of other Ets proteins, use of a library of cloned binding sites and several different Ets proteins revealed that these proteins do not have identical DNA binding specificities. This suggests that the regulation of target gene expression in the face of several Ets proteins may be achieved by subtle differences in Ets binding sites resident in their promoters. Using a set of amino terminal and carboxy terminal deletion mutants of PEA3 we have mapped the DNA binding and activation domains of PEA3. An 83 amino acid segment comprising the ETS domain was sufficient for sequence-specific DNA binding. PEA3 appears to possess several activation domains as well as domains that negatively influence its activity. Interestingly, a truncated version of PEA3 composed of the carboxy terminal 164 amino acids efficiently activated transcription. This region contains the ETS domain and flanking residues. Efforts are currently underway to map this activation domain more precisely.

B 351 **The isolation of a novel mitogen-inducible nuclear orphan receptor** - Cyrus V. Hedvat and Steven G. Irving, Dept. of Pathology, Georgetown University School of Medicine, Washington, DC 20007

Cellular proliferation is normally a tightly regulated process, with key controls exerted as a series of sequential and dependent biochemical events. Studies that block the expression of specific gene products which are normally inducible by mitogens at the G_0 or early G_1 stages of the cell cycle have demonstrated the necessary role of these products in the progression of the genetic program initiated by the mitogenic stimulus. We have previously described the isolation of a number of inducible gene clones from PHA+PMA-stimulated human T cells using subtractive cloning and the characterization of their expression patterns in response to various stimuli. We now report the isolation of a previously undescribed member of the steroid/thyroid nuclear receptor family, termed mitogen inducible nuclear orphan receptor (MINOR). The expression of MINOR in response to PHA+PMA or PMA+Ca^{++}-ionophore in Jurkat T cells, and in response to serum stimulation of quiescent fibroblasts results from de novo transcriptional activation. Interestingly, the induced expression of the MINOR gene in Jurkat T cells, but not fibroblasts, is strongly inhibited by cyclosporin A treatment.

The amino acid sequence of MINOR is similar to that of other orphan receptors (e.g., NGF I-B/nur 77), primarily in the Zn^{++}-finger/DNA binding domain with lesser homology in the putative ligand binding and transactivating domains.

We are currently examining the binding activity of MINOR and its ability to activate reporter gene expression. In light of the possibility for heterodimerization between members of the RxR/orphan receptor subgroup among themselves and with other nuclear receptor peptides, the expression of an additional member of this superfamily may result in novel functional interactions in appropriately stimulated cells.

B 352 **RECESSIVE AS WELL AS DOMINANT MUTATIONS IN THE YEAST TRANSCRIPTIONAL ACTIVATOR CHA4 CAN LEAD TO CONSTITUTIVE EXPRESSION OF *CHA1*.** Steen Holmberg, P.Schjerling, J.Ø.Pedersen, M.Prætorius & T.Nilsson-Tillgren, Institute of Genetics, University of Copenhagen, Denmark.

Expression of the *CHA1* gene in yeast is transcriptionally induced by serine (80-100 fold) and to a lesser extent by threonine. *CHA1*, encoding the catabolic L-serine (L-threonine) deaminase, allows the yeast to grow on media with serine or threonine as sole nitrogen source. Deletion analysis has identified a *cis*-acting element in the *CHA1* promoter, UAS_{CHA}, needed for induction. This sequence confers serine induced expression to a heterologous promoter.

We have isolated a recessive mutation, designated *cha4-1*, that prevents cells from utilizing serine/threonine as sole nitrogen source. The *cha4-1* mutation causes non-inducible expression of *CHA1* and reduces basal level expression (aspargine as nitrogen source) 8-10 fold. Furthermore, serine induced expression of a heterologous promoter through UAS_{CHA} depends on the presence of *CHA4*.

The *CHA4* gene has been cloned and sequenced. The CHA4 protein has a deduced length of 648 amino acids, including an N-proximal (residue 43 to residue 70) motif known as the $Zn(II)_2Cys_6$ binuclear cluster, suggesting that *CHA4* encodes a DNA binding protein. Furthermore, four acidic regions of 25 - 40 amino acids can be recognized. Gel retardation experiments employing an *E. coli* produced truncated version of CHA4 (residue 1 to residue 174) and wild-type and mutated forms of UAS_{CHA} demonstrate that CHA4 specifically binds to UAS_{CHA} *in vitro*.

Induction of *CHA1* leads to suppression of *ilv1*, i.e. the catabolic deaminase is able to substitute for the anabolic threonine deaminase encoded by the *ILV1* gene. We have identified a dominant mutation, *SIL3*, and a recessive mutation, *sil2*, that both suppress *ilv1* by inducer independent, constitutive transcription of *CHA1*. Gap-repair analysis has demonstrated that *sil2* and *SIL3* both are alleles of *cha4*. We have cloned six alleles of *SIL3* and two alleles of *sil2* and are in the process of localizing the mutated site in the CHA4 protein in order to relate domaine to functional role in transcriptional regulation.

B 353 **Identification of a negative cis-acting element and its binding proteins in EGF receptor gene promoter**

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Overexpression of the epidermal growth factor (EGF) receptor (c-erb B) proto-oncogene is a frequent occurrence in human carcinoma. Identification of the factors controlling transcription of the EGF receptor gene is essential for understanding the mechanisms of EGF receptor gene regulation and its role intumorigenicity. In this study, we have identified a 61-base-pair proximal element (-911 to -850 relative to the AUG translation initiation codon) in the EGF receptor 5' promoter region that functions as a negative *cis*-acting element. We demonstrate by gel mobility-shift, methylation interference, DNase I footprinting that a *trans*-acting factor specifically binds a ATTTC (N_5) ATTTC (-890 to -875 relative to AUG translation initiation codon) direct repeat and protects a region from -900 to -869 . Southwestern (DNA-protein) blot analysis shows that this element binds a 128-kDa polypeptide in Hela cells, which express normal levels of EGF receptor. In A431 human epidermoid carcinoma cells, which overexpress EGF receptor, this element specifically binds two factors-the 128-kDa polypeptide and another smaller 28-kDa polypeptide. These data suggest that specific transcription factors negatively regulate EGF receptor gene expression , and the nature of these regulatory factors depends upon the specific cell type.

B 354 THE CELL-SPECIFIC PROMOTER OF HOMEOBOX Cdx-1 GENE: CHARACTERIZATION OF THE POSITIVE AND NEGATIVE REGULATORY ELEMENTS, Yinling Hu[1], Jan Kazenwadel [2]and Robert James[2], [1]Melbourne Tumour Biology Branch, Ludwig Institute for Cancer Research; [2]Department of Medicine, Melbourne University, Parkville , Victoria, PO. Royal Melbourne Hospital, 3050, Australia

The murine gene, Cdx-1, encodes a homeodomain containing protein that is expressed in the epithelial cells of the intestine. In order to study its regulation, we have isolated the Cdx-1 gene and found that it contains three exons and two introns. Sequence analysis of the gene reveals the presence of a typical TATA box motif at nucleotides -31, which is located within a CpG island. The promoter region of the Cdx-1 gene from nucleotides +72 to -1040 was functionally mapped by transient transfection assays, in which a series of chimeric constructs containing the deletion promoter fragments and the bacterial chloramphenicol acetyltransferase (CAT) gene were transfected into two colonic carcinoma cell lines (LIM1899 and LIM2099) and a fibroblastic NIH 3T3 cell line. The expression level of the CAT gene is much higher in the two colonic carcinoma cell lines than in the NIH 3T3 cell line. The results of this analysis show that nucleotides +72 to -47 have a basal promoter function which can be enhanced by the addition of sequences -48 to -380. Inclusion of sequences between -381 to -887 represses transcription of the CAT gene. This repression can be relieved by addition of nucleotides from -888 to -1040. Using gel shift assays, we observed cell specific protein-DNA complexes in the fragments from +72 to -47 and from -381 to -589.

Furthermore, transfection experiments show that the sequences from -381 to -589 in both orientations can repress expression of the SV40 promoter in both LIM1899 and 3T3 cell lines, suggesting that this region may act as a silencer for regulation of the Cdx-1 gene. DNA footprinting and gel shift experiments indicate that at least two district proteins bind to specific sequences within this silencer element. The findings suggest that the binding proteins may be be relevant for the regulation of the Cdx-1 gene.

B 355 ANALYSIS OF THE PREEXISTING AND NUCLEAR FORMS OF NF-AT (NUCLEAR FACTOR OF ACTIVATED T CELLS)
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The nuclear factor of activated T cells (NF-AT) is an inducible DNA-binding protein which is essential for transcriptional induction of the Interleukin 2 gene during T cell activation. NF-AT is thought to consist of two components: a ubiquitous, inducible nuclear component which we have identified as Fos and Jun proteins, and a preexisting, T cell-specific component ($NF\text{-}AT_p$) which is the target for the immunosuppressive agents cyclosporin A and FK506. We have previously shown that nuclear extracts from activated T cells form two inducible NF-AT complexes with an oligonucleotide corresponding to the distal NF-AT site of the murine IL2 promoter, while hypotonic extracts of unstimulated T cells form a single complex containing $NF\text{-}AT_p$. Here we show that the ability to detect $NF\text{-}AT_p$ in a gel shift assay, which is essential for purification and biochemical studies of this protein, is strikingly dependent on the precise sequence of the NF-AT oligonucleotide used as the labelled probe. Moreover we present evidence that the component which forms the faster-migrating ("lower") nuclear NF-AT complex is derived by a calcium-dependent, cyclosporin-sensitive, posttranslational modification of $NF\text{-}AT_p$, and that Fos and Jun proteins stabilise its interaction with DNA. The results are discussed in the context of a model relating the two nuclear NF-AT complexes to $NF\text{-}AT_p$.

B 356 THE EFFECT OF DNA METHYLATIONON PROTEIN BINDING TO THE STAGE SELECTOR ELEMENT OF THE γ-GLOBIN GENE PROMOTER. S.M. Jane, D.L. Gumucio, P.A. Ney, J.M. Cunningham, and A.W. Nienhuis. Clinical Hematology Branch, NHLBI, Bethesda, MD and Depart. of Anatomy and Cell Biol., University of Michigan, Ann Arbor, Michigan.

The human γ-globin gene promoter contains a stage selector element (SSE) as shown in transient transfection assays (EMBO J. 1992 8:2961-9). A protein with fetal and erythroid specificity (-50γ) binds to this element. Sp1 also binds in this region of the γ-promoter. The contact nucleotides for the -50γ protein include two adjacent CpG dinucleotides known to be hypomethylated in fetal red cells but fully methylated in adult erythroid cells. We have examined the effects of methylation on protein binding to the SSE. Gel mobility shift assays using nuclear extracts from K562 cells (which contain both Sp1 and the -50γ protein) demonstrate preferential binding of the -50γ protein to the SSE under conditions in which the probe is limiting. Methylation of the CpG residues reverses this preference with a marked increase in the binding of Sp1 at the expense of the -50γ protein. Purified Sp1 binds with 10-fold higher affinity to the methylated versus non-methylated probe. An additional binding site for the -50γ protein is created by the -202 C→G HPFH mutation. This "de-novo" site is also adjacent to an Sp1 binding site. In functional assays using reporter genes, a hybrid ß-globin gene promoter containing the -202 HPFH sequence was 10-fold more active than either the wild-type ß-promoter or a hybrid containing the wild-type -202 sequence when transfected into K562 cells. The -202 HPFH mutation creates a CpG dinucleotide, but methylation of this cytosine residue has no effect on the relative affinities of Sp1 or -50γ. Because binding of the -50γ protein to the SSE correlates with enhanced promoter function, we infer that methylation-induced preferential binding of Sp1 with reduced binding of -50γ may be part of the mechanism by which γ-genes are repressed in normal adult erythroid cells. In contrast, in cells containing the -202 HPFH mutation, the inability of Sp1 to displace -50γ in the methylated state may explain the persistence of γ-promoter activity and γ-gene expression observed in adults with this mutation.

B 357 **SP1 IS REQUIRED FOR PLACENTAL ENHANCER- BUT NOT BASAL-MEDIATED TRANSACTIVATION OF THE HUMAN CHORIONIC SOMATOMAMMOTROPIN PROMOTER IN JEG AND BEWO CELLS**, Shi-Wen Jiang, Allan R. Shepard and Norman L. Eberhardt, Departments of Medicine and Biochemistry/Molecular Biology, Mayo Clinic, Rochester, MN 55905.

The human growth hormone (hGH) and chorionic somatomammotropin (hCS) genes are duplicated genes with ~95% sequence identity that are expressed exclusively in the pituitary and placenta, respectively. Cell-specific expression of the hGH gene is regulated by the pituitary-specific factor GHF-1. Placental-specific expression of the hCS gene may be controlled by an enhancer (CSEN) that appears to be related to the SV40 enhancer. The enhancer located 3′ to the hCS-2 gene was inserted into vectors containing the hCS-1 or hGH-1 5′-flanking DNA (nts -496/+2) fused to the luciferase (LUC) gene. Constructs with and without CSEN were transfected into choriocarcinoma cells (BeWo and JEG-3), rat anterior pituitary somatotrophs (GC) and HeLa S3 cells. CSEN stimulated LUC activity from the hCS and hGH promoters about 16- and 12-fold, respectively in BeWo and JEG-3 cells up to the comparable levels of expression observed for both the hGH and hCS promoters in GC and HeLa S3 cells. CSEN did not affect hGH or hCS promoter activity in GC and HeLa S3 cells. hCS promoter constructs containing site-specific mutations of the Sp1 and proximal, distal or both GHF-1 sites with and without CSEN were examined. GHF-1 mutations were without effect on CSEN-stimulated or basal hCS promoter activities in BeWo and JEG-3 cells. The Sp1 mutation abolished CSEN-stimulated activity, but basal activity was not affected. Thus Sp1 is required for mediating CSEN activity; however, other factors mediate basal hCS promoter activity in choriocarcinoma cells. The ability of CSEN to efficiently stimulate the hGH promoter suggests that other factors are required to suppress hGH promoter activity in the placenta.

B 358 REGULATION AND CHARACTERIZATION OF THE SIS/PDGF INDUCIBLE FACTOR, Leisa Johnson, P. Sanjeeva Reddy, and Brent H. Cochran. Center for Cancer Research and Dept. of Biology, M.I.T., Cambridge, MA, 02139

We have previously identified a PDGF-inducible DNA-binding protein, which binds to a conserved sequence approximately 346 bp upstream of the c-*fos* gene. We have called this factor SIF, for *sis*/PDGF-inducible factor. This DNA-binding activity is rapidly activated upon treatment of quiescent cells with *sis*/PDGF and occurs in the absence of new protein synthesis. PDGF-BB and PDGF-AA can both induce *SIF* binding activity, but the induction by the AA isoform is weaker. CSF-1 can also induce SIF in NIH 3T3 cells that express c-*fms*. Neither deletions of the kinase insert domain, nor a Tyr to Phe mutation at 809 of the receptor block the ability of CSF-1 to induce SIF binding activity. When a single copy of the SIF recognition element is placed upstream of a truncated, uninducible c-*fos* promoter, c-*sis*/PDGF inducibility is restored even in the absence of the c-*fos* serum response element.

To identify molecular clones of the SIF gene(s), we have developed a strategy to clone mammalian DNA-binding factors by screening cDNA libraries in yeast. A reporter strain was constructed by stably integrating the *CYC1/lacZ* gene in which the upstream activation sequence of the *CYC1* promoter has been replaced with multiple copies of a high affinity SIF binding site. The reporter strain was then transformed with a mammalian cDNA library that was constructed so as to fuse the activation domain of GAL4 to each cDNA. GAL4-cDNA fusion proteins that are capable of binding to the SIF element will activate the reporter gene and result in a blue colony. Upon screening about 1.5×10^6 independent clones, four cDNAs were isolated that were derived from two distinct genes. Partial sequence analysis reveals that both genes encode class I type zinc finger domains. Further characterization of these cDNA clones is underway to determine whether either of these genes encode the SIF factor.

B 359 5' REGULATORY REGION OF THE ICSBP GENE: AN INTERFERON-γ RESPONSIVE ELEMENT CONSERVED IN GENES OF THE IRF FAMILY

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A member of the family of the interferon (IFN) response factor (IRF), ICSBP, is expressed predominantly in lymphoid tissues, and is induced preferentially by IFN-γ. We analyzed the 5'upstream region of the ICSBP gene to investigate the mechanism controlling its expression. An upstream region from -351 to -151 relative to the transcription start site contained a functional interferon response element (IRE) which conferred IFN-γ selective induction on CAT reporter genes. This region included a palindromic motif, TTTCNNGGAAA, which also exists in the regulatory region of the IRF-1 gene. By gel shift assay, we detected an IFN-γ inducible, cycloheximide sensitive binding activity specific for this motif. The canonical ISREs of the MHC class I, ISG54 and GAS (IFN-γ response element) of GBP genes failed to compete for the binding. The results suggest that this palindromic IRE motif binds an as yet unidentified factor specifically induced by IFN-γ, and that a common regulatory mechanism exists for the ICSBP and IRF-1 genes.

B 360 SEQUENCES DOWNSTREAM OF THE TRANSCRIPTION INITIATION SITE ARE IMPORTANT FOR HTLV-I BASAL GENE EXPRESSION, Fatah Kashanchi, Janet F. Duvall, Paul F. Lindholm, Michael F. Radonovich and John N. Brady, Laboratory of Molecular Virology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Sequences which control basal HTLV-I transcription likely play an important role in initiation and maintenance of virus replication. We have identified and analyzed a 45 nucleotide sequence (DRE 1) at the boundary of the R/U5 region of the LTR which is required for HTLV-I basal transcription. The basal promoter strength of constructs that contained deletions in the R/U5 region of the HTLV-I LTR were analyzed by CAT assays following transfection of Jurkat T-cells. We consistently observed a ten-fold decrease in basal promoter activity when sequences between +202 to +246 were deleted. By RT/PCR RNA analysis, we confirmed that the drop in CAT activity was paralleled by a decrease in the level of steady-state RNA. DRE 1 did not effect the level of Tax_1 transactivation. Using a gel shift assay, we have purified a highly enriched fraction that could specifically bind DRE 1. This DNA affinity column fraction contained four detectable proteins on SDS/PAGE: p37, p50, p60 and p100. The affinity column fraction stimulated HTLV-I transcription approximately twelve-fold in vitro. No effect was observed with the HIV or AdML promoters. Following renaturation of the proteins isolated from an SDS gel, p37, but not the other protein fractions, was able to specifically bind to DRE 1.

B 361 **IN VITRO TRANSCRIPTIONAL ANALYSIS OF THE DROSOPHILA SUPPRESSOR OF HAIRY WING PROTEIN, Leslie A. Kerrigan, Deborah J. Frank, and James T. Kadonaga**, Department of Biology, University of California, San Diego, La Jolla, CA 92093.

We have purified suppressor of Hairy-wing [su(Hw)] to > 90% homogeneity from Drosophila embryos. su(Hw) contains an acidic domain and 12 copies of the zinc finger motif, which are characteristic of transcription factors. su(Hw) interacts specifically with the Drosophila transposable element gypsy in a 367 bp region that shows similarity to the octamer motif of mammalian transcriptional enhancers. Previous studies by V. Corces and others describe y^2, a well characterized gypsy-induced mutation in which the gypsy element is inserted 700 bp upstream of the transcriptional start site of the yellow gene. In y^2 flies, the wing and body enhancers (which are 1-2 kb upstream of the gypsy insertion) are unable to interact with the yellow promoter when su(Hw) is present. However, other tissue-specific enhancers of the yellow gene that do not have gypsy inserted between the enhancer and the start site are unaffected by su(Hw), and continue to function normally. We have attempted to reconstruct this phenomenon in vitro by placing the su(Hw) binding sites between activator binding sites and a promoter to simulate both short range and long range activation. In vitro transcription is performed with purified transcription factors, using both naked DNA and chromatin templates. Recent results will be presented.

B 362 PARAMETERS INFLUENCING THE EXPRESSION OF HUMAN HEMOGLOBIN IN TRANSGENIC PIGS, Anastasia Khoury, Jeannine Okabe, Michael Martin, Ajay Sharma, Stephen Pilder, John Logan and Ramesh Kumar, DNX, Inc., 303B College Road East, Princeton, NJ 08540

Various risk factors associated with human blood transfusions and the occasional scarcity of donated blood have contributed to the current interest in the development of a safe and effective blood-substitute. We are interested in producing human hemoglobin in a system that will ensure an abundant and inexpensive supply of this raw material for a blood-substitute. We have generated several transgenic pigs that carry human α and β globin genes linked to a locus control region (LCR). In these animals, the transgenes are expressed in a tissue specific manner and functional human hemoglobin is produced in the red blood cells. Initially, the expression level in our founder pigs is at or below 10% the level of the endogenous protein. For commercial development, it is desirable to have higher level expression of human globin in the transgenic pigs. We have undertaken detailed analysis of the factors involved in the regulation of the transgenic and endogenous globin genes in the pig. Our results indicate that in both high and low copy transgenic founders, the transcription of the human genes is less efficient than that of the endogenous globin genes. Presently, experiments are directed to understand the basis of this deficiency. In one approach, an in vitro transcription/translation system will be employed. Concurrently, we are designing additional expression constructs for improving hemoglobin expression. Results of these studies will be presented.

B 363 *CHARACTERIZATION OF THE MURINE T CELL* RECEPTOR Vδ1 PROMOTER, Laura J. Kienker and Philip W. Tucker, Dept. of Microbiology, Univ. of Texas Southwestern Medical Ctr., Dallas, TX 75235

T lymphocytes are divided into 2 classes (γδ and αß) based on the type of heterodimeric antigen receptor expressed on their surface. Each TCR gene locus consists of V,J, or V,D,J and C gene segments which undergo somatic rearrangement during development to generate functional TCR genes. TCR -ß, -γ, and -δ genes are rearranged and expressed by day 14 of mouse gestation; TCR-α genes by day 17. Further, within the γ and δ genes, distinct variable gene segments are rearranged and expressed in a programmed fashion. Vγ3, Vγ4, and Vδ1 are utilized in early fetal stages, whereas Vγ2 and Vδ5 are frequently rearranged and expressed in adults. The tissue and stage specificity of TCR gene rearrangements may be regulated by transcriptional activity at the germ-line loci controlling their accessibility to the recombinational machinery. Studying the regulation of a TCR gene can therefore provide insight into how tissue and lineage specific gene expression are achieved as well as how gene rearrangement might be regulated during T cell development. Toward this end, we have begun characterizing the Vδ1 promoter region of the TCR δ chain. Vδ1 is a variable gene segment used exclusively in γδ T cells. S1 nuclease protection identified the δ chain mRNA start site ~90 bp upstream from the initiator ATG. Transient transfections into Molt-13 cells of constructs containing various portions of the Vδ1 promoter driving CAT reporter gene transcription revealed that -88 to +361 is the region important for promoter activity. Sequencing identified several consensus binding sites in this region for T cell- and lymphoid-specific transcription factors (e.g. GATA-3, TCF-1, and Ets-1). Currently, proteins binding to the minimal Vδ1 promoter are being identified and characterized using gel mobility shift and in vitro MPE-Fe(II) footprinting.

B 364 MOLECULAR CLONING OF THE DNA BINDING DOMAIN OF THE SV40 LATE TRANSCRIPTION FACTOR GENE, Chung Han Kim[1] and Pitchai Sangan[1,2]. [1]Department of Molecular Biology, Coriell Institute for Medical Research, Camden, NJ 08103. [2]Present address; Department of Molecular Biophysics and Biochemistry, Yale University, School of Medicine, 06510.

Late simian virus 40 transcription factor (LSF) is a protein present in mammalian cells which binds to two distinct sites within the SV40 promoter region. LSF specifically activates the transcription of SV40 late genes. We have cloned a partial cDNA of about 872 bp which encodes LSF, by screening human HeLa cell cDNA expression library in λgt11 using labelled catenated synthetic oligonucleotide duplex (GC1-3, nt 38 to 73). GC1-3 contains the SV40 GC-motifs 1, 2, and 3. The β-galactosidase-LSF fusion protein was purified to homogeneity by anti-β-galactosidase antibody immunoaffinity chromatography, and analyzed by SDS-PAGE. A single band appeared at 150 kDa. DNA-binding specificity of the fusion protein was studied by different methods. Southwestern blot analysis indicated that the 150 kDa fusion protein formed a DNA-protein complex with both GC1-3 and LSF-280 labelled probes. Competition band mobility shift analysis showed that the fusion protein formed a DNA-protein complex with GC1-3 and LSF-280 DNAs and not with SP1 and AP1 binding site DNAs. Methylation interference studies revealed that the guanine residues located at position 53, 58, 63, and 68 in GC1-3 and 273, 279, and 283 in LSF-280 DNAs were the close contact points with the fusion protein. These data strongly suggest that a partial cDNA has been cloned which encodes the DNA-binding domain of LSF.

B 365 THE STRUCTURE OF THE N-ALKYL-SIDECHAIN IN 2-PHENYLINDOLE-BASED ANTIESTROGENS DETERMINES THE ANTAGONISTIC EFFECT ON ESTROGEN-DEPENDENT TRANSCRIPTION, Ronald Koop+*, Stefan Leichtl* Eggehard Holler+ and Erwin von Angerer*, +Institut für Biophysik und physikalische Biochemie, *Institut für Pharmazie, Universität Regensburg, 8400 Regensburg, Germany

The new nonsteroidal compound ZK 119.010 (2-(4-hydroxyphenyl)-3-methyl-1-[6-(1-pyrrolidinyl)-hexyl]-indol-5-ol) [1] showed strong antiestrogenic effects in immature mice and ovariectomized adult rats but has only marginal estrogenic effects [2]. From cotransfection experiments with estrogen receptor-expression vectors and an estrogen-dependent luciferase-reporter plasmid in HeLa cells it became evident that only the ligand-inducible transcription-activating-function 2 (TAF-2) of the estrogen receptor (ER) was inhibited, whereas the constitutive TAF-1 was apparently not influenced. The partial antiestrogen 4-hydroxytamoxifen (OHT) has similar characteristics, what was also shown by other investigators [3].

Replacement of the N-alkyl-sidechain of ZK 119.010 by the 7α-sidechain of the "pure" antiestrogen ICI 164.384 [4] creates a new compound L 19 (N-n-butyl-11-[2-(4-hydroxyphenyl)-3-methyl-5-hydroxyindole-1-yl]N-methyl-undecamide) which, like ICI 164.384, did not exert any TAF-1 activity in the cotransfection experiments.

Inhibition of estrogen-dependent transcription by these antiestrogens seems not to be based on disruption of ER-binding to estrogen-responsive-elements (ERE). Gel-shift-assays revealed specific ER-ERE interactions in the presence of estradiol, OHT, ZK 119.010, L 19 and ICI 164.384, although under these *in vitro* conditions quantitative differences could be observed.

Therefore, it seems likely, that the N-alkyl-sidechain is mainly responsible for the antagonistic properties, whereas the 2-phenylindole structure provides the ER specificity.

Estrogen receptor-expression vectors were kindly provided by Prof. P. Chambon (Straßbourg, France).

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B 366 REGULATION OF ANP GENE EXPRESSION BY DIFFERENT MEMBERS OF THE JUN AND FOS FAMILIES. Kovacic-Milivojevic, B. and Gardner, D.G. Metabolic Research Unit, University of California at San Francisco, CA. 94143.

Individual members of the jun gene family (i.e. c-jun, jun-B and jun-D) bind to the TPA response element (TRE) as homodimers or heterodimers with c-fos or other members of the fos family (i.e. fosB, fra-1 and -2). Employing transient transfection analysis in neonatal rat ventricular cardiocyte cultures, we have demonstrated that overexpression of either c-jun or jun-B results in a dose-dependent activation of the human (h) ANP gene promoter. In comparative terms, jun-B is less potent than c-jun in effecting this activation while jun-D is essentially devoid of activity in this context. Selective mutation of a TRE located 235 bp upstream from the transcription start site, suppressed basal activity significantly and truncated c-jun and jun-B activation of the promoter. Gel shift analysis confirmed that this region of the hANP promoter associated with c-jun/c-fos or jun B/c-fos complexes in sequence-specific fashion. Interestingly, cotransfection of c-jun and jun-B into ventricular cardiocytes resulted in a synergistic activation of hANP promoter activity. In atrial cardiocytes jun-B did not activate hANP gene transcription. Furthermore, when cotransfected with c-jun, it functioned as an inhibitor, rather than an amplifier, of c-jun dependent activation.

Cotransfection of the same hANPCAT reporter with a c-fos expression vector resulted in an unpredicted inhibition of basal as well as c-jun-stimulated reporter activity. On the other hand, overexpression of fra-1 had little or no effect on basal hANP gene promoter activity, while in combination with c-jun, it effected a strong activation of this promoter. Neither the junB/fra-1 nor the jun-D/fra-1 combinations resulted in productive trans-activation of this promoter.

Taken together these results imply that the ANP gene is a physiological target for jun- and fos-dependent activity. Individual members of the jun and fos family can be distinguished based on their relatively unique activities at the level of this gene's promoter, implying distinct functional roles for each. Such diversity would serve to broaden the repetoire of responses which this gene exhibits following exposure to different environmental stimuli.

B 367 **IDENTIFICATION OF REGULATORY ELEMENTS WITHIN THE MINIMAL PROMOTER REGION OF THE HUMAN ENDOGENOUS ERV9 PROVIRUSES: ACCURATE TRANSCRIPTION IS CONTROLLED BY AN Inr-LIKE ELEMENT, La Mantia, G. Strazzullo, M., Majello, B., Di Cristofano, A. and Lania, L.** *Dipartimento di Genetica, Biologia Generale e Molecolare. Università degli studi Federicoll. via Mezzocannone,8 80134 Napoli.Italy*

We have recently identified human endogenous retroviral sequences (ERV9) that are preferentially expressed in undifferentiated embryonal carcinoma cells NT2/D1. Whereas about 50 different ERV9 loci containing pol-related sequences are present in the human genome, we have extimated that ERV9 LTR sequences are at least 4000 copies. It seems therefore that ERV9 LTRs can represent an important reservoir of potentially functional promoter sequences that could affect the pattern of expression of a number of cellular genes. Moreover, different ERV9 LTRs are capable to drive expression of a reporter gene in transient expression assays.

We have analyzed the minimal promoter region located within the ERV9 LTR. Using the transient CAT expression assay we have identified the minimal promoter region, which includes sequences spanning from -70 to +6 relative to the major transcription start site. One region located between -70 to -39 acts as a transcriptional activating sequence and contains an Sp1 binding site. The second region from -7 to +6, which resembles an initiator element (Inr), was necessary for the correct transcription start site utilization, and binds to a regulatory protein. Cross-competition experiments using various Inr elements have indicated that the protein that binds to the ERV9 Inr element can be competed by the HIV-1 and TdT Inr sequences.

In the attempt to establish a more precise correlation between factor binding and proper site selection and to determine the relationship between the ERV9 Inr binding protein and the other known Inr binding factors we have undertaken a detailed site-directed mutational analysis of the Inr sequences and swapping experiments between the ERV9 Inr sequences and theTdT and HIV-1 Inr sequences Finally , to establish if sequences upstream of -7 are also involved in positioning the start site we have constructed plasmids in which the -7/+6 sequences are moved to new locations. The results of in vivo and in vitro transcriptions assays will be discussed.

B 368 **THE EXPRESSION OF THE PDGF B-CHAIN GENE IN MALIGNANT MESOTHELIOMA CELL LINES IS REGULATED AT THE TRANSCRIPTIONAL LEVEL**

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Elevated PDGF B-chain mRNA expression was observed previously in human malignant mesothelioma cell lines as compared to normal mesothelial cells. Therefore, the regulation of expression of this gene was studied in normal and malignant mesothelial cell lines. The PDGF B-chain expression level in both cell types was not increased by the protein synthesis inhibitor cycloheximide, whereas, in a run off assay nuclear PDGF B-chain mRNA levels in normal and malignant cells varied to the same extent as on Northern blots. So the elevated steady-state PDGF B-chain mRNA level in malignant mesothelioma cell lines is probably caused by increased transcription in the malignant cell type. Differences in degradation seem to be less important. DNAse I hypersensitive sites (DH's) were found in and around the PDGF B-chain promoter in both normal and malignant mesothelial cells. Preliminary results indicate promoter-induced CAT expression in both cell types, whereas the methylation pattern in the promoter region was shown to be similar. In the first intron of the gene two DH's were found in malignant and not in normal mesothelial cells. However, in CAT assays these regions did not seem to increase the basic promoter-induced CAT expression.

These results suggest that a difference in transcription rate is the decisive factor for the elevated PDGF B-chain mRNA expression in malignant mesothelioma cell lines, but that the promoter and the first intron do not seem to be the most important regions in this respect.

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B 369 FUNCTIONAL AND MUTATIONAL ANALYSIS OF THE DROSOPHILA KRÜPPEL TRANSCRIPTIONAL REPRESSOR

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The *Drosophila Krüppel* (*Kr*) protein is a DNA-binding repressor of transcription in mammalian cells. (*Nature* 346: 76-9,1990). The transcriptional repression and DNA-binding activities of the *Kr* protein can be dissociated and the repression function of *Kr* can be transferred to a heterologous DNA-binding protein, the *lac* repressor. Fusion genes between portions of the N-terminal region of the *Kr* protein and the *lac* repressor were assayed *in vivo* for their ability to repress transcription from a reporter gene containing *lac* operators upstream of the herpes thymidine kinase (*tk*) promoter. We thus localized a repression region between amino acids 60 and 90 of the *Kr* protein. This portion of the *Kr* protein is predicted to form an alpha-helix with several hydrophobic faces. A *Kr* mutant missing amino acids 28-213 was still able to repress transcription from a reporter gene containing *Kr* binding sites, suggesting the presence of a second repression region within the C-terminal portion of the protein. Supporting this hypothesis, a fusion between the *lac* repressor and amino acids 345-466 of *Kr* repressed transcription from a *lac* operator containing reporter gene. In accordance with our prediction that alanine-rich regions of other *Drosophila* transcriptional factors would mediate transcriptional repression, a *lac* repressor *even-skipped* fusion was three-times more potent a transcriptional repressor than *lac-Kr*. The presence of a stretch of 11 alanines in a row in the *lac-eve* proteins was required for repression. *Kr* represses transcription when bound at kilobase distances upstream or downstream from the start site of *tk* transcription, suggesting function through protein-protein interactions with factors bound to the *tk* promoter. Low amounts of *Kr* co-transfected with a *Kr* binding site containing reporter gene do not lead to activation and a monotonic decrease in transcription is observed with increasing amounts of *Kr* expression plasmid. We examined the ability of the *Kr* protein to repress transcription activated by different *GAL4* fusion activators. *Kr* selectively repressed transcription activated by a *GAL4*-Sp1, glutamine-rich activator protein but could not repress transcription mediated by the acidic activator *GAL4*. We currently believe that *Kr* functions to quench activation by glutamine-rich activation domains, perhaps by a direct protein-protein interaction, rather than interfering with some component of the basal transcriptional machinery required for all transcriptional activation events.

B 370 TRANSACTIVATION OF PROENKEPHALIN GENE EXPRESSION BY HTLV-1 TAX IS DEPENDENT UPON ENKCRE-2/AP-1 BINDING ELEMENT, Kenneth G. Low, Gwynn M. Daniels†, Michael H. Melner† and Michael J. Comb, Laboratory of Molecular Neurobiology, Massachusetts General Hospital, Charlestown, MA 02129, and †Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006

Human T-cell leukemia virus type 1 (HTLV-1) is responsible for adult T-cell leukemia. Transactivation of viral and cellular genes by HTLV-1 is dependent upon interactions between a viral *tax* protein and cellular transcription factors. In transactivation assays using human Jurkat T-lymphocytes, Tax transactivates basal expression of the proenkephalin (PENK) gene 3-fold. Treatment with 7 μg/ml concanavalin A, 500 μM cpt-cAMP or 10 ng/ml TPA enhanced Tax-dependent transactivation of PENK gene transcription between 40- to 70-fold. Deletion analysis of the human PENK gene promoter suggests that a region between -104 to -86 (relative to the start site of transcription initiation), which contains two consensus cAMP-responsive elements (CREs), mediates Tax-dependent trans-activation of PENK gene transcription. Further deletion analysis of this region identifies ENKCRE-2 (-92 to -86)(also a consensus AP-1 binding element) as responsible for mediating the Tax-dependent transactivation. Transactivation assays with CAT reporter constructs comprising multiple copies of ENKCRE-1 and/or ENKCRE-2 confirms that ENKCRE-2 but not ENKCRE-1 is responsible for mediating the effects of Tax. These results implicate an involvement of members of the AP-1 family of transcription factor proteins in mediating the transactivation of PENK gene transcription by Tax during HTLV-1 leukemogenesis.

B 371 THE VP16 TRANSCRIPTION ACTIVATION DOMAIN IS FUNCTIONAL WHEN TARGETED TO A PROMOTER PROXIMAL RNA SEQUENCE, Steven J. Madore, Laurence S. Tiley, Michael H. Malim and Bryan R. Cullen, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710

Among eukaryotic transcriptional *trans*-activators, the Human Immunodeficiency Virus Type 1 (HIV-1) Tat protein is exceptional in that its target site, TAR, is an RNA rather than a DNA sequence. We have confirmed that fusion of Tat to the RNA binding domain of the HIV-1 Rev protein permits the efficient activation of an HIV-1 long terminal repeat (LTR) promoter in which critical TAR sequences have been replaced by RNA sequences derived from the HIV-1 Rev Response Element (RRE). An RRE target sequence as small as 13 nucleotides was shown to form an effective *in vivo* target for Rev binding. Remarkably, a fusion protein consisting of Rev attached to the VP16 transcription activation domain was also observed to efficiently activate the HIV-1 LTR from this nascent RNA target. Like Tat, activation by the Rev VP16 fusion protein requires that the RNA target site be promoter proximal. These data demonstrate that *trans*-activation of transcription by acidic activation domains does not require a stable interaction with the promoter DNA and suggest that VP16, like Tat, can act on steps subsequent to the formation of the HIV-1 LTR preinitiation complex. The finding that the activation domains of VP16 and Tat are functionally interchangeable raises the possibility that these apparently disparate viral *trans*-activators may nevertheless act via similar mechanisms.

B 372 **CRE-BPa, a Novel Member of the Gene Family Encoding the cAMP Response Element-binding Protein CRE-BP1.**

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The cAMP response element (CRE) was first identified as being an inducible enhancer of genes that can be transcribed in response to an increased cAMP level. Multiple CRE-binding proteins such as CREB, CRE-BP1 (1), and ATFs have been identified by cDNA cloning and characterized. All of these proteins have a so-called "B-ZIP" structure which is found in a group of enhancer-binding proteins such as Jun.

Among multiple CRE-binding proteins, CRE-BP1 (also designated ATF-2) has two unique characteristics: it mediates the adenovirus E1A-induced transactivation and forms a heterodimer with c-Jun. Two structures, a putative metal finger and a leucine zipper, in CRE-BP1 are responsible for these capacities (2, 3). Identification of other proteins containing similar structure is useful to understand the mechanism of E1A- or other non DNA-binding proteins-induced transactivation. As a new member of a CRE-BP1 family that has similar metal finger and leucine zipper structures, we have isolated cDNA clones of CRE-BPa by cross-hybridizaion with CRE-BP1 cDNA. CRE-BPa protein consists of 508 amino acids and has a molecular weight of 56,840. CRE-BPa protein is highly homologous with CRE-BP1 in four regions: two of them are the regions containing the putative metal finger or the DNA-binding domain. Like CRE-BP1, CRE-BPa binds to CRE with higher affinity than to the TPA response element as a homodimer or a CRE-BPa/c-Jun or CRE-BPa/CRE-BP1 heterodimer.

Recently, we demonstrated that an interaction between CRE-binding protein(s) and c-Jun may be involved in the glucose-induced transactivation of the human insulin gene (4). It will be interesting whether CRE-BPa can mediate the transactivation by the non DNA-binding proteins such as E1A or Rb, or whether CRE-BPa is also involved in the glucose-dependent regulation of gene expression.

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B 373 A DNA-BINDING AND TRANS-ACTIVATING PROTEIN FROM *SACCHAROMYCES CEREVISIAE* RESEMBLING HUMAN E2F, Bernard Mai and Martin Lipp, Institut für Biochemie der Ludwig-Maximilians-Universität München, Am Klopferspitz 18a, D-8033 Martinsried, Federal Republic of Germany

The promoter of the human proto-oncogene MYC has been the first cellular target subject to regulation by the E2F transcription factor. E2F mediates basal transcription and transactivation by E1A of the human MYC promoter. E2F also has binding sites in a variety of other promoters regulated by cell proliferation and during the cell cycle. Since a variety of eukaryotic transcription factors are evolutionary highly conserved, we have analyzed the budding yeast *Saccharomyces cerevisiae* for the presence of an E2F-analogous protein. GAL1-based promoter constructs carrying the E2F binding site of the MYC or the adenovirus E2 promoter instead of the UAS element showed significant transcriptional activity in yeast cells. A DNA-binding factor of ≈12 kDa, designated YE2F, presumably mediating this activity was partially purified from yeast extracts specifically binding to the E2F consensus sequence. YE2F showed the same contact points within the MYC binding site as authentic E2F protein purified from mammalian cells. Furthermore yeast genomic Southerns, probed with an recently published human E2F-like cDNA, demonstrate the occurance of a homologous gene in the *S.c.* genome. The results suggest the existence of an E2F-analogous protein in the yeast *S. cerevisae*.

B 374 TRANSCRIPTIONAL REGULATION OF THE HUMAN S-ADENOSYLMETHIONINE DECARBOXYLASE PROMOTER BY FOS, JUN AND ANDROGEN RECEPTOR

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The proto-oncogenes c-fos and c-jun belong to the family of immediate-early responsive genes. Their protein products, Fos and Jun can form homo- and heterodimeric "leucine-zipper" transcriptional regulator (AP-1). AP-1 complex can interact with other proteins which do not belong to AP-1 protein families, like the glucocorticoid receptor, and will determine whether transcription of a given gene will be enhanced or repressed. The human S-adenosylmethionine decarboxylase (AdoMetDC) gene encodes a keyenzyme in polyamine biosynthesis. AdoMetDC protein and mRNA concentrations are regulated in a tissue-specific manner by multiple stimuli, such as steroid hormones. For example, AdoMetDC mRNA and protein levels are androgen-inducible in rodent accessory sex organs, with the maximum levels of mRNA expression being reached within 24-48 h of steroid administration. We have isolated and sequenced the 5'-flanking region of the human AdoMetDC gene and found the region for basal and induced transcription to contain juxtaposition of the AP-1 binding site (-66 to -58) and androgen responsive element (ARE) sites starting at position -63. Gel retardation assays were performed with a DNA fragment from the proximal promoter and with oligonucleotide containing the putative AP-1 site. Using both HeLa cell nuclear extracts and recombinant Fos and Jun proteins along with anti-Fos and anti-Jun antisera, we have shown specific binding of these two proteins, mainly as heterodimers, to the AP-1 site of the human AdoMetDC promoter. Using baculovirus expressed androgen receptor (AR), as well as specific AR peptide antibodies, we can detect AR-DNA specific complex formed with the hAdoMetDC oligonuclotide (-63 to-48). AR antibodies for N-terminal region stabilized dimer formation of AR and enhanced binding of AR to hAdoMetDC ARE site. In orther to further define the transcriptional regulation of the promoter, we prepared -96 and -45 promoter-CAT constructs and we are currently testing them in the transient expression in HepG2 and CV-1 cell lines. Juxtaposition of the AP-1 and ARE elements suggest the composite AP-1/ARE element. Further experiments are in progress to determine the interaction between AP-1 and AR, as transcriptional regulators of the hAdoMetDC promoter.

B 375 THE YEAST *SGC1* GENE ENCODES A NOVEL BASIC-HELIX-LOOP-HELIX PROTEIN INVOLVED IN TRANSCRIPTIONAL ACTIVATION OF THE YEAST ENOLASE GENES, Craig A. Martens, Chang Seo Park, Kayoko Nishi-Schnier and Michael J. Holland, Department of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616

The positive regulatory gene *GCR1* is required for high level expression of most yeast glycolytic genes. Stains carrying a *gcr1* null mutation are viable, but grow poorly in media containing glucose as carbon source. Transcription of the yeast enolase genes *ENO1* and *ENO2* is reduced approximately 50-fold in a *gcr1* strain. A dominant suppressor mutation, designated *SGC1-1*, was isolated that restored *ENO1* and *ENO2* transcription and growth in a medium containing glucose to near wild-type levels in a *gcr1* strain. Both the wild-type and dominant mutant *SGC1* alleles were cloned and sequenced. The deduced amino acid sequence of the SGC1 protein contains a region of strong similarity to the basic-helix-loop-helix motif present in the *myc* oncogene family as well as a number of other transcription factors. The *SGC1-1* mutant allele contains a single amino acid substitution within the basic helix region shown to be involved in DNA binding for several basic-helix-loop-helix proteins. Strains carrying an *sgc1* null mutation and a wild-type *GCR1* gene are viable, however, *ENO1* and *ENO2* expression was reduced approximately 5-fold showing that the *SGC1* gene product is involved in enolase gene expression. Like the *SGC1-1* mutant allele, multiple copies of the wild-type *SGC1* gene suppressed the growth and transcriptional defects caused by a *gcr1* null mutation. This latter observation suggests that the *SGC1-1* gene product is a more potent activator of enolase gene expression than the wild-type *SGC1* gene product. An epitope tag was introduced at the C-terminus of the *SGC1* coding sequences. Epitope tagged SGC1 protein was overproduced in yeast and purified to homogeneity. Gel mobility supershift analysis using a monoclonal antibody directed against the epitope tag showed that SGC1 protein binds to DNA sequences within the 5' regulatory regions of the *ENO1* and *ENO2* genes. The relationship between *GCR1*-dependent and *SGC1*-dependent activation of expression of the yeast enolase genes will be discussed.

B 376 INDUCTION MECHANISMS OF TH2 SPECIFIC LYMPHOKINES, INTERLEUKIN-4 (IL-4) AND INTERLEUKIN-5 (IL-5) GENES

Ikuo Matsuda[1,2], Akio Tsuboi[1], Hyun Jun Lee[2], Naoko Koyano-Nakagawa[2], Junji Nishida[3], Takashi Yokota[2], Naoko Arai[1] and Ken-ichi Arai[2] 1.Department of Molecular Biology, DNAX Research Institute, Palo Alto, CA U.S.A., 2.Department of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, Tokyo, JAPAN., 3.Jichi Medical School, Tochigi, JAPAN.

IL-4 and IL-5 are type 2 helper T cell (Th2)-specific cytokines. An 11 base-pair (bp) element (P element; 5'-$^{-79}$ CGAAAATTTCC-^{69}-3') is a PMA/calcium ionophore (A23187) responsive element in the 5' regulatory region of the human IL-4 gene[1]. The DNA binding of P element binding proteins [NF(P)] as well as the transcriptional activation via the P element requires the 3' half site of the P element (-TTTCC-). This 3' half is almost identical to the recognition consensus sequence of NF-κB. We compared several properties of NF(P) with those of NF-κB. The binding of NF(P) to the P element is stimulation-independent and was competitively inhibited by an excess amount of kB oligonucleotide from mouse Ig enhancer. P65 and/or Rel activated P element-dependent transcription when their cDNAs were cotransfected. Rabbit antisera against p50 did not inhibit the binding of NF(P) to the P element. By UV-crosslinking of NF(P) to the P element, we demonstrated that NF(P) contain at least two components, p150 (150K) and p80 (80K).
IL-5 promoter responds weakly to either PMA or cAMP signals and is markedly activated by combination of PMA and cAMP in EL-4 cells. Several prominent DNA binding motifs such as GATA-3, CLE1 and CLE0[2] whose sequence is shared between GM-CSF, IL-4 and IL-5 enhancer sequences, are found in the regulatory region of the mouse IL-5 gene. The mapping of cis-acting DNA element(s) which mediates the response to PMA/cAMP signals is in progress. 1. Abe et al. (1992) PNAS 89: 2864, 2. Miyatake et al. (1991) MCB 11: 5894

B 377 CELL-TYPE SPECIFICITY OF THE LAMININ B1 GENE PROMOTER. Takashi Matsui and Ryusuke Okano, Department of Mol. Biol., University of Occup. & Environ. Health, Japan

The laminin B1 gene is known to be induced at transcriptional level during differentiation of F9 cells. We have identified the core promoter of the murine laminin B1 gene. The core promoter lacked a TATA box, but was consisted of at least three regions between the cap sites and -100. The most distal region (-89 ~ -69) contained three GC boxes, the second region (-62 ~ -47) contained a direct repeat of TG(C/A)GCA motif, and the proximal region (-45 ~ -11) contained another direct repeat. The core promoter directed an efficient transcription in vitro with F9, but not with HeLa nuclear extracts. The cell type specific difference in the core promoter activity was also observed in vivo. β-RARE or TRE, which was placed upstream to the core promoter, activated the transcription in F9 cells, but not in HeLa cells. The protein binding analyses showed that an ubiquitous factor present both in F9 and HeLa cells binds to the TG(C/A)GCA repeat, an element of the core promoter. How the core promoter of the laminin B1 gene is inactivated in HeLa cells is underway.

B 378SEPARATION OF THE TRANSCRIPTIONAL ACTIVATION AND REPLICATION FUNCTIONS OF THE BOVINE PAPILLOMAVIRUS E2 PROTEIN. Alison A. McBride and Patricia Winokur, Laboratory of Tumor Virus Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Replication of bovine papillomavirus DNA requires both the viral E1 protein and the full-length E2 protein. The 48kD E2 protein is a site specific DNA binding protein that binds to several sites which lie adjacent to the origin of replication. The C-terminal domain contains the specific DNA binding and dimerization properties of the E2 protein and the N-terminal domain is required for transcriptional activation. Both domains are well conserved among the E2 proteins of different papillomaviruses. The functional domains are separated by an internal hinge region which is not conserved. A series of mutations were generated within the E2 protein that deleted various regions of the DNA binding domain, the transactivation domain and the internal hinge. Two mutated proteins that lack portions of the DNA binding domain were shown to support DNA replication but were unable to function as transcriptional transactivators. Conversely, two proteins containing large deletions of the hinge region were able to activate transcription but were defective for replication. Thus, the transactivation and replication properties of the E2 proteins are separable.

B 379 ARE INITIATOR ELEMENTS SIMPLY SEQUENCES PREFERRED BY RNA POLYMERASE II AS START SITES FOR TRANSCRIPTION? Janet E. Mertz, Richard Kraus, Nancy Zink, Elizabeth Murray, and Karla Loritz. McArdle Laboratory for Cancer Research, University of Wisconsin Madison, WI 53706.

The SV40 major late promoter (SV40-MLP) contains three genetically important sequence elements that control transcription at the major late initiation site at nt 325(+1). One maps approximately 30 bp upstream, one spans the initiation site itself, and one maps approximately 30 bp downstream of the +1 site(Ayer and Dynan Vol.8, M.C.B., 1988 p.2021.) Recently, we have shown that the upstream element, while not an obvious TATA box motif, functionally binds TFIID (Wiley et al.,P.N.A.S. Vol.89, 1992 p.5814). We have attempted to purify positive-acting factors that bind the genetically important initiator element of the SV40-MLP. Instead, we identified an initiator binding protein, "IBP", that represses initiation from the SV40 late promoter when template copy number is low. (Mertz et al.,these abstracts). Thus, the SV40-MLP initiator may not be recognized by a novel protein that functions in the formation of initiation complexes. Rather, it may simply be a sequence recognized by RNA polymerase II itself. To test this latter possibility, we performed a saturation mutagenesis of the SV40-MLP initiator. Each mutant was analyzed (i) *in vitro* with a HeLa cell nuclear extract transcription system and (ii) *in vivo* by transfection into monkey cells. With nuclear extract, base changes at -3 and -2 showed minimal effects on transcription initiation at +1. Base changes at -1 either lowered (T→A,T→G) or elevated (T→C) activity. All base changes from A at +1 significantly depressed activity. A T→G change at +2 resulted in a two fold increase, while other changes at this site exhibited minimal effects. Similar responses were seen *in vivo*. Our data are consistent with the hypothesis that initiator elements may simply be sequences preferred by RNA polymerase II as transcriptional start sites, with an optimal inr from -2 to +3 being one similar to TCAGT in sequence. Data from preliminary *in vitro* experiments performed with highly purified RNA polymerase II in the absence of other proteins provide additional support for this hypothesis.

B 380 STRUCTURE AND EXPRESSION OF THE MURINE IL-3 RECEPTOR α SUBUNIT GENE

Ikuko Miyajima, Takahiko Hara and Atsushi Miyajima. DNAX Research Institute, Palo Alto, CA94304

Interleukin 3 (IL-3) is a potent hematopoietic growth factor that stimulates various lineages of hematopoietic cells as well as very early hematopoietic progenitors. The high affinity murine IL-3 receptor is composed of two subunits, α and β. The α subunit binds IL-3 with low affinity and forms high affinity receptos with either of the two homologous β subunits that were initially designated as AIC2A and AIC2B. The α subunit is expressed in various hematopoietic cell lines including mast cells, myeloid progenitors, proB cell lines and very early progenitors, but is not expressed in T cells and fibroblasts. To further study the cell type specific expression of the IL-3 receptor subunits, we cloned the genomic gene for the α subunit. The exon-intron organization is similar to those of other members of the cytokine receptor super family. The transcription initiation site was mapped by primer extension and the 5' flanking region was found to contain TATA-like, CAT-like, GC-box, NF-kB, and GATA motifs. Promoter activity was detected in the 750 bp 5'-flanking sequence by using the luciferase gene as a reporter. We are currently identifying the sequence responsible for promoter activity and cell type specific expression.

B 381 CO-ORDINATE EXPRESSION OF A PROMOTER-BINDING FACTOR FOR THE PROGESTERONE-INDUCED UTEROGLOBIN GENE. C. Molloy[1], B.S. Chilton[2], J.C. Rayner[1], and D.W. Bullock[1], Centre for Molecular Biology, Lincoln University, Canterbury, New Zealand[1], and Department of Cell Biology and Anatomy, Texas Tech Health Sciences Center, Lubbock, TX 79430[2].

Progesterone-induced expression of the rabbit uteroglobin (UG) gene is accompanied by the appearance of a specific promoter-binding factor (UGPB) detected by gel-shift assays with UG200 (-194/+9). Heparin-purified UGPB gave partial DNase I-protection over an 82-bp region of the coding strand (-167/-85). Truncated oligonucleotides spanning this region indicated that the sequences -170/-100 and -154/-85 both gave a similar gel-shift to UG200. Southwestern blotting of UGPB using a UG200 probe revealed two proteins of MW 110K and 70K that were present in nuclei from progesterone-stimulated but not untreated rabbits. Probing Southwestern blots with different oligonucleotides suggested that p110 bound to upstream sequences and p70 to downstream sequences within the DNase I-protected region. A λgt11 expression library prepared from polyA$^+$ RNA from progesterone-stimulated endometrium was screened with UG200. One candidate clone detected a single mRNA species of approximately 5.2 kb on Northern blots of polyA$^+$ RNA from endometrium stimulated with progesterone but not from unstimulated uteri. Sequencing up to 2.3 kb of the cDNA has shown an open reading frame coding for a peptide with no matches in current database sequences. Thus progesterone appears to induce a promoter-binding factor(s) concomitant with its induction of UG expression in the uterus. [Supported by grants from the NZHRC to DWB and from NSF (INT-890019) and NIH (HD-00704) to BSC.]

B 382 ACTIVATION OF THE HUMAN c-fos PROMOTER BY THE HUMAN PAPILLOMAVIRUS EARLY GENES. Alexei Morosov (1), Min-Ho Lee (1), William C. Phelps (2) and Pradip Raychaudhuri (1). (1) Dept. of Biochemistry (M/C 536), University of Illinois, Box 6998, Chicago Il 60680; (2) Div. of Virology, Burroughs Wellcome Co. NC 27709.

The human papillomaviruses (HPVs) have been etiologically associated with of genital carcinomas, and over 80% of the human cervical cancers have been found to harbor HPV DNA. The cancer tissues, harboring HPV-DNA, regularly express E6 and E7 genes of the virus. In tissue culture, the E6 and the E7 genes cooperate to immortalize keratinocytes, the natural host cells of the papillomavirus. Recent studies suggest that part of the oncogenic properties of E6 and E7 involves inactivation of cellular growth suppressor proteins such as the retinoblastoma protein (Rb) and p53. E6 and E7 also possess transcriptional regulatory properties, and for E7, the transcriptional regulatory property has been linked to the interaction between E7 and Rb. The proto-oncogene c-fos is transcriptionally activated by a variety of growth signals as well as viral oncogenes. Overexpression of the c-fos gene product results in cell transformation. We find that the papillomavirus E6 and E7 genes can activate transcription from the c-fos promoter. This activation by E6 and E7 depends on DNA sequences between -99 and -53 in the c-fos promoter. We are in the process of determining the DNA element(s), within that region of the c-fos promoter, responsible for the activation by E6 and E7.

B 383 A UNIQUE TRANSCRIPTIONAL ACTIVATION MOTIF RESTRICTED TO A CLASS OF HELIX-LOOP-HELIX PROTEINS IS FUNCTIONALLY CONSERVED IN BOTH YEAST AND HUMANS, *Cornelis Murre, Melanie W. Quong, Mark E. Massari, and Ronald Zwart, Department of Biology, 0016 and *Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093

The helix-loop-helix (HLH) proteins are a growing family of proteins that contain a conserved structural motif (HLH) which mediates DNA binding and dimerization[1-4]. These proteins play a major role in the control of cell-type differentiation[1-4]. One particular class of HLH proteins (Class I) are closely related (70-90% identity in the HLH region), are ubiquitously expressed, and include as its members: E12[1-4,9], E47[1-4,5,9], E2-2[5], HEB[8], and daughterless[6,7]. E12 and E47 are encoded by the same gene (E2A), but arise through differential splicing[10]. The E2A gene products are involved in B cell, muscle, and pancreatic specific gene expression[11-14]. The E2A gene is translocated in acute lymphoblastoid leukemias (ALL). In pro-B ALL, containing a t (17;19) translocation, the E2A N-terminal region is fused to a leucine zipper like domain[16]. In pre-B ALL, containing a t (1;19) translocation, the E2A N-terminal domain is fused to a homebox-like domain[15]. The involvement of the E2A N-terminal domain in two translocation events both resulting in leukemia is puzzling and suggests an important function for this portion of the protein. We report here the identification of two conserved boxes in the E2A N-terminal domain, termed box 1 and box 2, that have extensive identity within the transactivation domains of E12, E47, E2-2, HEB, and daughterless. We show that together, both boxes are crucial for transactivation and have the potential to form a new motif, that of a loop linked to an amphipathic helix, designated the LH motif. We propose that the LH motif represents a novel transactivation domain, distinct from those containing acidic, proline-rich and glutamine-rich activation motifs and is restricted to a class of HLH activators.

B 384 AP-1 MODULATORY ACTIVITY IN THE CYTOPLASM Yoshikuni Nagamine, Janet S. Lee and Birgitta Kiefer
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In the regulation of urokinase-type plasminogen activator (uPA) gene expression in LLC-PK_1 cells, an AP-1-like site located at 2 kb upstream of the transcription initiation site plays a pivotal role. The site is essential and sufficient to mediate the inductive effect of cytoskeletal reorganization, okadaic acid and TPA. They activate the uPA gene through distinct signaling pathways but converge on the same AP-1-like site. The sequence of the AP-1-like site , ATGAGGTCAT, is similar but not identical to the consensus AP-1 binding sequence, TGAGTCA, and is well conserved in human, pig and mouse uPA genes. AP-1-like site is recognized by homo- or dimer of c-Jun/c-Fos families. c-Jun is the main transcription factor that interacts with the AP-1-like site of the uPA gene in LLC-PK_1 cells and is modulated differently by different inducers. As c-Jun is a nuclear protein, there should be communication between the cytoplasm and nucleus during the activation of c-Jun by these inducers. In the course of the study of cytoplasm-nucleus communication in DNA mobility shift assays, we found that the cytoplasm contains a factor that can enhance AP-1 binding activity in the nucleus. Enhancing activity was not seen when an oligo to which LFB3 binds was used as a probe, suggesting that the factor is specific to AP-1. The enhancing activity was abrogated when the cytoplasm was incubated at 60 °C for 10 min, suggesting that the enhancing activity is exerted by a proteinous factor. It is not that a nuclear factor activated the cytoplasmic, latent AP-1 complexed with an inhibitor, because Western blot analysis showed no signal of c-Jun and c-Fos in the cytoplasm.

B 385 PROMOTER ANALYSIS OF AN AUXIN-REGULATED GENE IN TRANSGENIC PLANTS. Ronald T. Nagao, Robert E. Wyatt, Virginia H. Goekjian, W. Michael Ainley, Timothy W. Conner and Joe L. Key. Botany Department, University of Georgia, Athens, GA 30602.

The application of auxin causes a rapid increase in the expression of specific sets of genes. Auxin-responsive genes from soybean (Aux22 and Aux28) and their corresponding homologs from *Arabidopsis* (AtAux2-11 and AtAux2-27) have been isolated. In order to better understand auxin-regulated gene expression in a homologous system, promoter deletions of the *Arabidopsis* AtAux2-11 gene (approximately -3.0, -1.5, -0.6, -0.5, and -0.4 kb) were fused to the β-galactosidase (LacZ) reporter gene and transformed into *Arabidopsis*. The five promoter deletions tested conferred tissue-specific expression patterns correlated with processes associated with auxin action and were active at several developmental stages of S_2 transgenic plants, from seedling to flowering. The similar expression pattern of large scale promoter deletion constructs (-3.0, -1.5, and -0.6 kb) analyzed in transformed *Arabidopsis* suggests that relevant regulatory sequences of the AtAux2-11 gene are located within 0.6 kb of 5' flanking sequence. To more precisely locate these relevant regulatory sequences, 70 bp 5' linker scan deletions and 70 bp fragment replacement mutations from approximately -600 to -57 were constructed with LacZ as the reporter gene and transformed into *Arabidopsis*. Analyses of transgenic plants containing 5' deletion constructs showed that deletions downstream of -337 drastically reduced activity. Fragment replacement analyses showed that two regions (-603 to -548 and -407 to -338) appear to be necessary for any promoter activity, while replacement of two other regions (-267 to -198 and -127 to -57) appeared to have little or no effect on activity. Disruptions of the other 70 bp regions of the AtAux2-11 promoter had intermediate effects on reporter gene expression. These analyses suggest that the 70 bp replacement regions may contain multiple functional motifs and that multiple 70 bp regions of the promoter are necessary for auxin-inducible expression.

B 386 CONTROL OF *E. COLI* LYSYL-tRNA SYNTHETASE EXPRESSION BY LEUCINE-RESPONSIVE REGULATORY PROTEIN. Yoshikazu Nakamura, Koichi Ito and Koichi Kawakami, Institute of Medical Science, University of Tokyo, P.O. Takanawa, Tokyo 108, Japan

Lysyl-tRNA synthetases of *E. coli* are synthesized from two distinct genes, *lysS* and *lysU*, which are regulated differentially. *lysS* is expressed constitutively, while *lysU* is normally silent and is induced by heat shock and several metabolites such as L-leucine. To investigate the mechanism of differential regulation and the physiological significance of these two genes, we have constructed a null mutation of *lysS* which causes cold-sensitive lethality, and used it to acquire and characterize several bypass mutations called *als* (abandonment of *lysS*). Cold-resistant survivors all caused derepression of *lysU* transcription. Two *als* mutations which were linked to *lysU* contained IS2 insertions upstream of the *lysU* promoter. Consistently, deletion mutations upstream of the promoter also restored growth of *lysS1*. These results suggest that transcription of *lysU* is negatively controlled by a cis-regulatory element located upstream of the promoter. All *lysU*-unlinked *als* mutations mapped in the *lrp* gene which encodes the leucine-responsive regulatory protein (Lrp). Lrp is a bifunctional transcription factor which activates or represses several genes involved in branched chain amino acid metabolism. Therefore, *lysU* is part of the leucine regulon and is negatively controlled by Lrp. Structural and functional features of LRP will be presented.

B 387 ANGIOGENIC FACTOR AND RAS REGULATION OF HUMAN CYCLOOXYGENASE-2 GENE TRANSCRIPTION. Karen Neilson, Susan Appleby, Javier Barriocanal and Timothy Hla, Department of Molecular Biology, Holland Laboratory, American Red Cross, Rockville, MD 20855

Cyclooxygenase catalyzes the rate-limiting step in the synthesis of inflammatory prostaglandins. Both forms of cyclooxygenase, hCox-1 and hCox-2, are expressed by human umbilical vein endothelial cells (HUVEC). However, only the level of hCox-2 mRNA is greatly increased in the presence of phorbol myristic acetate (PMA), an inducer of HUVEC differentiation and fibroblast growth factor-1 (FGF-1), a potent angiogenic factor. In quiescent rat embryo fibroblasts (REF), the hCox-2 mRNA is not detected by Northern blot analysis on total RNA preparations. However, in REF cell lines stably-transfected with the oncogenic H-ras gene, hCox-2 mRNA is expressed. Furthermore, the hCox-2 mRNA levels are induced strongly if the cells are treated with PMA. In contrast, in revertant REF cells that are expressing the Adenovirus Ela oncogene, the PMA response is blunted. These studies suggest that the transcription of the hCox-2 gene is stimulated by PMA, FGF and oncogenic ras and is inhibited by Ela. To understand the transcriptional regulation of the hCox-2 gene, we have isolated and sequenced the 5-flanking region of the gene. Primer extension analysis suggests that the hCox-2 gene possesses two transcription start sites. Sequence homology searches of the 0.8 kb, 5' flanking region revealed several regulatory regions including motifs similar to AP-2, CRE, SP1, NFkB and NFIL6/CREB. Interestingly, the known PMA responsive element AP-1 was not found. Transfection of NIH 3T3 cells with the chloramphenicol acetyl transferase (CAT) reporter gene under the control of 0.8 kb of hCox-2 5'-flanking region resulted in the elaboration of CAT activity which was induced by PMA, suggesting that the PMA-responsive elements are present in the construct. Since cyclooxygenase plays a key role in the synthesis of prostaglandins, understanding the transcriptional regulation of the cyclooxygenase gene will lead to further insight into the inflammatory processes including angiogenesis.

B 388 ICSBP A MEMBER OF THE IRF FAMILY SUPPRESSES IFN-INDUCED GENE TRANSCRIPTION Nancy Nelson, Michael S. Marks, Paul H. Driggers, Keiko Ozato, Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md. 20892

ICSBP is a member of the interferon response factor (IRF) family that binds to the interferon (IFN) response elements of several IFN-regulated genes as well as the virus inducible elements of IFN genes (Driggers et al, 1990). To study a functional role of ICSBP, transient co-transfection experiments have been performed using an ICSBP expression plasmid and IFN-responsive reporter genes in human embryonal carcinoma cell line, N-Tera2. These cells were shown not to express ICSBP or IRF-2, thus allowing functional analysis of transfected cDNAs. Co-transfection of ICSBP into cells treated with retinoic acid (RA) or any of the IFNs repressed expression of CAT reporter driven by the major histocompatibility complex (MHC) class I gene promoter. Similarly, ICSBP repressed expression of CAT reporters driven by the ISREs of the 2'-5' oligoadenylate synthetase, guanylate binding protein and ISG-15 genes in IFN-treated cells. Deletion analysis showed that the putative N-terminal DNA binding domain of ICSBP by itself is capable of mediating the repression. Using the same co-transfection conditions as ICSBP, a similar repression of these reporters was observed with IRF-2. Finally, ICSBP repressed the IRF-1 mediated induction of MHC class I and IFN-β reporters in the absence of IFN or RA. Gel shift analyses are currently being carried out to elucidate the mechanism of the repression.

B 389 STRUCTURE OF THE PROMOTER REGIONS OF THE V1.1 AND V1.2 GAMMA TCR GENES Itai Novick and Yacob Weinstein, Dept. of Microbiology and Immunology, Faculty of health sciences, Ben-gurion University, Beer-sheeba, Israel.

T lymphocytes recognize Antigen via the T cell receptor (TCR), which is a heterodimer formed of $\alpha\beta$ or $\gamma\delta$ chains. Each TCR chain is composed of a variable and a constant regions. We previously have shown that IL3 dependant lines express transcripts of different γTCR isotypes, originating from the embryonic unrearranged γTCR loci. Moreover, we found that IL3 regulates the expression of the γTCR genes in these lines. In order to further study this regulation, we decided to characterize the V1.1 and V1.2 promoters, which are only 2.9 Kb apart in opposite transcriptional orientations.

We sequenced the 2.9 Kb region between the V1.1 and V1.2 genes. No substantial homologies to consensus promoter elements were found in the region upstream to the V1.1 startsite. However, an octamer a kE2 and a MAT a1/α2 consensus sites were found -730 to -470 upstream to the ATG site of the V1.2 gene. Comparison of the two regions upstream of the Vγ genes, showed *diverging* homology: almost all of the 550 bp region upstream to the V1.1 is contained in the 1.7 Kb upstream to the V1.2 gene, but this region also contains two insertions between the homologous regions. In the sequenced region, we also found an Alu-like B1 mouse repetitive element and a 150 bp region homologous to the Vγ exon. These findings suggest a common origin for the two genes with evolutionary changes that occurred recently in the regulatory units. The V1.1 and V1.2 genes share over 95% similarity in their coding regions. In contrast, The large sequence difference that was found between these genes upstream to the ATG, suggests different transcriptional regulation for each gene.

Using S1 nuclease mapping assay, we found in EL4 cells multiple initiation sites for the V1.2 gene, located between 162 and 306 bp from ATG site. Inserts cloned from the upstream region of the V1.2 gene showed only minor promoter activity in a CAT assay.

B 390 MECHANISM OF REGULATED GENE EXPRESSION BY THE HUMAN T-CELL LEUKEMIA VIRUS TRANS-ACTIVATOR PROTEIN TAX. Jennifer K. Nyborg, Audrey A. Franklin, Martine N. Uittenbogaard, Allison P. Armstrong, Mark F. Kubik, Anne M. Brauweiler. Department of Biochemistry, Colorado State University, Fort Collins, CO 80523

Human T-cell leukemia virus type 1 (HTLV-1) was the first identified human retrovirus. HTLV-I is associated with a variety of clinical syndromes, including adult T-cell leukemia (ATL), tropical spastic paraparesis (a demyelinating neurological disease) and a form of rheumatoid arthritis.

The HTLV-I encoded transcriptional activator protein Tax is strongly implicated in the pathogenesis attributed to HTLV-1. Tax is essential to the life cycle of the virus, and has been shown to increase the rate of HTLV-I transcription. It is well established that three imperfect 21 bp repeat enhancer elements, located in the transcriptional control region of HTLV-I, are necessary for Tax dependent transcriptional activation. Tax does not bind to these DNA sequences directly, but appears to interact with cellular transcription factors that recognize these sites.

Research in our laboratory and others have shown that the cellular factors that bind the HTLV-I 21 bp repeats are members of the CREB/ATF protein family. CREB and ATF-2 are major components of a highly purified preparation of T-cell proteins that bind the 21 bp repeats. Purified recombinant ATF-2 and CREB specifically bind each of the three 21 bp repeat elements, and activate transcription of the HTLV-I promoter dependent on these sequences. Tax further stimulates RNA synthesis in the presence of CREB and ATF-2. These studies suggest that ATF-2 and CREB likely play a central role in regulating HTLV-I gene expression, and that they may mediate transcriptional activation by Tax.

We have used *in vitro* DNA binding experiments to identify the interaction between Tax and ATF-2 and CREB. Using Tax purified to near homogeneity from baculovirus or E. coli, we have shown that Tax causes a dramatic increase in the site-specific DNA binding activity of both CREB and ATF-2 to the 21 bp repeats of HTLV-I. Tax affects the DNA binding activity of native ATF-2 and CREB purified to homogeneity from cultured human cells, as well as with the recombinant proteins expressed and purified from E. coli. Significantly, Tax appears to be highly pleiotropic, affecting the DNA binding activity of many cellular transcription factors, consistent with the observation that Tax transactivates a wide range of cellular genes *in vivo*.

B 391 SPECIFIC RECOGNITION OF NUCLEOTIDES BY HISTONE H1 AND THE CHROMATOSOME, Jouko Oikarinen, Natalja Yli-Mäyry, Tatu Tarkka and Riitta-Maaria Mannermaa, Collagen Research Unit, Biocenter and Department of Medical Biochemistry, University of Oulu, Kajaanintie 52A, SF-90220 Oulu, Finland

We have reported previously that histone H1 is capable of binding nucleotides. We present here evidence using labeling with [^{14}C]acetyl-CoA and 8-azido-[^{32}P]ATP that the ability to bind nucleotides in a specific manner is a characteristic of histone H1 among the histone proteins; phosphate analogs such as AlF_4^- counteract the labeling of histone H1 while they do not compete for that of histones H2A, H2B, H3 and H4 or bovine serum albumin. When using 8-azido-[^{32}P]ATP, nucleotides appeared to compete for the labeling of histone H1 in a similar manner to that of heat shock protein-90 (hsp90), an ATP/GTP-binding protein with autophosphorylation activity

The site of nucleotide interaction was located in histone H1 using radioactive labeling with nucleotide analogs or [^{14}C]glucose and endoproteinase Glu-C digestion. The experiments pinpointed a Gly-rich region that displays homology with protein kinases, and as synthetic polypeptides corresponding to this region were capable of binding nucleotides in a specific manner, the histone H1 nucleotide binding site may be considered similar to those in protein kinases.

Nucleoside triphosphate display higher affinity towards histone H1 when it is part of the chromatosome than in the absence of the nucleosome. Nucleotide binding specificity of histone H1 complexed with the nucleosome appeared to be similar to casein kinase II (CKIIα). In the presence of the reconstituted nucleosome the k_D values for ATP and GTP ranged from 0.4 to 30 μM depending on the presence of Mg^{2+}. The capability of nucleotide binding is thus a characteristic of the chromatosome, and may provide a mechanism for the regulation of eukaryotic gene expression.

B 392 **TAT-ACTIVATED HIV-1 TRANSCRIPTION IS REPRESSED BY LBP-1, Camilo A. Parada, Jong-Bok Yoon and Robert, G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021**

HIV-1 Tat strongly activates the HIV-1 promoter by binding to the transactivation response element (TAR) located at the 5' end of all HIV-1 mRNAs (between +14 to +44). Although several observations suggest that Tat functions at the level of initiation and elongation, little is know about the mechanisms by which Tat function is regulated.

Our laboratory has previously shown that the cellular DNA binding protein LBP-1 represses HIV-1 transcription by preventing the binding of TFIID to the promoter (H.Kato *et al.* (1991) Science 251, 1476-1479) and a human cDNA encoding LBP-1 activity has been isolated (J-B. Yoon, G. Li and R.G. Roeder). We also have developed a HeLa cell-derived *in vitro* system in which recombinant Tat protein activates elongation of HIV-1 transcripts 10-fold, in conjunction with TFIIF (H.Kato *et al.* (1992) Genes & Dev. 6, 655-666). Here we have analyzed the effect of recombinant LBP-1 on the *in vitro* activation of HIV-1 transcription by Tat. We found that the addition of LBP-1 specifically inhibits HIV-1 transcription under conditions where Tat stimulates elongation. Since the preinitiation complex was allowed to form before the addition of LBP-1 (a condition that blocks LBP-1 repression) this result suggested that LBP-1 was inhibiting the Tat response by an alternative mechanism. To study how LBP-1 was preventing Tat function we developed a "pulse-chase" assay that involves the isolation of preinitiation complexes by gel filtration, followed by incubation under selected conditions that would allow transcription either to position 13 ("pulse") or 600 ("chase"). We found that during the "chase" RNA pol II pauses (with a half-life of 1 min) just downstream of the TAR sequences. This suggested a mechanism in which TAR is formed and then bound by Tat with cellular factors, thus allowing the Tat effect on elongation. We studied the effect of LBP-1 on the pausing and found that addition of LBP-1 during the "pulse" did not block formation of the 13-mer, but that during the "chase" it strongly inhibited elongation of the transcripts such that the TAR RNA structure could not be synthesized.

Our results strongly suggest that LBP-1 may play a crucial role in the repression of HIV-1 gene expression under certain condition since it has the potential to repress both initiation and elongation of HIV-1 transcripts.

B 393 THE MECHANISM BY WHICH THE HUMAN APO-LIPOPROTEIN B GENE REDUCER OPERATES INVOLVES BLOCKING OF TRANSCRIPTIONAL ACTIVATION BY HEPATOCYTE NUCLEAR FACTOR 3. *Bernhard Paulweber, *Friedrich Sandhofer, and #Beatriz Levy-Wilson. *First Department of Internal Medicine, General Hospital of Salzburg, 5020 Salzburg, Austria, #Gladstone Institute of Cardiovascular Disease, and Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94141-9100.

Recently we demonstrated that a DNA fragment extending from -3067 to -2734 of the human apolipoprotein B gene reduces transcriptional activity of the apo B promoter segment containing sequences from -139 to +121 by about 10-fold in cultured colon carcinoma cells (CaCo-2), but not in cultured hepatoma cells (HepG2). We postulated that this reducer operates by a mechanism involving active repression of a transcriptional activator that binds to the segment (-111 to -88) of the apo B promoter (B.Paulweber and B.Levy-Wilson, J.Biol.Chem. 266: 24161-24168,1991). We now report that a 24 base pair sequence from -2801 to -2778 of the apo B gene containing a binding site for the negative regulatory protein ARP-1 is essential and sufficient for the reducer effect. Furthermore, we demonstrate that the transcription factor HNF-3α binds to the sequence 5'-TGTTTGCTTTTC-3' (-95 to -106) of the apo B promoter, to stimulate transcription. Transcriptional activity of a CAT construct containing HNF-3 binding sites upstream of a minimal apo B promoter fragment (-30 to +121) is repressed, when the reducer sequence is inserted immediately upsteam of the HNF-3 binding sites, suggesting a mechanism by which the reducer-bound protein(s) block(s) activation promoted by HNF-3. From the results of cotransfection experiments in which ARP-1 was overexpressed in the absence of its binding site, we conclude that ARP-1 interacts either directly or via a meditator protein with proteins recognizing the HNF-3 site and that this interaction is sufficient to repress transcriptional activation by HNF-3. Since transcriptional activation promoted by Sp1 is not perturbed by the reducer, a direct interaction between the reducer-bound protein(s) and basic components of the transcriptional machinery is unlikely.

B 394 TRANSCRIPTIONAL REGULATION OF HIV

Matija Peterlin, Alicia Alonso, Andreas Baur, Subir Ghosh, Xiaobin Lu and Ying Luo, Howard Hughes Medical Institute, Departments of Medicine, Microbiology, and Immunology, University of California, San Francisco, San Francisco, CA 94143-0724.

Long terminal repeats (LTRs) of HIV-1 and 2 are transcriptionally activated following T cell or macrophage activation, growth, and proliferation. Cellular transacting factors that participate in this response include nuclear factors κB (NF-κB) and of activated T cells (NF-AT) in HIV-1 and NF-κB and AP-3 in HIV-2. Serine/threonine and tyrosine phosphatases modulate cytoplasmic to nuclear translocation and activities of these regulatory proteins. Following activation, HIV LTRs are transactivated by their respective transactivators (Tats). Tat interacts with an RNA stem-loop called TAR, which is located 3' to the site of initiation of viral transcription. For optimal interactions between Tat and the bulge in TAR RNA, cellular TAR RNA-binding proteins are required. The defect in rodent cells that leads to low levels of transactivation by Tat and that is complemented by human chromosome 12 maps to these TAR RNA-binding proteins. Tat modifies an unstable to a stable (i.e. elongation competent, processive) transcription complex. Orther DNA-binding proteins, i.e. NF-κB, SP-1, and LBP-1, are responsible for efficient loading of RNA polymerase II at the HIV TATA box. By fusing Tat to the MS2 coat protein, a prokaryotic RNA binding protein, and replacing TAR with the MS2 operator, activation and RNA binding domains of Tat were mapped. Besides determining differences in modes of action of Tat presented by RNA and DNA, we also constructed a minimal functional lentiviral Tat of only 25 amino acids. Here, 15 and 10 aa consitute activation and RNA-binding domains, respectively. This minimal Tat was subjected to structural studies. Mixing and matching different strains of the HIV LTR and Tat, i.e. constructing chimeric targets and effectors, revealed structural features of these LTRs and Tats that might explain distinct clinical courses of HIV-1 and HIV-2 infections. Based upon these observations, we developed a very sensitive test of viral burden and replication in infected individuals.

B 395 MOLECULAR ANALYSES OF MOUSE CELL LINES WITH HEAT SHOCK NON-RESPONSIVE HSP70 GENES,

Benette Phillips and Jacek Gorzowski, Dept. of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL 60611

Transcriptional activation of the hsp70 gene in response to heat shock, a nearly universal response, fails to occur in mouse erythroleukemia (MEL) cells, although gel retardation assays using an oligomer corresponding to the heat shock element (HSE) of the mouse hsp70 promoter indicate activation of heat shock transcription factor (HSF) in these cells. We are using genomic footprinting analyses to distinguish between two hypotheses: 1) HSF does not stimulate hsp70 transcription because it is unable to bind to the HSE in the endogenous hsp70 promoter, 2) HSF binds to the hsp70 promoter but fails to activate transcription. Our results support the first hypothesis. The hsp70 gene in the mouse plasmacytoma line MPC-11 has also been reported to be refractory to activation by heat shock. We have detected an HSE-binding activity in extracts of heat shocked MPC-11 cells as well. The stability of HSF-HSE complexes formed using extracts of MEL and MPC-11 cells is comparable to that of complexes formed in extracts of cells with activatable hsp70 genes. Surprisingly, the hsp86 gene, which is coordinately induced with hsp70 in mouse lines exhibiting a conventional heat shock response, is transcriptionally activated in both MEL and MPC-11 cells. We are currently trying to understand the basis for the differential responsiveness to heat shock of these two genes in MEL and MPC-11 cells.

B 396 A NUCLEAR PROTEIN WHICH PREFERENTIALLY BINDS THE HIV-1 PROMOTER WHEN THE DNA IS METHYLATED,

Karen Pratt and Peteranne Joel, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405

Loss of AIDS latency requires transcription of integrated HIV-1 proviral DNA. Transcription is directed by promoter elements in the LTR. It has been shown that DNA methylation in the promoters of many genes represses transcription in vivo and that DNA methylation of HIV LTR is correlated with HIV-1 latency. Our studies indicate that a novel protein, found in nuclear extracts of several mammalian cell lines, binds to specific methylated DNA sites in the HIV-1 promoter. We can distinguish between this protein and three other mammalian proteins so far discovered which preferentially bind methylated DNA sequences on the basis of binding site competition, molecular weight, binding conditions, and sequence of the binding site. HMBP (HIV-1 Methyl Binding Protein) binds to a subset of sequences to which the general transcription factor Sp1 binds. Binding by Sp1 has been shown to activate the expression of many genes. HMBP is not Sp1 nor a modified Sp1 protein since the Sp1 consensus binding site does not compete with the HMBP binding site for HMBP binding and since HMBP can easily be separated from Sp1 by column chromotography. Considering the role that DNA methylation plays in gene repression, HMBP may mediate repression of genes under the control of the HIV-1 LTR promoter in vivo thus mediating AIDS latency. Furthermore, HMBP may repress expression of the many other genes which contain these Sp1 sites in their promoters including some oncogenes.

B 397 CREB IS DIRECTLY INVOLVED IN BOTH BASAL EXPRESSION AND HORMONE-MEDIATED INDUCTION OF THE PEPCK GENE,

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Transcription of the PEPCK gene is induced by cAMP and glucocorticoids. To investigate possible interactions between CREB, glucocorticoid response unit (GRU) binding proteins, and other trans-acting factors, block mutations were introduced into well defined factor-binding sites and WT or mutant plasmids were transfected into H4IIEC rat hepatoma cells -/+ a PKA catalytic subunit expression vector. PKA or DEX induced PEPCK-CAT activity by 7- or 5-fold, respectively, and PKA+DEX mediated a 22-fold induction. The effects of PKA and DEX, separately or together, were significantly decreased in CREmut and ΔGRU ($P<0.005$), suggesting functional interaction between these pathways. Mutation of other factor-binding sites located between the CRE and the GRU also decreased induction by DEX. However, induction by PKA+DEX was reduced only by mutation of the IRS within AF2 of the GRU. The DNA binding domain of GAL4 was substituted for that of CREB (CRG) and the CRE was replaced with a GAL4 binding site (G4PEPCK-CAT). In the absence of CRG, G4PEPCK-CAT behaved the same as CREmut. CRG elevated basal activity and restored responsiveness to PKA. Induction by DEX and DEX+PKA increased in proportion to basal activity. Therefore, CREB (CRG) clearly contributes to the level of transcription activation, but it does not appear to mediate the interaction produced by DEX+PKA. Concomitant treatment with CRG plus PKA and PKA inhibitor peptide, or mutation of the PKA phosphorylation site were equivalent to CRG alone, indicating that CREB possesses a basal activation capacity that does not require phosphorylation for its expression.

B 398 MUTAGENS ENHANCE THE HIV-1, INTERLEUKIN-1 (IL-1), AND INTERLEUKIN-6 (IL-6) EXPRESSION THROUGH TRANSCRIPTIONAL OR ,POST-TRANSCRIPTIONAL MECHANISMS, Ileana Quinto, Massimo Mallardo, Maria R. Ruocco, Francesca Baldassarre, Vincenzo Giordano and Giuseppe Scala, Department of Biochemistry and Medical Biotechnology, 2nd Medical School, University of Naples, Naples, Italy.

Mutagens can affect the transcription of viral and cellular genes. This effect seems to be limited to a number of genes and requires cellular cofactors. The expression of HIV-1, the causative agent of AIDS, and of IL-1 and IL-6 was studied in human lymphocytes and monocytes after treatment with the *alkylating* agents mitomycin C, methyl methanesulfonate, and ethyl methanesulfonate. The expression of HIV-1 was monitored by measuring the CAT activity following the transfection of the cat gene under the trascriptional control of wild type or mutants HIV-1 LTR. The mutagen treatment significantly enhanced the CAT activity in the transfected cells. The effect was abolished by the deletion of the κB sites in the HIV-1 LTR, or it was halved by the deletion of Sp1 or TAR regions. An increased κB DNA binding activity was revealed in nuclear extracts from mutagen-treated cells by gel retardation assay. The expression of IL-1 and IL-6 genes was studied in primary human monocytes by northern blotting analysis of total RNA, or by run on assay in isolated nuclei. The DNA alkylating agents enhanced the expression of IL-1 α, IL-1 β and IL-6 genes at 30 min after treatment, reaching the maximum effect at 3 hours. This enhancement was not due to transcriptional activation of the interleukin genes since it was not observed in run-on transcription assays. By using the actinomycin D transcriptional inhibitor, it was shown that mutagens acted by stabilizing the interleukin mRNAs. Moreover, the effect was not abolished by cycloheximide pretreatment, thus indicating that "ex novo" synthesized cellular factors were not required to stabilize the interleukin transcripts. These data indicate that mutagens may affect the transcription of viral or cellular genes by activating both cellular transacting factors or cellular factors stabilizing specific mRNAs. This work was supported by CNR, AIRC and V Research Project on AIDS.

B 399 SOMATOSTATIN AND EPINEPHRINE INHIBIT INSULIN GENE TRANSCRIPTION IN THE HIT CELL, **J. Bruce Redmon, Howard C. Towle, and R. Paul Robertson, Diabetes Center and Division of Endocrinology and Metabolism, Department of Medicine and Department of Biochemistry, University of Minnesota Medical School, Minneapolis, MN 55455**

We recently observed in the HIT cell, a clonal insulin-secreting cell line, that somatostatin and epinephrine lower insulin mRNA levels and intracellular insulin content in addition to the well-recognized effect of these hormones to inhibit insulin secretion. To determine whether these inhibitory hormones regulate insulin synthesis at the level of insulin gene transcription, we transfected HIT cells with a reporter gene consisting of 327 base pairs of the 5'-flanking region of the human insulin gene linked to the bacterial gene chloramphenicol acetyl transferase (CAT). This insulin-CAT construct was responsive to glucose in a dose-dependent manner, and increased nearly three-fold as the glucose concentration increased from 0.4 to 11.0 mM. When HIT cells were cultured for 48 hours in the presence of somatostatin (10^{-6} M) or epinephrine ($5x10^{-5}$ M), HIT cell expression of the insulin-CAT reporter gene was inhibited to 66 ± 10% (p=0.03) and 54 ± 5% (p=0.002) of control, respectively. In dose-response experiments, somatostatin and epinephrine inhibited HIT cell expression of the insulin-CAT gene in a dose-dependent manner which paralleled dose-dependent inhibition of insulin secretion. These studies suggest that the effect of somatostatin and epinephrine to lower insulin mRNA levels is at least in part mediated by an inhibitory effect on insulin gene transcription. Thus, inhibition of insulin synthesis may be coupled to inhibition of insulin secretion via common regulatory mechanisms within the ß cell. Such a coupling may allow the ß cell to match insulin supply to secretory demand.

B 400 IDENTIFYING P53 TRANSACTIVATION TARGETS USING SUBTRACTIVE HYBRIDIZATION, Valerie Reinke and Guillermina Lozano, Department of Molecular Genetics, M.D. Anderson Cancer Center, Houston , TX 77030

The function of the p53 protein as a tumor suppressor is dependent on its function as a transcription factor. This is indicated by various p53 mutants which, unable to activate transcription, are also unable to suppress transformation. To understand the process by which p53 exerts its growth regulation on the cell, it is necessary to identify cellular targets of p53 transactivation. The focus of this project is to isolate p53-transactivated genes by subtractive hybridization using a specific cell line, A1-5's. The A1-5 cell line is a rat embryo fibroblast cell line transformed with *ras* and a mutant p53 allele that is temperature sensitive. At 32°C, the A1-5 cells behave as if the p53 were wild type; that is, the cells stop growing. At 39°C, p53 is in a mutant conformation and the cells grow very well. The p53 in this cell line is also temperature sensitive for transactivation. This was tested using a previously identified target of p53 transactivation: p53 itself. We have shown that p53 positively regulates its own promoter. By transfecting the p53 promoter fused upstream of the luciferase reporter gene into A1-5's, I have shown temperature sensitive transactivation by this mutant p53. At 32°C, the transfected p53 promoter is upregulated, whereas it is not at 39°C. This indicates that the cellular targets of p53 transactivation will be present at 32°C and not at 39°C. Using subtractive hybridization between the two temperatures is an excellent method for isolating these targets. Identification of targets will aid in elucidation of the cascade of events leading to tumor suppression by p53.

B 401 METALLOTHIONEIN GENE REGULATION: ROLE OF METAL-RESPONSIVE ELEMENTS AND INTRACELLULAR THIOLS IN BASAL AND HEME-HEMOPEXIN-INDUCED METALLOTHIONEIN EXPRESSION. Yafei Ren, Richard D. Palmiter* and Ann. Smith, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64110, *Howard Hughes Medical Institute Laboratory, University of Washington, Seattle, WA 98195.

Hemopexin (HPX) binds heme and delivers it to cells by receptor-mediated endocytosis. Hemopexin-mediated heme transport in mouse hepatoma (Hepa) cells stimulates the expression of metallothionein (MT-1) primarily at the level of initiation of transcription. MT mRNA is also extensively induced by cobalt-protoporphyrin (CoPP)-hemopexin which binds to the hemopexin receptor without intracellular uptake of CoPP. Moreover, inhibition of protein synthesis by cycloheximide augments the heme-hemopexin-mediated accumulation of MT-1 mRNA. The research presented here is designed to further delineate the mechanism of heme-hemopexin-mediated MT gene regulation. Transient transfection was used to define the regions of the mouse MT-1 promoter required for heme-hemopexin-mediated regulation. The potential role of intracellular signalling pathway(s) involving reactive oxygen species as intermediates in basal and induced MT gene expression was also investigated. Our results show transcriptional activation of metallothionein gene expression by heme-hemopexin requires at least 600 base pairs (-600 to +64) of 5'-flanking region of MT-1 promoter and is independent of the metal responsive elements of the MT-1 promoter. Both the basal and induced MT mRNA levels are decreased and abrogated, respectively, by the antioxidant and radical scavenger N-acetyl-L-cysteine (NAC), providing evidence that intracellular thiol and/or reactive oxygen radicals are involved in the transcriptional activation of MT gene by heme-hemopexin. NAC also prevents the CoPP-hemopexin-mediated induction of MT gene expression, showing that occupation of the hemopexin receptor is a step in a pathway to generate or trigger a series of events that generate free radicals. In addition, the phorbol ester 12-myristate 13-acetate (PMA), an activator of protein kinase C, causes a dramatic increase in MT mRNA level when incubated with Hepa cells and simultaneous addition of NAC abolishes this induction. The mechanism of these effects is currently under investigation. (Supported by USPHS grant, DK-37463)

B 402 MECHANISM OF METHYLATION-MEDIATED TRANSCRIPTIONAL SUPPRESSION OF THE MURINE α1(I) COLLAGEN GENE, Katherine Rhodes, Richard A. Rippe, Akihiro Umezawa, Michael Nehls, David A. Brenner, and Michael Breindl, Department of Biology, San Diego State University, San Diego, CA 92182 and Department of Medicine, University of California, San Diego, CA 92161

The molecular mechanisms by which DNA methylation may affect transcription of the murine α1(I) collagen gene have recently been under investigation in our laboratories. We have previously shown that methylation of a region located in the first exon of the α1(I) gene, but not of the promoter itself or of regulatory elements in the first intron is inversely correlated with transcription of the gene. Gel shift assays with methylated and unmethylated oligonucleotides have shown that none of the trans-acting factors so far identified as binding to the promoter region and first exon are sensitive to DNA methylation. Since comparative footprint and gel shift assays with extracts from collagen-producing and non-producing cells indicate that the factors interacting with the regulatory elements in the promoter, first exon and first intron are ubiquitous factors - factors present also in non-producing cells - either the critical factor(s) has not yet been identified or other mechanisms are involved. One alternative mechanism is suggested by transfection experiments using methylated and unmethylated α1(I) promoter-reporter gene constructs which showed that transcriptional activity of the promoter is inhibited by methylation in murine cell lines, but not in Drosophila cells. Because invertebrate DNA is not methylated and the Drosophila cells therefore presumably do not contain methyl-CpG-binding proteins, this finding suggests that the methylation-dependent transcriptional suppression is mediated by methyl CpG-binding proteins. Thus, methylation of this region may lead to transcriptional suppression of the α1(I) promoter by an indirect mechanism, e.g., an alteration of the local chromatin structure induced by methyl-C-binding proteins that silences the gene by rendering the promoter inaccessible to trans-acting factors. The observation that a DNase-hypersensitive site in the α1(I) promoter region is present only in collagen-expressing cells further supports this alternative. We are presently testing this hypothesis with co-transfection and oocyte-injection experiments using unspecific, CpG rich, highly methylated competitor oligonucleotides.

B 403 SUPERINDUCTION OF CYTOKINE GENE TRANSCRIPTION BY CIPROFLOXACIN,

Kristian Riesbeck, Erik Högbom, and Arne Forsgren, Department of Medical Microbiology, Lund University, Malmö General Hospital, S-214 01 Malmö and Immunology Unit, Department of Medical Microbiology, Lund University, Sölvegatan 21, S-223 62 Lund, Sweden.

Certain antibiotics act as biological response modifiers as a complement to their antibacterial function. Ciprofloxacin, a fluorinated 4-quinolone frequently used in clinical practice, upregulates interleukin 2 (IL-2) and gamma-interferon production in phytohemagglutinin (PHA) stimulated peripheral blood lymphocytes. The accumulation of cytokines is due to increased mRNA levels. However, the precise mechanism by which ciprofloxacin performs its action in eucaryotic cells is poorly understood.

To investigate whether other cytokine mRNAs than IL-2 and gamma-interferon were affected by ciprofloxacin, RNA was isolated from PHA stimulated lymphocytes and Northern blots were performed. Ciprofloxacin increased mRNA levels for IL-1α, IL-2 and its receptor, gamma-interferon, IL-3, IL-4, granulocyte-macrophage colony-stimulation factor, tumor necrosis factor α, and lymphotoxin in PHA stimulated peripheral blood lymphocytes. In contrast, IL-6 mRNA was unaffected. The level of nuclear mRNA precursors was also increased in ciprofloxacin treated cells. In addition, Jurkat cells were transfected with a plasmid containing the IL-2 promoter and enhancer region linked to the chloramphenicol acetyltransferase (CAT) reporter gene. Analysis of CAT activity revealed that ciprofloxacin enhanced IL-2 gene induction. Our results imply a regulatory pathway in common for these cytokines, which is influenced by ciprofloxacin.

B 404 BRAIN-PREFERENTIAL EXPRESSION OF THE HUMAN HPRT GENE: AN INTERPLAY OF POSITIVE AND NEGATIVE REGULATORY ELEMENTS, Diego E. Rincon-Limas[1], Robert S. Geske[2], Felipe Amaya-Manzanares[1], Juan-Juan Xue[3], Chung Y. Hsu[3], Paul A. Overbeek[1,4], and Pragna I. Patel[1], [1]Institute for Molecular Genetics, [2]Center for Comparative Medicine, [3]Division of Restorative Neurology and Human Neurobiology, and [4]Dept. of Cell Biology and Howard Hughes Medical Institute, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

Total deficiency of hypoxanthine phosphoribosyltransferase (HPRT) in humans causes the neurological disorder, Lesch-Nyhan syndrome. Partial deficiency is associated with a severe form of gouthy arthritis. Although HPRT is constitutively expressed at basal levels in all tissues, expression occurs at several-fold higher levels in the brain, the relevance and control of which is unknown.

To investigate the molecular mechanisms responsible for the tissue-differential expression, we generated transgenic mice carrying different sequences of the hHPRT promotor fused to the bacterial *lacZ* gene. We found that the hHPRT core promotor element (-233 to -122) is apparently not sufficient for constitutive expression. Additionally, sequences between -1681 and -122 directed high levels of beta-galactosidase expression to several areas of the forebrain. RT-PCR and *in situ* hybridization studies have shown that expression of the endogenous HPRT gene closely follows that of the reporter gene. We identified a 182 bp element (hHPRT-NE) which is involved not only in repressing transgene expression in non-neuronal tissues but also in conferring neuronal specificity. Trans-acting factors which interact with this regulatory element, both brain-specific and ubiquitous, have been identified. We also found that hHPRT-NE is one of at least two sequences required to direct prominent beta-galactosidase expression to the central nervous system. These studies provide the first molecular insights into increased HPRT expression in the brain.

B 405 COOPERATION AMONG MULTIPLE SIGNAL TRANSDUCTION PATHWAYS INDUCING GENE EXPRESSION VIA A COMMON ENHANCER ELEMENT. K. D. Rodland, P. Lenormand, D. Pribnow, and B. E. Magun. Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland OR 97201.

Transcriptionally active members of the VL30 family of defective murine retroviruses display many of the characteristics of immediate early genes, including rapid transcriptional induction in response to a variety of proliferative and transforming stimuli. A single transcriptionally active VL30 gene transferred into Rat-1 fibroblasts by pseudovirion-mediated transfection is transcriptionally induced following stimulation with either epidermal growth factor, endothelin-1, protein kinase C-activating phorbol esters, dibutyryl cAMP, or elevated intracellular calcium. Genetic analysis indicates that a triple repeat element within the VL30 long terminal repeat is capable of functioning as a classic enhancer element in conjunction with heterologous promoters linked to a chloramphenicol acetyl transferase reporter gene. Full induction of gene expression mediated by this VL30 triple repeat required concurrent stimulation of multiple signal transduction pathways, as exemplified by the dual elevation of intracellular calcium and diacylglycerol levels following endothelin-1 treatment. Simultaneous treatment with thapsigargin, an agent which stimulates the release of intracellular calcium, and either EGF, dibutyryl cAMP, or phorbol esters produced a synergistic increase in gene expression from the VL30 triple repeat. An additional 100% increase in gene expression was observed following treatment with the protein phosphatase inhibitor okadaic acid. Mutational analysis of the VL30 triple repeat indicated that a novel response element encompassing about 15 base pairs was required for the calcium-dependent induction of gene expression in response to either EGF, cAMP, or activation of protein kinase C. Mutations in this region also abrogated the binding of nuclear proteins to the VL30 triple repeat, as measured in gel mobility shift assays. Gel mobility shift experiments with *in vitro* translated FOS/JUN proteins indicated that none of these proteins was capable of specific binding to the response element present in the VL30 triple repeat. Furthermore, complexes between the VL30 enhancer and proteins present in Rat-1 nuclear extracts were not competed by a large molar excess of oligonucleotides representing consensus binding sites for AP-1, CREB/ATF, and E4F/E4BP4. The mutational analysis and gel shift experiments indicate that expression from the VL30 triple repeat in response to multiple signal transduction pathways is mediated by a common novel response element.

B 406 **INTERACTIVE EFFECTS OF FOS WITH THE AH RECEPTOR IN WILD-TYPE MOUSE HEPATOMA CELLS,** **J.C. Rowlands, X. Wang and S.H. Safe,** Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas, 77843-4466

Mouse hepatoma Hepa 1c1c7 cells have been extensively used as models for investigating the molecular mechanism of induction of CYP1A1 gene transcription by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. Recently, several reports have demonstrated that the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) can inhibit the aryl hydrocarbon (Ah) receptor-mediated transcriptional activation of the Hepa 1c1c7 CYP1A1 gene by TCDD. To further investigate this inhibition we cotransfected Hepa 1c1c7 cells with a mouse *c-fos* expression vector and a chloramphenicol acetyl transferase (CAT) reporter plasmid that contains a consensus dioxin responsive element (DRE). In cells transfected with reporter plasmid alone, TCDD caused a 3.2 fold increase in CAT activity compared to solvent-treated control cells. The CAT activities in cells cotransfected with the TCDD-inducible plasmid and 5, 10 and 20μg of the *c-fos* expression plasmid were 1.5, 0.8 and 0.2 fold respectively, compared to the control cells. Incubation of nuclear liganded Ah receptor complex from these cells with ^{32}P-labeled DRE in a gel shift assay gave a retarded band associated with the receptor-DRE complex. In cells transfected with the c-fos expression plasmid there were differences in the receptor-DRE complex levels and when these nuclear extracts were incubated with an anti-fos antibody this significantly altered the gel shift profiles. This data indicates that overexpression of fos down-regulates the Ah receptor-mediated *trans*-activation of transcription from CYP1A1 gene regulatory elements and the significance of this interaction will be discussed.

B 407 DIFFERENT NF1-LIKE BINDING SITES DIRECT EITHER NEGATIVE OR POSITIVE REGULATORY EFFECTS, René J. Roy, Steeve Leclerc, *Winnie Eskild, *Vidar Hansson and Sylvain L. Guérin, Molecular Endocrinology Laboratory, Laval University Medical Center, Québec, Canada, G1V 4G2 and *Institute of Molecular Biochemistry, University of Oslow, 0317 Oslow 3, Norway.

We demonstrated that the presence of 2 homologous negative regulatory elements are involved in the cell-specifique repression of the rat growth hormone gene (rGH) in non-expressing cells. Those elements, which contain an almost perfect consensus DNA-binding site for the transcriptional activator Nuclear Factor 1 (NF1), have been refered to the rGH silencer-1 and silencer-2 sequences. We also identified similar NF1 target sequences in the promoter region of the human Cellular Retinol Binding Protein-1 (hCRBP1). These elements were designated as Fp1 and Fp5. Our results demonstrate that the four elements (NF1-like binding sites from both rGH silencer-1 and -2 and hCRBP1 Fp1 and Fp5) bind a common rat liver nuclear factor (NF1-L) which reacted to an antiserum raised against the DNA-binding domain of the the rat liver form of NF1. Surprisingly, when double-stranded oligonucleotides of similar lengths containing each individual element where ligated upstream from the basal promoter of the p12 gene, different activities were obtained upon transient transfections. While the NF1-L binding site from both rGH silencer-1 and -2 repressed CAT gene expression (up to 33-fold), the homologous hCRBP1 Fp5 element had nearly no effect and the Fp1 element strongly activated (up to 38-fold) the expression of that same reporter gene. These results strongly suggested that little differences in the target sequence bound by a common nuclear protein can probably induce conformational changes that will ultimately switch a negative regulatory activity to a positive one.

B 408 ACTIVATION OF HEAT SHOCK GENE TRANSCRIPTION BY HSF1 INVOLVES OLIGOMERIZATION, ACQUISITION OF DNA BINDING ABILITY, AND NUCLEAR LOCALIZATION AND CAN OCCUR IN THE ABSENCE OF STRESS, Kevin D. Sarge, Shawn P. Murphy, and Richard I. Morimoto, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Il, 60208.

The existence of multiple heat shock transcription factor (HSF) genes in higher eukaryotes has prompted questions regarding the functions of these HSF family members, especially with respect to their roles in the stress-induced transcription of heat shock genes. To address these questions, we have used rabbit polyclonal antisera raised against mouse HSF1 and HSF2 to examine the biochemical, physical, and functional properties of these two factors in both unstressed and stressed mouse and human cells. We have identified HSF1 as the mediator of stress-induced heat shock gene transcription. HSF1 displays stress-induced DNA binding activity, oligomerization, and nuclear localization, while HSF2 does not. Also, HSF1 undergoes phosphorylation in cells exposed to heat or cadmium sulfate, but not in cells treated with the amino acid analogue L-azetidine-2-carboxylic acid, indicating that phosphorylation of HSF1 is not essential for its activation. Interestingly, HSF1 and HSF2 overexpressed in transfected 3T3 cells both display constitutive DNA binding activity, oligomerization, and transcriptional activity. In addition, HSF1 overexpressed in transfected cells is constitutively localized to the nucleus in a pattern identical to that found in stressed cells. These results demonstrate that HSF1 can be activated in the absence of physiological stress, and also provide support for a model of regulation of HSF1 and HSF2 activity by a titratable negative regulatory factor.

B 409 C/EBPβ AND MYC/MAX HETERODIMERS BOTH SPECIFICALLY RECOGNIZE THE EFII ENHANCER SEQUENCE IN THE ROUS SARCOMA VIRUS LTR, Rosalie C. Sears[1] and Linda Sealy[1,2], [1]Dept. of Cell Biology, [2]Dept. of Molecular Physiology, Vanderbilt University, Nashville, TN 37232

We have identified three protein complexes in chick embryo fibroblasts, called EFIIa, EFIIb and EFIIc, which bind to a 38 bp sequence in the 5' end of the RSV LTR enhancer extending from nucleotides -229 to -192, which we refer to as the EFII *cis* element. This *cis* element contains two near direct repeats which we have shown are not equivalent for either transcriptional activity in vivo, or factor recognition in vitro. The upstream 14 bp repeat is both necessary and sufficient for strong enhancer activity and high affinity recognition by EFIIa, EFIIb and EFIIc. The upstream repeat contains a C/EBP consensus binding site which is recognized by C/EBPβ. Using nuclear extracts from various cell types it appears that both the long form of C/EBPβ (LAP) and the short form (LIP) bind the EFII *cis* element, depending on the ratio of LAP/LIP expression in the cell type tested. We have also observed, in collaboration with Steve Hann[1], that the transcriptional activity of the EFII *cis* element is repressed by v-Myc expression and activated by c-Myc-1 expression. Using recombinant proteins we have demonstrated that Myc/Max heterodimers specifically bind the EFII *cis* element. Furthermore, the upstream 14 bp repeat in the EFII *cis* element appears to be sufficient for repression and activation by v-Myc and c-Myc-1 in vivo, and Myc/Max recognition in vitro.

B 410 C-JUN RECRUITS CYTOSOLIC PROTEINS OF T LYMPHOMA CELLS INTO NFAT-1 LIKE FACTOR COMPLEXES, Edgar Serfling, Christof Schomberg, Isolde Pfeuffer, Elke Schorr and Sergei Chuvpilo, Institute of Virology and Immunobiology, University of Würzburg, Versbacher Str. 7, D-87 Würzburg, Germany.

Cyclosporin A (CsA) and FK506 are potent immunosuppressives which block T cell activation by the selective inhibition of the inducible transcription of lymphokine genes. One prominent target of CsA and FK506 is NFAT-1, an inducible and lymphoid-specific DNA binding factor which is involved in the transcriptional induction of Interleukin 2 (Il-2) and Interleukin 4 (Il-4) genes. Within the promoter/enhancer regions of both genes, NFAT-1 binds to the so-called purine box motifs which share conserved motifs for the binding of Ets-like factors and closely linked, non-canonical AP-1 binding sites. It has recently be shown that in Jurkat T lymphoma cells NFAT-1 may consist of (at least) two components, a ubiquitous nuclear and a lymphoid-specific cytosolic component. We show here that NFAT-1 contains AP-1- and Ets-like proteins since (i) proteins of these factor groups, such as c-Jun and Ets-2, bind to the purine boxes, (ii) canonical AP-1 and Ets binding sites specifically compete for the binding of NFAT-1 to the purine boxes, (iii) the activity of purine boxes is dramatically transactivated in a CsA-dependent manner by co-transfection with cDNAs coding for AP-1 (JunB and FosB) proteins, and (iv) bacterially synthesized c-Jun is able to form NFAT-1-like factor complexes in mixing experiments using cytosolic proteins from Jurkat T lymphoma cells. The ability of c-Jun to recruit cytoplasmic, Ets-like proteins into NFAT-1-like factor complexes which specifically bind to the purine box motifs of the Il-2 and Il-4 control regions demonstrates that c-Jun (or other members of Jun family) interact with Ets-like, lymphoid-specific proteins in the generation of the CsA-sensitive factor NFAT-1.

B 411 ZINC FINGER FUNCTION IN TRANSCRIPTION FACTOR IIIA, David R. Setzer*, Samuel Del Rio*, Martha B. Rollins+, Angela L. Galey+, Sandra R. Menezes*, and Matthew T. Andrews+, *Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH 44106, and +Department of Genetics, North Carolina State University, Raleigh, NC 27695

We have analyzed a collection of TFIIIA mutants containing histidine-to-asparagine substitutions in one of the Zn^{++} ligands of individual zinc fingers. These mutations result in structural disruption of the mutated finger with little or no effect on other fingers. The energetic contribution of each finger to the overall binding energy of the TFIIIA-5S RNA gene interaction has been assessed by measuring the equilibrium binding constants of each mutant protein with the 5S RNA gene. The results indicate that each finger contributes to the DNA-binding energy, but to widely varying extents; furthermore, the binding of various fingers is energetically interdependent, in that loss of binding by one finger can be partially compensated by increases in the affinity of interactions elsewhere in the complex. The sites of individual zinc finger interaction with the DNA template have been approximated by identifying sites at which protection from nuclease digestion is lost when a single zinc finger is mutated. The results suggest a more complex mode of binding than has been proposed previously. Analysis of mutant proteins in in vitro transcription assays indicates that the transcriptional activity of TFIIIA is independent of its DNA-binding affinity in binary complexes, even when this affinity is reduced 25-fold relative to wild-type. Surprisingly, mutations in any one of the three C-terminal zinc fingers of TFIIIA result in reduction or loss of transcriptional activity, a result that implicates these three fingers in higher order interactions in complete transcription complexes. Analysis of *Xenopus* embryos expressing mutant forms of TFIIIA reveals that disruption of zinc finger 6 leads to hyperactivation of 5S RNA genes at levels well in excess of the level of activation conferred by wild-type TFIIIA. This surprising property can be reproduced in vitro if transcription is carried out in the presence of 5S RNA, suggesting that feedback regulation of 5S RNA synthesis occurs in vivo, and that disruption of zinc finger 6 results in a form of TFIIIA that exhibits reduced susceptibility to feedback inhibition by 5S RNA.

B 412 ANALYSIS OF cis-ELEMENTS OF THE CHO APRT PROMOTER, Bin-Ru She and Milton W.Taylor, Department of Biology, Indiana university, Bloomington, IN47401

The APRT gene encodes adenine phosphoribosyltransferase which catalyzes the condensation of adenine and PRPP to form AMP. The 5' region of the CHO aprt gene is 60% G+C without TATA or CAAT boxes. Transcription of the gene initiates at multiple sites with the most distal site located 80 nucleotides upstream of the translation initiation codon. One of the transcription initiation sites, denoted as +1, is 64 nucleotides upstream of the ATG codon. Based on the results of mutation analysis 3 functional cis-elements of the promoter have been defined. Element I is located in the -101 to -53 region. Elements II and III are located in the -33 to +19 and +56 to +85 regions respectively. Linker-scan mutations in element II decrease gene expression to 14-50 %. Deletion of the entire region reduces gene expression to 33%. Transcription of this deletion mutant starts at novel sites (-80 and downstream). As the deletion is further extended upstream or downstream, bringing element I and element III closer, gene expression increases accordingly. When elements I and III are adjoined, the gene expression is restored to 100%. Element I combined with element III is sufficient to carry out transcription when fused to a promoterless CAT gene, while niether element I nor III alone is sufficient . Element I contains 2 adjacent SP1 binding sites and a putative SV40 T-Ag binding site. The linker-scan mutations in element I, including mutations on Sp1 or SV40 T-Ag binding sites, have little affect on the gene expression. However deletion of entire element I reduces gene expression to 40 %. It is probable that the sequences within this element possess redundant function. Transcription initiation sites in the absence of the intervening sequence between elements I and III, including element II, are found from -80 downstream. In the absence of the intervening sequence the most upstream initiation site is at -16. Hence element II could modify the interaction between element I and element III and alter the transcription initiation sites, or element II may contain its own initiator and be able to drive transcription alone. The later possibility is being tested by fusing element II alone to a promoterless CAT gene and checking its ability to drive transcription. In this work we identify 3 functional cis-elements of CHO APRT promoter. Some of them may have redundant function or one element may become functionally cryptic in the presence of the other elements.

B 413 **TRP-185 AND TAR DEPENDENT TRANS-ACTIVATION BY THE HIV-1 TAT PROTEIN**

Christian T. Sheline, Lawrence H. Milocco, and Katherine A. Jones, The Salk Institute, La Jolla, CA 92037

Transcriptional activation by the HIV-1 Tat protein requires specific residues in the hexanucleotide loop and trinucleotide bulge of the TAR RNA stem-loop structure found in the 5'-untranslated leader of all viral transcripts. Tat directly contacts residue U^{22} in the bulge, and is thought to act in concert with cellular factors bound to the loop. We find that Hela nuclear extracts contain two specific TAR RNA-binding proteins, designated TRP-1 and TRP-2, which compete for binding to the upper portion of the TAR hairpin. Analysis of point mutants in TAR RNA reveals that TRP-1 contacts residues in the loop which are important for trans-activation, whereas TRP-2 contacts the bulge, including the same residue (U^{22}) that is required for the Tat-TAR interaction. Glycerol gradient sedimentation and UV cross-linking experiments indicate that TRP-1 is a large heteromeric complex containing a 185 kDa RNA-binding protein, whereas TRP-2 activity derives from a family of 110-70 kDa proteins. Interestingly, both TRP-1 and TRP-2 promote TAR-dependent transcription *in vitro* in the presence of Tat, even though mixing experiments indicate that each of the three proteins must bind independently to TAR RNA. These findings suggest that the TAR element is recognized by two different nuclear RNA-binding proteins that affect transcriptional regulation by Tat. *In vitro* transcriptions show that Tat basic domain mutants and TAR bulge mutants retain near wildtype levels of trans-activation, but large Tat truncations or TAR loop mutants are not able to transactivate. This suggests that the loop of TAR and regions in the N-terminus of Tat are the most crucial determinants at least for *in vitro* transactivation. The loop binding protein TRP-185 (TRP-1) appears to have some interesting properties which will be discussed.

B 414 THE INITIATOR FOR THE HUMAN TGFα PROMOTER, Tae Ho Shin and Jeffrey E. Kudlow, Departments of Cell Biology and Medicine, University of Alabama at Birmingham, Birmingham, AL 35294

The promoter for the human transforming growth factor-alpha (TGFα) gene does not contain a canonical TATA box, yet transcription occurs predominantly at unique position. To identify the promoter elements important for the proper transcription initiation, we have introduced various mutations near the natural CAP site of the gene. Transient transfection assays indicate that the sequences immediately upstream and downstream of the CAP site are required for the accurate basal level transcription. In a number of viral and cellular promoters, the element called initiator (Inr) has been found to be important for the CAP site selection. As an analogy, the newly-found element was named the TGFα Inr. By electrophoretic mobility shift assay, at least seven protein-DNA complexes are detectable in the TGFα Inr. The low-mobility complexes can be disrupted either with high salt or the TATA box oligonucleotide suggesting that these complexes contain multiple proteins. A family of TGFα Inr-binding proteins (TGFα IBPs) are present in both MDA468 and HeLa nuclear extracts. However, TFII-I, which functions through the adenovirus major late promoter (AdMLP) Inr, appears to be distinct from the TGFα IBPs since the protein-DNA complexes are not competed with the AdMLP Inr sequence. The TGFα IBPs may assist the formation of preinitiation complex at the TATA-less TGFα promoter.

B 415 REGULATION AND FUNCTION OF THE HUMAN T-CELL ENHANCER BINDING PROTEIN GATA3

Virginia M. Smith, Joseph Marine, Perry Lee and Astar Winoto. Department of Molecular and Cell Biology, University of California-Berkeley, Berkeley, CA 94720

Expression of the T-cell receptor chain genes (α, β, γ, and δ) during T cell development is regulated by their respective promoters and enhancers, and by the proteins which bind to them. We have studied a common TCR enhancer element (A/T)GATA(G/A), and the ability of Gata3 to bind to and regulate expression through this sequence. Although the GATA consensus sequence was present in all four TCR enhancers, in-vitro transcribed and translated Gata3 bound only to the α, β and δ enhancers. This binding was consistent with the activity of each GATA TCR enhancer element in transient transfection assays.

To study the role of Gata3 in T cell development, 60 kb of both mouse and human Gata3 genomic sequence have been cloned. Seven regions of homology were found outside the coding region between the mouse and human Gata3 genes. The human Gata3 promoter has been isolated and characterized. However, the promoter alone does not confer the restricted pattern of expression of Gata3. Current work is focused on identifying the role of these conserved regions (enhancers and/or silencers) in the regulation of Gata3.

A direct role for Gata3 in T cell development is being examined by creating a dominant negative mutation. In doing so, we have identified two regions outside the DNA binding domain required for human Gata3 activity. These putative activation domains are being further characterized.

B 416 CHARACTERIZATION OF THE MECHANISMS USED BY THE HUMAN CYTOMEGALOVIRUS IE2 86 KDA PROTEIN TO ACTIVATE EARLY GENE TRANSCRIPTION, Marvin Sommer, Ruth Schwartz, Kevin Klucher, James Kadonaga, and Deborah H. Spector, Dept. of Biology, Univ. of Calif., San Diego, La Jolla, CA 92093-0116.

To define mechanistically how the human cytomegalovirus (HCMV) major immediate early (IE) proteins induce early gene transcription, the IE1 72 kDa protein, the IE2 55 kDa protein, and the IE2 86 kDa protein were analyzed for their ability to activate transcription from an HCMV early promoter *in vivo* and *in vitro*. In transient expression assays in U373MG astrocytoma/glioblastoma and HeLa cells, only the IE2 86 kDa protein was able to activate the HCMV early promoter to high levels. Stimulation of this promoter required not only the TATAA box but also sequences located between 58 and 160 nucleotides upstream of the start site. Removal of 37 aa from the carboxy terminus of the IE2 86 kDa protein or an in frame internal deletion of aa 291 to 369 abrogated its ability to function as an activator, but an internal deletion of aa 136 to 291 had only a modest effect on its stimulatory activity. The results of the *in vitro* transcription experiments correlated well with those obtained *in vivo* with respect to requirements for *cis*-acting sequences and *trans*-acting factors. The basal activity of the promoter was minimal in both the HeLa and U373MG extracts, but was stimulated 6 to 10-fold by the IE2 86 kDa protein. With a histone H1-deficient extract from *Drosophila* embryos, the HCMV early promoter was quite active and was stimulated 2- to 4-fold by the IE2 86 kDa protein. Addition of histone H1 significantly repressed basal transcription from this promoter. However, the IE2 86 kDa protein, but none of the other IE proteins, was able to counteract the H1-mediated repression and stimulate transcription at least 10 to 20-fold. By itself the IE2 86 kDa protein binds only weakly to the HCMV early promoter, suggesting that protein-protein interactions are essential for IE-mediated transcriptional activation. In this regard, we have found that the IE2 86 kDa protein binds efficiently to TFIID and weakly to TFIIB. However, analysis of TFIID binding by various mutants of the IE2 86 kDa protein suggests that this interaction is not sufficient for transactivation. To further investigate the requirements for activation by the IE2 protein, we performed experiments with partially purified general transcription factors. The basal activity of the HCMV early promoter was high in these extracts, but the IE2 86 kDa protein could not further stimulate this activity nor could it counteract the H1-mediated repression. These results coupled with our finding that a minimal HCMV early promoter containing only a TATAA box is non-responsive to the IE2 protein suggest that upstream sequences as well as factors that do not copurify with the general factors are needed for the activation and antirepression functions of the IE2 86 kDa protein.

B 417 IDENTIFICATION OF A NEGATIVE REGULATORY ELEMENT LOCATED IN A M-BCR INTRON, Mark J. Stewart, Gregory Cox, Ann Reifel-Miller, and David Leibowitz. Department of Medicine, Indiana University, Indianapolis, IN 46202.

The Philadelphia (Ph) chromosome translocation activates the ABL proto-oncogene by fusing it with sequence from the BCR gene on chromosome 22. The BCR/ABL fusion gene which results is transcribed from the normal BCR promoter. In chronic myelogenous leukemia (CML) the chromosome 22 breakpoints fall within introns between 3 small exons of the BCR gene located in a region termed the breakpoint cluster region (M-bcr). A 427 bp AvaI fragment from the M-bcr contains binding sites for a number of sequence-specific DNA binding proteins. Transfection analysis indicates that this fragment markedly depresses transcription (15-fold) from the BCR promoter in K562 (CML) cells. Assays in a variety of other cell lines have shown that this suppression is lineage specific, since fibroblast and lymphoid lines do not exhibit the same effect. Gel-mobility shift assays demonstrate a different pattern with nuclear extracts from the different cell lines, correlating with the differences in transcriptional suppression. DNase I footprinting and methylation intereference assays have localized the binding activities to three specific regions within the 427 bp fragment. Analysis with clones containing mutations in two of the regions, which eliminate factor binding, indicate that both of these regions are required, but not sufficient to see potent transcriptional suppression of the BCR promoter. Further evidence that this fragment functions as a transcriptional suppressor is shown by its ability to inhibit transcription from the SV40 early promoter. The presence of lineage-specific transcriptional regulatory sequences within the M-bcr provides a mechanism by which the location of the breakpoint in an individual patient could be related to the course of CML, and defines a new area of BCR promoter regulation.

B 418 **Multiple Interacting Muscle-Type-Specific Positive and Negative Enhancer Elements Regulate Temporal and Tissue-Specific Expression of the Tropomyosin I Gene in *Drosophila*,** Robert V. Storti, Linda Gremke, Pat C. W. Lord, Leah Sabacan and Shu-Chun Lin, Dept. of Biochemistry, University of Illinois college of Medicine, Chicago, Il 60612

Muscle development involves the coordinated transcriptional regulation of muscle-type specific proteins during myogenesis. In *Drosophila* there are two phase of myogenesis (embryonic and pupal) and two phases of transcriptional control in which different muscle is synthesized *de novo*. We show here that transcription regulation of the *Drosophila* tropomyosin I (*TmI*) gene during both phases of myogenesis is under the control of a muscle enhancer region located within the first intron of the gene. This enhancer region consists of a complex set of multiple muscle-type specific positive and negative cis-acting enhancer elements which together contribute towards full expression of the gene. One of these enhancer regions is contained within a 355 bp fragment and is sufficient to direct high levels of temporally regulated expression from an heterologous promoter during both stages of myogenesis and in all muscle of transgenic flies. Dissection of this enhancer region into smaller fragments has allowed us to identify a positive acting cis-element(s) for expression in visceral and most somatic muscle of the embryo/larva and adult and an enhancer for adult IFM and TDT muscles. Furthermore, we show that the element(s) directing expression in both embryonic/larval and adult visceral and somatic muscle (non IFM and TDT) can be repressed through a negative control region, suggesting that regulation of expression in these muscles is under dual control during both phases of myogenesis. We propose a model in which transcriptional regulation of the *Drosophila* tropomyosin genes is controlled by cooperative interaction among multiple muscle-type specific positive and negative cis-acting regulatory elements

B 419 EFFICIENT TRANSCRIPTION OF A U1 snRNA GENE IN VITRO REQUIRES TBP AND TWO PROXIMAL CIS-ACTING ELEMENTS WITH STRINGENT SPACING REQUIREMENTS, William E. Stumph, Zulkeflie Zamrod and Yuru Song, Department of Chemistry and Molecular Biology Institute, San Diego State University, San Diego, CA 92182.

The promoter of a Drosophila U1 snRNA gene has been functionally analyzed in cell-free extracts prepared from 0-12 hour embryos. Two promoter elements essential for efficient initiation of transcription by RNA polymerase II were identified. The first, termed PSEA, is located between positions -41 and -61 relative to the transcription start site, is crucial for promoter activity, and is the dominant element for specifying the transcription start site. PSEA thus appears to be functionally equivalent to the Proximal Sequence Element (PSE) present upstream of vertebrate snRNA genes. The second element, termed PSEB, is located at positions -25 to -32, and is required for an efficient level of transcription initiation, as mutation of PSEB, or alteration of the spacing between PSEA and PSEB, severely reduced transcriptional activity relative to the wild type promoter. Although the PSEB sequence does not share any obvious sequence similarity to a TATA box, conversion of PSEB to a canonical TATA sequence increased the efficiency of the U1 promoter 4 to 5 fold and simultaneously relieved the requirement for the upstream PSEA. Despite these effects, introduction of the TATA sequence into the U1 promoter had no effect on the choice of start site nor on the RNA polymerase II specificity of the promoter. Finally, antibody inhibition studies indicate that the TATA box binding protein (TBP) is required for transcription from the wild type U1 promoter as well as from the TATA-containing U1 promoter.

B 420 THE LEU3 PROTEIN: A METABOLITE-DEPENDENT TRANSCRIPTIONAL ACTIVATOR THAT IS ALSO A REPRESSOR, Ji-Ying Sze, Eumorphia Remboutsika, and Gunter B. Kohlhaw, Department of Biochemistry, Purdue University, West Lafayette, IN 47907

The Leu3 protein of yeast (Leu3p) interacts with a *cis*-acting element (UAS_{LEU}) found upstream of several genes involved in branched-chain amino acid biosynthesis and in nitrogen metabolism. It was recently demonstrated using *in vitro* transcription assays that transcriptional activation by Leu3p is totally dependent on α-isopropylmalate (α-IPM), an intermediate in leucine biosynthesis (J. Sze, M. Woontner, J. Jaehning, and G. Kohlhaw, *Science*, in press). It was also shown that, in the absence of α-IPM, Leu3p *inhibits* transcription from templates containing the UAS_{LEU} element, *i.e.*, acts as a repressor. We now report that different regions of the Leu3 molecule are responsible for activation and repression. The activation function is contained within the C-terminal 30 amino acid residues, a region that is both necessary and sufficient for transcriptional activation. A mutant protein lacking the activation domain still acts as a repressor, as does a severely truncated Leu3 protein that contains little more than the DNA binding region of Leu3p. We postulate that repression is a direct consequence of the interaction between Leu3p and UAS_{LEU} and may be brought about by a conformational change of the DNA.

We also report that highly purified Leu3p exists in two forms that can be converted into one upon treatment with calf intestinal phosphatase. Whether the phosphorylated and non-phosphorylated forms of Leu3p have different functions is not known at this time. Supported by NIH Grant GM15102 and by a Purdue Research Foundation Grant (J.S.).

B 421 STRUCTURAL CHARACTERISATION OF THE HUMAN CARBOXYPEPTIDASE H GENE, Alice W.N. Tay, Duncan R. Smith and Louis Lim, Institute of Molecular and Cell Biology, National University of Singapore, Kent Ridge, Singapore 0511

Carboxypeptidase H (CPH) is a metallocarboxypeptidase implicated in the processing of peptide hormones. To study the regulation of expression of the human CPH gene, we have analysed its genomic structure and characterised its promoter. A map of the gene was constructed by using 20 clones isolated from 2 human genomic libraries. The gene spans more than 70 kb and consists of 9 exons. Comparison with the rat CPH gene reported by Fricker et al* revealed a high degree of conservation of the exonic sequences as well as the exon-intron junctions. The 5' flanking region of the human CPH gene is very GC rich and there is an absence of TATA or CCAAT box like motifs. There is however a sequence similar to the 'Initiator' sequence first defined in lymphocyte-specific terminal deoxynucleotidyltransferase. Primer extension and S1 mapping experiments have indicated the presence of at least 5 start sites.Expression of chloramphenicol acetyltransferase reporter fusions containing 3 kb of the putative promoter was observed in C6 and HepG2 cells.

* Y.K.Jung, C.J.Kuncz, R.Pearson, J.E.Dixon and L.D.Fricker, Molecular Endocrinology, vol 5 no 9, pp 1257 - 1268, 1991.

B 422 OKADAIC ACID INDUCES CYTOKINE GENE EXPRESSION AND κB BINDING ACTIVITY IN MURINE PERITONEAL MACROPHAGES, Julie M. Tebo and Thomas A. Hamilton, Section of Immunology, Cleveland Clinic Foundation, Cleveland, OH 44195

The potential contribution of phosphatases in the mechanism of LPS transcriptional control of proinflammatory genes has been investigated. We examined the effect of a specific phosphatase inhibitor, okadaic acid (OA), on TNFα and IP-10 mRNA expression either alone or in combination with LPS. OA alone induces expression of both TNFα and IP-10 mRNA by 8 h; however, the mechanism of LPS induced expression of IP-10 and TNFα mRNAs can be distinguished based on the effect of OA. OA inhibits the LPS induced expression of IP-10 mRNA at 8 h whereas there is enhanced expression of LPS induced TNFα mRNA by 2 h. OA induced expression of TNFα and IP-10 gene expression appears to be mediated at the level of transcription as measured by nuclear run-on. Treatment of PE macrophages with OA results in expression of proteins that specifically recognize a κB sequence within the IP-10 gene. Functional studies illustrate the importance of κB sequences for IP-10 and TNFα expression. Therefore, these findings suggest that the activation of κB binding proteins by OA may be important, but not necessarily sufficient, for the induction of these proinflammatory genes.

B 423 THE β_3 SUBUNIT OF THE INTEGRIN $\alpha_v\beta_3$ IS REGULATED TRANSCRIPTIONALLY AND POST-TRANSCRIPTIONALLY BY BOTH 1,25(OH)2D_3 AND RETINOIC ACID, Steven L. Teitelbaum, Xu Cao, Mirei Chiba, Hirohide Mimura and F. Patrick Ross, Jewish Hospital at Washington University Medical Center, St Louis, MO, 63110.

The first and probably rate-limiting step in bone resorption is the attachment to the matrix of the osteoclast, the major bone resorbing cell. We have shown that the integrin $\alpha_v\beta_3$ plays a central role in the process of attachment of osteoclasts to bone. The hormone $1,25(OH)_2D_3$ (D_3) is known to stimulate bone resorption and we postulated that it may be involved in regulating the synthesis and expression of $\alpha_v\beta_3$ on the surface of the osteoclast or its precursor. Using a 1.9 kb cDNA probe coding for avian β_3 (obtained from a cDNA library generated from avian osteoclast precursors) we showed that treatment of precursor cells with either (D_3) or retinoic acid (RA) results in a time- and dose-dependent increase in steady state β_3 mRNA levels. By a combination of nuclear runon studies and Northern analysis of cells treated with actinomycin D we proved that both D_3 and RA increase transcription and β_3 mRNA stabilization. A full length cDNA was obtained which contained a 42 bp untranslated region, whose integrity was established by RACE. A probe based on this sequence was used to screen a genomic library, leading to the isolation of 27 kb of genomic DNA containing the 42 bp of untreated sequence. Primer extension and S1-nuclease protection assays confirmed the presence of a transcriptional start site. Fragments of the 5' flanking region of the avian β_3 gene were cloned, sequenced and inserted into the cloning site of a luciferase reporter plasmid. Transfection of this plasmid into competent cells led to enhanced expression of luciferase activity, confirming that the sequence contains a functional promoter. 1.3 kb of this flanking region was sequenced and shown to contain consensus sequences for D_3 and RA response elements (VDREs and RAREs). We are using gel shift and co-transfection experiments to confirm that these putative elements function to mediate specific hormonal activation of β_3 gene expression.

B 424 **INTRODUCTION OF A FUNCTIONAL HUMAN ESTROGEN RECEPTOR RESTORES THE FUNCTION OF THE AH RECEPTOR IN THE HUMAN BREAST CARCINOMA CELL LINE MDA-MB-231,** **J. S. Thomsen, X. Wang, R. N. Hines and S. H. Safe,** Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas, 77843-4466

The induction of *CYP1A1* gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is mediated by the interaction of the liganded nuclear aryl hydrocarbon (Ah) receptor complex with genomic Ah responsive elements (AhRE's) present in several copies in the 5'-flanking region of the *CYP1A1* gene. Treatment of the MDA-MB-231 human breast cancer cell line with TCDD resulted in the formation of the nuclear Ah receptor complex which bound to a synthetic AhRE in a gel shift assay, but *CYP1A1* gene expression was not induced. Transient transfection of MDA-MB-231 cells with the plasmid, pRNH11c, containing the intact human *CYP1A1* regulatory region from -1140 to +2434 fused to the bacterial gene, chloramphenicol acetyl transferase (CAT), showed no TCDD-induced CAT-expression. This indicates that the lack of *CYP1A1* inducibility by TCDD is due to altered transcription factors. Recently, several cell lines with non-functional Ah receptors have also been reported to contain non-functional estrogen receptors. This phenomenon is also observed in MDA-MB-231 cells, and in order to elucidate whether the function of the estrogen receptor is a prerequisite for the function of Ah receptor mediated responses, the cells were cotransfected with pRNH11c and a vector containing the human estrogen receptor cDNA, ΔhER. The presence of ΔhER-plasmid did not change the level of basal CAT activity. However, in cells treated with 10 nM TCDD, a significant increase in CAT-activity was observed. The TCDD-induced CAT-activity could be further increased by increasing the amount of ΔhER-plasmid transfected into the cells. These results indicate that a functional estrogen receptor is necessary for a functional Ah receptor in this human breast carcinoma cell line. The regulatory mechanism underlying the interaction between the two receptors is currently being investigated.

B 425 INTESTINE-SPECIFIC DNA-BINDING PROTEINS, ISP1 AND ISP2, ACT IN COORDINATION WITH HNF-1 TO INITIATE TRANSCRIPTION OF THE SUCRASE-ISOMALTASE GENE Peter G. Traber, Gary D. Wu, Kristina Forslund, and Wei Wang. Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Understanding the mechanisms that regulate transcription of intestine-specific genes may elucidate the processes of intestinal development and differentiation. We have chosen to study sucrase-isomaltase (SI), an enterocyte-specific gene that exhibits complex developmental and adult patterns of expression. We previously showed that intestinal cell line-specific transcription of the SI gene is directed by an evolutionarily conserved 183 base pair promoter. Furthermore, we showed that 3424 bases of the 5'-flanking region of the human SI gene directed intestine-specific expression in transgenic mice. In the current studies, we characterized three promoter elements, and their cognate DNA binding proteins, that act as positive regulators of SI transcription, SIF1, 2 and 3 (SI Footprint). Electrophoretic mobility shift assays (EMSA), using oligonucleotides containing mutations in SIF1, demonstrated that two intestine-specific proteins bind to this element, ISP1 and ISP2 (Intestine Specific Protein). Furthermore, while the binding of ISP2 is dependent on ISP1, ISP1 is able to bind alone. We demonstrated that SIF2 and 3 bound hepatocyte nuclear factor-1 (HNF-1) by showing that DNA-protein complexes were "supershifted" on ENMSA in the presence of anti-HNF-1 antiserum. Cotransfection of a construct containing the SIF3 element, linked to SIF1, with an expression vector for HNF-1 demonstrated that the HNF-1 protein was able to transactivate the SIF3 regulatory element. However, HNF-1 was unable to activate transcription of the SI promoter in cell lines that lacked ISP1 and ISP2. In conclusion, we have shown that intestine-specific transcription of the SI gene promoter is dependent on two novel intestine-specific proteins, that we have named ISP1 and ISP2. Furthermore, HNF-1, previously described as a transcription factor for liver genes, acts to augment SI transcription in the presence of ISP1 and ISP2.

B 426 USE OF ALTERED-SPECIFICITY MUTANTS TO STUDY SRF FUNCTION. Richard Treisman, Caroline Hill, Stephen Dalton, Richard Marais, and Judy Wynne. Transcription Laboratory, ICRF, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

Serum Response Factor (SRF) binds regulatory elements found in the promoters of both growth factor-regulated and muscle-specific genes. At the c-fos Serum Response Element (SRE), SRF forms a ternary complex with either of two Ets-domain accessory proteins, Elk-1 and SAP-1. Although these accessory proteins do not bind the SRE alone, in the ternary complex they contact DNA next to the SRF binding site. In contrast, at muscle-specific promoters, SRF binding sites act as constitutive promoter elements. We used information from DNA binding studies to generate mutations in SRF that alter its DNA binding specificity. The DNA sequence bound by these mutants is inactive in both growth-factor regulated and muscle-specific contexts, but its activity can be partially restored by expression of the mutant SRF protein. Experiments using this approach to map function domains of SRF *in vivo* wil be presented. Using a related approach we have also generated altered-specificity derivatives of Elk-1 and SAP-1, whose binding remains SRF-dependent but contact different DNA sequences. We are using these proteins to investigate the function of the SRF ternary complex *in vivo*.

B 427 **IDENTIFICATION OF PROTEINS THAT INTERACT WITH THE TRANSCRIPTION ACTIVATION DOMAINS OF HUMAN P53, HSV VP16 AND ADENOVIRUS E1A**, Ray Truant, Hua Xiao, C.James Ingles and Jack Greenblatt, Banting and Best Department of Medical Research and the Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, CANADA M5G 1L6

The human tumor suppressor p53, Herpesvirus virion protein VP16 and Adenovirus E1A all have potent transcriptional activation domains that contain acidic residues. VP16 and E1A have previously been shown to interact with TBP, the TATA box-binding subunit of the general factor TFIID. As well, VP16 has been shown to interact with TFIIB. We have now used protein affinity chromatography to show that the p53 activation domain directly binds TBP. The p53 and VP16 activation domains also bind TFIIH, a general factor which phosphorylates the CTD of the largest subunit of RNA polymerase II. This interaction involves no other general factor and may also be direct. Columns containing immobilized p53 and VP16 do not bind RNA polymerase II or the remaining general factors TFIIE, TFIIF or TFIIJ.

The activating regions of VP16, p53 and E1A also all bind the protein product of the human MDM-2 oncogene. There is, however, poor correlation between the effects of activator protein mutations on transcriptional activation and their effects on binding of MDM-2 protein. This suggests that binding of MDM-2 has an effect on transcriptional activation that is negative, rather than positive, perhaps because binding of MDM-2 to the activation domains of VP16, p53 and E1A interferes with the binding of TFIID and/or TFIIH.

B 428 THE HUMAN PAPILLOMAVIRUS 16-E2 *TRANS*-ACTIVATOR REPRESSES TRANSCRIPTION OF THE VIRAL E6-E7 ONCOGENE PROMOTER BY BLOCKING Sp1 BINDING AND COOPERATION WITH BASAL PROMOTER FACTORS, Lubomir P. Turek[1] Sinikka Parkkinen[1], Yasushi Yamakawa[1], Masato Ushikai[1], Takaoki Ishiji[1], Michael J. Lace[1], James Anson[1], Stephen P. Kahanic[1,2], and Thomas H. Haugen[1], Departments of [1]Pathology and [2]Medicine, VAMC and The University of Iowa College of Medicine, Iowa City, IA 52242, USA.

HPV-16 E6 and E7 oncogene promoter, P97, is repressed by papillomaviral E2 gene products interacting with two adjacent proximal E2 sites 5' to a TATAA box. We found that P97 response to cellular enhancer factors required the cooperation between TFIID, Sp1 and possibly additional, as yet undefined factor(s) binding to precisely spaced motifs. The HPV-16 E2 *trans*-activator increased P97 mRNA levels at low concentrations, but repressed P97 transcription at higher levels, suggesting a possible autoregulatory mechanism for HPV-16 early gene expression. E2 binding did not displace human TFIID (TBP) from the TATAA box in vitro, and spacer insertions between the TATAA and E2 sites did not abolish E2 repression in vivo. In contrast, the HPV-16 E2 protein was found to block Sp1 binding to a motif partially overlapping an E2 site in DNase I footprinting. The insertion of a spacer sequence duplicating this site overlap allowed E2 and Sp1 to bind simultaneously in vitro and relieved E2 repression in vivo. Like a classical repressor, the E2 *trans*-activator thus inhibits HPV-16 P97 response to cellular activators by blocking Sp1 binding and disrupting the cooperation between enhancer proteins and basal promoter factors. These profound effects of the E2 gene product on P97 activity may explain the apparent selection against intact E2 in integrated HPV fragments in cervical carcinomas expressing the viral E6 and E7 oncoproteins.

B 429 PROTEIN AND DNA ELEMENTS INVOLVED IN THE TRANSCRIPTIONAL TRANS-ACTIVATION OF BLV, Ildiko Unk, Saowakon Paca-uccaralertkun, Endre Kiss-Toth, Imre Boros, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, 6701 Szeged, P.O.B. 521. Hungary

The Bovine Leukemia Virus (BLV) encodes a regulatory protein, tax, which enhances transcription from the viral promoter and several cellular promoters. Although tax itself does not bind DNA, a 21bp repeat present in three copies in the virus LTR has been shown to serve as tax responsive element. Most probably the recognition of these motifs by protein-protein complexes of cellular factors and tax results in tax activation. To study what is the role of particular sequences in BLV tax specificity and what are the cellular factors involved in this process we synthetised mutant 21bp repeats. These oligos were cloned upstream of the BLV core promoter and their tax responsiveness were determined in transient assays. In paralel, wild type and mutant BLV 21bp repeats and HeLa nuclear extract were used in footprinting and gelshift experiments to detect specific DNA-protein interactions. On the basis of these results we concluded that at least two cellular proteins form specific complexes with the 21bp repeat. One interacts with the CRE-like element in the center of each 21bp repeats, the other - most probably AP-4 - with a CAGCTG motif present in two 21 bp repeats. The interaction of these proteins with the tax responsive elements may indicate their involvement in tax trans-activation. Experiments to further analyse their role are in progress.

B 430 STRUCTURAL CHARACTERISTICS OF BINDING TO CELLULAR GENE PROMOTERS BY TRANSCRIPTION FACTOR E2F. Michael Wade[1], Kristian Helin[2], Ed Harlow[2] and Jane C. Azizkhan[2]. Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599[1] and Massachusetts General Hospital Cancer Center, Building 149, Charlestown, MA 02129[2].

Transcription factor E2F has been implicated in both transcription regulation and proliferation control, in part because of its interactions in DNA-binding complexes with Rb, cyclin A, $p33^{cdk2}$ and p107. We have been been studying the role of E2F in basal, serum-induced and human cytomegalovirus (HCMV)-induced control of the dihydrofolate reductase (DHFR) gene, and have recently shown that HCMV induces DHFR transcription through a highly conserved overlapping dyad E2F sequence at the major transcription start and also induces a heteromeric DNA-binding, E2F-containing multiprotein complex similar or identical to that associated with S phase. Although a single E2F "half-site" is sufficient for these effects, several gene promoters contain dyad E2F sites similar to that in the DHFR promoter. We have found that mutations abolishing only one of the two half-sites, which still allow E2F binding, reduce basal transcription, decrease the half-life of binding and subtly change protein-DNA interactions at the binding site. The recent cloning of E2F has allowed us to study the functional significance of the dyad binding motif with regard to both protein-DNA and protein-protein interactions.

B 431 SP1 BINDING ELEMENTS OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 PROMOTER ARE ESSENTIAL FOR TRANSACTIVATION BY A HUMAN *HERPESVIRUS 6 TRANSFORMIG GENE SEGMENT*, Jinhai Wang[1], Clinton Jones[2], Michael Norcross[1], Ernst Bohnlein[3], Abdur Razzaque[1], [1]Center for Biological Evaluation and Research, 8800 Rockville Pike, Bethesda, MD 20892
[2]University of Nebraska, Center for Biotechnology, Department of Vaterinary Science, Lincoln, NE 68583, [3]Vienna International Research Cooperation Center(VIRCC), University of Vienna, Austria

The transactivation of HIV LTR by different transactivators have been well documented. These transactivators are capable of transactivating HIV LTR through TAR, NF-kB, or other regulatory binding elements. Here we report that a human herpesvirus-6 (a potential cofactor of HIV-1) gene segment, pZVH14, which can neoplasticcally transform NIH 3T3 and human keratinocytes, is capable of transactivation of HIV LTR in a Sp1 binding site dependent way in CAT assays. This transactivation is dramatically reduced by cotransfection with oligos designed to form triplex with HIV LTR Sp1 binding sites. Since several viral genes have been reported to transactivate homologous or heterologous promoters by synergism with Sp1, such as BPV E2 and HIV-1 tat, we are currently investigating whether there is synergism between HHV-6 encoded gene products and Sp1 in transactivation of HIV LTR.

B 432 **MECHANISM OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) MEDIATED DOWN-REGULATION OF ESTROGEN RECEPTOR IN HUMAN BREAST CANCER MCF-7 CELL LINE,** **X. Wang, W. Porter and S.H. Safe.** Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas, 77843-4466

TCDD is a widespread environmental contaminant and has been shown to elicit a diverse spectrum of biological and toxicological responses. For example, TCDD is an antiestrogen in both cultured cells and animal models, but the molecular mechanism of this response is not understood. Most TCDD-mediated responses are mediated through initial binding to the aryl hydrocarbon (Ah) receptor. MCF-7 human breast cancer cells are routinely used as an *in vitro* model for mammary cancer and these cells express the estrogen and Ah receptors. In this study, we show that TCDD (1 nM) causes a time-dependent decrease of estrogen receptor (ER) levels, as determined by velocity sedimentation analysis. Within three hours after treatment with TCDD, ER levels decreased by 50% but no further decrease was measured for up to 24 hours after treatment with TCDD. Gel retardation analysis using an estrogen responsive element (ERE) consensus oligo demonstrated that TCDD causes a time-dependent decrease in ER-ERE binding. This inhibition of ER-ERE binding occurs as early as 1 hour after TCDD treatment, and is maximal after 12 hours. Co-treatment with α-naphthoflavone (αNF), a TCDD antagonist, inhibited TCDD down-regulation of both ER levels and ERE binding, and co-treatment with cycloheximide did not block the effects of TCDD on ER-ERE binding. Northern blot analysis showed that the ER mRNA steady state levels were not affected by TCDD treatment at any of the time points measured. This data indicates that TCDD down-regulation of ER levels and ER-ERE binding is Ah receptor mediated. However, this decrease does not involve changes in ER gene transcription.

B 433 RETINOIC ACID RESPONSE ELEMENT RECEPTOR SELECTIVITY IS DETERMINED BY SEQUENCES IN HALF SITES, THEIR SPACING AND SEQUENCES IN THE SPACER Yisheng Wang and Sotirios K. Karathanasis, Lederle Laboratories, Pearl River, NY 10965.

Previous studies in our laboratory have shown that a regulatory element, site A, in the upstream region of the apolipoprotein AI (apoAI) gene is a retinoic acid (RA) response element (RARE) that responds preferentially to the recently identified RA receptor RXRα over the previously characterized receptors RARα and RARβ. To investigate the molecular basis for this preference several site A mutants were inserted proximal to the apoAI basal promoter fused in a chloramphenicol acetyltransferase (CAT) reporter construct and their ability to stimulate CAT activity in response to RA in the presence or absence of RXRα was evaluated by transient cotransfection experiments in CV-1 cells. The results showed that the minimal RARE within site A is composed of two pentanucleotide repeats (half sites), TGAAC and TGACC, separated by a trinucleotide (spacer) CCT. Insertion of three additional nucleotides in the spacer, CCTCGG, did not change responsiveness to RXRα but deletion of the spacer completely eliminated responsiveness. However, alteration of the nucleotide sequence of the spacer from CCT to TTT resulted in RA responsiveness even in the absence of cotransfected RXRα, suggesting that this RARE responds to endogenous RA receptors (eRARs) present in CV-1 cells. When both half sites are TGACC responsiveness to RXRα is maintained if the spacer is CTT while responsiveness to eRARs is observed if the spacer is TTT. In contrast when both half sites are TGAAC responsiveness to RXRα is eliminated irrespective of whether the spacer is CCT or TTT and a low responsiveness to eRARs is observed when the spacer is TTT. Taken together these findings indicate that the combined contribution of the primary sequence in the half sites, their spacing and the nucleotide sequence in the spacer determine preferential responsiveness of site A to RXRα. We speculate that a combination of variations in these three parameters are fundamental to the generation of RARE's with distinct responses to different RA receptors.

B 434 IDENTIFICATION OF PROMOTER SEQUENCES RESPONSIBLE FOR THE DIFFERENTIAL EXPRESSION OF SAA4 AND SAA1 OR SAA2.
G. Watson, S. Coade and P.Woo. Division of Molecular Rheumatology, Clinical Research Centre, Watford Road, Harrow, Middlesex, UK

Serum amyloid A (SAA) is a group of acute phase proteins produced in response to infection, inflammation or trauma. Continued high levels of SAA, such as occurs in rheumatoid arthritis, leads to a fatal complication known as amyloidosis. SAA has also been implicated in the reverse cholesterol transport process. SAA1 and SAA2 form the major circulating SAA proteins. A third gene, SAA3, is a pseudogene. We have recently identified a fourth gene, SAA4 and sequenced the exonic regions of this gene. Analysis of the predicted SAA4 protein structure and comparison with the other SAA protein structures revealed significant differences and we predict that this protein has a distinct function from the other SAAs.

SAA4 was reported by others to be a constitutive protein and to show no response to the inflammatory cytokines. Recently, however, there has been some evidence to indicate that SAA4 does respond to induction by IL1 and IL6, but to a much lesser extent than the reponse of SAA1 and SAA2, which involves NFkB. We have now sequenced a genomic clone of SAA4, including the promoter region. The sequence information has revealed important differences in the promoter region of SAA4 relative to SAA1 and SAA2. Using promoter - reporter constructs and a variety of cell lines, we have identified differences in the promoter sequences which are responsible for the differential expression of SAA4 in comparison to SAA1 and SAA2.

B 435 TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF *pim-1* GENE EXPRESSION IN HEMATO-LYMPHOID CELLS, Denise Wingett, Raymond Reeves and Nancy S. Magnuson, Departments of Microbiology and Biochemistry, Washington State University, Pullman, WA 99164-4233

The *pim-1* proto-oncogene encodes a serine/threonine protein kinase that is expressed in cells of hemato-lymphoid origin and in the germ cell lineages. We have observed that in spite of containing a G/C-rich housekeeping promoter, that the *pim-1* gene is inducible by a variety of stimuli. We have shown that *pim-1* gene expression is rapidly induced in both mitogen-stimulated primary lymphocytes and in hemato-lymphoid cell lines and that *pim-1* gene expression is regulated at the post-transcriptional level. These post-transcriptional regulatory mechanisms involve the modulation of *pim-1* mRNA stability and are thought to be mediated, at least in part, by sequences located in the 3′ untranslated region of the *pim-1* gene including an A/U-rich motif. Based on nuclear run-ons and half-life experiments in PMA/ionomycin stimulated Hut-78 cells, a human T-cell line, our findings suggest that transcriptional regulation alone cannot account for the increases in *pim-1* mRNA observed in theses cells following stimulation. To further delineate the importance of 3′ untranslated sequences in the expression of *pim-1* mRNA in these cells, we are examining various deletions in the 3′ untranslated region of the *pim-1* gene and their corresponding effects on RNA expression and stability. This work was funded in part by NIH grant AI-26356 and USDA grant 91-37206-6867.

B 436 TWO DISTINCT NEGATIVE CONTROL REGIONS MEDIATE REPRESSION OF THE IL-3 GENE IN T-CELLS INFECTED WITH HTLV. M. Wolin, C. Hong, M. Kornuc, L. Dote, K. Kwan, R. Lau, and S. Nimer. UCLA School of Medicine. Los Angeles, CA
Interleukin-3 (IL-3) is expressed in activated but not resting T-cells and although HTLV-infected T-cells phenotypically resemble activated T-cells, they do not express IL-3 either constitutively or following mitogenic stimulation. The transcriptional control of IL-3 expression was compared in HTLV-infected and uninfected T-cells. Constructs consisting of IL-3 5′ sequences linked to a reporter gene (chloramphenicol acetyl transferase [CAT]) were transfected into HTLV-infected and -uninfected T-cells to identify transcriptional regulatory regions. DNase I footprinting of the IL-3 upstream sequences was performed to look at differences in DNA-protein interactions in cells infected with HTLV compared to uninfected cells. Using nuclear extracts from uninfected MLA 144 and primary human T-cells, a footprint region was identified which extends from bp -165 to -128. Extracts from the HTLV-infected S-LB-1 cell line demonstrated a footprint which protects the same nucleotides but extends 3′ to bp -103 and includes the lymphokine consensus sequences, CK-1 and CK-2. Deletion of sequences from bp -128 to -103 from reporter gene constructs demonstrated a 6-fold increase in basal and inducible CAT activity above the corresponding wild type construct in S-LB-1 cells, but no difference in uninfected Jurkat T-cells. Our transfection experiments also identified a repressive region, located between bp -690 and -315, which is functional in both uninfected and infected T-cells. These studies demonstrate the importance of the -128 to -103 region (which contains the lymphokine consensus sequences) in the repression of IL-3 gene expression in HTLV-infected T-cells.

B 437 REGULATION OF TRANSCRIPTIONAL ELONGATION WITHIN THE C-MYC AND C-FOS PROTOONCOGENES
Stephanie Wright * and J. Michael Bishop†, *Wellcome/CRC Institute of Cancer and Developmental Biology, Tennis Court Road, Cambridge, CB2 1QR, England, and †Departments of Microbiology and Immunology and the George Williams Hooper Foundation, University of California, San Francisco CA94143.

The expression of eukaryotic genes may be regulated via the modulation of transcriptonal elongation. "Blocks" to transcriptional elongation within a gene may be shown either by run-off transcription analysis in isolated nuclei or by the detection of discrete premature termination events upon expression of cloned genes microinjected into Xenopus oocytes. Alterations in the degree of transcriptional elongation blockage within a gene may be used to control its expression according to different physiological states of the cell.

We have previously defined DNA sequences within the mouse c-myc, c-myb and c-fos genes which mediate premature termination of transcription when placed downstream from a heterologous promoter and assayed by microinjection into Xenopus oocytes. Comparison of the nucleotide sequence of these elements revealed a common G-rich sequence. We have shown by gel mobility shift analysis that a common factor(s) binds to this G-rich element within the different genes and are currently assessing the role of this factor(s) in regulating the expression of the c-myc and c-fos genes.

B 438 MOLECULAR CLONING OF A TRANSCRIPTIONAL REPRESSOR PROTEIN (SIRP-1) WHICH BINDS TO THE INTESTINE-SPECIFIC PROMOTER REGION OF THE SUCRASE-ISOMALTASE GENE Gary D. Wu, Wei Wang, and Peter G. Traber. Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

The cAMP response element (CRE) is able to transcriptionally activate certain eukaryotic genes through transactivation by several DNA binding proteins of the ATF/CREB family. More recently, several DNA binding proteins in this family have be cloned which negatively regulate transcription from CRE (MCB; 12: 3070-3077 and PNAS; 89: 4820-4824) The functional significance of this emerging family of negative transcriptional regulators on eukaryotic gene transcription is unknown. In order to elucidate the molecular mechanisms which direct the temporal, spatial, and developmental patterns of intestinal gene expression, we have recently cloned and characterized the 5' flanking region for the intestinal disaccharidase sucrase-isomaltase (SI). Deletional analysis and DNAse 1 footprinting of the intestine-specific promoter for this gene revealed the presence of a negative cis-acting element, SIF-R (SI Footprint Repressor), which consists of an evolutionarily conserved core sequence CATTTATGTAAACTA. EMSA demonstrated that this element specifically binds a protein(s) which was expressed in all cell lines and tissues tested. A two base pair mutation of this core sequence (SIF-Rm) eliminated DNA-protein binding by EMSA. A southwestern blot using SIF-R and SIF-Rm oligos identified specific binding to a 92 kd protein in both liver and Caco-2 nuclear extracts. Southwestern screening of a mouse liver lamda gt11 cDNA library identified several specific clones. One clone, which we have named SIRP-1 (SI Repressor Protein), appears to be the mouse homolog of a recently identified bZIP human transcriptional repressor, E4BP4, cloned using the ATF viral binding domain of the E4 promoter (MCB; 12: 3070-3077). Although transfection studies will be required to determine the functional significance of this protein, its ability to bind to an ATF binding domain suggests that SIRP-1 may play a role in the cAMP mediated negative regulation of SI mRNA expression in Caco-2 cells described previously (J. Cell. Phys; 141: 627-635). Further investigation will be required to determine the role SIRP-1 and the other SIF-R clones play in the transcriptional regulation of SI during development and in the adult intestinal tract.

B 439 REDOX ACTIVATION OF FOS-JUN DNA BINDING ACTIVITY IS MEDIATED BY AN ENZYME INVOLVED IN DNA REPAIR. Steven Xanthoudakis[1], Graham Miao[1], Feng Wang[2], Yu-Ching E. Pan[2] and Tom Curran[1]. [1]Department of Molecular Oncology & Virology, Roche Institute of Molecular Biology and [2]Department of Protein Biochemistry, Hoffmann-La Roche Inc. Nutley, NJ 07110

The phorbol ester inducible transcription factor, AP-1, is a heterodimeric leucine zipper complex containing the protein products of the *fos* and *jun* protooncogenes. The DNA binding activity of Fos and Jun is regulated *in vitro* by a posttranslational mechanism involving reduction-oxidation. Redox regulation is mediated through a conserved cysteine residue located in the DNA binding domain of Fos and Jun. Reduction of this residue by chemical reducing agents or by a ubiquitous nuclear redox factor (Ref-1) purified from Hela cells stimulates AP-1 DNA binding activity *in vitro*, whereas oxidation or chemical modification of the cysteine has an inhibitory effect on DNA binding activity. Here we demonstrate that the protein product of the cloned *ref*-1 gene stimulates the DNA binding activity of several redox regulated transcription factors in addition to AP-1. Ref-1 is antigenically unrelated to AP-1, but copurifies with AP-1 binding activity through several chromatographic steps. Protein-DNA interactions between Fos/Jun complexes and the human metallothionine AP-1 motif are not altered by Ref-1 as determined by *in vitro* footprinting analysis. In addition, immunodepletion studies indicate that Ref-1 is the major AP-1 redox activity in Hela nuclear extracts. Interestingly, Ref-1 is a bifunctional protein; it also possesses an apurinic/apyrimidinic (AP) endonuclease DNA repair activity. However, the redox and DNA repair activities of Ref-1 can be physically and biochemically distinguished. Experiments are in progress to determine the precise nature of Ref-1 - AP-1 interactions *in vitro* and *in vivo*.

B 440 MOLECULAR CLONING OF A TRANSCRIPTION FACTOR REGULATING HIV-1 GENE EXPRESSION, Jong-Bok Yoon, Gen Li and Robert G. Roeder, Laboratory of Molecular Biology and Biochemistry, Rockefeller University, New York, NY 10021

LBP-1 is a group of proteins, with apparent molecular sizes of 63 to 68 KD, which bind strongly to sequences at and just downsream of the HIV-1 initiation site and weakly over the TATA box. We have previously shown that LBP-1 represses HIV-1 transcription by inhibiting the binding of TFIID to the TATA box (H.Kato *et al.* (1991) Science 251, 1476-1479).

Here we report the cloning and molecular characterization of LBP-1. We purified this factor from HeLa cells by affinity chromatography and Lys-C digested peptides were microsequenced. A specific probe was derived by PCR and used to isolate cDNA clones of LBP-1. Restriction enzyme mapping and DNA sequencing revealed four similar but distinct groups of cDNAs, which were termed LBP-1a, b, c, and d. LBP-1a and LBP-1b cDNAs are differentially spliced products of one gene, while LBP-1c and LBP-1d are differentially spliced products of another. LBP-1a and LBP-1c share 72 % amino acid identity. Interestingly, comparison of the amino acid sequence of LBP-1a with the available protein data bases revealed homology with D. melanogaster Elf-1, an essential transcriptional activator that functions during embryogenesis. However, computer analysis did not reveal any well defined DNA binding motifs.

In vitro translated LBP-1 proteins bind to the LBP-1 site in a sequence specific fashion. Antisera raised against recombinant LBP-1a recognize native LBP-1 from HeLa nuclear extract in a gel retardation assay, further demonstrating that the cloned cDNAs encode bona fide LBP-1. In addition, recombinant LBP-1 proteins specifically decreased transcription from the wild type HIV-1 template in a cell free transcription system. However, no represson was observed for the mutant template which lacks LBP-1 binding sites.

B 441 THE 22 kDa α-COIXIN PROMOTER IS TRANSACTIVATED BY THE MAIZE O2 PROTEIN, José A. Yunes, Germano Cord N., Marcio J. Silva, André L. Vettore, Adilson Leite & Paulo Arruda, Centro Biologia Molecular Engenharia Genetica, Universidade Estadual Campinas, 13081, Campinas, São Paulo, Brasil.

The maize opaque-2 mutation specifically reduces the accumulation of 22 kDa α-zeins at the level of transcription. Recently the product of the O2 locus was identified as a bZIP protein which, by interacting with the sequence TCCACGTAGA, present only in promoters of the 22 kDa α-zein genes, activates its transcription. Since genes encoding coixins, the prolamins of Coix, has been shown to present structural and regulatory sequences highly homologous to zeins, it seemed likely that an O2-like gene was also conserved in Coix during evolution. This hypotesis was confirmed by the isolation of an O2-homologous cDNA from Coix nearly identical to the maize O2 at the bZIP region. The 22 kDa-like α-coixin do not presents an O2 target sequence identical as the one identified in the 22 kDa α-zeins. However, transient expression assays in tobacco mesophyl protoplasts, using the α-coixin promoter fused to the GUS reporter gene showed an increment over 200 fold of GUS activity, when cotransformed with a maize O2 expressing vector. The same level of expression was obtained with the α-zein promoter. Interestingly, an α-coixin deleted promoter lacking the homologous O2 box, presented no decrease of GUS activity in both tobacco protoplasts and bombarded immature maize endosperms. Whereas, with a similarly deleted α-zein promoter, the GUS activity drops 6 fold. Our results indicates that the genes coding for the O2 regulatory protein are conserved in these cereals and that in the α-coixin promoter, there are other sequence downstream the proposed maize-O2 box that can serve as target site for the O2 protein.

This work was financed by grants to P.A. from FINEP, CNPq/PI and FAPESP/PTE-90/3808-5.

B 442 THE HIV-1 PROMOTER LACKS A SIMPLE INITIATOR ELEMENT, BUT CONTAINS A NOVEL, BIPARTITE ACTIVATOR AT THE TRANSCRIPTION START SITE, Beatrice Zenzie-Gregory and Stephen T. Smale, Howard Hughes Medical Institute and Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024

The HIV-1 core promoter region, extending from nucleotides -40 to +80 relative to the transcription start site, contains a complex array of putative regulatory elements, including a TATA box, an initiator element, an element between the TATA box and start site, binding sites for LBP/UBP, the TAR element, and others. However, because of this elaborate architecture, the actual boundaries and functional roles of most of the individual elements have not been defined. To facilitate a detailed analysis of the core promoter region, we employed in vitro transcription studies to identify the simplest control elements that activate transcription in the context of a synthetic, heterologous promoter. We anticipated finding a typical initiator as an important basal element because of previously-reported results, including 1) the detection of sequence homology between the HIV start site and known initiator elements, 2) a demonstration that mutations at the HIV-1 start site diminish promoter strength, and 3) a demonstration that an initiator-binding protein, called TFII-I, binds to the HIV-1 start site. Despite the previous observations, we have demonstrated that the HIV-1 core promoter does not contain a simple initiator that is structurally and functionally analogous to those found in the terminal transferase and adenovirus major late promoters. In its place, we identified a bipartite element, located between -6 and +30, that activates specific transcription initiation. Unlike a strong initiator element, however, the activity of this element is dependent on the presence of a TATA box. The first domain of the element, which overlaps the start site, requires appropriate positioning relative to the TATA box. The positioning of the second domain, between nucleotides +21 and +30, is more flexible, relative both to the TATA box and to the transcription start site. In addition, this downstream domain is active only in the context of the HIV-1 start-site domain, and is completely inactive in the context of a typical initiator element. Further analysis of this unique element and of additional control elements in a well-defined, heterologous context might provide a route towards a detailed understanding of HIV-1 transcription.

B 443 MOLECULAR ANALYSIS OF THE TESTIS ACE PROMOTER, Yudong Zhou and Kenneth E. Bernstein, Department of Pathology, Emory University School of Medicine, Atlanta, GA 30322

A unique isozyme of angiotensin converting enzyme (ACE) is made by developing male germ cells and is called tACE. Analysis of transgenic mice has shown that tissue specificity is encoded by a promoter region encompassing DNA -91 to +16 relative to the transcription start site of tACE. DNA sequence analysis, in vitro transcription and DNase protection studies suggest that the tissue specificity is dependent on a TATA box (TCTTATT at position -25) and a CRE-like element (TGACCTCA at position -44). Each of these two DNA motifs has now been systematically mutated or deleted. We are analyzing each construct for relative efficiency of transcription by in vitro transcription using a rat testis nuclear extract. In vitro transcription in the presence of anti-CREMτ antibody suggests that this trans-activating factor is important in tissue specific expression of tACE.

B 444 DETERMINANTS FOR DNA BINDING SITE RECOGNITION BY THE GLUCOCORTICOID RECEPTOR, Johanna Zilliacus[1], Anthony P. H. Wright[1], Ulf Norinder[2], Jan-Åke Gustafsson[1] and Jan Carlstedt-Duke[1], [1]Center for Biochemistry and Department of Medical Nutrition, Karolinska Institute, NOVUM, S-141 57 Huddinge, Sweden and [2]Karo Bio AB, Box 4032, S-141 04 Huddinge, Sweden

The glucocorticoid receptor is a member of the nuclear receptor family of ligand-inducible transcription factors. The receptor binds with high specificity to glucocorticoid response elements, palindromic DNA sequences consisting of two half sites, discriminating them from other closely related binding sites, such as estrogen response elements. Three amino acids in the recognition helix of the DNA binding domain of the receptor are primarily responsible for this specific DNA binding activity. We have analysed in detail how these residues determine the specific DNA binding by studying a series of mutant glucocorticoid receptor DNA binding domains containing all combinations of glucocorticoid and estrogen receptor specific residues at these positions. DNA binding activity was measured directly using DNA binding domains purified from *E. coli* expression lysates and by using a transactivation based assay in yeast cells. Statistical analysis of the results enables us to create models describing the association between amino acids and base pairs. The results indicate that discrimination between the DNA binding sites by the receptors results from at least three general strategies. First, the proteins seem to use individual amino acids for both positive interactions with correct binding sites and negative interactions with incorrect binding sites thus increasing the discriminative power of each residue. Second, discrimination is accomplished by reducing binding affinity to all sites but predominantly to non-cognate binding sites. Third, binding efficiency is not simply the sum of interactions between individual amino acids and base pairs; amino acid combinations appear to act synergistically to augment discrimination between binding sites.

Cell Cycle

B 500 RAN, A RAS-LIKE PROTEIN INVOLVED IN CELL CYCLE REGULATION, IS HIGHLY CONSERVED BETWEEN HUMAN, S. POMBE, AND PLANTS, Robert A. Ach and Wilhelm Gruissem, Department of Plant Biology, University of California, Berkeley, CA 94720

Unlike animal cells, plant cells do not move during plant growth and development, and plant morphology is thus determined only by cell division and expansion. Cell division in plants is highly regulated, occurring mostly in specialized regions called meristems, and is regulated by plant hormones, as well as by environmental signals such as light and gravity. In order to begin to understand the regulation of cell division in plants, we are interested in isolating genes involved in plant cell cycle control.

We report here the cloning of two cDNAs from tomato which encode ran-like proteins. Ran is a 25 kDa ras-related protein which is very highly conserved between humans and S. pombe, sharing about 80% amino acid identity. Ran has been found to form a stable noncovalent complex with the chromatin-binding RCC1 protein, a negative regulator of mitosis. In S. pombe, a temperature-sensitive mutation of the pim1 gene, a pombe RCC1 homolog, causes premature induction of mitosis, and this mutation can be suppressed by overexpression of the spi1 gene, which encodes a pombe ran homolog. The tomato ran genes encode proteins which are approximately 80% identical to both the human and pombe proteins. Characterization, expression, and a functional analysis of the tomato ran cDNA clones will be reported.

B 501 IDENTIFICATION OF TWO CODING REGION SUBSEQUENCES REQUIRED FOR HIGH EXPRESSION OF A REPLICATION-DEPENDENT MOUSE HISTONE GENE, T.Bowman[1] and M. Hurt[2], Dept. of Chemistry[1] and Dept. of Biol. Sciences[2], Florida State University, Tallahassee, FL 32306.

We have previously identified an element in the protein-encoding sequence of two highly expressed replication-dependent mouse histone genes, a H2a.2 and a H3.2 respectively. The coding region activating sequence (CRAS) of both genes is responsible for a 20-fold drop in expression when deleted (Mol. Cell. Biol., June 1991, p. 2929-2936). Both the H3 and H2a CRAS elements have been shown to bind nuclear proteins and compete with each other for binding of the same nuclear proteins. Oligonucleotide-directed mutagenesis studies have identified two subsequences, box 1b and box 4, in the H3 CRAS which together are responsible for a 20-fold activation of expression of the mouse gene in stably transfected CHO cells. Both H3 subsequences were previously identified by homology studies with the H2a CRAS. The importance of these two subsequences in interactions with nuclear proteins has been confirmed by comparing gel mobility shift assays of oligonucleotide duplexes containing the H3.2 subsequences as well as duplexes containing sequences from the same region from a replication-independent H3.3 gene. Synthetic duplexes containing the two H3.2 subsequences independently bind nuclear proteins from logrithmically growing mouse cells. The box 1b and box 4 duplexes compete for binding with the intact CRAS and have identified the same specific protein complexes formed with the intact CRAS. This binding activity is greatly increased in S phase nuclear extracts when compared to G-1 phase extracts prepared from populations of cells synchronized by mitotic shake-off. Identical results are obtained when using the intact H3 CRAS or the box 1b and box 4 synthetic duplexes in binding assays. DNase I footprint analysis of the box 4 subsequence has identified the exact nucleotides required for protein binding. Purification of the protein(s) which bind the H3 CRAS is under way. Preliminary experiments indicate that two different protein complexes bind the box 1b and box 4 subsequences. Presence of a key phosphate group on the box 4 binding proteins is required for DNA binding activity. Additionally, state of phosphorylation is important in binding of the box 1b subsequence by nuclear proteins.

B 502 ORNITHINE DECARBOXYLASE IS A TRANSCRIPTIONAL TARGET OF *c-myc*, John L. Cleveland, Concha Bello-Fernandez and Graham Packham, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis TN 38101.

Growth factor deprivation of cells results in a rapid down-regulation of *c-myc* expression and subsequent arrest in the G1 phase of the cell cycle. Constitutive *c-myc* expression suppresses cell cycle arrest and promotes entry into S phase. Constitutive *c-myc* expression also results in the growth factor-independent expression of ornithine decarboxylase (ODC). This enzyme is rate-limiting for polyamine biosynthesis and is required for cells to enter S phase. The ODC gene contains a conserved repeat of the *myc* binding site, CACGTG, in intron 1. *c-myc* is a very potent transactivator of ODC promoter-reporter gene constructs in fibroblasts and myeloid cells which requires the CACGTG repeat. Purified *Myc* and *Max* proteins efficiently bind these elements *in vitro* and when fused to a heterologous promoter, this element can function as a *myc*-responsive enhancer. These criteria indicate that *c-myc* is a transcription factor and suggest that ODC is one of its transcriptional targets. Deletion and point mutants were used to define domains of *c-myc* that were required for transactivation. While predictable domains of *c-myc* were required for transactivation (including the B-HLH domain), removal of others did not abolish transactivation. The most surprising region which was dispensable was a large portion of the LZ domain, suggesting that *myc*, at least in the context of transactivation of the ODC promoter, can function independent of its identified heterodimeric partner. Consistent with this possibility, *max* expression vectors fail to influence *c-myc* transactivation of the ODC promoter in co-transfection assays. This raises the possibility that *c-myc* may interact with novel dimerization partners through other HLH-HLH or HLH-LZ interactions to transactivate ODC. Evidence for *max*-independent, but *myc*-specific, complexes from nuclear extracts which bind to these elements will be presented.

B 503 CHARACTERIZATION OF THE *DROSOPHILA* HOMOLOGUE OF THE p53 ANTI-ONCOGENE AND ITS RESPONSE TO DNA DAMAGE, Ruth L. Dusenbery and F. Michael Yakes, Department of Chemistry, Wayne State University, Detroit, MI 48202

Loss of normal p53 gene function is associated with neoplastic transformation *in vitro* and a wide variety of tumors *in vivo*. Evidence links p53 function to transcriptional control of the G1/S transition in the cell cycle. Failure of the normal arrest of cells at the G1/S boundary following DNA damage results in replication of DNA containing unrepaired lesions. This increases levels of mutations and chromosome rearrangements, thus increasing the probability of neoplastic transformation. It is difficult to study these effects *in vivo* in mammalian systems. Previous investigations have shown a high degree of conservation of p53 sequence elements among vertebrates. We report the first evidence of a non-vertebrate p53 homologue in *Drosophila melanogaster*, a system amenable to further biochemical and genetic analysis. Initial identification of the Drosophila p53 homologue was made by its cross reactivity with a monoclonal antibody PAB421, which recognizes both mutant and wild type human p53 sequences. PAGE analysis of S^{35}-labeled proteins immunoprecipitated from Drosophila cell lines yields a major band in the 53kD range, and minor bands at 70kD and 22kD. Western blots of proteins extracted from both cell lines and intact animals, confirms that the 53kD protein reacts directly with the anti-p53 PAB421. PCR cycle sequencingof genomic DNA, using oligo nucleotide primers derived from a highly conserved region of the p53 coding sequence, produces a nearly perfect match to the human p53 sequence. Immunofluorescent detection of the PAB421-p53 complex, localizes the Drosophila p53 homologue to a small region of the nucleus in the majority of cells in an unsynchronized population grown under normal conditions. Significant amounts of p53 were also identified in several tissues of post-embryonic developmental stages, especially in polytene nuclei. However, very little p53 was detected in the nuclei of early embryo stages, indicating a non-maternal origin for the gene product. Unsynchronized cells grown in 1% serum for extended periods exhibit a much lower nuclear level of the p53 homologue. This level increases following transfer to 10% serum-containing media. Heat shock at 37°C for 30 min produced a dramatic alteration in the hsp70 heat shock protein, but does not change the level or distribution of the p53 homologue. Irradiation with 25 J/m^2 UV light, which eliminates S-phase DNA synthesis, modifies the distribution of the p53 signal and increase the level of p53 in the cells for several hours.

B 504 ISOLATION AND CHARACTERIZATION OF MURINE E2F cDNAs. Peggy J. Farnham and Yue Li, Department of Oncology, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

A mouse cDNA library was screened with a probe consisting of the C-terminal half of the human E2F-1 cDNA. Several related cDNAs were obtained, two of which are homologous to the full length human E2F-1 cDNA. These two mouse clones are 87% identical at the nucleotide level to the human clone in the DNA and RB binding domains and 95 - 98% identical at the amino acid level to the human clone in these regions. Sequences 5' of the DNA binding domain and between the DNA and RB binding domains were only 50-60% identical at the amino acid level to the human clone. Other clones were obtained which were more divergent from human E2F-1 and are presently being further characterized.

Northern blotting analysis indicates that there are two E2F-1 mRNAs of 2.7 and 2.2 kb. These two E2F mRNAs are seen in mouse 3T3, L, and macrophage cells lines, and in mouse liver samples. The levels of both E2F mRNAs increase near the G1/S phase boundary, although the timing of appearance of the two is not exactly the same. This results in a change in the ratio of bands at different points in the cell cycle. We are currently investigating the basis of the length difference in these E2F mRNAs. It is our goal to elucidate the mechanism by which the E2F mRNAs are cell cycle regulated. Towards this goal, we are currently sequencing murine E2F genomic clones.

B 505 YY1/UCRBP IS UP-REGULATED IN DIVIDING CELLS IN-VIVO AND IN-VITRO BY A POST-TRANSCRIPTIONAL MECHANISM. James R. Flanagan[1] and John W. Kasik[2]. [1]Departments of Internal Medicine, the University of Iowa and VA Medical Center, Iowa City, IA 52246. [2]Departments of Pediatrics, Case Western University and CMGH, Cleveland, OH.

The ubiquitous transcription regulator YY1, which we reported as UCRBP, has been found to regulate transcription of numerous viral and cellular genes and also binds and interacts with E1A protein. While another E1A-binding, anti-oncogene protein, Rb, may regulate mitotic cycling via a qualitative change in the Rb gene product, here we show that YY1 has properties consistent with cell cycle regulation by quantitative change of YY1. That is, we find increased steady-state levels of YY1 (examined by Northern and/or RNAse protection and nuclear extract protein) in six paired models having one thing in common: cells undergoing frequent cell division compared with those in which few cells are dividing. In vitro models that demonstrate this include: NIH3T3 log-phase growth cells express 5 fold more YY1 RNA than confluent cells; undifferentiated HL60 cells express 5-10 fold more than the non-dividing DMSO-differentiated HL60; rat primary fibroblasts serum-stimulated have 2-3 fold more than serum-starved. In vivo models include: cell lines (NIH3T3, L cells, B and T cell lines) have over 10 fold more YY1 RNA and YY1 protein than adult mouse tissues; placenta has 5-10 fold more YY1 RNA than uterus or other maternal tissues from a pregnant mouse; fetal liver has 5 fold more YY1 RNA than maternal or male liver. A nuclear run-on assay comparing the NIH3T3 confluent vs dividing cells (similar results will be shown for the HL60 and fibroblast models) demonstrates little or no difference in rate of YY1 gene transcription despite the difference in steady-state levels. This implication of post-transcriptional regulation is interesting in view of the unusually high conservation (between human and mouse) of the 3' untranslated (3'UT) region of the YY1 cDNA. We will also present data on the effect of the 3'UT on RNA stability in heterologous context. We propose that increased YY1 synthesis is essential for regulation primarily in dividing cells.

B 506 TRANSCRIPTIONAL REGULATION OF THE DELAYED EARLY GROWTH RESPONSE GENE ORNITHINE DECARBOXYLASE. M.A. Flanagan[1], J.R. Cooper[1], M. Skunca[2], C.W. Woods[1], and J.A. Moshier[2], [1]Marion Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215 and [2]Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI 48201.

Maximal activity of the growth-obligatory enzyme ornithine decarboxylase (ODC) is seen in mid-G_1 of the cell cycle following serum-stimulation of quiescent cells. In this study it was shown in IMR90 cells, normal human fibroblasts, that this increase can be accounted for by increased transcription of the gene. The sequences in the ODC promoter binding to nuclear protein isolated from IMR90 cells were determined using the DNase I protection assay. No qualitative differences in the protein binding pattern were found between quiescent and serum-stimulated cells, but quantitative differences were detected using the electrophoretic mobility shift assay (EMSA). The four distal sequences in the ODC promoter binding to nuclear protein contain GC-boxes, putative Sp1 binding sites. The most abundant protein binding to these sites has been identified by EMSA as Sp1. It was shown that Sp1 binding activity is low in quiescent cells, and that it returns to log phase values when quiescent cells are serum-stimulated. It is postulated that Sp1 acts to enhance transcription of the ODC gene in response to serum-stimulation. The proximal sequence in the ODC promoter binding to protein, directly adjacent to the TATA box, was shown to form three nucleoprotein complexes, FPIA-1,-2 and -3. None of the proteins binding in these complexes were competed by the somatostatin cAMP responsive element (CRE), although this region of the promoter contains a putative CRE in all mammalian ODC genes sequenced to date. The proteins binding in the complexes FPIA-2 and -3 were competed by the human c-fos serum response element (SRE), while the protein binding in complex FPIA-1 was not. Point mutations of the SRE were used to tentatively identify the proteins binding to FPIA-2 and -3 as YY1 ($p62^{DBF}$) and a proteolytic fragment of YY1, respectively. It was found that the formation of one of these complexes, FPIA-2, was reduced when quiescent cells were serum-stimulated. The formation of complex FPIA-1 was unaffected. Recently, YY1 was shown to compete with the serum response factor (SRF) for binding to the SRE *in vitro* and to repress serum-inducible and basal expression from the c-fos promoter when over-expressed *in vivo* (Gualberto *et al.* (1992) *Mol. Cell. Biol.* **12**: 4209). Taken together with the results of the present study, this suggests that YY1 may act to repress transcription of the ODC gene in quiescent cells, and that this repression is reversed by serum-stimulation.

B 507 TRANSCRIPTIONAL DOWN REGULATION BY THE RETINOBLASTOMA PROTEIN IS MEDIATED THROUGH E2F, ATF AND OTHER SITES. Ullrich Graeven, Nobuo Horikoshi, & Roberto Weinmann. The Wistar Institute, Philadelphia, PA 19104.

Inactivation of the retinoblastoma susceptibility gene product (pRB) has been linked to a variety of human tumors. pRB negatively regulates cellular proliferation by controlling the passage through the cell cycle in late G1. The interaction between pRB and the transcription factor E2F has led to the notion, that pRB might function by modifying the activities of transcription factors. We examined the effect of pRB overexpression on the activity of different promoter constructs. Adenovirus E2- and histone 2a promoter constructs showed reduced promoter activities in the presencce of pRB in pRB negative 5637 bladder carcinoma cells. Similar results were obtained in J82 bladder carcinoma cells (exon 21 pRB deletion mutant). In pRB positive cells, expressing SV40 T (FM516SV) or papillomavirus E7 (Hela), overexpression of pRB also reduced promoter activities. Analysis of sites involved in this down regulation, identified E2F sites as targets in all cell lines. ATF sites mediated down regulation in both bladder carcinoma cell lines and to a lesser degree in Hela cells, whereas no effect was observed in FM516SV cells. SP1 sites were also down regulated by pRB. The variability of the response to pRB in different cell lines suggests that cell specific levels of transcription factors may influence the pRB responsiveness of these promoters.

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B 508 E2F IS RELATED TO THE ETS FAMILY OF TRANSCRIPTION FACTORS: IMPLICATIONS FOR EARLY GENE TRANSCRIPTION, Scott W. Hiebert, Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN 38101.

E2F is a cellular transcription factor targeted by the E1A proteins of Ad5 for the stimulation of the adenovirus E2 promoter. Recent evidence suggests that this stimulation is brought about by the dissociation of E2F from protein-protein interactions involving the product of the retinoblastoma gene (RB1) and the pRb related p107 protein. The recent determination of the amino acid sequence of E2F-1 suggested no similarities with other known transcription factors. However, comparison of the DNA binding sites of E2F and the ets family of transcription factors suggest that they may bind similar sites and thus contain similar motifs within their respective DNA binding domains. Visual comparison of these domains identified a short region of near perfect identity. Gel mobility shift analysis using a GST-ets-2 fusion protein indicates that ets-2 can bind the Ad5 E2 promoter, the minimal c-myc promoter and the minimal DHFR promoter all containing the E2F binding motif.

To determine whether the conserved residues in E2F are required for DNA binding, a small fragment of E2F (residues 95-191) was subcloned by PCR amplification into pGEX-2T to create a small GST-E2F fusion protein. This protein not only binds DNA but forms multimeric protein-DNA complexes suggesting that E2F binds DNA as a dimer. Further gel mobility shift analysis of this fusion protein suggests that two E2F dimers can interact with the dyad E2F binding site from the c-myc and DHFR promoters (TTTCGCGCCAAA). Substitution of the conserved domain in E2F with the corresponding domain of ets-2 eliminated the ability of E2F to bind DNA suggesting that the overall domain structure is important for E2F/DNA interactions.

The relevance of these findings to the regulation of cell cycle responsive genes containing E2F binding sites, including c-myc and DHFR, will be discussed.

B 509 MODULATION OF THE ACTIVITY OF THE HUMAN E2F TRANSCRIPTION FACTOR BY VIRAL ONCOPROTEINS AND DURING THE CELL CYCLE, P. Jansen-Dürr, M. Eilers[1], M. Pagano[2], D. Spitkovsky, J. Botz, A. Schulze, S. Joswig; Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum and [1]ZMBH and [2] EMBL; D-6900 Heidelberg/FRG.

We are interested in the modulation of the transcriptional activity of cellular genes by viral transforming proteins. In particular we study the effects exerted by the E7 gene products of human papillomaviruses on a subset of cellular genes, the expression of which is correlated with increased cell proliferation. The transcription factor E2F, controlling activity of these genes, has been shown to form complexes with pRB as well as with cyclin A. We show that binding of E2F to pRB but not to cyclin A is abolished by E7. One function of cyclin A is to target the cdk2 kinase subunit to the E2F protein during S phase, concomitant with cell cycle-regulated activation of this transcription factor. Evidence for a positive role of cyclin A in the process of E2F activation is provided by our observations that i) activation of MYC induces E2F by increasing the levels of cyclin A and ii) that cotransfection of a cyclin A expression vector directly activates E2F-dependent transcription.

B 510 HUMAN PAPILLOMAVIRUS E6 AND E7 PROTEINS ABROGATE TUMOR SUPPRESSOR-MEDIATED TRANSCRIPTIONAL REPRESSION. Laimonis A. Laimins, David H. Mack, and Mark S. Lechner, Department of Molecular Genetics and Cell Biology, Howard Hughes Medical Institute, 920 E. 58th Street, The University of Chicago, Chicago, Illinois 60637.

Evidence has been provided that p53 may act as a transcriptional regulator and that this activity may play a role in transformation. We have observed that in transient transfection assays coexpression of wild-type human p53, but not a mutant p53, results in a dose-dependent repression of gene expression from multiple promoters, including the human papillomavirus (HPV) type 18 early gene promoter. In cotransfection experiments with a fixed amount of reporter plasmid together with increasing amounts of expression plasmid encoding wild-type human p53, CAT activity was repressed by as much as 90%. In similar experiments using a plasmid encoding a mutant p53 no significant effect on CAT expression was observed. Of the promoters tested, wild-type p53 had no effect on the human proliferating cell nuclear antigen (PCNA) or human ras promoter driving CAT expression. Interestingly, all sequences unaffected by p53 expression lack a characteristic TATA box. This category of promoters is often involved in expression of genes involved in cell growth control. To determine if HPV E6 proteins could influence the inhibitory effect of wild-type p53 on gene expression, we cotransfected increasing amounts of HPV11, -16, or -18 E6 expression vectors with a fixed amount of reporter and p53 expression plasmid in human keratinocytes. Data will be presented which demonstrate that binding of E6 to p53, but not its enhanced degradation, is sufficient to relieve the inhibitory effect on gene expression in transient assays.

In addition to p53, we find that cotransfection of human Rb expression plasmid with the HPV18 URR reporter construct also results in transcriptional repression in a dose-dependent manner in human keratinocytes. We believe that this repression is mediated through the c-fos gene product which is involved in AP-1 activation of HPV-18 expression. We demonstrate that coexpression of HPV18 E7 protein in this system abrogates the inhibitory effect of Rb on HPV18 expression. Taken together, these data indicate that as a consequence of their interactions with tumor suppressors, E6 and E7 may contribute to the transcriptional regulation of the HPV18 URR.

B 511 CONTROL OF TRANSCRIPTION FACTORS BY MU-BETA-GALACTOSIDE BINDING PROTEIN, A NEGATIVE REGULATOR OF CELL REPLICATION. Livio Mallucci, Valerie Wells, *Gerard I. Evan, *Trevor D. Littlewood, **Nicholas B. La Thangue, **Lasantha R. Bandara. Laboratory of Cellular and Molecular Biology, Microbiology Division, Guy's Medical School, London SE1 9RT; *Imperial Cancer Research Fund Laboratories, London EC1A 7BE; **National Institute for Medical Research, London NW7 1AA, UK.

Murine beta-galactoside binding protein (GBP) is an autocrine negative growth factor which has three key properties: (i) cell stage specificity, (ii) receptor mediated action and (iii) cytostatic effect. It binds with high specific affinity to 5 x 10^4 specific cell surface receptors and both as a regulatory molecule and as a cytostatic factor, it controls exit from GO and traverse through late S-G2 into mitosis (Wells and Mallucci: Cell 1991, 64, 91-97; B.B.A.1991, 1089, 54-60; B.B.A.1992, 1121, 239-244; Genomics, 1992, in press). We have examined the effect of GBP on early growth response genes and their cognate proteins, Fos, Jun, Myc and Egr-1/NGF-1A, and on pRB, p107 and E2F/DRTF-1. Our data show that treatment with GBP correlates with absence of Fos expression and that it affects E2F/DRTF-1, a cellular transcription factor regulated by pRB and by p107, both in the exit from GO and during progression towards G2. Different transduction pathways may be used by GBP in the control of G0/G1 transition and of S/G2 transition.

B 512 THE HUMAN PAPILLOMAVIRUS E7 ONCOPROTEIN AND THE CELLULAR TRANSCRIPTION FACTOR E2F BIND TO SEPARATE SITES ON THE RETINOBLASTOMA TUMOR SUPPRESSOR PROTEIN, Karl Münger, Erica W. Wu*, Karen E. Clemens and Donald V. Heck*, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda MD 20892

The ability of "high risk" and "low risk" HPV encoded E7 oncoproteins to disrupt complexes of the retinoblastoma tumor suppressor protein pRB and the cellular transcription factor E2F was studied. The ability of E7 to disrupt this transcription factor complex concured with the different pRB binding efficiencies of the "high risk" and the "low risk" HPV encoded E7 proteins. The pRB binding site was the sole determinant for these observed differences. The phosphorylation status of the casein kinase II site that is immediately adjacent to the pRB binding site in E7 had no marked effect on this biochemical property of E7. Peptides consisting of the pRB binding site of E7, however, were not able to disrupt the pRB/E2F complex. These data suggest that additional carboxy terminal sequences in E7 are also required for the efficient disruption of the pRB/E2F complex and that E7 and E2F may interact with non-identical sites of pRB.

*E.W.W. and D.V.H were supported by the Howard Hughes Medical Institutes - National Institutes of Health Research Scholars Program.

B 513 SUBTRACTIVE CLONING IDENTIFIES A HEAT-SHOCK GENE AS A MYC-INDUCED cDNA, Linda J.Z. Penn, Jasmine Daksis and Rich Lu, Department of Microbiology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada, M5G 1X8

Our understanding of precisely which genes are regulated by Myc or to what biological end remains unclear. To address this issue, we have cloned Myc-induced genes by a subtractive hybridization approach with a Myc-inducible Rat-1 cell line. The inducible c-myc system is that of the "Myc/ER" fusion protein which carries out Myc-specific functions in an estrogen-dependent manner. In quiescent Rat-1 cells, induction of Myc/ER induces progression from the G1 to S phase of the cell cycle and subsequent apoptosis. Therefore, genes involved in both the positive and negative growth regulatory activities of Myc should be effected in these cells. We conducted subtractive hybridization of confluent, quiescent control Rat-1 cells with Rat-1 cells expressing the activated Myc/ER fusion protein for 6h, and have identified six cDNAs whose elevated expression is an early consequence of Myc activation in Rat-1 cells.

Nucleotide sequence analysis of a small portion (250bp of 4kb) of one of the cDNA clones representing a Myc-induced gene (MIG), MIG 36, revealed a putative open reading frame which when compared with those in the GenBank sequence data base, was shown to possess 91.7% sequence homology to mouse heat shock protein (HSP) 89-alpha. Further analysis showed that RNA expression of other members of the heat shock gene family, was not affected by Myc/ER activation. Moreover, DNA slot blot and Southern blot analysis showed MIG36 to cross-react with HSP 89-alpha, but not HSP 89-beta, HSP 70 or HSC 70 DNA. This data strongly suggests MIG-36 is related to the rat HSP 89-alpha gene. Comparison of additional nucleotide sequence data and protein analysis is required to firmly establish the relationship of MIG-36 to the HSP 89-alpha gene.

Kinetic analysis of MIG36 RNA expression in activated Rat-1 MYC/ER cells, shows an induction within 1h which proceeded to gradually increase over the following 24h. Although Myc is an early growth-response gene, elevated expression of MIG36 was not evident following serum-treatment of nutrient-starved control Rat-1 cells. This result indicates the serum-induced expression of endogenous Myc does not, in turn, lead to MIG36 induction, which further suggests MIG36 may not be involved in the positive growth-regulatory activities of Myc, but may have a role in the apoptotic pathway. The transcriptional nature of this regulation will be discussed.

B 514 REGULATION BY SERUM RESPONSE FACTOR *IN VIVO*, Ron Prywes and Finn-Eirik Johansen, Dept. of Biological Sciences, Columbia University, New York, NY 10027.

Transcription of the c-fos proto-oncogene is activated rapidly by growth factors in mammalian cells. The Serum Response Element (SRE) in the c-fos promoter can mediate this activation. Serum Response Factor (SRF), which binds specifically to the SRE, has been shown to be required for serum activation of the fos gene. We have investigated how cellular signalling pathways cause SRF to activate fos transcription. No changes, however, have been found in SRF's cellular localization, DNA binding activity, phosphorylation, or ability to complex with other factors.

We have now tried to map critical domains of SRF *in vivo* to determine regions essential for growth factor regulation. We have made chimeric GAL4-SRF fusion genes since all cells tested contain endogenous SRF. The fusion gene was tested for activation of a minimal promoter containing GAL4 binding sites. While we have not been able to render this promoter growth factor responsive, this system has allowed us to identify SRF's transcriptional activation domain (amino acids 339-508 in Hela cells and 414-508 in NIH3T3 cells) and two domains which appear to inhibit the function of the activation domain. (These domains have ends in the region of the DNA binding and dimerization domains between amino acids 140 and 171 and 172 and 204, respectively.)

We have developed a system where SRF does render a reporter gene responsive to epidermal growth factor (EGF). We have used a low affinity SRF binding site on a reporter gene. This construct was only weakly EGF-responsive when transfected into Hela cells. Overexpression of SRF, however, caused expression from this reporter gene to be strongly induced by EGF. An interesting mutant of SRF was isolated which caused constitutive expression of the reporter gene. In this mutant, we exchanged 31 amino acids of SRF's DNA binding domain (amino acids 135 to 165) with that of MCM1, a yeast gene which is similar to SRF in this region. This result suggests that this domain of SRF may be a target for regulation in response to EGF. The effects of other mutations of SRF in this assay will be discussed.

B 515 CHOLERA TOXIN POTENTIATES TPA-DRIVEN MITOGENIC RESPONSES AND C-FOS INDUCTION. Beth Runnels+*, Miriam Smyth+, Walker Wharton+*; +Los Alamos National Laboratory, Life Sciences Division, Los Alamos, NM, 87545; *University of New Mexico, Dept. of Pathology, Albuquerque, NM, 87131.

Mitogen-stimulated signal transduction pathways and their downstream targets have proven to be extremely complex. Using a Balb/c 3T3 fibroblast-derived cell line, A31T6, we have studied the role of specific signaling pathways and second messengers in mitogenesis. In the presence of insulin, A31T6 cells showed a weak mitogenic response, as measured by ^{3}H-thymidine incorporation, to cholera toxin, a stimulator of adenylate cyclase activity, and a strong response to phorbol ester (TPA), an activator of PKC. However, addition of both cholera toxin and TPA synergistically enhanced the response significantly beyond the added individual responses. To determine if this mitogenic synergism could be seen at the gene expression level, we investigated expression of the c-fos gene, an early-intermediate gene which is known to respond within 15 minutes to mitogenic induction. In the presence of cycloheximide, endogenous c-fos gene expression in A31T6 cells, as measured by RNAase protection assays, was minimally induced by cholera toxin, significantly induced by TPA and synergistically induced by the combination of the two mitogens in a manner paralleling the mitogenic response. Cycloheximde by itself or in conjunction with either CT or TPA had no effect beyond that seen with each mitogen alone. This induction pattern could be seen in both density-arrested cells and in proliferating cells. Further studies characterizing the mechanism of synergistic response by the fos promoter are currently being conducted.

B 516 TRANSCRIPTIONAL REPRESSION OF CYCLIN A,B1 AND CDC2 GENES AT NONPERMISSIVE TEMPERATURE IN TSBN462 CELLS.
Takeshi Sekiguchi,Toshiro Hayashida,Torahiko Nakashima, Takeharu Nishimoto,·Department of molecular Biology,Graduated school of medical science, Kyushu University, Maidashi, Fukuoka, 812, Japan
Human CCG1 gene complements tsBN462, a ts mutant of BHK21, defective in the progression of G1 phase. The excurtion point of the mutation was found to be around 10hr after release from serum starvation. To know whether there could be specific proteins which would *disappear* at a non-permissive temperature(39.5°C), we performed the two dimensional gel electrophoresis of the ^{35}S-Met pulse labelled proteins of tsBN462 protein at 39.5°C and 33.5°C. An intensity of protein spots at 39.5°C at 6-8 hr after serum stimulation did not change when compared with that at 33.5°C, but at 13-15hr, the intensity of almost all protein spots decreased. This result suggests that the mutation affect the expression of the genes of G1/S boundary. So, we studied the expression of the immediate early genes, fos, jun, myc and G1/S boundary genes, cyclin A,cyclin B1,cdc2 and cdk2. The results of northern blottings showed that immediate early genes and cdk2 genes expressed at 39.5°C, but other genes did not express. From these results, we suppose that the CCG1 gene is involved for expression of a set of G1/S boundary genes, such as cyclin A,B1 and cdc2, but not of other set of genes, such as cdk2.

B 517 **E2F PLAYS A CRITICAL ROLE IN THE CELL CYCLE CONTROL OF DHFR.** Jill Slansky and Peggy Farnham, Department of Oncology, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

Progression of cells through the cell division cycle is regulated by interconnecting signal transduction pathways that result in the transient activation and repression of growth-responsive genes. We are using the dihydrofolate reductase (dhfr) gene, which is involved in nucleotide biosynthesis, as a model system to study cellular events controlling the entrance into the S phase of the cell cycle. Analysis of RNA from serum starved and restimulated NIH 3T3 cells has demonstrated that the increase in DHFR mRNA at the G1/S phase boundary requires protein synthesis. This suggests that a protein(s) is lacking in G1 cells that is required for DHFR transcription. Our previous results have shown that the HIP/E2F element at the DHFR transcription initiation site is necessary for DHFR cell cycle regulation. We have also demonstrated that the HIP/E2F site alone, in the absence of all other promoter elements, is sufficient for cell cycle-regulated promoter activity. Our recent results suggest that E2F is a limiting component in G0 and G1 cells. First, Northern analysis reveals that murine E2F-1 mRNA is not present in G0 or G1 cells. The levels of murine E2F-1 mRNA increase at the G1/S phase boundary in a protein synthesis-dependent manner. Second, an E2F-1 expression plasmid cotransfected with the DHFR promoter fused to a reporter plasmid results in a 22-fold increase in reporter activity in quiescent cells. Third, constitutive expression of E2F-1 abolishes cell cycle regulation from the DHFR promoter. In summary, we have demonstrated the importance of E2F in the cell cycle regulation of DHFR and are now investigating the cellular consequences of altered E2F expression.

B 518 DEFINITION OF MID- AND LATE G1 PHASE EVENTS IN DMSO-ARRESTED LYMPHOID CELLS INDUCED TO RE-ENTER THE CELL CYCLE: DIFFERENTIAL REGULATION OF THE CYCLIN-DEPENDENT KINASES cdc2 AND cdk2. Kozo Takase, Naohiro Terada, Joseph J. Lucas, Hirobumi Teraoka and Erwin W. Gelfand. National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado, 80206, and Tokyo Medical and Dental University, Tokyo 113, Japan.
Dimethyl sulfoxide (DMSO) has been shown to induce arrest of several lymphoid cell lines in a G0-like or very early G1 phase. Using the Raji Burkitt's lymphoma cell line, we determined that greater than 90% of cells accumulated with a 2N content of DNA following 96-120 hr of treatment with 1.5% DMSO. After removal of DMSO, entry into S phase was first observed at about 24 hrs. Because of the synchronous manner in which a high portion of the cells entered and progressed through the cell cycle, the system proved useful in delineating events occurring throughout the G1 phase. Two major stages of G1 phase progression could be delineated. The first was characterized by the increased synthesis and activation of the cdk2 kinase and the initial phosphorylation of Rb, the product of the retinoblastoma susceptibility gene. These events occurred in mid-G1 phase, about 15 hrs after release from DMSO-induced arrest. A second set of events, including the accumulation of PCNA (proliferating-cell nuclear antigen) and the first appearance of the cdc2 kinase and cyclin A , occurred later in G1 phase, closer to entry into S-phase. It was clear in this synchronized system of cells exiting the resting (G0-like) state that phosphorylation of Rb was initiated well before the first appearance of the cdc2 kinase. *In vitro* studies confirmed that recombinant Rb protein could serve as a good substrate for the cdk2 kinase, prepared by immunoprecipitation using antibodies specific for this enzyme. The system provides an excellent means for defining the specific roles of cdc2 and cdk2, and their associated cyclins, in human B lymphocyte cell cycle entry and progression.

B 519 THE DIFFERENTIAL REGULATORY CAPACITIES OF C-MYC AND MAX, Sean V. Tavtigian, Sonya D. Zabludoff, and Barbara J. Wold. Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125

The process of emergence from G_0 growth arrest and progression across G_1 to the restriction point is the major control point in regulation of vertebrate cell proliferation. Among cellular oncogene/transcription factors that are conspicuously up-regulated at the G_0/G_1 transition, c-myc stands out because its conditional expression can drive a large fraction of G_0 arrested cells through G_1 into S-phase.

We have cloned a group of cDNAs that are either up- or down-regulated at a mid-G_1 point in the serum response (mid-SR) with the expectation that these would include direct and indirect targets of immediate early regulators. Using conditional c-myc- and conditional Max-expressing cell lines, we have identified target genes among the immediate early and mid-SR transcript classes and characterized their pattern of expression in response to one, the other, or both regulators.

Conclusions drawn from this work add three new elements to current thinking about how c-myc and its co-regulator Max work: (1) Max probably plays a more complex role than merely facilitating the function of myc family proteins. (2) We observed that a subset of the c-myc:Max target transcripts (among the panel of mid-G_1 markers) were regulated in opposite directions during mitogen- versus c-myc:Max-driven $G_0 \rightarrow$ S-phase progression. These data suggest that both the similarities and the differences between these two stimulation regimes contribute to the biological consequences of deregulated c-myc expression. (3) The assymetric dimerization specificities of the c-myc and Max proteins, taken together with the biological consequences of their overexpression, suggest that the phenotypes associated with c-myc overexpression are the consequence of simultaneous overexpression of c-myc:Max heteromers and underexpression of Max homodimers.

B 520 THE TRANSCRIPTION OF THE DELAYED EARLY GENE T1 IS REGULATED BY C-FOS AND C-MYC

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The T1 mRNA was initially found after expression of the oncoproteins Ras or Mos in mouse fibroblast cells (NIH 3T3). Transcription of T1 can also be stimulated by growth factors in serum. Peak levels of T1 mRNA are reached in 4 to 6 hrs, following serum stimulation of quiescent cells. The induction is dependent on protein synthesis.
We identified an 80 bp long sequence which stimulates the expression of the transcription. It contains one TRE (TTAGTCA), two E-Boxes (CACATG) one OctA and one Egr site. The sequence lies between positions - 3631 and - 3552 bp upstream of the transcription initiation site of the T1 gene. This 80 bp fragment was cloned in front of a TK-minimal-promotor CAT-construct (pBLcat2). This construct (XB-CAT) strongly stimulates transcription in transient transfection assays. Point mutations were introduced into the TRE, or into one or both E-boxes. The original and the mutated forms of XB-CAT were tested in transient transfection assays. All mutated forms showed a significantly decreased activation of transcription compared to XB-CAT in transient transfection assays. Co-transfection of a c-Fos expression plasmid stimulates transcription as strongly as serum. To test the effect of Myc on the T1 promoter, we co-transfected either a dominant negative mutation of the c-Myc or a c-Myc expression plasmid with XB-CAT. The dominant negative mutation represses the transcription of the CAT-gene, whereas the expression plasmid increases the transcription rate.

B 521 ALTERNATIVE *max* mRNAs AND PROTEINS

Imre Västrik[1], Päivi J. Koskinen[1], Riitta Alitalo[2], Tomi P. Mäkelä[1] and Kari Alitalo[1], [1]Laboratory of Cancer Biology, Departments of Virology and Pathology, University of Helsinki, and [2]Transplantation Laboratory, Helsinki University Central Hospital, Haartmaninkatu 3, 00290 Helsinki, Finland

The *max* gene encodes a heterodimeric partner of Myc. We have recently identified an alternative *max* mRNA (Δ*max*; Mäkelä et al., 1992), which contains an additional internal exon introducing an in-frame translational termination. Thus ΔMax lacks the nuclear localization signal and putative regulatory region in the C-terminus. Still, ΔMax has retained the ability to heterodimerize with Myc and bind to the CACGTG motif. Northern blotting analysis of human *max* mRNAs revealed the presence of four other bands in addition to the major 2.4 kb mRNA form. Our results indicate that these bands represent differentially spliced mRNA forms, which contain altogether three open reading frames (Västrik et al., 1993). In addition to the previously identified Max and ΔMax proteins, sequence analysis of a 3.5 kb mRNA form predicted a protein that resembled ΔMax in structure. Similarly to ΔMax this protein enhanced the number of transformed foci in the *ras-myc* cotransformation assay. Although the 3.5 kb mRNA represents a minor form in actively proliferating cells, a shift from the major 2.3 kb mRNA to the 3.5 kb form was observed in response to acidification of the growth medium. Our results indicate the presence of several differentially spliced mRNA forms of the *max* gene, and suggest a possible mechanism for the production of functionally distinct Max proteins.

Mäkelä, T.P., P.J. Koskinen, I. Västrik and K. Alitalo. 1992. Alternative forms of Max as enhancers or suppressors of Myc-Ras cotransformation. *Science* 256: 373-377.

Västrik, I., P.J. Koskinen, R. Alitalo and T.P. Mäkelä. 1993. Alternative mRNA forms and open reading frames of the *max* gene. *Oncogene*, in press.

B 522 CELL GROWTH AND CELL CYCLE REGULATION OF THE MAMMALIAN TRANSCRIPTION FACTOR LSF

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The DNA-binding activity of the mammalian transcription factor LSF (H.-C. Huang, et al, Genes Dev. 4:287-298, 1991) has been examined as a function of the growth state of the cell. Growth regulation from resting cells was examined in two systems. In human peripheral T cells, a dramatic stimulation (5 to 10-fold) of LSF DNA-binding activity was observed after mitogenic stimulation of the resting T cells. In A31 tissue culture cells, LSF DNA-binding activity slowly decreased upon starvation of the cells in low serum. Shortly after stimulation of the resting cells with high levels of serum, LSF DNA-binding activity increased several fold. Upon examination of the protein during the G0 to G1 transition, both by immunoprecipitation and Western analyses, the electrophoretic mobility of LSF decreased upon stimulation with serum, due apparently to a phosphorylation event. This modification is likely to be the cause of increased DNA-binding activity, since dephosphorylation of LSF *in vitro* decreases its DNA-binding activity. Upon examination of LSF DNA-binding activity through the cell cycle, we reproducibly observed a peak of activity corresponding with late G1 phase. These data derive in part from elutriation experiments, in which a striking peak of activity was observed corresponding to fractions containing decreasing numbers of cells in G1 and increasing numbers of cells in S. Synchronization of cells in early G1 with the drug lovastatin and release of cells from the block allowed a more precise determination of the point of peak LSF DNA-binding activity, as being in late G1. In all cases, supershift experiments using LSF-specific antibodies proved that the altered levels of DNA-binding activity were due to LSF. Thus, increases in DNA-binding activity of LSF are observed both upon stimulation of resting cells to grow and in cycling cells in late G1. LSF binds cellular promoters activated around these stages in the cell cycle: the *c-fos* and thymidylate synthase promoters.

B 523 IDENTIFICATION OF A p53-REGULATED GENE INVOLVED IN A CELL-CYCLE CHECKPOINT ACTIVATED BY DNA DAMAGE.

Qimin Zhan, Michael B. Kastan*, France Carrier, M. Christine Hollander, and Albert J. Fornace, Jr., Lab. of Molecular Pharmacology, N.I.H., Bethesda, MD, and Johns Hopkins Oncology Center, Baltimore, MD.

Cell-cycle checkpoints can enhance cell survival and limit mutagenic events following DNA damage. In mammalian cells the G_1 checkpoint activated by ionizing radiation (IR) has recently been found to be dependent on the p53 tumor suppressor[1]. p53 is a sequence-specific DNA-binding protein that can activate transcription when the consensus binding site is linked to a reporter gene[2]. We have now determined that the IR-responsiveness of the human *GADD45* is dependent on a *wt* p53 phenotype. This gene has previously been found to be IR-inducible in human cells, and its expression has been associated with certain types of growth arrest[3]. After IR, *GADD45* mRNA rapidly increased in 6 cell lines that have *wt* p53 genes; of the 5 tested, all show an intact G_1 cell-cycle delay after IR. In contrast, 5 cell lines with mutant or absent p53 genes lacked appreciable induction after IR and also lacked the G_1 checkpoint. This included a previously *wt* p53 cell line that had been transfected with mutant p53. Both the human and hamster *GADD45* genes contain a conserved element corresponding to the previously[2] determined p53 consensus sequence. A p53-containing nuclear factor, which bound this element, was detected in extracts from irradiated cells. This is the first demonstration of a cellular gene regulated by p53 and also the first demonstration of sequence-specific binding for normal cellular p53. A p53-mediated signal transduction pathway, controlling cell cycle arrest, is proposed involving the induction of *GADD45* and possibly other cellular growth-arrest genes. Abnormalities in this pathway probably contribute to tumor development. Features of *GADD45* regulation will be discussed.

1 Kuerbitz S.J., Plunkett, B.S., Walsh, W.V., and Kastan, M.B.: Proc. Natl. Acad. Sci. *89*: 7491-5, 1992.
2 El-Deiry, W.S., Kern, S.E., Pientenpol, J.A., Kinzler, K.W., and Vogelstein, B.: Nature Genetics *1*: 45-9, 1992.
3 Papathanasiou, M.A., Kerr, N., Robbins, J.H., Mc Bride, O.W., Alamo, I., Jr., Barrett, S.F., Hickson, I., and Fornace, A.J. Jr.: Induction by ionizing radiation of the *gadd45* gene in cultured human cells: lack of mediation by protein kinase C. Molec. Cell. Biol. *11*: 1009-1016, 1991

Rel/NF-kB Family

B 524 INTERACTION OF MCM1 WITH MEMBERS OF THE κB/REL FAMILY, Cyrille Alexandre and Michael Gilman, Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, NY 11724.

The SRE is a multifunctional element localized at -300 in the c-*fos* promoter. This element is the binding site for several DNA binding proteins including SRF, SAP1, ELK1, YY1, and ZBP. Since the multifunctional nature of the SRE cannot be attributed solely to the binding of SRF, it is suspected that protein-protein interactions between SRF and accessory factors are required for the transcriptionnal activation of c-*fos*. Recently, an SRF homolog, termed MCM1, has been identified in yeast. Like SRF, MCM1 binds to a similar site and acts in conjunction with other accessory factors like Matα1, Matα2 and STE12. In both cases, these multiprotein complexes are required for gene induction by extracellular signals. Because SRF and MCM1 are closely related in structure and function, we have used a genetic approach in yeast to identify novel accessory proteins important for MCM1/SRF function. We have constructed a yeast strain in which the expression of a selectable marker (*HIS3*) is dependent on the MCM1/Matα1 complex. We have deleted Matα1 from this strain and selected for human cDNAs that restore the His^+ phenotype. Restriction analysis and sequencing revealed that 6 of the 11 clones contained a cDNA derived from the gene encoding the transcription activator p65/NFκB. p65/NFκB is a member of the rel/dorsal family and is one subunit of the heterodimeric p50-p65 NFκB transcription factor. After transformation of yeast strains carrying the HIS3 gene under the control of 3 NFκB sites or a mutated MCM1 binding site with p65, we were able to show that (i) p65 cannot activate the transcription of the HIS3 gene containing 3 NFκB sites and (ii) the mutation in MCM1 binding site drastically reduced the activation of transcription by p65. These results strongly suggest that p65 does not bind DNA directly and cooperates with MCM1 for activation of the HIS3 gene. Mutagenesis of p65 has revealed that the rel domain is necessary and sufficient for the activation of the HIS3 gene. Other members of the rel family have also been tested: c-rel and dorsal are capable of activating the HIS3 gene, while the p50 NFκB is not. Further experiments are now underway (i) to characterize more precisely the MCM1-p65 interaction in vitro and in vivo and subsequently the putative SRF-p65 interaction and (ii) to study the possible role of p65 in the regulation of the c-*fos* gene in mammalian cells.

B 525 A BRAIN-SPECIFIC BETA-LIKE κB-BINDING FACTOR. Georgy Bakalkin,Tatjana Yakovleva and Lars Terenius, *Department of Drug Dependence Research, Karolinska Institute, S-10401 Stockholm, Sweden*

Three main κB-binding factors I, II and III, presumably p65/c-Rel heterodimer, NF-κB and p50 homodimer, respectively, were identified in embryonal and neonatal rat brain and one (factor II) in adult rat brain with a band shift assay and by UV cross-linking. Factors I and II were activated by DOC. A minor κB-specific factor A, distinctive to the brain in the adult rat, showed very low gel mobility and was sensitive to DOC treatment. Therefore, it may be identical to a previously described brain-specific factor, BETA [M. Korner et al., Neuron 3 (1989) 563-572]. Expression of this factor was stable in the rat brain during development. It was also found in embryonal, but not in adult, rat liver. Seven human cell lines studied (neuroblastoma, small cell and large cell lung carcinomas and lymphoblast-like cells) also produced one to four minor κB-specific factors with low electrophoretic mobility similar to factor A. Factor A is homo-oligomeric, composed of a 116 kDa subunit, the others are hetero-oligomeric, composed of p116 in complex with other κB-specific subunits, that seem identical to p50, p65 and c-Rel, in different combinations. The κB-binding p116 subunit may be identical to a 115 kDa protein coimmunoprecipitated with c-Rel [T.Kochel and N.R. Rice, Oncogene 7(1992) 567-572]. Different combinations of κB-proteins with p116 could allow for selective regulation via individual κB-motifs.

B 526 IN VIVO MECHANISMS INVOLVED IN THE CYTOPLASMIC RETENTION AND RELEASE OF NF-κB FROM IκB, Amer A. Beg[1,2] , Timothy S. Finco[1,3] and Albert S. Baldwin, Jr.[1,2,3], [1]Lineberger Comprehensive Cancer Center, [2]Department of Biology, [3]Curriculum in Genetics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599.

NF-κB is an inducible transcription factor comprised of a 50 kD (p50) and a 65 kD (p65) subunit. Induction of NF-κB activity, which is a critical event in many signal transduction pathways, involves release from a cytoplasmic retention protein, IκB, followed by translocation of the active transcription factor complex into the nucleus. We have recently demonstrated that the conserved nuclear localization sequences (NLSs) of NF-κB and c-Rel are the targets for IκB/MAD-3 interaction (in collaboration with S. M. Ruben and C. A. Rosen). We propose that IκB/MAD-3 masks the NLSs of NF-κB and c-Rel and that this constitutes the mechanism for cytoplasmic retention of these proteins.

We are currently studying the mechanisms by which NF-κB is released from IκB. Our results have indicated that both p50/65 heterodimers and p65/65 homodimers are complexed with IκB/MAD-3 in the cytoplasm. Furthermore, stimulation of cells with TNFα leads to the release and translocation of both homo- and heterodimers into the nucleus. Preliminary experiments have indicated that the nuclear levels of p50/65 are different from that of p65/65. While the levels of the heterodimers remain relatively constant over several hours, the levels of homodimers are down-regulated after one hour of stimulation. These results suggest that p65 homodimers are also responsible for transcriptional activation of NF-κB responsive genes.

Our studies indicate that more than one form of IκB may be bound to NF-κB in a cell-type specific manner and that stimulation of cells with various inducers of NF-κB, e.g; TNFα and PMA/PHA, can lead to the modification and subsequent degradation of IκB/MAD-3 as well as to the phosphorylation of p65. The time-course of the degradation of IκB/MAD-3 following stimulation correlates with the appearance of nuclear NF-κB. Interestingly, synthesis of new IκB/MAD-3 rapidly re-establishes the inactive NF-κB pool making the responses transient and ensuring that the cell returns to equilibrium.

B 527 LOCALIZATION OF DETERMINANTS FOR DNA BINDING SPECIFICITY OF THE p50 AND p65 SUBUNITS OF NF-κB, T.A. Coleman, C. Kunsch, S.M. Ruben and C. Rosen, Dept. of Gene Regulation, Roche Institute of Molecular Biology, Nutley, NJ 07110.

The NF-κB transcription factor complex is a pleiotropic activator composed of two subunits designated, p50 and p65. The amino terminus of both subunits shares considerable homology with the *rel* oncogene product. The *rel* homology domain (RHD) is essential for both multimerization (formation of homo- and heterodimers) and DNA binding. We have recently identified DNA binding sites that bind selectively to homodimers of either p50 or p65. To define regions within the RHD involved in multimerization and DNA binding, we constructed a series of p50/p65 fusion proteins. Proteins were assayed first for their ability to bind the κB motif present in the immunoglobulin κ light chain enhancer, and subsequently for their ability to bind the p50- or p65-selective κB motifs. Because multimerization is prerequisite to DNA binding, we further assessed the specificity of these fusion proteins by selective co-immunoprecipitation analysis. Two fusion proteins that have the ability to bind DNA as homodimers to the Ig enhancer κB motif, but which have altered specificities in either selective DNA binding or multimerization were identified. One fusion protein which contains 34 amino acids of p50 and 270 amino acids of p65 bound both the κB and p50-selective motifs but not the p65-selective motif. Similarly, a fusion protein that maintains the DNA binding specificity of p65 was generated. Of most significance, a single point mutation within the p50 domain of the p50-selective p50/p65 fusion protein altered the binding specificity to favor interaction with the p65-selective motif. Our findings demonstrate that p50 specificity can be conferred onto p65 by inclusion of 34 amino acids, and furthermore, that a single amino acid change within the p50 sequence can alter the specificity back to that of p65 within the context of this fusion protein.

B 528 ECI-6, AN IκB-LIKE GENE, IS INDUCED IN ENDOTHELIAL CELLS BY PROINFLAMMATORY AGENTS: INVOLVEMENT OF NFκB IN ITS REGULATION, Rainer de Martin, Bernard Vanhove, Qi Cheng, Johann Winkler, Erhard Hofer and Fritz H. Bach, Vienna International Research Cooperation Center (VIRCC), Brunnerstr. 59, A-1235 Vienna, Austria, Tel.: 43-1-866-34-620, Fax: 43-1-866-34-623.

During inflammation, endothelial cells (EC) upregulate a number of different genes leading to "activation" of the cell. This activation process is accompanied by a change in the function of the EC from maintaining anticoagulation to promoting procoagulation. Using differential screening and subtractive hybridizations, we have identified several cDNAs, some with no sequence homology to previously described genes or proteins, that are upregulated by treatment of porcine aortic endothelial cells with IL-1 or TNFα. One of them, ECI-6, showed strong homology with members of the IκBβ family of proteins (pp40, MAD-3 and RL/IF-1) that have been reported to bind NFκB. ECI-6 mRNA is induced 10-15 fold within four to six hours after stimulation of EC with either IL-1α, TNFα or lipopolysaccharide (LPS), in a cycloheximide independent way.

In order to investigate the regulation of the ECI-6 gene, we have isolated corresponding clones from a porcine genomic library. The transcriptional start site was mapped by primer extension and RNAse protection, and two potential NFκB binding sites were detected in the upstream regulatory region.

This represents the first report that an IκB-like gene is upregulated coincident with EC activation. The finding of potential NFκB binding sites in the region 5' to the gene suggests that NFκB may function to regulate its own inhibitor, an interesting example of feedback control. More detailed data on the ECI-6 promoter region relevant to this hypothesis will be presented.

B 529 MAPPING OF THE TRANSACTIVATION DOMAINS OF RELB: ROLE OF THE PUTATIVE LEUCINE ZIPPER, Pawel Dobrzanski, Rolf-Peter Ryseck and Rodrigo Bravo.Department of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O.Box 4000, Princeton, New Jersey 08543-4000.

RelB, a member of the Rel family, differs from the other Rel related proteins by not forming stable homodimers. However, it forms stable heterodimers with p50 and p50B, which bind well to κB binding sites. These heterodimers, in contrast to p50 and p50B homodimers transactivate κB-dependent transcription in vivo. This data indicate that RelB, like p65, provides the heterodimer with an activating domain. To analyze the RelB regions which contribute to this transactivation, different deletion mutants of RelB and RelB/p50 chimeras were generated, and their activating potential was tested by cotransfection into F9 cells. For full activation with p50, RelB requires both, the N-terminal 100 amino acids preceding the rel homology region and the last 180 C-terminal amino acids. RelB molecules containg only one of these domains exhibited a weak transactivating potential. High levels of activation were observed when the two domains were provided either in cis or trans. Since the long N-terminus (which is unique feature of RelB) contains a putative leucine zipper-like structure (LZ), various constructs were made in which the LZ was mutated. All mutants displayed a 3 to 4 fold weaker ability to transactivate than the corresponding wild type molecules. This indicates that the LZ is functional and may contribute to the activating potential of RelB.

B 530 THE DNA-BINDING ACTIVITY OF Rel-FAMILY PROTEINS IS REGULATED BY DISCRETE RESIDUES MAPPING TO THE Rel-HOMOLOGY REGION, Céline Gélinas[1,2], Xiang Xu[1,2], Arnold B. Rabson[1], and Sushant Kumar[1,2], [1]Center for Advanced Biotechnology and Medicine; [2]Department of Biochemistry - Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway NJ 08854-5635

Rel oncoproteins bind to oligonucleotides containing κB motifs, form heterodimers with other members of the Rel-family, and modulate expression of genes linked to κB motifs. Here we report that the RxxRxRxxC motif conserved in all Rel/κB proteins is absolutely required for protein/DNA contact and for the resulting transforming activity of v-Rel. Serine substitution of the cysteine residue conserved within this motif enabled v-Rel to escape redox control, promoting overall DNA-binding. Mutant proteins with a serine residue at this position retained the ability to competitively inhibit κB-mediated transcriptional activation of the HIV LTR, but failed to efficiently transform chicken lymphoid cells both *in vitro* and *in vivo*. Our results indicate that reduction of the conserved cysteine residue in the RxxRxRxxC motif is required for optimal DNA/protein interactions and for biological activity. Further mutagenesis in the Rel-homology region has revealed discrete residues critical for the binding affinity of Rel-family members. Experiments are currently in progress to determine the effect of these sequences on the binding specificity of Rel proteins, and the extent to which they contribute to their respective biological functions.

B 531 POSITIVE AUTOREGULATION OF THE MURINE C-REL PROMOTER BY REL PROTEINS IS NOT MEDIATED THROUGH NF-κB ELEMENTS INVOLVED IN CONTROLLING CONSTITUTIVE TRANSCRIPTION IN B CELLS, Steve Gerondakis, Imogen B. Richardson and Raelene Grumont, The Walter and Eliza Hall Institute of Medical Research Post Office, The Royal Melbourne Hospital, Parkville, Victoria 3050, Australia.

The c-rel proto-oncogene, a member of a transcription factor family that includes NF-κB, displays a complex pattern of gene expression. In most cell types, expression is normally low or undetectable and can be rapidly induced in a transient fashion by a wide variety of signals. In contrast, B cells express c-rel at high constitutive levels. To understand the basis of this expression, the regulatory region upstream of the murine c-rel transcription start sites has been cloned and characterised. Transcription initiates at multiple sites downstream of a GC-rich region conserved in the chicken c-rel promoter. This conserved region contains consensus transcription factor binding sites for HIP-1, SP-1 and NF-κB (kB3 site) and is sufficient for basal expression in Jurkat T cells. In contrast, two additional NF-κB sites (kB1 and kB2) and an octamer binding site, all located upstream of the conserved region, are required for expression of promoter-reporter gene constructs in the B cell line, 129B. NF-κB sites kB1 and kB3 bind p50/65 and p50 homodimers, while kB2 binds a distinct complex. The consensus octamer site while only able to bind Oct1 and Oct2 with low affinity appears to overlap with a binding site for a novel protein expressed in 129B cells. Co-transfection studies show that $p75^{c\text{-}rel}$ and a carboxyl terminal truncated c-rel protein that lacks the known transactivating domain both upregulate the c-rel promoter in 129B cells via a mechanism independent of the NF-κB motifs.

B 532 REGULATION OF THE DNA BINDING ACTIVITY OF THE TRANSCRIPTION FACTOR NF-κB. Ronald T. Hay, Naomi Wakasugi, Jean-Louis Virelizier and James Matthews, School of Biological and Medical Sciences, University of St. Andrews, Fife KY16 9AL, Scotland and Institut Pasteur, Paris, France.

Activity of the p50 and p65 subunits of NF-κB is modulated by association with IκB molecules α, β and γ. To study the activity of p50, p65, MAD3 and IκBγ *in vitro* (and for antibodies) these proteins have been expressed in and purified from bacteria. Large scale purification of p50 has yielded sufficient protein to initiate structural and biochemical approaches to determine the molecular basis for DNA binding specificity. Limited proteolysis has defined a minimal DNA binding domain and cleavages within the DNA binding domain have been mapped by N-terminal sequencing and mass spectrometry. This information has allowed proteolysis to be used to map sites of interactions with other proteins, such as IκB. Interactions between p50 homodimers and IκBγ have been studied by native gel electrophoresis and indicate that two molecules of IκBγ bind to each p50 homodimer. MAD3 binds to p50 with similar stiochiometry but the effect on DNA binding activity is less pronounced. A combination of immunoprecipitation and Western blotting is being used to study the composition and pattern of phosphorylation of protein complexes containing p50 and IκB *in vivo* when cells are subjected to different activation regimes. We have previously shown that p50 is regulated by a RedOx mechanism. Mutagenesis of conserved cysteine residues in the p50 subunit identified amino acid 62 as being important in this process, as a serine substitution at this position renders the protein insensitive to -SH modifying agents. Quantitative analysis of the interaction of purified mutant proteins with DNA indicated that the amino acid 62 mutant bound to DNA with reduced specificity. These data indicate that cysteine 62 is in close proximity to the bound DNA and this was confirmed by substrate protection experiments with ^{14}C iodoacetate (collaboration with W. Kasubska, Geneva). DNA binding activity of the wild type protein but not the amino acid 62 mutant was also stimulated by thioredoxin and cotransfection of a plasmid expressing human thioredoxin and an HIV LTR driven reporter construct resulted in an NF-κB dependent increase in expression of the reporter gene. Thus modification of p50 by thioredoxin, a gene induced by stimulation of T-lymphocytes in parallel with NF-κB translocation, is a likely step in the cascade of events leading to full NF-κB activation.

B 533 POSITIVE AND NEGATIVE CONTROL OF THE HUMAN INTERFERON β PROMOTER BY NF-κB/rel AND IRF TRANSCRIPTION FACTORS, John Hiscott, Ivy Kwan, Normand Pepin, Judith Lacoste and Anne Roulston. Lady Davis Institute for Medical Research and Dept. of Microbiology and Immunology, McGill University, Montreal, Canada H3T 1E2.

Multiple regulatory domains within the interferon β promoter control the inducible response of the interferon gene to virus infection. The sequence element PRDII (-64 to -55) interacts with members of the NF-κB/rel family *in vitro*. In co-transfection experiments using various NF-κB subunit expression plasmids and two copies of PRDII/NF-κB linked to a CAT reporter gene, expression of p65, p85 (c-rel), or combinations of p50/65 and p65/85 stimulated NF-κB dependent transcription, whereas expression of the p50 subunit alone did not activate the reporter gene. Co-expression of IκBβ/MAD3 or a naturally occurring splicing variant p65Δ completely abrogated p65, c-rel, or p65/p50 induced gene activity. Based on *in vitro* binding studies using E.coli produced NF-κB subunits, IκB and p65 Δ could either form non-DNA binding heterodimers in solution or could cause rapid dissociation of NF-κB protein-DNA complexes. When the entire IFN-β promoter (-281 to +19) was used in co-expression studies, the combination of p50 and p65 only weakly increased IFN gene activity. Synergistic stimulation of IFN-β promoter activity was obtained when NF-κB subunits were co-expressed together with IRF-1, a transcription factor that binds to adjacent PRDI (-79 to -64) and PRDIII (-94 to -78) domains. The PRDI and PRDIII domains also interact with a structurally similar, but functionally distinct protein IRF-2 that normally represses IFN gene expression. Following induction of cells with cycloheximide and double stranded RNA or virus, IRF-2 was processed to a 14kDa, DNA-binding protein that lacked the repressive activity associated with IRF-2. Thus, multiple regulatory events - including activation of DNA binding NF-κB heterodimers, synergistic interaction between IRF-1 and NF-κB, and processing of the IRF-2 repressor - are all required for the transcriptional activation of the interferon β promoter.

B 534 LONG TERM NUCLEAR EXPRESSION OF NF-kB IS INDUCED IN PURIFIED HUMAN RESTING T LYMPHOCYTES ACTIVATED VIA THE CD2+CD28 ADHESION MOLECULE PATHWAY. Jean Imbert, Régis Costello, Carol Lipcey, Michèle Algarté, Chantal Cerdan, Yves Martin and Daniel Olive, INSERM U119, 27 bd Leï Roure, 13009 Marseille, FRANCE

Human T lymphocytes can be activated by mAbs directed against two adhesion molecules, CD2 and CD28. We have demonstrated previously that the simultaneous activation by CD2 and CD28 of purified resting T cells led to a long lasting (>3 weeks) proliferation independent of accessory cells. This monocyte-independent proliferation could be explained by a high and prolonged T-cell synthesis of cytokines normally produced by accessory cells such as IL-1α, TNF-α and CSF-1. CD2+CD28 costimulation regulates cytokine and associated receptor genes both at the transcriptional and post-transcriptional levels. We are currently investigating which *cis*-acting regulatory elements and *trans*-acting factors are associated with CD2+CD28-mediated transcription activation. Among the transcription factors potentially involved, recent attention has focused on the role of NF-kB in the expression of inducible genes activated during T cell growth. In an attempt to determine the putative role of NF-kB in the adhesion molecule pathway, we have performed a kinetic study of its activation in purified human T lymphocytes in presence of mAbs anti-CD2 and anti-CD28. Our results showed a long term induction of NF-kB nuclear expression in accordance with the time-course of T-cell activation. Futhermore, we have identified the different NF-kB subunits forming the kB-specific protein-DNA complexes revealed by EMSA and UV-crosslinking. Experiments are in progress in order to analyze NF-kB functional role.

B 535 REQUIREMENT OF ANKYRIN REPEATS FOR THE FUNCTION OF IkB proteins,

Jun-ichiro Inoue[1], Lawrence D. Kerr[2], Inder M. Verma[2] and Okio Hino[1],[1] Department of Experimental Pathology, Cancer Institute,Tokyo 170 Japan, [2]MBVL, The Salk Institute, La Jolla, CA 92037

IkB proteins, which regulate the DNA binding activity and subcellular localization of rel/NFkB transcription factors, contain several ankyrin repeats. To elucidate the mechanism by which pp40/IkB inhibits the DNA binding activity of rel/NFkB proteins, we have investigated the role of ankyrin repeats on the function of pp40/IkB. Four out of five ankyrin repeats were essential for the association with rel protein and the inhibiton of DNA binding. However, ankyrin repeats were not sufficient and the C-terminal region of pp40/IkB was also required.[1)]

To further investigate the role of ankyrin repeats, we have cloned the cDNA for 2.6kb NFkB mRNA which encodes C-terminal half of p110 NFkB precursor including eight ankyrin repeats.[2)] Since a 70kd protein expressed from this mRNA inhibits DNA binding activity of p50/p65 heterodimer, p50 homodimer and rel protein, we called this protein IkBγ.

These results indicate that ankyrin repeats are common structure of IkB family and form a structure required for the specific association with the rel-related proteins.

1) Inoue,J.,et.al. Proc.Natl.Acad.Sci.USA 89,4333-4337(1992)
2) Inoue,J.,et.al. Cell 68,1109-1120(1992)

B 536 **THE PROTO-ONCOGENE BCL-3 ENCODES AN IκB PROTEIN RELATED TO IκB-α.** Lawrence D. Kerr, Penny Wamsley, Colin Duckett, Qiang Zhang. Gary Nabel, Timothy McKeithan, Patrick A. Baeuerle, and Inder M. Verma. The Salk Institute, Mol. Biol. and Vir. Lab., 10010 N. Torrey Pines Road, San Diego, CA 92138.

The bcl-3 gene product, overexpressed in breakpoint translocations t(14:19) in chronic lymphocytic leukemia (CLL) patients is a member of the IκB family. The 56 kD bcl-3 protein is able to inhibit the DNA binding and transactivation of authentic NF-κB heterodimers p50/p65, p49/p65, as well as p50 and p49 homodimers. The bcl-3 protein does not inhibit either the DNA binding of Rel protein or its ability to transactivate genes linked to the κB site. A human 37 kD protein (IκB-α), previously identified as member of the IκB family, is also unable to inhibit the DNA binding activity of Rel proteins. However, unlike bcl-3, the 37 kD (IκB-α) protein has no effect on the DNA binding activity of p50 or p49 homodimers. Two dimensional phosphotryptic peptide maps of human bcl-3 and human 37 kD (IκB-α) protein reveal that the phosphopeptides from 37 kD (IκB-α) are nested within the bcl-3 protein. Furthermore, bcl-3 antisera immunoprecipitates an *in vitro* radiolabeled 37 kD (IκB-α) protein. A 56 and 38 kD protein can be identified in Hela cells stimulated with PMA and immunoprecipitated with bcl-3 antisera. Comparison of tryptic peptide maps of p56, p38, and bcl-3 protein synthesized *in vitro* show that they are all structurally related. Removal of the amino terminal sequences of the bcl-3 protein generates a protein which inhibits the DNA binding of p50/p65 heterodimers, but like the 37 kD (IκB-α) protein, is no longer able to inhibit the binding of the p50 and p49 homodimers with κB DNA. We propose that bcl-3 and 37 kD (IκB-α) proteins are related members of the IκB family.

B 537 DETERMINATION OF DNA-BINDING SPECIFICITY OF NF-κB p50 AND p65 MUTANTS BY A YEAST GENETIC ASSAY, Charles Kunsch, P.A. Moore, T.A. Coleman, S.M. Ruben and C.A. Rosen, Department of Gene Regulation, Roche Institute of Molecular Biology, Nutley, NJ 07110.

Nuclear factor κB (NF-κB) is an inducible transcription factor complex that binds to, and mediates transcription from, *cis*-acting DNA sequences in the regulatory region of several viral and cellular genes. Although several κB-DNA binding sequences have been identified we have examined the preferred DNA binding sequences of several of the κB-related proteins by amplifying from a pool of random oligonucleotides those sequences that bound preferentially to homodimers of p50, p65 or c-Rel. In addition to obtaining novel DNA sequences recognized by each of the individual proteins, sequences were obtained that bound selectively to only p50, p65 or c-Rel. These selective binding sites were used to identify regions of p50 and p65 that confer specificity of DNA binding by the use of a yeast transcriptional activation assay in which the specific DNA binding motifs confer transcriptional activation in *Saccharomyces cerevisiae.* Chimeric p50/p65 proteins were constructed which contain minimal regions of either p50 or p65 in the heterologous background and fused to the acidic transcriptional activator VP-16. Using this assay, we have confirmed initial in vitro observations identifying minimal regions in p50 and p65 that confer specificity of DNA binding. Furthermore, both in vitro and yeast transcriptional activation studies indicated that a single amino acid change in the minimal region of p50 (in the context of the p50/p65 chimeric protein) was sufficient to alter the specificity of p50 to that of p65 for a particular DNA sequence. Localized random mutagenesis is being performed to select for additional altered specificity mutants of p50 and p65. Our studies indicate the utility of a yeast genetic assay to examine the DNA binding properties of the NF-κB family of transcriptional activators.

B 538 NF-κB/rel AND Tax : INTERACTIONS BETWEEN ONCOGENIC PROTEINS, Judith Lacoste, Ivy Kwan, Normand Pépin, Lyly Le, Jacqueline Lanoix and John Hiscott. Lady Davis Institute for Medical Research and Dept. of Microbiology and Immunology, McGill University, Montréal, Canada H3T 1E2.

Oncoproteins of the NF-κB/rel family are involved in the transcriptional regulation of many polypeptides important to the immune response. In T cells stably expressing the HTLV-I Tax oncogene, an endogenous κB-regulated gene (GM-CSF) and NF-κB binding activity were constitutively expressed; also, increased basal level expression of transfected κB-regulated promoters was observed in these cells. Northern blot analysis revealed higher basal level expression of NF-κB p50 and IκB RNAs in Tax expressing cells, while levels of p65 RNA were not significantly altered by constitutive expression of Tax. Transfection studies were performed in Jurkat T cells using expression vectors for the various NF-κB subunits and a κB-regulated reporter plasmid. Significant induction of transcription was obtained when p65, c-rel or combinations of p50/p65 and p65/c-rel were used in transient expression assays. These transcriptional activities were inhibited by co-transfecting expression vectors encoding the inhibitor IκBβ/MAD3 or p65Δ, a naturally occuring splicing variant of p65.In prelimirary studies, Tax co-expression was found to abrogate the inhibitory effects of IκB on p65- or p50/p65-induced transcription when co-transfected along with the inhibitory subunit. We are now investigating the interactions of recombinant NF-κB p50, p65, p65Δ and c-rel with Tax protein. Interestingly, a report recently demonstrated that Tax interacts with the C-terminus of the p50 precursor, p105. Therefore we will also examine the possibility that Tax interacts with ankyrin repeat structures such as those found in IκB and in the C-terminus of p105. These studies may provide information about how two types of oncoproteins (Tax and NF-κB/rel) interact together and alter the expression of growth regulated genes.

B 539 THE A20 ZINC FINGER PROTEIN: TRANSCRIPTIONAL ACTIVATION BY DISTINCT PATHS INVOLVING NF-κB

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Apoptosis is an active cell suicide process of fundamental importance in inflammation, development, and lymphocyte selection. Originally identified as a primary tumor necrosis factor α (TNFα) responsive gene in endothelial cells, the A20 gene encodes an inducible zinc finger protein which confers resistance to TNFα cytotoxicity. A survey of cell lines revealed that A20 expression correlated with lymphocyte activation and monocyte differentiation. A20 was also constitutively expressed in lymphocytes expressing the viral proteins Human T cell leukemia virus type I Tax or Epstein-Barr virus LMP1. Thus, the induction of protective genes such as A20 may represent a common mechanism by which these viral transforming proteins render host cells resistant to apoptosis and ultimately cause transformation. Transfection studies demonstrated that stimulation of A20 transcription by TNFα, PMA, Tax or LMP1 was mediated by two distinct κB elements. Accordingly, DNA band shift assays confirmed inducible binding of NF-κB to A20 κB elements. Both κB sites were required for TNFα or PMA activation of the A20 promoter; however, Tax required only one κB site. Overexpression of NF-κB subunit combinations p49 + p65 or p50 + p65 activated the wild type A20 promoter but not mutated forms containing single κB sites. Thus, Tax activation of A20 transcription occurs through a mechanism distinct from PMA and TNFα, possibly due to differential activation of NF-κB complexes or transcriptional cofactors. Given A20's role in protecting cells from TNFα cytotoxicity, these observations suggest a direct role for NF-κB in the regulation of programmed cell death and offer an example of the pleiotropic role NF-κB plays in cellular activation.

B 540 ACTIVATION OF THE NF-KB/C-REL SYSTEM BY HTLV-I TAX PROTEIN. Paul Lambert, Mandy Ludford, Nicholas Deacon and Richard Doherty, Macfarlane Burnet Centre for Medical Research, Yarra Bend Road, Fairfield, Victoria 3078, Australia.

The transactivator of human T cell leukemia virus type I, tax, is capable of activating a number of host cell genes, among them those controlled by proteins of the NF-kB/c-rel family. While some investigators have found that activation of NF-kB/c-rel by tax occurs in the absence of de novo protein synthesis[1], others have seen increased levels of NF-kB and c-rel mRNAs in cells which stably express tax[2]. As the appearance of the different proteins is at least biphasic[3], and as it appears that at least one member of the NF-kB/c-rel family participates in its own regulation[4], we were interested to see which components of the system are directly activated by tax, and which are activated as a secondary response. We also asked whether a direct response to tax occurred at the pre-translational level as well as post-translationally, that is, whether tax causes activation of NF-kB/c-rel genes by pathways that do not involve presynthesized NF-kB/c-rel proteins.

1. Lindholm, P.F., et al., J.Virol. 66(3), 1294-1302 (1992).
2. Arima, N., et al., J.Virol. 65(12), 6892-99 (1991).
3. Molitor, J.A., et al., Proc. Natl. Acad. Sci. USA 87, 10028-32 (1990).
4. Ten, R.M., et al., EMBO J. 11(1), 195-203 (1992).

B 541 THE NF-κB-p50 PROTEIN INTERACTS WITH REL, I-κB-γ, NF-IL6 AND OTHER PROTEINS. Kenneth P. LeClair, Division of Immunology, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215

Studies to understand the regulation of eukaryotic gene expression are increasingly concerned with understanding the interactions that occur between protein transcription factors. Biochemical analyses revealed that the NF-κB transcription factor is a heterodimer of two proteins, p50 and p65. The cloning of the p50 and p65 cDNAs revealed that each contains a domain with homology to the Rel oncoprotein and to the product of the Drosophila *dorsal* gene. The subcellular distribution and biological activity of Rel family proteins is regulated by association with the I-κB family of inhibitor proteins (I-κB-α, I-κB-β, and I-κB-γ). A recent report indicates that the 3' end of the p105 cDNA encodes the I-κB-γ protein, which associates with NF-κB-p50 (Inoue *et al.*, Cell 68, p. 1109).

To investigate its interactive potential, the human NF-κB-p50 protein was expressed in a T7 polymerase-driven bacterial expression system with a 15 amino acid peptide engineered onto its amino terminus. These amino acids introduce an antigenic epitope and a site for *in vitro* ^{32}P-phosphorylation by heart muscle protein kinase. The NF-κB-p50 fusion protein binds NF-κB and related DNA sequence elements in gel mobility shift assays, and homodimerizes or heterodimerizes with other Rel proteins by both Western blot and immune precipitation analyses. When ^{32}P-labeled NF-κB p50 protein was used as a probe of a λgt11 expression library, partial cDNAs of the c-Rel protein and the NF-κB-p105 protein (the precursor of NF-κB-p50) were obtained. Analysis of the NF-κB-p105 cDNA revealed that a termination codon in the 5' untranslated region and a frame shift induced by apparent aberrant splicing would preclude synthesis of an intact Rel domain. Expression of the 3'-end fragment of the cDNA confirms that I-κB-γ protein is produced from this cDNA, and as expected, has the ability to block DNA binding of NF-κB-p50 protein.

In addition to Rel and I-κB-γ, other cDNAs were obtained using this interactive screening method, including the cDNA for the NF-IL6 protein (LeClair, Blanar, and Sharp, P.N.A.S. 89, in press). Further characterization of two other cDNAs isolated (one for an HMG domain protein and the other for a novel protein with striking homology to the Drosophila *trithorax* gene product) is in progress.

B 542 DIFFERENTIAL EXPRESSION OF REL FAMILY MEMBERS IN HTLV-I INFECTED CELLS: EVIDENCE FOR TRANSCRIPTIONAL ACTIVATION OF C-REL BY TAX PROTEIN, Chou-Chi H. Li[1], Francis W. Ruscetti[2], Nancy Rice[3], Eying Chen[1], Ning-Sun Yang[4], Judy Mikovits[1] and Dan L. Longo[2]. [1]Biological Carcinogenesis and Development Program, Program Resources, Inc./DynCorp, [2]Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, [3]Laboratory of Molecular Virology and Carcinogenesis, BRI-Basic Research Program, NCI-FCRDC, Frederick, MD 21702, [4]Agracetus, WI.

The Tax protein of the human T-cell leukemia virus type I (HTLV-I) has been shown to induce nuclear expression of the Rel family NF-κB-binding proteins (Arima et al. J. Virol. 65, 6892-6899, 1991). We used specific immunologic reagents capable of distinguishing individual members of the Rel family proteins to show that only the c-rel, but not NF-κB p50 or p65, is induced in HTLV-I infected cells. The c-rel induction was detected at both protein and RNA levels. In addition to the full-length 80-kD c-rel protein, a novel truncated form of p60 was also detected in the HTLV-I infected cells. The induced c-rel expression was detected in cells stably transfected with the Tax cDNA, further correlating the c-rel induction with the viral Tax expression. The expression of nuclear binding form of the c-rel family protein was also examined using specific antisera in electrophoretic mobility shift assays. Consistent with the c-rel induction at the steady-state protein level, the nuclear-binding form of c-rel protein is also preferentially induced by Tax. An increase in c-rel mRNA is seen within 3 hours after induction of Tax expression suggesting that this effect is at least partially regulated at the level of transcription. Furthermore, using a particle bombardment method to transfer genes into cells, we show that Tax can activate the c-rel promoter.

B 543 **Regulation of NF-κB Activity by Differentiation Signals and Extracellular Stimuli**

Hsiou-Chi Liou and David Baltimore, Rockefeller Universtity, 1230 York Avenue, New York, NY 10021.

NF-κB is a transcription factor which regulates the expression of the Ig κ light chain gene among others. Although its activity was originally detected in B cells, the inactive NF-κB can be detected in most cells in a complex with an inhibitor protein, termed IκB (Inhibitor of NF-κB).

NF-κB activity is tightly regulated by differentiation signals. In B lineage cells, nuclear NF-κB activity is undetectable in immature B cells, whereas constitutive nuclear activity is enriched in mature B cells. The inactive form of NF-κB in immature B cells, however, can be activated by LPS or IL-1 which also induces Ig κ chain rearrangement.

Post-translational modification such as phosphorylation has been suggested to play an important role in the regulation of NF-κB activity. Here we showed that IκBα is a good substrate for c-Abl-tyrosine kinase, protein kinase A (PKA) and protein kinase C (PKC) by in vitro phosphorylation studies. Phospho-peptide mapping and phospho-amino acid analyses of the IκBα protein phosphorylated by PKA and PKC was also performed. The results showed that PKA and PKC phosphorylate IκBα at different sites. Furthermore, PKA phosphorylated IκBα on serine residues exclusively.

By co-transfection of a 3x κB-luciferase reporter construct (containing three tandem repeated Ig κB sites) with constructs carrying either oncogenic or transdominant-negative mutant cDNAs encoding signalling molecules, the effect of these signalling molecules on NF-κB activity were addressed. The phosphorylation status of NF-κB and IκBα in response to differentiation signals and extracellular stimuli in vivo will also be discussed.

B 544 CHARACTERIZATION OF THE NF-κB p49(100) PROMOTER. Susanne Liptay, Roland M. Schmid, Elizabeth G. Nabel, Gary J. Nabel, Howard Hughes Medical Institute, University of Michigan Medical Center, Departments of Internal Medicine and Biological Chemistry, Ann Arbor, MI 48109-0650

NF-κB has been shown to be constutively active in mature B-lymphocytes, monocytes and macrophages, and is present in an inactive complex in the cytoplasm in T cells and a variety of other celltypes. Regulation of NF-κB can occur by post-translational events e. g. causing nuclear translocation of the NF-κB complex. However, maintenance of the NF-κB activity required ongoing protein synthesis and continuous stimulation suggesting a regulation at a pre-translational level. To characterize the transcriptional regulation of the p49(100) subunit of NF-κB, human genomic clones were isolated. A potential promotor region was identified. This region lacks classical TATA or CAAT elements, but contains multiple κB motifs. Two major transcription start sites were determined by primer extension analysis. Transient transfection assays using chloramphenicol acetyltransferase reporter plasmids demonstrated that the p49(100) NF-κB promoter has a strong basal activity in B cells and monocytes. In T cells, the promotor can be activated by PMA, TNF-alpha or the tax gene product of HTLV-I. Co-transfection of NF-κB p49(100) promoter reporter plasmids with different NF-κB subunits showed that the NF-κB p49(100) promoter is activated most strongly by a combination of p49 and p65 in comparison to p50 and p65. These data suggest that NF-κB participates in the regulation of its own expression in an autoregulatory fashion.

B 545 MAPPING OF THE INTERACTION REGIONS OF THE TRANSCRIPTON FACTOR NF-κB WITH ITS INHIBITOR PROTEIN IκB. James R. Mathews, Louise Crawford, Elizabeth Watson and Ronald T. Hay, School of Biological and Medical Sciences, University of St. Andrews, Fife KY16 9AL, Scotland.

The DNA binding activity of the p50-p65 NF-κB heterodimer and p50 homodimer can be repressed by association with the inhibitor proteins IκB α, β, and γ. To allow extensive in vitro studies, the p50 and IκB γ proteins have been expressed and purified from bacteria. Previous studies have suggested the involvement of the conserved cysteine 62 in the redox modulation of p50 homodimer DNA binding activity - substrate protection experiments with ^{14}C iodoacetate have recently confirmed that cysteine 62 is intimately involved in the p50 DNA binding site (collaboration with W.Kaszubska, Geneva). To extend the identification of amino acid residues in the DNA binding site of the p50 homodimer, photoreactive radiolabelled κB site oligonucleotides are bound to p50, cross-linked by exposure to UV light and after proteolysis the peptides linked to the oligonucleotide isolated and sequenced.

A similar substrate protection strategy is being used to identify the regions of intimate contact between the p50 homodimer and IκB γ using glutathione S-transferase-p50 and -IκB γ fusion proteins bound to glutathione-agarose matrices, with binding of the complementary protein species followed by specific side chain chemical modification. Release of the complementary protein species from the complex and modification with radiolabelled reagents identifying amino acid residues important for the interaction.

Partial proteolysis is being used to define the points of contact of the p50 homodimer with the glutathione S-transferase-IκB γ fusion protein carrying a C-terminal antigen tag allowing monoclonal antibody recognition. Partial proteolysis of the p50-IκBγ-fusion protein complex followed by precipitation of the complex with anti-p50 antiserum and western blotting of the partial digest with either anti-tag or anti-glutathione S-transferase antibodies identifies those regions of IκB γ necessary for its interaction with the NF-κB p50 homodimer.

B 546 MULTIPLICITY OF NUCLEAR NF-κB/REL COMPLEXES: TISSUE-SPECIFICITY, EFFECTS OF THE SEQUENCE CONTEXT OF THE EXTENDED κB SITE AND RELATION TO PROTEIN-INDUCED DNA BENDING, S.A. Nedospasov, R.L. Turetskaya, I.A. Udalova, N.R. Rice and D.V. Kuprash, Engelhardt Institute of Molecular Biology, Moscow, 117984 Russia; LMI, BRMP and LMVC, ABL-BRP, NCI/FCRDC, Frederick MD 21702.

We used high resolution EMSA and a panel of monospecific antibodies against distinct members of NF-κB/rel family to study complexes formed *in vitro* on a variety of synthetic κB probes. Protein composition of distinct complexes has been determined. First, we observed considerable heterogeneity among complexes. For example, in extracts from some cell types the major NF-κB complex formed on many of the κB probes could be resolved into p65-p50 and p65-p50B/lyt10/p49; in other cases, c-rel-p65 and c-rel-p50 could be identified. Second, we observed that the DNA sequence context of the extended κB probes is important for selecting distinct combinations of NF-κB/rel proteins. In extracts from LPS-activated murine macrophages some κB probes would bind predominantly p50-p65; others in addition would bind p50-p50, while a distinct subgroup of κB sites predominantly bound c-rel-p65 and p65-p50. Due to the previously demonstrated threshold effect in transcriptional activation by NF-κB, quantitative changes in the affinities may result in dramatic effects on transcription. The functional significance of these combinatorial effects was suggested by transfection experiments in which reporter genes under the control of synthetic κB ehnancers were introduced into various cell lines either alone or in combination with NF-κB/rel expression constructs. Third, the binding of all NF-κB/rel complexes resulted in strong complex-specific DNA bending at the κB site, suggesting that one of the activating effects of NF-κB/rel may be mediated through changes in geometry or topology.

B 547 THE BCL-3 PROTO-ONCOGENE IS A NUCLEAR-RESIDENT IκB-LIKE MOLECULE THAT PREFERENTIALLY REGULATES NF-κB P50 AND P49 DNA-BINDING ACTIVITY IN A PHOSPHORYLATION-DEPENDENT MANNER. Garry P. Nolan[1], Takashi Fujita[1], Kishor Bhatia[2], Conrad Huppi[2], Hsiou-Chi-Liou[1], Martin L. Scott and David Baltimore[1].

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The product of the putative proto-oncogene Bcl-3 is an IκB-like molecule with novel inhibitory properties specific for a subset of the rel family of transcriptional regulators. In vitro, Bcl-3 protein specifically inhibited the DNA-binding of both homodimeric NF-κB p50 subunit and a closely related homologue, p49 to Igκ NF-κB DNA motifs. Bcl-3 protein was capable of actively inhibiting the DNA binding of p50 to certain NF-κB motifs. At concentrations that give complete inhibition for p50 Bcl-3 did not inhibit the specific DNA-binding of reconstituted heterodimeric NF-κB (p50:p65), a DNA-binding homodimeric form of p65, or homodimers of c-rel. Transduced Bcl-3 associated with p50 in vivo and was localized to the nucleus of NIH3T3 cells; this correlates with the previously observed constitutive nuclear localization of p50 homodimers. Phosphatase treatment of Bcl-3 inactivated its inhibitory properties, implicating a role for phosphorylation in the regulation of Bcl-3 activity. These properties distinguish Bcl-3 from classically defined IκB that regulates the subcellular localization of heterodimeric NF-κB p50:p65 through specific interactions with the p65 subunit. Bcl-3 therefore appears to be a nuclear IκB-related molecule that regulates the activity of homodimeric nuclear p50.

B 548 DISTINCT NF-κB BINDING ACTIVITIES INDUCED IN RESPONSE TO PHORBOL ESTER AND VIRAL INFECTION OF MYELOMONOBLASTIC CELLS.

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The effects of viral infection on induction of NF-κB/rel binding activity has been examined using a myelomonoblastic cell line (PLB-985) chronically infected with HIV-1 (PLB-IIIB). Mobility shift analysis was performed using NF-κB binding sites from the IFN-β PRD II domain (P2), HIV enhancer, and IL-2Rα promoters. UV crosslinking analysis has identified an inducible NF-κB complex consisting of 40 and 50kDa proteins in PLB-985 cells following PMA or TNFα treatment. In PLB-IIIB cells, a novel complex consisting of 70, 90 and 100kDa subunits was constitutively present; the 70 kDa protein was further inducible by PMA or TNFα treatment. This complex appeared as early as 5 days after *de novo* HIV infection and strikingly, the same complex was strongly induced in PLB-985 following a 6 hr. Sendai virus infection. The 40/50kDa species bound HIV>P2>IL-2Rα while the virally induced 70/90/100kDa complexes bound P2 preferentially. Analysis with NF-κB/rel subunit specific antisera has shown that the PMA/TNFα inducible complex in the PLB-985 cell line consists predominantly of the p50 subunit. The inducible 70kDa protein in PLB-IIIB cells corresponds to p65; the 90/100kDa proteins are unknown but do not correspond to p50, p65, c-rel or p105 NF-κB/rel subunits. To examine the functional consequences of these changes in protein-DNA interactions, CAT reporter constructs driven by the HIV or P2 NF-κB elements were transfected into normal and HIV infected cells. NF-κB dependent promoters were inducible by PMA in PLB-985 cells but in HIV-infected cells high basal levels of activity and low inducibility were observed. Finally, PLB-IIIB cells show increased IFNβ and IL-1β mRNA expression in response to secondary stimuli compared to PLB-985 cells. Thus, viral infection induces distinct NF-κB subunits with unique sequence specificity, which may subsequently alter NF-κB mediated gene expression.

B 549 SUBCELLULAR LOCALIZATION OF PROTEINS IN THE NF-κB TRANSCRIPTION COMPLEX REVEALS THAT THE CYTOSOLIC INHIBITOR, IκB, IS A NUCLEAR PROTEIN.

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NF-κB is a transcriptional activator originally identified as being involved in the tissue specific control of the mouse κ light chain enhancer. The DNA κB binding site has now been identified in the regulatory elements of cytokine, cytokine receptor, MHC class I and II antigens, inflammatory and acute phase response genes, and viral enhancer elements. NF-κB is composed of a heterodimer of the p50 and p65 subunits, both members of the *rel*-family of proteins. This family of proteins contains a region of approximately 300 AA at the amino terminal end important for DNA binding, protein dimerization and nuclear localization. In most cells NF-κB is complexed in the cytoplasm with the repressor, IκB. Upon activation of the cells by a variety of stimuli, NF-κB shuttles to the nucleus, a process thought to involve phosphorylation of IκB. To gain an understanding of the mechanism of IκB function, the subcellular localization of proteins in the NF-κB complex was determined by indirect immunofluorescence in COS7 cells. Both p65 and p50 were localized in the nucleus, although the subnuclear localization was quite different. When IκB was co-transfected with p65, p65 was excluded from the nucleus. In addition p50 was also excluded from the nucleus following co-transfection with IκB even though IκB fails to inhibit p50 binding in vitro. Co-immunoprecipitation studies also demonstrated an interaction of IκB with both p65 and p50. Mutational analysis demonstrated that the nuclear localization signal of p65 was critical for interaction with IκB. Also, addition of a heterologous nuclear localization signal inhibited the ability of IκB to exclude p65 from the nucleus. Surprisingly, IκB was also localized in the nucleus. When p50 and p65 were expressed with IκB, it was observed in the cytoplasm. These results suggest that IκB may have a role within the nucleus. The role of phosphorylation on localization and function will be discussed.

B 550 ANALYSIS OF FUNCTIONAL DOMAINS OF RELB, A TRANSCRIPTIONAL FACTOR OF THE REL FAMILY, Rolf-Peter Ryseck, Pawel Dobrzanski, and Rodrigo Bravo, Department of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000, USA

We have identified a serum-inducible gene, *relB*, which encodes a protein of 558 amino acids containing a region of 300 amino acids, the Rel homology domain (RHD), with high similarity to c-Rel and other members of the Rel family. In contrast to other members of this family RelB is not able to form stable homodimers and does not bind with high affinity to NF-κB sites. However, it forms heterodimers with p50-NF-κB and p50B-NF-κB that do bind to different NF-κB-binding sites with a similar or higher affinity to that shown by p50-NF-κB homodimers. We identified the minimal length of the RHD of RelB required for its dimerization with p50-NF-κB.

We have found that the transcriptional activation of RelB/p50-NF-κB heterodimers is suppressed by IκBα due to inhibition of effective DNA binding. Therefore we have initiated studies to identify the regions of RelB, p50-NF-κB, and IκBα necessary for this interaction. The results indicate that the RelB/p50-NF-κB complex in contrast to RelB and p50-NF-κB alone strongly associates with IκBα. Furthermore, a complex of p50-NF-κB with only the RelB RHD is weakly susceptible to the inhibiting effect of IκBα, although it can strongly bind to DNA. Whereas the presence of the N-terminal region of RelB has no influence on the IκBα interaction C-terminal located sequences outside the RHD are required for a complete inhibition of DNA binding. These observation have been confirmed by co-immunoprecipitation experiments.

Hybrid molecules between RelB and p50-NF-κB were constructed to better determine the region of RelB interacting with IκBα.

B 551 Functional and Physical Associations of NF-κB With Regulators of Cell Growth. Scheinman, R.I., Beg, A.A., and Baldwin, A.S. Lineberger Cancer Center, UNC at Chapel Hill, Chapel Hill, NC 27519.

NF-κB, a regulator of immune system and inflammation genes, consists of a 50 kDal protein (p50) and a 65 kDal protein (p65). The cloning of p50 and p65 revealed a homology with the proto-oncogene c-*rel* and expanded the model of NF-κB function to include the possible regulation of cell growth.

We have observed that an NF-κB like activity is transiently induced by serum stimulation of quiescent 3T3 fibroblasts. We now show that the p65 subunit of NF-κB is capable of transactivating gene expression from the c-*myc* promoter in transient transfections. We have mapped two p65 responsive sites; each site separately contributing a 3-4 fold stimulation, increasing to10-20 fold with both sites present. One site is located 1100 bp 5' of the P1 start site while the second site is located 3' of the P2 start site within exon 1 in a region responsible for transcriptional attenuation. In addition, we have preliminary evidence that fos is able to interact synergistically with p65 to activate c-*myc* over 100 fold above basal activity. We are currently addressing the importance of these NF-κB sites *in vivo*.

Recently a novel oncogene, *lyt*-10 (also known as p100/p49), was cloned from a translocation breakpoint in a B cell lymphoma and identified as a member of the *rel* family. We have identified a DNA binding protein with properties similar to *lyt*-10 while characterizing the EMSA complex, H2TF1, from HeLa and Raji extracts. We observe three EMSA complexes, primarily in cytoplasmic fractions, which have different binding specificities when tested with a panel of NF-κB motifs. UV crosslinking studies has identified a single 125 kDal DNA/protein complex derived from each of these EMSA complexes. This 125 kDal crosslinked complex is immunoprecipitated by a number of antisera raised against NF-κB, *rel*, and *lyt*-10 epitopes while an antibody specific for *lyt*-10 homodimers only supershifts the EMSA complex corresponding to H2TF1. These data suggest that cytoplasmic *lyt*-10 may be found either as homodimers or complexed with NF-κB and *rel* proteins, thus raising the possibility that *lyt*-10 functions either by sequestering NF-κB and blocking NF-κB function or by modifying NF-κB mediated gene expression.

B 552 STRUCTURE/FUNCTION ANALYSIS OF NF-κB P49(100). Roland M. Schmid, Susanne Liptay, Neil Perkins, Gary J. Nabel, Howard Hughes Medical Institute, University of Michigan Medical Center, Departments of Internal Medicine and Biological Chemistry, Ann Arbor, MI 48109-0650

p50/p(105) and p49/p(100) are highly related molecules of the NF-κB multigene family. They are synthesized as high molecular weight precursors and have ankyrin repeats in their C-termini. Homodimeric p49 binds weakly to the HIV κB site but strongly to the palindromic H2 κB element in contrast to p50 which binds both sites with high affinity. In association with p65, the heterodimeric complex binds with greater affinity to the HIV κB element. In transfection experiments, the combination of p49/p65 is more potent in stimulating the HIV enhancer. To understand the structural basis for this difference, chimeric p49/p50 proteins were synthesized. DNA binding activity and transactivation were tested in combination with p65. Analysis of chimeric molecules indicate, that a conserved region in the N-terminal portion of the rel conserved domain contributes to the specificity DNA binding and function of p49 and p50. Chimeric molecules have been characterized with p50 DNA binding properties which transactivate the HIV-LTR in synergy with p65 like p49. These results stongly suggest that the preferential transactivation by p49/p65 heterodimers is an intrinsic property of p49 amino acid sequences and is not likely to be due to repression by p50 homodimers.

B 553 NF-κB AND C/EBP TRANSCRIPTION FACTORS INTERACT TO ACTIVATE THE G-CSF PROMOTER, M. Frances Shannon, Stephanie M. Dunn, Leeanne S. Coles, Robyn K. Fielke and Mathew A. Vadas, Division of Human Immunology, Institute of Medical & Veterinary Science, Frome Road, Adelaide, South Australia, 5000.

Granulocyte-colony stimulating factor (G-CSF) is an important cytokine in myeloid haemopoiesis and in inflammatory reactions. Its expression is tightly controlled, being induced in mesenchymal and myeloid cell types by inflammatory mediators. Transcription mediated by the G-CSF promoter region is also inducible by inflammatory cytokines.

We have found that a functional element in the G-CSF promoter, termed CK-1, can be transactivated by over expression of p65, a member of the NF-κB family of transcription factors. More significantly, expression of antisense RNA for the NF-κB proteins, p50 and p65, can block activation of this sequence by TNF-α and IL-1β implying a direct or indirect involvement of NF-κB in activation of G-CSF. Although we had previously stated that NF-κB could not bind to the G-CSF CK-1 sequence, we can now detect weak NF-κB, as well as synthetic p65 binding to this sequence under altered polyacrylamide gel conditions.

Detailed mutational analyses has shown that p65 transactivation of the G-CSF promoter is dependent on CK-1 as well as an overlapping sequence which can bind NF-IL6. NF-IL6 is a member of the C/EBP family of transcription factors. Both the CK-1 and NF-IL6 sites are also essential for TNF-α and IL-1β activation of the G-CSF promoter.

These data imply that the IL-1/TNF activation of the G-CSF promoter requires an interaction between NF-κB and C/EBP proteins which may lead to the cell specific expression of G-CSF.

B 554 NF-κB ASSOCIATES WITH MEMBERS OF THE C/EBP AND AP-1 FAMILIES OF TRANSCRIPTION FACTORS.

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NF-κB, AP-1 and C/EBP represent three distinct families of transcription factors that target unique DNA enhancer elements. The heterodimeric NF-κB complex is composed of two subunits, a 50 kD and a 65 kD protein. All members of the NF-κB family are characterized by their highly homologous N-terminal region, found also in the product of the proto-oncogene c-*rel*. This Rel homology domain mediates DNA binding, dimerization and nuclear targeting of these proteins. The AP-1 transcription factor complex is comprised of dimers of the c-*jun* and c-*fos* proto-oncogene products or closely related proteins. AP-1 and C/EBP are members of the bZIP family, characterized by two elements in the C-terminal half of the proteins, a basic region domain involved in DNA binding and a leucine zipper motif involved in dimerization. The C/EBP family consists of several related proteins, C/EBPα, β, γ and δ that can form homodimers and heterodimers with each other.

We now demonstrate the unexpected cross-coupling of NF-κB with members of the C/EBP and AP-1 family. NF-κB p50, p65 and c-Rel directly physically associate with the bZIP region of C/EBPβ through the Rel homology domain. This cross-coupling results in altered DNA binding *in vitro*, in inhibition of promoters with κB enhancer motifs and in synergistic stimulation of promoters with C/EBP binding sites. Similarly, NF-κB p65 functionally synergizes with Jun and Fos through a "mini-leucine zipper" motif in its C-terminal activation domain. This results in synergistic stimulation of promoters with κB enhancer and AP-1 enhancer motifs. In contrast, Jun and Fos do not cross-couple with NF-κB p50 or with c-Rel. Only the nuclear form of NF-κB is associated with Jun or Fos. The exact nature of this interaction and the composition of the NF-κB–Jun/Fos and NF-κB–C/EBP complexes remain to be characterized. The cross-coupling of NF-κB with C/EBP, Jun and Fos highlights a mechanism of gene regulation involving the interaction between distinct transcription factor families.

B 555 INVOLVEMENT OF REL/NF-κB PROTEINS IN ACTIVATION OF GM-CSF GENE IN T CELLS

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Helper T cells produce a battery of lymphokines including GM-CSF when stimulated by antigens. This activation can be mimicked by a combination of phorbol ester (PMA) and calcium ionophore (A23187). We have identified two elements, GM2/GC box and CLE0, between positions -96 and -40 upstream of the mouse GM-CSF gene. Both of them are responsible for the maximal transcription activation, induced by the costimulation with PMA and A23187. The GM2 sequence (GGTAGTTCCC) is recognized by NF-GM2, whose binding activity is mainly induced by PMA. The CLE0 sequence appears to associate with a factor(s), NF-CLE0, which is optimally induced by PMA/A23187. We purified NF-GM2 from the nuclear extract of the stimulated Jurkat cells and found that NF-GM2 is composed of 50 kDa (p50) and 65 kDa (p65) polypeptides which are immunologically indistinguishable from NF-κB. We checked the involvement of human Rel/NF-κB proteins (p50 [p105], p50B [p100], p65, and c-Rel) in regulation of the GM-CSF gene by transient transfection assay in Jurkat cells. Among various combinations, cotransfection with p50 and p65 cDNAs stimulated optimally the transcription from the GM-CSF promoter harboring a $(GM2)_3$/GC-box sequence. The cotransfection with p50 and p65 cDNAs failed to activate the transcription from the GM-CSF promoter containing a single GM2/GC-box sequence, whereas it stimulated slightly the transcription from the promoter containing an Ig-κB (GGAAAGTCCC)/GC-box sequence, which shows a higher affinity to NF-κB (NF-GM2). The purified NF-GM2 stimulated the transcription from the Ig-κB enhancer, but not from the GM2/GC-box sequence of the GM-CSF promoter in the *in vitro* transcription assay. These results indicate that NF-κB alone cannot activate the transcription of the GM-CSF gene, suggesting that a cooperation with the GM2/GC-box and the CLE0 elements is required for the transcriptional activation. We are now characterizing the CLE0 element and the binding factor (NF-CLE0) in order to test this possibility.

B 556 **HIGH BASAL TRANSCRIPTION THROUGH THE NF-κB ELEMENT RESULTS IN ACCUMULATION OF MGSA/GROα IN HUMAN MELANOMA,** Lauren D. Wood, Rebecca L. Shattuck, and Ann Richmond, Departments of Cell Biology and Medicine, VA Medical Center (Nashville) and Vanderbilt University School of Medicine, Nashville, TN 37232

Genes encoding three isoforms of the chemokine, melanoma growth stimulatory activity (MGSA/GRO), have been identified. The three mRNAs are barely detectable in normal human melanocytes while the α isoform (but not β and γ) is highly prevalent in Hs294T melanoma cells. To characterize the regulation of expression of MGSA/GRO mRNA in Hs294T melanoma, the basal and cytokine induced transcription of MGSA/GRO were compared. The Hs294T melanoma cells exhibit a high basal transcription of the MGSAα 5' CAT fusion gene relative to non-malignant pigmented control cells. Mutation or deletion of the NF-κB element within this construct markedly decreased basal activity (>75%) in the melanoma cells. However, since all of the MGSA/GRO genes contain identical NF-κB elements, other potential sites may be involved in the elevated expression of MGSA/GROα in Hs294T cells. One potential co-regulatory site is the C/EBP-like element near the NF-κB element. MGSA/GROα mRNA is only modestly transcriptionally regulated by TNFα, IL-1α, TPA and cycloheximide as shown by nuclear run-offs and CAT assays, though gel mobility shift assays indicate strong activation by these factors of the NF-κB element in the Hs294T cells. Our findings suggest that deregulation of MGSA/GROα in Hs294T cells appears to involve the NF-κB element and that the 5' regulatory region of MGSA/GRO becomes transcriptionally insensitive to effectors requiring this element for enhancement of transcription.

B 557 REGULATION OF NF-kB PROTEINS BY ANKYRIN REPEAT CONTAINING INHIBITORS

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Regulation of the transcription factor NF-kB depends on the interaction between families of DNA binding proteins and cytoplasmic inhibitory proteins. The p50 subunit of NF-κB is synthesized as an 105 kdalton precursor protein(p105). Cytoplasmic processing of p105 releases the DNA-binding p50 domain. We show that p105 is able to inhibit DNA-binding of rel-factors in trans. This inhibitory function has been mapped to seven ankyrin-like repeats in the C-terminus of p105. In the cell, p105 is complexed with rel proteins in the cytoplasm, and thus serves as an anchor preventing nuclear translocation.

The ankyrin repeat domain of p105 is highly homologous to analogous domains in the cytoplasmic inhibitory proteins IkB/MAD-3 and pp40, as well as the candidate proto-oncogene bcl-3. bcl-3 is able to inhibit DNA-binding of NF-kB and displays asubunit specificity for p50 and p49. The ankyrin repeat domain of bcl-3 is necessary and sufficient for inhibition of p50. The region of p50 required for the interaction with bcl-3 has been mapped, allowing the definition of subdomains within the rel homologous region responsible for DNA-binding, dimerization, and inhibitor binding. The ability of bcl-3 to sequester NF-kB subunits in the cytoplasm has been demonstrated in a co-transfection assay.

Transcription Factors and Differentiation I

B 600 REGULATION OF C/EBP-RELATED GENES DURING ADIPOCYTE DETERMINATION AND DIFFERENTIATION.

Ingrid E. Akerblom and Charles P. Emerson, Jr., Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia,PA 19111. We have used the multipotential C3H10T1/2 cell line as a model system to examine genetic changes that occur when embryonic cells are determined to a specific differentiation pathway. These cells convert to stable muscle, adipocyte and chondrocyte lineages after treatment with DNA-demethylating agents. We have taken both molecular and genetic approaches in our initial efforts to identify genes important in the conversion of a multipotential cell to an adipoblast. Several novel adipocyte cell lines have been established by selection for 10T1/2 adipocyte variants that exhibit unique differentiation properties. Comparison of these adipocyte lines with both the parental 10T1/2 cells and with cells committed to an alternative lineage, the 10T1/2-derived 23A2 muscle cell line, has revealed differences in the expression of transcription factors known to be markers for adipocyte differentiation. Using western blot analysis, we have found that all differentiating 10T1/2 adipocyte lines express C/EBPα, a transcription factor which binds to regulatory regions of fat-specific genes and is required for differentiation in 3T3L1 adipocytes. In contrast, neither the parental 10T1/2 cells nor the 23A2 muscle cells express detectable C/EBPα in response to differentiation hormones. Therefore, one of the genetic programming changes which occurs during 10T1/2 conversion from a multipotential cell to a committed adipoblast is the ability to induce C/EBPα gene expression. Further work is being directed towards identifying elements on the C/EBPα gene which regulate this lineage-specific expression. Interestingly, 10T1/2 cells and 10T1/2-derived adipocytes share some common signal transduction properties as both express another marker of fat differentiation, C/EBPδ, in a similar manner during hormone treatment; 23A2 muscle cells, however, have lost the ability to express high levels of C/EBPδ. These results demonstrate that lineage choice in the 10T1/2 system involves both the acquisition and the loss of specific sets of transcription factors.

B 601 IDENTIFICATION OF LUNG-SPECIFIC ENHANCER AND CYCLIC AMP RESPONSE (CRE) ELEMENTS IN THE 5'-FLANKING REGION OF THE RABBIT SURFACTANT PROTEIN-A (SP-A) GENE, Joseph L. Alcorn, Erwei Gao, Margaret E. Smith, and Carole R. Mendelson, Depts of Biochem and Ob-Gyn, Univ Texas Southwestern Med Ctr, Dallas, TX 75235

The rabbit SP-A gene is expressed in pulmonary alveolar type II cells and is transcriptionally regulated by cAMP in fetal lung in culture. By electrophoretic mobility shift assay we identified two regions, termed "proximal binding element" (PBE, ~-100 bp) and "distal binding element" (DBE, ~-980 bp), that specifically bind type II cell nuclear proteins. The PBE corresponds to the localization of a lung-specific DNaseI hypersensitive site. To define regions of the SP-A gene involved in type II cell-specific and cAMP-regulated expression, fusion genes containing various amounts of DNA flanking the 5'-end of the rabbit SP-A gene linked to the human growth hormone (hGH) structural gene were introduced into transgenic mice and into primary cultures of type II pneumonocytes. Studies in transgenic mice indicate that -381 bp of SP-A 5'-flanking DNA is sufficient to promote lung-specific and appropriate developmental timing of hGH expression. In transfected type II cells in primary monolayer culture, we observed that -990 bp of SP-A 5'-flanking DNA is required for high levels of cAMP-inducible (30-40 fold) SP-A:hGH expression. When SP-A$_{-990}$:hGH fusion genes were transfected into two human adenocarcinoma cell lines (A549 and H358) which do not express SP-A and into cAMP-responsive adrenal Y1 cells, Bt$_2$cAMP had no effect to increase fusion gene expression. Deletion of the DBE (SP-A$_{-976}$:hGH) resulted in a >90% decrease in basal levels of fusion gene expression in type II cells, although a 2.5-fold induction in fusion gene expression by Bt$_2$cAMP was observed. The finding that basal expression of SP-A$_{-976}$:hGH fusion genes also was greatly reduced in H358, A549 and Y1 cells suggests that the DBE may act as a general enhancer. Mutagenesis of the putative CRE (TGACCTCA) at -260 bp resulted in a >95% reduction in basal and cAMP stimulated levels of SP-A$_{-990}$:hGH fusion gene expression in type II cells. Deletion of the PBE at -100 bp also caused a pronounced reduction in fusion gene expression in type II cells and abolished the stimulatory effect of cAMP. These findings suggest a synergistic interaction of *trans*-acting factors bound to the CRE, the DBE and PBE; the DBE may serve as a general enhancer, whereas, the PBE appears to be required for type II cell-specific cAMP-induced SP-A gene expression.

B 602 **EXPRESSION OF MULTIPLE HUMAN *fur* TRANSCRIPTS CONTAINING ALTERNATIVE 5' NONCODING EXONS**

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The ubiquitously expressed *fur* gene encodes the furin protein, a member of the novel mammalian family of subtilisin-like proprotein processing enzymes with cleavage specificity for pairs of basic amino acids. Furin is shown to possess cleavage specificity for the consensus motif Arg-X-Arg/Lys-Arg. Other members of this family, such as PC1/PC3 and PC2, cleave C-terminally of simple pairs of basic amino acids. In gene transfer experiments, furin has been shown to accurately process various substrates, such as the precursors of von Willebrand factor, ß-nerve growth factor, albumin, complement C3 and influenza virus hemagglutinin. There is only limited knownledge about the regulation of expression of the *fur* gene. Three alternative 5'-noncoding exons have been detected and characterized in cDNAs from human, mouse and rat tissues, indicating that the presence of mRNA isoforms is a conserved characteristic of *fur* gene expression. Results of our studies point towards the involvement of three alternative promoters in the regulation of *fur* gene expression. The transcription start sites for the alternative *fur* promoters were determined using primer extension analysis. Exon 1A had an extremely heterogenous start site while exons 1 and 1B had a major start site and some minor ones. The regions upstream of exons 1A and 1B have characteristic features of the promoter regions of so-called "housekeeping genes". They lack typical TATA and CAAT boxes, have a high G+C content, multiple Sp1-binding sites and transcription is initiated at heterogenous sites. The region upstream of exon 1 contains a TATA element and is not G+C rich. Interestingly, the TATA box seems to be located downstream of the transcription start site as determined by primer extension analysis. We are currently characterizing the three putative promoter regions for their possible involvement in the differential regulation of the *fur* gene in different tissues and developmental stages.

B 603 COMPLEX REGULATORY STRUCTURE OF THE HUMAN EMBRYONIC β-GLOBIN GENE PROMOTER, Margaret H. Baron and William L. Trepicchio, The Biological Laboratories, Harvard University, Cambridge, MA 02138.

During its ontogeny, the human red blood cell expresses first an embryonic, then a fetal, and finally an adult globin gene ("hemoglobin switching"). Very little is known about the regulatory elements which control embryonic β-globin gene (ε) expression. We have prepared a series of promoter mutants by exonuclease III digestion and have analyzed their behavior in several different cell lines. Comparison of expression patterns of the various promoter constructs in mouse erythroleukemia (MEL), GM979 (MEL cell variant that expresses both embryonic and adult globins), K562 (human embryonic erythroid), and Hela cells suggests the presence of at least three positive and at least four negative regulatory elements within the 860 base pairs just upstream of the transcriptional start site. An embryonic erythroid-specific positive regulatory element is of particular interest. To our knowledge, it is the first stage-specific positive control element to have been identified for any of the mammalian globin genes. Erythroid-specific expression of the embryonic β-globin gene appears to be mediated in part by two other positive regulatory elements, presumably in combination with the dominant regulatory "locus control region (LCR)" located far upstream. We have identified four (possibly five) negative elements, one of which probably corresponds to a previously reported silencer that is more active in nonerythroid than in erythroid cells. Thus, the organization of the human embryonic globin gene promoter region is more complex than previously believed. *In vivo*, the high level expression of this promoter in erythroid cells probably results from synergistic/cooperative interactions among the multiple (relatively weak) positive regulatory elements. Correct temporal regulation of the gene must reflect a balance between antagonistic and synergistic interactions among the negative and positive elements. DNA-binding studies are now in progress to identify the regulatory protein(s) that are responsible for the embryonic expression pattern of the earliest-transcribed β-globin gene.

B 604 INVOLVEMENT OF A C/EBP-LIKE PROTEIN IN THE ACQUISITION OF RESPONSIVENESS TO GLUCOCORTICOID HORMONES DURING CHICK NEURAL RETINA DEVELOPMENT, Sara Ben-Or[1] and Sam Okret[2], [1]Department of Physiology, Hebrew University, Hadassah Medical School, P.O.B. 1172, Jerusalem 91010, Israel and [2]Department of Medical Nutrition, Karolinska Institute, Huddinge University Hospital F60 Novum, S-141 86 Huddinge, Sweden

The glucocorticoid receptor (GR) in chick embryonic (E) neural retina is expressed early in ontogeny, yet the tissue's response to the glucocorticoid hormone, i.e. induction of glutamine synthetase (GS) develops later, only during the second week of ontogeny. Transient transfection of embryonic day 7 (E7) retinal cells, which are nonresponsive to glucocorticoids, with chimeric plasmids containing the chloramphenicol acetyltransferase (CAT)-reporter gene under the control of glucocorticoid responsive promoters, demonstrated that GR in E7 cells is a functional transactivating factor. We show that the limiting transcription factor that controls the developmental acquisition of responsiveness to glucocorticoids is a member of the CCAAT enhancer binding protein (C/EBP) family. This protein recognizes a sequence in the cloned chick GS promoter, which is required for eliciting the glucocorticoid response. Retinal C/EBP-like protein was not detected in the glucocorticoid nonresponsive (E7) proliferating glioblasts but was found to be present in glucocorticoid responsive (E12) post-mitotic cells. Premature expression of C/EBP in the nonresponsive E7 cells by transfection was shown to enhance the developmental acquisition of responsiveness to the glucocorticoid hormone, as deduced from the level of GS inducibility.

B 605 STEROID RESPONSE ELEMENTS ARE INVOLVED IN THE TRANSCRIPTIONAL INDUCTION OF THE EPO GENE BY HYPOXIA Kerry L. Blanchard¥, Anthony M. Acquaviva, Deborah Galson, H. F. Bunn, Department of Medicine, Division of Hematology/Oncology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, and ¥Louisiana State University Medical Center, Shreveport, LA.

Oxygen is vital for growth and development of mammalian cells. During physiologic and pathologic stress (e.g. ischemia, neoplasia, respiratory failure, and anemia), cells are subjected to low oxygen tensions. Changes in gene expression are induced by hypoxia. A striking example of a gene regulated by hypoxia is erythropoietin (Epo), the hormone that controls the oxygen carrying capacity of the blood. Epo transcription increases 50-100 fold in response to hypoxia *in vivo* and in an *in vitro* system based on the cell line Hep3B. We have investigated the cis-acting elements necessary for this response by transient expression in Hep3B cells of a vector containing fragments of the Epo promoter and enhancer upstream of a luciferase reporter gene. We have defined short regions of the promoter and enhancer that cooperate in the hypoxic induction of expression. Both regions contain steroid receptor response element half-sites, and the enhancer contains a direct repeat of half-sites with a 2 bp gap (DR-2). A factor(s) that bind(s) the DR-2 in the enhancer has been identified by electrophoretic mobility shift assay. DNA binding has been characterized by DNase I footprinting and methylation interference. The ability of the DR-2 region to direct transcriptional induction is destroyed by site-specific mutagenesis of the DR-2. Steroid hormones in physiologic concentrations do not affect the basal or inducible activity of the enhancer, suggesting the DR-2 may be activated by an orphan member of the steroid receptor superfamily.

B 606 The NFAT-1 DNA-BINDING COMPLEX IN ACTIVATED T CELLS CONTAINS FRA-1 AND JUNB, Lawrence H. Boise[1], Xiaohong Mao[2], Rodrigo Bravo[3], Jeffrey M. Leiden[4], and Craig B. Thompson[1,2,5]
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Interleukin-2 (IL-2) gene expression is activated upon stimulation of T cells through the signal transduction pathway associated with the CD3/T cell receptor complex. Activation of IL-2 gene expression is transcriptional and mediated through a 320 bp enhancer that is upstream of the gene. One site within the enhancer which is a good model of T cell gene regulation is the NFAT-1 site, since, like the IL-2 enhancer its activity is specific to activated T cells and sensitive to cyclosporin A. We have previously reported that the NFAT-1 site binds a complex of at least two proteins and that the 5' portion of the site can function as a binding site for members of the Ets protooncogene family. In the current studies we demonstrate that the 3' portion of the NFAT-1 site is a variant form of an AP-1 site. NFAT-1 binding can be competed specifically by a consensus AP-1. Mutation of the AP-1 site within the NFAT-1 sequence blocks the ability for complex formation in gel shift assays and the ability of NFAT-1 to function as an inducible enhancer in transfection assays. To determine if specific AP-1 dimers are part of the NFAT-1 DNA-binding complex, antisera against different Fos and Jun family members were used in gel shift assays. Supershifts were seen with antisera specific to Fra-1 and JunB. An explanation for the specificity of the presence of Fra-1 and JunB in the complex is currently under investigation. Interestingly the expression of *jun*B mRNA was shown to be completely dependent on T cell activation. Current theories on complex formation of NFAT-1 suggest that a newly synthesized ubiquitous component complexes with a modified T cell-specific component. Thus, formation of a Fra-1/JunB heterodimer may represent the newly synthesized portion of the NFAT-1 DNA-binding complex.

B 607 **RNA EXPRESSION AND LOCALIZATION OF THE NUCLEAR HORMONE RECEPTOR ST-59 (NUR77/NGF-B/N10) IN HUMAN LUNG,** Gregory P. Bondy, Shaun Granleese, Simon Bicknell, John-David Aubert, James C. Hogg and Tony R. Bai, UBC Pulmonary Research Laboratory, St. Paul's Hospital, Vancouver, British Columbia, Canada, V6Z 1Y6.

ST-59 is an inducible gene that has the structure of a ligand activated nuclear hormone receptor gene (Cell Growth Diff. 2:203-208,1991). It is normally not expressed in quiescent cells but is rapidly induced by a variety of external stimuli including growth factors, serum, heat shock, and seizures. The function of ST-59 in cells is unknown. In order to improve our understanding of ST-59's function in cells, we have been examining the expression of ST-59 in normal and pathological human tissues. ST-59 mRNA was found to be persistently and highly expressed in peripheral human lung tissue. The expression of other "early response" genes (fos, jun, myc) was low or not detectable in lung tissue. In situ hybridization histochemistry localized the ST-59 mRNA expression to bronchiolar and alveolar lining cells. ST-59 mRNA expression was examined in a number of pathological conditions. Expression of ST-59 in lung carcinomas was decreased when compared to adjacent non-neoplastic lung tissue. The decrease in expression of ST-59 in lung tumors was not due to gene deletion or re-arrangement. Studies with the A549 cell line demonstrate that high levels of ST-59 expression are induced in lung cancer cells following treatment with serum and/or second messanger analogues (phorbol esters, forskolin). Variable expression of ST-59 was observed in lung tissue obtained from patients with asthma and chronic obstructive lung disease. A very high level of expression was seen in a 4 yr old child who died of acute asthma. These findings demonstrate that ST-59 is highly expressed in the lung and suggest that the level of ST-59 expression is modulated by external signals.

B 608 IDENTIFICATION OF AN ESTROGEN - RESPONSIVE ELEMENT IN THE HUMAN GFAP GENE.

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In this work we demonstrate that 17β –estradiol can increase the level of glial fibrillary acidic protein (GFAP) mRNA in the human glioma cell line U - 343 MGa 31 L. We have determined the promoter sequence of the human GFAP gene and studied the promoter activity with and without 17β –estradiol stimulation. In the proximal promoter region between -250 and +1 bp we also identified several regulatory motifs including an Estrogen Response Element (ERE) (GGGCACAGTGACC) showing only one mismatch from the ERE consensus sequence (GGTCAXXXTGACC). The functionality of this element has been analysed by gel retardation and DNase I footprinting experiments.

B 609 FUNCTIONAL ANTAGONISM BETWEEN RETINOIC ACID AND VITAMIN D3 IN THE REGULATION OF CD14 AND CD23 EXPRESSION DURING MONOCYTIC DIFFERENTIATION OF U-937 CELLS

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1,25α-dihydroxicholecalciferol (VitD3) and retinoic acid (RA) are important regulators of proliferation and differentiation in many cell systems. We show that the expression of the monocyte-macrophage antigen CD14 and the low affinity Fc receptor for IgE, CD23, were inversely regulated by VitD3 and RA during monocytic differentiation of U-937 monoblasts. Phorbol-myristate-acetate (PMA) induced the expression of both CD14 and CD23. VitD3, on the other hand, rapidly induced CD14 mRNA and protein while suppressing the basal, PMA- and RA induced CD23 levels in a concentration dependent manner. In contrast, U-937 cells induced by RA strongly upregulated the CD23 expression, whereas they completely lacked CD14. Furthermore, the VitD3- and PMA inducible CD14 expression was gradually inhibited as a temporal consequence of RA induced differentiation. The mechanisms behind the inverse regulation of these genes in U-937 cells are not known. However, the similarities between the VitD3 and RA signaling pathways, i.e. similar nuclear receptors, common co-receptors and related gene target sequences, provide several possible levels of interaction that could explain the functional antagonism between RA and VitD3 with respect to CD14 and CD23 gene regulation. The effects on CD14 and CD23 further implicate VitD3 and RA as important regulators of monocyte differentiation and activation during inflammatory responses and normal monopoiesis.

B 610 AN ALTERNATIVE AP-2 PROTEIN IS A NEGATIVE REGULATOR OF TRANSACTIVATION, Reinhard Buettner*, Axel Imhof*, Reinhard Bauer*, Peri Kannan+, and Michael A. Tainsky+, *Department of Pathology, University of Regensburg, D-8400 Regensburg, Germany; +Department of Tumor Biology, M.D. Anderson Cancer Center, Houston, TX 77030

AP-2 is a retinoic acid-sensitive and developmentally regulated transcription activator. We have cloned an alternative AP-2 transcript (AP-2B) from the human teratocarcinoma cell line PA-1, which encodes a protein differing in the C-terminus from the previously isolated AP-2 protein (AP-2A). Analysis of overlapping genomic clones spanning the entire AP-2 gene proves that AP-2A and AP-2B transcripts are alternatively spliced from the same gene. Both transient and stable transfection experiments show that AP-2B inhibits AP-2 transactivator function as measured by an AP-2 sensitive CAT reporter plasmid. Furthermore, constitutive AP-2B expression causes a retinoic acid resistant phenotype and leads to anchorage independent growth in soft agar, phenotypes that have been associated previously in PA-1 cells with the effect of transformation by oncogenes.

To determine the mechanism by which AP-2B exerts its inhibitory function, we have purified bacterially expressed proteins A and B. While bacterial AP-2B does not bind an AP-2 consensus site, it strongly inhibits binding of the endogenous AP-2 present in PA-1 nuclear extracts. In contrast, bacterially expressed AP-2A binds the DNA consensus site, but is not competed by AP-2B. We conclude that inhibition of AP-2 by the protein AP-2B requires an additional factor or modification present in nuclear extracts.

B 611 A COMPLEX REGULATORY ELEMENT IS REQUIRED FOR CO-OPERATION BETWEEN A PEA3/AP-1 ELEMENT AND AN AP-1 SITE DURING PHORBOL ESTER INDUCTION AND GLUCOCORTICOID REPRESSION OF THE ENHANCER ELEMENT OF HUMAN UROKINASE GENE.

Anna Caracciolo, Dario de Cesare, Claus Nerlov, Francesco Blasi, Morten Johnsen and Pasquale Verde, University Institute of Microbiology, Øster Farimagsgade 2A, 1353 Copenhagen K, Denmark and International Institute of Genetics and Biophysics, Via Marconi 12, 80125 Naples, Italy.

We have analyzed the enhancer of the human urokinase-type plasminogen activator (uPA) gene. uPA is a serine protease involved, like collagenase and stromelysin, in the degradation of the extra-cellular matrix. We have found that the enhancer sequence, located at about 2 kb upstream of the transcription start site of the uPA gene, is required both for growth factor-dependent induction of the gene, which can be mimicked by phorbol esters, and for transcriptional repression by glucocorticoids. In vivo and in vitro mutational analysis showed the role of multiple binding sites for nuclear factors, which interact with a 100 bp DNA region.We have characterized a canonical AP-1 site and a PEA3/AP-1 element, which contains a spurious AP-1 sequence. This AP-1-like site interacts in vitro with a factor related to, but distinct from the jun-fos heterodimer. The other binding sites form a regulatory element, that we designated co-operation mediator (COM), since it is required for co-operation between the PEA3/AP-1 element and the AP-1 site. Both the PEA3/AP-1 element, the AP-1 site and the COM are required for phorbol ester induction and for glucocorticoid-mediated repression of transcription from the uPA promoter in the HepG2 hepatoma cell line. Mutational analysis showed that COM function requires the presence of multiple overlapping binding sites for nuclear proteins, designated uPA enhancer factors 1 through 4 (UEF-1 to -4). We have identified putative binding sites for UEF-1, -2 and -3. Several lines of evidence suggest that the UEF factors might play a more general role, in addition to their function in the context of the urokinase enhancer. In fact, we have shown that the COM is able to mediate co-operation between different inducible trans-acting factors. We have also shown that UEF-3 binds to the NIP-element, previously characterized as a regulatory element in the human interleukin-3 and stromelysin promoters, suggesting that it plays a role in regulation of a variety of genes.

B 612 TWO NUCLEAR SIGNALLING PATHWAYS FOR VITAMIN D, Carsten Carlberg, Clinique de Dermatologie, Hôpital Cantonal Universitaire, CH-1211 Genève 14, Switzerland

1,25-dihydroxyvitamin D3 (VD) mediates a biological response by binding to intracellular VD-receptors (VDR) that belong to the steroid receptor superfamily. These receptors act as ligand-dependent transcription factors which bind to specific DNA sequences. We have identified two classes of VD response elements that are either activated by the VDR alone or by heterodimers of VDR and the retinoid-X-receptor α (RXR). Arranging the motif GGGTGA either as a direct repeat with a spacing of 6 nucleotides or as a palindrome or inverted palindrome with 0 and 12 nucleotides spacing, respectively, confers VD inducibility that is mediated by VDR alone. Conversely, a second class of VD response elements composed of directly repeated pairs of motifs (GGTTCA, AGGTCA, or GGGTGA) spaced by 3 nucleotides is synergistically activated by RXR and VDR, but only in the presence of both ligands. Thus, the nature of the response element determines whether a nuclear receptor is co-regulated by RXR.

B 613 **REGULATION OF THE C/EBP-RELATED GENE, *gadd153*, BY GLUCOSE DEPRIVATION,** Sara G. Carlson, Timothy W. Fawcett, Nikki J. Holbrook; Laboratory of Molecular Genetics, National Institute on Aging, Baltimore, MD 21224.

gadd153 encodes a C/EBP-related protein which heterodimerizes with other C/EBPs but does not bind DNA. *gadd153* may therefore function as a negative regulator of C/EBP transcription factors. Since it has been proposed that C/EBPalpha may be a central regulator of genes encoding proteins involved in energy metabolism, we have examined the role of glucose in the control of *gadd153* expression, and have compared the expression of *gadd153* with that of other C/EBP genes. Glucose deprivation induced *gadd153* mRNA levels 14.4 and 15.4 fold in HeLa and 3T3-L1 cells, respectively. This induction was reversible, as addition of D-(+)-glucose resulted in the rapid loss of *gadd153* mRNA. A similar induction and reversal of *gadd153* expression in response to glucose deprivation was observed at the protein level. Nuclear run-on assays determined that the induction is transcriptionally mediated. Because the C/EBPs appear to play an important role in the induction and/or maintenence of adipogenesis, we examined the expression of *gadd153* in relation to other C/EBPs during differentiation of 3T3-L1 cells, and as a function of glucose utilization. As per the standard differentiation scheme, differentiation was induced by hormonal stimulation (dexamethasone, insulin, methylisobutylxanthine) for 2 days, followed by medium changes every 2 days thereafter. As expected, under the standard conditions, C/EBPs beta and delta were induced early (days 1 and 2) in response to the hormone treatment, followed by C/EBP alpha (day 3) as the cells became more differentiated. *gadd153* was not significantly expressed until after C/EBPalpha (day 4). Because adipocytes metabolize glucose at a much higher rate than preadipocytes, we suspected that glucose depletion may influence the expression of gadd153 during differentiation. To test this, we altered glucose levels by either refeeding the cells with fresh medium or adding D-(+)-glucose to the medium every 8 hours. When glucose concentrations were maintained at high levels by either refeeding or adding glucose, *gadd153* expression was significantly reduced in contrast to the other C/EBPs. These results show that *gadd153* expression is not essential for the process of differentiation, and is significantly influenced by the availability of glucose to the cell.

B 614 REACTIVATION OF A MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II GENE IN MOUSE PLASMACYTOMA CELLS AND MOUSE T CELLS, Cheong-Hee Chang, William L. Fodor, and Richard A. Flavell, HHMI, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

Terminally differentiated plasma cells and mouse T cells do not express Major Histocompatibility Complex (MHC) class II genes although class II gene expression is observed in pre B cells and mature B cells as well as in activated human T cells. Transient heterokaryons were prepared and analyzed to investigate the mechanisms of inactivation of MHC class II gene in mouse plasmacytoma cells and mouse T cells. The endogenous MHC class II genes in both mouse plasmacytoma cells and mouse T cells can be reactivated by factors present in B cells. This reactivation of class II gene is also observed by fusion with a human T cell line which express MHC class II genes but not with a class II negative human T cell line. It appears that the loss of MHC class II gene expression during the terminal differentiation of B cells or T cell lineage is due to absence of positive regulatory factor(s) necessary for class II transcription. Cloning of this factor(s) will be discussed.

B 615 CONVERGENT REGULATION OF NF-IL6 AND OCT-1 SYNTHESIS BY INTERLEUKIN-6 AND RETINOIC ACID SIGNALLING, Selina Chen-Kiang and Wei Hsu, Molecular Biology Center, Mt Sinai Medical School, New York, N.Y. 10029

Among the mammalian genes encoding POU domain-containing proteins, the oct-1 gene is ubiquitously expressed and has been shown to be regulated post-translationally. We present evidence here that it can also be regulated at the levels of mRNA and protein synthesis by two diverse signals, interleukin-6(IL-6) and retinoic acid (RA) in human embryonal carcinoma and T cells. NF-IL6, a human transcription factor of the C/EBP family and an intermediate in the IL-6 signal transduction pathway, can confer this regulation. Furthermore, the abundance and the molar ration of the three forms of NF-IL6, corresponding to the activator and repressor proteins initiated in frame from different AUG's, are regulated by IL-6 and by RA. These results suggest that the two signal transduction pathways overlap and that Oct-1 may be downstream of NF-IL6 in the shared regulatory cascade.

We have previous shown that NF-IL6 can substitute for the adenovirus E1A proteins in transactivating E1A-respnsive promoters in transfection and in complementing E1A-deletion mutants in viral infection. Enhanced Oct-1 synthesis was found to correlate with one of the functions of Oct-1: stimulation of adenovirus DNA replication. This result provides an example of the functional consequence of IL-6 and RA signalling that is mediated by NF-IL6 and Oct-1 regulation.

B 616 INDUCTION OF DIFFERENTIATION OF 3T3-L1 FIBROBLASTS TO ADIPOCYTES BY 3-DEAZAADENOSINE OR INSULIN REQUIRES C-*FOS* PROTO-ONCOGENE. Chiang, P. K., Dave, J. R., Nicholson, D. E., *Rhodes, C., *Yamada, Y., and Zeng, G. C., Walter Reed Army Institute of Research, and Walter Reed Army Med. Ctr., Washington, DC 20307-5100, and *National Institute of Dental Research, NIH, Bethesda, MD 20892.

3-Deaza-adenosine (3-deaza-Ado) is an inhibitor of S-adenosylhomocysteine (AdoHcy) hydrolase. In cells treated with 3-deaza-Ado, AdoHcy accumulates, leading to an inhibition of methylation reactions. Confluent 3T3-L1 fibroblasts treated with either 3-deaza-Ado or insulin differentiate into adipocytes [Science 211, 1164 (1981)]. Both 3-deaza-Ado and insulin produced in confluent 3T3-L1 cells a rapid but transient expression of mRNA for proto-oncogenes c-*fos* and c-*jun* within 30-60 min, after which the mRNA of both proto-oncogenes became undetectable. In comparison c-myc proto-oncogene became detectable after 2 h, and then declined after 4 h. Electrophoretic mobility shift assays showed an increase in the levels of transcription factors AP-1 and AP-2, 1 h following treatment with either 3-deaza-Ado or insulin. In cells stably transfected with antisense c-*fos*, neither 3-deaza-Ado nor insulin could induce cellular differentiation response. In comparison, in cells transfected with sense c-*fos*, the differentiation to adipocytes proceeded as normal. Furthermore, in cells stably transfected with c-*fos*-CAT, the CAT activity was activated by 3-deaza-Ado or insulin in a dose-dependent manner, and there was no additive effect between 3-deaza-Ado and insulin. In cells stably transfected with H-*ras*, the CAT activity was not affected by 3-deaza-Ado or insulin.

B 617 TRANSCRIPTIONAL REGULATION OF JUNB PROMOTER ACTIVITY, Paul J. Coffer, Hubrecht Laboratory for Development Biology, Utrecht, The Netherlands.

The product of the *jun*B gene, is a component of the AP-1 transcription factor family. Although homologous to the proto-oncogene c-Jun, JunB differs in its ability to both bind DNA, transactivate target genes and in its response to extracellular stimuli.

JunB expression is itself modulated by a wide range of extracellular stimuli including serum, growth factors, TPA and protein kinase A activators. We have recently cloned the mouse *jun*B promoter and have analysed the response of promoter deletion constructs to mitogens.

We will present further data concerning the modulation of *jun*B promoter transcriptional activation and discuss the possible role of such regulation in differentiation of embryonal carcinoma cells and embryonic stem cells.

B 618 MULTIPLE MECHANISMS OF COUP-TF-DEPENDENT REPRESSION OF STEROID RECEPTOR TRANSACTIVATION.

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COUP-TF is a member of the steroid/thyroid hormone receptor superfamily, about which little is known of its functional role in the cell. However, it is able to repress hormonal induction of target genes by VDR, TR, and RAR. We have previously shown that COUP-TF can bind a wide variety of A/GGGTCA repeats. This promiscuous recognition of response elements correlates with the ability of COUP-TF I to repress other receptors that bind to A/GGGTCA repeats with different spacings between the half sites. Here we show that repression of transactivation by these receptors is a general phenomenon for the COUP-TF subfamily, as inhibition is also observed with COUP-TF II. This repression is dose-dependent on COUP-TF. Inhibition of VDR, TR, and RAR activities also occurs through natural physiological response elements found in the osteocalcin, myosin heavy chain, and βRAR promoters, respectively. In search of the mechanisms of repression by COUP-TF we show that it does not involve the formation of detectable functionally inactive heterodimers between COUP-TF and VDR, TR, and RAR. Instead, we show that the mechanism of repression could occur at three different levels : active silencing of transcription, and dual competition for, (a), occupancy of DNA binding sites, and (b), heterodimer formation with RXR, the coregulator of VDR, TR, and RAR. The silencing activity was localized to the putative ligand binding domain of COUP-TF. In addition, COUP-TF can inhibit RA induction of a reporter gene through endogenous RAR in P19 and F9 embryonic carcinoma cell lines. We postulate that COUP-TF may play a master role in regulating transactivation by VDR, TR, and RAR.

B 619 THE ROLE OF NUCLEAR FACTORS (MYB, SCL AND ID) IN MEL CELL DIFFERENTIATION,

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Friend-virus infected murine erythroleukemia (MEL) cells, a model for erythroid differentiation can be induced by various agents (HMBA and DMSO) to terminally differentiate into more mature erythroid cells expressing globin. During this terminal differentiation, the nuclear protooncogenes, c-*myc* and c-*myb*, are biphasically downregulated. Constituitive expression of either *myc* or *myb* blocks differentiation in a dose dependent manner. Mutational analysis of c-*myb* shows that its ability to block differentiation requires intact DNA binding and transactivation domains suggesting that *myb*, like *myc*, blocks differentiation through a transcriptional transactivation mechanism.

Negative regulatory nuclear factors may also control differentiation. In the muscle system, Id-1 dimerizes with E2A (a constituitively expressed HLH protein) preventing E2A from dimerizing with muscle specific MyoD to form a DNA binding complex. During differentiation, the expression of Id-1 decreases, correlating with an increase in the dimerization of E2A with MyoD. Id-1 and Id-2 are also down-regulated during lymphoid and erythroid differentiation. To test if Id is a negative regulator of MEL cell differentiation and up-regulation of Id-1 can block differentiation, we stably transfected MEL cells with Id-1 under the control of an inducible promoter. Preliminary results suggest that Id-1 does not block differentiation even with high Id protein expression.

In contrast to *myb* and *myc*, the expression of an erythroid specific nuclear factor SCL increases with MEL differentiation, and over-expression of stably transfected SCL increases the frequency of chemically induced and spontaneous differentiation. Preliminary data suggest that SCL may operate downstream from *myc* and *myb* because in MEL cells, blocked in differentiation by stably transfected anti-sense SCL, *myc* and *myb* were still down regulated. Conversely, in cells blocked by *myb* and *myc*, the expression of SCL did not increase. To test this hypothesis, MEL cell clones, blocked by stably transfected *myb*, are being super-transfected with vectors expressing SCL and the effect on chemically induced differentiation examined.

B 620 REGULATION OF GERMLINE TRANSCRIPTION WITHIN THE HUMAN T-CELL RECEPTOR Jα CLUSTER.

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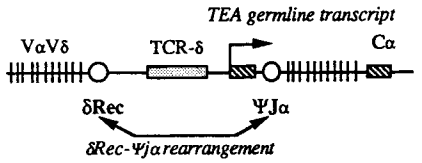
The TCR-α and TCR-δ loci are located on the same chromosome, but are independently rearranged and expressed during T cell ontogeny. The δRec-ΨJα rearrangement, which is preceded by the germline transcription of the ΨJα region (TEA transcript), leads to the site-specific deletion of the TCR-δ locus.



In order to get insight into the molecular events that may govern the accessibility of the Jα cluster to the recombinase machinery, we analyzed the regulation of the human TEA transcription. The human TEA transcript initiates from one major start site located 2.5 Kb upstream of the ΨJα recombination acceptor site. A DNase I hypersensitive site was found in the vicinity of the TEA transcription start site. Although this HS site was ubiquitous it was much stronger in lymphoid versus non lymphoid cells. This suggests the presence of tissue specific DNA-binding factors in this area. Indeed, several evolutionary-conserved DNA-binding sites for known lymphoid-specific transcription factors could be noted in the TEA promoter. The sequence analysis of the murine counterpart of TEA promoter revealed a region with 70% homology over 250 bp centered around the TEA start site. Transient transfection experiments established that a 140bp fragment upstream of the TEA initiation site had promoter activity in many cell types, but was dependant on the addition of a transcriptional enhancer. These results suggest that the germline transcription through the Jα cluster, under the control of the TEA promoter and the TCR-α and δ enhancers, may "unlock" the TCR-α locus for recombination. The understanding of the fine regulation of the TEA transcription might help elucidate the γδ versus αβ T cell differentiation pathways.

B 621 IDENTIFICATION OF SPECIFIC DNA BINDING SEQUENCES FOR A DIVERGED HUMAN HOMEOPROTEIN.

Yasuhiro Deguchi, Atsushi Muraguchi and John H. Kehrl, Dept. Immunol., Toyama Med. Pharm. Univ., Japan and NIAID, NIH, USA.

Homeobox genes encode specific DNA-binding proteins which have been implicated in the control of gene expression both in developing as well as in adult tissues. We have recently isolated a diverged human homeobox gene, HB24, which encodes a homeodomain which is 35-48% identical to the homeodomains of the major classes of mammalian and Drosophila homeobox genes. HB24 was found to be expressed in hematopoietic progenitors, but not in differentiated hematopoietic cells. Treatment of CD34 positive hematopoietic progenitors with IL3 and GM-CSF in vitro resulted in an initial increase in HB24 mRNA transcripts followed by their down-regulation and disappearance after several days of culture. Furthermore, high levels of HB24 were found in cells from patients with acute myelogenous leukemia. These data suggest that HB24 play an important role in the proliferation and lineage commitment of hematopoietic progenitors and that dysregulated expression of HB24 may potentially contribute to leukemogenesis. In this study, the specific DNA binding sites of the HB24 protein were identified by polymerase chain reaction and cloning DNA fragments from binding complexes from repeated gel retardation assays using random oligonucleotide fragments. The HB24 protein used in this study was from in vitro translation of in vitro transcribed HB24 RNA. We have found that the HB24 protein specifically binds to nucleotide sequences similar to the target binding sites of Drosophila Antenapedia (Antp) homeotic selector protein. By using mutated oligonucleotides, the core binding site of ATTA was identified. Functional analysis using reporter-chloramphenicol acetyltransferase (CAT) constructs containing this target core binding site is in progress.

B 622 **TRANSCRIPTIONAL REGULATION OF INTERFERON ALPHA GENES, Carolyn Dent, Nigel Sharp, Sonya MacBride, and Dirk Gewert, Department of Cell Biology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, UK.**

We have recently commenced a series of experiments aimed at a more complete understanding of the induction of interferon-α by Sendai virus. In this poster we communicate our initial findings and discuss their relevance to induction. Our studies are aimed at the identification and characterisation of transcription factors (including IRF-1 and IRF-2) involved in the induction process, and comparing the relevance of these factors to the induction of different interferon alpha sub-types. Promoters from subtypes known to be differentially induced by Sendai virus in Namalwa lymphoblastoid cells have been cloned into an expression vector, ptkSAP. The levels of SAP (secreted alkaline phosphatase) activity driven by these promoters on induction can then be assayed and compared with the proteins found to be binding to the same promoters in gel retardation assays. This will then enable us to compare mutations that disrupt the binding of proteins with their ability to drive transcription, thus aiding us in the identification of the role of these proteins.

B 623 MOUSE MAMMARY TUMOR VIRUS DISPLAYS DISCRETE TRANSCRIPTIONAL REGULATION IN DIFFERENT CELLULAR ENVIRONMENTS, Stefan Doerre and Ronald B. Corley, Department of Immunology, Duke Medical Center, Durham, NC 27710

The expression of MMTV has been used as a paradigm of tissue specific transcriptional regulation, and the MMTV 5' LTR contains both positive and negative sequences that govern tissue specificity of proviral gene expression. MMTV transcripts are expressed in several normal tissues in addition to mammary tissue, including B lymphocytes. Expression in B cells has profound consequences for the developing immune system, since B cells present a viral gene, the superantigen encoded within the 3' open reading frame of the viral LTR, to T lymphocytes. We have been studying the regulation of MMTV expression in B cells, and find that it is constitutively expressed in B cells and transcriptionally upregulated by many stimuli that activate mature B cells, including LPS and the lymphokines IL-2 and IL-5. In contrast with its expression in tissues such as in mammary cells, MMTV expression in B cells is independent of hormone receptor/hormone response element interactions. Nevertheless, the U3 domain of the viral LTR is essential for its expression in B cells. We have mapped the transcription start sites in B cells and are currently defining the *cis* sequences within the LTR that are required for regulated expression in B cells, using transient and stable transfection of reporter constructs with the MMTV LTR as promoter elements. Interestingly, the region of the LTR that contains the HRE also appears to contain the essential elements for MMTV expression in B cells. Since the expression of the superantigen by B cells is known to be an important step in the life cycle of the infectious MMTV, these studies should provide unique insights into viral-host interactions. Moreover, they may contribute to our understanding of a "bifunctional" promoter, one which allows gene expression in two different cellular environments using at least partially non-overlapping factors to accommodate viral transcription. (Supported by NIH grant CA36642)

B 624 REGULATION OF max AND myc IN DIFFERENTIATION OF MURINE ERYTHROLEUKEMIA CELLS STABLY TRANSFECTED WITH max OR c-, N- AND L-myc. B.Dunn, T. Cogliati, C.Cultraro and S.Segal. NCI-Navy Medical Oncology Branch, NIH, Bethesda, MD 20889-5105. Myc and its dimerization partner Max are believed to regulate cell growth via Myc-Max heterodimers which transactivate and Max homodimers which suppress growth promoting genes. While two forms of the Max protein (short form (S) of 151 and long form (L) of 160 amino acids) were initially identified, multiple mRNAs due to alternative splicing are now known to exist. To investigate the role of Max in differentiation, C19 MEL cells were transfected with an expression vector containing wild type (wt) or basic region mutant (bm) max, S or L, under transcriptional control of the metallothionein promoter. With increasing amounts of transfected myc mRNA, there is a gradual decrease in expression of endogenous max species. When transfected max is highly expressed, endogenous myc mRNA appears to increase. Following HMBA induction of C19 cells, expression of the predominant endogenous 1.8 kb and 2.3 kb max mRNA species is downregulated in a pattern similar to that of myc mRNA. In contrast, the 3.1 kb max mRNA decreases initially, but is upregulated after 24 hours.C19 cells blocked in HMBA induced differentiation by large amounts of transfected c-, N- or L-Myc show no significant change in expression of endogenous max. In HMBA induced clones expressing transfected wt-max, the biphasic downregulation of the endogenous myc is delayed, which, in turn, is paralleled by a delay in the differentiation process. Lbm-max clones differentiate spontaneously. Following induction with HMBA, they show accelerated differentiation, although myc downregulation is normal.

B 625 **AN IMMUNOGLOBULIN LAMBDA LIGHT CHAIN GENE ENHANCER CONTAINS AN *ets* CONSENSUS BINDING SITE WHICH BINDS B CELL-RESTRICTED FACTORS AND IS REQUIRED FOR ENHANCER ACTIVITY, Charles F. Eisenbeis, Harinder Singh, and Ursula Storb, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637**

The murine immunoglobulin λ light chain gene locus contains two highly homologous transcriptional enhancers, each associated with a Jλ-Cλ cluster. In an effort to understand the cell type- and developmental stage-specific expression of the λ genes, we have begun a molecular analysis of the regulatory activity of these enhancers. A candidate protein binding site, λB, has homology to an identified site in the κE3' enhancer, and contains a near-consensus binding site for the *ets* family of transcription factors. In gel mobility shift assays using a myeloma nuclear extract, three B cell-specific complexes are formed on this site, at least two of which are multi-protein complexes containing PU.1, a B cell- and macrophage-restricted transcription factor. In addition, transient transfection assays using a CAT reporter have shown this site to be required for enhancer function. A second protein binding site 3' of the *ets* consensus is required, but not sufficient, for the formation of two of the complexes. We are currently studying the developmental regulation of λ gene expression, and its relationship to κ gene expression.

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B 626 DOMINANT REPRESSION OF THE RAT INSULIN I GENE IN HNF-1-CONTAINING CELL LINES, Leisha A. Emens and Larry Gene Moss, Departments of Cell Biology and Medicine, Baylor College of Medicine, Houston, TX 77030

Previous studies have defined a small region of the rat insulin I gene, the FF minienhancer, that confers cell-specific and glucose-inducible activity on a heterologous promoter. It is composed of two primary regulatory elements, the Far box (-241 to -232) and the FLAT element (-222 to -208), which interact to synergistically upregulate transcription. The FLAT element consists of two closely apposed regulatory sites; the activity of the positive upstream element, FLAT-F, is revealed only upon mutation of the neighboring repressor locus, FLAT-E. We have demonstrated that HNF1α, an important regulator of liver-specific genes, is both present in the FLAT-F binding complex in hamster insulinoma (HIT) cells and capable of upregulating rat insulin I gene transcription. To further elucidate the complex regulatory interactions mediated by the FF-minienhancer, constructs containing unidirectional multimers of the wild-type enhancer and various mutant derivatives upstream of -109 TK CAT were transfected into a variety of cell types. Transfection into hepatoma (HepG2), colon carcinoma (CaCo2), and kidney epithelial (MDCK) cell lines, demonstrated that the FF minienhancer is weakly active. Specific mutation of the FLAT-E locus resulted in dramatic upregulation of transcription, suggesting that FLAT-E functions as a dominant repressor locus in these non-insulin producing cell types. Furthermore, a construct containing the FLAT-E mutation specifically engineered into the full-length rat insulin I promoter exhibited significant transcriptional activity in these cells. Double mutation of the FLAT-F and FLAT-E sites abrogated activity, suggesting that the transcription is primarily HNF1 dependent. Further characterization of the basal and physiologic regulation of this modified promoter in HNF1-containing cells is critical as it may ultimately represent a useful tool for gene therapy in the management of insulin-dependent diabetes mellitus.

B 627 TRANSCRIPTIONAL REGULATION OF N-MYC BY IRON Christopher N.Frantz, Jaya Iyer, and Kevin K.Frick, Departments of Pediatrics, University of Maryland School of Medicine, Baltimore, MD 21201 and University of Rochester School of Medicine, Rochester, N.Y.

Expression of N-myc in human neuroblastoma may play an important role in the malignant behavior of this tumor. We explored N-myc regulation in LA-N-1 human neuroblastoma cells cultured in defined medium containing insulin, transferrin, putrescine, and progesterone and measured cellular N-myc protein content by Western blot. Removal of transferrin from the defined medium resulted in a decrease in N-myc, while deletion of the other single factors had no effect. Similarly, inhibition of transferrin binding to its receptor by monoclonal antibody 42/6 decreased N-myc expression. Deferoxamine (DFO), an iron chelator, decreased cellular N-myc protein in a time and dose-dependent manner. Antibody and DFO effects were prevented and reversed by soluble iron. DFO also decreased cell survival and increased neural differentiation, measured as percent of cells with extended neurites. The decrease in N-myc was not due to simple blockade of cell cycle progression in or to decreased ribonucleotide reductase activity because induction of cell cycle arrest with hydroxyurea (which also inhibits ribonucleotide reductase) had no effect on N-myc. The decrease in N-myc protein correlated with a decrease in N-myc mRNA. There was no effect of DFO on the half-life of N-myc protein or mRNA, and in nuclear run-on assays the iron starved neuroblastoma cells failed to transcribe N-myc although they transcribed actin at near-normal levels. Transcriptional regulation by iron of genes important for proliferation and differentiation has not been previously reported, and the mechanism requires further definition.

B 628 CHARACTERIZATION OF THE αB-CRYSTALLIN PROMOTER: EVIDENCE FOR A ROLE FOR HSF IN LENS EXPRESSION Peter Frederikse and Joram Piatigorsky, LMDB, NEI, NIH, Bethesda MD 20892

αA- and αB-crystallin are major constituents of the vertebrate lens, and are related to small heat shock proteins. Both αA- and αB-crystallin expression are strongly lens preferred. In contrast to αA-, αB-crystallin protein and RNA are readily detected in several non-lens tissues. In addition, αB-crystallin is induced by heat and osmotic shock, and oncogene expression (Klemenz and co-workers, and Carper and co-workers). We found a 4-fold increase of αB-crystallin expression upon heat shock at 42°C. Deletion analysis of the αB-crystallin promoter was performed to identify factors mediating the heat shock response in transfected cells. A comparison of consensus heat shock elements (HSE) in the αB-crystallin promoter with heat shock promoters from chicken, mouse, and human, revealed an overlapping dyad-element involved in directing lens preferred expression by several groups (Piatigorsky, Breitman, and Yasuda). We have compared the binding of factors from chicken lens, αTN4-1 (a mouse lens cell line transformed with SV-40 T-antigen), and HeLa nuclear extracts to oligonucleotides in EMSA assays. We found complexes cross-reactive with anti-heat shock transcription factor (HSF) antibodies, using sequences containing HSE, dyad-element, or HSE/dyad-element sites These results raise the possibility that an HSF has been co-opted for directing lens-preferred expression, together with the recruitment of a heat shock protein as a lens crystallin.

B 629 OPAQUE 2: A PLANT LEUCINE ZIPPER TRANSCRIPTIONAL ACTIVATOR, Philippe Gallusci, Jost Muth, Stephane Lohmer, Francesco Salamini and Richard Thompson, Max-Planck-Institut fuer Zuechtungsforschung, Carl Von-Linne-Weg 10, 5000 Koeln 30, Germany

The Opaque 2 (O2) gene product controls the synthesis of one of the two major classes of storage protein in maize endosperm, the 22 kda zeins, and of several other non-zein polypeptides, the most abundant of which is the endosperm albumin b32. The O2 gene has been recently cloned and shown to encode a protein which shares a high level of homology with transcriptional activators of the leucine zipper family[1] (b zip). It has been shown that the O2 protein is able to bind a series of 5 sites on the promoter of the b32 gene, and to transactivate it in a transient expression system using tobacco protoplasts.[2] The mutagenesis of these binding sites has allowed us to determine their relative contribution to this transactivation.

The transcription of the O2 gene is itself precisely controlled during endosperm development since its mRNA is first detected 10 days after pollination (DAP) and reaches a steady state level two or three days later. In order to analyse the mechanism involved in this control, a functional analysis of the O2 promoter has been performed using transgenic tobacco plants as an heterologous system. The O2 promoter is expressed in tobacco endosperm and is developmentally regulated. Furthermore a minimal promoter (160 bp long),which is similarly regulated has been defined .

Different sequence specific interactions occuring between the O2 promoter and endosperm nuclear proteins have been characterised. We are now analysing the function of these interactions in the control of the transcription of the O2 gene during endosperm development.

1) Maddaloni et al (1989). The sequence of the regulatory gene Opaque 2 (O2) of Zeas mays. Nucl. Acids Res. 17:7532

2) Lohmer et al (1991). The maize regulatory locus Opaque 2 encodes a DNA-binding protein which activates the transcription of the b-32 gene. EMBO J. 10:617-624

B 630 INDUCTION OF INTERFERON REGULATORY FACTOR-1 mRNA IN HEPATOCYTES STIMULATED BY CYTOKINES, David A. Geller, Stewart C. Wang, Mauricio Di Silvio, Andreas K. Nussler, Richard A. Shapiro, Dan Nguyen, Richard L. Simmons and Timothy R. Billiar, Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15261

Interferon regulatory factor-1 (IRF-1) is a transcriptional factor protein originally cloned from mouse fibroblasts and rat T cells which activates interferon-ß (IFN-ß) and other IFN-inducible genes. The cytokines TNF and IL-1 have been shown to stimulate IRF-1 production in fibroblasts, but the role of IRF-1 in the liver during inflammation is unknown. Since TNF and IL-1 are mediators of the hepatic response to sepsis, we hypothesized that IRF-1 may play a role in regulating gene expression in the liver during inflammation. Recently, we demonstrated the induction of IRF-1 mRNA in rat hepatocytes (HC) *in vivo* in models of sepsis and the acute phase response, but the specific stimuli that upregulate HC IRF-1 are unknown. To determine the signals which induce HC IRF-1 mRNA expression, normal rat HC were isolated by *in-situ* collagenase perfusion and stimulated *in vitro* with the cytokines IFNγ (100 U/ml), TNF (500 U/ml), IL-1 (5 U/ml), or lipopolysaccharide (LPS, 10 µg/ml). Total RNA was extracted from the HC following stimulation, and Northern blot analysis performed using an IRF-1 cDNA generated by polymerase chain reaction. Autoradiography revealed a single mRNA band at ~2.4 Kb. The HC IRF-1 mRNA levels were markedly upregulated (vs. control HC) 2 hours following *in vitro* stimulation with IFNγ (16-fold), TNF (2.6-fold), or IL-1 (1.8-fold). LPS had no direct effect. The addition of cytokines plus cycloheximide further increased IRF-1 mRNA levels, consistent with the role of IRF-1 as an "early-immediate" transcriptional factor. The results show that IRF-1 is upregulated not only in T cells and fibroblasts, but also in HC *in vitro* primarily in response to IFNγ, and to a lesser extent following TNF or IL-1 stimulation. This suggests that IRF-1 may play a role in regulating gene expression in the liver following inflammatory or septic stimuli.

B 631 IKAROS AN EARLY LYMPHOID RESTRICTED REGULATORY PROTEIN A PUTATIVE MEDIATOR FOR T CELL SPECIFICATION. Katia Georgopoulos, CBRC, Mass General Hospital, Boston, M.A. 02129

In search of the transcriptional regulators that mediate commitment and differentiation of an early hemopoietic stem cell into the T cell lineage we have isolated a cDNA encoding a unique zinc finger DNA binding protein (Ikaros).

The Ikaros protein is comprised of 431 amino acids with five $CX_2CX_{12}HX_3H$ zinc finger motifs organized in two distinct domains. The organization of zinc fingers in this protein is reminiscent of that in the Drosophila Gap gene Hunchback. Protein similarity searches revealed a 41% identity between the C terminal finger domain in the two proteins. The Ikaros protein binds to and activates the enhancer of the CD3δ the earliest definitive T cell differentiation antigen. In the adult mouse Ikaros expression is restricted in the spleen and the thymus with thymic expression being three fold higher. During mouse embryonic development Ikaros is first detected in the fetal liver at a time when hemopoietic progenitors are proliferating in this tissue and before development of the thymus. Expression of the Ikaros gene is detected in the early thymus which becomes very prominent through midgestation. The restricted pattern of expression of the Ikaros gene in T cells and their progenitors, and in sites of embryonic and adult thymopoiesis, and its ability to stimulate transcription of early T cell markers is suggestive of a determining role in T lineage specification. Furthermore, several cDNAs products of differential splicing at the Ikaros gene were isolated which encode for protein isoforms with distinct DNA binding domains. The role of these proteins in transcription in T cells will be discussed.

B 632 BRAIN 3.0: A CLASS IV POU-DOMAIN REGULATORY GENE WITH NOVEL DNA BINDING AND TRANS-ACTIVATION CHARACTERISTICS, Michelle Renée Gerrero, and Michael G. Rosenfeld. University of California, San Diego and the Howard Hughes Medical Institute, La Jolla, California 92093-0648

A member of the POU-IV class, Brn-3.0 is the mammalian homologue of unc-86, the POU-domain developmental regulator in C. elegans necessary for the determination of neuronal cell identity. Identification of the complete sequence of Brn-3.0 mRNA, and subsequent characterization of the Brn-3.0 protein has revealed several novel features of this POU-IV class member. In contrast to previously described POU domain proteins, Brn-3.0 binds ineffectively to octomer DNA binding motifs. Instead, Brn-3.0 binds with high affinity to a specific, highly restricted class of DNA sites. These sites include a site in the corticotrophin releasing factor (CRH) promoter which has been previously identified as a high affinity site for Brn-2. Mutation across the CRH site defined nucleotides essential for DNA binding. The data suggest that Brn-3.0 binds DNA in a highly restricted manner and is competent to bind a much more limited set of DNA sites than other members of the POU domain gene family. Brn-3.0 holoprotein as well as N-terminal truncated Brn-3.0 protein, consisting of only the POU domain, are directly able to transactivate upon binding to cis-active response elements. Isolated POU domains have generally proved to be ineffective transactivators. Brn-2, a member of the POU III class, binds the CRH sites with affinity levels equal to or higher than Brn-3.0, however, the Brn-2 POU domain was ineffective in transactivation while the Brn-3.0 POU domain was a highly effective transactivator. An attempt to isolate the specific POU domain region(s) conferring the observed ability to stimulate transcription will be made by co-transfection of Brn-2/Brn-3.0 chimeric POU domain proteins.

B 633 SPECIFIC MODULATION OF THE RETINOID SIGNAL BY A NOVEL ORPHAN NUCLEAR RECEPTOR

Vincent Giguère, Marco Tini, Gail Otulakowski and Grace Flock, Department of Molecular and Medical Genetics, University of Toronto, and Research Institute, Hospital for Sick Children, Toronto, Canada M5G 1X8

Nuclear receptors represent a large family of transcription factors that mediate complex effects on development, growth and homeostasis by selective modulation of gene expression via interactions with small hydrophobic ligands such as steroid and thyroid hormones, vitamin D and retinoids. This family also contains members referred to as orphan nuclear receptors for which no ligands has been identified.

We described the characterization of a novel member of the superfamily of nuclear receptors, here referred to as ROR, that show closest homology with the retinoic acid receptors (RARs). Within the DNA-binding region, ROR exhibits 67%, 59% and 48% amino acid identity with the RAR, T3R and VD3R, respectively. In particular, the amino acid sequence at the base of the first DNA-binding Zinc finger allows the classification of ROR into the subfamily of nuclear receptors that recognizes hormone response elements (HRE) containing the half-site consensus sequence AGGTCA. However, unlike RARs, T_3R, and VD_3R that bind to direct tandem repeats of the half-site motif AGGTCA as heterodimers with the retinoid X receptor (RXR), ROR binds as a monomer to a specific subset of HRE containing the half-site AGGTCA. Further analysis showed that specific recognition of an HRE by ROR is provided by the base pairs preceding the half-site at its 5' end. Based on this definition of the binding site for ROR, we predicted that ROR might bind to a newly identified retinoic acid response element (RARE) located in the promoter of the mouse γF-crytallin gene. In vitro studies showed that ROR binds with high affinity to the γF-RARE while in vivo studies revealed that ROR activates gene expression through the γF-RARE in a ligand-independent manner. Transient transfection of ROR leads to synergistic activation of the reporter gene upon retinoic acid treatment. These results provide novel evidence that interaction between orphan receptors and RARs can specifically modulate retinoid signaling.

B 634 ISOLATION OF AN AVIAN MEMBER OF THE INTERFERON REGULATORY FACTOR (IRF) FAMILY OF PROTEINS, Caroline E. Grant and Roger G. Deeley, Cancer Research Laboratories, Queen's University, Kingston, Ontario,Canada, K7L 3N6

The avian apoVLDLII gene is normally expressed exclusively in the liver of laying hens. However, birds of either sex can be induced to express the gene as early as day 7 of embryogenesis in response to estrogen. Previous studies have demonstrated that demethylation of specific sites 5' and 3' to the apoVLDLII gene takes place during the critical period between days 7 and 9. Specific protein interactions at a site -2.6kb upstream from the gene have been extensively characterized (Hoodless *et. al.*,MCB 10,154-164; Hoodless *et. al.*,1992,DNA Cell Biol., in press). This site, Site 1, includes an *MspI* recognition sequence that is demethylated between days 7-9, and methylation interference experiments indicate that the outer G's of *MspI* site are contact points in a DNA/protein interaction. We used a direct screening method in which a day 9 embryonic liver expression library was screened with a concatenated Site 1 oligonucleotide probe. One of the clones isolated hybridized to a broad band on Northern blots corresponding to mRNA species of 1.9-2.1 kb. The predicted amino acid sequence of the full length cDNA sequence was compared to the translated Genbank database and significant similarity was found to the DNA binding domains of ISGF2/IRF1, IRF2, ICSBP, and ISGF-3γ, proteins that compose the interferon regulatory factor family. Members of this family contain a highly conserved DNA binding domain that includes five tryptophan residues whose spacing and periodicity are reminiscent of the DNA binding domains of the c-*myb*-related proteins. The amino acid identity in this region is 37% between our avian sequence and the closest member of the family, ICSBP, and all five tryptophan residues are conserved. Expression of the avian IRF-like mRNA in a chicken hepatocyte cell line, LMH, is dependent on the presence of serum and is enhanced by cycloheximide. The growth factor dependence of this expression will be described.

B 635 SCL AND GATA-1 ARE COEXPRESSED IN MULTIPOTENTIAL IL3-DEPENDENT PROGENITORS AND DOWNREGULATED DURING GRANULOCYTE/ MACROPHAGE DIFFERENTIATION, AR Green+, A Murrell+, E-O Bockamp+, E Spooner*, C Heyworth* and M Cross*, +Dept of Haematology, University of Cambridge, MRC Centre, Hills Road, Cambridge CB2 2QH, UK and *Paterson Institute for Cancer Research, Wilmslow Road, Manchester M70 9BX, UK.

The SCL or TAL-1 gene encodes a putative transcription factor with a basic helix-loop-helix motif. SCL and GATA-1 are coexpressed in erythroid, mast and megakaryocytic cells but not in other differentiated haemopoietic lineages. We have previously suggested that SCL and GATA-1 may be expressed in multipotential haemopoietic precursors and that lineage-dependent modulation of their expression may occur during differentiation. Here we extend our previous observations and show that SCL and GATA-1 are coexpressed in all IL3-dependent murine cell lines examined. Furthermore, one murine multipotential IL3-dependent cell line was induced to undergo either erythroid or granulocyte/macrophage differentiation using appropriate mixtures of IL3, EPO or GM-CSF. Erythroid differentiation was accompanied by upregulation of SCL and GATA-1 whereas granulocyte/macrophage differentiation resulted in a loss of mRNA for SCL and GATA-1. Our results suggest that SCL and GATA-1 are coexpressed in multipotential progenitors. Moreover the final lineage-restricted pattern of expression of SCL and GATA-1 involves their lineage dependent down-regulation.

B 636 REGULATION OF INTERLEUKIN-2 GENE TRANSCRIPTION BY NUCLEAR HORMONE RECEPTORS, Alberto Gulino#°, Alessandra Vacca°, Maria P. Felli#, Daniela Meco°, Ugo de Grazia°, Marella Maroder°, Isabella Screpanti°, Stefano Martinotti# and Luigi Frati°, °Department of Experimental Medicine, University La Sapienza, 00161, Rome and #Department of Experimental Medicine, University of L'Aquila, Italy.

Interference between phorbol ester and calcium ionophore-induced activation of the interleukin-2 (IL2) promoter by two members of the steroid hormone receptor superfamily (retinoic acid and glucocorticoid receptors, RAR and GR) has been studied in T lymphocytes. Both RAR and GR inhibit the cis-regulatory activity of the IL2 enhancer (-317 bp to +47 bp). Functional analysis of CAT vectors containing either internal deletion mutants of the IL2 enhancer or multimerized cis-regulatory elements showed that RAR inhibits the phorbol ester/calcium-induced enhancer activity of an IL2-promoter octamer motif without affecting the levels of the cellular oct-1 DNA-binding protein. Transfection of wild type or deletion mutants or chimeric oct-2 proteins shows that RA is a strong inhibitor of oct-2-dependent transcription. In contrast, the GR-dependent inhibition of the IL2 enhancer involves two elements: the NFAT (-273/-263 bp) and the jun and fos-binding AP1 (-160/-150 bp) motifs. The AP1 element alone was not activated by phorbol ester and calcium whereas it synergistically cooperated with the NFAT motif. Interestingly, such a phorbol ester/calcium-induced functional cooperativity was selectively impaired by GR. Our data suggest multiple levels of transcriptional interference between nuclear receptors and phorbol esterand calcium-generated signals involving distinct transacting factors.

B 637 Retinoid Regulation of Bone Morphogenetic Protein 4 (BMP 4 or DVR 4): Analysis of the Mouse BMP 4 Gene Promoter by Transfection Into Primary Cultures of Fetal Rat Calvariae (FC) osteoblasts. Stephen E. Harris[1], J Feng[1], MA Harris[1], B Cristy[2], A Cooney[3], S Tsai[3], and MJ Tsai[3], J Wozney[4], and GR Mundy[1]. The University of Texas Health Science Center, Dept of Medicine, Division of Endocrinology, San Antonio, TX 78284; [2]Institute of Biotechnology, San Antonio, TX 78245; [3]Dept of Cell Biology, Baylor College of Medicine, Houston, TX; [4]Genetics Institute, Cambridge, MA.

Retinoic acid has a profound effect on bone cell growth and differentiation. Using primary cells from the fetal rat calvaria, which on long term culture for 10-15 days forms various kinds of "bone" like structures or mineralized nodules. We have found that retinoic acid stimulates "bone" formation at 10^{-6}M but completely inhibit bone formation at 10^{-7}M RA. However, the combination of recombinant BMP 2 plus RA at 10^{-7}M reverses this inhibition and allows in vitro bone formation to occur. We have been studying the biphasic nature of RA and RA plus BMP 2 on BMP 4 mRNA expression in this system and beginning to analyze the mouse BMP 4 promoter. RA at 10^{-6}M or BMP 2 at 60ng/ml plus 10^{-7}M RA stimulates BMP 4 mRNA, at 3 days in these long-term FRC bone forming cultures. However, RA at 10^{-7}M inhibits BMP 4 mRNA. Using a (-1067/+120) BMP 4-reporter CAT gene construct, transfected into FRC osteoblasts, we show that 10^{-7}M inhibits CAT activity. Further experiments are in progress. A zif-TF 268 response element at -4 in the BMP 4 gene has been shown to bind to in vitro translated zif 268-TF and bacterially produced zif 268. The zif 268 gene is regulated by RA in a variety of osteoblast cell lines and FRC cultures and is most likely playing a role in BMP 4 transcription. Also at -911 in the BMP 4 promoter is a DR-1 element (6 bp direct repeat with 1 base pair spacing), which specifically bind the COUP-TF and the RxRα -TF. We propose that the biphasic nature of RA on the BMP 4 gene, and bone formation itself, may, in part, be explained by RxR homodimers activating positively on the BMP 4 DR-1 elements, while RxR-COUP -TF or RxR-RAR heterodimers act negatively on the same DR-1 element.

B 638 MOLECULAR CHARACTERIZATION OF A MAST CELL SPECIFIC IL-4 INTRONIC ENHANCER, Greg Henkel and Melissa A. Brown, Departments of Microbiology/Immunology and Medicine, Oregon Health Sciences University, Portland, OR 97201

Interleukin 4 (IL-4) is a multifunctional cytokine with many diverse biological functions which have been defined by both *in vitro* and *in vivo* assays. These functions include mediating growth, activation and/or differentiation among various cells of the hematopoetic lineage. The most well documented *in vivo*-defined activities include the regulation of IgE production in B cells as well as anti-tumor activity. In general, the production of IL-4 is limited to a small subset of $CD4^+$ T cells and cells of the mast cell/basophil lineage. The physiologic expression of IL-4 in T and mast cells is transient and is activation-dependent. In contrast, it has been found that many transformed murine mast cells can express IL-4 constitutively. The constitutive expression of IL-4 in transformed mast cells provides a useful model to define *cis* and *trans*-acting regulatory elements that modulate mast cell IL-4 expression. Previously, DNAse I hypersensitivity analysis was used to map potential regulatory regions in the murine IL-4 gene in these cells. A hypersensitive site was located in the second intron of the mouse IL-4 gene. A 670 bp fragment, comprising the area of the hypersensitive site, was found to contain prototypic enhancer function using SV40 or IL-4 promoter /CAT reporter gene consructs. The enhancer was functional in IL-4 producing transformed mast cells, weakly operative in activated normal mast cells and inactive in stimulated T-cells. It was also inactive in IL-4 non-producing transformed mast cells. Sequence analysis identified three potential binding sites for SP-1-like transcription factors. Several deletions of 670 fragment were generated using restriction enzymes and/or limited treatment with Bal 31 exonuclease and tested for enhancer function. This analysis identified two regions (E1 and E2) within a 340 bp subsequence that were necessary for positive function. The SP-1 like consensus sites were located in E1. Preliminary gel shift analysis with an E1 region-containing DNA fragment showed nuclear extracts from IL-4 producing mast cells formed specific DNA-protein complexes of distinct mobilities compared to extracts from non-IL-4 producing mast cells. A third functionally important region contains a negative regulatory element (NRE) which, when deleted, results in a two to three-fold higher enhancer activity, compared to the parent construct. In contrast to the parent plasmid, this NRE minus construct demonstrates enhancer function in the IL-4 non-producing transformed mast cells.

B 639 REGULATION OF THE TCR δ ENHANCER BY PROTEIN BINDING TO ADJACENT MYB AND CORE SITES, Cristina Hernandez-Munain and Michael S. Krangel, Department of Immunology, Duke University Medical Center, Durham, NC 27710.

A T cell-specific transcriptional enhancer lies within the J-C intron of the human T cell receptor (TCR) δ gene. The minimal enhancer has been defined as a 30 bp element denoted δE3. The binding of a T cell-specific factor to the core sequence (TGTGGTTT) in the 5' end of δE3 is necessary for transcriptional activation by the δE3 element or a larger 370 bp enhancer fragment, but is not sufficient for activation. We recently identified a consensus Myb binding site (TAACGG) within the 3' portion of δE3 that is adjacent to the core site. Using EMSA we found that bacterially expressed v-Myb specifically binds to this site, but not a site with a mutation predicted to disrupt Myb binding. We detect proteins in Jurkat T cell nuclear extracts that bind with similar specificity and that are recognized by an anti-c-Myb serum in supershift experiments. A mutation in the Myb site abolishes enhancer activity of the δE3 element, and also abolishes the activity of the 370 bp enhancer fragment. Enhancer activity is also abolished by the introduction of either a 5 bp (half-turn) or 10 bp (full turn) insertion between the Myb and core sites. These data indicate that the interaction of proteins that bind to the Myb and core sites within δE3 is essential for transcriptional activation by the TCR δ enhancer, and argue that protein binding to these sites plays an important role in T cell development.

B 640 ANALYSIS OF TRANSCRIPTIONAL ELEMENTS REGULATING THE EXPRESSION OF HUMAN T CELL RECEPTOR GAMMA GENES, Thore Hettmann and Amos Cohen, Department of Immunology and Cancer Research, Hospital for Sick Children, Toronto, ON, Canada, M6G 1X8

Positive and negative transcriptional regulatory mechanisms are thought to play a major role in the expression of T cell antigen receptor (TcR) genes. Since the αβ and γd T cell heterodimers are expressed in a mutually exclusive fashion and since TcR genes are sequentially activated during T cell ontogeny, transcriptional activation and repression must at least in part determine T lineage-specific and developmental-specific expression of these genes. As a first attempt to identify these transcriptional regulatory elements, we have cloned and sequenced the 5' upstream regions of the human T cell receptor gamma (TRG) genes. A conserved heptamer (CTGCAGG) was found upstream from the translation initiation site of all TRG genes and was determined to be located 65 bp and 124 bp upstream from the cap sites of two V region genes. Furthermore, we have identified a transcriptional enhancer located 6.5 kb downstream from the human T cell receptor gamma (TRG) locus. The nucleotide sequence of the 301 bp enhancer core element shows strong sequence homology to the recently identified murine Cγ1 enhancer. The enhancer demonstrates T cell-specific activity, but not γδ sublineage-specificity in combination with either a heterologous or gene specific promoter. Additional regulatory elements may downregulate the expression of rearranged TRG genes in non-γδ T cells.

B 641 ***In-vivo*** **heterodimerisation between yeast GCN4 and the plant b-ZIP factor Opaque-2.** M.J.Holdsworth, J. Muñoz Blanco, [1]W. Schuch, M. Bevan. John Innes Centre for Plant Science Research, Colney Lane, Norwich, Norfolk, U.K.[1] ICI International Seeds Business, plc, Jealotts Hill Research Station, Bracknell, Berks, U.K.

Opaque-2 is a maize b-ZIP transcription factor that regulates the expression of seed storage proteins during endosperm development. We have analyzed the activation potential of opaque-2 in yeast using several opaque-2 *cis*-elements derived from storage protein gene promoters, in a two component effector (opaque-2)/reporter (*cis*-element-*LacZ* fusion) system. Opaque-2 activated reporter gene transcription more than 100-fold through one promoter fragment from a wheat seed gene promoter (designated *EB*). We have shown by *in-vivo* footprinting and gel retardation analyses that wheat endosperm contains two factors that bind two separate *cis*-elements within *EB*, and in transgenic plants these sequences confer endosperm-specific transcription. One of the *EB cis*-elements is very similar to the yeast *cis*-element GCRE, the binding site for GCN4. We have shown that in yeast opaque-2 interacts with GCN4 specifically on the GCRE-like element within *EB*, and this interaction results in opaque-2 activity in yeast: Growth of yeast in conditions that suppress GCN4 levels (high amino-acid availability) results in a drastic reduction in opaque-2 activity, and opaque-2 is inactive on *EB* in a *gcn4*$^-$ yeast strain. As both GCN4 and opaque-2 regulate nitrogen metabolism we speculate that wheat opaque-2 may interact with a GCN4 homolog, and this heterodimer may be involved in the control of storage protein gene transcription and regulation of seed nitrogen metabolism.

B 642 ANALYSIS OF CIS-ELEMENTS AND TRANSACTING FACTORS OF Na,K-ATPase ALPHA2 SUBUNIT GENE, Keiko Ikeda, Kiyoshi Kawakami and Kei Nagano,Department of Biology,Jichi Medical School, Minami-kawachi, Tochigi, 329-04 , Japan

Na, K-ATPase is an integral membrane protein which is essential for maintaining the Na^+ and K^+ concentration in all animal cells using energy from ATP hydrolysis. This enzyme plays a critical role for nerve excitation, various ion and nutrient transports and cell volume regulation. The enzyme is composed of two subunits named alpha and beta. The alpha subunit is the catalytic subunit and at least three isoforms (alpha1, alpha2, alpha3) have been identified. The alpha2 subunit gene is expressed mainly in skeletal muscle and brain and is thought to be involved in the specific function of these tissues. Moreover, the expression of the alpha2 subunit gene is known to be regulated through developmental stages. In new-born rats, the mRNA increases 90-fold in skeletal muscle by 15days (J.Orlowski et al., JBC263, pp.10436-10442,1988).

For understanding the regulatory mechanism of alpha2 subunit gene expression during myogenic differentiation, we analyzed cis-elements in the 5' flanking region of the gene using transient transfection assays in 10T1/2 cells, which were cotransfected with MyoD cDNA, and L6 myoblast cells. By 5'-deletion mutation analysis, the positive regulatory element of the promoter activity (8 to10 fold) was found between the region of -175 and -108 relative to the transcription start site as +1 in both cells. In this region, there are two E box motifs, one Sp1 consensus binding site and a GGGAGG sequence. Site-specific mutation with any one of proximal E box, Sp1 binding site or GGGAGG diminished the promoter activity in both cells. The distal E box acted as a negative regulatory element in L6 cells. Gel mobility shift assays were performed with a DNA fragment of ATP1A2 (-175 to -99) as a probe. Three major complexes (C1,C2 and C3) were observed in the nuclear extract of both cells. The formations of these complexes were competed with specific Sp1 consensus oligonucleotides. These results suggest that the binding factors in these complexes bind Sp1 consensus sequence. Using a mutated DNA fragment of mut34 (double mutant of Sp1 site and GGGAGG sequence) as a probe, one major complex (C4) was observed. The formation of this complex was competed with mut4 (single mutant of GGGAGG sequence) and mut34 but was not competed with wild type fragment and other mutated fragments which have intact GGGAGG sequence. These results suggest that the GGGAGG sequence interferes the formation of this complex.

B 643 MODULATION OF THE TRANSCRIPTION FACTOR AP-2 BY N-*ras* ONCOGENE AND RETINOIC ACID. Perry Kannan, Reinhard Buettner, Paul J Chiao and Michael A Tainsky. Department of Tumor Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.

Transcription factor AP-2 plays an important role in cell differentiation and development. Increased level of AP-2 expression was observed in retinoic acid-induced differentiation of the human teratocarcinoma cell line PA-1. N-*ras* oncogene-induced transformation blocks retinoic acid-induced differentiation of these cells and promotes cell growth. However, we also observed a several-fold increase in AP-2 mRNA level in N-*ras* oncogene transformed PA-1 cells. When assayed with an AP-2-tk-CAT reporter gene construct, retinoic acid induced AP-2 transactivation activity several-fold commensurate with changes in the mRNA level. On the contrary, N-*ras* oncogene inhibited AP-2 transactivation activity. Transfection of anti-sense N-*ras* into these cells restored AP-2 activity suggesting inhibition of AP-2 activity is a direct effect of N-*ras* oncogene. In mobility shift assays nuclear extracts of the N-*ras* transformed cells exhibited AP-2 specific DNA binding activity although AP-2 failed to activate transcription in these cells. We established stable transfectants of non-N-*ras* PA-1 cells that express AP-2 from an SV40 early promoter. These overexpressors were very similar to N-*ras* transformed PA-1 cells with high expression of AP-2 and reduced AP-2 transactivation activity. Similar to N-*ras* transformed cells AP-2 over-expression also promoted anchorage independent growth. In transient transfections the AP-2 transactivation activity was inhibited progressively when increasing amounts of AP-2 expressor plasmid construct were transfected. We call this phenomenon 'self-squelching'. A series of deletion and frame-shift mutations in the AP-2 over-expressor construct were made and the region that is involved in self-squelching was analyzed and mapped to the carboxy terminus of AP-2 protein. A DNA binding domain with an integral dimerization doamin are located in this region of AP-2 protein.

B 644 CHARACTERIZATION OF A B-CELL SPECIFIC PROMOTER OF J CHAIN GENE TRANSCRIPTION, Saoussen Karray, Jeffery Cox & Marian E. Koshland, Department of Molecular and Cell Biology, University of California, Berkeley 94720

During the differentiation of a B cell to an IgM-secreting plasma cell, a signal from the T-cell lymphokine, IL-2, induces synthesis of the J chain required for IgM assembly. Fine deletion analyses of the J chain gene promoter have identified an IL-2 responsive control element, JB, that has positive and negative regulator effects (Lansford et al. PNAS 89: 5966, 1992). Recently a second control element with a unique T-rich sequence, JA, has been located immediately upstream of the JB element. Gel shift mobility assays, have identified a nuclear factor that recognizes the JA element and shown that the protein is B cell specific and constitutively expressed at all B cell stages. Functional studies of the JA element were performed using a wild type or mutant JA oligonucleotide cloned upstream of a heterologous promoter and the CAT gene. In transient transfections, the JA element increased transcription of the CAT gene reporter in both J chain positive and negative B cell lines, but had no effect in other cell types. Mutations in the JA site completely suppressed its activity in B cells, thus demonstrating that JA element is critical for J chain gene transcription. Further characterization of the JA transcription factor and its potential interaction with other regulatory elements in J chain promoter are under investigation.

B 645 A NOVEL PATHWAY FOR ACTION OF RETINOIC ACID: DIFFERENTIATION OF F9 CELLS WITHOUT RECEPTOR-MEDIATED TRANS-ACTIVATION, Issay Kitabayashi and Kazushige Yokoyama, Tsukuba Life Science Center, RIKEN(The Institute of Physical and Chemical Research), Tsukuba, Ibaraki 305, Japan.

Retinoic acid (RA) has striking effects on vertebrate development and induces differentiation of several lines of cells including embryonal carcinoma F9 cells. During differentiation of F9 cells, transcription of the c-*jun* gene is strongly induced. Ectopic expression of the c-*jun* gene results in differentiation of F9 cells, suggesting that the induction of c-*jun* plays an important role in the differentiation process. Through the use of deletion, substitution and insertion analysis, the differentiation response element (DRE) has been defined as a 145 bp element between -190 and -46 in the 5'-flanking region of the c-*jun* gene. However, gel retardation analysis indicates that RA receptors (RARs) and Retinoid X receptors (RXRs) does not bind specifically to the DRE, suggesting that these receptors are unlikely to be involved in the activation of the c-*jun* gene. We now provide evidence that F9 cells can differentiate in response to RA without *trans*-activation by nuclear receptors. Irreversible differentiation of F9 cells and constitutive expression of the c-*jun* gene are induced by 18 h of exposure to RA with subsequent incubation in the absence of RA. This induction of differentiation is not blocked after incubation without any amino acids and with cycloheximide and α-amanitin for 22 h in totality from 1 h before 18 h of RA-treatment to 3 h after the treatment. During this treatment and subsequent incubation without retinoic acid and the inhibitors, however, the endogenous receptors fail to activate transcription of their primarily target promoters containing RA response elements such as βRARE and TREp. These results suggest that RA-induced differentiation of F9 cells can be determined without protein synthesis *de novo*, and that there is a novel pathway for RA-action that is independent of RAR-mediated *trans*-activation. Furthermore, differentiation of F9 cells is triggered by inhibitors of protein kinases such as K-252a, staurosporin and herbimycin A. During the commitment to RA-induced differentiation, at least five sets of four specifically phosphorylated proteins are altered in the absence of protein synthesis *de novo*. We will also report factors which specifically bind to the DRE.

B 646 TISSUE-SPECIFIC RNA SPLICING DISRUPTS A LEUCINE HEPTAD REPEAT THAT AFFECTS THE DIMERIZATION PROPERTIES OF THE bHLH PROTEINS REBα and REBβ, Elliott S. Klein and Michael G. Rosenfeld, Howard Hughes Medical Institute and University of California, San Diego, Department and School of Medicine, La Jolla, Ca 92093-0648

mRNAs encoding two rat bHLH proteins, refered to as REBα and REBβ have been identified as alternatively spliced transcripts derived from a single genomic locus. This RNA processing results in tissue-specific differences in the ratios of these two mRNAs. Although exhibiting a highly enriched level of expression in the developing neural tube, the REB gene is expressed at variable levels in many organs of the mature animal. REBα encodes a polypeptide containing a leucine heptad repeat situated N-terminal from its C-terminally located bHLH domain. REBβ is identical to REBα except for a 24 amino acid insertion in the leucine heptad repeat which is due to the inclusion of an additional 72 base pair exon in the REBβ transcript. As a consequence of this insertion, REBβ exhibits a markedly diminished capacity to bind to cognate E-box binding sites, and to form homodimers and heterodimers with other members of the bHLH gene family. It is proposed that this tissue-specific pattern of REB RNA splicing is involved in the determination of corresponding tissue-specific combinations of heterodimeric complexes of ubiquitous and tissue-restricted bHLH proteins. Thus, REBα and REBβ represent a novel example of a regulated disruption of a leucine heptad repeat in a bHLH protein that can mediate control of protein-protein interactions.

B 647 THE MOUSE TESTIS-SPECIFIC PDHEIα SUBUNIT: INTERACTION OF THE PROXIMAL PROMOTER WITH PUTATIVE TRANSCRIPTION FACTORS. Ismail Kola, Rocco Iannello and Heinrich H-M Dahl, Centre for Early Human Development, Monash University, Victoria, 3168, Australia

In mouse and human, two genes code for the E1α subunit of the pyruvate dehydrogenase (PDH) complex: an autosomal intronless gene which codes for the testis-specific E1α isoform (t-E1α) and the somatic X-linked gene. The testis-specific enzyme has an important function in the provision of energy to spermatozoa. In vivo, the expression of t-E1α is restricted to the testis. In this study we investigate the transcriptional regulation of the t-E1α gene.

Our data demonstrates that the expression of the t-E1α gene can be detected in somatic tissue when a proximal promoter fragment spanning the nucleotide sequences from -187 to +22 is ligated upstream of CAT. Furthermore, a 4-5X inhibition in CAT activity is obtained in somatic cells when other more upstream t-E1α promoter sequences are ligated to CAT. This data suggests that the restricted pattern of t-E1α is due to repressor elements upstream of -187 in somatic cells.

DNaseI footprinting of the [-187to+22] proximal promoter fragment using testis or brain extracts revealed 4 common regions of protection. One of these contain the consensus sequence for Sp1 and another the ATF/CREB binding site. The cis-sequences of the remaining two protected regions (MEP-1 & MEP-3; Mouse E1α Promoter) show no apparent consensus homology with other cis-elements. Thus, these MEP sequences represent novel transcription factor binding sites. Another novel binding site, MEP-2, is unique to the testis only.

Gel shift assays of the 4 common sites confirm that these interact with factors present in nuclei of both testis and brain in a specific manner. However, the MEP-2 site demonstrates binding to a specific protein found in the testis only. In addition, the MEP-2 protein interaction is first observed in the testis of 2 week old mice, correlating temporally with the transcription of this gene. This data therefore suggests (1) that MEP-2 binding factor is developmentally regulated and (2) interactions involving the MEP-2 binding factor with its binding site may be essential for t-E1α expression in the testis.

B 648 THE EVI-1 ZINC FINGER PROTEIN BLOCKS GATA-1 MEDIATED TRANSCRIPTIONAL ACTIVATION AND CAN ALTER THE RESPONSE TO ERYTHROPOIETIN (EPO)IN EPO-RESPONSIVE CELLS. Brent L. Kreider and James N. Ihle, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN, 38105.

Evi-1 was first identified as a gene associated with a common site of retroviral integration in murine myeloid tumors and encodes a zinc finger protein which is not normally expressed in hematopoietic cells. Recently, it has been shown that Evi-1 binds to DNA containing the sequence GATA or GACA, but it is not known if it can act as a transcriptional activator of such sequences. Another zinc finger protein, GATA-1, is a transcriptional activator which also recognizes the sequence GATA and has been shown to be absolutely required for maturation of the erythroid lineage. To determine the consequence of Evi-1 binding to its recognition sequence, transient co-transfection assays were performed using various CAT vectors harboring an Evi-1 binding sequence. In these assays, Evi-1 was not able to act as a transcriptional activator whereas GATA-1 activated all of the constructs tested. Additionally, when both GATA-1 and Evi-1 were cotransfected along with a reporter construct, Evi-1 acted as a transcriptional repressor of GATA-1 activation. The ability of Evi-1 to repress GATA-1 activation was dose dependent and also was influenced by the recognition sequence used for the reporter construct. Since Evi-1 was capable of interfering with GATA-1 mediated transcriptional activation in these transient assays, it was of interest to see what effect Evi-1 may have in a cell where GATA-1 is expressed. For this purpose, a retroviral construct was introduced into the 32D Epo1 cell line which requires either Epo or IL-3 for growth and expresses a number of erythroid specific mRNAs including those for GATA-1, Epo receptor, β-globin, and carbonic anhydrous I. Expression of Evi-1 in this cell line resulted in its inability to grow in response to Epo without altering its growth in IL-3.

B 649 RETINOIC ACID INDUCED DIFFERENTIATION OF HL-60 CELLS LEADS TO DIFFERENTIAL EXPRESSION AND ALTERNATIVE SPLICING OF HOX 3.5, Katharine M. Lang, Edbert Hsu and James D. Griffin, Division of Tumor Immunology, Dana-Farber Cancer Institute, Boston, MA 02115

Differentiation of hematopoietic cells is not well understood at the molecular level and the genes which control commitment of immature stem cells to different lineages have not been identified. The human HL-60 promyelocytic leukemia cell line can be induced to differentiate to either monocytes or neutrophils. In other tissues, members of the homeobox-containing gene family are known to control aspects of morphogenesis and tissue-specific differentiation. Using inverse PCR we cloned a number of homeobox-containing gene fragments whose expression patterns changed during retinoic acid (RA)-induced neutrophilic differentiation of HL-60 cells. The expression of one such gene, HOX 3.5 (3E), a deformed-related homeobox-containing gene, was unexpectedly complex on Northern blots. 10 HOX 3.5 cDNA clones were isolated, 5 from an uninduced HL-60 cDNA library and 5 from a RA induced 3-day HL-60 cDNA library. Sequencing the cDNAs revealed a complex pattern of alternative splicing involving a 485 bp segment just 5' to the homeobox, previously published as a single intron (Simeone *et al.* NAR 16:5379-5390). Three seperate regions (A,B and C) of this segment have been alternatively spliced to produce four different transcripts. Two cDNAs contain a putative leucine zipper (LZ) domain which would be in frame with the homeodomain (HD) in transcripts where regions A and B are included with region C spliced out. Transcripts containing this splicing pattern decrease dramatically with differentiation. Other cDNAs contain the putative "LZ" and an in frame stop codon preventing the translation of the homeobox (regions A, B and C included). The amount of these transcripts increase after RA induction. In addition, some cDNAs contain only region B in a different frame which also contains an in frame stop codon. Therefore, based on the cDNA sequences, four possible proteins can be predicted: 1) a protein containing only the HD (Simeone *et al.*); 2) a protein which contains both the "LZ" and the HD (transcripts which decrease after HL-60 induction); 3) a protein which contains the "LZ" but not the HD (transcripts which increase with differentiation); 4) and a protein which contains neither the "LZ" or the HD. Thus, the process of neutrophilic differentiation in HL-60 cells is associated with a complex pattern of alternative splicing of HOX 3.5 transcripts. This results in differential expression of a putative LZ domain and the HD, potentially altering the biological properties of the HOX 3.5 gene product.

B 650 TRANS-DOMINANT NEGATIVE GLUCOCORTICOID RECEPTOR MUTANTS

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We have studied the behavior of several mutations in the glucocorticoid receptor (GR) by expressing GR-cDNAs along with appropriate reporter genes and other non-ubiquitous transactivators in mammalian cells. The two mutations are: (1) a trans-dominant *negative* GR generated upon alteration of the translational reading frame of the amino-located OPA repeat and (2) a double point mutant GR which fully activates transcription in the presence of the antagonist RU486.

(1) The glucocorticoid receptor embodies more than one transactivation domain and this functional redundancy has not allowed the isolation of mutants which are unable to transactivate while being fully competent for DNA binding, nuclear translocation and hormone binding. In particular, no trans-dominant negative (TDN) mutant has been described so far. A systematic study on the role of monotonous aminoacid repeats in GR revealed different effects depending on the frame by which the repeated CAG-motif just upstream to the major activation domain is translated. By changing the reading frame of this OPA repeat to encode a poly-*Ala*-segment, we have been able to generate a mutant which displays inter- as well as intra-cistronic negative dominance when tested for transcription activation *in vivo*, whereas a shift to poly-*Ser* do not show these effects. This poly-*Ala*-containing GR acts as a trans-dominant negative (TDN) mutant. We can show that changes in the OPA-reading frame affect squelching capacity with respect to Oct2A and that other functions of this mutant are impaired as demonstrated with chimeras with other members of the steroid receptor superfamily. The potential general significance of this finding is outlined by the existence of several other factors containing either alanine rich or oligo-Ala stretches and displaying transcriptional repressing activities.

(2) Steroid receptors are ligand inducible transcription factors. One of the two described transcriptional activation function was localized in the hormone binding domain. It was shown that mutagenesis in the most carboxy terminal portion of this very conserved region can abolish ligand dependend transcriptional activation without apparent effect on steroid or DNA binding (Danielian et al., 1992). On the other hand, a progesterone receptor (PR) deletion mutant lacking the carboxy terminal 42 aa has been reported to be a unresponsive to progesterone, but efficiently activated by the potent antiprogestin RU486 (Vegeto et al., 1992). We reconstructed these carboxy terminal mutations by either replacement of a pair of hydrophobic residues (GR aa 770 and 771) with alanines or by deletion of this C-terminal part of the receptor and tested them for trans-dominance or for response to agonist/antagonist. We found that the substitution mutants have a differential response to dexamethasone/RU486, while the truncated form failed to react in the way anticipated from work with PR. None of these mutants appears to have TDN properties.

B 651 DNA-BINDING ACTIVITY OF MYC AND MAX DURING DIFFERENTIATION OF U-937 MONOBLASTS, Lars-Gunnar Larsson[1,2], Monica Pettersson[1] and Bernhard Lüscher[2], [1]Department of Pathology, University of Uppsala, University Hospital, S-751 85 Uppsala, Sweden, [2]Institut für Molekularbiologie, Medizinische Hochschule Hannover, 3000 Hannover 61, Germany.

Differentiation of U-937 monoblasts can be induced by various physiological and non-physiological agents and is accompanied by a reduced expression of c-Myc. We have previously shown that constitutive expression of v-Myc inhibits TPA-, vitamin D3- and retinoic acid-induced terminal differentiation of U-937. However, the differentiation programme is restored if the inducers are combined with interferon-γ (IFN-γ), despite a continous expression of v-Myc. The mechanism by which IFN-γ restores the differentiation is unknown. Now we show that IFN-γ alone or in combination with the inducers of differentiation increase the expression of Max, the heterodimeric partner of Myc. By using anti-Myc and -Max antibodies (Ab) which do not interfere with Myc/Max DNA binding, we show that the specific DNA-binding activity of *in vivo*-formed Myc/Max-containing complexes (precipitated by anti-Myc Ab under low stringency conditions) was rapidly decreased during TPA-, VitD3- and RA-induced differentiation in both v-Myc expressing and control U-937 cells. However, the DNA-binding was thereafter regained in the v-Myc cells but not in the control cells. In contrast the DNA-binding activity of anti-Max precipitated material remained constant or increased during U-937 differentiation. Preliminary results suggest that IFN-γ alone or in combination reduced the binding activity of anti-Myc precipitated immunocomplexes in U-937-v-Myc cells but at least transiently increased the binding of anti-Max complexes. The results points to the possibilty that external signals may regulate the DNA-binding activity of Myc and Max independent of their expression. This issue is being investigated at present.

B 652 REGULATION OF THE ACTIVITY OF RAT SERINE PROTEASE INHIBITOR GENE PROMOTERS BY GROWTH HORMONE AND GLUCOCORTICOIDS, Alphonse Le Cam, Laurent Paquereau and Marie José Vilarem, Centre CNRS-INSERM de Pharmacology Endocrinologie, 34094 Montpellier cédex 05, France

Three genes, spi 2.1, 2.2 and 2.3, encoding serine protease inhibitors are differentially expressed in rat hepatocytes, depending on the hormonal status of the animals. Transcription of spi 2.1 and 2.2 is tightly controled by growth hormone (GH) and, glucocorticoids (GC) that are inactive *per se*, strongly potentiate its action. In contrast, spi 2.3 escapes GH regulation and GC activate its transcription. The structural parts of these genes display a high degree of identity whereas major differences occur in the 5' flanking regions. Analysis of nuclear factors interacting with the first 200 bp of the promoters performed *in vitro* revealed the presence of three common binding sites that bound mainly proteins of the C/EBP family. However, the most upstream (5') site was found to specifically interact with a GH-inducible factor. Functional assays were performed with normal hepatocytes transfected by electroporation and maintained in culture for 24 h. They revealed the presence of two apparently independent GH-responsive regions located between positions -150 and +8 in spi promoters. The first one maps within the most upstream common protein binding site whereas the second one appears to be located in the close vicinity of the transcription start site. Inhibitory sequences were identified further upstream in the spi 2.3 promoter and may account for the GH refractoriness of this gene. Both positive and negative glucocorticoid-responsive regions were found in all spi promoters.

B 653 ANALYSIS OF RETINOID X RECEPTOR (RXRβ) FUNCTION BY AN IN VITRO TRANSCRIPTION ASSAY, Insong J. Lee, Paul H. Driggers, Jeffrey A. Medin and Keiko Ozato, Laboratory of Molecular Growth Regulation, NICHD, NIH, Bethesda, MD 20894

Our laboratory has previously cloned a cDNA encoding a retinoid X receptor (RXRβ). In the presence of its specific ligand 9-*cis* RA, RXRβ homodimer formation is increased as seen by band shift experiments and RXRβ stimulates transcription from promoters containing the appropriate response elements in transient transfection experiments. Our lab as well as others have shown that RXRβ can act as an auxilliary protein by heterodimerizing with a diverse group of nuclear hormone receptors as well as some orphan receptors to increase their binding affinity and affect transcription from promoters containing the appropriate response elements.

We are investigating the mechanism of transcriptional activation by RXRβ by using an in vitro transcription system. Recombinant RXRβ protein was prepared with a baculovirus expression system and nuclear extracts from embryonal carcinoma cells (N-tera2) and lymphoblastoid cells (Namalwa) were used to support transcription. The test templates we used contained either region II (from MHC class I gene H-2Ld promoter), TREp or CRBPII RXRE response elements connected upstream of an H-2Ld basal promoter fused to a G-free cassette. In N-tera2 nuclear extracts, addition of RXRβ increased transcription from the templates above in a ligand dependent manner. Transcription of control template containing only the basal promoter was not affected. Also, addition of nuclear extracts from wild type baculovirus infected Sf9 cells had no effect on transcription. Transcriptional activation by RXRβ was receptor concentration dependent and was ligand specific since all-*trans* RA had no effect on transcription. Preliminary order of addition experiments suggest that RXRβ increases transcription by stabilizing the pre-initiation complex. In Namalwa nuclear extracts, the transcriptional activation by RXRβ seen in N-tera2 nuclear extracts was not reproducible. The possible basis of this lack of activation is currently being investigated.

B 654 LIGAND-DEPENDENT CONFORMATIONAL CHANGES IN HOMODIMERS AND HETERODIMERS OF THYROID HORMONE AND RETINOIC ACID RECEPTORS, Xiaohua Leng, Sophia Y. Tsai, Bert W. O'Malley and Ming-Jer Tsai, Dept. of Cell Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

Like the steroids, thyroid hormone and retinoids exert their biological effects via specific interaction with their cognate nuclear receptors, thyroid hormone receptor (TR) and retinoic acid receptor (RAR), respectively. This group of receptors form strong heterodimers with retinoid X receptor (RXR) and function as transcriptional repressor in the absence of ligands. Upon binding ligands, they are activated and stimulate the target gene transcription. We have recently reported that steroid hormone, progesterone, induce distinct conformational changes in the progesterone receptor (PR). Those conformational changes then initiate heat shock proteins dissociation, receptor dimerization, DNA binding and transactivation. However, the exact role of this structural change in receptor activation is not clear. Utilizing partial proteolytic analysis, here we show that TR and RAR also undergo hormone-dependent conformational changes. Studies of various deletion mutants reveal that the major structural change centers upon the ligand binding domain of the receptor and renders the entire region resistant to protease digestion. The conformational change occurs in the absence of DNA and occurs independent of the other domains of the receptor. Heterodimerization between RXR and TR or RAR has little effect on receptor conformation in the absence of hormone but does enhance the ligand-dependent structural change. Interestingly, dual hormone treatment intensifies this enhancement. We suggest that the observed ligand-dependent conformational change is not required for the receptor to bind to the DNA or to heterodimerize with RXR, but instead, may be important for converting the receptor from a negative regulator to a positive activator.

B 655 POSITIVE AND NEGATIVE MODULATION OF JUN ACTION BY THYROID HORMONE RECEPTOR AT UNIQUE AP1 SITE. Gabriela N. Lopez, Fred Schaufele, Paul Webb, Jeffrey M. Holloway, John D. Baxter, and Peter J. Kushner. Metabolic Research Unit. University of California San Francisco. San Francisco C.A. 94143.

Unliganded thyroid receptor (TR) potentiates transcriptional activation of promoters by transfected jun at an AP1 site found in the backbone of pUC and related plasmids. Thyroid hormone, by contrast, changed potentiation into repression. AP1 site from the collagenase promoter, studied in parallel, failed to show potentiation of jun activation with TR or repression with thyroid hormone on the tk-promoter. The plasmid AP1 site thus has unique regulatory effects. Deletions of the ligand binding domain of the TR eliminated the ability of the receptor to boost jun activity, and deletion, mutation, or changes in specificity of the DNA binding domain eliminated both potentiation by unliganded receptor and repression by thyroid hormone.

Gel shift assays confirmed that jun/fos binds the operative fragment, and the presence of TR, even in excess, did not interfere with jun/fos binding activity.TR could also bind the fragment, at least in the absence of jun/fos,.and thyroid hormone reduced this ability. Protein interaction studies in the absence of DNA suggested that TR binds jun protein in solution either in presence or absence of hormone .This observations suggest a mechanism for synergy and repression by TR through modulation of jun activity by accompanying TR; positive when TR is unliganded, and negative when hormone is bound.

B 656 9-CIS RETINOIC ACID ACTIVATES RXRα IN YEAST

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The retinoid X receptor (RXRα) heterodimerizes with retinoic acid receptors (RARs), thyroid hormone receptor (TR) and vitamin D3 receptor (VDR) and stimulates both their DNA binding and transactivation properties. The stereoisomer 9-cis retinoic acid binds with high affinity to RXRα and transactivates basal promoters containing the retinoid X response element (RXREs) in mammalian cell transcient transfection studies. Because all mammalian cells contain endogenous RARs, it is not clear whether RXRα functions exclusively as heterodimer or also functions on its own. Here we report that RXRα expressed in yeast, which does not contain endogenous RARs transactivates efficiently a basal promoter containing an RXRE from the apolipoprotein AI gene (site A) in response to 9-cis RA. RXRα produced from yeast exhibits similar DNA-binding properties as those produced from mammalian cells. Thus, this yeast expression system provides a useful model to study the transcription activation pathways induced by retinoids.

B 657 PROTEIN KINASE PATHWAYS AND REGULATION OF HEPATIC APO E GENE EXPRESSION

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Apolipoprotein E (Apo E) is a plasma protein involved in the transport of cholesterol and other lipids among various tissues and organs through its interaction with low density lipoprotein-receptor and to the LDL-receptor related protein. Hepatic parenchymal cells are primarily responsible for apo E production within the liver.

We have previously demonstrated that liver apo E mRNA level is highly correlated with Insulin/Glucagon molecular ratio (I/G) in the plasma, during the development of fetal and young rats. Furthermore, injection of glucagon or db cAMP to young rats induced an increase in the apo E mRNA content of their liver. In various nutritional circumstances which modified the I/G in rat plasma, we also found a correlation with liver apo E mRNA levels which increased when I/G decreased.

In order to investigate the role of the cAMP/PKA pathway in the regulation of apo E gene expression in hepatocytes, we have submitted rat liver hepatocytes in primary culture to db cAMP and theophyllin treatment. We evidenced a two fold increase in apo E mRNA level paralleled by a three fold increase in [^{35}S] methionine incorporation into apo E. To examin the role of the apo E promoter in the db cAMP effect, we prepared by PCR the [-200/+1] region of the promoter and inserted it at the Hind III/Xho I site of pUC-SH-CAT. The CAT activities of Hep G2 transiently transfected by pUC-[-200/+1]-apo E-CAT was stimulated by two fold in presence of cAMP and theophyllin.

The actual target of db cAMP remains to be established since no CRE B sequence homology was observed between -200 and +1, which only contains a sequence highly homologous to AP_2 (CCCACCTC) in a region protected in footprint experiments. Preliminary results showed that TPA decreased, and interleukin 1 increased apo E mRNA level in Hep G2 cells. To identify the actual target of protein kinase pathways on apo E gene transcription, several pUC-CAT constructs of the 5' flanking region of the apo E gene are currently under investigation.

B 658 POSITIVE AND NEGATIVE REGULATION OF HUMAN *lck* TYPE I PROMOTER TRANSCRIPTION.
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The human *lck* gene, encoding a lymphoid-specific, cytoplasmic membrane-associated tyrosine kinase p56^{lck}, is transcribed from two structurally distinct promoters. The two *lck* promoters are expressed in a developmental stage-specific manner in T cells. The *lck* type II promoter is active in all T cells, whereas the *lck* type I promoter is active in immature thymocytes and is down-regulated during T cell maturation. In addition, several human colon and small cell lung carcinoma lines aberrantly express the *lck* type I promoter. To elucidate mechanisms controlling the expression of the human *lck* gene, we investigated the *cis*-acting DNA sequence requirements for transcription of the human *lck* type I promoter in the leukemic T cell line, Jurkat, and in the colonic tumor cell line, SW620. Transient transfection assays in Jurkat and SW620 cells revealed both negative and positive *cis*-acting regulatory regions. *Lck* type I promoter activity was only detected after removal of sequences between -570 and -480. Further 5' deletions showed that 128 bp of 5' flanking sequences of the type I promoter, containing an ETS-binding element (EBE, positions -98 to -91), a TCF binding site (positions -77 to -71), and a consensus helix-loop-helix transcription factor binding site (IgHE, positions -45 to -37), was sufficient to direct rabbit β-globin reporter gene transcription. This region of the *lck* type I promoter is highly conserved between man and mouse. *In vitro* assays demonstrated that both the EBE and the TCF binding site were specifically bound by ETS and TCF family members, respectively. Point mutation of the EBE abolished *lck* type I promoter activity in Jurkat and SW620 cells. In contrast, alteration of the TCF binding site led to an ~3-fold increase in *lck* type I promoter activity in Jurkat cells. Currently, we are investigating the effect of mutating the IgHE on *lck* type I promoter transcription.

B 659 9-CIS-RETINOIC ACID ALTERS THE DNA BINDING CHARACTERISTICS OF RECOMBINANT RXRß, Jeffrey A. Medin, Paul H. Driggers, and Keiko Ozato, Laboratory of Molecular Growth Regulation, Bldg. 6 Rm 416, NICHD, NIH, Bethesda, MD 20892
We have previously cloned the murine gene for Retinoid X Receptor ß (RXRß), a member of the steroid/thyroid subfamily of nuclear hormone receptors. RXRß has been shown, by us and others, to heterodimerize with a specific subset of nuclear hormone receptors and thus act as a potential convergence point for multiple hormone response pathways. The specific ligand for RXRß has been identified as the 9-cis form of retinoic acid (RA) and it has also been recently demonstrated that the addition of 9-cisRA induces RXRß homodimer binding to various elements, creating even greater potential response diversity.

In this study we examine the physical DNA binding characteristics of heterodimers and homodimers of recombinant RXRß in the presence or absence of 9-cisRA for a series of nonequivalent hormone response elements. It is shown that the addition of 9-cisRA does not affect the ability of heterodimers and homodimers to form in solution by co-immunoprecipitation assays and by direct protein-protein crosslinking analysis. The addition of specific ligand does increase the binding of homodimers to multiple hormone response elements, while heterodimer binding affinity is not affected. Nanomolar binding constants are calculated from quantitative gel shift analyses and reflect preferential binding of certain dimeric forms for different elements. DNA methylation interference patterns are also examined for heterodimers and homodimers formed in the presence and absence of the specific ligand.

B 660 CHARACTERIZATION OF RXRß DELETION MUTANTS AND ANALYSIS OF THEIR FUNCTION,
Saverio Minucci, Dina J. Zand, Michael S. Marks, Toshi Nagata and Keiko Ozato, Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892
Retinoic Acid Receptors (RARs) and Retinoid X Receptors (RXRs) mediate the cellular response to Retinoic Acid (RA). RARs bind to all-*trans* RA with high affinity, while the RA stereoisomer, 9-*cis* RA, was recently shown to be a good candidate for the role of physiological ligand for RXRs. RXRs form heterodimers with RARs and with several other members of the nuclear receptor gene superfamily, suggesting a central role for RXRs in the fine-tuning of multiple hormonal signaling pathways.
In order to further investigate the molecular anatomy of this system, a deletion analysis of murine RXRß was performed. Different C-terminal and N-terminal truncated RXRß polypeptides were synthesized *in vitro* and analyzed for their molecular characteristics. This analysis confirms the previous findings of the critical role of the C-terminal domain in the formation of heterodimers and show that the deletion encompassing the whole DNA binding domain, albeit to a lesser extent than the intact RXRß, still allows heterodimerization to occur. We established clones of P19 embryonal carcinoma cells expressing a deletion mutant of RXRß lacking the DNA binding domain. The functional analysis of these clones and transient transfectants have given results consistent with those obtained from *in vitro* heterodimerization experiments. This RXRß deletion mutant may serve as a useful tool to study physiological responses to RA controlled by RXR heterodimers.

B 661 TRANSCRIPTIONAL CONTROL OF ENDOGENOUS MOUSE MAMMARY TUMOR VIRUS IN B LYMPHOCYTES, Edwin C. Murphy III, Stefan Doerre and Ronald B. Corley, Department of Immunology, Duke Medical Center, Durham, NC 27710
It has been previously shown that levels of transcripts encoded by an endogenous mouse mammary tumor virus (MMTV) increase during both lipopolysaccaride (LPS) and interleukin 5 induced differentiation. This occurs both in normal B lymphocytes and in inducible B cell lymphomas. Induction of MMTV by LPS in B cells occurs via a glucocorticoid-independent pathway, and is at least partially distinct from mechanisms controlling immunoglobulin gene expression. Further, two transcription factors known to function in immunoglobulin upregulation, NF-kappaB and OTF-2, are not sufficient for MMTV upregulation, even though the viral LTR contains putative binding elements for each factor. Transient transfections have shown that MMTV upregulation results from transcriptional stimulation from the viral long terminal repeat (LTR), indicating the presence of specific and inducible transcription factors in B cells. The transcription of MMTV in B cells results in the expression of a virally encoded superantigen which is important in the viral life cycle. It is therefore of interest to define the elements involved in MMTV upregulation in B cells, and the factors that act on these elements. Electrophoretic mobility shift assays are now being used to define the elements in the viral LTR which may function to bind specific transcription factors and thus regulate MMTV transcription.
(Supported by NIH grant CA36642)

B 662 *Abstract Withdrawn*

B 663 RETINOID REGULATION OF THE HUMAN SKELETAL α- ACTIN GENE: IDENTIFICATION OF A RETINOIC ACID RESPONSE ELEMENT

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The retinoids, *all trans* retinoic acid (RA) and 9-cis RA have diverse regulatory roles in vertebrate development. The action of these ligands are mediated by the retinoic acid and retinoid X receptor (RAR and RXR) families. The RXRs have been shown to selectively modulate the binding of the thyroid hormone, vitamin D and retinoic acid receptors to their cognate elements, thus affording the potential of a wide and complex level of hormonal regulation.

We have used electrophoretic mobility shift assays (EMSA) and northern analyses to characterize the retinoid regulation of the skeletal α-actin gene. This gene is specifically expressed and pathophysiologically regulated in cardiac and skeletal muscle tissue. EMSA experiments have shown that bacterially expressed RARα and RXRα interact specifically with sequences between nucleotide positions -273 and -249 in the human skeletal α-actin gene; a region previously shown to mediate a response to T3. In addition, RXR enhances the dimeric and oligomeric binding of RAR to this element. This element conferred a RAR-dependent transcriptional response to an enhancerless SV40 promoter. In vitro competition utilizing mutant oligonucleotides spanning this region indicated that RAR interacts with a direct repeat of the **AGGTCA** motif with a 2 nucleotide spacing. These findings are consistent with the fact that most, if not all, T3 responsive genes are subject to multihormonal control. Northern analyses indicated that the various RAR and RXR isoforms are differentially expressed and predominantly down regulated during rodent myogenesis. We are currently over-expressing these receptors in muscle cell lines to evaluate their regulatory role during myogenesis.

B 664 MINIMAL DNA SEQUENCES WHICH CONTROL THE TISSUE-SPECIFIC ACTIVITY OF THE PROα2(I) COLLAGEN PROMOTER. Karen Niederreither, Tarja Helaakoski, Lee Ann Garrett, Rena N. D'Souza* and Benoit de Crombrugghe *Department of Molecular Genetics M. D. Anderson Cancer Center, *Department of Anatomical Sciences University of Texas Health Science Center Dental Branch, Houston, TX 77030.*

The pattern of expression of the proα2(I) collagen gene is highly tissue-specific in adult mice and shows its strongest expression in bones, tendons, and skin. Transgenic mice were generated harboring promoter fragments of the mouse proα2(I) collagen gene linked to the Escherichia coli β-galactosidase or firefly luciferase genes to examine the activity of these promoters during development. A region of the mouse proα2(I) collagen promoter between -2000 and +54 exhibited a pattern of β-galactosidase activity during embryonic development that corresponded to the expression pattern of the endogenous proα2(I) collagen gene as determined by in situ hybridization. A similar pattern of activity was also observed with much smaller promoter fragments containing either 500 or 350 bp of upstream sequence relative to the start of transcription. Embryonic regions expressing high levels of β-galactosidase activity included the valves of the developing heart, sclerotomes, meninges, limb buds, connective tissue fascia between muscle fibers, osteoblasts, tendon, periosteum, dermis, and peritoneal membranes. The pattern of β-galactosidase activity was similar to the extracellular immunohistochemical localization of transforming growth factor-β1 (TGF-β1). The -315 to -284 region of the proα2(I) collagen promoter was previously shown to mediate the stimulatory effects of TGF-β1 on the proα2(I) collagen promoter in DNA transfection experiments with cultured fibroblasts. A construct containing this sequence tandemly repeated 5' to a very short α2(I) collagen promoter (-40 to +54) showed preferential activity in tail and skin of 4-week old transgenic mice. The pattern of expresssion mimics that of the -350 to +54 proα2(I) collagen promoter linked to a luciferase reporter gene in transgenic mice. We are currently testing whether the -315 to -284 sequence functions upstream of a heterologous minimal promoter and are attempting to identify the transcription factors responsible for this tissue-specific expression.

B 665 REGULATION OF INTERFERON-INDUCIBLE GENES DURING MONOCYTIC DIFFERENTIATION OF U-937 CELLS: EFFECTS OF THE CONSTITUTIVE EXPRESSION OF v-Myc

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Extensive studies suggest a role for the *myc* proto-oncogene family in the control of cell proliferation and differentiation in vertebrates. We have utilized the human monoblastic U-937 cells constitutively expressing an exogenous v-*myc* construct to study the role of the *myc* gene during hematopoietic differentiation. The expression of v-Myc blocks the phorbol ester (PMA), 1,25α-dihydroxyvitamin D_3 ($VitD_3$) and retinoic acid (RA) induced terminal differentiation of U-937 cells. However, this block can be abrogated by the co-stimulation of these inducers with interferon-γ (IFN-γ). In contrast, the terminal differentiation cannot be restored by co-stimulation with IFN-α. Since IFN-γ and IFN-α differ in their capacity to restore terminal differentiation it was of interest to examine the effects of v-Myc expression on the response to these cytokines. Furthermore, the expression of several interferon-inducible genes, e.g. MHC class I and Fc receptors, are upregulated during differentiation of U-937 control cells, induced by PMA, $VitD_3$ and RA. To investigate these responses in further detail the regulation of the expression of six interferon-inducible genes, 6-16, 9-27, FcγRI, the transcription factor IRF-1, and the enzymes dsRNA kinase (p68) and 2'-5' oligoA synthetase, was studied in U-937 control cells and cells constitutively expressing v-Myc. Data on the effect of v-Myc on the regulation of the expression these interferon-inducible genes during terminal differentiation, and in the response to IFN-γ and IFN-α stimulation will be presented.

B 666 **TRANSCRIPTIONAL REGULATION OF THE HUMAN MIP GENE.** **Chiaki Ohtaka-Maruyama, LaShawn R. Drew, M. Michele Pisano and Ana B. Chepelinsky.** Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20892, U.S.A.

MIP, the major intrinsic protein of the lens fiber membrane, is specifically expressed in the ocular lens fiber cell membrane. MIP belongs to an ancient superfamily of putative transmembrane channel proteins and may play a role in cell-cell communication.

We cloned 2840 bp of 5' flanking sequence of the human MIP gene to study the *cis* regulatory elements responsible for the tissue specificity and developmental regulation of the MIP gene. Initially we analyzed the sequences proximal to the initiation site of transcription. We found that a DNA fragment containing 253 bp of 5' flanking sequence and 42 bp of exon one of the human MIP gene fused to the reporter gene chloramphenicol acetyltransferase (CAT) gene directs CAT gene expression to lens cells in transient assays and transgenic mice. We are presently focusing on other 5' flanking sequences of the MIP gene that may be required for appropiate tissue specificity. Two putative NF1 binding sites are present in one of the three Alu repeats present in the 5' flanking region of the MIP gene, at positions -2687 and -2460. The other two Alu repeats contain two CACCC boxes each, at positions -2072, -2080, -1750 and -1701. The most proximal Alu repeat, positions -1700 to -1994, contains a CpG island. The 5' flanking region between this Alu repeat and the transcription initiation site contains five CCAAT boxes, seven additional CACCC boxes, one Sp1 binding site, six additional NF-1 binding sites, and two glucocorticoid responsive elements; all of them known to function synergistically in the binding to the glucocorticoid receptor. To elucidate whether those motifs are involved in the regulation of MIP gene expression we are presently studying the effect of several factors on the in vitro transcription of the MIP gene using Drosophila and Hela nuclear extracts. Purified human Sp1 activates the in vitro transcription of the MIP promoter, suggesting its involvement in the regulation of transcription of the MIP gene. These studies will further our understanding of the role of general transcription factors on the tissue specific expression of the MIP gene.

B 667 **INVOLVEMENT OF A C/EBP-LIKE PROTEIN IN THE ACQUISITION OF RESPONSIVENESS TO GLUCOCORTICOID HORMONES DURING CHICK NEURAL RETINA DEVELOPMENT.** Sarah Ben-Or and Sam Okret, Dept. of Physiology, Hebrew Univerity Hadassah Medical School, Jerusalem, Israel and Dept. of Medical Nutrition, Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden.

The glucocorticoid receptor (GR) in chick embryonic (E) neural retina is expressed early in ontogeny, yet the tissue's response to the glucocorticoid hormone, i.e. induction of glutamine synthetase (GS) develops later, only during the second week of ontogeny. Transient transfection of embryonic day 7 (E7) retinal cells, which are nonresponsive to glucocorticoids, with chimeric plasmids containing the chloramphenicol acetyltransferase (CAT)-reporter gene under the control of glucocorticoid responsive promoters, demonstrated that GR in E7 cells is a functional transactivating factor. We show that the limiting transcription factor that controls the developmental acquisition of responsiveness to glucocorticoids is similar to a CCAAT enhancer binding protein (C/EBP). This protein recognizes a sequence in the promoter of the chick GS gene, which is required for eliciting the glucocorticoid response. Retinal C/EBP-like protein was not detected in the glucocorticoid nonresponsive (E7) proliferating glioblasts but was found to be present in the glucocorticoid responsive (E12) post-mitotic cells. Premature expression of C/EBP in the nonresponsive E7 cells by transfection was shown to enhance the developmental acquisition of responsiveness to the glucocorticoid hormone, as deduced from the level of GS inducibility.

B 668 CHRONIC EXPOSURE OF HIT CELLS TO HIGH GLUCOSE CONCENTRATIONS ALTERS BINDING OF INSULIN GENE REGULATORY PROTEIN AND PARADOXICALLY DECREASES INSULIN GENE TRANSCRIPTION, L. Karl Olson, J. Bruce Redmon, Howard C. Towle, and R. Paul Robertson, Diabetes Center and the Division of Endocrinology and Metabolism, Department of Medicine, and Department of Biochemistry and Institute of Human Genetics, University of Minnesota Medical School, Minneapolis, MN 55455

Chronically culturing HIT-T15 cells in media containing 11.1 mM glucose leads to decreased insulin mRNA levels, insulin content and insulin secretion, and these changes can be prevented by culturing the cells in media containing 0.8 mM glucose. This represents a paradoxical event since glucose is a physiologic stimulator of insulin gene transcription and stabilizes insulin mRNA. To determine the mechanism of this event we transiently transfected HIT cells with a chloramphenicol acetyl transferase (CAT) reporter gene controlled by the 5'-regulatory domain of the human insulin gene (INSCAT). Early passages of HIT cells readily expressed INSCAT, whereas late passages of cells chronically cultured in 11.1 mM glucose expressed only 14.1±3.3 % (mean±SEM) of the CAT activity expressed in early passages. In contrast, late passages of HIT cells chronically cultured in 0.8 mM glucose retained the ability to express INSCAT to the same extent as early passages. The decrease in INSCAT expression in late passages of HIT cells serially cultured in 11.1 mM glucose was associated with the absence of a specific nuclear protein-DNA complex. This specific protein-DNA complex could be preserved in late passages of HIT cells by serially culturing in 0.8 mM glucose. The binding site of the protein was determined to lie within the CT-II motif (-215/-210) of the human insulin promoter. This motif has been shown to bind the beta cell specific transcription factor, IUF, and is similar to a sequence found in the glucose responsive element of the rat 1 insulin gene. There were no other observable differences in protein-DNA complexes when the sequences between -326 and -131 of the human insulin gene were examined. These data suggest that chronic exposure of the beta cell to high glucose concentrations can paradoxically decrease insulin gene transcription, in part, by altering the interaction of a regulatory protein with the CT-II motif of the human insulin gene promoter.

B 669 THE LIGAND BINDING DOMAIN OF THE HUMAN RETINOIC ACID RECEPTORγ RECOGNIZES A CHIRAL LIGAND, Jacek Ostrowski, Kuo-Long Yu*, Roman Sterzycki*, Laura Hammer, Thor Roalsvig, Simon Chen, Joyce Phelan, Kevin Pokornowski, Patrick Spinazze, John Starrett, Muzammil Mansuri*, Peter R. Reczek, Department of Molecular Biology and *Retinoid Chemistry, Bristol-Myers Squibb PRI, Buffalo, NY 14213

Retinoic acid exerts its many biological effects by interaction with specific nuclear receptors (RAR). The human retinoic acid receptors are members of the steroid/thyroid hormone receptor superfamily. Of the three known receptors, RARγ is the predominant form in the skin.

Despite efforts to understand the binding properties of retinoic acid for its receptor, little is known about this interaction. Retinoids that bind selectively to each of the three receptor subtypes have only recently been described. We report here that several different classes of synthetic retinoids with a racemic hydroxyl group in the linker region are selective for the RARγ subtype. Further, traditional transactivation and direct binding assays using these synthetic compounds demonstrate that the receptor clearly prefers one enantiomer over the other. These results are correlated with the same preferences in *in vivo* experiments.

Taken together, these results suggest that RARγ is very sensitive to structural modifications in synthetic ligands and that the portion of the receptor that interacts with the linker region of these ligands is chiral.

B 670 DIFFERENTIAL GENE REGULATION IN RESPONSE TO LOSS OF TUMOR SUPPRESSOR GENE FUNCTION: COLLAGEN II (ALPHA 1) AND H19, Russell D. Owen and J. Carl Barrett, Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, D2-04, National Institutes of Health, P.O. Box 12233, Research Triangle Park, NC 27709

Loss of tumor suppressor gene function is essential to the multistep progression of cells to neoplasia. Immortalized, non-tumorigenic cells which were established by carcinogen treatment of Syrian hamster embryo (SHE) cells, retained the ability to suppress tumorigenicity in cell hybrids with highly malignant cells (termed suppressor-positive). Passage and subcloning of these cells allowed the isolation of variant cell lines that had lost tumor suppressor activity (termed suppressor-negative) but remained non-tumorigenic. The steady state mRNA levels for collagen II (alpha 1a), a chondrocyte differentiation marker, and H19, a developmentally-controlled gene, were found to be differentially regulated between suppressor-positive and suppressor-negative cells. Nuclear run-on analysis indicated that this differential gene expression was effected in cells by alterations of transcriptional and post-transcriptional regulation. Transient transfection experiments were used to identify a cis-acting transcriptional element resident in the rat collagen II 5' flanking sequences that directs differentially regulated gene transcription. Gel retention analysis demonstrated the presence of a nuclear DNA-binding factor(s) that specifically recognizes a DNA sequence common to both the mouse H19 enhancer and the rat collagen II gene. The emergence of two independent mechanisms that cause differential expression of collagen II and H19 in response to tumor suppressor loss, operating at both the transcriptional and the post-transcriptional levels, strongly suggests that the coordinate regulation of these genes, or other genes regulated by common mechanisms, may be important in maintaining tumor suppression.

B 671 EXPRESSION OF THE ACUTE PROMYELOCYTIC LEUKEMIA (APL) TRANSLOCATION PRODUCTS INTO HUMAN MYELOID CELL LINES. Pier Giuseppe Pelicci, Pier Francesco Ferrucci, Marta Fagioli, Myriam Alcalay, Giampaolo Talamo, Amedea Mencarelli, Fausto Grignani and Francesco Grignani, Istituto di Clinica Medica I, University of Perugia, Perugia, Italy 06100.

The APL 15;17 chromosomal translocation breakpoints lie within the RARα and PML loci. Two fusion genes, PML/RARα and RARα/PML, are formed as consequence of the translocation. Alternatively spliced PML/RARα transcripts encode PML/RARα fusion proteins and aberrant truncated PML proteins; the RARα/PML transcript encodes a fusion RARα/PML protein.

To investigate the contribution of these abnormal proteins to the leukemic phenotype, we constructed retroviral expression vectors for the PML/RARα, RARα/PML and aberrant PML proteins as well as for the wild type PML and RARα. We also generated retroviral vectors for engineered PML/RARα fusion proteins lacking the RARα and the PML DNA binding domains, respectively. We used these vectors to infect three human myeloid cell lines: HL-60, which is sensitive to RA-induced granulocytic terminal differentiation; HL-60R, an HL-60 subline defective for RARα and resistant to RA; U937, which is also sensititive to the RA-induced differentiation, but is committed to the monocytic lineage. For each cell line we have generated subclones expressing protein of the transduced gene. The effects of the expression of the 15;17 translocation aberrant proteins on the differentiation programme and the proliferation of these myeloid cell lines will be presented and discussed.

B 672 1,25 DIHYDROXYVITAMIN D3 INDUCTION OF A RETINOIC ACID RESPONSE ELEMENT BY A CHIMERIC RECEPTOR, S. M. Pemrick, L. J. Sturzenbecker, P. Abarzúa[1], Cl. Kratzeisen, M. S. Marks[2], J. A. Medin[2], Keiko Ozato[2], A. A. Levin, W. Hunziker[3], and J. F. Grippo, Depts. of Investigative Toxicol. and Oncology[1], Nutley, NJ, and Pharma Research[3], Basel Switzerland, Hoffmann-La Roche, Inc., and Laboratory of Cell Biology, Nat'l Cancer Inst., N.I.H., Bethesda, MD[2].

A chimeric receptor (RAR/VD) containing the DNA binding domain of the retinoic acid receptor (RAR α) and the ligand binding domain of the vitamin D receptor (VDR) mediated transcription of a retinoic acid (RA) response element (RARE) in the presence of 1,25 dihydroxyvitamin D3 (D3). Nucleosol fractions from COS-1 cells transfected with either VDR or RAR/VD bound [^{3}H]1,25 dihydroxy vitamin D3 with Kds of approximately 0.1 nM. In CV-1 cells, RAR/VDR mediated transcriptional activity of a RARE (β-RARE$_3$)-thymidine kinase (tk)-luciferase reporter gene, but not that of a retinoic acid X receptor, RXRE (ApoA1)-tk-luciferase reporter gene, in response to treatment with D3. Transcriptional activation by RAR/VDR was linearly dependent upon receptor plasmid concentration and exhibited half maximal activation at 0.7nM D3. Two additional chimeric receptors containing RAR α DNA binding domains and estrogen receptor (ER) ligand binding domains failed to mediate transcriptional activity on the RARE, although both were capable of binding [^{3}H] estradiol. Gel retardation assays indicated RAR/VDR, but not the RAR/ER chimeric receptors bound to the RARE. Co-precipitation assays with RXR antibody showed RAR and RAR/VDR, but not the RAR-estrogen chimeras, formed heterodimers with RXR. These results demonstrate that formation of a functional RAR chimeric receptor may require a ligand binding domain capable of interacting with RXR.

B 673 HOX 2.4 AND A MILIEU OF HIGH IL-3 CONCENTRATION DELIVER SYNERGISTIC SELF-RENEWAL SIGNALS. A.C.Perkins, J.M.Adams, S.Cory. Walter and Eliza Hall Institute of Medical Research, P.O.Box Royal Melbourne Hospital, Parkville, VIC., AUSTRALIA 3050.

Homeobox genes perform important managerial roles in embryonic development and subsequent cellular differentiation processes. Hematopoietic cells express a wide array of Hox genes, some of which may govern lineage commitment. However, the expression of Hox 2.4 in the murine myelomonocytic cell line, WEHI-3B is aberrant due to insertion of an endogenous intracisternal A-particle (IAP) into the 5' untranslated region of the gene. A similar IAP insertion leads to autocrine IL-3 production. We have previously demonstrated that the simultaneous expression of Hox 2.4 and IL-3 leads to the tumorigenic transformation of a subpopulation of bone marrow progenitor cells. In view of the fact that enforced autocrine IL-3 production alone can cause proliferation but cannot impede differentiation of naive bone marrow cells we have argued that Hox 2.4 is the immotalizing partner in the generation of the WEHI-3B leukemia. More recently we have confirmed that Hox 2.4 alone can immortalize a subpopulation of progenitors cells from bone marrow to yield factor-dependent, non-tumorigenic lines of myeloid, mast and megakaryocyte lineages. Many of the lines express surface markers of more that one lineage and some have stem cell features such as the ability to form cobblestone areas on marrow stromal lines and day 13 CFUs *in vivo* . Surprisingly , immortalization is dependent upon the continued presence of very high concentrations of IL-3. Its withdrawl results in terminal differentiation which can be biased towards granulocytes or macrophages with G-CSF or M-CSF respectively. Mice reconstituted with Hox 2.4 retroviral-infected marrow remain healthy presumably because the amounts of IL-3 available *in vivo* are limiting. Nevertheless self-renewal of the progenitor cell compartment was demonstrable as an increased number of CFCs which grew as compact colonies in the presence of high IL-3 concentrations. Many clonally identical immortalized lines were generated from independent colonies which argues for CFC self-renewal *in vivo*. In addition, Hox 2.4 expression within CFUs allows for their continued self-renewal during serial transplantation.The critical effect of Hox 2.4 expression appears to be increased self-renewal potential. It may mimic the action of another homeobox gene product that normally maintains stem cell self-renewal or alternatively, it may inhibit the function of one needed for terminal differentiation. The requirement for high concentrations of IL-3 to maintain self-renewal raises intriguing questions about signal pathways in hematopoietic stem cells.

B 674 THE ATF/CREB FAMILY OF TRANSCRIPTION FACTORS IS A TARGET FOR REGULATION BY ras TRANSFORMATION, Manoussos Perros, Steffen Faisst, Nathalie Spruyt and Jean Rommelaere, Oncologie Moléculaire, Institut Pasteur de Lille, BP245, 59019 Lille, France

Oncogenic transformation of mammalian cells causes modifications in the transcriptional activity of a wide variety of cellular genes. Although most nuclear oncoproteins can act as transcription factors, others appear to regulate gene expression by modulating the activity of cellular transcriptional complexes. In the present study, promoter P4 of parvovirus MVMp was used as a model system to identify transformation-responsive transcription factors. Indeed, ras transformation was previously shown to result in a striking stimulation of the activity of this promoter in rat cells. Protein-DNA complexes formed on the regulatory region of promoter P4 were mapped by in vitro and in vivo footprinting experiments. Among other transformation-dependent changes, a striking differential interaction was detected within a repeated sequence at the 5' end of the parvoviral promoter. Copper-orthophenanthroline footprinting and methylation interference assays narrowed this region down to a tandem-repeated motif that closely resembles the consensus sequence for the binding of the ATF/CREB family of transcription factors. Competitive gel retardation assays, UV-crosslinking experiments and incubation with specific antibodies were performed to characterize the nucleoprotein complexes that are formed within that region. Out of five complexes, three were found to be ras-sensitive and to consist of CREB/CREB, CREB/ATF1 and ATF1/ATF1 dimers. Although all three species could be detected in both normal and transformed cells, ras transformation appeared to favor the formation of ATF1 homodimers compared to CREB/CREB and CREB/ATF1 complexes. These data and other experiments under progress in our laboratory suggest that ras transformation can modulate transcription of target genes through members of the ATF/CREB familly.

B 675 SELECTIVE MYOD AND MYOGENIN mRNA EXPRESSION IN FAST AND SLOW MUSCLE IS UNDER NEURONAL CONTROL, Charlotte A. Peterson‡, Helen M. Blau†, and Simon M. Hughes*, ‡Departments of Medicine and Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences and John L. McClellan Veterans Medical Center, Little Rock, AR 72205, USA; †Department of Pharmacology, Stanford University Medical Center, Stanford, CA 94305-5332, USA; *Current address: MRC Muscle and Cell Motility Unit, King's College London, 26-29 Drury Lane, London, WC2B 5RL, UK.

In skeletal muscle, each of the myogenic helix-loop-helix transcription factors (MyoD, Myogenin, Myf-5, and MRF4) is capable of activating muscle-specific gene expression, yet distinct functions have not been ascribed to the individual proteins. One possibility is that their differential expression or activity may contribute to the distinct phenotypes characteristic of different muscles of the limb. We report here that MyoD and Myogenin mRNAs are selectively expressed in hindlimb muscles of the adult rat that differ in contractile activity: MyoD was prevalent in fast twitch and Myogenin in slow twitch muscles. Furthermore, within a single muscle, *in situ* hybridization showed that the distribution of MyoD and Myogenin transcripts correlated with the proportions of fast glycolytic and slow oxidative muscle fibers. Alteration of the fast/slow fiber type distribution by cross-reinnervation resulted in a corresponding alteration in the MyoD/Myogenin mRNA expression pattern: Myogenin transcripts declined in those regions of a slow muscle that were converted to express fast myosin heavy chain. These findings suggest that the content of specific myogenic helix-loop-helix regulators may control adult muscle fiber phenotype and mediate nerve-dependent changes in contractile protein expression.

B 676 FUNCTIONAL CHARACTERIZATION OF RAR/RXR DIMERIZATION, Jens Pohl, Thomas Bugge and Henk Stunnenberg, Gene Expression Programm, European Molecular Biology Laboratories, Meyerhofstrasse 1, D-6900 Heidelberg, Germany

Stable binding of retinoic acid receptors (RARs) to their response elements requires heterodimerization with the retinoid X receptor (RXR). To characterize the nature of the interaction, the C-terminal part of RARα and RXRα encompassing the ligand binding domain and nine short hydrophobic heptad regions presumably forming a coiled coil structure, were expressed in bacteria. The use of histidine tagged proteins faciliated purification of soluble proteins on Ni^{2+}-chelate NTA agarose. Co-expression of histidine-tagged RXRα ligand binding domain with non-tagged RARα ligand binding domain yielded a soluble heterodimeric RAR/RXR complex as shown by SDS-PAGE and western blotting using polyclonal antibodies against RARα and RXRα. This clearly demonstrated the importance of the ligand binding domain for the receptor interaction and that RXR and RAR are tightly associated in the absence of DNA. Purification of these heterodimeric complexes will facilitate studies of the dimerization interface by NMR and crystallography.

The response elements for RARs are composed of two direct repeats of GGTCA. To clearify the orientation of binding of the RAR/RXR heterodimers, we substituted the DNA binding domain of either RAR or RXR with that of the glucocorticoid receptor (GR). By binding to chimeric response elements having either the first or second direct repeat substituted with a consensus GRE half site, we conclude that RXR binds specifically to the first half site and RAR to the second.

B 677 DEVELOPMENTAL REGULATION OF THREE bHLH MYOGENIC REGULATORY GENES, M.E.Pownall and C.P.Emerson, Jr. Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901 and Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111.

qmf1, qmf2, and qmf3 are bHLH transcription factors that are involved in regulating gene expression during the establishment of the myogenic lineage. The expression of the quail bHLH myogenic factors themselves is precisely regulated during somite development. *qmf1* (the quail MyoD homologue) expression is detected earliest, in the immature somite, followed by *qmf3*. *qmf2* and contractile protein gene expression is not detected until the somite has matured. Expression of the *qmfs* and other muscle specific genes is restricted to the myotomal compartment of the mature somite. In the earlier, immature somite, *qmf1* and *qmf3* are expressed in the medial cells proximal to the neural tube. The expression patterns of these quail myogenic regulatory factors differs from that of the mouse homologues, suggesting that although the amino acid sequence conservation between homologues is high, they may have different roles in myogenic determination and differentiation. The activation of *qmf1* expression in the somite cells proximal to the neural tube supports the hypothesis that neural tube influences may regulate some initial events of myogenesis. We are testing the capacity of the neural tube to induce ectopic *qmf1* expression in immature somites. The sequential activation and co-localization of *qmf1*, *qmf2* and *qmf3* gene expression indicate that there may be a gene regulatory hierarchy. We are using a cell culture/ DNA transfection approach to identify regulatory sequences responsible for the observed sequential activation pathway in somite cells.

B 678 RETINOIC ACID-INDUCED ACTIVATION OF TRANSCRIPTION FACTORS HNF-3α AND HNF-3β UPON TERATOCARCINOMA CELL DIFFERENTIATION, Ronald R. Reichel[1], Shalini Budhiraja[1], Alexander Jacob[1], Xiaobing *Qian*[2] *and Robert* H. Costa[2], [1]Department of Pharmacology and Molecular Biology, The Chicago Medical School, North Chicago, IL 60064, [2] Department of Biochemistry, University of Illinois, College of Medicine, Chicago, IL 60612

We have investigated the regulation of HNF-3α and HNF-3β during differentiation of F9 teratocarcinoma cells. Addition of retinoic acid to F9 stem cells leads to differentiation into primitive endoderm, which is accompanied by a dramatic increase of the DNA binding activities of both HNF-3α and HNF-3β. Activation of HNF-3β occurs between 1 and 2 days after induction of differentiation and HNF-3β levels remain elevated up to 10 days post-differentiation. In contrast, HNF-3α is activated much earlier, between 4 and 8 hours after retinoic acid addition. Furthermore, HNF-3α levels peak at about 24hours post-differentiation and then decline to undetectable levels. The described kinetic behaviours indicate that these two members of the HNF-3 transcription factor family are involved in different aspects of the differentiation process. In addition, the relatively early induction of HNF-3α opens up the possibility that the HNF-3α gene is a direct target of retinoic acid receptor. We have also studied the fluctuation of HNF-3α and HNF-3β upon F9 cell differentiation into parietal endoderm, which is triggered by the combined addition of retinoic acid and cAMP level-increasing agents. Both HNF-3 factors are induced with kinetics that are virtually identical to the ones observed during primitive endoderm differentiation. With the help of specific DNA probes, we are currently investigating the regulation of both transcription factors at the RNA level. This will bring as closer to our long-term goal, which is the elucidation of the pathway that links retinoic acid action to the regulation of gene expression.

B 679 CLONING OF POTENTIAL TARGETS OF GATA-1 ACTIVATION DURING MAST CELL DEVELOPMENT, Paul M. Richardson, Paula Fraenkel, and Leonard I. Zon, Harvard Medical School, Division of Hematology, Children's Hospital, Boston, MA 02115.
GATA-1 is a cell-specific transcription factor which is required for normal erythroid development. GATA-1 is also expressed in the mast cell lineage. Northern blot analysis of several mast cell lines revealed abundant expression of GATA-1, except in the cell line p815. Although this line was derived from a mastocytoma tumor, during tissue culture the cells lost the ability to form metachromatic staining granules typical of mature mast cells. An expression vector containing the GATA-1 cDNA was stably transfected into p815 cells. Several resultant cell lines showed significant morphological differences compared to the parental line, most notably the appearance of granules. To identify genes which could be targets of GATA-1 action during the development of mast cells, a subtractive approach was initiated. First strand cDNA was obtained from polyA RNA from a GATA-1 expressing subline (designated pMG6) by reverse transcription, and hybridized to a 20-fold excess of photobiotinylated mRNA from the parent cell line (p815). Hybridized nucelic acids were removed with streptavidin. The resultant subtracted cDNA probe was randomly labelled and used to screen a mouse bone marrow-derived mast cell cDNA library. Of approximately 200,000 plaques screened, sixty independent clones were found to be expressed at a higher level in pMG6 than p815. Comparison of the sequences of these 60 clones with the Genbank database revealed that several have previously been isolated and characterized; these include the mouse mast cell carboxypeptidase A gene, which is known to be GATA regulated. Other differentially regulated genes include a non-classical histocompatibility gene, and the sex-limited gene, which is related to the C4 complement factor. Several of the cDNA's obtained had homology to known genes: these include the human *tre* oncogene, and a highly homologous fungi gene of unknown funtion. Based on preliminary analysis, we have isolated at least twelve novel cDNA's, including several which are hematopoietic-specific. The differential expression of these genes may result from direct activation by GATA-1, or may be downstream events in the mast cell program. Thus, GATA-1 appears to regulate many genes involved in mast cell development, consistent with its previously determined role in the erythroid lineage.

B 680 INDUCTION OF TERMINAL DIFFERENTIATION AND EFFECTS ON CELL CYCLE REGULATORY PROTEINS IN KERATINOCYTES, K.Hartmut Richter, Petra Rehberger and Friedrich Marks, Department of Tissue Specific Regulation, German Cancer Research Center, W-69 Heidelberg, GERMANY

Primary murine keratinocytes were prepared from neonatal mice acc. to Yuspa et al., fractionated on a Percoll gradient acc. to Fürstenberger et al. and cultured at 34°C in modified MEM with 10% fetal bovine serum containing only 0.06 mM Ca^{2+}. Terminal differentiation was induced by raising the Ca^{2+}-concentration up to 1.2 mM together with a medium change.
Differentiation was followed thereafter by measuring the amount of Coomassie blue stained covalently linked proteins (cornified envelopes) bound to regenerated cellulose and by determining the distribution of the cells in the cell cycle by cytoflow fluorometric measurements. The content of tumorsuppressor p53, $p110^{Rb}$, Cyclin A, Cyclin B, cdk2- and cdc2-kinase was monitored concomitantly by analyzing Western blots of cell lysates with corresponding antibodies by using the ECL method.

More than 80% of the cells accumulated within 30 hours in the G_1-phase of the cell cycle. In parallel to the increase of the G_1-arrested cells the amount of cdk2-, cdc2-kinase, Cyclin A and Cyclin B decreased and was hardly detectable 30 h after increasing the Ca^{2+}-concentration, whereas the content of p53 and $p110^{Rb}$ seemed to remain constant.
A 20mer antisense p53 deoxy-oligonucleotide of the 5'-end of the coding region at a concentration of 10 μM is in use to investigate the involvement of p53 in the process of differentiation. Uptake reached saturation levels after ca. 30 h of treatment with a half life in the medium of about 6 h.
In keratinocyte fractions isolated from neonatal murine skin followed by Percoll gradient centrifugation only the viable cells showed expression of cyclin A, cdk2 and cdc2, whereas the nonviable, differentiated cells did not. In contrast there were no significant differences in the cellular distribution of $p110^{Rb}$ and p53.

B 681 PROMOTER CHARACTERIZATION OF THE HUMAN MIP-1α GENE, Linda M. Ritter*, Margaret Bryans and Neil Wilkie, Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210

Hematopoietic stem cells derived from bone marrow are normally quiescent. They are induced to proliferate in response to as yet unknown signals when peripheral blood white cells are damaged by cytotoxic drugs. Macrophage from quiescent areas of marrow secrete an activity (SCI) which rapidly inhibits cell cycle progression of proliferating stem cells from regenerating bone marrow. Recently, it has been shown that the inflammatory cytokine MIP-1α is a major component of SCI. There is evidence that there is a switch in macrophage expression from SCI to a stem cell stimulatory activity in regenerating bone marrow. It is therefore important to identify the signals which regulate gene expression and secretion of the MIP-1α\SCI protein.

To identify tissue-specific transcription factors and factors involved in the transcriptional activation of the MIP-1α gene in activated macrophage, we have begun a molecular analysis of the huMIP-1α promoter. Promoter sequences have been linked to a luciferase reporter gene, and mutational analyses used to identify regions of the promoter necessary for transcription and increased transcriptional activity after macrophage activation. Probes spanning the promoter have been used in mobility shift assays with nuclear extracts from activated macrophages to identify possible sequence specific DNA binding proteins. The correlation between the results of these experiments have identified several regions containing sites for specific DNA-binding proteins which affect transcriptional activity. One region necessary for transcriptional activation contains a site(s) for macrophage specific factor(s). Methylation interference is being used to identify the specific DNA-binding site for this factor.

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B 682 **REGULATION OF A NOVEL TRANSCRIPT, C-jer IN HL-60 CELLS WITH HOMOLOGY TO THE C_2 REGULATORY DOMAIN OF PKC α** **Jane E. Rosen, Dept. of Physiology and Cellular Biophysics, Columbia University College of Physicians and Surgeons, New York, N.Y. 10032 Present address: The American Health Foundation, Valhalla, New York 10595**

A novel transcript, C-jer, has been identified in HL-60 cells. The gene has not been cloned and is reported here as a unique message of 1.4 kb. Approximately 350 base pairs of C-jer are homologous to the C_2 regulatory domain of the gene coding for PKC α. The plasmid pHPKCα-7 cut with BamHI released a 350 bp fragment which after being random primed to create the cDNA, hybridized to both PKC α (5.7 kb) and to the new 1.4 kb (C-jer) message in HL-60 cells according to Northern analysis. The C_2 regulatory domain of PKC α has been proposed to coordinate the binding of calcium. C-jer mRNA is down regulated after HL-60 induced differentiation with DMSO, PMA, RA and 1,25$(OH)_2$ Vitamin D_3 and is concomitantly down regulated with myeloblastin, a serine protease (aka proteinase 3 or Wegener's autoantigen) mRNA (1.3 kb)(Bories et al., 1989 Cell 59, 959). Myeloblastin mRNA was used as a control for differentiation since it is known to be down regulated after induced differentiation in the presence of DMSO, PMA, RA and 1,25$(OH)_2$ Vitamin D_3. Since growth arrest is always associated with HL-60 cell differentiation, serum starvation has been used to determine whether regulation of myeloblastin and C-jer mRNA is linked to growth inhibition of HL-60 cells through serum growth factors. A concomitant serum dependent up regulation of both myeloblastin and C-jer mRNA was observed after serum starvation. After exposure to the serum growth factors insulin or transferrin a concomitant up regulation of both messages was also observed. The fact that the regulation of C-jer mRNA is coincident with that of myeloblastin mRNA after induced differentiation and after exposure to serum, insulin and transferrin may reflect that C-jer is an important regulatory gene for growth (since myeloblastin is known to be down regulated during growth arrest in HL-60 cells and since the known 350 bp sequence of the pHPKCα-7 plasmid is related to a conserved region in the PKC gene family). Inhibition of myeloblastin mRNA expression with antisense caused growth arrest and induced differentiation of HL-60 cells. The known 350 bp sequence of the pHPKCα-7 plasmid which hybridizes to the 1.4 kb message has no homology to the 800 bp sequence of the myeloblastin gene. The goal is to clone the new gene C-jer, to discover whether calcium regulation by C-jer is critical to its role in the cell and to better understand its function with respect to myeloblastin down regulation during growth arrest in HL-60 cells.

B 683 THE CD18 PROMOTER DIRECTS LEUKOCYTE-SPECIFIC, MYELOID-INDUCIBLE EXPRESSION.
Alan G Rosmarin, David Caprio, Gina Trampe, and Robert Levy, Brown University/The Miriam Hospital, Providence, RI, 02906

CD18 is the ß chain of the leukocyte integrins LFA-1, Mo1, and p150/95. It is expressed exclusively by leukocytes, and CD18 cell surface and mRNA expression increase markedly during myeloid differentiation. CD18 transcription increases during phorbol ester (TPA) and retinoic acid induced myeloid differentiation of HL-60 and U937 cells. We cloned the CD18 gene in order to study the *cis* elements and *trans* acting factors which are responsible for myeloid differentiation. The CD18 promoter, like other leukocyte specific genes, lacks CCAAT and TATA boxes. It contains DNA sequences which correspond to the AP-1 (a TPA responsive element), CREB, and Oct elements. The CD18 promoter also contains potential binding sites for PU.1 - an *ets*-related transcription factor which is expressed exclusively by B lymphocytes and macrophages. We linked the 900 nucleotides upstream of the CD18 transcription initiation site to the luciferase reporter gene and transfected this construct [CD18(-0.9)/luc] into the U937 myeloid cell line. Basal expression from CD18(-0.9)/luc increases 3 to 5 fold following exposure to phorbol esters, suggesting that it contains a functional TPA responsive element. Luciferase expression increases following exposure to retinoic acid and when co-transfected with retinoic acid receptors, suggesting the presence of a retinoic acid response element. CD18(-0.9)/luc is expressed, but not phorbol responsive, following transfection into Raji and Jurkat cells (B and T lymphocyte lines, respectively). It is not expressed following transfection into HeLa (non-hematopoietic) cells. Thus, transfection of the CD18 promoter recapitulates the tissue specific and myeloid inducible expression of the gene *in vivo*. A region of the promoter which directs high level myeloid expression contains the PU sites. By DNAse I footprinting with nuclear extracts from myeloid cells, we have identified binding to the proximal PU site and to a neighboring region which does not contain a recognizable transcription factor binding site. We used the electrophoretic mobility shift assay to evaluate the tissue specificity of binding to the CD18 PU site. CD18 should serve as an important model for defining the *cis* elements and *trans* acting factors which regulate myeloid differentiation.

B 684 IDENTIFICATION OF A NOVEL MEMBER OF THE C/EBP FAMILY OF TRANSCRIPTION FACTORS, Aimee K. Ryan and Roger G. Deeley, Cancer Research Laboratories, Queen's University, Kingston, Ontario, Canada, K7L 3N6

Expression of the avian apoVLDLII gene is liver specific and completely estrogen dependent. Mutational analysis of the promoter identified a region (-231 to -248) that has a major effect on the ability to induce transcription in the presence of an estrogen receptor complex (Schrader et al., in prep.). This site corresponds to a genomic footprint (Site F) occupied in vivo when the gene is active (Wijnholds et al., 1991, Nucl. Acids. Res. 19:33-41). Its sequence does not resemble any known liver enriched or specific factors. Using mobility shift and methylation interference assays, we have identified two protein complexes that can form at this site, one of which is liver enriched. A day 9 embryonic chicken liver cDNA library was screened using concatenated Site F oligonucleotide. One of the isolated clones hybridizes to two mRNA species (approx. 2.3 and 1.8 kb) at moderate stringency (0.1xSSC/52°C), but only to the larger mRNA at higher stringency (0.1xSSC/65°C). Expression of the smaller mRNA is liver specific and increases during development. The larger mRNA is expressed in liver, lung, intestine, spleen, kidney and gizzard. In the liver, expression of the larger mRNA is highest early in embryogenesis (day 7-9) and then gradually declines until day 20 at which point the level of expression has decreased by 75%. Expression of both mRNAs increases upon activation of the apoVLDLII gene with estrogen. The cloned sequence shows significant homology to C/EBPα at the nucleotide and amino acid level (90% identity to the basic domain of C/EBPα). At moderate stringency (0.1xSSC/52°C) the 2.3 kb mRNA is recognized by a portion of rat C/EBPα cDNA encoding the bzip domain but not by the portion which encodes the NH_2-terminal trans-activation domains. Our data suggests that this clone is not the avian homologue of C/EBPα but is a member of the C/EBP family. Studies to address the functional significance of the two closely related mRNAs and the ability of the cloned protein to promote/suppress transcription are underway.

B 685 DNA-PROTEIN INTERACTIONS ON A gPAL2 PROMOTER FRAGMENT WHICH DRIVES DEVELOPMENTALLY REGULATED EXPRESSION IN TOBACCO, Robert W. M. Sablowski, Diane Shufflebottom and Michael Bevan, IPSR Cambridge Laboratory, Norwich, UK, NR4 7UJ.

Phenylalanine-ammonia-lyase (PAL) is the first enzyme in the plant phenylpropanoid pathway, which participates in the synthesis of lignin, pigments and antimicrobial substances. The promoter of one of the bean PAL genes (gPAL2) drives a complex pattern of reporter gene expression in tobacco. Deletion analysis defined a 104 bp fragment of this promoter which drives expression in tobacco xylem and petals. Tobacco nuclear proteins binding to this promoter fragment were detected by EMSA assays. Their corresponding binding sites were determined by methylation interference and binding site mutation experiments. One of these binding activities is petal-specific and binds to a sequence similar to a cis-element involved in UV-induced expression in parsley cells. Another binding activity has a wide tissue distribution and binds to a G-box -like sequence. A probe containing this G-box sequence was used to screen a tobacco cDNA expression library in lambda gt11. Two cDNA clones were isolated which bound specifically to the probe. One of them is highly similar to the tobacco transcription factor TAF-1, whereas the other clone shows no striking homology to any sequence in the EMBL database. The role of these DNA-binding proteins in the control of expression from the gPAL2 promoter is being investigated.

B 686 **TRANSCRIPTIONAL REGULATION OF *C-FMS* GENE EXPRESSION BY GLUCOCORTICOIDS IN BREAST CAR CINOMA CELLS.** Eva Sapi and Barry M. Kacinski, Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT 06510.

Regulation of *c-fms* (CSF-1R) mRNA expression was investigated in breast carcinoma cells. Specifically, we tested whether a transcriptional regulation exists, in addition to a known post-transcriptional regulation of the *c-fms* transcript during monocytic differentiation. The *c-fms* transcript and protein expression in breast carcinoma cell lines is dramatically increased by dexamethasone, a glucocorticoid which is a potent modulator of normal mammary epithelial cell differentiation. Measurement of the *c-fms* mRNA half-life was not significantly different in resting or dexamethasone induced breast carcinoma cells. Analysis of the time-course of *c-fms* mRNA induction by dexamethasone revealed a two-fold increase within 60 min after the addition of the agent. The nuclear run-on transcription assay demonstrated that this early change in mRNA abundance corresponds to changes in transcriptional activity of the *c-fms* gene. The major *c-fms* transcripts arising from different initiation sites were measured by RNase protection assay. The dexamethasone-treatment increased the expression of various *c-fms* transcripts with markedly different kinetics.

Our data indicate that glucocorticoids can influence both the activity and the utilization of *c-fms* promoters. The shift in promoter utilization raises the possibility that binding of a single transcription factor (e.g. the glucocorticoid receptor) to *c-fms* promoters alters their relative activity and utilization in human breast carcinoma cells.

B 687 DIFFERENTIATION-SPECIFIC EXPRESSION OF TRANSGLUTAMINASE TYPE I IN SQUAMOUS DIFFERENTIATING EPITHELIA: REGULATION BY RETINOIDS. Nicholas A Saunders, Susan H Bernacki, Margaret D George and Anton M Jetten. Lab. Pulmonary Pathobiology, NIEHS, RTP, NC, 27709

Although many epithelia can undergo squamous differentiation, the transcriptional events regulating this pathway are unknown. An understanding of the events regulating squamous differentiation can be obtained by studying mechanisms regulating the expression of squamous specific marker genes such as, transglutaminase type I (TGI). Isolation of a genomic clone encoding a functional TGI promoter revealed several putative regulatory elements. A TATA-like element and an SP1 site were found within 100 bp 5' of the transcription initiation site. Putative response elements for c-myc, Ker1/AP2, AP1, AP2 and CK-8-mer were also present. Transfection of rabbit tracheobronchial epithelial (RbTE) cells with a 2.9 kb TGI promoter/luciferase reporter construct resulted in 2.2 fold more luciferase activity in differentiated vs undifferentiated cells. Differentiation-specific activity was localised to a 1.2 kb region containing putative AP1 and Ker1/AP2 sites. The 2.9 kb promoter also exhibited differentiation-specific expression in human epidermal keratinocytes (HEK) induced to differentiate by phorbol ester treatment (8 fold greater than undifferentiated cells). This induction was inhibited 5 fold by bryostatin I. Differentiation-specific luciferase activity of the 2.9 kb construct in RbTE cells was suppressed 82% by RA, a known regulator of squamous differentiation. All deletion mutant constructs were suppressed by RA in RbTE cells as was phorbol ester induced luciferase activity of the 2.9 kb construct (2.5 fold) in HEK cells. These data indicate that response elements mediating differentiation-specific expression and retinoid sensitivity of the TGI gene are contained within the proximal 2.9 kb of the promoter.

B 688 BIOCHEMICAL CHARACTERIZATION OF REGION I OF THE RAT CC10 PROMOTER REVEALS COMPLEX BINDING OF GENERAL AND LUNG SPECIFIC TRANSCRIPTION FACTORS FROM THE AP-1, OCTAMER AND HNF-3 FAMILIES. Patricia L. Sawaya, Charlene D. McWhinney and Donal S. Luse, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0524

The CC10 gene product is the major secretory protein of lung Clara cells, a non-ciliated bronchiolar epithelial cell. DNase I footprinting has demonstrated that nuclear extracts prepared from NCI-H441 (a Clara cell-like human tumor line), HeLa, or rat liver cells bind region I of the rat CC10 promoter (-86/-128) in cell-specific patterns (JBC 267, 14703). By utilizing specific antibodies and competitor oligonucleotides in electrophoretic mobility shift assays, the proteins binding CC10 region I in HeLa extracts have been identified as Oct-1 and members of the AP-1 family, while in rat liver extracts an HNF-3 class factor has been found to have two binding sites within CC10 region I. Proteins from NCI-H441 cells which bind to region I are either considerably enriched in H441 cells or absent altogether in HeLa, rat liver and fetal sheep lung cells. (The fetal sheep lung preparation consisted primarily of alveolar, not bronchiolar cells). An AP-1-like activity present in H441 cells but not in HeLa, rat liver, or fetal sheep lung has been found to bind the AP-1 site within CC10 region I. This same AP-1 activity has been found to bind the promoter of the surfactant protein B gene. An HNF3-like activity greatly enriched in H441 extracts relative to HeLa extracts, and absent in rat liver and fetal sheep lung extracts, binds to one and possibly both of the HNF-3 sites found in CC10 region I.

B 689 **ON THE MOLECULAR MECHANISM OF IL-4 IMMUNOSUPPRESSION,** Edward M. Schwarz and Barry R. Bloom, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461

IL-2 and IL-4 appear to be produced by different functional T-cell subsets, and are reciprocally negatively involved with the different subset. In order to elucidate the molecular mechanism of IL-4 immunosuppression we chose to study its inhibitory effects on an essential T cell growth factor, IL-2. IL-4 pretreatment of Jurkat cells for 24hr followed by stimulation with PMA and ionomycin results in a 4 fold decrease in IL-2 mRNA production, as determined by Northern analysis. The addition of the CD28 second signal eliminates this inhibition. Since the IL-2 gene promotor has been extensively studied, we were able to test the effect of IL-4 on the individual enhancer elements, using β-galactosidase as a reporter. One of these enhancer elements, NFIL-2B, showed a decrease of 50% β-galactosidase activity when the transformed cells were pretreated with IL-4. In electrophoretic mobility shift assays, using a DNA oligomer containing the NFIL-2B binding site, it was determined that pretreatment with IL-4 inhibits the formation of the NFIL-2B complex. The NFIL-2B site has most recently been characterized as merely an AP-1 binding site. Here we show that the NFIL-2B complex contains at least one other factor which is distinct from AP-1 and is inhibited in IL-4 pretreated cells. These data lead us to speculate that IL-4 may inhibit factors required for transcription of genes involved in immune responses.

B 690 INDUCTION OF MHC CLASS I GENES IN RETINOIC-ACID TREATED NTERA2 CELLS: CO-ACTIVATION OF RETINOID RECEPTORS AND P50/P65-NFκB FACTORS, James Segars, Toshi Nagata, Paul Drew, Vincent Bours, Jeff Medin, Jiabin An, Kevin Becker, David Stephany, Ulrich Siebenlist and Keiko Ozato, National Institutes of Health, Bethesda, MD 20892
Human embryonal carcinoma NTera2 cells respond to retinoic acid by differentiation along a neuronal cell lineage. We show that retinoic acid induces expression of major histocompatibility complex (MHC) class I and $ß_2$-microglobin molecules in these cells. This induction was accompanied by increased levels of MHC class I mRNA, which was attributable to an increase in MHC class I enhancer activity. The enhancement was mediated by a conserved upstream regulatory element that contains two enhancers, region I and region II. Mutation of either of the two enhancer elements resulted in a decrease in enhancer activity. This induction of enhancer activity was accompanied by expression of a nuclear factor binding activity specific for region I which was induced following retinoic acid treatment. We show that the induced binding activity results from heterodimeric binding of P50/P65 to the region I enhancer element. Further, P50 mRNA was found to increase after retinoic acid treatment, suggesting that the induction of region I binding activity is caused by activation of the P50 gene. Region II binding activity, although present in undifferentiated cells, was also increased after retinoic acid differentiation. We show that increased region II binding activity consists of the retinoic acid X receptor ß (RXRß) and a second heterodimer partner which was induced following retinoic acid treatment. The increased region II binding was not due to an increase in RXRß mRNA or protein, but rather was caused by retinoic acid induction of the heterodimer partner. These results indicate that the mechanism responsible for induction of MHC class I genes in NTera2 cells upon retinoic acid induced differentiation involves the induction and co-activation of retinoid receptors and NFκB-like nuclear factors.

B 691 INDUCTION OF AP-1 BINDING PROTEINS POSITIVELY REGULATES THE EXPRESSION OF HLA-DRA IN B-CELLS, Niclas Setterblad, B, Matija Peterlin*, and Göran Andersson, Department of Medical Genetics, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden; *Departments of Medicine, Microbiology and Immunology, Howard Hughes Medical Institute, University of California at San Francisco, San Francisco, CA 94143-0724, USA.
We have examined the transcriptional regulation of the human major histocompatibility HLA class II DRA gene during protein kinase C dependent signal transduction in the B-lymphoblastoid cell line Raji. Transcription of the DRA gene is tightly regulated during B cell development. High expression is restricted to mature B cells, whereas DRA expression is repressed in pre-B cells and plasma B cells. Treatment of Raji cells with the phorbol ester 12-O tetradecanoylphorbol 13-acetate (TPA), increased mRNA expression of the DRA gene and the activity of the DRA promoter in transient transfection analyses. Gel mobility shift assays using an oligonucleotide corresponding to the TPA-response element (AP-1 box; TGCGTCA), at position -97 to -91 in the DRA promoter, showed induced AP-1 binding. The induced complex consisted of h-XBP-1/c-Fos heterodimers. Although c-Jun is expressed, c-Jun binding was undetectable in Raji nuclear extracts even after TPA treatment. In order to determine the *cis*-promoter elements involved in the TPA response, transfection assays were performed with wild type and mutated DRA promoter constructs. In these studies, mutation of the AP-1 box caused reduction in DRA expression as well as in TPA induction. Therefore, we conclude that the DRA AP-1 box is a major component for PKC dependent high DRA gene expression and that induced c-Fos-h-XBP-1 complexes may be responsible for this phenotype in Raji B-lymphoblasts.

B 692 MOLECULAR CLONING OF A PROTEIN BINDING TO THE PROMOTER OF Ig Cγ1 GERM LINE IN THE MOUSE, Eva Severinson, Mats Lundgren, Tatsunobu-Ryushin Mizuta, Janet Stavnezer, Tasuku Honjo and Akira Shimizu, Department of Cell and Molecular Biology, Karolinska institutet, Stockholm, Sweden, Center for Molecular Biology and Genetics, Kyoto university, Japan and Department of Molecular Genetics and Microbiology, University of Massachusetts, USA
Resting B lymphocytes activated by mitogens and interleukins divide and differentiate to high rate Ig secreting cells. When cells secrete other classes than IgM, DNA recombination has normally occured, resulting in a juxtaposition of the VDJ region and the CH gene to be expressed. The mechanism of class switching is unknown, but activation of germ line Ig transcripts prior to the recombination event has been implicated as one necessary requirement. It is of importance to identify factors which may regulate the above mentioned transcription.
cDNA from murine B lymphocytes activated by lipopolysaccharide plus interleukin 4 was prepared and cloned into λgt11. The expression-library was screened in southwestern hybridization using a radioactive concatenated probe of 70 bp, corresponding to the minimal promoter of germ line Cγ1 transcripts. One clone out of 7×10^4 were positive. Sequence-analysis indicate that the cDNA encode a protein with zinc-finger motif, with homology to early growth response genes. We are at present trying to isolate full length clones and investigate the expression pattern.

B 693 A METHOD FOR THE IDENTIFICATION OF RETINOID-RESPONSIVE GENES EXPRESSED DURING MOUSE DEVELOPMENT, Mary Shago and Vincent Giguère, Department of Molecular and Medical Genetics, University of Toronto, and Research Institute, Hospital for Sick Children, Toronto, Canada M5G 1X8
Our understanding of the molecular mechanisms of morphogenesis is severely limited by the paucity of genes known to regulate this intricate process. Retinoic acid (RA), a biologically active metabolite of vitamin A, has been implicated as a putative morphogen in mouse development and is a powerful teratogen in mammals. The developmental effects of RA are mediated through interaction with nuclear proteins (referred to as RARs and RXRs) that belong to the superfamily of steroid hormone receptors. Nuclear receptors are transcription factors that control cell functions and developmental processes by regulating gene expression in a ligand-dependent manner. Thus, the interaction between RA and its receptors is believed to induce of a cascade of regulatory events that results from the activation of specific gene networks by the RA-receptor complexes.
In order to identify genes whose expression is under the control of RA during cell differentiation, we have developed a method whereby small genomic DNA fragments that are contained within CpG-rich islands and not associated with bulk inactive chromatin are selected for the presence of RAR/RXR binding sites by electrophoretic mobility shift assay (EMSA). Chromatin present in intact nuclei obtained from F9 cells, a mouse teratocarcinoma RA-responsive cell line, or mouse embryos at different stages of development, is first subjected to digestion with restriction enzymes whose recognition sites contain the dinucleotide CpG. The specific subsets of non-nucleosomal (NN) fragments obtained by this procedure are then isolated from bulk chromatin by gel electrophoresis. NN fragments are incubated with RAR/RXR and DNA-receptor complexes are selected by EMSA. Subsequent to enzymatic amplification, cloned DNA fragments are tested for their ability to confer RA-responsiveness to a basal promoter driving the luciferase reporter gene, using F9 cells as vehicles for transient transfection experiments. The DNA fragments shown to be functional in this assay are then used as probes to clone the genomic sequences surrounding them and, subsequently, larger DNA probes derived from these loci used to search transcripts whose expression is regulated by RA. These genes will be subjected to detailed molecular analysis and their role in development analyzed using reverse genetics.

B 694 THE TRANSCRIPTION FACTOR AP-2 REGULATES TRANSCRIPTION OF U6 snRNA GENES, Anne-Christine Ström and Gunnar Westin, Department of Medical Genetics, Biomedical Center, Box 589, Uppsala University, S-751 23 Uppsala, Sweden

Recently it has been found that the TATA-binding protein (TBP) and the transcription factor Oct-1 are involved in both RNA polymerase II and pol III transcription. We will present evidence that transcription factor AP-2 is also involved in regulation of U6 snRNA (pol III) transcription. The inducible transcription factor AP-2 is involved in the process of signal transduction and cell-specific expression and may play a role in the control of developmentally regulated gene expression. We have found that affinity purified AP-2 binds adjacent to the octamer site in the mouse U6 snRNA gene enhancer. The functional importance of this AP-2 site was demonstrated in a transient expression assay using U6 maxi genes with wild type- and mutated enhancer sequences. Furthermore, preliminary results suggest that U6 maxi gene transcription is TPA-inducible in BJA-B cells and that the induction is dependent on an intact AP-2 site. The presence of an AP-2 binding site in the U6 snRNA gene enhancer might thus be a way for a cell to control transcription of this highly expressed class of RNA polymerase III transcribed genes. The AP-2, TBP and Oct-1 factors are all involved in both pol II and pol III transcription and this points towards a unifying mechanism for eukaryotic transcription initiation.

B 695 INTERACTION BETWEEN TWO CELLULAR SIGNALLING PATHWAYS IN T LYMPHOCYTES MEDIATED BY cAMP REGULATION OF TPA RESPONSIVE ELEMENT (TRE)-BINDING PROTEINS

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Activators of adenylate cyclase or cell permeable cAMP derivatives inhibit T cell activation by interfering with the antigen receptor (TCR)-linked phosphoinositide turnover. We found that dibutyryl cAMP (dbcAMP) also inhibits T cell activation by TPA + Ionomycin which directly activate PKC and bypass the requirement for TCR perturbation and phosphoinositide hydrolysis. dbcAMP inhibited also the TPA + Ionomycin-mediated induction of c-jun but induced increased trans-cription of c-fos, FosB, Fral and Fra2. Homodimers of JUN or hetero-dimers of JUN and FOS or any of the FOS-related proteins function as transcription factors (AP1) that bind unique regulatory DNA sequences termed TPA responsive elements (TRE). To test whether the dbcAMP-mediated inhibition of c-jun transcription is accompanied by a parallel reduction in AP-1 proteins, we performed a gel mobility shift assay using oligonucleotide probes possessing the consensus AP-1 recognition site. We found that dbcAMP, similar to TPA + Ionomycin, induced increased TRE-binding activity that could be blocked by anti-JUN-B, JUN-D and FOS Abs. Recent data have indicated that members of the JUN family differ with regard to their regulatory functions and that JUN-B inhibits c-JUN-mediated activation of genes that possess TRE-containing promoters. In our system dbcAMP increased the transcription of junB. Thus, binding of JUN-B/FOS heterodimers to the AP1 binding site on the c-jun promoter may suppress c-jun transcription even in the presence of TPA + Ionomycin. The data suggest that increased cAMP levels inhibit early activation genes possessing the AP-1 recognition site, apparently, induction DNA binding protein that inhibit transcryption- regulation via the TRE.

B 696 ANALYSIS OF THE RETINOIC ACID RECEPTOR-ß.

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Molecular Medicine Unit, Beth Israel Hospital, Boston, MA 02215

Retinoic acid receptor-ß (RAR-ß) is a retinoic acid (RA) dependent trans-acting transcription factor, which belongs to the steroid-thyroid hormone receptor superfamily. The molecular details of signal transduction involving RAR-ß remain obscure. To approach this question, we have raised and affinity-purified poly-clonal antibodies to the N- and C- termini of RAR-ß, respectively. These antibodies specifically recognize both overexpressed and endogenous murine or human RAR-ß. Using these antibodies, we have identified RAR-ß as a 55kDa serine phosphoprotein in immunoprecipitation and immunoblotting assays. Immuno-precipitates of RAR-ß run as a doublet on sodium dodecyl sulfate polyacrylamide gels. Alkaline phosphatase treatment converts the doublet to a single, more rapidly migrating species, indicating that the posttranslational modification occuring is phosphorylation. Pulse-chase analysis reveals that RAR-ß is initially synthesized in an underphosphorylated state, then is rapidly phosphorylated to a slower migrating species. Moreover, RAR-ß appears to be relatively unstable, with a half-life of approximately fifteen minutes, at least in NIH-3T3 cells stably overexpressing the protein. Cyanogen bromide cleavage and phosphotryptic peptide analysis show that RAR-ß is phosphorylated at multiple sites; currently these techniques are being used to determine if phosphorylation is regulated by RA or other agents.

B 697 TRANSCRIPTION FACTORS PU.1 AND Sp1 ARE ESSENTIAL FOR MYELOID SPECIFIC EXPRESSION OF CD11b, Daniel G. Tenen, Heike L. Pahl, Hui-min Chen, Dong-Er Zhang, Richard A. Maki, and Renate J. Scheibe, Division of Hematology/Oncology, Beth Israel Hospital, Harvard Medical School, Boston, MA 02215

The myeloid cell surface marker CD11b is expressed exclusively on mature monocytes, macrophages, granulocytes and natural killer cells, and is transcriptionally upregulated during retinoic acid-induced differentiation of myeloid cells. We have recently demonstrated that 1.7 kb of 5' flanking DNA constitute a functional CD11b promoter which directs myeloid-specific expression of reporter genes in both tissue culture and transgenic mice. Deletion analysis shows that a promoter containing only the first 92 bp of 5' flanking DNA, although less active than the 1.7 kb fragment, still retains complete tissue specificty in transient transfection assays; the same 92 bp fragment contains the element responsible for retinoic acid inducibility. Here we show that the proto-oncogene and macrophage transcription factor PU.1 binds the 92 bp CD11b promoter *in vitro* and *in vivo*, and that mutation of the PU.1 site reduces CD11b promoter activity 4 fold in myeloid cells, but does not effect low level expression in non-myeloid cells. This is the first report of a macrophage target for PU.1. Although PU.1 is thus a major determinant of the myeloid specific expression of CD11b, the promoter still retains significant activity after mutation of the PU.1 site. Binding of a second transcription factor, Sp1, is essential for CD11b promoter activity. Mutation of the Sp1 site at bp -60 reduces promoter activity 20 fold. Both EMSA and *in vitro* footprinting show that the ubiquitous transcription factor Sp1 binds the CD11b promoter when nuclear extracts from either U937 or HeLa cells are used. However, *in vivo* footprinting demonstrates that Sp1 binds specifically in myeloid cells (U937), not in non-myeloid cells (HeLa) *in vivo*. Thus, both PU.1 and Sp1 represent important determinants of the myeloid specific expression of CD11b. Furthermore, since there is no retinoic acid receptor binding site in the 92 bp CD11b promoter, these or other factors binding in this region appear to mediate the retinoic acid-induced upregulation of CD11b during the course of myeloid maturation.

B 698 DIFFERENTIAL SPLICING OF THE *GHF1* PRIMARY TRANSCRIPT GIVES RISE TO A GHF-1 ISOFORM WITH A PROMOTER-SELECTIVE ACTIVATION DOMAIN,

Lars E. Theill[1], Kazue Hattori[2], José-Luis Castrillo[2], and Michael Karin[2], [1]Department of Cell and Molecular Biology, Amgen, Thousand Oaks, CA 91320 and [2]Department of Pharmacology, Center for Molecular Genetics, School of Medicine, University of California at San Diego, La Jolla, CA 92093-0636

The POU-domain protein GHF-1 has a critical role in generation, proliferation, and phenotypic expression of three pituitary cell types. GHF-1 functions in part by binding to and transactivating the promoters of both the growth hormone (*GH*) and prolactin (*PRL*) genes and that of the *GHF1* gene itself. We describe a naturally occurring isoform of GHF-1, GHF-2, in which additional 26 amino acids are inserted into the activation domain of the protein as a result of alternative splicing. GHF-2 retains the DNA-binding activity of GHF-1 and can activate the *GH* promoter but has lost the ability to activate the *PRL* and *GHF1* promoters. These results suggest that GHF-2 may function in differential target gene activation during differentiation of the somatotrophic lineage. Both GHF-1 and GHF-2 transcripts are specifically expressed in the anterior pituitary. Analysis of the genomic *GHF1* gene shows that most of the distinct functional domains of GHF-1 (and GHF-2) are encoded by separate exons. Gene segment duplication and exon shuffling may have contributed to the evolution of this cell type-specific transcriptional regulatory gene.

B 699 **DISSOCIATION BETWEEN CELL PROLIFERATION AND *c-myc* EXPRESSION IN PHYSIOLOGICALLY INDUCED HYPERPLASIA IN BROWN ADIPOSE TISSUE,**

Petr Tvrdik[1,2], Anders Jacobsson[1], Dipnady Sugiaman[1], Josef Houstek[2] and Jan Nedergaard[1], [1]The Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, S-106 91 Stockholm, Sweden; and [2]Institute of Physiology, Czechoslovak Academy of Sciences, Prague.

In order to investigate the significance of proto-oncogene expression for physiologically induced cell proliferation, *c-myc* and *c-fos* mRNA levels were examined in brown adipose tissue. Onset of cell proliferation in brown adipose tissue was induced by exposing mice to cold. We found a large (> 30-fold) but transient increase of the *c-fos* mRNA level 30 min after the mice had been transferred to the cold. In contrast, there was no significant change in the *c-myc* mRNA level. As cold-induced cell proliferation in this tissue has been demonstrated to be regulated via ß-adrenergic receptors, the effect of injection of mice with adrenergic agents was examined. In mice injected with norepinephrine, the mRNA levels of both oncogenes was elevated: *c-fos* about 100-fold with a maximum after 30 min and *c-myc* about 3-fold after 1.5 h. However, pharmacological analysis revealed that the *c-myc* response was mediated via α-adrenergic receptors. The pharmacological characteristics of the norepinephrine-induced *c-myc* expression were thus different from those of norepinephrine-induced cell proliferation. Furthermore, in cell cultures of brown-fat cell precursors, norepinephrine and 8-Br-cAMP (which have been demonstrated to induce cell profileration in these cultures) could not induce *c-myc* expression, whereas the protein kinase C activator TPA was able to induce *c-myc* expression. – We conclude that an enhanced *c-myc* level is not a regulatory step during physiologically induced cell proliferation in brown adipose tissue and that physiologically induced cell proliferation in this tissue does not proceed via stimulation of a protein kinase C dependent pathway. Thus, an enhanced *c-myc* expression is not necessarily a mediator in conditions of physiological induction of cell proliferation.

B 700 **REGULATION OF AFP TRANSCRIPTION IN THE MOUSE SMALL INTESTINE.**

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In the fetal mouse, transcription of α-fetoprotein (AFP) is activated in the yolk sac, liver and intestine. In the intestine, highest levels of expression are detected shortly before birth when most epithelial cells lining the small intestine express AFP mRNA. In the adult, AFP expression is found only in enteroendocrine cells that represent less than 1.0% of the total epithelial cells. We have investigated the mechanisms underlying repression of AFP transcription in the remainder of the intestinal epithelial cell types by examining expression of AFP minigene constructs in transgenic mice. We have found that repression occurs in a temporal gradient along the length of the intestine from the duodenum to the ileum, with expression decreasing first in most epithelial cells of the duodenum. Deletion of cis-acting sequences that lie between -838 and -250 of the AFP gene results in high level AFP minigene transcription in additional intestinal epithelial cells in adult animals, primarily of the goblet cell lineage. This suggests that cis-acting sequences not included within the -838 to -250 deletion and still present in the minigene, may mediate repression in the absorptive cell lineage, the major epithelial cell type. Alternatively, absorptive cells may lack the positive factors that are necessary for directing AFP transcription in the absence of repressor binding. We have used in situ hybridization to determine if expression of the HNF-1 transcription factors that regulate AFP transcription is restricted to specific cell types in the gut. HNF-1α and β are expressed in multiple cell types along the length of the intestine, with highest levels in the intestinal crypts where proliferation and cell differentiation take place. Thus, HNF-1 activity does not appear to be responsible for the cell type specific expression of the AFP gene and minigenes in the small intestine.

B 701 TRANSCRIPTIONAL REPRESSION OF BAND 3 AND CAII IN V-*ERBA* TRANSFORMED ERYTHROBLASTS ACCOUNTS FOR AN IMPORTANT PART OF THE LEUKEMIC PHENOTYPE,

Sally Fuerstenberg[1], Irene Leitner[2], Gunilla Wahlström[1)], Christian Schroeder[2], Björn Vennström[1] , and Hartmut Beug[2]. [1)]Department of Molecular Biology, Karolinska Institute, Box 60400, Stockholm, [2)] Institute for Molecular Pathology, Dr. Bohrgasse 7, Vienna.

The v-*erbA* oncogene confers two prominent properties to transformed erythroblasts: a block of spontaneous differentiation and tolerance to wide variations in the pH or ionic strength of culture medium. V-*erbA* acts as a constitutive repressor of erythrocyte-specific gene transcription, arresting the expression of least three different erythroid genes: the erythrocyte anion transporter (band 3), carbonic anhydrase II (CAII), and δ-aminolevulinate synthase (ALA-S). To test whether or not the v-*erbA* induced repression of these genes is causally related to the v-*erbA* induced leukemic phenotype, we have reintroduced the genes for band 3 or CAII into transformed erythroblasts via retrovirus vectors. We show here that such erythroblasts, expressing v-*erbA*, require the same narrow range of medium pH and ion concentration for growth as do transformed erythroblasts lacking v-*erbA*, i.e. the v-*erbA* induced tolerance to pH variation was abrogated. The v-*erbA* induced differentiation block, however, remained unaffected by the re-expression of band 3 and was only slightly affected by the re-expression of CAII. Our experiments show that the two v-*erbA*-related "erythroblast transformation parameters" are separable: suppression of band 3 and CAII accounts for one parameter (pH/ion tolerance), while the second parameter (differentiation block) must involve v-*erbA* regulation of a different set of target genes.

We next studied the heterodimerization partners with which the v-erbA protein associates to achieve transcriptional repression in the erythroblasts. Nuclear extracts from transformed cells were analyzed by a novel "shift-western" technique, a combination of the gel retardation and Western blotting techniques. The results sugggest that intranuclear v-erbA protein is associated with at least 2 distinct proteins belonging to the RXR group of nuclear receptors.

B 702 **PROTEIN FACTORS THAT INTERACT WITH AND REGULATE TRANSCRIPTIONAL ACTIVATION BY E47 HELIX-LOOP-HELIX PROTEIN, Anna Voronova and Frank Lee DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304-1104**

The protein products of the E2A gene, E47 and E12, are members of the family of B-HLH transcription factors which are involved in cell growth control and carcinogenesis, neurogenesis, sex determination, muscle differentiation, and pancreatic expression. Furthermore their cognate DNA sequence motif CANNTG (E box) is present in the regulatory regions of a variety of genes. The E12 and E47 proteins form both homo- and heterodimers which recognize E box motif present in the enhancers of the immunoglobulin genes. Enhancer-binding activity of E47 results in the transcriptional activation of the immunoglobulin locus and plays a critical role in B lymphocyte development. E12 and E47 form heterodimers with myogenic HLH proteins, MyoD and myogenin, and are necessary for induction of myogenesis. We investigated the nature of the *in vivo* oligomeric interactions of E12/E47 proteins in various cell types as to elucidate the involvement of E47 in the developmental pathways of other lineages. Electrophoretic mobility shift assay (EMSA) in conjunction with analysis using specific antisera revealed heterodimers between E12/E47 molecules and another B-HLH protein, *tal*-1. The ability of *tal*-1 to heterodimerize with E47 *in vitro* was previously reported. We now demonstrate the presence of E2A/*tal*-1 heterodimers in myeloid cells including mast cells and monocyte cells, as well as in some T-ALL. Although *tal*-1 gene is not normally expressed in T lymphocytes, it is rearranged and transcriptionally activated in 30% of human T-ALL. We examined whether *tal*-1 regulates transcriptional activation by E47*in vivo*. Transient expression and CAT assays demonstrated the inhibition of the E47 trans-activation by *tal* -1 *in vivo*. Interaction of other cellular proteins with E47 resulting in modulation of transcriptional activity by will be presented.

B 703 *Abstract Withdrawn*

B 704 **Tissue Specific Regulation of the Human Phenylalanine Hydroxylase Gene, Wang, Y., Hahn, M. T., Tsai, S. and Woo, S. L. C., Howard Hughes Medical Institute and Department of Cell Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA.**

Human hepatic phenylalanine hydroxylase (PAH) is specifically expressed in liver where it catalyzes the hydroxylation of phenylalanine to tyrosine. A deficiency of this enzyme results in Phenylketonuria (PKU), which can cause mental retardation among affected children. Human PAH gene has a TATA-less promoter with multiple transcriptional initiation sites. An approximately 9 kilobase DNA fragment from 5' end of the human PAH gene is sufficient to confer tissue- and development-specific expression to a reporter gene in the transgenic mice. In transient expression assays, a 120 bp promoter region still retains the basal hepatic specific activity, while the deletion of the PAH gene promoter to -54 bp can abolish its basal activity in hepatoma cells. Two protein binding domains were identified within the region from -120 bp to -54 bp, including a CAGGTGACCTG palindromic sequence and a direct repeat with CCCTCCC motif. The initial characterization of the protein factors interacting with these *cis* elements indicated that the palindromic sequence was recognized by an ubiquitous protein while the direct repeats interacted with multiple factors. These results suggest that the tissue specific expression of the human PAH gene is at least in part regulated by its basic promoter involving multiple *cis*- and *trans*-acting regulatory elements.

B 705 **SCREENING OF AN EXPRESSION LIBRARY WITH ^{32}P-LABELED CREB PROTEIN IDENTIFIES cDNAs THAT ENCODE INTERACTING PROTEINS.**

Andreas Wechsler, Cox Terhorst, Kenneth P. LeClair, Division of Immunology, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

Research has shown that protein transcription factors modulate eukaryotic gene expression by binding to specific regulatory DNA elements in gene enhancers and promoters. Transcription factor proteins possess multiple structural elements that confer DNA binding function as well as the ability to interact with other proteins. These protein-protein interactions may play a role in the regulation of the function of a given transcription factor. One key transcription factor known to be expressed in different tissues at different times is the cAMP response element binding protein (CREB). The CREB protein, along with other transcription factors, plays an important role during T-cell development by controlling expression of genes encoding T-cell specific proteins, such as CD3ε, TCR-α and -β.

As an approach to study interactions between the CREB protein and other proteins, human CREB was produced in a bacterial expression system as a fusion protein with 2 additional epitopes. The 15 amino acid fragment added to the CREB amino terminus contains an antigenic epitope as well as a specific substrate for heart muscle kinase, allowing ^{32}P-labeling by *in vitro* phosphorylation. This modified CREB protein was purified by immunoaffinity chromatography and was shown to bind the CRE DNA element in gel shift analysis. Also, use of the ^{32}P-labeled CREB as molecular probe for Western blotting demonstrated that CREB forms homodimers and heterodimerizes with a differential splicing product of the CREB gene, termed CREB.B. Additionally, a limited number of other bands are seen after probing cellular extracts with the ^{32}P-labeled CREB protein.

When a λgt11 expression library was screened with the CREB protein probe, 6 distinct cDNA fragments were isolated. Preliminary results from sequencing one of these cDNAs reveals a nucleotide sequence distinct from CREB with many 3rd position codon changes but encoding a stretch of protein identical to CREB. Further characterization of this potentially novel CREB-related cDNA and of the other cDNAs encoding interactive domains is in progress.

B 706 BOTH POSITIVE AND NEGATIVE-ACTING 5' ELEMENTS REGULATE IL-4 GENE TRANSCRIPTION IN MURINE MAST CELLS, Deborah L. Weiss, David Tara and Melissa A. Brown, Departments of Medicine and Microbiology-Immunology, Oregon Health Sciences University, Portland, OR 97201.

Interleukin 4 (IL-4) is a key immunoregulatory cytokine which exerts its effects on a variety of hematopoietic cells. Originally defined as a T cell-derived cytokine, it is clear that cells of the mast cell-basophil lineage are also important sources of IL-4. IL-4 has a major role in regulating B cell production of IgE and is a mast cell growth factor. Thus, mast cell production of IL-4 may be a dominant influence in allergic reactions in which high IgE titers and high mast cell numbers are found. An increase in the transcriptional activity of the IL-4 gene was previously demonstrated in ionomycin-stimulated, growth factor-dependent mast cell lines. To identify 5' *cis* and *trans* acting factors that regulate IL-4 transcription in activated mast cells, a series of 5' deletion mutants of an IL-4/CAT reporter gene fusion construct were tested in transiently transfected, ionomycin-stimulated CFTL 15 mast cells. These experiments defined two functionally important regions. One region, between -87 and -70, is required for inducible expression of IL-4. This is the same region that is critical for IL-4 expression in activated T cells. Mobility shift assays demonstrated that nuclear extracts from unstimulated and stimulated mast cells contain proteins which specifically bind this region. Activated cell extracts contain an additional stimulation-dependent DNA -protein complex of lower mobility. Interestingly, all mast cell complexes showed marked differences in mobility compared to those observed using T cell extracts. These results suggest that either different proteins or differentially modified proteins interact with the IL-4 inducible response element in T and mast cells. In addition, a strong mast cell-specific negative regulatory element is located 5' of -306. An oligonucleotide spanning -326 to -302 forms similar specific DNA-protein complexes with nuclear extracts from unstimulated and stimulated mast cells. No such specific interaction was observed with T cell extracts. Taken together, these data support the hypothesis that T and mast cell production of IL-4 is differentially regulated.

B 707 IDENTIFICATION OF THE ENHANCER/SILENCER ELEMENTS OF THE SCAVENGER RECEPTOR GENE THAT IS REGULATED BY TPA

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Transcription factors that control gene networks during terminal differentiation of the macrophages have been poorly characterized. To address this problem, we are studying the mechanisms regulating the expression of the macrophage scavenger receptor, which is markedly upregulated during the final stages of macrophage development. Studies using the monocytic cell line, THP-1, have demonstrated that the phorbol ester, TPA induces macrophage differentiation and maximally stimulates scavenger receptor gene transcription 16hr after treatment. Three functional elements have been identified that regulate scavenger receptor gene expression in transient transfection experiments. Promoter sequences (-696-+46bp) are sufficient for tissue-specific expression of the scavenger receptor in monocyte/macrophage cells, but confer only a fraction of maximal TPA-dependent induction. A silencer element, located more than 3.5 kb upstream of the transcription start sites, suppresses the basal promoter activity by 70%-80%. An enhancer element containing two AP-1 consensus sequences resides between the silencer and the promoter elements and is required for maximal transcriptional activation by TPA. Deletion of either of the AP-1 sites within the enhancer abolishes the TPA responsiveness. Multiple cis-active elements that are widely spaced are therefore required for appropriate control of the scavenger receptor gene. These studies should provide further insights into mechanisms responsible for macrophage-specific responses to protein kinase C activation.

B 708 EFFECT OF 9-CIS RETINOIC ACID ON HUMAN PRIMARY MELANOCYTES AND METASTATIC MELANOMA CELLS. Douglas T. Yamanishi, Edward A. Wagner, Joel E. Voboril, Richard A. Heyman[2], Marcus F. Boehm[2], and Frank L. Meyskens Jr., Clinical Cancer Center, U.C. Irvine, Irvine, CA 92717 and [2]Ligand Pharmaceuticals Inc., San Diego, CA 92121

Our previous studies have determined that high doses of all-trans retinoic acid (tRA, ID50 > 1 μM) or 13-cis retinoic acid (13-cis RA, ID50 > 1 μM) were required to inhibit the growth of human metastatic melanoma cells. We have determined that the basal and tRA-induced RNA transcript expression levels of the retinoic acid receptors (RAR) did not correlate to the cell growth studies obtained with tRA or 13-cis RA. These studies would suggest that another retinoic acid metabolite may be involved in the growth inhibition of human melanoma cells. We investigated the effect of 9-cis retinoic acid (9-cis RA) on cell growth in primary melanocytes and metastatic melanoma cell strains using cell proliferation assays. Primary melanocytes were slightly stimulated following treatment with 9-cis RA (10^{-10} - 10^{-6} M). However, we observed a decrease in cell proliferation following 9-cis RA treatment in three of four melanoma cell strains (ID50 ≈ 10^{-7} M). The other melanoma cell strain was only slightly inhibited (<30% for all doses). Current studies are underway to determine the basal and 9-cis RA induced RNA transcript expression levels of the RXR isotypes in human primary melanocytes and metastatic melanoma cells.

B 709 ***Drosophila*** **Ultraspiracle Modulates Ecdysone Response via Heterodimer Formation**

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The vertebrate retinoid X receptor (RXR) has been implicated in the regulation of multiple hormonal signalling pathways through the formation of heteromeric receptor complexes which bind DNA with high affinity. We now demonstrate that ultraspiracle (usp), a *Drosophila* RXR homologue can substitute for RXR in stimulating the DNA binding of receptors for RA, T3, VitD and peroxisome proliferator activators. Ultraspiracle has been shown to be a pleiotropic factor which is required at multiple developmental stages and tissues. The pleiotropic phenotype of usp mutant may reflect its ability to interact with multiple factors which are important in the *Drosophila* development. These observations led to the search and ultimate identification of the ecdysone receptor (EcR) as a *Drosophila* partner of usp. Together, usp and EcR bind DNA in a highly cooperative fashion and the heterodimer shows a DNA binding specificity correlated well with the ecdysone response in vivo. Cotransfection of both EcR and usp expression vectors is required to render cultured mammalian cells ecdysone responsive. These results implicate the functional ecdysone receptor is a binary complex consisting of the products of both *EcR* and *usp* locus and offers a molecular mechanism by which the complex ecdysone response can be achieved. By demonstrating that receptor heterodimer formation precedes the divergence of vertebrate and invertebrate lineages and its involvement in the important aspects of the animal development and homeostasis, these data underscore a central role for RXR and its homologue usp in the evolution and control of the nuclear receptor based endocrine system.

B 710 C-FMS DEPENDENT HL-60 CELL DIFFERENTIATION AND REGULATION OF RB GENE EXPRESSION, Andrew Yen, Mary E. Forbes, Mark L. Tykocinski, Richard K. Groger, and Joseph D. Platko, Cancer Biology Laboratory, Department of Pathology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

The dependence of induced myelo-monocytic cell differentiation, and regulation of the RB tumor suppressor gene during this process, on the c-fms gene product, the CSF-1 lymphokine receptor, was studied in HL-60 promyelocytic leukemia cells. Adding a monoclonal antibody with specificity for the c-fms gene product to cells treated with various inducers of myelo-monocytic or macrophage differentiation, including retinoic acid and 1,25-dihydroxy vitamin D3, inhibited the rate of differentiation. During the period of inducer treatment usually preceding onset of differentiation, longer periods of antibody exposure caused greater inhibition of differentiation. In a stable HL-60 transfectant over-expressing the CSF-1 receptor at the cell surface due to a constitutively driven c-fms trans gene, the rate of differentiation was enhanced compared to the wild type cell, consistent with a positive regulatory role for the CSF-1 receptor. The anti-fms antibody caused much less inhibition of differentiation in the transfectants than in wild type cells, consistent with a larger number of receptors causing reduced sensitivity. During the induced metabolic cascade leading to differentiation, the typical early down regulation of RB gene expression was inhibited by the antibody. The antibody itself caused an increase in RB expression per cell, which offset the decrease normally caused by differentiation inducers (1,25-dihydroxy vitamin D3 and retinoic acid). Thus the metabolic cascade leading to myelo-monocytic differentiation of HL-60 cells appears to be driven by a function of the c-fms protein. Inhibiting that process by attacking this receptor impedes differentiation as well as compromises the down regulation of RB tumor suppressor gene expression which normally precedes differentiation. These findings provide additional support for a potential role for down regulating RB expression in promoting cell differentiation, and suggest the possibility that RB may be either a target or intermediate mediator of CSF-1 actions.

B 711 HORMONE-DEPENDENT GROWTH INHIBITION OF ESTROGEN RECEPTOR-TRANSFECTED HUMAN MAMMARY EPITHELIAL CELLS REQUIRES THE DNA BINDING DOMAIN OF THE RECEPTOR, Deborah A. Zajchowski and Lynn Webster, Department of Cell Biology and Immunology, Berlex Biosciences, Alameda, CA 94501

Constitutive expression of the cloned human estrogen receptor (ER) in ER-negative immortal, non-tumorigenic (184B5) as well as tumor-derived human mammary epithelial cells (21T and MDA-231) enables estrogen-responsive gene expression [1]. Estrogen treatment also results in cell cycle perturbation and marked growth inhibition. Postulated mechanisms for such an anti-proliferative effect include: (a) direct interaction between the estrogen-bound ER and target genes to induce expression of inhibitory proteins or block expression of growth enhancing factors; (b) indirect inhibition of growth-related gene expression by ER competition for ancillary transcription factors essential for the transcription of those genes ("squelching"). Since ER binding to Estrogen Responsive Elements in specific genes is required for mechanism (a), 184B5 cell lines that stably express ER mutants in the DNA binding domain (HE27, HE34, [2,3]) were constructed. These cells were not inhibited by estrogen treatment even though receptor levels were comparable to those found in growth-inhibited wild-type ER transfectants and ER nuclear localization was verified. On the contrary, ER mutants which lack the N-terminal A/B domain (HE19) were inhibited to the same extent as wild-type transfectants, while cells expressing ER with C-terminal deletions of the hormone binding domain (HE15) showed no change in growth rate. These data suggest that the negative growth regulatory effect of estrogen-bound receptors is mediated by DNA interaction and that the transcriptional activation function in the A/B domain is not essential.

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Phosphorylation of Transcription Factors

B 800 Ras-dependent transcriptional activation of NF-IL6

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NF-IL6 was initially identified as a nuclear factor binding to a 14 bp palindromic sequence(ACATTGCACAATCT) within an IL-1 responsive element in the human IL-6 gene. The cloned NF-IL6 contained a region highly homologous to the C-terminal portion of C/EBP. NF-IL6 recognizes the same nucleotide sequences as C/EBP. In contrast to the tissue-specific expression of C/EBP, NF-IL6 is an inducible and ubiquitously expressed transcriptional activator. NF-IL6 is also known as LAP, AGP/EBP, IL-6DBP, rNFIL-6, and C/EBPβ. In this study we demonstrated that the transactivating activity of NF-IL6 is stimulated by coexpression of oncogenic ras. Recently the Ras protein has been implicated in signal transduction pathways mediated by tyrosine kinases and functions upstream of the serine/threonine kinases such as MAP kinases and c-Raf. In fact, we noticed that NF-IL6 harbors the optimal consensus sequence for MAP kinases. The mutation of the sequence resulted in loss of the ras-dependent activation of NF-IL6. Taken together, these findings suggest that the activation of NF-IL6 is mediated through phosphorylation by ras-regulated MAP kinases.

B 801 ROLE OF THE SEQUENTIAL PHOSPHORYLATIONS OF CREB IN THE REGULATION OF ITS TRANSCRIPTIONAL RESPONSE TO cAMP, J.S.Williams, C.J. Fiol, P.J. Roach and O.M. Andrisani,Dept. of Vet. Physiology,Purdue University and Dept. of Biochemistry & Mol. Biology, I.U. School of Medicine, Indianapolis, IN

The transcriptional activity of the cAMP-response-element binding protein CREB is regulated by the cAMP-dependent protein Kinase A. Phophorylation occurs at $Ser_{133/119}$ of $CREB_{341/327}$, respectively. We observed that phosphorylation by PKA is required for a secondary phosphorylation of CREB by the Glycogen Synthase Kinase-3. Based on the recognition determinants for GSK-3 phosphorylation, -SXXXS(P)-, Ser-115/129 of CREB was predicted as the target for GSK-3. A peptide was synthesized containing these phosphorylation sites. Ser-115 in the model peptide was phosphorylated by GSK-3 but again only after previous phosphorylation of Ser-119 by PKA. Similarly, employing recombinant $CREB_{341/327}$ proteins it was shown *in vitro* that sequential phosphorylation at the GSK-3 site of the native CREB, requires prior phosphorylation *in vitro* by PKA.

To assess the functional role of the sequential phosphorylation of CREB *in vivo*, we constructed CREB mammalian expression vectors, containing Ser → Ala substitution of at the GSK-3 site. The mammalian expression vectors encode a chimeric CREB protein fused to the DNA binding domain of $Gal4_{(1-147)}$. Two types of chimeric proteins were constructed: 1) the $Gal4_{1-147}$ portion was fused to the NH_2-terminus of CREB and 2) the $Gal4_{1-147}$ replaced the bZip of CREB. The indicator plasmid is the somatostatin promoter (+50 to -750), containing the Gal4 binding site in place of the CRE motif. Transient transfection assays in PC12 cells of either expressor construct show that the $CREB_{341}$ mutant containing Ser → Ala substitution at the GSK-3 consensus site is transcriptionally inactive in response to forskolin. The corresponding $CREB_{327}$ mutant retains ≈ 30% of its transcriptional activity. *In vivo* labeling studies employing S^{35}-methionine confirmed the synthesis of the CREB-Gal4 proteins. These results support the importance of the sequential phosphorylations of CREB in its transcriptional response to cAMP, in PC12 cells.

B 802 PHOSPHORYLATION OF PU.1 CAN CONTROL PROTEIN INTERACTION, DNA BINDING, AND TRANSCRIPTIONAL ACTIVATION BY NF-EM5, Michael L. Atchison[1], Sujatha Nagulapalli[1], Charles Van Beveren[2], Michael J. Klemsz[2], Richard A. Maki[2], and Jagan M. R. Pongubala[1]. [1]Department of Animal Biology, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA 19104, [2]Cancer Research Center, La Jolla Cancer Research Foundation, La Jolla, CA 92037

PU.1 is an ets-related transcription factor that binds to the immunoglobulin kappa (Ig_κ) 3' enhancer (κE3'). Binding of PU.1 to the κE3' enhancer enables the binding of a second B cell-specific factor, NF-EM5. Binding of NF-EM5 to the enhancer requires protein-protein interaction with PU.1 as well as specific DNA contacts. We show here that phosphorylation of PU.1 is necessary for interaction with NF-EM5. PU.1 prepared by in vitro translation is capable of interacting with NF-EM5, but not PU.1 produced in bacteria. In addition, dephosphorylated PU.1 can bind to DNA but is incapable of interacting with NF-EM5. However, treatment of bacterially made PU.1 with purified casein kinase II modified it to a form capable of interacting with NF-EM5. Analysis of serine to alanine mutations in PU.1 indicated that serine 148 is crucial for protein-protein interaction. This serine residue is phosphorylated in vivo and lies within a consensus sequence for casein kinase II. To determine whether PU.1 interaction with NF-EM5 is important for enhancer activity in vivo, transfection experiments were performed with plasmids expressing either the wild-type PU.1 cDNA or the serine 148 mutant form. Wild-type PU.1 increased expression of a reporter construct containing the PU.1 and NF-EM5 binding sites nearly 6 fold, while the serine 148 mutant form only weakly activated transcription. In addition, a reporter containing the PU.1 binding site, but lacking the NF-EM5 binding site was only weakly activated by the wild-type PU.1 protein. These results demonstrate that phosphorylation of PU.1 at serine 148 is necessary for interaction with NF-EM5 and suggest that this phosphorylation can regulate transcriptional activity.

B 803 CHARACTERIZATION AND PHOSPHORYLATION PROPERTY OF THE HUMAN GCF, A TRANSCRIPTIONAL REGULATOR OF THE EGF RECEPTOR GENE.
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The human EGF Receptor (EGF-R) is a proto-oncogene whose over-expression is implicated in the development of several types of human tumors. Transcriptional regulation of EGF-R gene is complex. EGF-R promoter binds several transcriptional activators, like Sp1 and ETF, and one repressor protein named GCF, cloned for its ability to bind to EGF-R promoter GC rich sequences and shown to repress its transcription *in vitro*. GCF is composed of a basic amino-terminal region which constitute the DNA binding region, two leucine-zipper motifs and contains several putative nuclear localization signals and potential phosphorylation sites. To characterize GCF protein and to address its role we have developed antibodies against a bacterial expressed GCF-fusion protein. GCF antibodies recognize GCF synthetized *in vitro* and in cell extracts from human cells. In cell extracts, the major form of GCF has a molecular weight of approximately 97Kdalton. In addition other less abundant species with a slightly higher apparent molecular weight are specifically recognized, suggesting extensive post-translational modification. GCF shows a wide variety of expression in cultured human cells and human tissues and is present both in EGF-R negative human cell lines (HUT-102, U266) and in lower amounts in EGF-R expressing cells (KB, A431, AGS). GCF is a stable protein with a relatively long half-life. Cell fractionation studies indicate that the major form of GCF is localized in the nucleus and the association with the nuclear fraction is quite stable. In addition, GCF is a phosphoprotein and the phosphorylated form is associated mainly with the nuclear compartment both in HUT-102 and KB cells. Phosphorylation occurs on serine and threonine residues and is stimulated by Okadaic acid, PMA and cAMP but not vanadate or EGF. Interestingly, PMA and Okadaic acid effects are not additive suggesting stimulation of a similar pathway. Studies will be presented on the role of different cellular kinases in GCF phosphorylation.

B 804 A NOVEL TRANSCRIPTION FACTOR REGULATES HIV-1 LTR-DIRECTED TRANSCRIPTION IN RESPONSE TO SIGNALS FROM TYROSINE KINASES
Brendan Bell and Ivan Sadowski, Dept. of Biochemistry, University of British Columbia, Vancouver, Canada, V6T 1Z3.

To investigate the mechanisms that alter the transcriptional machinery in response to extracellular signals we have identified and begun characterizing a transcription factor whose activity is increased by signals from the v-fps protein-tyrosine kinase. We used a Rat-2 cell line that expresses a temperature sensitive mutant of $P130^{gag\text{-}fps}$ to screen for promoters that are stimulated by increased kinase activity of $P130^{gag\text{-}fps}$. The HIV-1 LTR was shown to be stimulated 10 fold by v-fps in this system. Deletion analysis of the HIV LTR revealed a novel fps responsive element (FRE) residing mainly 119 to 160 nucleotides upstream of the transcriptional start site. Stimulation of the HIV LTR occurs in the HIV permissive human monocyte cell line U937 by v-fps and in Jurkat T cells by $p56^{lck}$. Nuclear extracts from U937, Jurkat with and Rat-2 cells contain proteins that form specific complexes with oligonucleotides from the FRE in mobility shift assays. Moreover, the mobility of these complexes are altered upon increasing v-fps kinase activity in Rat-2 cells. This altered mobility is due to a change in the phosphorylation status of the FRE binding protein(s) (FREBP), since it can be reversed by addition of a non-specific phosphatase to the nuclear extract. Signals from membrane associated fps are coupled to nuclear FREBP by ras and the cytoplasmic raf kinase as determined by cotransfection experiments with constitutive and dominant negative ras and raf mutants. FREBP thus represents a novel transcription factor that responds to signals from tyrosine kinases via ras and raf and may play a role in transcriptional regulation of the HIV-1 virus. Work to isolate and characterize cDNAs coding for FREBP is in progress.

B 805 STRUCTURE/FUNCTION STUDIES OF THE PHO85 GENE PRODUCT, A PROTEIN KINASE INVOLVED IN REPRESSION OF ACID PHOSPHATASE TRANSCRIPTION IN *SACCHAROMYCES CEREVISIAE*,
Lawrence W. Bergman, Reynaldo Santos and David L. Johnson, Department of Microbiology and Immunology, Hahnemann University School of Medicine, Broad & Vine, Philadelphia, PA 19102.

The PHO85 gene product is required for the transcriptional repression of the yeast phosphate-repressible acid phosphatase (encoded by the PHO5 gene), under conditions of excess inorganic phosphate and is involved in some aspects of general carbon metabolism. The sequence of the coding region of PHO85 is highly homologous (approximately 52% identical) to the CDC28 gene of *Saccharomyces cerevisiae*, suggesting that PHO85 encodes a protein kinase. Mutation of a conserved Lys residue, involved in ATP binding in other protein kinases, causes constitutive synthesis of PHO5. This implicates the kinase activity of PHO85 in the repression of PHO5 transcription. Due to the high due of homology, we have constructed a series of fusions between the CDC28 gene and the PHO85 gene. These fusions were then assayed for their ability to complement a *pho85* mutation and repress PHO5 expression. These studies may allow the localization of the domain which confers the specificity to the PHO85 kinase. Finally we have utilized a selection scheme to isolate mutants which constitutively express acid phosphatase. Genetic studies have mapped some of these mutations to the PHO85 gene. These mutant PHO85 genes are being cloned and the DNA sequences determined to provide information concerning the mode of action of the PHO85 gene.

B 806 PHOSPHORYLATION OF c-Jun DURING THE CELL CYCLE, Biedenkapp, H. and Hunter, T., Department of Molecular Biology and Virology, The Salk Institute, 10010 Torrey Pines Road, La Jolla, CA 92037

The c-Jun oncoprotein is a nuclear phosphoprotein that acts as a transcription factor. Recent studies have shown that both the DNA-binding activity as well as the transactivation activity of c-Jun are regulated by protein phosphorylation.
In order to investigate possible changes in Jun activity during cell proliferation we have studied the phosphorylation of the human c-Jun protein during the cell cycle.

Phosphorylation of bacterial expressed human c-Jun protein *in vitro* reveals that c-Jun serves as a substrate for cyclinB/p34^{cdc2} isolated from mitotic Hela cells. Tryptic phosphopeptide mapping and phosphoamino acid analysis show that cyclinB/p34^{cdc2} phosphorylates c-Jun at sites that are also phosphorylated *in vivo*. To study the *in vivo* phosphorylation of those sites during the cell cycle we first analyzed tryptic phosphopetide maps of c-Jun isolated from Nocodazole blocked Hela cells. Preliminary data suggest that the sites phosphorylated by cyclinB/p34^{cdc2} *in vitro* are also phosphorylated in mitotic cells. To analyze possible changes in c-Jun phosphorylation during the cell cycle in more detail, we are currently examining c-Jun from Hela cells at different times after release from Nocodazole or Aphidicolin block.

B 807 TRANSLATIONAL CONTROL OF C-JUN: REPRESSION BY UNTRANSLATED SEQUENCES
Timothy J. Bos, Anil Sehgal, and Ankita Patel. Department of Microbiology and Immunology, Eastern Virginia Medical School, P.O. Box 1980, Norfolk, VA 23501.

The Jun proto-oncogene has been widely studied for its role as a transcription factor and oncogene. The Jun protein is a sequence specific DNA binding protein that recognizes the consensus target sequence TGACTCA. It was first characterized as a major component of the mammalian transcription complex, AP-1. Like many transcription factors, Jun activity is under tight regulatory controls. Jun message is induced rapidly and transiently by a variety of growth and differentiation factors as an immediate early response gene. In the absence of protein synthesis inhibitors, the *jun* message is rapidly turned over. Jun activity is also modulated at the protein level. Jun protein can be activated in response to tumor promoters as well as other oncogenes such as Ras by post translational modification. The phosphorylation state of the Jun protein plays a critical role in determining if it can bind DNA or activate transcription. In addition, Jun activity is affected by a variety of protein - protein interactions. Through a structural domain called the leucine zipper, Jun is able to dimerize with a number of proteins including those in the Jun family (c-Jun, Jun D, and Jun B), the Fos family (c-Fos, Fra-1, Fra-2, FosB and FosB-SF) and the CREB family. The affinity for AP-1 target DNA varies according to the dimerization partner utilized. Thus, regulation of Jun activity is complex and exists at a number of levels.

Here we describe another level of regulation of Jun activity - the translational level. Although rapid increases in c-Jun mRNA are a common response to a variety of stimuli, an equal rise in Jun protein does not always follow. The chicken c-*jun* message contains a 5' leader over 300 bases in length with a high GC content. This leader sequence has the potential to form a long and stable hairpin loop structure. Similar structures in other oncogenes have been implicated in translational repression. We have assayed the affect of this 5' leader sequence on Jun translation *in vitro* and found it to repress protein synthesis several fold. A mutational analysis of this region has been initiated, the results of which will be presented.

B 808 MAZ, A ZINC FINGER PROTEIN WHICH POSITIVELY REGULATES THE c-*MYC* PROTO-ONCOGENE, Steven A. Bossone*, Amanda J. Patel#, Marco A. Passini#, Timothy J. Sellati#, Claude Asselin@ and Kenneth B. Marcu*#, Department of Pathology* and Biochemistry and Molecular Biology#, SUNY at Stony Brook, Stony Brook, NY 11794; Department of Anatomy and Cell Biology@, University of Sherbrooke, Sherbrooke, Quebec.
MAZ encodes a zinc finger protein which possesses a proline-rich domain and several polyalanine tracts which are thought to mediate transcriptional activation and/or repression. It binds double-stranded DNA in a sequence-specific manner with a minimal core-binding site of GGGAGGG. MAZ protein binding sites have been associated with transcriptional initiation and termination in the c-*myc* gene and with C2 gene termination. MAZ displays a generally ubiquitous expression pattern in most tissues and cell lines tested except for its absence in human kidney and the human promyelocytic leukemia cell line HL60 after treatment with dimethyl sulfoxide (DMSO). DMSO effectively consigns HL60 cells to their differentiation pathway concomitant with a rapid downregulation of c-*myc* mRNA. The effect of constitutive MAZ expression on c-*myc* regulation in differentiating HL60 cells will be discussed. In addition to its predominant 2.7 kilobase mRNA, multiple forms of MAZ RNA were observed and an alternatively spliced cDNA has been obtained which lacks one of its putative transcriptional effector domains. The functional significance of this observation remains to be determined.
Proper transcription of the c-*myc* gene was shown to be dependent upon a functional MAZ protein binding site. Furthermore, MAZ was able to transactivate *myc*-chloramphenicol acetylytransferase (CAT) chimeric constructs in COS-1 cells under transient conditions as determined by CAT assay. Transcription of constructs containing multiple MAZ protein binding sites was dramatically induced in the presence of enforced MAZ expression. The role of MAZ in c-*myc* expression will be further refined by employing MAZ-specific monoclonal antibodies and in both *in vitro* and *in vivo* systems.

B 809 IDENTIFICATION OF A NOVEL PROTEIN THAT INTERACTS WITH C-*MYC*. Brent H. Cochran and P. Sanjeeva Reddy. Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Room E17 - 517, 77 Mass. Ave., Cambridge, MA 02139

We have used the yeast two-hybrid method of screening for protein-protein interactions to look for mammalian genes that interact with c-*myc*. After screening over a million cDNAs from a hybridoma cell library, 16 positive clones were found. All the cDNA clones isolated were derived from a single gene. To score positive in the yeast assay, the hybrid proteins must interact with the c-*myc* helix-loop-helix-leucine zipper domain. The clones do not interact with an Rb-LexA fusion protein indicating that the interaction does not occur through the LexA portion of the fusion protein. A common region of all the cDNAs isolated contained a predicted leucine zipper motif. This gene which we are calling *Myp* for myc interacting protein, is distinct from *max* and has no predicted helix-loop-helix domain immediately adjacent to the leucine zipper. However, the complete sequence of this gene has yet to be determined. In the *in vivo* yeast interaction assay *myp* gives a stronger signal, than does Max. Work that is in progress will address the ability of *myp* to bind *myc* in vitro, the activity of *myp in vivo*, and the domain of *myc* required for the interaction.

B 810 EFFECTS OF PHOSPHORYLATION ON THE HOMEODOMAIN-CONTAINING PROTEIN *fushi tarazu*, John W.R. Copeland, L.-H. Hung, B. Strome, E. Burgess[1] and H. Krause, Banting and Best Dept. of Medical Research, University of Toronto, Toronto, Ontario, Canada, M5G 1L6, [1]Dept. of Biology, York University, Toronto, Ontario, Canada

The *fushi tarazu* (*ftz*) protein of *Drosophila* is a homeodomain-containing transcription factor which plays a role in segmentation, neurogenesis, and, perhaps, gut development. *ftz* protein (Ftz) is phosphorylated differentially during its different phases of expression, suggesting that these modifications may alter tissue specific activities of the protein. Notable differences are observed in the partial proteolysis profiles of phosphorylated Ftz expressed in the Baculovirus system compared with unphosphorylated protein expressed in bacteria, suggesting that phosphorylation alters Ftz conformation. Phosphatase treatment of Baculovirus-expressed Ftz also reduces the ability of the protein to bind DNA in a gel retardation assay. Scatchard analyses of gel retardation data indicates that different Ftz phosphoisoforms have different affinities for the tested sequence. Many of the phosphorylation sites of the Baculovirus-expressed Ftz have been mapped. Some of these sites have been altered by site-directed mutagenesis. Interestingly, several of these mutants show altered transcriptional activity when expressed in *Drosophila* Schneider cells.

B 811 DOWN-REGULATION OF THE DROSOPHILA MORPHOGEN BICOID BY THE TORSO RECEPTOR MEDIATED SIGNAL TRANSDUCTION CASCADE. Nathalie Dostatni[1], Elettra Ronchi[1], Jessica Treisman [1,2], Gary Struhl[3] and Claude Desplan[1]. Howard Hughes Medical Institute, 1. The Rockefeller University, 1230 York Ave, New York, NY 10021. 2. Department of Molecular and Cell Biology, University of California, Berkeley 94720 3. Center for Neurobiology and Behavior, Columbia University College of Physicians and Surgeons, New York, NY 10032.

Most aspects of anterior body pattern in *Drosophila* are specified by the graded distribution of the Bicoid protein (Bcd) which is presumed to bind with different affinities to subordinate regulatory genes such as *hunchback (hb)* and *orthodenticle (otd)*, leading to their transcriptional activation in distinct anterior domains. However, transcription of these target genes is subsequently repressed at the anterior pole in response to the activity of the receptor tyrosine kinase *torso (tor)*. We show that both activation by *bcd* and repression by *tor* can be reproduced using an artificial promoter consisting solely of Bcd binding sites placed upstream of a naive transcriptional start site. Repression depends on the function of the *Drosophila* homologue of the serine-threonine kinase *raf (D-raf)*, but not on either *tailless (tll)* or *huckebein (hkb)*, two transcription factors whose local activity was previously thought to constitute the output of the *tor* signalling system. Further, the addition of heterologous transcriptional activation domains to Bcd renders it insensitive to *tor*-mediated repression. Thus, the activity of the Bcd protein appears to be down-regulated by input from the *tor* signalling cascade, possibly by D-raf-dependent phosphorylation of the Bcd structural domain normally required for activating transcription. We are currently analysing the molecular basis of this observation.

B 812 INDUCIBILITY OF JUN D BY A POST-TRANSCRIPTIONAL MECHANISM IN T CELL, [1]Antonietta R. Farina, [2]Terri Davis-Smith, [2]Kevin Gardner and [2]David L. Levens. [1]Dept. Medicina Sperimentale, University L'Aquila, Italy. [2]Lab. of Pathology, NCI, NHI. Bethesda MD.

Multiple chromatographically distinct complexes interacting with the AP1 site of the GALV-Seato LTR are separable from MLA144 gibbon T-cell and TPA induced human T-cell line Jurkat nuclear extracts. The most abundant of these complexes (T-AP1) possesses novel biochemical properties and is different from the fos/jun complex. The T-AP1 complex, composed of at least two dissociable protein components, CORE and FT , is induced in Jurkat cells following activation with TPA. The purified core component was identified as jun D by biochemical and immunological methods. As TPA induction of T-AP1 binding was not modified by protein synthesis inhibitors and the core factor was preexistant in Jurkat cells, we propose that the jun D component is activated by a protein synthesis independent mechanism. GALV-TRE enhancer activity was also increased in vivo following TPA treatment in the presence of protein synthesis inhibitors. Transcription, in vitro, indicated that purified T-cell jun D activated GALV-TRE driven RNA synthesis. Jun D binding to GALV-TRE was highly dependent on the presence of a separate cofactor, Flow Through (FT). The protein synthesis independent nature of TPA dependent T-AP1 activation may suggest that this transcription factor is involved in the earliest stages of T cell activation.

B 813 MAX AND MYC/MAX COMPLEXES ACTIVATE TRANSCRIPTION IN VIVO, Fiona Fisher*, D.H. Crouch, Colin R. Goding*, P.-S. Jayaraman*, W. Clark and D.A.F. Gillespie, *Marie Curie Research Institute, The Chart, Oxted, Surrey, UK RH8 0TL, Cancer Research Campaign, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsdon, Glasgow, UK G61 1BD.

Despite the large body of evidence implicating the Myc oncoprotein in the control of proliferation, differentiation and transformation, little advance was made until recently in understanding its biochemical function. The observation that Myc contains structural motifs common to the basic helix-loop-helix leucine zipper (bHLH-LZ) family of transcription factors, and can interact specifically with the DNA sequence CACGTG as a heterodimer with an unrelated bHLH-LZ protein, Max, strongly suggests a role for Myc and Max in transcriptional regulation. To date however, there has been no direct demonstration of the ability of Myc or Max to activate or repress transcription. In Mammalian cells analysis of Myc/Max function is complicated by the presence of endogenous Myc and Max as well as multiple other CACGTG-binding transcription factors. We have therefore used the yeast, *Saccharomyces cerevisiae*, to express Myc and Max and assay their ability to regulate transcription *in vivo*. Our results show that both Max and Myc can bind to DNA and activate transcription, but that Myc can only do so in conjunction with Max. Furthermore, the ability of mutant Myc proteins with lesions in the bHLH-LZ domain to activate transcription in yeast, complex with Max and bind DNA, correlates with their neoplastic transforming capacity for higher eukaryotic cells.

B 814 TRANSACTIVATION OF GENE EXPRESSION BY MYC IS INHIBITED BY A POINT MUTATION AT THE MAP KINASE PHOSPHORYLATION SITE. Shashi Gupta, Alpna Seth, and Roger J. Davis, Program in Molecular Medicine, and the Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center , Worcester, MA 01605.

The c-Myc protein is a short-lived nuclear phosphoprotein that has an important functional role during cellular proliferation, differentiation, and neoplasia. The structural organization of Myc indicates the presence of sub-domains that are found in several transcriptional regulators; Basic-helix-loop-helix (bHLH) and leucine zipper (Zip) motifs at the COOH terminal region and a transcriptional activation domain at the NH_2-terminal region. We demonstrate that the specific Myc DNA binding site CACGTG placed up-stream of a luciferase reporter gene confers Myc-stimulated expression of the reporter gene. Over-expression of Max inhibited the Myc-activated gene expression.

The c-Myc protein is phosphorylated *in vitro* by MAP kinases within the NH_2-terminal transactivation domain at Ser^{62}, which may be a regulatory site of phosphorylation. Here, we show that Ser^{62} is a major site of Myc phosphorylation *in vivo*. Thr^{58} was identified as another site of *in vivo* phosphorylation of Myc. A mutational analysis was performed to investigate the role of the phosphorylation sites Thr^{58} and Ser^{62} during transactivation of gene expression by Myc. Mutation at Thr^{58} caused no significant change in Myc function, but the replacement of Ser^{62} with Ala markedly inhibited the observed Myc-dependent transactivation of gene expression. In contrast, the replacement of Ser^{62} with an acidic residue (Glu) caused only a small inhibition of transactivation. Together, these data demonstrate that the NH2-terminal phosphorylation site Ser^{62} is required for high levels of transactivation of gene expression by Myc.

B 815 Cyclin Expression Overcomes Promoter Repression by $p110^{RBI}$.

Paul A. Hamel[1] and Joan Hanley-Hyde[2].
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$p110^{RBI}$ is a nuclear, phosphoprotein which becomes phosphorylated in a cell cycle dependant manner and which exhibits characteristics of a regulator of the cell cycle. Recently, evidence has suggested that $p110^{RBI}$ acts as a transcription factor, potentially regulating genes required for normal progression through the cell cycle, such as c-*myc*. One complex in which $p110^{RBI}$ appears to participate as a negative regulator of transcription contains the transcription factor, E2F. When examined in the context of this complex, $p110^{RBI}$ is found in the hypo-phosphorylated and, presumably, active form. The transcriptional repression activity of $p110^{RBI}$ was determined in promoter assays and that the active form of $p110^{RBI}$ was the hypo-phosphorylated form supported using a mutant protein refractory to hyperphosphorylation.

We now demonstrate that over expression of cyclin A or E can overcome the repression of $p110^{RBI}$ on the c-*myc* and adenovirus early promoter, EIIaE. Transient co-transfection assays were performed using P19 cells where either cyclin A or E were titrated against $p110^{RBI}$. Both cyclins could overcome repression by $p110^{RBI}$ in a dose dependent manner. When cyclin D was tested, reversal of $p110^{RBI}$ repression of EIIaE occurred, while repression of the c-*myc* and N-*myc* continued. However, the action of cyclin D appears to be independent of $p110^{RBI}$ or E2F since a c-*myc* promoter construct, defective in the E2F site (mycΔE2), was still repressed by cyclin D.

Our data suggest that cyclins A and E are able to overcome transcriptional repression by $p110^{RBI}$ by acting directly on $p110^{RBI}$ while cyclin D acts on other elements or initiation factors independent of $p110^{RBI}$.

B 816 A PROTEIN TYROSINE KINASE IS INVOLVED IN THE RAPID ACTIVATION OF THE TRANSCRIPTION FACTOR APRF BY INTERLEUKIN-6, Friedemann Horn, Jan Buschmann, Claudia Lütticken, Ursula M. Wegenka, Juping Yuan, and Peter C. Heinrich, Institute for Biochemistry, RWTH Aachen, Klinikum Pauwelsstrasse 30, D-5100 Aachen, Federal Republic of Germany

We have shown previously that interleukin-6 (IL-6) and leukemia inhibitory factor (LIF) both trigger the rapid activation of acute phase response factor (APRF), a transcription factor involved in the regulation of various acute phase protein genes in hepatocytes. APRF is different from other cytokine-regulated transcription factors of the C/EBP and NF-κB/rel families, exhibits an apparent molecular weight of about 110 kDa as estimated by Southwestern blotting and gel filtration, and binds with highest affinity to the palindromic sequence TTCCNGGAA. Since APRF occurs ubiquitously, it is likely to play an important role in the regulation of gene expression by IL-6 and LIF in many tissues.

We studied the mechanism of APRF activation in the human hepatoma cell line HepG2. APRF activation does not depend on ongoing protein synthesis and thus occurs posttranslationally. Within one minute after stimulation with IL-6, active APRF can be observed in cytosolic fractions indicating that the activation takes place in the cytoplasm followed by translocation of the factor to the nucleus. Genistein and tyrphostin, specific inhibitors of protein tyrosine kinases, prevent the IL-6-induced APRF activation. Preliminary evidence from studies with anti-phosphotyrosine antibodies indicates that APRF is phosphorylated at tyrosine residues in response to IL-6. Furthermore, APRF could also be activated *in vitro* using cellular homogenates and subcellular membrane fractions. This process was dependent on the presence of ATP and could be blocked by protein tyrosine kinase inhibitors as well.

The results from these studies lead to the conclusions that (1) IL-6 rapidly activates a cytoplasmic protein tyrosine kinase which in turn phosphorylates and thereby activates the transcription factor APRF, and (2) that these events are likely to occur in close association with the plasma membrane.

B 817 A NOVEL TRANSCRIPTION FACTOR, OB2-1, IS UPREGULATED IN BREAST TUMOUR LINES WHICH OVEREXPRESS c-*erb*B-2, Helen C. Hurst and Donal P. Hollywood, Gene Transcription Laboratory, ICRF Oncology Group, Hammersmith Hospital, Du Cane Road, London W12 0NN, U.K.

The receptor tyrosine kinase protooncogene, c-*erb*B-2, is overexpressed in 20-30% of mammary carcinomas and correlates with poor prognosis. It is known that although the c-*erb*B-2 gene is often amplified in breast lines and tumours, overexpression can occur from a single copy gene. Furthermore, whether or not the gene is amplified, overexpressing cells produce 4-8 fold more c-*erb*B-2 mRNA per gene copy than low expressing cells. We have examined the mechanism behind these observations and have found that the c-*erb*B-2 gene is more heavily transcribed and the promoter is more active in overexpressing cells. By combining promoter deletion assays with DNase footprinting we have identified a novel factor, OB2-1, which mediates at least some of these effects. The binding activity of OB2-1 is low or absent in control breast lines, but readily detected in all lines which overexpress c-*erb*B-2. However, our preliminary experiments have lead us to suspect that the absolute abundence of the factor does not vary greatly between the two types of cell line. Instead, we believe that the binding activity of OB2-1 is regulated by its phosphorylation state and that the protein is constitutively active in the lines which overexpress c-*erb*B-2, thus contributing to the overaccumulation of mRNA and protein observed in these tumour lines. We hope to clone a gene for OB2-1 and to investigate the differences in its regulation between control and overexpressing mammary cells.

B 818 MOLECULAR CHARACTERISATION OF THE DNA DEPENDENT PROTEIN KINASE, Stephen Jackson, Tanya Gottlieb and Katharine Hartley, Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR, U.K.

The DNA-dependent protein kinase (DNA-PK) phosphorylates Sp1 and a variety of other transcription factors. The kinase is only active when bound to DNA. For efficient Sp1 phosphorylation, GC boxes must be present on the DNA. By template competition studies, we have shown that the DNA-PK and Sp1 must be colocalised on the same DNA molecule for efficient phosphorylation to occur. Whilst determining where the kinase binds in relation to Sp1, we made the surprising discovery that the DNA-PK interacts with ends of DNA molecules. Furthermore, we found that the DNA-PK requires DNA termini for activity. Another protein that binds specifically to DNA ends is Ku. This protein, originally identified as an antigen recognised by various human autoimmune sera, consists of two polypeptides of approximately 70 and 80 kDa in a 1:1 complex. Because of their similar DNA binding modes, and because Ku co-purifies with DNA-PK activity, we tested for a possible relationship between Ku and the DNA-PK. Through a combination of biochemical fractionation and immunological approaches, we have demonstrated that the DNA-PK consists of two functional components. One, the catalytic subunit, corresponds to a polypeptide of approximately 350 kDa. The other specifies DNA binding by the kinase and corresponds to Ku. Our studies have therefore provided a function for the enigmatic Ku protein, and have shown the DNA-PK to be a multiprotein complex.

As a step towards further characterisation of the DNA-PK, we have collaborated with Dr. Carl Anderson to isolate a series of overlapping partial p350 cDNA clones. Together, the cloned region spans approximately 9 kb, and a single open-reading-frame has been identified running throughout this region. RNase H mapping studies indicate that a further 4.5 kb of cDNA remains to be cloned. We are currently expressing various portions of p350 in bacteria to determine whether isolated regions of the protein display kinase activity, DNA binding, or an ability to interact with Ku.

B 819 INTRACELLULAR INTERACTIONS BETWEEN MYC, MAX AND ΔMAX

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Max is a basic/helix-loop-helix/leucine zipper protein, which forms a sequence-specific DNA-binding complex with the Myc family proteins (1). We have recently identified an alternatively spliced *max* cDNA, which produces a carboxyterminally truncated protein termed ΔMax (2). ΔMax has retained the ability of Max to form DNA-binding heterodimers with Myc, but is exclusively cytoplasmic in the absence of Myc, since it lacks the nuclear localization signal. In rat embryo fibroblasts, ΔMax enhances transformation by Myc and Ras, whereas Max causes a decrease in the number of transformed foci. Thus, the *max* gene encodes both a negative (Max) and a positive (ΔMax) regulator of Myc function. The intracellular interactions between Myc, Max and ΔMax will be discussed in the light of our recent results on the properties of these proteins.

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B 820 REGULATION OF C-JUN PHOSPHORYLATION IN HEMATOPOIETIC CELLS, Andrew S. Kraft, Christopher Franklin, Tino Unlap, and Victor Adler, Division of Hematology/Oncology, University of Alabama, B'ham, Ala. 35294.

We have shown that the addition of phorbol esters to U937 leukemic cells induces differentiation of these cells and stimulates the phosphosphorylation of the c-Jun on serines 63 and 73. This increase in phosphorylation was demonstrated by transfecting a plasmid encoding a c-Jun protein truncated after amino acid 234. Now we have investigated the location of this c-Jun Δ234 by both cell fractionation and immunofluorescence and find that it is in the cytoplasm. Time course studies demonstrate that c-Jun Δ234 is phosphorylated over the same time course as endogenous c-Jun which is found in the nucleus. To examine the location of the amino terminal c-Jun protein kinase (c-JAT-PK), we used a fusion protein between GST and amino acids 5-89 of the c-Jun protein as a substrate for kinase reactions. This substrate was equally phosphorylated by both nuclear and cytosolic extracts. As reported previously the c-JAT-PK binds strongly to beads which have glutathione-GST-Jun attached. We have eluted this protein kinase from the beads and by passing it over a molecular seive sizing column demonstrate a M.W. of approximately 60 kDa. Unlike c-Jun, we have shown that v-Jun is not phosphorylated by phorbol ester treatment of U937 cells. c-Jun contains the δ domain, a.a. 40-66, which is not found in v-Jun, suggesting that these amino acids are important for the regulation of phosphorylation. To examine this possibility we have synthesized the δ domain peptide and linked it to sepharose beads. We find that iodinated c-JAT-PK which has been eluted from the sizing column binds tightly to the δ domain containing beads. These results suggest that the protein kinase phosphorylating c-Jun has an approximate M.W. of 60 kDa, is found in the nucleus and cytoplasm, and is capable of direct binding to the δ domain.

B 821 THE FUNCTIONAL VERSATILITY OF CREM IS DETERMINED BY ITS MODULAR STRUCTURE, Brid M. Laoide, Nicholas S. Foulkes, Florence Schlotter and Paolo Sassone-Corsi, Laboratoire Génétique Moléculaire des Eucaryotes du CNRS, U184 de l'INSERM, 11 rue Humann, 67085 Strasbourg Cedex, France.

The CREM gene (cAMP-responsive element modulator) generates both activators and repressors of cAMP-induced transcription by alternative splicing (1,2). We determined the exon structure of the CREM gene and have identified new isoforms. CREM isoforms with different structural characteristics are generated by the shuffling of exons to produce proteins with various combinations of functional domains. CREM proteins bind efficiently to CREs; the various isoforms heterodimerize in vivo with each other and with CREB. The two alternative DNA binding domains of CREM, which are differentially spliced in the various isoforms, show distinct binding efficiencies, while CREMα/CREB heterodimers exhibit stronger binding than CREMβ/CREB heterodimers to a consensus CRE in vitro. We identify the protein domains involved in activation function and find that the phosphorylation domain and a single glutamine-rich domain are sufficient for activation. A minimal CREM repressor, containing only the b-Zip motif, efficiently antagonises cAMP-induced transcription. In addition, phosphorylation may reduce repressor function, as a CREMβ mutant carrying a mutation of the serine phosphoacceptor site (CREMβ68) represses more efficiently than the wild-type CREMβ.

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B 822 MODULATION OF NUCLEAR FACTOR AP-1 BY pX INVOLVES BOTH AN INCREASE IN DNA BINDING AND CHANGES IN c-JUN TRANSCRIPTIONAL ACTIVITY, G Natoli, ML Avantaggiati, C Balsano, M Artini, P Chirillo, MS Bonavita, M Levrero - Fond. A. Cesalpino and I Clinica Medica-Rome-Italy.

The HBV X gene product (pX) activates in trans several regulatory sequences of viruses and of cellular genes by modulating defined nuclear transcription factors. We used in vitro DNA/protein interaction assays to study the mechanism of the pX induced activation of the transcription factors AP-1. AP1 binding activity is clearly increased in nuclear extracts from HepG2 cells infected with a pX expressing vaccinia virus. The same results were obtained in Hela Cells but not in Alexander, CV1, and murine L cells, in which, anyway, pX is able to activate AP1. The mechanism of this pX induced increase of AP1 binding was further investigated using in vitro translated pX, fos and jun proteins in gel retardation assays. pX does not bind canonical or atypical AP1/TRE sites, does not form functional heterodimers with c-fos, does not modify the binding of either c-jun homodimers or fos/jun heterodimers and does not modify the binding of AP1 complexes when added to nuclear extracts: all these data suggest that pX needs or acts through additional cellular factors that are not ubiquitous. The role of phosphorylation events is suggested by the blocking effect of PKC inhibitors. Cotransfection experiments conducted in F9 cells show that pX can increase the intrinsic ability of c-Jun to activate transcription, thus explaining, at least in part, the ability of pX to increase the TRE-directed transcription in cells in which it is unable to augment the AP1 binding.

B 823 **NF-AT_p: A T cell DNA-binding protein that is a target for calcineurin and immunosuppressive drugs.** Patricia G. McCaffrey, Brian A. Perrino , Thomas R. Soderling and Anjana Rao, Division of Tumor Virology, Dana Farber Cancer Institute, Department of Pathology, Harvard Medical School, and Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, Oregon.

The nuclear factor of activated T cells (NF-AT) is essential for transcription of the interleukin 2 (IL2) gene upon T cell activation. Here we use a technique involving elution and renaturation of proteins from SDS gels to identify a DNA-binding component of NF-AT (NF-AT_p) that is present in hypotonic extracts of T cells prior to activation and appears in nuclear extracts when T cells are activated. NF-AT_p is present in resting T cells predominantly in a form migrating with an apparent molecular weight of 110-140 kDa, while NF-AT_p from nuclear extracts of activated T cells migrates with a lower apparent molecular weight (90-125 kDa). This difference is likely to reflect dephosphorylation of NF-AT_p, since treatment of NF-AT_p with calf intestinal phosphatase or the calcium- and calmodulin-dependent phosphatase calcineurin *in vitro* results in a similar decrease in its apparent molecular weight. We show that NF-AT_p is dephosphorylated in cell lysates by a calcium-dependent process that is blocked by inclusion of EGTA or a specific peptide inhibitor of calcineurin in the cell lysis buffer. Moreover, dephosphorylation of NF-AT_p is inhibited by treatment of T cells with the immunosuppressive drugs cyclosporin A (CsA) or FK506, which inhibit the phosphatase activity of calcineurin when complexed with their specific binding proteins, cyclophilin and FK506 binding protein. This work identifies NF-AT_p as a DNA-binding phosphoprotein and a target for the drug/immunophilin/calcineurin complexes thought to mediate the inhibition of IL2 gene induction by CsA and FK506.

B 824 A COMPARATIVE STUDY OF BACULOVIRUS EXPRESSED FOS AND JUN FAMILY POLYPEPTIDES BY AN *IN VITRO* TRANSCRIPTION ASSAY., Richard Metz and Rodrigo Bravo, Department of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O.Box 4000, Princeton, New Jersey 08543-4000.

Each of the Fos family polypeptides, c-Fos, FosB, Fra-1, and Fra-2, are able to form heterodimeric complexes with members of the Jun family and bind to a TPA response element (TRE). The transcriptional activation properties of the various heterodimeric complexes have been studied mostly by transient transfection assays. In order to directly characterize the transcriptional activation properties of the various Fos and Jun molecules, we expressed the polypeptides in Sf9 cells using the baculovirus expression system. All of the proteins were expressed as fusion polypeptides with six histidines to enable their purification by nickel affinity chromatography.

The fosB gene is a member of the immediate early gene family, whose differentially spliced mRNA encodes two polypeptides, FosB and FosB/SF. Recently reported transient transfection experiments with FosB and FosB/SF suggest that FosB/SF functions as a negative regulator of AP1 activity. However, our lab and others have demonstrated that both forms of FosB, when complexed with c-Jun can activate transcription through a TRE element. In order to demonstrate directly the ability of FosB and FosB/SF to activate transcription, purified FosB and FosB/SF proteins were complexed with a truncated c-Jun molecule [c-Jun short form (c-Jun/SF)] which contains only its b-zip domain. Both FosB-cJun/SF and FosB/SF-c-Jun/SF can activate a collagenase TRE containing template in an *in vitro* transcription assay. In contrast, the control c-Jun/SF homodimers can not activate transcription. Therefore both FosB and FosB/SF contain at least one functional activation domain. Currently, the activity of the Fos and Jun family members are being characterized *in vitro*, and experiments are underway to determine whether these molecules function through independent biochemical pathways to activate transcription. The outcome from these studies will be presented.

B 825 CELL TRANSFORMATION BY THE ONCOGENIC TRANSCRIPTION FACTOR C-FOS REQUIRES AN EXTENDED PERIOD OF EXPRESSION. Graham Gang Miao[1,2] and Tom Curran[1]. [1]Department of Molecular Oncology & Virology, Roche Institute of Molecular Biology, Nutley, NJ 07110 and [2]Deptartment of Biological Sciences, Columbia University, New York, NY 10027.

The proto-oncogene c-*fos* is the cellular counterpart of the viral oncogene v-*fos* carried by the FBJ and the FBR murine osteogenic sarcoma viruses. Induction of oncogenesis by *fos* is a consequence of the deregulation of gene expression. Continuous, high level expression of this transcription factor results in neoplastic transformation of fibroblasts in culture and induction of tumors in mice. The protein product of the *fos* oncogene (Fos) forms a heterodimeric complex with the product of the *jun* oncogene (Jun) that functions as a component of the mammalian transcription factor AP-1. Both *fos* and *jun* are members of the class known as cellular immediate-early genes whose expression is induced rapidly and transiently by an array of extracellular signals associated with cell proliferation, differentiation and neuronal excitation. Despite the general role played by c-Fos as a nuclear signaling molecule involved in signal transduction processes, the pathological effect associated with deregulation of *fos* expression is limited to bone and cartilage tumors. In an attempt to unravel the molecular basis of *fos*-induced tumorigenesis and to identify target gene(s) controlled by this oncogenic transcription factor, we have established a conditional cell transformation system. Rat fibroblasts were transformed by a c-*fos* gene under the control of LacI-VP16 activator protein (LAP) which can be regulated by isopropyl-β-D-thiogalactoside (IPTG). Stable cell lines were established in which morphological transformation could be induced or reversed depending upon the absence or presence of IPTG. While the regulation of *fos* expression by IPTG was extremely rapid, phenotypic transformation or reversion required a much longer period of time. This indicates that the products of the target genes regulated directly or indirectly by *fos* must reach a critical threshold to affect cell morphology. The system will allow an analysis of the molecular events that occur during the transition of normal fibroblasts to the transformed state in response to Fos expression.

B 826 INHIBITION OF BASAL TRANSCRIPTION FROM THE V-*FOS* PROMOTER BY V-*MOS* PROTEIN DEPENDS ON V-*MOS* SEINE KINASE ACTIVITY. Lydia A. Bishop and Edwin C. Murphy, Jr.. Dept. of Tumor Biology, Section of Molecular Virology, The University of Texas M. D. Anderson Cancer Center, Houston, TX. 77030.
We have studied the effect of P37 $^{v\text{-}mos}$ expression on transcription from the human c-*fos* promoter in NIH 3T3 cells. In these experiments, the v-*mos* gene linked to an SV-40 promoter (termed v-*mos*) and c-*fos* promoter sequences linked to the CAT gene (termed fosCAT) were co-transfected into NIH 3T3 cells, and the CAT activity assessed 36 hrs later. Relative to controls, v-*mos* expression was observed to inhibit CAT production by 4-10 fold. This inhibitory activity was found to depend on v-*mos* serine kinase activity since a v-*mos* "kinaseless" mutant containing a inactivating mutation in the kinase domain was unable to inhibit fosCAT production. Moreover, using a series of v-*mos* mutants with progressively larger N-terminal deletions, the inhibitory effect was not lost until the deletions invaded the v-*mos* ATP-binding site. To determine the basis for the inhibition of the c-*fos* promoter, co-transfection assays were done using a series of progressively truncated c-*fos* promoters linked to the CAT gene. The inhibitory effect of v-*mos* on CAT expression persisted until the region of dyad symmetry responsible for basal transcription from the c-*fos* promoter about 100 nucleotides upstream of the c-*fos* transcriptional start site was deleted; at this point the inhibitory effect was lost. In gel shift assays, DNA probes containing this region were retarded by a protein(s) found in cells transfected with the v-*mos* construct. This protein was found in much lower abundance in cells transfected with the "kinaseless" v-*mos* mutant, arguing that the kinase activity of v-*mos* necessary for cell transformation was involved in the formation of the complex. The region of the c-*fos* promoter responsive to v-*mos* inhibition contains a tract very closely related to the E2F transcription factor recognition sequence. Mutation of this sequence to one less closely related to the E2F recognition sequence caused the c-*fos* promoter to lose its sensitivity to c-*mos* inhibition and, in parallel, its ability to be retarded in gel shift assays by the proteins(s) found in nuclei of cells expressing v-*mos*. We conclude that the depressive effect of v-*mos* expression on the c-*fos* promoter proceeds through phosphorylation of the E2F transcription factor or other proteins that can complex either with E2F or the same region of the c-*fos* promoter.

B 827 CAMP-DEPENDENT PROTEIN KINASE A REGULATES THE ACTIVITY OF THE THYROID TRANSCRIPTION FACTOR TTF1 Musti A.M., Gallo A., Zannini S., Avvedimento V.E., Gottesman. M.E. C.E.O.S. del C.N.R. II facolta di Medicina ,80131 Naples, Italy. I.C.R, Columbia University, N.Y.N.Y.10032
In thyroid cells (FRTL) expression of thyroid specific function is dependent on the cellular response to TSH. TTF1 is an homeodomain protein that binds to the thyroglobulin (Tg) promoter and it is required for the tissue-specific expression of the gene. We have recently show that the DNA binding activity of TTF1 present in FTRL nuclear extracts is phosphatase sensitive and is restored by in vitro treatment with cAMP dependent protein kinase A (PKA) . In this study we show that either TSH or forskolin induce TTF1 binding activity, in FRTL cells. Immunoprecipitation experiments, using TTF1 antibody, show that forskolin induces TTF1 phosphorylation. To estabilish a causal link between PKA phosphorylation and the capacity of TTF1 to trans-activate Tg promoter we initiated a series of transient expression experiments in the PKA deficient cell line $A_{126\text{-}1B2}$. We show that a TTF1-expressing plasmid trans-activates Tg-CAT fused gene only if cotransfected with a plasmid expressing the catalitic subunit of PKA. We propose that TTF1 activity is regulated by PKA phosphorylation , and plays an important role in the cAMP regulation of Tg gene expression.

B 828 CALCINEURIN REGULATES THE ACTIVITY OF MULTIPLE TRANSCRIPTION FACTORS IN T CELLS, E.A. O'Neill*, B. Frantz-Wattley*, E. Nordby*, J.N. Parsons*, M.J. Tocci*, R.L. Kincaid† and S.J. O'Keefe*; *Dept. of Molecular Immunology, Merck Research Labs, Rahway, NJ 07065 and †Immunology Sect., NIAAA/NIH, Rockville, MD 20852.

T-cell proliferation requires induction of interleukin-2 (IL-2) gene transcription via signal transduction pathways which involve activation of protein kinase C and a rise in intracellular Ca^{2+}. The immunosuppressants, Cyclosporin A (CsA) and FK-506, selectively inhibit a Ca^{2+}-dependent pathway. We have recently demonstrated that the Ca^{2+}/calmodulin dependent protein phosphatase calcineurin (phosphatase 2B) is the FK-506- and cyclosporin A-sensitive protein required for T-cell-receptor-mediated activation of interleukin-2 gene transcription. To identify transcription factors activated by calcineurin in T cells we have examined the effects of calcineurin on various promoter elements. We report that the IL-2 enhancer elements IL-2A (recognized by Oct-1 and OAP) and IL-2E (recognized by NF-AT and Ets-factors), and binding sites for either NFkB or AP-1, all respond to calcineurin in Jurkat T cells, each in a unique manner. We demonstrate that maximal activation of the IL-2A and IL-2E elements require not only PMA and calcineurin but also an additional Ca^{2+} signal. The NFkB site requires only PMA and calcineurin for maximal activity. The AP-1 element requires PMA and a Ca^{2+} signal for full activity but this activation is inhibited by calcineurin. These data demonstrate that calcineurin can activate at least three distinct transcription factors and inhibit activation of a fourth. The data also suggest the existence of a non-calcineurin, Ca^{2+}-dependent promoter activation pathway in T cells. These observations indicate a broad role for calcineurin in T cell transcription regulation.

B 829 TRANSCRIPTIONAL REGULATION OF PROTEIN KINASE C BY PHORBOL ESTERS, Lina M. Obeid and Yusuf A. Hannun, Departments of Medicine and Cell Biology, Duke University Medical Center, Durham, NC 27710.
Protein kinase C is a family of closely related but distinct isoenzymes that has an important role in differentiation and development. We have shown that PKCß is transcriptionally upregulated in leukemia cell differentiation (J. Biol. Chem. 265, 2370-2374). Here we show that phorbol ester induced differentiation of K562 erythroleukemia cells is accompanied by a 4 fold increase in RNA for PKCß. In order to explore the mechanism of this upregulation we have cloned the PKCß 5' region and identified its promoter. PKCß has a GC rich TATA-less promoter that is upregulated up to 20 fold in response to phorbol esters. This regulation is independent of AP1 as deletion of an AP1 site does not abolish the upregulation. Several deletion constructs narrow promoter activity to a 150 bp fragment that contains 2 SP1 sites and an octamer binding motif. This fragment is also sufficient to induce the PMA effect. Gel shift analysis shows that phorbol esters cause loss of octamer binding protein. These effects are late starting at 6 hours and coincide with down regulation of PKCß protein. This represents a novel mechanism of PKC autoregulation at a transcriptional level.

B 830 CHANGES IN THE PHOSPHORYLATION STATUS OF MYOD ALTER DIMERIZATION SPECIFICITY AND DNA BINDING ACTIVITY. Bruce M. Paterson, Kazuhiro Mitsui, and Masaki Shirakata, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892
The members of the MyoD family of transcriptional regulators are nuclear phosphoproteins but little is know about the role of phosphorylation in the regulation of their function. In order to study this question we have developed a convenient variation on the Baculovirus expression system that has allowed us to express reasonable amounts of phosphorylated protein for study. Our preliminary studies have looked at the phosphorylation of the avian MyoD homolog, CMD1. The phosphopeptide pattern for CMD1 produced in primary muscle cultures is very similar, if not identical, to that of the Baculo-CMD1 protein. Phosphoamino acid analysis of both proteins identifies only phosphoserine. Changes in the phosphorylation status of the protein alters its dimerization specificity as well as its DNA binding activity. Identification of the phosphorylated sites will help us to identify potential kinases involved in the modulation of CMD1 function. Models describing the CMD1 homodimer and the heterodimer with E12 will be presented with a view towards understanding the role of these phosphate groups in HLH protein function.

B 831 CONTROL OF INTRACELLULAR LOCALIZATION AND DEGRADATION OF c-FOS PROTEIN, Marc Piechaczyk, Serge CARILLO, Pierre ROUX, Ann-Muriel STEFF, Maryse ETIENNE-JULAN, Jean-Marie BLANCHARD and Philippe JEANTEUR, *Laboratoire de Biologie Moléculaire, UA CNRS 1191 "Génétique Moléculaire", place E. Bataillon -34095 - Montpellier Cedex 05 -France; Fax : (33) 67 14 33 93.*

c-fos proto-oncogene is a master switch converting short-term stimulations to long term-responses such as proliferation and differentiation. It is the cellular homolog of oncogenes carried by two tumorigenic murine retroviruses, FBR and FBJ. It encodes a short-lived transcription factor interacting with the members of the jun family into the AP1 transcription complex. Its regulation involves numerous transcriptional, post-transcriptional and post-translational levels of repression, the multiplicity of which likely explains why it has not yet been found constitutively expressed in natural tumors.

We have recently pointed to the existence of two new gene activity regulation levels :
(1) the transport of c-fos protein from the cytoplasm, where it is synthesized, into the nucleus, where it plays its part, is not constitutive but subjected to tight control by extracellular signals.
(2) c-fos protein is extremely instable into the cytoplasm where its degradation is initiated by calcium-dependent proteases, a situation that departs from the nuclear one.

Interestingly, FBR and FBJ viral proteins are constitutively transported into the nucleus and display lower sensitivity to cytoplasmic degradation, thus revealing two new mechanisms contributing to their tumorigenic potential.

B 832 LIGAND- AND PHOSPHORYLATION-DEPENDENT HETERODIMERIZATION AND DNA BINDING ACTIVITY OF THE bHLH DIOXIN RECEPTOR AND THE bHLH Arnt CO-REGULATOR. Lorenz Poellinger, Murray Whitelaw, Ingemar Pongratz and Anna Wilhelmsson; Department of Medical Nutrition, Karolinska Institute, Huddinge University Hospital F-60, Novum, S-141 86 Huddinge, Sweden
The intracellular basic region-helix-loop-helix (bHLH) dioxin receptor mediates signal transduction by dioxin and functions as a ligand-activated DNA binding protein directly interacting with target genes by binding to dioxin response elements. The purified, ligand-bound receptor showed poor, if any, affinity for target DNA. In contrast, efficient DNA binding by the receptor could be induced by addition of the structurally related bHLH factor Arnt. While Arnt exhibited no detectable affinity for the dioxin response element in the absence of the dioxin receptor, it strongly promoted the DNA binding function of the ligand-activated but not the ligand-free receptor forms. The ligand-free receptor form is characterized by stable association with hsp90 which is important for maintainance of a ligand-binding configuration of the receptor. Importantly, co-immunoprecipitation experiments showed that Arnt physically interacted with the ligand-activated dioxin receptor but failed to heterodimerize with the ligand-free, hsp90-associated receptor form, indicating that the dimerization interface may be blocked by hsp90. Thus, the dioxin receptor system also provides the first example of signal-controlled dimerization of bHLH factors. Dephosphorylation experiments indicated that both heterodimerization of the receptor and Arnt and the DNA binding activity of the heterodimeric complex is regulated by phosphorylation. In line with this model, down-regulation of PKC activity in vivo by TPA resulted in inhibition of both the DNA binding activity of the ligand-activated receptor and the function of the dioxin response element, suggesting a critical role of PKC in receptor function. (This work was supported by the Swedish Cancer Society).

B 833 UV-C INDUCED IMMEDIATE ACTIVATION OF MAP-2 KINASE AND C-JUN PHOSPHORYLATION
Adriana Radler-Pohl, Christoph Sachsenmeier, Stephan Gebel, Peter Herrlich, Hans J.Rahmsdorf
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Exposure of mammalian cells to growth factors and tumor promoters induces the transcription of the group of immediate early genes. Induced transcription does not require the synthesis of regulatory proteins and depends entirely on the posttranslational modification of transcription factors such as AP-1 (c-Jun/c-Fos). Posttranslational modification is caused by a signal transduction cascade, that starts at the cell membrane with the activation of receptors, proceeding to GTP-binding proteins and to cytoplasmic and nuclear protein kinases, such as raf and MAP-2 kinase. Here we show that UV-C also feeds in this signal transduction chain. Several UV-C inducible genes are controlled by AP-1 dependent promoters. We therefore analyzed UV-C dependent posttranslational modification of AP-1 and compared it to the modifications seen after phorbol ester treatment. The two agents, although both activating AP-1, did not only induce similar (e.g. phosphorylation of peptides x and y, dephosphorylation of peptide a and in c-Jun), but also different modifications. The phosphorylation of the peptides x and y may be due to MAP-2 kinase activation; the activated form of this kinase, which depends on threonine and tyrosine phosphorylation, is transiently found in TPA treated cells. MAP-2 kinase is also transiently activated in UV-C radiated cells. Thus the TPA and UV-C induced pathways must merge prior to MAP-2 kinase. The modifications found after UV-C may be due to the involvement of a growth factor loop in UV-C induced signal transduction: an inhibitor of growth factor/receptor interactions inhibits UV-C but not TPA induced signal transduction, and interleukin-1α (a growth factor secreted by UV treated cells) and UV-C induce identical posttranslational modifications of the c-Jun protein.

B 834 **DNA-BINDING AFFINITY OF NONHISTONE PROTEIN HMG-I/Y IS MODULATED BY $p34^{cdc2}$ KINASE PHOSPHORYLATION,** Raymond Reeves[+], Thomas A. Langan[#] and Mark S. Nissen[+]; [+]Dept. Biochem./Biophys., Washington State Univ., Pullman, WA 99163; [#]Dept. Pharmacol., Univ. Colorado Sch. Med., Denver, CO 80262.

Members of the HMG-I/Y family of nonhistone proteins are the first *mammalian chromatin proteins demonstrated to specifically bind to* the minor groove of A·T-rich regions of DNA both *in vitro* and *in vivo*. The HMG-I/Y proteins are of considerable interest because they have been experimentally implicated in a variety of important nuclear functions. These include participation in transcriptional regulation of a number of different genes whose promoter/enhancer regions contain HMG-I/Y binding sites and in the establishment of specialized chromatin structures in regions of A·T-rich DNA. Recently we determined the peptide domain(s) of the HMG-I/Y proteins that mediate *in vitro* A·T-DNA binding. Both computer modeling and two-dimensional ^{1}H NMR solution studies of the structure of synthetic DNA-binding domain (BD) peptides suggest that they represent a new peptide motif (the "A·T-hook") that specifically recognizes the structure of the narrow minor groove of A·T-DNA, rather than its sequence. We have also demonstrated that the DNA-binding, A·T-hook peptide domains of intact HMG-I/Y proteins are specific sites of phosphorylation by purified mammalian cdc2 (aka, histone H1) kinase *in vitro* and have further demonstrated that these same protein site(s) are phosphorylated *in vivo* in a cell cycle dependent manner by a cdc2 kinase-like enzyme. Quantitative *in vitro* studies indicate that the DNA-binding affinity of cdc2 kinase phosphorylated HMG-I/Y proteins is reduced greater than 20-fold compared to unphosphorylated proteins. Together these results strongly support the assertion that HMG-I/Y proteins are natural substrates for mammalian cdc2 kinase *in vivo* and that their cell cycle-dependent phosphorylation by this enzyme(s) significantly modulates their DNA-binding affinity thereby possibly altering their biological functions.

B 835 TYROSINE PHOSPHORYLATION IS REQUIRED FOR ACTIVATION OF AN INTERFERON-α-STIMULATED TRANSCRIPTION FACTOR, ISGF3, Nancy C. Reich, Christopher Daly and Michael J. Gutch, Department of Pathology, State University of New York at Stony Brook, Stony Brook, NY 11974

The signal transduction pathway of interferon-α utilizes tyrosine phosphorylation to transmit a signal generated at the cell surface to the transcriptional machinery in the nucleus. Activation of the interferon pathway initiates with the binding of interferon-α to its cell surface receptor. The ligand-receptor complex signals the activation of a latent cytoplasmic transcription factor. The active form of the interferon-stimulated gene factor (ISGF3) is phosphorylated on tyrosine residues. ISGF3 subsequently translocates to the nucleus and binds to a DNA sequence, the interferon-stimulated response element, found within the promoter of inducible genes. ISGF3 is a multicomponent factor consisting of four proteins of 113-kDa, 91-kDa, 84-kDa and 48-kDa. Three proteins of 113-kDa, 91-kDa and 84-kDa are phosphorylated on tyrosine residues after stimulation by interferon-α. Tyrosine phosphorylation is essential for activation of ISGF3. Genistein, a tyrosine kinase inhibitor, blocks the appearance of ISGF3, and blocks the transcriptional stimulation of interferon-induced genes. Tyrosine phosphorylation provides a link between the interferon-receptor complex at the plasma membrane and specific activation of gene expression in the nucleus. In addition, activation of the interferon-stimulated tyrosine kinase appears to occur in the absence of interferon during adenovirus infection or dsRNA transfection.

B 836 TYROSINE PHOSPHORYLATION REGULATES THE DNA BINDING ACTIVITY OF A HELA CELL NUCLEAR PROTEIN, Anne E. Reifel-Miller and Brian W. Grinnell, Departments of Diabetes and Cardiovascular Research, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN. 46285

We have previously shown that the enhancer of BK virus P2, BKV-P2, is repressed in HeLa cells and that the oncoproteins of adenovirus, the early region 1A proteins (E1A), induce further repression of the enhancer. In addition, we have demonstrated that a 98-kDa HeLa cell nuclear protein referred to as BEF-1 (BK virus enhancer factor 1) is responsible for both the endogenous and E1A-induced repression and that phosphorylation of this protein is required for its DNA binding activity (Reifel-Miller et al., 1991). We demonstrate here that dephosphorylation of BEF-1 with either potato acid phosphatase or calf intestinal alkaline phosphatase inhibits its DNA binding activity and that the inhibition of binding is reversed by sodium orthovanadate, a specific inhibitor of phosphotyrosyl-protein phosphatases. Using gel mobility retardation assays, we show that polyclonal or monoclonal phosphotyrosine-specific antibodies block binding of the repressor protein to the BEF-1 site. Transfection studies in HeLa cells demonstrate that E1A-induced repression can be relieved in a dose dependent manner with the tyrosine kinase inhibitor genistein. Therefore, a phosphotyrosine on the 98-kDa repressor protein, BEF-1, regulates its DNA binding activity and thus regulates repression of the BK virus enhancer.

B 837 **SOMATIC CELL MUTANTS IMPLICATE A THAPSIGARGIN-SENSITIVE CALCIUM CHANNEL AS AN ESSENTIAL SIGNALLING ELEMENT IN T CELL ACTIVATION,** **Andrew T. Serafini[*†], Richard S. Lewis[^†], Steve Fiering[*†], Leonard A. Herzenberg[*†] and Gerald R. Crabtree[#†], [*]Department of Genetics, [^]Department of Molecular and Cellular Physiology, [#]The Howard Hughes Medical Institute, [†]Beckman Center for Molecular and Genetic Medicine, Stanford University, Stanford CA 94305-5125**

A sustained rise in the concentration of intracellular calcium (Ca^{2+}) regulates a wide variety of intracellular processes, including the activation of early genes essential to T cell proliferation and acquisition of immune-effector function. The regulatory mechanisms controlling the increase in Ca^{2+} during T cell activation are poorly understood. We report a novel system to select cellular mutants in previously uncharacterized areas of the T cell activation pathway. The system utilizes a human Jurkat T cell line stably transfected with an NF-AT responsive diphtheria toxin construct. Upon activation by the calcium ionophore ionomycin and the phorbol ester PMA, wildtype cells of this line produce diphtheria toxin A chain *(dipA)* and die. Cells with mutations in the T cell activation pathway do not produce *dipA* and survive. Two of the selected mutant clones have a specific defect in Ca^{2+}-dependent signalling. Both mutant clones fail to make IL-2 and do not translocate the cytosolic component of NF-AT to the nucleus, however they do induce the IL-2 receptor normally. Unlike wildtype cells, depletion of intracellular Ca^{2+} stores in the mutants by treatment with thapsigargin fails to activate Ca^{2+} entry across the plasma membrane. However, transfection of the mutants with a construct expressing a constitutively active Ca^{2+}-independent calcineurin gene can overcome the signalling defect and reconstitute normal NF-AT activity. These results suggest that activation of thapisgargin-sensitive Ca^{2+} channels is a critical step in the regulation of sustained Ca^{2+} signals during T cell activation and that calcineurin is the major regulator of calcium-dependent early events in T lymphocytes.

B 838 PKA-Dependent Repression of Myogenic Differentiation and Activity of the Muscle-specific Transcription by Myf-5 and MyoDI. Barbara Winter, Thomas Braun and Hans-Henning Arnold, Department of Cell and Molecular Biology, TU of Braunschweig, Konstantin-Uhde Str.5, 3300 Braunschweig, FRG

The human muscle determination factor Myf 5 like MyoDI and the other members of the family of skeletal muscle-specific regulatory proteins, contains a highly conserved putativ basic helix-loop-helix (bH-L-H) domain. This motif is required for the myogenic capacity in C3H mouse 10T1/2 fibroblasts and other nonmuscle cells as well as for transcriptional activation of muscle genes. Site-directed mutagenesis defines two clusters of basic amino acids within the conserved basic region and two amphipathic helices within the adjacent H-L-H domain that are essential for sequence specific DNA binding and hetero-oligomerization., respectively. Transcriptional activation by Myf 5 requires two additional domains located in the amino- and carboxy-terminus of the protein. The two domains apparantly co-operate since deletion of either one results in inactivation. The biological activity of the Myf-5 protein is subject to negative regulation by serum components and peptide growth factors, such as bFGF and TGFβ. Pathways potentially involved in conveying these signals from the cell surface to the nucleus were probed by modulating the intracellular cAMP level in myoblasts. Addition of dibutyryl-cAMP and overexpression of cAMP-dependent protein kinase (PKA) repress myoblast differentiation and Myf-5 directed activation of muscle-specific reporter genes. This repression is mediated through the bH-L-H domain and the E-box sequence to which Myf-5 binds. Myf-5 constitutes a substrate for PKA-dependent phosphorylation in vitro suggesting that this modification may play an important role for regulation of this myogenic determination factor. In addition to the inactivation of Myf-5 and MyoDI by a posttranslational mechanism, we observed that the transcription of the myogenin gene is also repressed by cAMP and PkA. The present data suggest that phosphorylation of Myf5, either directly of indirectly modifies the transactivating capacity of this protein and therefore may play an important role for the regulation of the myogenic determination factors.

B 839 CREB IS DIRECTLY INVOLVED IN BOTH BASAL EXPRESSION AND HORMONE-MEDIATED INDUCTION OF THE PEPCK GENE, L.P. Xing and P.G. Quinn, Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

Transcription of the PEPCK gene is induced by cAMP and glucocorticoids. To investigate possible interactions between CREB, glucocorticoid response unit (GRU) binding proteins, and other trans-acting factors, block mutations were introduced into well defined factor-binding sites and WT or mutant plasmids were transfected into H4IIEC rat hepatoma cells -/+ a PKA catalytic subunit expression vector. PKA or DEX induced PEPCK-CAT activity by 7- or 5-fold, respectively, and PKA+DEX mediated a 22-fold induction. The effects of PKA and DEX, separately or together, were significantly decreased in CREmut and ΔGRU ($P<0.005$), suggesting functional interaction between these pathways. Mutation of other factor-binding sites located between the CRE and the GRU also decreased induction by DEX. However, induction by PKA+DEX was reduced only by mutation of the IRS within AF2 of the GRU. The DNA binding domain of GAL4 was substituted for that of CREB (CRG) and the CRE was replaced with a GAL4 binding site (G4PEPCK-CAT). In the absence of CRG, G4PEPCK-CAT behaved the same as CREmut. CRG elevated basal activity and restored responsiveness to PKA. Induction by DEX and DEX+PKA increased in proportion to basal activity. Therefore, CREB (CRG) clearly contributes to the level of transcription activation, but it does not appear to mediate the interaction produced by DEX+PKA. Concomitant treatment with CRG plus PKA and PKA inhibitor peptide, or mutation of the PKA phosphorylation site were equivalent to CRG alone, indicating that CREB possesses a basal activation capacity that does not require phosphorylation for its expression.

B 840 PHOSPHORYLATION OF THE E2F TRANSCRIPTION FACTOR

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The E2F transcription factor is a target of regulation by signals that control cell growth. During the cell cycle, E2F forms a complex with regulatory proteins that have been shown to be important for cell cycle progression. Several laboratories have demonstrated that E2F exists in a complex with p107, cyclin A and cdk-2. As a first step towards examining the functional relevance of this E2F complex, we investigated whether the E2F complex could be reconstituted and if E2F itself could be a substrate for cdk-2. E2F was overexpressed in bacteria and purified (generous gift of Wen-Hwa Lee and Bill Kaelin). Cyclin A, cdk-2, and p107 were produced by overexpression with baculovirus. Using these cloned and overexpressed components, we find that E2F can be reconstituted with p107, cyclin A and cdk-2 in vitro. As a result, E2F becomes phosphorylated in vitro by cdk-2. Phosphoamino acid analysis indicates that both serine and threonine residues are phosphorylated.

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Transcription Factors and Differentiation II

B 900 THE HUMAN CARDIAC TROPONIN I GENE : A MODEL FOR CARDIAC-SPECIFIC REGULATION.

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In contrast to the situation in skeletal muscle, the mechanisms regulating gene expression in cardiac muscle are poorly understood. No cardiac-specific transcription factors and, notably, no cardiac equivalents of the MyoD class of basic-helix-loop-helix (bHLH) factors which regulate gene expression and myogenesis in skeletal muscle, have been identified to date. One approach to identifying cardiac transcription factors is to examine the regulation of a gene expressed exclusively in cardiac myocytes. Genes encoding components of the contractile apparatus in striated muscle have been extensively used to investigate muscle gene regulation, but few of these exhibit cardiac-specific expression during development. We have chosen to examine the regulation of the human cardiac Troponin I (TnIc) gene as evidence suggests that this gene is indeed expressed only in cardiac myocytes, both during development and in the adult.

Analysis of TnIc protein and mRNA accumulation show that the human TnIc gene is expressed at a low level throughout cardiac development and that it is up-regulated at birth. We have isolated and sequenced the human TnIc gene including 1.1kb of the promoter and are currently analysing the regulatory elements of promoter region by cell transfection techniques, using a variety of cell lines and primary cultures of cardiac myocytes, in an attempt to identify regions which are required for expression and which direct tissue-specific transcription. Sequence analysis has identified several putative regulatory elements in the proximal promoter including an atypical TATA-box centred within a consensus RSRF-binding site sequence and, intriguingly, an E-box (bHLH binding)-like element.

B 901 FUNCTIONAL AND BIOCHEMICAL ANALYSIS OF A NEURAL-SPECIFIC PROMOTER. Landin Boring, Nerville Koeiman, Harald Haugen, Debbie Mineo, and Christopher Clegg, Department of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121.

Cyclic AMP-dependent protein kinase (cAPK) is a signal transduction enzyme that regulates cellular responses to numerous hormones and neurotransmitters. Expression of the type Iβ regulatory (R) subunit of cAPK is restricted to neurons of the CNS and pachytene-stage spermatocytes. To identify DNA sequences responsible for neural-specific expression, we are analyzing RIβ promoter/LacZ fusion constructs in transgenic mice and the Neuro-2A neuroblastoma cell line. In transgenic mice, tissue-specific expression is retained with as little as 700 bp of 5' sequence. This 5' region is rich in consensus sequences for known DNA binding proteins, including basic helix-loop-helix (bHLH) transcription factors. Gel-retardation assays demonstrate protein binding by extracts from brain nuclei, but not by proteins from non-neuronal cell nuclei. Mutational analysis confirms that the sequence CANNTG (bHLH consensus) is responsible for the protein binding activity. Expression of the RIβ/LacZ fusion genes in N2-A cells suggest that transcription is regulated by both positive and negative elements within the 700 bp 5' region. Further studies should allow the precise identification of relevant neural-specifc enhancer elements within the RIβ promoter.

B 902 PROTEINS INVOLVED IN THE CONTROL OF c-MYC TRANSCRIPTION DURING DIFFERENTIATION OF HEMATOPOIETIC CELL LINES, Linda M. Boxer, Department of Medicine, Stanford University School of Medicine and Veterans Administration Medical Center, Stanford, CA 94305

c-myc expression decreases during the differentiation of certain hematopoietic cell lines such as HL-60 and K562. Initially a block to transcription elongation is responsible for the decrease in c-myc message. In vivo footprinting by ligation mediated polymerase chain reaction has been performed to identify binding sites of proteins that may be involved in the regulation of c-myc transcription during differentiation. This technique allows one to examine protein-DNA interactions in the intact cell. A protein footprint located near DNase hypersensitive site II_2 disappears during differentiation of HL-60 and K562 cells. There is also a change in a protein footprint near DNase hypersensitive site III_2 in the region between the two promoters. This region has been shown to be required for the block to transcription elongation. Other regions in the c-myc promoter and the 3' end of the first exon and the 5' region of the first intron where the block to transcription elongation is located are also being examined for protein footprints.

B 903 INOSITOL TRISPHOSPHATE RECEPTOR GENE TRANSCRIPTION IN HORMONE AND CYTOKINE-TREATED HUMAN MYELOID LEUKEMIC CELLS, **Peter G. Bradford, Youyu Jin, and Pei Hui, Department of Pharmacology, SUNY at Buffalo, Buffalo, NY 14214**

The calcium-mobilizing inositol trisphosphate ($InsP_3$) receptor is essential for chemoattractant and neurokinin-stimulated secretion from human neutrophils and monocytes. Precursor myeloid cells and undifferentiated leukemic cell lines (K562 and HL-60) express only minimal levels of signal transduction proteins including the $InsP_3$ receptor and exhibit diminished functional capacities, facts contributing to the morbidity and mortality associated with myeloid leukemias. Retinoic acid (RA) and 1,25-dihydroxyvitamin D_3 ($VitD_3$) drive the granulocytic and monocytic differentiation of HL-60 cells with concurrent acquisition of signal transduction functions and appropriate cellular responsiveness. $InsP_3$ receptor expression and Ca^{2+}-mobilizing activity are increased following either RA or $VitD_3$ treatment through a mechanism that involves the enhanced transcription of the $InsP_3$ receptor gene. The transcriptional enhancing effects of RA and $VitD_3$ at the $InsP_3$ receptor gene do not require protein synthesis suggesting that these effects are mediated directly by hormone-specific response elements within the $InsP_3$ receptor gene. Transcript levels and functional expression of the $InsP_3$ receptor are also increased by IL-3 and GM-CSF in the more pluripotent K562 myeloblast cells, suggesting the existence of additional $InsP_3$ receptor gene transcription factors which are regulated by cell surface receptor-dependent phosphorylation dephosphorylation mechanisms. The mapping of these and other promoter/enhancer sequences in the $InsP_3$ receptor gene will be presented.

B 904 IDENTIFICATION OF ZINC FINGER AND SRY-LIKE TRANSCRIPTION FACTORS EXPRESSED IN HUMAN HEART. Nigel J. Brand, Nina Dabhade, Pankaj K. Bhavsar, Magdi H. Yacoub and Paul J. R. Barton. Department of Cardiothoracic Surgery, National Heart and Lung Institute, London SW3 6LY, UK.

Though many of the genes encoding contractile proteins of striated muscle have been cloned, little is known about the regulatory mechanisms controlling their expression in cardiac muscle. In order to understand how cardiac gene regulation is achieved, we are attempting to clone novel transcription factor cDNAs from cardiac muscle by (i) polymerase chain reaction (PCR) cloning using degenerate oligonucleotide primer sets specific for conserved domains of known transcription factor gene families and (ii) direct screening of cardiac cDNA libraries. Using PCR we have cloned six factors expressed in human heart which contain a region of homology with the recently described testis-determining factor SRY/sry. These factors, named SOX for SRY-box (1), are expressed in both adult atrial and ventricular muscle. We have also screened human cardiac cDNA libraries for C_2H_2 zinc finger factors using radiolabelled oligonucleotide and cDNA probes. Several clones have been identified, including the human homologue of the murine gene *mkr5*. Also, we have cloned (in collaboration with Dr. Arthur Zelent, Leukaemia Research Fund Centre, Chester Beatty Institute, London) a novel C_2H_2 cDNA called PLZF (for *P*romyelocytic *L*eukaemia *Z*inc *F*inger), which is related in sequence to the Drosophila factor *kruppel*. PLZF was identified as a fusion gene in promyelocytic leukaemia caused by a t(11:17) chromosomal translocation which fuses, in frame, the 5' part of the PLZF gene to part of the retinoic acid receptor α (RAR-α) gene, resulting in the expression of a novel *fusion transcript* (2). We are currently characterising the expression of these genes in cardiac and other tissues.

1. P Denny, S Swift, N Brand, N Dabhade, P Barton and A Ashworth (1992) NAR 20:2887.
2. Z Chen, NJ Brand, A Chen, S-J Chen, J-H Tong, Z Wang, S Waxman and A Zelent (1992) Submitted for publication.

B 905 INTERFERON-γ INDUCIBLE AND CONSTITUTIVE TRANSCRIPTION OF THE HUMAN MHC CLASS II INVARIANT CHAIN GENE: *IN VIVO* FOOTPRINT AND FUNCTIONAL ANALYSIS. Adrienne M. Brown, Kenneth L. Wright, Bernd Stein and Jenny P.-Y. Ting. Department of Microbiology and Immunology, University of North Carolina-Chapel Hill, Chapel Hill, N.C. 27599

MHC class II molecules and class II-associated invariant chain (Ii) are physically associated within the cell, functionally related, coordinately regulated at the level of transcription and yet Ii is encoded outside the MHC locus on a separate chromosome. Coordinate expression is detected at the level of cell type specificity and induction by IFN-γ. The MHC class II promoter regions share well defined elements, W/S, X and Y which are all necessary for maximal levels of basal expression as well as IFN-γ induced expression. In addition many of the DNA-binding proteins that interact with the MHC class II promoter have been identified and their genes cloned.

Ii and the class II genes share some mechanisms of transcriptional regulation and not others. We have demonstrated that similar to other class II genes, the human Ii X and Y elements are necessary for constitutive expression in B lymphocytes and that Ii S, X and Y are required for IFN-γ induced expression in the glioblastoma cell line U373-MG. These regulatory elements function in a parallel manner with those of the class II gene HLA-DRA. The *in vivo* genomic footprint of Ii correlates with these functional elements and displays a pattern strikingly similar to that of HLA-DRA. Ii footprint reveals protein interactions on the X and Y elements in B cell lines and in class II-expressing T cell lines. In addition IFN-γ treatment clearly upregulates binding at the X box while all other contacts remain the same.

The Ii *in vivo* footprint also delineates two NF-κB binding sites, a motif absent in the HLA-DRA promoter. Surprisingly, functional analysis of the proximal NF-κB element revealed it to exert a positive regulatory effect in B cells and the T cell line, Jurkat, but to have negative regulatory function in the T cell line H9. The mechanism for this dual activity is under investigation.

B 906 KID-1: A NOVEL POU GENE EXPRESSED IN THE HUMAN FETAL KIDNEY, Christopher R. Burrow, Michael G. Kauffman, Patricia D. Wilson, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The discovery of transcription factors which direct epithelial differentiation from mesodermal progenitor cells is a critical step in the identification of molecular control mechanisms regulating specification of the diverse, and segmentally organized, cell phenotypes of the renal tubular epithelium during kidney organogenesis. The potential importance of a tissue specific POU gene in renal development was suggested by the discovery of OCT-K, a novel octamer binding protein present in nuclear extracts prepared from 16 week human fetal kidney. OCT-K has an electrophoretic mobility similar to OCT-2 but is not recognized by OCT-2 specific antisera. Systematic cloning of all POU gene cDNA's expressed in the human fetal kidney at this developmental stage using a mixed degenerate primer PCR approach identified two genes, OCT-1 and a new member of the POU-III subclass, KID-1. A comparison of the predicted amino acid sequence of KID-1 with two closely-related human POU genes, BRN-1 and BRN-2, revealed multiple substitutions in predicted helical regions I and II of the POU homeodomain establishing KID-1 as a newly identified human POU gene. In situ hybridization analysis localizes KID-1 expression exclusively to proliferative epithelial cells in the fetal kidney collecting ducts and suggest a role for this POU gene in the regulation of epithelial differentiation at this site. This segment of the embryonic nephron gives rise to two functionally distinct tubular epithelial cells, the principal cell and the intercalated cell, a developmental specification event which may be influenced by KID-1 expression.

B 907 A NOVEL TRANSCRIPTIONAL ENHANCER IS INVOLVED IN THE PROLACTIN- AND EXTRACELLULAR MATRIX-DEPENDENT REGULATION OF ß-CASEIN GENE EXPRESSION. G.F. Casperson+, C. Schmidhauser*, C. A. Myers*, K. T. Sanzo+, S. Bolten+, and M. J. Bissell*, *Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, U. of California, Berkeley, California 94720; +Monsanto Corporate Research, AA3C, Chesterfield Village Parkway, Chesterfield, MO 63198

Lactogenic hormones and extracellular matrix (ECM) act synergistically to regulate ß-casein expression in culture. We have developed a functional subpopulation of the mouse mammary epithelial cell strain COMMA-1D (designated CID 9), which expresses high level of ß-casein, forms alveolar structures when plated onto the EHS tumor-derived matrix, and apically secretes ß-casein. We have further shown that ECM- and prolactin-dependent regulation of ß-casein occurs mainly at the transcriptional level and that 5' sequences play an important role in these regulations. To address the question of the nature of the DNA sequence requirements for such regulation, we analyzed the bovine ß-casein gene promoter in these cells. We now have located a 160-bp transcriptional enhancer (BCE1) within the 5' flanking region of the ß-casein gene. Using functional assays, we show that BCE1 contains responsive elements for prolactin- and ECM-dependent regulation. BCE1 placed upstream of a truncated and inactive ß-casein promoter (the shortest extending from -89 to +42 bp with regard to the transcription start site) reconstitutes a promoter even more potent than the intact promoter, which contains BCE1 in its normal context more than 1.5 kb upstream. This small fusion promoter also reconstitutes the normal pattern of regulation, including a requirement for both prolactin and ECM and a synergistic action of prolactin and hydrocortisone. By placing the milk promoter with a heterologous viral promoter, we show that BCE1 participates in the prolactin- and ECM-mediated regulation.

B 908 IDENTIFICATION AND CHARACTERIZATION OF HLH PROTEINS EXPRESSED DURING ORGANOGENESIS,

Anne E. Chiaramello, Dan H. Shain, Tomaas Neuman and Mauricio X. Zuber, Department of Biochemistry and Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523

Helix-loop-helix (HLH) transcriptional factors are known to regulate many developmental processes in Drosophila and vertebrates. We have cloned members of the class A HLH transcription factors family from the developing mouse nervous system. Sequence analyses of the cDNAs show the homology to E12/E47 to reside exclusively in the helix-loop-helix region. Our data on gene analysis suggets that as a result of alternative splicing, there are four different transcripts encoding HLH proteins with different physical and biochemical characteristics, one of these named ME1a corresponding to the murine form of HTF4 or HEB (Nucl. Acids Res. (1991) **19**, 4555; Mol. Cell. Biol. (1992) **12**, 1031-1045). Northern blot and in situ hybridization analyses show different temporal and spatial pattern consistent with a role as developmental regulator. Various inducible expression plasmids were constructed that program *Escherichia coli* to produce recombinant ME1 HLH derivatives. Their binding activities were studied using electrophoretic mobility shift assay (EMSA). These studies were extended to compare binding abilities of homo and heterodimers to different E-box binding sites in vitro. The different forms of ME1 HLH proteins have been cloned in eukaryotic expression vectors to analyze their in vivo characteristics.

B 909 THE IL3/GM-CSF LOCUS IS REGULATED BY AN INDUCIBLE CYCLOSPORIN A SENSITIVE ENHANCER

Peter N. Cockerill, M. Frances Shannon, Andrew G. Bert, Gregory R. Ryan and Mathew A. Vadas, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide 5000, Australia

Interleukin-3 (IL3) and granulocyte-macrophage colony stimulating factor (GM-CSF) are pleiotropic haemopoietic growth factors whose genes are located just 10 kb apart on human chromosome 5. Both genes are induced in T lymphocytes upon activation of the T cell receptor, in a cyclosporin A (CsA)-sensitive fashion. We observed, however, that the proximal promoters of these genes responded poorly to signals normally delivered via the T cell receptor, and that they were not suppressed CsA. We therefore mapped DNase I hypersensitive (DH) sites across the locus to identify additional regulatory elements which might account for the observed regulation of each gene. We located an inducible DH site, 3 kilobases upstream of the GM-CSF gene, which was suppressed by CsA, and appeared just before the onset of IL3 and GM-CSF transcription. This DH site functioned as a strong CsA-sensitive enhancer of both the GM-CSF and IL3 promoters. The enhancer is a likely target for protein kinase C and Ca^{2+} mediated signals resulting from activation of the T cell receptor. Three sites in the enhancer associate with the CsA-sensitive T cell-specific transcription factor NFAT, but represent a new class of NFAT site since they also associate with the transcription factor AP-1 in the absence of other CsA-sensitive components of NFAT. These NFAT sites may, therefore, also support the activation of the enhancer in cell types other than T cells, which express GM-CSF and AP-1 but not NFAT. We provide additional evidence that AP-1 is an essential component of NFAT. We propose that the intergenic enhancer described here mediates the correctly regulated activation of both GM-CSF and IL3 gene expression in T cells, and that it is responsible for the CsA-sensitivity of the GM-CSF/IL3 locus. Future studies will determine whether the enhancer functions as a locus control region, and whether it is also required for expression of the nearby IL4 and IL5 genes.

B 910 THE REGULATION OF GENE EXPRESSION IN MULTIPOTENT HAEMOPOIETIC PROGENITOR CELLS,

Michael Cross, Reinhard Henschler, Ian Hampson, Clare Heyworth, Erika deWinter and T. Michael Dexter, Department of Experimental Haematology, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Manchester M20 9BX, U.K.

Our aim is to examine the mechanisms by which differentially expressed genes are regulated at the transcriptional level during haemopoiesis, and to investigate ways in which changes in the transcriptional environment are involved in the processes of lineage committment and maturation.

In response to defined combinations of haemopoietic growth factors, the FDCPmix (Factor Dependent Cell Paterson mixed potential) cell lines can be made either to proliferate and maintain a multipotential phenotype, or to embark upon programmes of differentiation which lead over a period of about one week to mature, post-mitotic erythroid or granulocyte/macrophage cells. As such they present an attractive system in which to analyse the molecular processes governing self renewal and differentiation.

Differential screening of FDCPmix cDNA libraries has led to the identification of a number of cDNA species which are down-regulated upon the induction of granulocyte/macrophage differentiation. We have optimised an electroporation procedure for the introduction of DNA into the FDCPmix cells, and are using the technique to identify transcriptional regulatory sequences controlling the differentially expressed genes. An analysis of the regulation in these cells of the CCPI (granzyme B) gene will be presented.

B 911 ANALYSIS OF CIS-ACTING ELEMENTS AND DNA-BINDING PROTEINS REGULATING TRANSCRIPTION OF MOUSE AND CHICKEN αA-CRYSTALLIN GENE,

Ales Cvekl, Marc Kantorow, John F. Klement, Christina M. Sax and Joram Piatigorsky, LMDB, NEI, NIH, Bethesda, MD 20892.

αA-crystallin is a highly abundant protein found in the ocular lens. The expression of this gene is developmentally and spatially regulated during formation of the lens. There are significant differences in the expression pattern of the αA-crystallin in the mouse and chicken. Previous transfection and transgenic mouse experiments identified a lens-preffered promoter from -111 to +46 of the mouse αA-crystallin gene; the chicken gene needs at least 162 bp upstream from the transcription initiation site for its activity in transfected lens epithelial cells. Footprinting and linker scanning analysis of the mouse αA-crystallin promoter have identified four protein binding sites responsible for function of this promoter: DE1 (-111/-99), αA-CRYBP1 (-72/-55), PE1 (-35/-12) and PE2 (+24/+43). The DE1 site (5'CTGCTGACGGTGC 3') is homologous to the binding site for the ATF/CREB family of factors. Gel-shift data and partial protein purification suggest interaction of this family of factors with the DE1 site. Although a strong homology was noted between the mouse and chicken αA-crystallin promoter up to position -111, the linker scanning analysis of -160/+3 region of the chicken αA-crystallin promoter revealed the importance of -144/-103 sequence as well as of the TATA box (-33/-22). The chicken -144/-103 sequence is composed of at least three protein binding sites: DE2A (-144/-134), DE2B (-128/-119) and DE1A (-114/-103). None of these sites is homologous to any known cis-element of the mouse gene. These data show that a highly conserved gene may have considerable species-dependent differences in its regulation.

B 912 REGULATION OF THE ACTIVITY OF THE TRANSCRIPTION FACTOR GATA-1, Wei Dai and *Martin J. Murphy, Jr.*, Hipple Cancer Research Center, 4100 S. Kettering Blvd., Dayton, OH 45439-2092

Phorbol myristate acetate (PMA) induces the expression of megakaryocyte and/or platelet proteins during terminal differentiation of human erythroleukemia HEL cells. However, it is not established whether the megakaryocytic differentiation is accompanied by the down-regulation of the major erythroid transcription factor GATA-1. In addition, little is known about the mechanism regulating the activity as well as the expression of GATA-1. Using gel mobility shift assays, we have obtained the following evidence concerning the effects of PMA and other chemical agents on the target affinity of GATA-1: (i) Upon PMA treatment, HEL cells dramatically decrease the expression of GATA-1 activity which is accompanied by a loss of the erythroid-specific protein glycophorin A expression. In contrast, the DNA binding activities of AP-1 and SP-1 transcription factors are up-regulated by PMA treatment of HEL cells. The coordinated negative regulation of GATA-1 activity and glycophorin A mRNA after PMA treatment suggests that down-regulation of GATA-1 activity is at least partly responsible for the loss of the erythroid phenotype during megakaryocytic differentiation; (ii) The DNA binding affinity of GATA-1 can be reduced in vitro by treatment of cell nuclear extracts with the nucleotide ATP and the non-hydrolyzable ATP analog AMPP(NH)P but not with AMP, suggesting that GATA-1 is at least partly regulated through allosteric mechanisms; (iii) Cytosolic fraction of cell lysates also contains significant amounts of GATA-1 activity and this activity is not due to nuclear contaminations during isolation as determined by other marker protein expression. The non-ionic detergent Chaps enhances both cytoplasmic and nuclear GATA-1 activity in vitro, suggesting that a detergent sensitive inhibitor(s) may be involved in regulating its target affinity and thus erythroid gene expression during cell differentiation.

B 913 SEVERAL TRANSCRIPTION FACTORS WITH A LEUCINE ZIPPER STRUCTURE CAN PARTICIPATE IN THE MODULATION OF THE RAT α-FETOPROTEIN GENE EXPRESSION

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The α-fetoprotein (AFP) gene offers a very powerful model to gain insights into the molecular mechanisms which govern liver-specific gene expression in the course of development and cancerogenesis.

In the present work we have tested the effect on the functioning of the rat AFP regulatory elements of several transcription factors which share in common a dimerization domain rich in leucines. For this, plasmids allowing expression of these transcription factors were cotransfected together with vectors carrying the CAT gene under the control of rat AFP gene regulatory elements into human HepG2 hepatoma cells .

We observed that expression of the liver-enriched factors C/EBP α, LAP (C/EBPβ) and DBP stimulated (5 to 7 fold) the CAT activity driven by the AFP promoter. By opposition, LIP, a truncated form of LAP obtained by an alternative use of a translation initiation site had a negative effect on the activity of the AFP promoter. These results suggest that a combination of these proteins, whose concentrations greatly vary in the course of liver development and which can specifically bind to two critical regions of the AFP promoter, might be used to modulate the transcription of the AFP gene in the course of development.

We showed that Jun proteins are able to down regulate (5 to 8 fold) the CAT activity driven by the AFP promoter ; c-Jun was more efficient than Jun D ,while Jun B and Fos alone had no effect. In agreement with these results, phorbol esters (TPA) also down regulated the AFP promoter .The specificity of these effects was assessed by using constructs in which the CAT gene is governed by the Tk promoter and a functional or a mutated TRE as controls. Jun/Fos complexes can thus be intermediates in cascades of different cellular events initiated by growth factors and hormonal stimuli specific of the differentiation and of the proliferation status of the liver cell and which result in the modulation of the AFP gene expression. Whether the negative regulation of the AFP promoter acitivity by these proto-oncogenes in the HepG2 cells involves direct binding of an AP1 complex to the AFP promoter or not is presently under investigation.

Thanks are due to Drs B. Binetruy, M. Karin, S.McKnight, U. Schibler and M. Yaniv for their gifts of plasmids .This work was supported by the CNRS, and by grants from the ARC, the FRM and the INSERM to J-L.D.

B 914 IDENTIFICATION OF GENOMIC SEQUENCES REQUIRED FOR THE INDUCTION OF STROMELYSIN BY NERVE GROWTH FACTOR (NGF) AND BASIC FIBROBLAST GROWTH FACTOR (bFGF), Sunita deSouza, Cindy M. Machida, Janis E. Lochner and Gary Ciment, Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201

In this study, we examine NGF- and bFGF-responsive DNA regulatory sequences in the 5'-untranscribed region of the stromelysin (ST-1) (a.k.a. transin) gene in PC12 cells. We found that both NGF and bFGF induces ST-1 mRNA expression at least 1000-fold over initially undetectable levels in PC12 cells. We found, moreover, that this induction was due to *de novo* transcription and was sensitive to cyclohexamide, indicating that ST-1 is a "late" gene. Transient transfection assays using a plasmid containing a 750 base pair region of the 5'-untranscribed region of the ST-1 gene fused to CAT reporter gene indicated that this region contained NGF- and FGF-responsive cis-acting elements. We have performed transient transfection assays using various manipulations of the ST-1 promoter region. We find that mutations of an AP1 sequence within this promoter is necessary for both basal and growth factor-induced levels of CAT gene expression. Using 5' promoter deletion mutants, we also find other, as yet unidentified, DNA sequences necessary for growth factor-responsiveness of ST-1. Gel-shift analysis is currently being performed to determine wether transcriptional proteins bind to these regions in a growth factor-specific fashion. These studies suggest that the induction of the ST-1 gene in PC12 cells may be a useful system for characterizing and comparing the NGF and bFGF intracellular signalling pathways.

B 915 TTF-1, AN HOMEODOMAIN CONTAINING PROTEIN, AND PAX-8, A PAIRED DOMAIN CONTAINING PROTEIN, BIND TO OVERLAPPING SITES IN TWO THYROID SPECIFIC PROMOTERS AND ACTIVATE TRANSCRIPTION, Roberto Di Lauro§, Mario De Felice§, Helen Francis-Lang¶ and Mariastella Zannini§, §Stazione Zoologica A. Dohrn, Villa comunale, 80121, Naples, Italy, ¶ICRF, Developmental Biology Unit, South Parks Rd, Oxford OX1 3PS, Great Britain.

TTF-1 is a member of a new family of homeobox genes. The TTF-1 protein binds to the thyroglobulin and thyroperoxidase promoters, two genes exclusively expressed in thyroid cells. Expression of TTF-1 in some non thyroid cells in colture results in transcriptional activation of co-transfected thyroglobulin and thyroperoxidase promoters. Such activation depends on the structural integrity of two coarsely defined TTF-1 domains, located at the amino and the carboxy terminal side of the homeodomain. Using artificial promoters containing one or several binding sites for TTF-1, it can be shown that TTF-1 requires multiple binding sites in the target promoter to function as transcriptional activator.

In rats, TTF-1 is expressed in thyroid, lung and restricted areas of the brain. In thyroid, TTF-1 expression begins at the onset of thyroid morphogenesis, five days before the expression of thyroglobulin and thyroperoxidase. These data suggest that additional mechanisms are required for transcriptional activation of thyroid specific promoters by TTF-1.

Thyroid cells also contain the paired domain containing protein Pax-8. Binding sites for Pax-8 are present in the promoters of thyroglobulin and thyroperoxidase. In both promoters the binding site of Pax-8 overlaps with a TTF-1 binding site, the closest to transcription initiation. Pax-8 also can activate transcription from both promoters. The possible functional implications of this arrangement of binding sites are at present under investigation.

B 916 CHARACTERIZATION OF A NOVEL DNA/PROTEIN TRANSCRIPTION COMPLEX ON THE PROMOTER OF THE RAT ALPHA-1-ACID GLYCOPROTEIN GENE.

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The alpha-1-acid glycoprotein gene in rat liver is transcriptionally activated by glucocorticoid and interleukins 1 and 6. Glucocorticoids act through a GRE (Glucocorticoid Responsive Element) which is centered around -110. 35 nucleotides upstream this sequence is present a strong element (URE, Upstream Regulatory Element, -143-155) which bind nuclear proteins essential for the activity of the glucocorticoid receptor. This sequence show a perfect dyad symmetry. Deletion mutants and point mutation analysis that block the binding of transcription factors to this element completely inhibit glucocorticoid transcription activation of a -215+1 promoter fragment linked upstream the CAT gene.

Footprint experiments and EMSA (Electophoresis Mobility Shift Assay) revealed the binding of at least three different proteins. Column fractionation of rat liver nuclear extract allowed the distinction between the transcription factor C/EBPß and a protein we named ANF-4 (AGP Nuclear Factor 4). The binding sites of these two protein are partially overlapping and share their accomodation on the URE with a third protein probably related to IL-6 REBP, a protein active on the alpha-2-macroglobulin promoter during the acute phase reaction.

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B 917 EXPRESSION OF Fork Head GENES DURING EARLY Xenopus DEVELOPMENT, Marie Luise Dirksen, Andra Miller, and Milan Jamrich, Laboratory of Molecular Pharmacology, CBER, FDA, Bethesda, MD 20892

Using PCR technology we have isolated cDNA clones which belong to a new Xenopus gene family of transcriptional factors - those containing a fork head DNA binding domain. This gene family appears to have more than twenty members. They are expressed throughout the entire embryonic development. Some of the genes are expressed maternally, others in early development, and others in adult tissues. Their expression pattern suggests that they are involved in pattern formation and cellular differentiation. We are specifically concentrating on fork head genes which are activated at the beginning of zygotic transcription in Xenopus embryos. Our experiments indicate that some of these genes are involved in regionalization of the neural plate during gastrula and early neurula stage of Xenopus development.

B 918 CHARACTERIZATION AND TARGETED DISRUPTION OF THE MURINE TEF GENE, D.W. Drolet, R.J. McEvilly, K.A. Kalla, K.J. Jenne, and M.G. Rosenfeld, HHMI, Dept. Medicine, University of California, San Diego, La Jolla, CA 92093-0648.

The anterior pituitary gland is composed of five phenotypically distinct cell types that are distinguished by the peptide hormone they express. These hormones regulate many different physiological systems including milk production, growth, and basal metabolic rate. The thyrotroph cells of the anterior pituitary are responsible for the production of thyroid-stimulating hormone which controls the levels of T_3 and T_4 produced by the thyroid gland. Investigation into the DNA-binding proteins expressed in pituitary cells from rats has led to the identification and characterization of thyrotroph embryonic facrtor, TEF, a basic-leucine zipper (bZIP) transcription factor (*Genes Dev.* 1991. 5:1739-1753). In addition to the bZIP domain, TEF contains a proline and acidic rich (PAR) domain that is amino-terminally adjacent to the bZIP domain. Highly conserved PAR regions are also found in the bZIP proteins DBP (rat) and HLF (human) as well as in the TEF homologue, VBP (chicken). The role of the PAR domain, however, has not been elucidated. In the rat, TEF gene expression is initially detected in the rostral tip of the anterior pituitary on embryonic days 14 to 16. This expression pattern corresponds both temporally and spatially to the onset of thyroid-stimulating hormone gene expression. Later in development, however, TEF is expressed in many cell types throughout the animal.

Here we report the characterization of the murine TEF-gene including the promoter sequence and location of intron-exon boundaries. A single exon was found that contained only the PAR domain. In order to gain insight into the developmental and regulatory role(s) of TEF, we have initiated the production of TEF-deficient mice using targeted homologous recombination in embryonic stem cells. A replacement-type targeting vector containing 8.4 Kbp of the murine TEF gene was constructed with the neomycin resistance gene inserted in place of sequences coding for the PAR and bZIP domains of TEF. After positive-negative selection, 19 out of 145 individual clones were determined to be homologous recombinants based on DNA-blot analysis. Several of these clones have been injected into blastocysts and chimeric mice have been generated. These mice are being bred in order to score for the presence of germ line chimeras.

B 919 DEVELOPMENTAL REGULATION OF XENOPUS LAEVIS HISTONE H2A AND H2B GENE TRANSCRIPTION, Heithem M. El-Hodiri and W. Michael Perry, Department of Biochemistry and Molecular Biology, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Box 117, Houston, Texas 77030.

Histone gene transcription is DNA replication-independent in Xenopus oocytes and early embryos, but becomes replication-dependent later in embryogenesis. One of the goals of our laboratory is to identify promoter elements involved in this switch. We are currently focusing of the histone H2A and H2B genes. In the xlh3 histone H2A and H2B are transcribed in opposite directions from initiation sites located approximately 250 base pairs apart. The promoter region is relatively simple, containing several known transcriptional control elements, including two TATA motifs, three CAAT motifs, an octamer-like motif, and an ATF/CREB binding site. To study the contributions of different promoter elements in the developmental control of H2A and H2B transcription, genes with mutated or deleted promoter regions are microinjected into oocytes and embryos. Analysis of mRNA isolated from injected oocytes and embryos will lead to the identification of acting elements involved in DNA replication-dependent and independent expression of these two genes, as well as the regulation of their separate or coordinate transcription.

B 920 LEUCINE ZIPPER-DEPENDENT INHIBITION OF CREB ACTIVITY IN cAMP UNRESPONSIVE CELLS. EVIDENCE FOR AN INHIBITOR OF CREB DNA BINDING.

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Cell lines vary considerably in their ability to activate transcription through promoters that contain a cAMP response element (CRE). For example, in differentiated F9 cells and JEG cells a transfected somatostastin promoter responds to increased intracellular cAMP, whereas in HeLa cells, COS cells and undifferentiated F9 cells it does not. The basis for this difference is not variation in the levels of the cAMP response element binding protein CREB or protein kinase A, which are present in all these cell types at similar levels. Since overexpression of these factors in normally unresponsive cells leads to activation of the somatostatin promoter, it appears that CREB activity can be negatively regulated by a titratable inhibitor (inhibitor of CREB or ICR)[1].

Further evidence for this inhibitor was obtained by fusing CREB to the DNA binding domain of GAL4. In cAMP unresponsive cells, a GAL4-CREB fusion only weakly activates a GAL reporter construct. However, fusions which contain mutations in the leucine zipper domain of CREB show significantly enhanced activity, suggesting that the inhibitor interacts with this region. This inhibition is reflected at the level of DNA binding in extracts from unresponsive cells which have been transfected with clones which encode these fusions. Thus, the presence of the CREB leucine zipper interferes with DNA binding to both a CRE and a GAL UAS, indicating that ICR acts as a general inhibitor of DNA binding.

[1]Masson, N., (1992). Mol. Cell. Biol. 12: 1096-1106.

B 921 TRANSCRIPTIONAL REGULATION OF INDUCIBLE IMMUNE GENES IN DROSOPHILA. Ylva Engström, Gunnel Björklund, Erik Roos and Latha Kadalayil, Department of Molecular Biology, Stockholm University, S-106 91 Stockholm, Sweden.

The *Drosophila Cecropin (Cec)* gene family encodes a set of powerful anti-bacterial peptides, synthesized as part of an inducible immune response. The main sites of *Cec*-gene activity after induction are localized to the fat body and to hemocytes (1). Our aim is to characterize the *cis*- and *trans*-acting control elements of *Cec* gene regulation, necessary for proper spatial and inducible expression. Different sets of promoter-reporter (*Cec-lac Z)* fusion genes were constructed and the expression of ß-galactosidase (ß-gal) was analyzed, both in a *Drosophila* hemocyte cell line by transient expression and in stably transformed *Drosophila* larvae and flies, after P-element mediated transformation.

Several of the *Cec-lac* Z fusion gene constructs were indeed effectively activated after the induction of an immune response by (LPS) lipopolysaccharide. The presence of a conserved 40 bp sequence was crucial for inducible reporter gene expression in hemocytes and in fat body. Interestingly, this 40 bp element contains a κB-like motif, which is the binding site of the mammalian transcription factor NF-κB (2). A trimer of the κB-like motif, but not of a mutated version, confers high levels of inducible reporter gene expression. An immunoresponsive factor, DIF, binding to such κB-like motifs was recently isolated from *Hyalophora cecropia*, and shown to share many characteristics with mammalian NF-κB (3). We have identified an inducible nuclear factor from *Drosophila* cell extracts that specifically binds to the κB-like motif and we have named this new factor *Drosophila* immunoresponsive factor, DIF.

(1) Samakovlis, C. et al. (1990), EMBO J. **9**, 2969-2976.
(2) Leonardo, M.J. and Baltimore, D. (1989), Cell **58**, 227-229.
(3) Sun, S.-C. and Faye, I. (1992), Eur. J. Biochem. **204**, 885-892.

B 922 REGULATION OF THE TATA-LESS TERMINAL TRANSFERASE GENE IN DEVELOPING LYMPHOCYTES, Patricia Ernst, Kiersten Lo, Ben Hahm, and Stephen Smale, Howard Hughes Medical Institute and Department of Microbiology and Immunology, U.C.L.A. School of Medicine, Los Angeles, California 90024

A fundamental question in the study of gene expression is how upstream activators communicate with the general transcription machinery to regulate cell-type specific transcription. We are addressing this question for a class of promoters which lack TATA boxes using the terminal deoxynucleotidyltransferase (TdT) gene regulatory elements as a paradigm. Interestingly, many genes that are expressed transiently during B or T cell development, including TdT, λ5, VpreB, and lck, do not contain TATA boxes in their promoters. This unusual promoter architecture may be essential for the appropriate regulation of these lymphocyte stage-specific genes. Therefore, the study of lymphocyte-specific elements as well as basal elements will allow us ultimately to understand how tight regulation of these genes is accomplished.

Deletions and mutations in a region termed D' (located from -79 to -49 relative to the transcription start site) result in dramatically reduced transcript levels in lymphoid cells as compared to non-lymphocytes. A novel protein called LyF-1 binds to this functionally important site and has been purified from T cells. LyF-1 is enriched in lymphocytes and binds to the promoters of a number of other TATA-less, lymphoid-specific genes. In addition, members of the ets family of DNA-binding proteins, including ets-1 and fli-1, bind tightly to a site directly overlapping that of LyF-1. Since fli-1, ets-1, and LyF-1 are all present at high levels in cells that express TdT, this suggests that TdT expression may require the simultaneous activity of LyF-1 and an ets family protein, and suggests also that there may be a physical interaction between LyF-1 and an ets-related protein. Using a series of 5 bp mutations which span the D' site, we have begun a detailed characterization of the binding of ets family proteins and LyF-1 to the TdT promoter, and have correlated binding of these putative transcription factors with TdT transcription in vivo. A model incorporating this functional data will be presented.

B 923 TGFβ1 INDUCES *c-ets-1* EXPRESSION IN ENDOTHELIAL CELLS. Fafeur, V., Vercamer, C., Raes, M.B., Stéhelin, D., and Vandenbunder, B. CNRS URA 1160. Laboratory of Molecular Oncology. Pasteur Institute of Lille. Lille. France

The expression of the proto-oncogene *c-ets-1* is detected in various physiological situations that involve extensive cell proliferation and migration, such as neural crest migration, new bone formation and new blood vessel formation (angiogenesis) (Vandenbunder et al., Development, 107: 265-274, 1989; Wernert et al., Am. J. Pathol., 140:119-127, 1992). The role of *c-ets-1* during these processes is unknown. By investigating the regulation of *c-ets-1* expression by angiogenic factors in endothelial cells, we found that tumor necrosis factor-alpha (TNFα), basic fibroblast growth factor (bFGF) and transforming growth factor-beta type 1 (TGFβ1) increase *c-ets-1* mRNA expression, with TGFβ1 being the most potent of these factors. The fact that *c-ets-1* expression is induced both under growth stimulatory (bFGF-induction) and growth inhibitory (TNFα- and TGFβ1-induction) conditions suggests that *c-ets-1* is probably not involved in controlling cell proliferation. TGFβ1 is also the most potent stimulator of extra-cellular matrix formation and this effect involves the stimulation of the expression of various genes, including fibronectin and plasminogen activator inhibitor-type 1. After 18 h of TGFβ1 stimulation, both an increase of expression of these genes and of *c-ets-1* was observed, whereas the expression of urokinase-plasminogen activator was unaffected. These data and the fact that *c-ets-1* encodes a nuclear protein which is a transcription factor, suggest that the role of *c-ets-1* during angiogenesis might be to control the expression of genes involved in controlling extra-cellular matrix production.

B 924 Structural and Function of the Mouse Bone Morphogenetic Protein 2 (BMP 2) Gene: Analysis of the Promoter/Enhancer Region in Fetal Rat Calvariae (FRC) Osteoblast Primary Cultures.
Nandini Ghosh-Choudhury[1], B. Christy[2], M. A. Harris[1], G. R. Mundy[1] and S. E. Harris[1]. [1]University of Texas Health Science Center, Department of Medicine, Division of Endocrinology, San Antonio, TX, 78284-7877; [2]Institute of Biotechnology, San Antonio, TX 78245.

Recombinant human BMP2 by itself can induce ectopic bone formation in vivo. We are studying expression of BMP2 in primary cultures of fetal rat calvarial (FRC) osteoblasts. BMP2 expression is found to be temporal in long term "bone" forming FRC cultures, peaking at 8-10 days. BMP 2 expression is coincident with the expression of the osteoblast differentiation markers, such as, alkaline phosphatase, osteopontin and osteocalcin. We are analyzing the mechanisms of regulation of BMP2 expression in these differentiating bone cells. A 10.8kb mouse BMP2 transcription unit and its 5' flanking sequences have been cloned and sequenced. Near the transcription start site for the gene, as mapped by primer extension technique, contains 8 out of 9 bp similar to DNA binding sequences for the transcription factor Zif268. By gel retardation assay we have shown that this sequence specifically binds to the Zif268 protein. We have constructed a number of CAT constructs with variable lengths of mBMP2 5' flanking sequences. Transient transfection studies using these constructs in primary FRC cultures, have led us to identify regions upstream of mBMP2 transcription unit that are modulated by various factors, such as, BMP2, TGFβ and 1,25 $(OH)_2D_3$. One region positively responded to exogenously added BMP2 (autoregulation), and the BMP 2 response region is negatively moduled by 1,25$(OH)_2D_3$. We will discuss these results in terms of a model in which 1,25$(OH)_2D_3$ acts as an "organizer" of well formed in vitro "bone". We are presently in the process of fine mapping these response elements, and analyzing their interactions.

B 925 **Id-REGULATED TRANSCRIPTION FACTORS ARE NOT INVOLVED IN MATURATION OF VASCULAR SMOOTH MUSCLE CELLS.** David J. Grainger, Paul R. Kemp, Peter L. Weissberg & James C. Metcalfe. Department of Biochemistry, University of Cambridge, England.

We have identified several stable clonal types of vascular smooth muscle cells (VSMCs) in culture. These include type C VSMCs with cobblestone morphology which predominate in cultures from neonatal rats, type A VSMCs with spindle morphology which predominate in cultures from adult rats and type E cells which are multinucleate, do not proliferate and whose proportions increase with the age of the rat. These observations suggested a VSMC lineage. In support of this, clones of type C VSMCs can be can be converted to type A in culture by treatment with TGFβ and type A VSMCs convert to type E when plated at low cell densities. By contrast, type C VSMCs are unaffected by low cell density. A number of features in addition to morphology distinguish type C from type A VSMCs. For example, more than 50% of type A VSMCs transfected with the MyoD gene form myotubes in the absence of serum while type C VSMCs similarly transfected show no change.

By analogy to skeletal muscle myogenesis, we hypothesized that the maturation lineage of type C converting to type A may be controlled by a smooth muscle equivalent of MyoD acting as a heterodimer with E2A. In skeletal muscle, E2A activity is suppressed by the action of the helix-loop-helix protein Id. Thus constitutive expression of Id in type C VSMCs may prevent conversion of type C VSMCs to type A.

To test this hypothesis, VSMCs from a type C VSMC clone were transfected, by electroporation, with a dexamethasone-inducible antisense Id gene. We have shown that this construct will induce myogenesis in MyoD-expressing type A cells in the presence of serum. After addition of dexamethasone (1μM) for 48h all the cells retained the cobblestone morphology typical of type C VSMCs, even when plated at low cell densities. Furthermore, ectopic expression of MyoD did not induce any myotube formation. Thus no maturation of the type C VSMCs had occurred. We conclude that Id-regulated transcription factors are not involved in VSMC maturation.

B 926 **CHARACTERIZATION OF THE DISTAL α-FETOPROTEIN ENHANCER, A STRONG, LONG DISTANCE, LIVER-SPECIFIC ACTIVATOR,** Elyse Groupp and Joseph Locker, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

The stage-specific expression of α-fetoprotein (AFP) in developing hepatocytes is regulated by 6 kb upstream of the cap site. Of the 3 enhancers within this region, the most distal ("Complex 3", at -6 kb) is the strongest in $HepG_2$ cells. Computer studies suggested multiple transcription factor binding sites, and footprint analysis, using $HepG_2$ nuclear extracts, confirmed binding over these sites. High resolution deletion demonstrated 4 regions in the enhancer. Enhancers with regions I or II deleted, though weaker, could stimulate the AFP promoter from long distance. However, Region I was essential for stimulation of the tk promoter. Region III increased enhancer strength but was not essential, while region IV complemented some deletions, but had no other obvious effects. 1. Complex 3 can stimulate transcription not only through the AFP promoter, but also the heterologous albumin and tk promoters; with these promoters, the enhancer functions in hepatic cell lines where the AFP gene is silent. 2. Deletion analysis mapped a core activity to a 340 base pair region between -5780 to -6122. Progressive deletion of either the 5' or 3' end identified 84 base pairs which accounted for 90% of the activity associated with the enhancer. Gel shift and oligonucleotide competition suggest that region I has a strong HNF3 binding site, with weaker sites for HNF4, SRF and an unidentified factor. Region II binds C/EBP- and SP1-like factors. Region III analysis is in progress. Complex 3 is thus a strong unique liver-specific enhancer that resembles several liver-specific promoters. (Supported by NIH Grant CA 43909).

B 927 PHYLOGENETIC FOOTPRINTING OF THE HUMAN ε GLOBIN GENE: 46 NUCLEAR PROTEINS ARE DETECTED BY 20 CONSERVED SEQUENCE PROBES, Deborah L. Gumucio, David A. Shelton, Wendy L. Bailey, Kenji Hayasaka, Jerry Slightom and Morris Goodman, Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616.

The human ε gene is expressed at high levels in embryonic erythrocytes and completely repressed in fetal and adult erythoid cells. The same dramatic changes in transcriptional activity are exhibited by the orthologous ε genes of all mammalian species. Thus, the *cis* sequences which control this process are likely to be evolutionarily conserved. Using "phlyogenetic footprinting", 20 conserved sequence elements upstream from the ε gene have been identified and the nuclear proteins which bind to oligonucleotide probes spanning these elements have been characterized. Among the 46 proteins detected are eight binding sites for the YY-1/NF-E1/CSBP-1 repressor. In other systems, this protein acts as a "switch" protein, mediating a change from gene repression to activation and is involved in the developmental stage-specific repression of another tissue-specific gene (Igκ). Seven sites for a novel protein (CSBP-2), and five binding sites for the putative stage selector protein, -50γ were also detected. In addition to the detection of individual binding proteins, this analysis also revealed several interesting binding patterns. Six of the 7 probes which bound to CSBP-2 also bound CSBP-1; 7 of 9 probes binding GATA-1 also bound Oct-1. Several additional examples of this co-detection of Oct-1 and GATA-1 were detected in an earlier analysis of the γ promoter. Furthermore, examination of the sequences of the probes which bind GATA-1 revealed that relatively high affinity binding is observed with probes which do not contain good matches for the canonical GATA site. It seems highly likely that the regulatory mechanisms which control ε expression are extremely complex and that multiple control elements will eventually be found. Characterization of the proteins which bind at or near evolutionarily conserved sites provides a means to begin to dissect this complexity in a way that cannot be approached by gross deletional mutagenesis. The highly conserved cis sequences identified here represent potentially important elements which have been identified by binding characteristics rather than transcriptional function. It is possible, with the information we now have, to design direct functional tests to analyze the contribution of individual binding proteins, to probe possible interactions among binding proteins or to determine the relevance of binding sites with sequences which deviate from the consensus.

B 928 TRANSCRIPTIONAL ACTIVITY OF THE MOUSE ENGRAILED PROTEINS, Mark Hanks, Frederick Jones* and Alexandra Joyner, Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, 600 University Avenue, Toronto, Ontario, Canada. *Scripps Research Institute, North Torrey Pines Rd, La Jolla, CA 92037.

The *engrailed* (*en*) genes have been cloned from a number of vertebrate and invertebrate species. Amino acid sequence comparisons between members of the *en* family reveals five highly conserved regions, which we have designated EH1-5, including the 60 amino acid homeodomain (EH4). Mutant analysis in *Drosophila* reveals that the *en* gene is involved in conferring posterior compartment identity and expression studies suggest an additional role in neurogenesis. There is also considerable genetic and biochemical evidence to indicate that the *en* protein is a transcriptional repressor. In the mouse, mutant analysis has shown that the *En-2* gene is required (at least) for patterning in the cerebellum, but the role of the *En-1* gene is only now being addressed.

We have shown by southwestern blotting that the mouse En-2 homeodomain is capable of binding to the same sequence (NP6) recognized by the *en* protein of *Drosophila*. However, in contrast to the *Drosophila en* protein (Jaynes and O'Farrell, 1991), in transient transfection assays we have found that *En-1* and *En-2* are unable to act as active transcriptional repressors of reporter genes containing the NP6 sequence. A caveat to the simple interpretation of these data is that the cells (P19 and COS) used for these transfections may lack the protein co-factors necessary for *En* repressor function. However, such factors may have been present in Schneider cells which were used for the *Drosophila en* transfections mentioned above and we are in the process of testing the mouse *En* proteins in the Schneider cell system.

Direct evidence that the *En-1 and En-2* proteins are transcriptional regulators stems from our observations that both of the *En* proteins will activate transcription from *LacZ* reporter genes driven by the chick cytotactin (tenascin) promoter in transient cotransfections into P19 and NIH 3T3 cells. This promoter contains a number of potential homeodomain binding sequences within a 4 kb 5' flanking region. However, deletion analysis of this 5' flank indicates that the sequences sufficient for the activation are located within 1kb of the TATA box, a region which contains only one potential homeodomain binding site. We intend to identify the *En* binding site(s) in this 1kb region to determine if the activation is a direct result of *En* protein binding. This system can then be used to carry out a structure function analysis of the *En* proteins and to determine the functional significance of the conserved protein domains mentioned above.

B 929 MODULATION OF COLONY STIMULATING FACTOR-1 EXPRESSION BY CTF/NF1, Maureen A. Harrington, Bruce Konicek and Xiao-ling Xia, Department of Medicine, Biochemistry and Molecular Biology and the Walther Oncology Center, Indiana University School of Medicine, Indpls, IN.

Monocyte/macrophage cell activity is regulated in part an interaction between circulating and tissue specific monocytes/macrophages and stromal elements which include fibroblasts, adipocytes, reticular and endothelial cells. One role of the stromal cell elements is to release cytokines like colony stimulating factor-1 (CSF-1) which functions to stimulate the proliferation, differentiation and activity of monocytes. The observation that fibroblasts produce and do not respond to CSF-1 whereas monocytes can both produce and respond in a proliferative manner to CSF-1 prompted us to examine whether there are tissue specific differences in the control of CSF-1 expression. With heterologous reporter constructs we determined that deletion of a region between nt -152 and -88 results in a 4-fold decrease in CSF-1 promoter activity in proliferating fibroblasts and monocytes. Competitive band shift assays were used to demonstrate specific DNA binding activity to a fragment containing this region in nuclear extracts isolated from fibroblasts, that was not due to SP1 or AP1 which have putative binding sites in this region. Results of DNase I protection assays with nuclear extracts from proliferating fibroblasts or monocytes revealed distinct as well as overlapping regions of protein binding in the region between nt -283 and +17. Protein binding to the region spanning nt -90 to -68 was detected in both fibroblasts and monocytes. Synthetic oligonucleotides containing this region used in competitive band shift and methylation interference assays strongly suggest CTF/NF1 is the cognate trans-acting factor. Results of our studies suggest multiple trans-acting factors may regulate CSF-1 expression, some of which may be tissue specific while others such CTF/NF1 are shared in common.

B 930 SIGNAL TRANSDUCTION BY KINASE C THROUGH CLASSICAL CRE'S, Jonathan H. Hecht, Joachim Altschmied and Pamela L. Mellon, Department of Reproductive Medicine, University of California, San Diego, School of Medicine, La Jolla, CA 92093-0674

Gonadotropin-releasing hormone (GnRH), which acts through a seven-transmembrane receptor coupled to the kinase C pathway, activates transcription of the genes for the gonadotropin hormones, follicle stimulating hormone and luteinizing hormone, which are heterodimers composed of a shared α chain and a unique β chain. The mechanism by which GnRH regulates the common α gene was studied in LβT202 cells, an immortalized pituitary gonadotrope cell line made by targeted oncogenesis in transgenic mice. Our studies detected three DNA elements which contribute to pituitary expression of the α gene: a gonadotrope-specific element (GSE) centered at -220, a GATA element centered at -146, and two cAMP-response elements (CRE's) located at -141 and -123. Transfecting a series of truncations revealed that the deletion from -152 to -118, eliminating the CRE's and the GATA site, reduced GnRH induction from 6.9 fold to 1.2 fold. Point mutations which inactivated the CRE, GATA, or both elements, reduced induction from 5.2 fold to 1.7, 2.5, and 1.0 fold, respectively. Isolated DNA elements cloned upstream of the thymidine kinase promoter were also tested for GnRH responsiveness. Two CRE's activated transcription 6.3 fold and two GATA sites activated transcription 4.3 fold, indicating that both sites can confer GnRH responsiveness, a surprising result since GnRH activates protein kinase C, but not kinase A. Two TPA-response elements (TRE's) were induced 5.7 fold, indicating that GnRH activated AP-1 as expected. Gel retardation assays showed that LβT202 nuclear extracts contained proteins that shifted the CRE, producing three complexes. After treating cells with GnRH, the major complex became significantly more abundant. The induced component of this complex was specifically competed by a TRE. Though CREB was also present, it was not changed in GnRH-treated extracts. To test whether the regulated component may be AP-1, the TRE was shifted with LβT202 nuclear extracts. After one hour of GnRH treatment, TRE binding activity increased. An anti-fos antibody supershifted a portion of this TRE binding activity. Northern hybridization indicated that *fos* mRNA is present 30 min after GnRH treatment and peaks after 1-2 hours of treatment. Since fos is a component of AP-1, our data indicate that the GnRH signal transduction pathway may activate transcription of the α gene through a member of the AP-1 family binding to CRE's.

B 931 A DNA ELEMENT THAT REGULATES EXPRESSION OF AN ENDOGENOUS RETROVIRUS DURING F9 CELL DIFFERENTIATION IS E1A-DEPENDENT, Chin C. Howe, Bruce T. Lamb, Kapaettu Satyamoorthy, Davor Solter, Amitabha Basu, Roberto Weinmann and Mei Q. Xu, The Wistar Institute of Anatomy and Biology, 3601 Spruce Street, Philadelphia, PA 19104

The retinoic acid-induced differentiation of F9 cells into parietal endoderm (PE)-like cells activates transcription of the endogenous mouse retrovirus, the intracisternal A-particle (IAP). To investigate the elements that control IAP gene differentiation-specific expression, we used methylation interference and Southwestern and transient transfection assays, and identified the IAP proximal enhancer (IPE) element that directs differentiation-specific expression. We find that the IPE is inactive in undifferentiated F9 cells and active in differentiated PE-like PYS-2 cells. Three proteins of 40, 60, and 68 kDa in size bind to the sequence GAGTGAC located between nucleotides -53 and -47 within the IPE. The 40- and 68-kDa proteins from both the undifferentiated and differentiated cells exhibits similar DNA binding activities. However, the 60-kDa protein from differentiated cells has greater binding activity than that from undifferentiated cells, suggesting a role for this protein in F9 differentiation-specific expression of the IAP gene. The IAP gene is negatively regulated by the adenovirus E1A proteins, and the E1A sequence responsible for repression is located at the N-terminus between amino acids 2 to 67. The DNA sequence that is the target of E1A repression also maps to the IPE element. Co-localization of the differentiation-specific and E1A-sensitive elements to the same protein binding site within the IPE suggests that the E1A-like activity functions in F9 cells to repress IAP gene expression. Activation of the IAP gene may result when the E1A-like activity is lost or inactivated during F9 cell differentiation, followed by binding of the 60 kDa-positive regulatory protein to the enhancer element.

B 932 MULTIPLE ZINC FINGER FORMS RESULTING FROM DEVELOPMENTALLY REGULATED ALTERNATIVE SPLICING OF A TRANSCRIPTION FACTOR GENE, Tien Hsu, Joseph A. Gogos, Dennis L. King and Fotis C. Kafatos, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

A *Drosophila* transcription factor CF2 has been shown to regulate the temporal transcription of a chorion (eggshell protein) gene. *In situ* antibody staining suggests that CF2 may also play roles in late embryonic development. Transcripts encoding CF2 are subject to three alternative modes of splicing, which are under strict developmental control and result in three isoforms that differ in the number of zinc fingers. One testis-specific isoform includes a frame-shifted segment of drastically different amino acid sequence. The other two isoforms bind *in vitro* to distinct target sequences and different promoters, as predicted by the modularity of DNA recognition by zinc finger motifs. The temporally and spatially specific expression of different CF2 isoforms underlies their multiple roles during development, especially in male and female gonads. Thus, because of alternative splicing, a single gene generates *in vivo* multiple distinct DNA-binding proteins, which could act as developmental switches, regulating different gene sets in different tissues and developmental periods.

B 933 DEVELOPMENTAL REGULATION OF A SILKMOTH GENE ENCODING "GATA-1" TYPE TRANSCRIPTION FACTORS BY ALTERNATIVE SPLICING. K. Iatrou, J. Drevet and Y.A.W. Skeiky. Department of Medical Biochemistry, Faculty of Medicine, University of Calgary, Calgary, Alberta, T2N 4N1, Canada.

Band shift and DNA footprinting assays have been used to identify proteins that bind specifically to conserved promoter elements of late silkmoth chorion genes. The binding site of a major chorion promoter DNA-binding factor, BCF1, contains a perfect match to the consensus binding site of the transcription factors known collectively as "GATA-1" factors. We have isolated a genomic DNA clone, ZF1, whose sequence, upon conceptual translation, shows extensive similarities to those of the GATA-1 factors. The GATA-1-like zinc finger motifs in ZF1 are separated by a single intron. Southern hybridizations have established that ZF1 is a single copy gene, while Northern analyses revealed the presence of a 3.5 kb transcript in follicular cells. RT-PCR amplification of follicular RNA using primers that border the zinc finger domain of ZF1 resulted in the isolation of two cDNA sequences, ZF1S and ZF1L, which differ from each other by the presence (absence) of a strech of 14 amino acids in the spacer region separating the two zinc fingers. Considering that only one ZF1 gene exits in *Bombyx*, we conclude that the expression of ZF1 is regulated by alternative splicing. ZF1 hnRNA processing is developmentally regulated: while ZF1L mRNA is constitutively present in follicular cells, ZF1S mRNA accumulates preferentially in choriogenic follicles which express late chorion genes. The zinc finger domains of ZF1S and ZF1L mRNA were also over-expressed and used in band shift assays. Both domains are able to bind specifically to the BCF1 recognition sequences of the chorion gene promoters. A polyclonal antibody recognizing the short zinc finger domain of ZF1 was used to confirm that factor BCF1 contains the ZF1S domain and to show that ZF1 (presumably BCF1) present in follicular cells has a molecular weight of 60 kD. Nucleotide sequence analysis of ZF1 cDNA clones is in progress.

B 934 ROLE OF bHLH PROTEINS IN ANTERIOR PITUITARY DIFFERENTIATION, Stephen M. Jackson, Arthur Gutierrez-Hartmann and James P. Hoeffler, University of Colorado Health Science Center, Denver, CO 80262

Members of the basic-helix-loop-helix (bHLH) class of transcription factors are involved in the expression of many cell- and tissue-specific genes. These factors appear to share the ability to bind to DNA containing the loose consensus sequence CANNTG and can form heterodimers with other HLH family members quite readily. This study takes advantage of these shared characteristics to begin to address whether or not bHLH proteins are involved in the establishment of the highly differentiated cells which populate the anterior pituitary gland. Gel shift analysis using an oligonucleotide containing a CANNTG sequence demonstrates that a number of factors present in αT3 gonadotrope, GH3 somatolactotrope and αTSH thyrotrope pituitary cell nuclear extracts bind specifically to this probe, suggesting that bHLH proteins are indeed present in these cells. To further examine whether bHLH proteins are involved in differentiation, RNA derived from adult rat pituitary tissue and the above cell lines was examined for the presence of Id, an inhibitor of bHLH activity. Id mRNA was found in each of the cell lines but was absent in the adult rat pituitary. Far-western analysis of pituitary cell nuclear extracts using radioiodinated Id as a probe demonstrates that Id interacts with at least 6 proteins in these cells, ranging in size from 70kD to 190kD. In addition, southwestern analysis using the CANNTG oligonucleotide reveals at least 7 proteins which can bind to this probe. Comparison of far- and southwestern data reveals proteins of 70kD, 120kD and 190kD which can bind to both probes. To determine whether Id has an effect on the phenotype of pituitary cells, transient cotransfection experiments were undertaken using α-subunit promoter/luciferase fusions and an Id overexpression vector in pituitary cells. Overexpression of Id specifically reduces the expression of the α-subunit reporter gene in αT3 and αTSH cells up to 4-fold. Similar experiments in GH3 cells using growth hormone- and prolactin-luciferase constructions showed Id had little specific effect on the expression of these genes. Taken together, these data suggest that bHLH proteins are present in pituitary cells and that their activity is regulated by Id. Since the α-subunit gene is the first known pituitary marker to appear in developing tissue, its expression may be activated by lineage-specific factors. Because Id inhibits expression of α-subunit in pituitary cells, it is possible that one of these lineage-specific factors could be a member of the bHLH family.

B 935 REPRESSION OF TRANSCRIPTION BY THE ENGRAILED HOMEODOMAIN PROTEIN IN CELL CULTURE AND IN EMBRYOS, James B. Jaynes, Dept. of Microbiology and Immunology, Thomas Jefferson Univ., Philadelphia, PA 19107

The Engrailed homeodomain protein is required in Drosophila embryos for specification of cell fates in posterior compartments and in the nervous system. Engrailed functions in cultured cells as an "active" or dominant repressor of transcription of target genes which contain Engrailed binding sites. This action does not appear to require displacement of activators from the DNA, since the activity is observed over considerable distances, and since DNA binding is not sufficient for the repression activity. An in vivo functional assay, involving retargeting of Engrailed repression sequences via a homeodomain swap, provides evidence that such repression is one function of Engrailed in the embryo.

B 936 CELL TYPE-DEPENDENT REGULATION OF GENE EXPRESSION OF A NONMUSCLE MYOSIN HEAVY CHAIN, Sachiyo Kawamoto, Rajesh K. Chopra and Gregory Kitagawa, Laboratory of Molecular Cardiology, NHLBI, NIH, Bethesda, MD 20892

The myosins found in both muscle and nonmuscle cells comprise a family of proteins having similar native structure, subunit composition and functional properties. We previously identified two gene products for nonmuscle myosin heavy chains (MHCs) and demonstrated tissue and cell type-dependent expression for the two MHC mRNAs, as well as changes in mRNA expression associated with cell growth and differentiation.

To understand the mechanisms responsible for regulating the expression of nonmuscle MHC genes, we cloned the 5' portion of a human MHC gene. The promoter region lacks consensus sequences such as TATA and CAAT, but is rich in GC sequence. Transcriptional activities have been monitored using luciferase as a reporter. Following transfection of various luciferase constructs into NIH 3T3 fibroblasts, a fragment of ~175 bp (115 bp upstream and 60 bp downstream from the major transcriptional start site) was found to possess core promoter activity, which was equivalent to the known viral core promoter activity. The fragment containing the core 175 bp as well as an additional ~150 bp of downstream sequence shows a 10-fold increase in luciferase activity following transfection into NIH 3T3 cells. In contrast to its effect in NIH 3T3 cells, this downstream sequence does not activate luciferase expression in differentiated skeletal muscle cells (mouse C2C12 myotubes). In these cells, the endogenous mRNA for this gene is also down-regulated. Gel shift assay using the 150 bp DNA fragment demonstrated a different type of complex with NIH 3T3 nuclear proteins compared to C2C12 myotube nuclear proteins.

B 937 CHARACTERIZATION OF THE MOLECULAR MECHANISMS INVOLVED IN THE INDUCTION OF HEMATOPOIETIC TRANSCRIPTION FACTORS Clair Kelley and Leonard Zon. Harvard Medical School, Division of Hematology, Children's Hospital, Boston, MA

The zinc-finger DNA binding proteins GATA-1 and -2, and the helix-loop-helix transcription factor SCL (tal-1), serve as useful markers for studying blood formation during development. The Xenopus embryo is ideally suited for the study of early embryonic inductive events. Blood is derived from the most ventral mesoderm and is histologically evident at 36 hours after fertilization. By in situ analysis, all three genes are expressed in ventral regions of the embryo several hours before globin gene expression. GATA-2 is expressed at high levels during early gastrulation, and is an excellent early marker of ventral mesoderm. It is not detected in embryos "dorsalized" by treatment with LiCl, and is expressed in a radially symmetric pattern in embryos "ventralized" by ultraviolet light. SCL, which has been proposed to be activated by the GATA DNA binding proteins, is expressed in a similar distribution to and slightly later than GATA-2. Animal pole cells from blastula stages are fated to be ectoderm, but can be induced to form mesoderm with peptide growth factors. Using a quantitative RT-PCR assay, GATA-1 is detected at low levels in uninduced animal pole explants. However, the currently available mesoderm inducing factors are not capable of inducing physiologically relevant levels of GATA-1, or subsequent blood formation in animal pole explants. Ventral marginal zone (VMZ) and dorsal marginal zone (DMZ) regions were explanted at the early gastrula stage and cultured until blood island formation. In situ hybridization of GATA-1, SCL, and globin indicates that the expression of these genes is localized within a distinct region in VMZ explants. Western blot analysis demonstrates the protein products are expressed at high levels in VMZ, but not DMZ explants. Therefore, the VMZ region from the early gastrula stage embryo contains an activity, which induces and maintains hematopoiesis. Further characterization and localization of this activity requires the ability to distinguish induction in the VMZ from induction in multipotential animal cap cells. For this purpose, a sensitive assay was developed to examine the ability of VMZ regions to induce blood formation. X. laevis and X. borealis interspecies recombinants were generated between animal pole and VMZ explants, and tested for the induction of GATA-1 and globin transcripts using species-specific PCR primers. Preliminary experiments indicate that the inducer is spatially and temporally regulated within the ventral marginal zone. Our results demonstrate an active induction of mesoderm to form hematopoietic tissue, as opposed to a model implicating blood formation as the "default pathway" for mesoderm formation.

B 938 **Id GENE EXPRESSION ANTAGONISES MyoD-INDUCED MYOTUBE FORMATION IN VASCULAR SMOOTH MUSCLE CELLS,** Paul R.Kemp, David J.Grainger, James C.Metcalfe and Peter L.Weissberg. Department of Biochemistry, Cambridge University, Cambridge. CB1 2QW

We have previously shown that the Id gene is expressed in vascular smooth muscle cells (VSMCs) in culture in response to stimulation by serum but is very weakly expressed in quiescent cells in serum-free medium. The product of the Id gene is thought to be a negative regulator of specific transcription factors for skeletal muscle and immunoglobulin genes through its ability to form heterodimers with the products of the E2A gene. The E2A gene products have been shown to dimerise with the transcription factor MyoD and these heterodimers trans-activate skeletal muscle-specific promoters in many cell types in which MyoD is endogenously or ectopically expressed. Id is therefore assumed to prevent MyoD function by competing for E2A and forming inactive heterodimers. We examined whether Id is a negative regulator of MyoD function in VSMCs using MyoD-dependent formation of myotubes as an assay for MyoD activity.

We infected adult rat aortic VSMCs with a retrovirus carrying the MyoD gene (MDSN) and selected cells which had incorporated the virus with geneticin. A small percentage of these cells formed myotubes (4±0.6%) in medium containing 10% foetal calf serum (FCS). This proportion was increased to 51.2±5.6% by the removal of serum, when expression of the Id gene is greatly reduced. To investigate further the involvement of the Id gene in the regulation of MyoD activity, the VSMC/MDSNs were transfected by electroporation with a sense or antisense Id gene under the control of a dexamethasone-inducible promoter. Approximately 10% of the cells which survive this electroporation process are transfected with the constructs.

	10% FCS		serum free	
dexamethasone	-	+	-	+
untransfected	4.0±0.6	1.1±0.6	51.2±5.6	35.8±9.7
antisense Id	4.3±1.1	9.7±2.7*	51.5±6.9	54.5±4.0*
sense Id	4.0±0.6	1.6±0.7	49.5±10.1	36.9±9.7

(Data are % nuclei in myotubes ± SEM, *p<0.05 t test vs untransfected)

These data suggest that Id expression in VSMCs in the presence of serum may prevent myotube formation in cells expressing MyoD and that Id may therefore be a negative regulator of E2A function in these cells.

B 939 EXPRESSION OF POU DOMAIN TRANSCRIPTION FACTORS IN A NEURONAL CELL LINE

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Certain members of the POU domain family of transcription factors are believed to play an important role in the development of the nervous system. In this regard He *et al.* (Nature **340**, 35-42) used PCR, in conjunction with degenerate oligonucleotides corresponding to conserved regions of the POU domain, to isolate several novel POU domain transcription factors expressed in the rat brain. Using the same approach as He *et al.* we have isolated four distinct sets of POU domain cDNAs from an ND7 cell line. This cell line was obtained by fusing post mitotic sensory neurons, derived from rat dorsal root ganglia, with the mouse N18 neuroblastoma cell line. Three of these sets of clones correspond to the previously described Oct-1, Oct-2 and Brn-3 (Brn-3A) POU domain transcription factors. In addition to this, however, a fourth clone was isolated that encodes a novel POU domain protein (Brn-3B) that is identical to Brn-3A with the exception of seven amino acid substitutions throughout the POU domain.

To date eight differentially spliced mRNAs encoding different isoforms of Oct-2 have been identified in the mouse. Using PCR to detect the different isoforms of Oct-2 mRNA we demonstrate that neuronal cells, when compared to B-cells, contain elevated levels of the Oct-2.4 and Oct-2.5 isoform mRNA. In addition to this, using PCR to specifically detect the two related forms of Brn-3 mRNA, we show that Brn-3A is present at high levels in mature sensory neurons, whilst Brn-3B is barely detectable in this tissue. Furthermore, whilst the level of Brn-3A mRNA increases from a basal level during the *in vitro* differentiation of ND7 cells, the levels of Brn-3B mRNA decreases. The implications of this distinct, yet overlapping expression pattern of POU domain transcription factors in neuronal cells will be discussed.

B 940 TWO ELEMENTS WHICH ARE INACTIVE ON THEIR OWN CONFER CELL SPECIFICITY TO THE POMC PROMOTER, Thomas Lamonerie, Marc Therrien, Christian Lanctôt and Jacques Drouin, Laboratoire de génétique moléculaire, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA H2W 1R7

The pro-opiomelanocortin (POMC) gene is expressed in a subset of hormone producing cells of the pituitary gland. Previous studies from our laboratory have shown that a 543 bp fragment of the rat POMC gene is sufficient to confer cell-specific expression in transgenic mice and in transfection studies. Analysis of this promoter by footprinting, mutagenesis, and gel retardation experiments have revealed a complex organization in which at least 10 different regulatory elements binding as many different factors are required for transcription. However, when tested individually, most of these elements were found to be transcriptionally active in various cell lines, including the POMC expressing AtT-20 cells, the growth hormone-expressing GH_3 pituitary cells and L cells. None of these elements tested individually as oligonucleotides fused to a minimal promoter exhibited cell-specific activity in AtT-20 cells. Interestingly, two elements located far apart in the promoter and which are almost transcriptionally inactive on their own showed a marked synergistic activity only in AtT-20 cells. One of these elements located in the distal region of the promoter appears to be composite and to contain three protein binding sites. The most active part of this element contains a CANNTG motif typical of binding sites for HLH transcription factors. Single nucleotide mutagenesis correlated the cell-specific activity of the element with integrity of this motif. Consistent with the hypothesis that HLH factors are involved, overexpression of the dominant negative HLH protein Id or of the ubiquitous Pan-2 HLH protein resulted in decreased and augmented POMC promoter activity, respectively. *In vitro* protein DNA binding experiments have revealed the presence of nuclear proteins which appear unique to AtT-20 cells, which have properties expected of HLH factors and the binding of which correlates with the activity of mutants in the CANNTG motif. Thus, it appears that cell-specific HLH factors are present in POMC-expressing cells and that these factors are required for cell-specific transcription of POMC in the pituitary.

B 941 CARDIAC AND SKELETAL MUSCLE M-CAT BINDING FACTOR IS ENCODED BY TEF-1 HOMOLOGUES.
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MyoD-independent activation of cardiac troponin T (cTNT) and other muscle-specific genes, including the ß myosin heavy chain, the cardiac troponin C and skeletal α actin genes, is dependent upon the presence of one or more M-CAT *cis*-regulatory element(s). M-CAT binds a nuclear factor (MCBF) found to be related to the human transcription factor TEF-1. This factor is known to be involved in SV40 and papillomavirus-16 early gene activation, via the GTIIC element and other elements related to the MCAT site, but a cellular target for TEF-1 has yet to be identified. We describe here the cloning of avian TEF-1 cDNAs and present evidence that they encode the activity identified as MCBF. TEF-1 message is found in both cardiac and skeletal muscle at high levels relative to non-muscle tissues, which correlates with the relative levels previously shown for MCBF activity. In vitro transcribed/translated avian TEF-1 encodes proteins capable of binding an M-CAT element. Co-transfection of avian TEF-1 squelches M-CAT-dependent expression of a cTNT-CAT reporter gene in a quail muscle cell line, as reported for overexpression of human TEF-1 on transcription of GTIIC-driven chimeric reporters in HeLa cells. Avian TEF-1 cDNAs initiate translation at isoleucine, as does human TEF-1. Experiments are in progress to investigate the possible involvement of coactivators in TEF-1 mediated transactivation.

B 942 REGULATION OF TCR δ GENE REARRANGEMENT IN TRANSGENIC MICE, Pilar Lauzurica and Michael S. Krangel, Department of Immunology, Duke University Medical Center, Durham, NC 27710.

T cell receptor (TCR) genes rearrange in a highly ordered pattern intrathymically. We are studying the role of the δ enhancer in the control of the time- and lineage-specific rearrangement of the TCR δ gene.

A human TCR δ locus was constructed by fusing DNA germline fragments containing the Vδ1, Vδ2, Dδ3, Jδ1, Jδ3 and Cδ gene segments. A stop codon was introduced in both Vδ coding regions to abort the expression of any possible rearranged transgene and therefore avoid possible interference in the normal development of the transgenic mice. Four transgenic founder mice were obtained containing very few copies of the transgene. TCR δ gene VDJ rearrangements are detected by PCR. VDJ rearrangements of the human transgene were detected in thymus and spleen but not in non lymphoid tissues. Rearrangements are detected in 1 to 5% of the thymocytes. The V-D rearrangements detected are restricted to T-lymphocytes. Rearrangements were detected in splenocytes enriched for T lymphocytes but not in splenocytes enriched for B lymphocytes. A construction removing the δ TCR enhancer was generated and transgenic mice were obtained. The study of rearrangements in these mice will determine the role of the δ enhancer in the regulation of TCR δ gene rearrangement.

B 943 STRUCTURAL AND FUNCTIONAL ANALYSIS OF GLUTAMINE-RICH PROTEINS, Chuan Li and Philip W. Tucker, Department of Microbiology, Genetics and Development Graduate Program, The University of Texas Southwestern Medical Center, Dallas, Texas 75235

A number of known and suspected transcription factors contain glutamine-rich segments and poly-glutamine repeats. It has been shown that the glutamine-rich domains serve as transcriptional activation domains and the poly-glutamine repeat of hTFIID is potentially important for the transcriptional activation mediated by SP1 and GAL4-VP16. By using a CAG repetitive sequence (CAG encodes for glutamine) as a probe, we have cloned a dozen potential transcription factors from a B-cell line cDNA library. Four of them have been sequenced and characterized. We tentatively named these factors Plasma-Cell-Transcription-Factors (PCTFs). PCTF-1 is a fkh DNA-binding-domain-containing protein. Its message is preferentially expressed at the plasma cell stage of B-cell differentiation and highly expressed in skeletal muscle but not in other tissues examined. Like hTFIID, it contains 37 poly-glutamine residues in the N-terminus and five imperfect Pro-X-Thr repeats. This is probably the first transcription factor cloned sharing certain structural features with hTFIID. We propose a structural model for the fkh DNA binding domain which we demonstrate can bind specifically to immunoglobulin gene promoter probes. Preliminary data indicate that PTF-1 is a transcription activator. PCTF-2 is a plasma-cell-specific nuclear factor that contains a potential helix-turn-helix DNA binding domain. It is also expressed at far lower level in testis, brain, spleen, thymus, and liver. PCTF-3 is a B-cell specific factor which is expressed highly in mature splenic B-cells but undetectable in pre-B or plasma cells. PCTF-4 localizes to the nucleolus of T-cells with a domain homologous to the Myb-family DNA binding domain. It also contains acidic amino acid stretches which are a characteristic of other nucleolus transcription factors.

B 944 TRANSCRIPTIONAL ACTIVATION OF THE CORTICOTROPIN RELEASING HORMONE GENE BY POU-DOMAIN PROTEIN BRN-2 Peng Li, Xi He and Michael G.Rosenfeld, University of California, San Diego and the Howard Hughes Medical Institute, La Jolla, CA 92093-0648

Brn-2, a member of the large POU-domain gene family has been cloned and localized to paraventricular hypothalamic neurons which produce corticotropin-releasing hormone (CRH). The co-expression of Brn-2 and CRH suggests the possible functional role of Brn-2 in the activation of CRH gene expression. We evaluate this possibility by examining the binding specificity of Brn-2 on the CRH promoter. We observed that Brn-2 binds to several sites on CRH promoter and activates it in heterologous cells. Further characterization of the DNA binding specificity of Brn-2 a member of the POU-III class, Pit-1 (POU-I class) and Brn-3 (POU-IV class) led to the identification of conserved DNA sites for the bipartite binding of POU proteins . The POU domain of all classes binds to the consensus site $CATN_nTAAT$, N representing the variable region which we call spacing. The spacing defines the specificity of different classes within the POU superfamily. The consensus and spacing may function as a code that accounts for the selective patterns of POU-protein transcriptional activation.

B 945 CHARACTERIZATION OF THE STEROID SULFATASE (STS) PROMOTER: A GENE WHICH ESCAPES X INACTIVATION, X-M Li, E. Schultz, E. Salido, P. Yen*, and L. Shapiro. Department of Pediatrics, UCSF School of Medicine, San Francisco, CA 94143. *:Division of Medical Genetics, Harbor-UCLA Medical Center, Torrance, CA 90502.

STS 5' sequences contain no TATA-like elements nor CG rich regions. We have used primer extension and S1 nuclease protection experiments to identify at least four distinct transcription initiation sites for this gene scattered over a 90 base-pair region. Deletion analysis with reporter constructs indicates that the STS promoter consists of 90bp of sequence immediately adjacent to the major site of STS transcription initiation. In addition, an approximately 300bp element located 500bp 5' of the promoter functions as an enhancer of transcription. Although the STS gene is ubiquitously expressed, transcript levels are 30-fold more abundant in placental syncytial trophoblast cells than in fibroblast cells. Plasmids containing various portions of STS 5' flanking sequences linked to the chloramphenicol acetyl transferase (CAT) gene showed no expression in COS cells. However, promoter activity was detected in JEG choriocarcinoma cells. Gel shift assays using crude unclear extracts from JEG, COS and HELA cells suggest negative regulatory element(s) in COS and HELA cells. STS is one of the growing number of genes which escape X inactivation and the first one to have its promoter analyzed. Further studies will reveal the relationship of the STS promoter machinery and the ability of this gene to escape X inactivation.

B 946 **ERP, A NEW MEMBER OF THE ETS GENE FAMILY, IS DIFFERENTIALLY EXPRESSED IN B CELLS.** Towia A. Libermann, Peter Oettgen, Ulrich Dendorfer, and Monica Lopez, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

In our approach to understand the molecular mechanisms underlying B lymphocyte development we have recently focused on the role of specific transcription factors in the regulation of immunoglobulin heavy chain (IgH) gene expression. We have previously identified a novel IgH enhancer element, designated π, whose activation appears to be linked to B cell-specific expression of the IgH gene at early stages of B cell development prior to immunoglobulin κ light chain gene expression. Since the π enhancer element shows striking similarity to binding sites for transcription factors of the Ets gene family, we have explored the possibility that yet unidentified members of the Ets gene family are expressed at the preB cell stage.

We have used a PCR approach with degenerate oligonucleotides to isolate Ets related genes expressed in preB lymphocytes and have cloned the gene for a new transcription factor, ERP (Ets Related Protein) from a murine preB cell line. The ERP protein contains a region of striking homology with the Ets DNA binding domain common to all members of the Ets transcription factor/oncogene family. Two additional smaller regions show homology to the ELK-1 and SAP-1 genes, a-subclass of the Ets gene family. Multiple transcripts of ERP appear to be expressed in a variety of tissues. However, in the B cell lineage, ERP is highly expressed only at early stages of B lymphocyte development, and expression declines drastically upon B cell maturation.

Involvement of ERP in IgH gene regulation and B cell differentiation will be discussed.

B 947 DIFFERENTIAL EXPRESSION OF THE HOMEOBOX GENE, HOX 2.3, CHANGES THE RESPONSE OF HL60 CELLS TO CHEMICAL INDUCERS OF DIFFERENTIATION. M. Lill,* S. Ellis,* R. Herzig,* G.M. Crooks,* D. Kohn,* and J. Gasson. Division of Hematology-Oncology, Departments of Medicine and Biological Chemistry, UCLA School of Medicine, Los Angeles, CA; Division of Research Immunology, Children's Hospital, Los Angeles.

Homeobox genes encode DNA-binding proteins that can regulate several aspects of morphogenesis. Many of the features of embryogenesis are recapitulated in the hematopoietic system of the adult animal, in which pluripotent self-renewing stem cells undergo a hierarchical process of proliferation and eventual terminal differentiation. During this process, cells become highly specialized and express many unique proteins. The role of homeobox genes in hematopoiesis remains unclear, despite their implication in the development of human diseases, such as lymphoma and acute leukemia, resulting from either abnormal expression of a homeobox gene or from expression of an abnormal homeobox-containing fusion gene.

Previous work has correlated expression of Hox 2.3 with macrophage cell lines. Anti-sense oligonucleotides were used to inhibit expression of the human homeobox gene, Hox 2.3, during induction of differentiation of HL-60 cells by chemical inducing agents. The response of HL-60 cells to differentiation-inducing agents was assessed using FACS scanning and standard cytochemical techniques. Inhibition of expression resulted in a failure of monocyte differentiation in response to vitamin D3. In the reciprocal experiment, we used a retrovirus vector to overexpress Hox 2.3 in HL-60 cells. Overexpression of Hox 2.3 resulted in pronounced changes in expression of differentiation markers in response to DMSO.

These results imply a role for Hox 2.3 in the monocyte differentiation pathway of HL-60 cells and suggest that overexpression of Hox 2.3 may subvert the normal response to granulocyte inducing agents.

B 948 cAMP REGULATION OF THE MOUSE ANDROGEN RECEPTOR GENE: INTERACTIONS WITH ANDROGENS, Jonathan Lindzey, Michael Grossmann, Donald Tindall, Depts of Urology, Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN 55905

The androgen receptor (AR) is one member of a large family of ligand dependent nuclear transcription factors. AR mediate the widespread effects of androgens on sexual differentiation and reproductive physiology in adult male vertebrates and, thus, factors regulating expression of this transcription factor are critical to normal male development and reproductive physiology. We recently cloned a 1.5kb portion of the 5'flanking region of the mouse AR gene that contains a GC rich promoter, putative CRE, AP1 and ARE. Transfection of the full-length 1.5kb-CAT construct into QT6 cells demonstrated that forskolin (FSK), 8-bromocAMP, and IBMX stimulated 4-5, 2-3 and 5 fold increases in CAT activity, respectively. Truncations of the 5' and 3' ends of the 1.5kb clone indicate that the putCRE may partially mediate the effects of cAMP pathways on this gene. Bandshift analysis indicates the putCRE forms specific, competable DNA-protein complexes with nuclear proteins. However, comparison with bandshifts using recombinant CREB indicates the nuclear protein(s) are not CREB.

Further experiments demonstrate that cotransfection of varying amounts of a mouse AR expression vector and treatment with dihydrotestosterone (DHT) results in a dose-dependent suppression (range 0-60%) of both basal and FSK induced CAT activity of the 1.5kb-CAT construct. These data indicate an interplay or "cross-talk" between cAMP-pKa paths and androgens in regulating expression of the AR gene.

B 949 SEQUENCE-SPECIFIC DNA BINDING BY HEPATIC LEUKEMIA FACTOR, A. Thomas Look, Toshiya Inaba, Linda H. Shapiro, Tesunori Funabiki, and Bart Jones, Department of Experimental Oncology, St. Jude Children's Research Hospital, Memphis, TN 38105, and the University of Tennessee College of Medicine, Memphis, TN 38163

Genes encoding transcription factors are the targets of specific chromosomal translocations in human leukemic cells, but the mechanisms by which they promote aberrant growth and differentiation are still largely undefined. Hepatic leukemia factor (HLF) is a newly identified sequence-specific DNA-binding protein of the basic-region/leucine-zipper (bZip) superfamily. A t(17;19)(q22;p13) chromosomal translocation in childhood B-lineage acute lymphoblastic leukemia (ALL) results in an *E2A-HLF* fusion gene, which encodes a chimeric protein that retains the amino-terminal transcriptional activation domain of E2A, but not its basic helix-loop-helix domain, which is replaced by the bZip DNA-binding and dimerization domain of the HLF protein. Using a glutathione S-transferase HLF fusion protein produced in bacteria, we screened a random oligomer pool and used the polymerase chain reaction to amplify oligomers that bound to HLF in a sequence-specific manner. Alignment of the sequences identified by this technique revealed a 10 bp consensus sequence 5'-GTTACGTAAT-3', which contains a core dyad-symmetric motif, consistent with the "scissors-grip" model for DNA binding by bZip proteins. A probe containing this consensus sequence was shown to bind chimeric E2A-HLF proteins in nuclear lysates of a leukemic cell line (UOC-B1) containing the t(17;19), by electrophoretic mobility shift analysis demonstrating "supershifted" complexes in the presence of antibodies specific for amino-terminal epitopes of E2A or carboxyl-terminal epitopes of HLF. In addition, the chimeric E2A-HLF protein was shown to function as transcriptional *trans*-activator in transient transfection assays. These results indicate that the E2A-HLF protein expressed by leukemic cells is able to bind to DNA in a sequence-specific manner and transactivate the expression of an artificial target gene, suggesting that this hybrid protein may subvert transcriptional programs that normally control the growth and differentiation of hematopoietic cells.

B 950 REGULATION OF MAIZE 82 AND 18kD HEAT SHOCK GENE TRANSCRIPTION DURING HEAT SHOCK AND DEVELOPMENT, Kathleen A. Marrs, Sherry A. Capitant and Ralph M. Sinibaldi, Sandoz Agro, Palo Alto, CA 94304.

Maize has a family of heat-inducible genes encoding proteins in the ~80 kD and 18 kD range. The promoter regions of these genes contain sequences corresponding to the consensus heat-shock element (HSE) consisting of multiple nGAAn repeats. The promoters of the hsp82, hsp81 and hsp18 genes were linked to a B-glucuronidase (GUS) reporter gene to study the heat shock response in transient expression assays. The hsp82 and hsp18 promoters directed strong heat-inducible GUS expression (10 to 80 fold), whereas GUS expression from the hsp81 promoter was only mildly heat-inducible. The promoters were analyzed for interactions with nuclear factors from maize cells by gel shift assays. The nuclear extracts contain factors which bind specifically either to HSE-containing regions of the promoters or to synthetic HSE oligos. The specificity of the complex formation between the promoter regions and the DNA-binding factors was determined by competition with synthetic HSE oligos. Unlabeled HSE oligos did compete for binding, but a mutated oligo did not. Footprinting experiments localized the binding to a region containing the HSEs. Gene specific probes were used to examine the regulation of the closely related hsp82 and hsp81 genes and the hsp18 gene during maize pollen development and embryogenesis. Distinct patterns of expression of all three genes were detected in developing embryos and during pollen development, suggesting distinct roles for these genes during development.

B 951 THE THYROID TRANSCRIPTION FACTOR- 1 (TTF-1) GENE IS A CANDIDATE TARGET FOR HOX PROTEINS. S. Guazzi[1], R. Lonigro[2], E. Boncinelli[1], R. Di Lauro[2] and F. Mavilio[1]. *1. Dept. of Biology and Biotechnology, Istituto Scientifico H. S. Raffaele, Milano, and 2. Stazione Zoologica "A Dohrn", Napoli, ITALY.*

Vertebrate homeobox-containing (HOX) genes are transcription factors which regulate antero-posterior axial identities in embryogenesis, presumably through activation and/or repression of downstream target genes. Although many cross- and auto-regulatory interactions exist among Hox genes, the number and identity of their downstream targets is still largely unknown. A few candidates have been recently proposed, all coding for molecules which might be directly involved in determining regional identities by regulating cell-cell interaction. However, no relationship has yet been demonstrated between Hox genes and other transcriptional regulators, directly involved in determining and/or maintaining tissue specificity. The Thyroid Transcription Factor-1 (TTF-1) is a homeodomain-containing protein required in thyroid gland development and differentiated thyroid cell lines for the expression of - at least - two different thyroid-specific genes, i. e., thyroglobulin and thyroperoxidase. A 800-bp 5' genomic fragment from the rat TTF-1 gene was able to direct cell type-specific expression of a luciferase reporter gene when transfected into rat thyroid FRTL-5 cell line. A shorter, 250-bp fragment, encompassing the TTF-1 major transcription start site was cotransfected in NIH3T3 and HeLa cells with eukaryotic expression vectors for a number of human HOX genes. Cotransfection with the HOX2G expression vector caused a specific, 4 to 6- fold increase in the TTF-1 promoter activity in these cells. HOX2G (=murine *Hox 2.7*) is expressed in the anterior neuroectoderm, brachial arches and their derivatives in early mammalian embryogenesis, including the thyroid primordia and thyroid gland, where TTF-1 is specifically expressed and exerts its regulatory functions. No effect on the TTF-1 promoter was instead observed with HOX gene expressed more posteriorly, e.g., HOX4B (*Dfd*-like), HOX3D (*Scr*-like), HOX4E (*abd-A*-like) and HOX4D (*Abd-B*-like). DNase I footprinting experiments on the 250-bp TTF-1 promoter with a bacterially-synthesized HOX2G homeodomain showed the presence of two strong HOX2G binding sites around positions -90 and +30 from the TTF-1 major transcription start site. These sites contain core ATTA sequences, frequently found in many Drosophila and vertebrate Hox binding sites. These data suggest that HOX2G might be a positive transcriptional regulator of the TTF-1 promoter. HOX2G belongs to the same paralogous group (*zen-pb*-like) of HOX 1E (=*Hox-1.5*), which is also expressed in embryonic thyroid. In *Hox-1.5*$^{-}$/*Hox-1.5*$^{-}$ knock-out mutant mice, a consistently reduced thyroid gland was observed. We suggest that Hox genes belonging to *zen-pb* paralogous group might be involved in regulation of TTF-1 gene expression in early embryos, thereby indirectly participating to thyroid gland development.

B 952 *FOS/JUN* REPRESSION OF CARDIAC-SPECIFIC TRANSCRIPTION IN QUIESCENT AND GROWTH - STIMULATED MYOCYTES IS TARGETED AT A TISSUE-SPECIFIC *CIS*-ELEMENT, Kevin McBride, Lynda Robitaille, Stéphane Tremblay, Stefania Argentin and Mona Nemer, Laboratoire de développement et différenciation cardiaques, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA H2W 1R7

Unlike skeletal muscle cells where growth and differentiation appear mutually exclusive, growth stimulation of cardiac cells is characterized by transient expression of early response nuclear protooncogenes as well as induction of several cardiac-specific markers. This observation led to the speculation that these protooncogenes, particularly c-*fos* and c-*jun*, might act as positive regulators of cardiac transcription. We have examined the role of c-*jun* and c-*fos* in basal and growth stimulated cardiac transcription, using the cardiac-specific atrial natriuretic factor (ANF) gene as a marker. The results indicate that c-*jun* and c-*fos* are negative regulators of ANF transcription. Inducers of *jun* and *fos* activity such as mitogens and growth factors inhibited endogenous ANF transcripts. In transient cotransfection assays, *jun* and *fos* were able to *trans*-repress the ANF promoter in both quiescent and α_1-adrenergic stimulated myocytes. This repression was specific to myocyte cultures and was not observed in non-muscle cells. Deletion analysis indicated that repression does not require typical AP-1 binding sites (TRE) or serum response elements but is targeted at a cardiac-specific element within the ANF promoter. Various *fos* related proteins including *fra*-1, *fos* B and v-*fos* were able to *trans*-repress ANF transcription. In addition, C-terminal c-*fos* mutants which no longer repress transcription of such early growth response genes as c-*fos* and EGR-1, retained the ability to repress ANF transcription. Repression by c-*jun* occurs via the N-terminal activation domain and does not require the DNA-binding domain suggesting that protooncogene repression involves interaction with one or more limiting cardiac-specific coactivator(s). Thus, this study provides evidence that in non-dividing myocytes, *jun* and *fos* protooncogenes are negative regulators of a cardiac-specific marker gene. Furthermore, it suggests that, by using non-dividing cardiac myocytes, it may be possible to dissociate protooncogene effects on differentiation from their role in growth and proliferation.

B 953 ISOLATION AND CHARACTERIZATION OF A NOVEL TRANSCRIPTION FACTOR EXPRESSED IN BRAIN AND MUSCLE, John C.McDermott, Yie-Teh Yu, M. Cristina Cardoso, Vicente Andrés, Roger E. Breitbart, Hanh Nguyen, Dana Leifer, Dimitri Krainc, Stuart A. Lipton, Vijak Mahdavi & Bernardo Nadal-Ginard. Howard Hughes Medical Institute and Dept. of Cardiology, Children's Hospital, Dept's of Cellular and Molecular Physiology, and Neurology, Harvard Medical School, Boston, MA 02115

A DNA binding site in the promoter region of many muscle specific genes has been identified as the myocyte enhancer-binding factor 2 (MEF2) activity. This binding activity is partially attributable to a recently cloned factor (Yu et al. Genes & Dev. In Press1992). Genomic southern blots revealed that several genes exist which contain homology to this factor. A skeletal muscle cDNA library was screened at low stringency with a DNA binding domain probe from the first MEF2 related gene, with the purpose of identifying additional members of the putative MEF2 family of transcription factors. We report the isolation and characterization of cDNA clones encoding a new MEF2 related factor, termed dMEF2, which is derived from a separate gene. The mRNA species transcribed from this gene are expressed abundantly in skeletal muscle and brain, and, through tissue specific alternative splicing, generate several distinct proteins. One of the alternate exons in this gene is highly brain specific. The products of this gene, termed dMEF2, activate transcription when the MEF2 binding site consensus is present and have a similar binding specificity to the previously isolated MEF2 related factors [CTA $(A/T)_4$TAG]. Immunofluorescence studies indicate that dMEF2 is developmentally up-regulated in the myoblast to myotube transition and is also present in a subset of neuronal cell nuclei. The strict tissue specific transcriptional regulation of this gene, in comparison to the more ubiquitous expression of the other MEF2 related factors, suggests a potentially important role for dMEF2 in tissue specific gene expression in skeletal muscle and brain.

B 954 DEVELOPMENTAL SWITCH OF CREM FUNCTION DURING SPERMATOGENESIS: FROM ANTAGONIST TO TRANSCRIPTIONAL ACTIVATOR

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The first cDNA clones which were characterised from the CREM gene (cAMP response element modulator) encode antagonists of cAMP-induced transcription (Foulkes et al., 1991). The CREM antagonists are able to bind to CRE (cAMP response element) sites as homodimers and also as heterodimers with the transcriptional activator CREB. It is postulated that either by blocking the CRE site for CREB binding or by forming inactive DNA-binding heterodimers, CREM exerts down-regulation. The CREM antagonists share extensive homology with CREB but they lack two glutamine-rich domains which in CREB have been shown to be necessary for transcriptional activation. Now it is clear that the CREM gene also encodes an activator of transcription (Foulkes et al., 1992). In the adult testis, an isoform, CREMτ, has been identified which resembles in structure one of the antagonist forms (CREMβ) but includes two exons that encode glutamine-rich domains. This form has been demonstrated to transactivate transcription from a CRE site. In adult testis, the CREMτ isoform is expressed alone and it constitutes an abundant mRNA in late spermatocytes and spermatids however in other tissues it is coexpressed with the other antagonist CREM forms. The CREM mRNA isoforms are a graphic illustration of how alternative splicing can modulate the function of a transcription factor in a tissue and developmental-specific manner (Foulkes and Sassone-Corsi, 1992). Using the seasonal-dependent regulation of testis function in the golden hamster as a model system, we have established that the switch in CREM expression is regulated by FSH (Foulkes et al., Submitted). In addition we demonstrate that use of an alternative polyadenylation site enhances CREMτ transcript stability.

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B 955 FUNCTIONAL ANALYSIS OF THE MOUSE FGF-3 GENE PROMOTER, Akira Murakami, Jane Thurlow, Daniel Grinberg, Gordon Peters and Clive Dickson, Imperial Cancer Research Fund, London WC2A 3PX.

The mouse *Fgf-3* gene is expressed at various stages of fetal development in a distinct spacio-temporal pattern. To understand the complex regulation of this embryonic signalling molecule we have used the embryonal carcinoma cell lines, F9 and PCC4, as an in vitro model. *Fgf-3* is transcriptionally activated in these cells following exposure to retinoic acid and dibutyryl cAMP, a treatment that causes cellular differentiation from primitive endoderm to predominantly parietal endoderm. A fragment of DNA encompassing the first 1.7kb of sequences upstream of the open reading frame was shown to confer this regulated response to the reporter gene CAT. DNase-I footprinting analysis of this region has revealed at least 15 potential protein binding sites, designated as IPS1 to IPS15. An analysis of deletion mutants lacking each binding site has disclosed five functionally important domains. Thus, IPS2 and IPS4 show a positive effect on transcription of the reporter gene in transient and stable transfection assays into F9 cells. The sites IPS2 and IPS4 contain Ets-1 and AP1 binding motifs respectively, and in a gel retardation assay, binding of a nuclear factor to these domains can be competed by the oligonucleotides containing the Ets-1 or AP1 motif respectively. Two other domains, IPS5 and IPS9 show a strong negative effect on transcription, since their deletion greatly enhances transcription of the reporter gene. The fifth region 1.4kb upstream of the major cap sites appears to control the retinoic acid/dibutyryl cAMP response. We are trying to identify the nuclear factors which bind to these domains and regulate the transcription of the *Fgf-3* gene during animal development.

B 956 FUNCTIONAL AND BIOCHEMICAL CHARACTERIZATION OF A LYMPHOCYTE SPECIFIC TRANSCRIPTION FACTOR, Markus Nabholz, Enric Espel, Anne-Laure Lattion, Patrick Reichenbach, Philipp Bucher and Cathrine Fromenthal[1], ISREC, CH-1066 Epalinges, Switzerland and [1]INSERM U184, F-67085 Strasbourg, France

NP-TC_{II} was discovered as a nuclear protein with specifictiy for the TC-II motif in the SV40 enhancer [Espel et al.(1990) EMBO J. 9:929-937]. Further characterization has shown that it is constitutively present in the nuclei of normal, unstimulated B- and T-lymphocytes and in lymphoid as well as in some non-lymphoid hematopoietic cell lines but not in non-hematopoietic cell types [Lattion et al.(1992) Mol.Cell.Biol., in press]. Renaturation experiments with material fractionated by SDS-PAGE have shown that NP-TC_{II} consists of single polypeptide chains that are very heterogeneous in size (100 to 150 KD apparent MW). Comparison of gel filtration and gradient sedimentation analysis indicates that NP-TC_{II} is an assymetric protein with a frictional ratio higher than 2. NP-TC_{II} is not affected by IκB or by antibodies against NF-κBp50 or *rel* proteins. Trypsin digestion yields two well defined DNA-binding fragments with apparent MW of 44 and 40 KD. The native protein and both trypsin fragments have the same fine specificity, which is different from that of NF-κB. We have compared the relative affinity of a number of oligonucleotides for NP-TC_{II} and used these data to define a binding sequence for NP-TC_{II} with a novel computer algorithm, CONSCOR. Comparison of the binding and the enhanson activity of different point mutations in the SV40 TC-II motif indicates that NP-TC_{II} is responsible for the constitutive enhanson activity of this motif in lymphoid cells. Comparison of the characterisitcs of NP-TC_{II} with other lymphocyte specific transcription factor indicates that this protein has not been identified previously. We are now in the process of purifying it in amounts sufficient for obtaining sequence information.

B 957 ADRENERGIC REGULATION OF THE EXPRESSION OF *c-fos* AND STRUCTURAL PROTEINS – AS WELL AS OF PROLIFERATION – IN BROWN ADIPOCYTE CULTURES, Jan Nedergaard, Stefan Rehnmark, Håkan Thonberg, Anders Jacobsson, Gennady Bronnikov and Barbara Cannon; The Wenner-Gren Institute, Stockholm University, S-106 91 Stockholm, Sweden.

In order to understand the differentiation and proliferation processes occurring in brown adipose tissue during the recruitment process, the ability of adrenergic stimulation to influence these processes in brown adipocytes proliferating and differentiating in culture from isolated precursor cells was studied. It was found that the expression of a series of transcriptional factors and structural proteins was under adrenergic control but showed individual receptor characteristics. *c-fos* expression was induced synergistically by α_1- and ß-adrenergic stimulation. Both α_1- and $ß_3$-adrenergic stimuli could affect the expression of the gene for the uncoupling protein thermogenin (UCP). – Lipoprotein lipase gene expression was also influenced by adrenergic stimulation.– Cell proliferation was followed as the rate of DNA synthesis and it was found that it was possible to induce DNA synthesis in serum-starved cells; this induction was apparrently mediated via $ß_1$-adrenergic receptors. It was concluded that adrenergic pathways are central for the control of both proliferation and differentiation processes in these cells but that the intracellular pathways may differ.

B 958 MYOD TETHERED TO ITF-1 ACTIVATES A MUSCLE-SPECIFIC ENHANCER IN UNDIFFERENTIATED MYOBLAST, Lisa A. Neuhold and Barbara Wold, Division of Biology, California Institute of Technology, 156-29. Pasadena, CA 91125

Regulatory molecules of the MyoD family play an important role in skeletal muscle development in diverse organisms. Their biological activities depend on their function as sequence-specific DNA-binding proteins, which, in turn, depend on their association, *via* protein:protein interactions, with other regulatory proteins of the same superfamily. These prospective partners share a related sequence motif essential for dimerization with each other or with MyoD family molecules. Some pairings act positively by enabling DNA-binding of the multimeric form, while others act negatively by preventing DNA-binding. Several different partners have been shown capable of pairing with MyoD class proteins in *in vitro* assays, and the properties of these heterodimers vary from one pairing to another. There are multiple potential partners present in myoblasts and in myocytes. To assess the functional significance of specific dimers we have generated covalently tethered basic helix-loop-helix polypeptides which are linked together *via* a flexible polylinker. This will increase the local concentration of a particular pair so that they are more likely to dimerize and not be influenced by the concentrations of other potential partners. To date, we have examined the role of MyoD linked to the immunoglobulin transcription factor, ITF-1 (or "E47") both in myoblast and in myocytes. Briefly, MyoD~ITF-1 is able to stimulate the muscle creatine kinase enhancer linked to the chloramphenicol acetyltransferase reporter gene in both undifferentiated and differentiated cells. This enables MyoD~ITF-1 to bypass normal amounts of repression such as that caused by Id.

B 959 *MULTIPLE CIS-REGULATORY ELEMENTS IN THE SECRETIN GENE ENHANCER.* Nishitani J, Wheeler M, Petry M, and Leiter A. GI Division, New England Medical Center, Tufts University School of Medicine, Boston, MA 02111

Expression of the gene encoding secretin is restricted to intestinal S cells and to B-cells of the developing pancreas. Previously we described a 122-bp enhancer in the secretin gene which confers cell-type specific transcriptional regulation. Here we characterize three potential transcription factor binding sites within this domain. The sequence, CAGCTG, located 130 bp upstream from the mRNA startsite, resembles the core CANNTG binding motif for the helix-loop-helix (HLH) family of proteins. HLH proteins have been implicated in the regulation of many tissue-specific genes including the expression of insulin and gastrin in islet cells. In addition, two GC-rich regions resembling SP1 binding sites are present at -122 and -68.

The potential role of the three consensus sequences in secretin gene expression was examined by site-directed mutagenesis. The transcriptional activity of mutation-bearing reporter gene constructions was measured by transfection into secretin-producing HIT cells for transient expression assays. Mutations in any single element alone reduced activity to 15% of the control. Introduction of mutations into both the CAGCTG and the adjacent GC-rich sequence further reduced activity to less than 5% of the control, suggesting that these two elements function independently.

The nature of the proteins in HIT cell nuclear extracts that interact with the three elements was examined by gel-mobility shift assays. Proteins specifically bound to each element but not to the transcriptionally inactive mutants. Competition studies indicated that the CAGCTG element interacted with a hamster homologue of the E2A gene products, PAN1/PAN2, like the insulin gene B-cell-specific enhancer and the GC-rich sequences interacted with multiple factors, including SP1.

We conclude that the mechanisms which limit secretin gene transcription to the apppropriate cell type involve the interaction of multiple transcription factors with different elements in the secretin enhancer. In the present study, we identified two of the proteins as PAN1/PAN2 and SP1. Our data suggests that additional proteins form complexes with this enhancer and may contribute to cell-specific transcriptional regulation of the secretin gene.

B 960 TRANSCRIPTION FACTORS THAT REGULATE THE INTERLEUKIN-2 GENE DURING THE RESTIMULATION OF ANERGIC T CELLS, Steven D. Norton and Jim Miller, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

T cell activation and IL-2 production requires two distinct signals. The first is provided by the interaction of the T cell receptor (TCR) with a complex of antigen and major histocompatibility complex encoded class II molecules found on the surface of an antigen presenting cell (APC). The second co-stimulatory signal can be delivered through CD28 by its ligand, B7, present on the surface of APC. Stimulation of type 1 T helper cell clones through the TCR in the absence of a co-stimulatory signal does not lead to IL-2 secretion or proliferation, and results in the induction of long-lasting, antigen-specific, unresponsiveness, or anergy. Regulatory regions in the IL-2 promoter/enhancer have been described which bind T cell-specific transcription factor(s). Several nuclear factors critical in the regulation of the IL-2 gene, including NF-AT and NFκB2, are not induced by T cells in the absence of co-stimulatory signals. However, all of the relevant transcription factors are expressed after restimulation of anergized T cells even though no IL-2 is produced. These data suggest additional mechanisms regulating IL-2 gene expression. Experiments are in progress to test whether a negative regulatory region may be involved in preventing IL-2 transcription in anergic T cells. In addition, although NF-AT is still present, we are testing whether the composition or activity of its components may have been modified in normal vs. anergic cells preventing transcriptional activity.

B 961 REQUIREMENT OF THE Z AND Y HOMEODOMAINS IN Z/Y REGULATION OF SCHIZOPHYLLUM DEVELOPMENT C.P. Novotny[1], Y. Luo[1], and R.C. Ullrich[2], Departments of Microbiology and Molecular Genetics[1] and Botany[2], University of Vermont, Burlington, Vt 05405

The Aα mating locus is one of four loci that regulate sexual development in the fungus Schizophyllum commune The locus consists of two dissimilar multiallelic genes Y and Z. There are nine alternative forms of Aα in nature. The Z polypeptides encoded by different alleles are 42% identical. The Y polypeptides exhibit 49-54% identity. The deduced Z and Y polypeptides have homeodomain motifs that may enable them to bind to DNA and thereby regulate the expression of developmental genes. Transformation experiments show that the A developmental pathway is activated when Z from one Aα interacts with Y from a different Aα

We made deletions and point mutations in the homeodomain regions of Z and Y and tested the mutagenized genes in transformation for their ability to activate the A developmental pathway. The results show that the homeodomain of Y is essential for Y/Z activation of development whereas the homeodomain of Z is not.

B 962 TYROSINE PHOSPHORYLATION INHIBITORS (TYRPHOSTINS) INHIBIT FSH MEDIATED EXPRESSION OF STEROIDOGENIC ENZYMES IN CULTURED RAT OVARIAN CELLS* Joseph Orly, Rei Ziv, Seri Gomberg-Malool, Yael Re'em, Israel Posner, Alexander Levitzki, Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

FSH induces expression of cholesterol side chain cleavage cytochrome P450 (P450scc) in rat ovarian granulosa cells. This enzyme catalyses the fiest and key regulatory reaction in the steroidogenic cascade leading to progesterone and estrogen production. By use of Western blot analysis and immunofluorescence assays, the present study reveals that the tyrphostin AG18, a member of novel protein tyrosine kinase inhibitors, can arrest the FSH induced synthesis of P450scc with an apparent IC_{50} of 30μM. Total inhibition of P450scc expression was achieved at 80μM AG18. Northern blot analysis showed that AG18 primarily inhibits the accumulation of P450scc mRNA. AG18 mediated inhibition of P450scc was also observed when the enzyme was induced by PGE2, forskolin or 8-br-cAMP. The drug did not affect FSH induced cAMP accumulation suggesting that it may interfere with the flow of FSH signal transduction at a site distal to intracellular accumulation of cAMP. Control experiments demonstrated that the inhibitory action of AG18 was reversible, and did not hamper total protein synthesis in the cells. A cell-free assay of cAMP-dependent protein kinase showed that the tyrphostin AG18 does not affect this enzyme activity up to concentrations above 200μM. When cells where firstly exposed to FSH for 48 hrs to accumulate high levels of P450scc mRNA, and then treated with AG18 and α-amanitin, a precipitous decline (half life 2 hrs) of P450scc mRNA level was observed, suggesting that the tyrphostin exerts its action at the level of transcription. These results suggest that a putative tyrosine kinase activity is involved in the gonadotropin signal transduction pathway leading to expression of functional genes in ovarian cells.

* This study was supported by the United States-Israel Binational Science Foundation, Grant 86-00065

B 963 **Hepatocyte Nuclear Factor 3 (HNF-3) and Drosophila Forkhead DNA Binding Domains Define a Novel Transcription Factor Gene Family.** D.G. Overdier, D. Clevidence, L. Pani, and R.H. Costa. The University of Illinois College of Medicine, 1853 W. Polk St., Chicago, IL, 60612.

The characterization of hepatocyte-specific promoters suggests that several liver genes are coordinately regulated by the combinatorial action of transcription factors that demonstrate cell-type restriction. The transcription factor HNF-3 exists as three distinct proteins in rat liver (α, β, γ). These proteins share 95% homology in their DNA binding domains and possess conservation in their activation domains. The HNF-3 DNA binding domain also shares homology with the Drosophila homeotic protein, forkhead, and with several other developmental regulatory factors. The isolation of an HNF-3 homolog in mouse brain (BF-1) suggests that a larger HNF-3 family may play a role in the regulation of tissue-specific gene expression. Using degenerate oligonucleotides corresponding to conserved residues in the HNF-3/forkhead DNA binding domain and cDNAs as templates for the polymerase chain reaction, we have isolated seven additional family members from different tissues. We have chosen to name these new members as HNF-3/forkhead homologs (HFH). The HFH DNA binding domains are conserved at the amino terminus whereas the carboxyl terminus displays significant divergence. This indicates that the HFH proteins may recognize different target sequences. Most of the HFH genes display a restricted expression pattern and are present in tissues other than the liver. The determination of the HFH DNA recognition sequence will allow identification of possible target promoters and allow the analysis of structure/function of HNF-3/forkhead DNA binding motif.

B 964 REGULATION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 GENE TRANSCRIPTION BY INSULIN IN DIABETIC RATS AND HEPATOCYTE PRIMARY CULTURE, C.-I Pao, P.K. Farmer, S. Begovic, S. Goldstein, B.C. Villafuerte, C.P. Thiessen, D.G. Robertson, G.-J Wu and L.S. Phillips, Departments of Medicine and Microbiology, Emory University School of Medicine, Atlanta, GA 30303

Although circulating levels of insulin-like growth factor binding protein-1 (IGFBP-1) appear responsive to insulin status in man and animals, and IGFBP-1 secretion and mRNA levels fluctuate in response to added insulin in vitro, underlying mechanisms are poorly understood. To examine regulation by insulin, we evaluated IGFBP-1 gene transcription in normal and diabetic rats, and in normal rat hepatocytes in primary culture. Transcription of the IGFBP-1 gene was measured as incorporation of [α-^{32}P] UTP into pre-initiated message in isolated nuclei, quantitated by densitometric scanning, and expressed relative to transcription of β-actin. Streptozotocin-diabetic animals had a 400% rise in levels of IGFBP-1 mRNA ($p < 0.005$); with insulin treatment, levels of IGFBP-1 mRNA fell below values in normal animals ($p < 0.01$). IGFBP-1 gene transcription rates rose up to 10-fold in animals given 144 mg/kg streptozotocin ($p < 0.002$), and fell 94% below control levels with insulin treatment ($p < 0.001$). In normal and diabetic animals, levels of IGFBP-1 mRNA were strongly correlated with IGFBP-1 gene transcription rates ($r = 0.91$, $p < 0.001$). To examine regulation of gene transcription under more controlled conditions, effects of insulin were evaluated in hepatocyte primary culture. Provision of insulin at 10^{-9} M lowered IGFBP-1 gene transcription rates 60% below transcription rates with 10^{-11} M insulin. Effects of regulatory factors on IGFBP-1 gene transcription were rapid: dexamethasone 10^{-7} M raised IGFBP-1 gene transcription 300% above control levels (10^{-10} M) within 30 min, and addition of phorbol ester (PMA) decreased IGFBP-1 gene transcription 45% within 30 min. In separate experiments, an 80% decrease in IGFBP-1 gene transcription could be detected within 15 min after adding insulin 10^{-6} M, and IGFBP-1 gene transcription fell 90% after 30 min; suppression of IGFBP-1 gene transcription by insulin was unaffected by the presence of cycloheximide.

CONCLUSION: In normal and diabetic animals, strong correlations between levels of IGFBP-1 mRNA and IGFBP-1 gene transcription rates support the hypothesis of regulation at the level of gene transcription. In cultured hepatocytes, regulation of IGFBP-1 transcription is responsive to provision of physiologic levels of insulin, and appears to be independent of ongoing protein synthesis.

B 965 ALTERNATE 5'-UNTRANSLATED EXON/PROMOTER USAGE RENDERS TISSUE-SPECIFIC TRANSCRIPTIONAL REGULATION OF THE HUMAN ACIDIC FIBROBLAST GROWTH FACTOR GENE (aFGF), Robert A. Payson, Maqsood A. Chotani, Rene L. Myers, Stephen E. Harrist, and Ing-Ming Chiu, Department of Internal Medicine, The Ohio State University, Columbus, OH, 43210 and †Department of Medicine, University of Texas, San Antonio, TX 78249

There now appears to be at least four 5' non-coding exons for the human aFGF gene. These exons and their associated promoters confer tissue-specific and cell-specific expression. We have previously isolated two different aFGF cDNA clones from kidney and brain, designated aFGF 1.A and 1.B respectively. During the characterization of aFGF mRNA in glioblastoma cells, we found that aFGF mRNA in U1242MG and D65MG glioblastoma cells contain 5'-untranslated regions different from those of 1.A and 1.B. By reverse transcription and polymerase chain reaction (RT-PCR) we have isolated two novel cDNA clones-aFGF 1.C and 1.D from U1242MG cells and D65MG cells respectively. Promoter 1C has extensive sequence homology to the hamster aFGF gene promoter which was shown by chloramphenicol acetyltransferase reporter gene assays (CAT) to respond to androgen stimulation. RNase protection and primer extension analysis have been used to map the transcription start site of the 1.C aFGF transcript. Its cis-acting regulatory sequences, especially those which share >85% sequence homology to the hamster equivalent aFGF promoter, are currently being characterized. RT-PCR using 1.D-specific primers showed that kidney, brain and prostate do not express 1.D mRNA even though kidney and brain are the most abundant source for aFGF protein. RNase protection analysis further showed that 1.D mRNA is the predominant aFGF RNA transcript in D65MG glioblastoma cells and in NFF-6 neonatal foreskin fibroblast cells.

B 966 **TISSUE SPECIFIC AND DEVELOPMENTAL REGULATION OF TRANSCRIPTION OF QR1: A GENE SPECIFICALLY EXPRESSED IN POST-MITOTIC QUAIL NEURORETINA CELLS AND NEGATIVELY REGULATED BY THE V-SRC GENE PRODUCT. Alessandra Pierani, Celio Pouponnot and George Calothy, Institut Curie-Biologie, Centre Universitaire, 91405 ORSAY Cedex, France.**

The embryonic avian neuroretina (NR) is part of the central nervous system and is composed of various cell types: photoreceptors, neuronal and Müller (glial) cells. Precursor cells derive from proliferating neuroectodermal precursors which differentiate after terminal mitosis and become organized in cell strata.

To understand the mechanisms which regulate neural cell growth and differentiation we isolated several cDNA clones corresponding to genes specifically expressed in post-mitotic NR cells. Transcriptional levels of these genes are strongly reduced in NR cells induced to proliferate by RSV. In particular, transcription of one of them, QR1, is tightly cell type-specific (Müller cells) and strongly downregulated by the *v-src* gene product. Furthermore, expression of QR1 takes place only during late phase of retinal development and is shut off abruptly at hatching. Moreover, the QR1 gene encodes a protein with significant sequence similarity to that of extracellular matrix proteins, suggesting a possible role in the organization of the cell strata in the developing NR.

To understand the transcriptional control of the QR1 gene during development and upon v-*src* expression we have isolated the cis-acting transcriptional elements. To functionally characterize these elements we have linked up to 8.5 kb and serial deletions of the 5' flanking sequences to a reporter gene (CAT). By transfection of these constructs on normal QNR or QNR cells infected with a ts mutant of RSV, we have identified several regions ' hich are involved in transcriptional control of QR1.

Transcriptional repression by *v-src* involves at least two superimposing cis acting elements. The first binds a putative repressor which is specific to the *v-src* regulation. The second binds a putative activator which is common target also for the regulation during development and is probably induced by quiescence. None of the factors seem to be a known possible target of pp60$^{v\text{-}src}$.

Transcriptional control during development involves two elements. The positive factor described previously and a second box located around -1000 which binds one (or more) neurospecific and stage-specific factor(s) of the POU family. One factor would be responsible for repression of transcription in proliferating precursor neuroretina cells; the second would be an activator in differentiated post mitotic cells.

B 967 TRANSCRIPTIONAL ACTIVATION OF ERYTHROID-SPECIFIC GENES IS IMPAIRED IN cAMP-DEPENDENT PROTEIN KINASE-DEFICIENT ERYTHROLEUKEMIA CELLS. Renate B. Pilz, Tom P. Nguyen, Arlene D. Garingo and Carol Kent. Dept. of Medicine, Univ. of Calif., San Diego, La Jolla, CA 92093-0652

We have recently shown that chemically induced differentiation of murine erythroleukemia (MEL) cells is severely impaired when cAMP-dependent protein kinase (A-Kinase) activity is repressed by transfected genes (J.Biol.Chem.267: 16161-16167, 1992). In MEL cells, chemical inducers like hexamethylene bisacetamide (HMBA) and dimethylsulfoxide transcriptionally activate globin genes and several genes encoding for heme synthetic enzymes. We have now demonstrated by nuclear run-off analysis that the transcriptional activation by HMBA of several of these erythroid-specific genes is impaired in the A-Kinase-deficient MEL cells while transcription rates of several housekeeping genes are normal. After treatment with HMBA, steady-state mRNA levels of ß-globin, δ-amino-levulinic acid synthetase and porphobilinogen deaminase increased to a much lesser degree in A-Kinase-deficient cells as compared to parental cells. Consistent with these findings, the intracellular concentrations of both heme and globin chains were reduced in HMBA-treated A-Kinase-deficient cells; supplementing the culture media with δ-aminolevulinic acid partially restored the cells' ability to produce heme and globin chains. These data, in agreement with previous observations, indicate that δ-amino-levulinate synthetase may be rate-limiting for heme synthesis during erythroid differentiation and the synthesis of heme may be a prerequisite for globin chain synthesis. It will be important to define the role of A-Kinase in the regulation of erythroid-specific genes by examining transcription factors such as GATA-1 and NFE-2 in A-Kinase-deficient cells.

B 972 PITUITARY CELL-SPECIFIC REGULATION OF THE POU-DOMAIN TRANSCRIPTION FACTOR *PIT-1* GENE, Simon J. Rhodes, R. Chen, G. DiMattia, K. Scully, S. Lin, V. Yu, K. Kalla, M.G. Rosenfeld, HHMI, Dept. Medicine, University of California, San Diego, La Jolla, CA 92093-0648.

The mammalian anterior pituitary is a principal endocrine gland producing peptide hormones that regulate sexual development, thyroid activity, milk production, and growth. The mature gland contains five distinct hormone-producing cell types: gonadotrophs, corticotrophs, thyrotrophs, lactotrophs, and somatotrophs. Regulation of anterior pituitary function is critical to mammalian growth and homeostasis. The homeodomain transcription factor Pit-1/GHF-1 is a key regulator of prolactin and growth hormone gene activity within the lactotroph and somatotroph cell lineages. During pituitary development *pit-1* gene expression is activated prior to the appearance of the lactotroph and somatotroph lineages. In addition, the pituitaries of dwarf mice harboring defective *pit-1* genes lack thyrotroph, lactotroph and somatotroph cell types, indicating that functional Pit-1 protein expression is required for the maintenance of these three cell lineages. Mutations within the DNA-binding (POU) domain of Pit-1 are associated with dwarfism and pituitary disease in humans. Pit-1 therefore is a tissue-specific regulator of anterior pituitary activity.

We describe the organization and function of *cis*-active elements of the *pit-1* gene locus involved in the cell-specific expression and regulation of the gene. Autoregulatory and other complex interactions with constitutive and cell-restricted *trans*-acting factors will be discussed.

B 973 EVIDENCE FOR THE INVOLVEMENT OF TWO DISTINCT TRANSCRIPTION FACTORS IN THE cAMP RESPONSIVENESS OF THE PHOSPHOENOLPYRUVATE CARBOXYKINASE GENE, William J. Roesler, Pamela J. McFie and Debra M. Puttick, Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

The gene encoding phosphoenolpyruvate carboxykinase (GTP) (PEPCK) is expressed to significant levels in both kidney and liver, but its responsiveness to cAMP is much more robust in liver. Previous work had implicated a region of the promoter which bound liver-enriched proteins as necessary for its cAMP responsiveness. We show that the robust cAMP responsiveness of the PEPCK promoter is mediated by a 'cAMP response unit'. This response unit can be synthesized by simply linking the typical CRE component, located at position -85, with a region of the promoter extending from -300 to -230 which contains multiple binding sites for liver-enriched nuclear proteins. All other regions of the promoter can be omitted without affecting the strength of cAMP response unit. Additionally, the activity of the 'liver-specific region' (LSR) in this synthetic promoter can be substituted for by linking in tandem three copies of just one protein binding site present in that region. This suggests that the binding of three molecules of a single liver-enriched factor is what forms this component of the cAMP response unit. The other component of the cAMP response unit, the CRE, has been shown previously to be bound by these same liver-enriched factors as well as by CREB, leading to some debate as to the identity of the protein mediating the cAMP response through this element. By two different experimental approaches, we show that CREB appears to be the protein involved. Thus, the robust cAMP responsiveness demonstrated by the PEPCK promoter in liver appears to result from the synergistic activity of two distinct transcription factors, a ubiquitous CREB-like factor and a liver-enriched protein.

B 974 GM-CSF AND IL-3 ACTIVATE HUMAN EARLY RESPONSE GENE (EGR-1) TRANSCRIPTION THROUGH BOTH OVERLAPPING AND DISTINCT UPSTREAM REGULATORY SEQUENCES, Kathleen M. Sakamoto, Julie H.J. Lee, Judith C. Gasson, Division of Hematology-Oncology, Departments of Pediatrics, Medicine and Biological Chemistry, UCLA School of Medicine, Los Angeles, CA

GM-CSF and IL-3 exert overlapping and unique biological effects on the proliferation and maturation of myeloid progenitor cells. Both of these growth factor receptors share a common beta subunit which is responsible for signal transduction. GM-CSF or IL-3 can induce the rapid and transient expression of the primary response gene, EGR-1, in a human factor-dependent myeloid leukemia cell line, TF-1. Therefore, we sought to determine whether the molecular signals inducing transcriptional activation of the human EGR-1 gene in response to GM-CSF and IL-3 were convergent or divergent. We isolated and mapped the human EGR-1 gene and sequenced approximately 700 nucleotides (nt) of the 5' upstream region, which contains several putative regulatory elements, including an EGR-1 binding site (EBS), serum response-like elements (SRE) and a cAMP-responsive element (CRE). We have prepared recombinant constructs containing different fragments of this promoter subcloned into a vector containing the chloramphenical acetyltransferase (CAT) reporter gene. These constructs were transiently transfected into TF-1 cells and stimulated with GM-CSF or IL-3. Our results demonstrate that the promoter region between nt -116 to -7 contains sequences which are responsive to both GM-CSF and IL-3, while the region between nt -600 to -480 includes sequences which are stimulated by GM-CSF but not IL-3. Oligonucleotides containing previously described transcription factor-binding sequences were subcloned into a vector containing the thymidine kinase promoter and CAT reporter gene. One or two copies of the EBS, SRE or CRE did not confer GM-CSF or IL-3 responsiveness in transient transfection assays, although they did respond appropriately to control stimuli. These results suggest that GM-CSF and IL-3 mediate their actions through recognition sites of novel transcription factors. Experiments utilizing further deletion and site-directed mutants of GM-CSF-responsive sequences are currently in progress. By characterizing early nuclear events following GM-CSF or IL-3 stimulation of factor-dependent cells, we can begin to define and compare signal transduction pathways which regulate myeloid cell proliferation.

B 975 TRANSCRIPTIONAL REGULATION OF XMYODB, Jon B. Scales, Eric N. Olson and W. Michael Perry, Dept. of Biochemistry and Molecular Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

XMyodB is one of two genes present in *Xenopus laevis* which encode homologs of MyoD (Scales, J.B. et al, Mol. Cell. Biol., 10:1516-24, 1990). The two proteins are 91% similar in primary structure. They are also very similar in their biological properties, i.e. myogenic conversion of non-muscle cells, DNA binding and transactivation (Scales, J.B. et al, Cell Growth & Diff., 2:619-29, 1991). Despite their similarity of function, the two genes are differentially expressed during early development; XMyoDa is maternally expressed, while XMyodb is expressed in an exclusively zygotic pattern. The biological significance of this expression pattern is not known however, it poses an interesting question about how myogenic regulatory factors are themselves regulated. Initial experiments examined transcription levels from deletion constructs containing varying amounts of genomic sequence from 8000bp to 130bp upstream of the start site placed upstream of b-galactosidase. These constructs were injected into oocytes and embryos as well as transfected into primary cultures of chicken embryonic myoblasts. Several potential regulatory elements can be identified within a 200bp region of flanking sequence. In addition to deletional analysis, site specific mutants have been generated to assay the individual contibution of these potential regulatory elements. In conjunction with these functional assays, binding of factors present in the embryonic myoblasts to these elements has also been investigated.

B 976 STAGE-SPECIFIC CHANGES IN TRANSCRIPTION FACTOR EXPRESSION DURING NORMAL B CELL DEVELOPMENT, Peggy Scherle, Kevin Holmes* and Louis Staudt, Metabolism Branch, National Cancer Institute, and National Institute of Allergy and Infectious Diseases,* Bethesda, MD 20892

A number of transcription factors have been suggested to be important during B cell development based on their restricted expression in lymphoid cell lines and/or the identification of potential binding sites in promoters and enhancers of lymphoid-specific genes. The expression pattern and role of these transcription factors during normal B lymphopoiesis, however, has not been carefully studied. To address this issue, we studied a progenitor cell population which can be induced to differentiate to mature B and myeloid cells in vitro. The progenitor cell population, representing 1% of murine bone marrow, was isolated by FACS sorting for cells which are negative for B220 and MAC-1 but which express a novel surface marker, LIP-6. LIP-6 is expressed on immature and mature myeloid and B lymphoid cells in the bone marrow and spleen. When these cells were cultured on the clonal stromal cell line, S17, under Whitlock-Witte conditions, they gave rise to pre-B and B cells. Culture on S17 under Dexter conditions gave rise to immature and mature myeloid cells. The LIP-6+ progenitor cells had not undergone rearrangement of immunoglobulin heavy and light chain genes. Using a quantitative RT-PCR assay, we demonstrated that Rag-1, Rag-2, TdT and sterile μ transcripts were expressed at low or undetectable levels in the progenitors but were dramatically upregulated when the cells were cultured under lymphoid but not myeloid condition. We further examined the expression pattern of transcription factors thought to be important for immunoglobulin transcription in the various cell populations. Two transcription factors, oct-2 and LEF-1, were expressed at only low levels in the progenitors but were dramatically upregulated in lymphoid but not myeloid cultures. This implies a potential involvement of these factors in commitment to the lymphoid lineage. Other factors such as Pu.1 and ets-1 were expressed in the progenitors and did not show a significant increase in the differentiated cells. Interestingly, expression of the HLH protein, E47, was low in the progenitors and high in both myeloid and lymphoid cultures. Previous studies of transformed cell lines had suggested that E47 had a widespread expression pattern. Our results suggest that upregulation of E47 may be required to allow differentiation of progenitor cells to the lymphoid or myeloid lineages.

B 977 **Function of *jun*B and c-*jun* in the Differentiation of Neurons and PC-12 Tumor Cells**

Karl-Hermann Schlingensiepen, Wilhelm Gerdes, Wilfried Seifert and Wolfgang Brysch
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Two homologues of the proto-oncogene c-*jun* have been identified in mammals. We have recently shown that in contrast to c-*jun*, *jun*B negatively regulates cell proliferation[1]. This and the different expression patterns of c-*jun* and *jun*B[2] raise the possibility that *jun*B may play a role in cell differentiation. We have used antisense phosphorothioate oligodeoxynucleotides[3,4] (S-ODN) to specifically inhibit expression of c-*jun* and *jun*B in neuronally differentiating PC-12 tumor cells and in primary neuronal cell cultures from rat hippocampus. Western blot analysis revealed specific reductions in the respective jun protein levels by more than 90% after application of 2μM S-ODN.

In neuronal cell cultures, neurite outgrowth was strongly inhibited after inhibition of *jun*B expression, but was enhanced after application of anti c-jun-S-ODN. Even more drastic changes were observed in neuronally differentiating PC-12 tumor cells. NGF-induced differentiation was completely inhibited in PC-12 cells after application of anti *jun*B-S-ODN, but again enhanced after the use of anti c-*jun*-S-ODN. These findings suggest that *jun*B plays a crucial role in cell differentiation, while c-*jun* appears to inhibit differentiation. Thus differential activity of different *jun* genes may be a key element involved in changing cell programs *e.g.* from proliferation to differentiation.

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B 978 EXPRESSION OF C/EBP-RELATED TRANSCRIPTION FACTORS CORRELATES WITH THE LINEAGE SWITCH OF TRANSFORMED B CELLS, Richard C. Schwartz[1], James D. Bretz[1], Simon Williams[2] and Peter F. Johnson[2], [1]Department of Microbiology, Michigan State University, East Lansing, MI 48824 and [2]NCI, ABL-Basic Research Program, P.O. Box B, Frederick, MD 21701

Transformed B cells can undergo a lineage conversion to a monocyte/macrophage phenotype. Cells that undergo this conversion become adherent, gain an extensive vacuolated cytoplasm, express high levels of Mac-1 and α-naphthyl acetate esterase, and acquire the capacity to phagocytose, process and present antigen. This process is accompanied by the down regulation of c-*myc* and c-*myb*, two proto-oncogenes that act as transcription factors. Here we report the up regulation of the CRP2 and CRP3 transcription factors in "lineage switch" cells. CRP2 (also reported as NF-IL6, LAP, IL-6DBP and AGP/EBP) and CRP3 belong to a C/EBP-like protein family, and exhibit DNA-binding and leucine zipper dimerization specificities that are nearly identical to those of C/EBP. Previous studies suggested that CRP2 is a regulatory component of the acute phase response in hepatocytes. In accordance with this role, treatment of cells with interleukin 1 or 6 was seen to elicit an increase in CRP2 binding activity. Although Northern blot analysis revealed that CRP2 and CRP3 RNAs are expressed in a wide variety of tissues, we show that its expression within the hematopoietic lineages is restricted to monocytes, macrophages, and neutrophils. CRP2 protein expersion has also been detected in "lineage switch" macrophages, as well as CRP2 and/or CRP3 gel shift species upon incubation of nuclear extracts with C/EBP binding motifs. The strict correlation between CRP2 and CRP3 mRNA expression and the monocyte/macrophage phenotype, observed in several independent cell lines, suggests that these transcription factors may also have a function in determining macrophage differentiation. Experiments are underway that examine the consequences of ectopic expression of CRP2 and CRP3 in B lymphoid cells.

B 979 MOLECULAR STRATEGIES IN T CELL DEVELOPMENT Jyoti Sen, Steven J Burakoff and Ranjan Sen, Dana Farber Cancer Institute, Department of Pathology, Harvard Medical School, Boston, MA 02115 and Rosensteil Center, Brandeis University, Waltham, MA 02254

Thymocytes develop into functionally mature T cells in response to cues from the thymic microenvironment. As the thymocytes mature they display varying combinations of CD4, CD8 and the T cell Receptor (TCR) on the cell surface. Several lines of evidence suggest that signals transduced via these receptors provide critical signals for T cell maturation. To study the effects of signalling via the TCR and coreceptors, we have analyzed the induction of nuclear factors in thymocytes. Specifically, we have investigated NFKB, NFAT, AP-1 and CREB induction in various thymocyte populations in response to signalling through individual or combinations of receptor molecules. These studies will be complemented by a corresponding analysis of inducible DNA binding proteins in fetal thymocytes at different developmental stages.

B 980 Differential Expression and Structure of the Human Annexin VI Gene.

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The annexins constitute a large family of Ca^{2+}/phospholipid binding proteins. Annexins are defined by an internally repetitive conserved sequence of around 70 amino-acids which appears 8 times in annexin VI but four times in all other members of the family. Here we report immunohistochemical analysis of the expression of human annexin VI and the structure of the human annexin VI gene. Most tissues investigated expressed annexin VI but the protein was usually restricted to highly specific cell types within each tissue. There was good correlation between expression and hormone secreting cells in the pancreas, testes and adrenal cortex. Differential expression was observed in the ductal epithelia of the mammary gland, expression being restricted to non-lactating tissue. Annexin VI expression is developmentaly regulated in B- and T-lymphocyte differentiation, with negative staining in the proliferating B cells of the germinal centre of the lymph nodes, but strong staining in the mature small lymphocytes of the cortex, mantle zone and paracortex. We also report the structure of the human annexin VI gene, it is around 60 kb long and consists of 25 translated exons and at least one untranslated exon. Exon 18 is alternatively spliced in a manner which seems to be dependent upon the proliferative status of the cell.

B 981 IDENTIFICATION OF *CIS*-ACTING ELEMENTS OF THE MOUSE *En-2* GENE, Dong-li Song, C. Logan, G. Cado, W. Khoo, and A. L. Joyner; Samuel Lunenfeld Research Institute of Mount Sinai Hospital, 600 University Avenue, Toronto Ontario, M5G 1X5 Canada.

The expression of the mouse *engrailed*-like gene, *En-2* , is temporally and spatially regulated during embryogenesis. To identify the *cis*-acting regulatory elements that specify the expression pattern of this homeobox-containing gene we have analyzed the *En-2* promoter region using *Escherichia coli lacZ* reporter constructs in transgenic mice. Five transgenic lines were established that express *lacZ* in an *En-2* manner using a construct containing 9.5 kb of *En-2* genomic DNA. The transgene is first expressed in five somite embryos in a band of cells in the anterior neuroepithelium and is maintained in this region, which later marks the mid/hindbrain junction, at least up to 15.5 d of gestation. The *lacZ* expression is also detected in the mandibular arch from 9.5 d to 15.5 d. Deletion analysis has identified two enhancer elements which can specify the expression to either the mid/hindbrain or mandibular region, respectively. A 1 kb fragment 5 kb 5' of the start of transcription is sufficient to direct *lacZ* expression to the embryonic mid/hindbrain from either the *En-2* or mouse heat shock promoter. This mid/hindbrain enhancer as well as 0.5 kb its corresponding region from the human EN2 gene were sequenced. Sequence comparison revealed a high degree of sequence similarity (73% in 0.4kb) between the two sequences. The functional significance of the conserved region is currently being studied in transgenic mice and using band shift analysis with embryonic brain extracts.

B 982 TGF-β INHIBITION OF 3T3 T PROADIPOCYTE DIFFERENTIATION: EFFECTS ON C/EBP AND DIFFERENTIATION-DEPENDENT GENE EXPRESSION, Rodney L. Sparks, V. A. Manga, and B. J. Allen, Department of Anatomy, Molecular and Cellular Biology Graduate Program, Tulane Medical School, New Orleans, LA 70112

The objectives of our studies are to further define the regulatory mechanisms, both positive and negative, that control the differentiation of normal and transformed stem cells. CCAAT Enhancer Binding Protein α (C/EBPα) is a basic leucine zipper transcription factor whose expression: 1) increases during adipocyte differentiation; 2) transactivates several differentiation-specific genes; and 3) causes growth arrest. Recently discovered members of the C/EBP family (β and δ) increase shortly after the differentiation stimulus and decrease prior to increases in "fat" gene or C/EBPα expression, suggesting an early regulatory role. We are attempting to elucidate their role in our model system--differentiation of BALB/c 3T3 T proadipocytes. Transforming growth factor β (TGF-β) is known to inhibit differentiation of 3T3 T cells as well as various other proadipocyte models. We have found by northern analyses that TGF-β inhibits the differentiation-specific increase in C/EBPα expression, a similar effect to that which we have seen with "fat" genes, such as Lipoprotein lipase and Glycerol-3-phosphate dehydrogenase. The mechanism(s) of action is not known. TGF-β only inhibits the expression of C/EBPα and the differentiation-specific genes if added prior to their induction. This suggests that TGF-β may not inhibit 3T3 T differentiation by directly blocking expression of C/EBPα or the differentiation-specific genes, but rather by blocking a regulatory gene(s) [analogous to myoD]. The C/EBP family of genes might be involved in a similar regulatory cascade in adipocytes. More specifically, we show here that while TGF-β inhibits C/EBPα expression when added early in the differentiation regimen--its effects on C/EBP β and δ are more complicated. Contrary to C/EBPα, there is a decrease in expression of both C/EBPβ and C/EBPδ in fat cells. In fact, their expression appears to be regulated shortly after the differentiation stimulus yet prior to any increase in induction of differentiation-specific gene expression. We are also studying the expression of these genes in transformed proadipocytes with various growth and differentiation defects. We have found aberrant expression patterns for C/EBPα, β, and δ in growing clones of transformed 3T3 T cells as well in transformed cells exposed to the differentiation stimulus. (Funded by NCI- CA46683 and Fraternal Order of Eagles to RLS).

B 983 DNA BINDING PROPERTIES OF BF-1, A TELENCEPHALON RESTRICTED MEMBER OF THE HNF-3/FORK HEAD GENE FAMILY, Wufan Tao, Gabriela Balas and Eseng Lai, Division of Endocrinology and Program of Cell and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

BF1 (brain factor-1), a new member of HNF-3/*Fork Head* gene family, was isolated from a rat brain cDNA library. Expression of BF-1 is highly restricted in the developing neural tube to its rostral end which gives rise to the telencephalon. Its expression is easily detected as early as was examined, E10 in the rat, reaches peak at E17 and then declines gradually. BF-1 is most highly homologous to another recently reported member of HNF-3/*Fork Head* gene family from Drosophila, sloppy paired 2, which functions in segmentation. These results suggest that BF-1 may play an important role in the establishment of the regional subdivision of the developing brain and in the development of the telencephalon.

BF-1 has distinct DNA binding properties from those of HNF-3 proteins. BF-1 only shows weak binding activity to one of HNF-3 binding sites (B2). In order to study the function of BF-1 and the interaction between BF-1 protein and its DNA binding sites, a consensus sequence to which BF-1 shows a high binding activity was identified from random oligonucleotides by a site selection protocol. Our results suggest that BF-1 has a greater sequence recognition specificity than HNF-3 proteins. A detailed investigation of interaction between BF-1 protein and different binding sites is in progress.

B 984 CHARACTERIZATION OF THE CIS-REGULATORY ELEMENT MEDIATING TGF β STIMULATION OF COLLAGEN TRANSCRIPTION IN NORMAL FIBROBLASTS AND SPONTANEOUS UPREGULATION OF COLLAGEN TRANSCRIPTION IN SCLERODERMA FIBROBLASTS, Maria Trojanowska, Christoph Hartl, Takeshi Tamaki and E. Carwile LeRoy, Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston, SC 29425

Scleroderma is a disease characterized by excessive deposition of connective tissue in the dermis and internal organs. Increased levels of collagen mRNAs have been demonstrated in scleroderma skin by in situ hybridization studies and in cultured fibroblasts derived from scleroderma lesions.

Recently, we have found that TGFß stimulates collagen α2(I) (COL1A2) transcription in normal skin fibroblasts, while in scleroderma fibroblasts basal COL1A2 transcription level is elevated and resistant to further stimulation by TGFß. We used deletions and specific nucleotide substitutions to define a cis-regulatory element (termed α2-TAE) located at -318 to -294 in human COL1A2 promoter responsible for mediating the TGFß stimulation in normal fibroblasts. In gel retardation assays, we showed that normal fibroblast nuclear extract contains a binding activity to α2-TAE and that this activity is enhanced after TGFß stimulation. Interestingly, although α2-TAE resembles NF-1 binding site, consensus NF-1 sequence does not compete for binding with human α2-TAE, suggesting that α2-TAE binding protein is not the same as NF-1 proteins characterized in HeLa cells. Scleroderma fibroblasts, when compared to closely matched normal controls, show higher specific binding activity for α2-TAE, which is not significantly stimulated by TGFß. These results suggest that activation of the α2-TAE binding protein may be involved in TGFß stimulation of COL1A2 transcription in normal fibroblasts and constitutive upregulation of COL1A2 transcription in scleroderma fibroblasts.

B 985 THE EXPRESSION OF *ETS1* TRANSCRIPTION FACTOR DURING MURINE EMBRYOGENESIS SUGGESTS A ROLE IN HAEMATOPOIESIS, ORGANOGENESIS AND BRANCHING MORPHOGENESIS. M. Tymms, A. Seth, S. Brookes, T. Green, S. Chu, R. Thomas, T.S. Papas and I. Kola, Institute of Reproduction and Development, Monash University.

The *ETS* family represent a novel class of transcription factors. These transcription factors bind a purine-rich core found in the promoters of various cellular and viral genes. However, the biological roles of the various family members are largely unknown. In this study we investigate the expression of *Ets1* and *Ets2* in murine embryos.

Our data demonstrates that *Ets1* is expressed at high levels in the yolk sacs of day 8-10 mouse conceptuses. This pattern of expression correlates with the presence of haematopoietic stem cells in the yolk sac. Furthermore we also show, using gel shifts, that the *Ets1* protein binds sequences derived from the promoters of a number of and growth factor genes involved in haematopoiesis. These data suggest that *Ets1* may play an important role in haematopoiesis by regulating the expression of several genes involved in the process.

We also find that the expression of *Ets1* is widespread during the formation of organs in murine embryonic development. The expression of the gene is not detected in organs derived from later fetal stages, except in organs that are undergoing branching morphogenesis and in lymphoid organs. In neonatal and adult mice the expression of *Ets1* is restricted mainly to lymphoid organs. *Ets1* transactivates metalloproteinase genes such as stromelysin, collagenase and urokinase plasminogen activator which are important in extracellular matrix degradation. Thus, *Ets1* may have an important role in regulating the expression of genes involved in organ formation and modelling.

Our data demonstrates that the expression of the *Ets1* transcription factor is regulated in a temporal and tissue specific manner during murine embryogenesis. *Ets1* is expressed during haematopoiesis, organogenesis and branching morphogenesis, and binds sequences derived from the promoters/enhancers of other genes involved in these processes.

B 986 CHARACTERIZATION OF CIS-ELEMENTS AND TRANS-ACTING FACTORS INVOLVED IN THE REGULATION OF THE RAT OXYTOCIN PROMOTER, Hermien van Schaick, Roger A.H. Adan, Sofia Lopes da Silva and J. Peter H. Burbach, Rudolf Magnus Institute, Utrecht University, Vondellaan 6, 3521 GD Utrecht, The Netherlands

The oxytocin (OT) gene is expressed in the hypothalamo-neurohypophyseal system (HNS) and is regulated during development, hyperosmolality, pregnancy, lactation and the estrous cycle. To understand the molecular mechanisms underlying the regulation of the OT gene, studies in search for regulatory elements in the 5' flanking region of the gene and the transcription factors using these elements have been initiated. Two classes of transcription factors have been considered: members of the steroid/thyroid hormone receptor family and POU domain proteins. In transient heterologous expression systems the rat OT promoter was stimulated by the estrogen receptor (ER), retinoic acid receptor (RAR) and thyroid hormone receptor (T3R). Analysis of 5'-deletion mutants in transient expression systems and of protein-DNA interactions revealed that these receptors act through a common element located between nucleotides -172 and -148. This element integrates a direct and an inverted repeat of the motif TGACC. Mutagenesis indicated that the inverted repeat is the ERE, while the direct repeat that is of the DR0 type mediates responses to T3 and RA. The orphan receptor COUP-TFI also bound to this element and repressed induction of the OT promoter by E, T3 and RA. The class III POU domain proteins Brn-1, Brn-2 and Brn-4 are expressed in the HNS (ref. He et al., Nature 340:35 (1989); Le Moine and Young, PNAS 89:3285 (1992)). Brn-1 did not influence the activity of the rat OT promoter (nucleotides -363 to +16) in the heterologous expression systems. This and other class III POU domain proteins are now being investigated for interaction and transactivation of the OT promoter using longer regions of the gene and neuronal expression systems.

B 987 RESTRICTED EXPRESSION OF ISLET CELL ZINC FINGER PROTEINS. Henrik Vissing, Anette A. Pedersen, Birgitte Michelsen, *Esper Boel and Lizzi Aagaard. Hagedorn Research Institute, 2820 Gentofte, Denmark and * Pharmaceutical Research, Novo Nordisk, Bagsvaerd, Denmark.

The Islet of Langerhans have an important role in the control of metabolism. They produce several hormones which include insulin, glucagon, pancreatic polypeptide and somatostatin. Each islet contains some 3000 hormone producing cells, 70 percent of which are insulin producing β–cells, the remaining being α–, δ– and PP cells. Destruction of the β-cells results in insulin deficiency, ultimately leading to type I diabetes. We are working towards identifying specific factors that are involved in determining the phenotypic properties of the cells in the Islet of Langerhans.

Zinc finger proteins of the Cys_2-His_2 type have been isolated from both human and rat tissue. Approximately 100 clones were isolated from a human insulinoma cDNA library and 30 clones from a growth hormone stimulated rat islet cell cDNA library. A randomly selected subset from the two groups of the clones was further analysed. The DNA sequence has been determined by automated fluorescent sequencing and out of 34 ZFPs analysed so far only one match was found in GenBank/EMBL. All ZFPs exhibit tandem multi-fingered structures and a few have additional scattered fingers. In addition to the zinc finger domain, additional conserved motifs are present, such as KRAB domain structures. Northern blot analysis revealed a high variation in the expression profile of the different ZFPs. Some are ubiquitously expressed and others are differentially expressed to a varying degree e.g. islet/testis, islet/brain/lung. The expression profile is further being investigated by in situ hybridisation.

B 988 *IN VIVO* IDENTIFICATION OF A HOX 1.3 TARGET, Peter Vos, Shang-Ding Zhang, and Ward F. Odenwald, Neurogenetics Unit, LNC, NINDS, NIH, Bethesda, MD 20892

Homeodomain proteins have been implicated as regulatory factors that control and coordinate the expression of target genes. However, our inability to selectively control the expression of individual homeobox genes *in vivo* has made it difficult to identify candidate targets for homeotic gene products in vertebrate systems. Hox 1.3, a murine homologue of the *Drosophila Antennapedia* class homeotic gene, *sex combs reduced*, is a phosphoprotein which binds DNA in a sequence specific manner. To test the *in vivo* function of Hox 1.3, we generated transgenic mice in which the transgene consisted of Hox 1.3 cDNA linked to an interferon (IFN) inducible promoter, Mx1. In the absence of deliberate induction, these mice appeared normal. Baseline transgene expression was undetectable by Northern analysis. However, exogenous IFN administration lead to a rapid induction of Hox 1.3 RNA and protein expression in many organs (e.g., CNS, lung, and liver).

Many of the currently known targets for *Drosophila* homeodomain proteins are transcription factors. Gel-mobility shift assays and Northern analysis were used to determine if ectopic Hox 1.3 expression modulated the steady state levels of other known transcription factors. One of the transcription factor families examined was hepatocyte nuclear factor-3 (HNF-3). Liver extracts from both transgenic and wild-type mice were compared for their levels of HNF-3-like DNA binding activity eighteen hours after IFN administration. This DNA binding activity was decreased in liver extracts from the transgenic mice, but not in extracts from the wild-type mice. Liver RNA from both transgenic and wild-type mice were then compared for levels of HNF-3 expression six hours after IFN administration. HNF-3 RNA expression was decreased in the transgenic mice, but not in the wild-type mice. We conclude Hox 1.3 can negatively regulate HNF-3 expression in these transgenic mice.

B 989 PURIFICATION OF A MAMMARY GLAND SPECIFIC NUCLEAR DNA BINDING FACTOR (MGF) FROM LACTATING SHEEP.

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The expression of milk proteins is regulated both at the transcriptional and the post-transcriptional level. It has been shown that insulin, glucocorticoids, and prolactin are necessary for the synthesis and the accumulation of the casein mRNA in rat and mouse mammary gland cells. We have chosen the ß-casein gene as a model for the study of the multihormonal control of gene expression. The analysis of the 5' flanking region of the casein gene family revealed a highly conserved sequence in their promoters. To this sequence, 5'-TTCTTRGAATT--AA-3' binds a trans-acting factor. The activity of this factor increases during pregnancy and reaches maximum at birth judged from gel retardation assay. During lactation, its level is kept constant, however, once pups are withdrawn, no factor is detected. This indicates that the suckling is also very important for the DNA binding activity. Our previous studies revealed that this factor is indispensable for the responsiveness of the ß-casein gene promoter to the lactogenic hormones, glucocorticoid and prolactin. This factor is mammary gland specific. We observed its homologue in bovine and in sheep mammary gland. In order to elucidate the molecular mechanisms by which these hormones regulate the expression of the ß-casein gene, we purified this mammary gland specific factor (MGF). We initially purified MGF from lactating rats. The amount of proteins we acquired was, however, too small to get the internal amino acid sequence. We have changed the source for the purification and simplified the method to purify MGF. A combination of Red A Sepharose and sequence specific DNA affinity column chromatography was sufficient to yield highly purified proteins. MGF was purified more than 2400 fold compared to the whole nuclear extract with a 60 % of recovery. Analysis of the purified fraction on SDS polyacrylamide gel electrophoresis revealed that it has been purified to near homogeneity, with an apparent molecular weight of 92 kD and 90 kD. These two peptides were consistently present after the combination of a mutated and a wild type DNA affinity column chromatography. Purified fraction was sensitive to phosphatase treatments.

B 990 IDENTIFICATION OF NUCLEAR PROTEINS FROM DIFFERENTIATING CHONDROCYTES WHICH BIND TO THE ENHANCER OF COLLAGEN II GENE AND REGULATE ITS TRANSCRIPTION, Liqun Wang, Richard Balakir, Patricia Precht and Walter E. Horton, Jr., Gerontology Research Center, NIA/NIH, 4940 Eastern Ave., Baltimore, MD 21224

The first intron of the rat collagen II gene contains a region that has the characteristics of a transcriptional enhancer. We have recently identified a *cis*-acting sequence (5'-CACAATGCAT-3') located within this region that is required for the enhancer activity. We have shown that binding of nuclear extracts prepared from differentiating chondrocytes to the enhancer correlated well with the differentiation process. In order to purify the binding proteins and study their function in the cell type-specific transcription, a DNA-affinity column was prepared by using Sepharose CL-2B resin and the *cis*-acting DNA sequence. Nuclear extracts prepared from 2 expressing cell types (chick sternal chondrocytes and rat chondrosarcoma-chondrocytes) were fractionated according to their binding property. Three distinct proteins of similar size (~ MW = 32 kD) were isolated from the chick sternal chondrocytes, while two proteins (~ MW = 32 kD & 18 kD) were isolated from the chondrosarcoma chondrocytes. The column-purified proteins retained binding activity as evidenced by both mobility-shift assay and Southwestern analysis. Currently, we are attempting to further purify these proteins by HPLC and to obtain partial amino acid sequence for the cloning of their genes. In addition, the partially purified protein fraction has been used to reconstitute the transcription activity *in vitro*.

B 991 THE CHICKEN E12/E47 PROTEINS ARE NUCLEAR PHOSPHOPROTEINS WHICH ARE UPREGULATED DURING MYOGENESIS AND ARE ASSOCIATED WITH CMD1 IN VIVO. Qin Wei and Bruce M. Paterson, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

We have isolated two different cDNA clones corresponding to transcripts from the chicken E2A gene homolog by virtue of their weak hybridization to the human E12 cDNA clone. Nucleotide sequence analysis and amino acid comparison define these clones as the avian counterparts to E12 and E47 with identies greater than 80%. The E2A gene transcripts are expressed in a variety of tissues at different levels but, suprisingly, expression levels are highest in chicken embryonic muscle as compared to brain and liver. During myogenesis in primary muscle cultures the level of the E2A gene products increase measurably and accumulate in the nuclei of newly formed muscle fibers. Immunoprecipitation studies indicate chicken E12/E47 proteins are phosphorylated and associated with CMD1, the avian MyoD homolog. Cotransfections in primary muscle cultures with the chicken E12/E47 expression vectors and either an [E2-E5] CAT or CKTK-CAT reporter indicate these E2A proteins can enhance the activation effects of CMD1 on the CK enhancer. The role of the E2A gene products in the differentiation of muscle is under investigation.

B 992 A NEW IN VITRO MODEL SYSTEM FOR ANALYSIS OF METANEPHRIC MESENCHYME TO EPITHELIAL DIFFERENTIATION , Patricia D. Wilson and Christopher R. Burrow, Division of Nephrology, Johns Hopkins School of Medicine, Baltimore, MD 21205.

The molecular regulation of commitment of mesodermal blast cells to an epithelial fate has been little studied. A model system has been developed to study this phenomenon during human renal development, in which mesenchymal cells of the metanephric blastema are induced to proliferate and differentiate into several characteristic tubular and glomerular epithelial cell types that populate a mature nephron. Nephroblast-rich areas of 14-16wk human fetal kidneys are microdissected from the outer cortical rim, plated on gelatin in defined medium, passaged and grown in continually proliferating suspension culture in the presence of serum-free conditioned medium from a Wilms' tumor cell line (WT-CM). A secreted soluble peptide factor(s) (NB-GF) is essential to sustain proliferation of these undifferentiated nephroblasts in vitro. Replacement of WT-CM with 10% serum-containing medium and addition of collagen IV matrix, resulted in differentiation of the small round nephroblasts into attached foci of larger, flattened polygonal cells, characterized as epithelia by their increased cytoplasmic volume and organelle complexity, polarized distribution of membrane proteins, intercellular desmosomes and specific marker analysis of cytoskeletal and adhesion proteins, matrix receptors and enzymes. Immunostaining showed that conversion from blast to epithelial morphology was accompanied by loss of vimentin and syndecan but acquisition of E-cadherin, alkaline phosphatase and networks of cytokeratin, characteristics that mirror the changes observed in vivo during nephrogenesis. This characterized system will allow for the identification of transcription factors important in the molecular control of proliferation and differentiation of renal progenitors.

B 993 THE CREM GENE IS RAPIDLY AND TRANSIENTLY INDUCED BY cAMP, Paul I. Woloshin, Robert P. Rehfuss, Richard H. Goodman and Roger D. Cone, Vollum Institute, Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201.

CREM, a member of the CRE binding protein (CREB) family of transcription factors, produces several mRNA isoforms through differential splicing and multiple start sites. Depending on the isoform, CREM can be a transcriptional stimulator or repressor. At present, all identified CREB family members have been shown to be constitutively transcribed and regulated post-transcriptionally via phosphorylation. In contrast, we show that the CREM gene is transcriptionally regulated via the cAMP mediated pathway in several cell types. Treatment of rat thyroid FRTL5 cells with thyroid stimulating hormone (TSH), which elevates cAMP and is required for normal growth and differentiation, produced a rapid expression of CREM mRNA. CREM first appeared in 2 hours, peaked at 4 hours and then decreased. Treatment with 5 μM forskolin mimicked both the time course and degree of TSH induced expression. Four separate mRNA species were expressed, ranging from approximately 1.0kb to 2.0 kb in size. Treatment with TPA, EGF or A23187 produced little induction of CREM. CREM was also found to be induced by cAMP in Y1 adrenocortical, Cloudman S91 melanoma, SY5Y neuroblastoma, and Balb/c 3T3 fibroblast cells, as well as in primary foreskin fibroblasts. The time course of expression was similar, but different mRNA species were induced by cAMP in the Balb/c 3T3 cells. While forskolin treatment induced CREM in Balb/c 3T3 cells, serum stimulation did not. CREM has been previously shown to be an inhibitor of cAMP mediated c-fos gene expression in cells transfected with a c-fos CRE reporter plasmid. The time course of cAMP induced c-fos expression in FRTL5 cells showed that c-fos peaked at 60 minutes post-TSH treatment and dropped to zero by 2 hours. This is precisely the point at which CREM expression comes on, providing *in vivo* evidence to support the hypothesis that in some cell types CREM may be acting as a suppressor of c-fos expression.

B 994 MOLECULAR CLONING AND CHARACTERIZATION OF FACTORS THAT REGULATE THE ACTIVITY OF THE MEF-2 SITE IN THE XENOPUS MYOD PROMOTER. Mee-wa Wong and Michael Perry, Department of Biochemistry and Molecular Biology, U.T. MD. Anderson Cancer Center, 1515 Holcombe Blvd., Box 117, Houston, TX 77030.

A conserved DNA sequence, CTA(A/T)$_4$TAG, is a transcriptional regulatory element in promoters and enhancers of a variety of muscle-specific genes, such as the MyoD and myogenin promoters and the muscle creatine kinase (MCK) and myosin light chain 1/3 enhancers. A muscle-specific factor, termed MEF-2 interacts specifically with this regulatory motif. To examine the potential role of MEF-2 in the regulation of MyoD transcription during early development, we have analyzed the appearance of MEF-2 binding activity in developing frog embryos using the electrophoretic mobility shift assay with a probe containing the MEF-2 site in the XMyoDa promoter. Two genes were isolated from a *X. laevis* stage 24 cDNA library that encode factors that bind the XMyoDa MEF-2 site. Both genes are highly homologous to each other and belong to the MADS (MCM1-Arg 80-agamous-deficiens-SRF) protein family. The proteins encoded by both genes contain a conserved N-terminal MADS box required for binding to DNA. The temporal and tissue-specific expression of these two genes is being investigated by ribonuclease protection. We plan to test the functional roles of these proteins during early development by analyzing the effects of ectopic expression of these genes in frog oocytes and embryos.

B 995 NGF-REGULABLE OCT-2 ISOFORMS IN RAT DRG NEURONS DEFINED BY SPECIFIC ANTISERA.

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A variety of mRNA transcripts for POU domain proteins, which play an important role in tissue specific gene expression, have been detected in the developing and adult nervous system. In adult rat peripheral sensory neurons, mRNA transcripts encoding an octamer binding protein, Oct-2, originally identified in B cells, have been detected by PCR and in situ hybridisation. In addition, DNA mobility shift assays using octamer motifs have identified transcription factors in nuclear extracts of sensory neurons which co-migrate with B-cell Oct-2. Both the levels of mRNA encoding Oct-2 and functional protein as measured in band shift assays are upregulated by treatment of adult sensory neurons with Nerve Growth Factor. These results are particularly interesting with respect to the observation that Oct-2 is capable of blocking immediate early HSV transcription and may thus play a role in the establishment of latent sensory neuron infections. A total of 8 related forms of differentially spliced Oct-2 mRNAs have been identified in the mouse. In order to identify the Oct-2 isoforms expressed and regulated by NGF in sensory neurons, we have generated specific rabbit polyclonal antisera to a unique dodecapeptide sequence CSAAPMLPSPGK that is present 3' of the POU homeobox in all known isoforms of Oct-2. The sera were affinity-purified on immobilised peptide columns and used to probe western blots of adult rat sensory neurons cultured in the presence or absence of NGF. A single broad band of immunoreactive material migrating at 60 kDa was detected in neuronal extracts and quantitated with ^{125}I anti-rabbit antisera. NGF caused a 2-3 fold increase in immunoreactivity. Higher resolution gels developed with alkaline phosphatase-conjugated antisera showed the presence of a major band of apparent molecular weight 61kDa and two minor bands migrating above (64kDa) and below (59kDa) this band, all of which were induced by NGF. These molecular weights are similar to those found in purified Oct-2 fractions from human B cells , and may correspond to murine Oct 2.1, 2.2 and 2.3 isoforms. Monospecific antisera to Oct-2 should thus provide a useful reagent for the detection, quantitation and comparison of tissue-specific Oct-2 isoform protein expression.

B 996 $p21^{ras}$ function is important for T cell antigen receptor and protein kinase C regulation of NFAT (nuclear factor of activated T cells) M. Woodrow, S. Rayter*, J. Downward*, and D. Cantrell. Lymphocyte Activation Laboratory and *Signal Transduction Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London, WC2A 3PX England

NFAT(nuclear factor of activated T cells) is a transactivating factor critical to the regulation of the interleukin-2 gene. The intracellular signalling mechanisms that control the expression of NFAT have been examined, and it is evident from a number of studies that protein kinase C(PKC) and increases in intracellular calcium concentration synergize to induce NFAT. It is now understood that the NFAT complex is at least partly composed of fos- and jun-related proteins, which are responsive to PKC initiated signals. Recent work has demonstrated that the T cell antigen receptor (TCR) and PKC regulate the activity of the guanine nucleotide binding proteins, $p21^{ras}$, suggesting that $p21^{ras}$ may mediate PKC and TCR functions in T cells. We have explored the role of $p21^{ras}$ in the regulation of NFAT using a transient transfection assay. Cotransfection was performed using i) a plasmid bearing multimers of the NFAT site linked to a reporter gene, and ii) a construct constitutively expressing either active or dominant inhibitory ras mutants. Data show that expression of activated ras proteins can synergize with calcium and PKC signals to regulate NFAT. The introduction of dominant inhibitory ras blocks both TCR and PKC regulation of NFAT. This suggests a role for ras as a mediator of TCR and PKC in T cells.

B 997 REGULATION AND FUNCTION OF CREB IN B LYMPHOCYTES, Huijuan Xie, Thomas C. Chiles and Thomas L. Rothstein, Departments of Microbiology and Medicine, Boston University Medical Center, Boston, MA 02118.
The cyclic AMP response element (CRE) binding protein CREB was previously characterized as a constitutively expressed nuclear factor whose transcriptional activity is regulated by phosphorylation. In the present study, the regulation and function of CREB was examined in B lymphocytes in order to begin to elucidate the role of cAMP derived signals in B cell activation. CRE-binding activity detected by the electrophoretic mobility shift assay (EMSA) was found to be constitutively expressed in nuclear extracts of primary murine splenic B cells stimulated in a variety of ways. This activity was shown to be specific by competition analysis and to represent CREB or a closely related molecule on the basis of a "supershift" in the mobility of the EMSA-detected nucleoprotein complex induced by anti-CREB antiserum (kindly provided by Dr. Mark Montminy, Salk Institute, La Jolla, CA). The function of B cell CREB was assessed by transient transfection of the sIg-responsive murine B lymphoma cell line, BAL17, with a CRE-dependent CAT construct that contains a portion of the somatostatin promoter. Forskolin, which markedly induced CAT expression in PC12 cells transfected with this construct, failed to stimulate CAT activity in transfected BAL17 B cells. However, cross-linking of the sIg receptors of transfected BAL17 B cells produced a 3-fold induction of CAT activity. Interestingly, anti-Ig was found to act in synergy with forskolin and other cAMP raising agents to produce greatly enhanced CAT activity. A phosphoprotein of appropriate molecular size was immunoprecipitated from anti-Ig plus forskolin treated BAL17 B cells by anti-CREB. These results suggest that CREB is present in primary B cells and that CREB mediated gene expression is regulated by sIg either alone or in synergy with cAMP; the latter implies cross-talk between intracellular signaling pathways acting at the level of CREB.

B 998 TARGET SPECIFICITY AND PROTEIN-PROTEIN INTERACTIONS IN THE REGULATION OF TRANSCRIPTION BY HOX GENE PRODUCTS. V. Zappavigna and F. Mavilio, *Department of Biology and Biotechnology, Istituto Scientifico H. S. Raffaele, Milano, ITALY.*

Considerable evidence has been gained for a causal relationship between vertebrate Hox gene expression and morphogenetic events during embryogenesis. Nevertheless, vertebrate Hox genes are still poorly characterized with respect to their specificity and mode of action as transcriptional regulators. We have recently shown that murine and human Hox gene products are able to modulate transcription of HOX promoters through binding to specific target sequences in transient cotransfection assays in cultured cells. By analyzing Hox genes belonging to different paralogy groups, we were able to show both positive and negative regulatory interactions. The products of the human HOX4C and HOX4D (both *Abd-B*-like) genes enhance transcriptional activity through different target sequences in HOX4C (HCR sequence) and HOX3D promoters. In contrast, the product of the murine *Hox-4.3* (*abd-A*-like) gene is a positive transcriptional regulator only of the HOX3D promoter, whereas it is inactive on the HCR sequence in the HOX4C promoter. Moreover, the *Hox-4.3* gene product specifically antagonizes the activating functions of the HOX4C and HOX4D gene products on the HCR sequence. The dual capacity of the *Hox-4.3* gene product, namely to activate transcription on one target and to negatively modulate the activity of other Hox gene products on a different target, was further analyzed. In particular, we explored the possibility that the *Hox-4.3* negative modulatory effect might be mediated through direct protein-protein interactions between the HOX gene products, as described for POU-homeobox proteins. For this purpose, a series of deletions were generated in the *Hox-4.3* protein, involving the homeodomain and surrounding regions, together with constructs in which the homeodomains were swapped between the HOX4C and *Hox-4.3* genes. We could show that substitution of the entire *Hox-4.3* homeodomain with that of HOX4C is sufficient to confer to the *Hox-4.3* protein the ability to activate transcription through the HCR sequence. Furthermore, we could show that constructs involving a deletion of the recognition helix of the *Hox-4.3* homeodomain were still able to antagonize HOX4C transcriptional activity, while constructs bearing a complete deletion of the homeodomain or the HOX4C homeodomain were not. These data show that the antagonizing function of the *Hox-4.3* protein is not dependent on its DNA-binding function, and suggest a role for regions in the homeodomain outside the recognition helix in mediating protein-protein interactions between Hox gene products.

B 999 THE ROLE OF COSTIMULATION IN IL-2 GENE REGULATION. Linda A. Zuckerman and Jim Miller, Committee on Immunology, University of Chicago, Chicago, IL 60637. Most T cell proliferate to IL-2, a growth factor secreted by the Th1 subset of CD4+ and some CD8+ T cells. IL-2 gene expression is under strict control of a complex array of transcription factors that may be dependent upon the type of stimulation the T cell receives. For example, we have shown that: 1) chemically fixed spleen cells fail to induce proliferation of CD4+ T cell clones and 2) stimulation by fixed spleen cells results in a lack of NF-AT and NF-κB binding factors to the IL-2 enhancer. These results suggest that chemically fixed spleen cells may be lacking a costimulatory signal and that this signal may directly regulate the presence/binding of specific transcription factors necessary for IL-2 gene expression. In addition, lack of a costimulatory signal may also result in anergy, or a long-lived nonreposnsiveness of T cells.

We have developed a model system to study the role of costimulation in T cell activation using live APCs and antigen dependent T cell clones. Several accessory molecules including B7, HSA, ICAM, and CD2 have been suggested to provide a costimulatory signal. Therefore we have developed a panel of MHC class II postive cell lines that differ in their expression of B7, ICAM, and HSA. Using these cell lines we can determine whether the presence of specific costimulatory molecules on APCs correlate with T cell proliferation and the presence of specific transcription factors for IL-2 gene transcription. Using both live APCs that differ in their expression of accessory molecules and mAbs with costimulatory capabilities we hope to better define the factors that regulate IL-2 gene transcription during T cell activation.

Signal Transduction and Disease

B 1000 *FLT-4* RECEPTOR TYROSINE KINASE IN CHROMOSOME 5q35: INVOLVEMENT IN THE t(2;5), t(3;5) AND t(5;6) TRANSLOCATIONS AND EXPRESSION IN LEUKEMIA CELLS Elina Armstrong, Kumar Kastury*, Olga Aprelikova, Anne Polvi, Florencia Bullrich*, Christian Nezelof¤, John J. Wasmuth**, Kari Alitalo, Steven Morris*** and Kay Huebner*, Cancer Biology Laboratory, Departments of Virology and Pathology, University of Helsinki, 00290 Helsinki, FINLAND, Jefferson Cancer Institute*, Philadelphia, Department of Biological Chemistry**, UC Irvine, St. Jude Hospital***, Memphis, Pediatric Pathology¤, Necker Hospital, Paris, FRANCE

Receptor tyrosine kinases (RTKs) are an important group of proto-oncogenes which can be activated by chromosomal rearrangements. We have recently reported a novel member of class III RTK, *FLT-4*, characterized by seven immunoglobulin loop-like domains in its extracellular domain. *FLT-4* is expressed in human placenta, lung, heart and kidney. We have mapped *FLT4* to chromosome 5q33-qter in somatic cell hybrids and radiation-reduced hybrids. Interestingly, at least three class III RTKs map to the 5q chromosomal arm. In addition, the 5q arm contains a cluster of other growth factor and receptor genes. We have now refined the localization of the *FLT4* gene to band 5q35 and explored its relationship to the t(2;5) and t(5;6) translocations of Ki-1 lymphomas, as well as t(3;5), occasionally found in ANLL. *FLT4* expression occurs primarily in erythroid and megakaryoblastoid cell lines among the leukemia cell lines studied. In contrast to related *FLT1* and KDR/*FLK-1* genes, *FLT4* is not expressed in endothelial cell lines. *FLT4* clones were also found in analysis of a bone marrow cDNA library. These results suggest that *FLT4* has a role in hematopoiesis.
Cancer Res.. 52: 746, 1992; ***Genomics*** , 1992, in press; ***Cytogenet. Cell Genet.***. 46: 147,1987.

B 1001 GENE FUSION WITH AN ETS DOMAIN CAUSED BY CHROMOSOME TRANSLOCATION IN HUMAN TUMORS Delattre O.[1], Zucman J.[1], Plougastel B.[1], Desmaze C.[1], Melot T.[1], Peter M.[1], Dejong P.[2] , Aurias A.[1], and Thomas G.[1] [1] I. Curie, FRANCE; [2] LLNL, LIVERMORE, U S A.

Ewing sarcoma (ES) and related subtypes of primitive neuroectodermal tumors share a recurrent, specific and cytogenetically identical t(11;22)(q24;q12) chromosome translocation. The cloning of the breakpoints and the identification of phylogenetically conserved sequences in their viccinity have allowed identification of transcribed sequences and have shown that a hybrid transcript was generated by the translocation. Cloning of normal and fusion cDNAs has been achieved. On chromosome 22, the normal gene, termed *EWS*, encode a 656 aminoacids protein with homologies with RNA binding proteins. On chromosome 11, the normal gene encodes a 452 aminoacids protein with homologies with the different members of the ETS family of transcription factors. More precisely, this gene represents the human homologue of the murine *Fli-1* gene. In ES, a fusion transcript can be detected by Reverse transcriptase-PCR. Sequences of amplification products show that, in each case, the fusion is in-frame and alters the open reading frame of *EWS* by substituting the sequence encoding the putative RNA-binding domain for that encoding the DNA binding domain of the *FLI-1* gene. Depending on the positions of the breakpoints with respect to the location of exons on each chromosomes, different types of fusion transcript can be generated.

B 1002 PML-RAR, A Fusion Protein in Acute Promyelocytic Leukemia. *Akira Kakizuka, & #Ronald M. Evans
*Dept. of Pharmacology, Faculty of Medicine, Kyoto University. Japan
#Gene Expression Lab, The Salk Institute, La Jolla, CA. 92037. U.S.A.

Karyotypic changes, including chromosomal translocation, are consistently found in certain types of cancer. The balanced t(15;17)(q22; q12-21) translocation is highly correlated with acute promyelocytic leukemia (APL), and is often the only visible karyotypic aberration present. This translocation is detected in as many as 90% of APL patients and has become the definitive marker for the disease. The chromosome 17 breakpoint in APL was recently mapped to the *retinoic acid receptor (RAR) α* gene, and Northern blotting analyses of RNAs from APL patients revealed aberrant RARα mRNAs when probed with RARα cDNA. These results suggest that the t(15;17) translocation produces RARα-fusion gene products and that the fusion products contribute to the disease as the pathogenic agents.
The aberrant RARα cDNAs from a t(15;17) positive APL patient were isolated and characterized. The translocation fuses the RARα gene with a *novel gene from chromosome 15, referred to as PML*. The resulting fusion product is a cDNA encoding the DNA binding and ligand binding domain of RARα with an additional 394 amino acids in its amino-terminus from PML. The PML portion contains a striking cysteine-rich motif which is also conserved in several putative DNA-binding proteins, including the product of the recombination activating gene 1 (RAG-1). PML may represent a novel transcription factor. The structural features of PML-RAR suggest that the aberrant PML-RAR fusion protein interferes with either the PML or RAR transcription pathways. Coupled with the observation that patients with APL can be induced into remission with high dose *all-trans* retinoic acid (RA) therapy, we propose that the non-liganded PML-RAR is a new class of oncogene product. Supporting the model, PML-RAR displays altered transcriptional response to retinoic acid in cell-type and promoter-specific manner in transient transfection assays. Accordingly, we propose that the transforming activity of PML-RAR may be reversed by *all-trans* RA treatment, resulting in myeloid differentiation to a non-leukemic state.

B 1003 THE E2A-PBX1, t(1;19) TRANSLOCATION PROTEIN INDUCES A BLOCKED-DIFFERENTIATION PHENOTYPE IN NORMAL MARROW MYELOID PROGENITORS. Mark P. Kamps and Dwaine Wright. University of California School of Medicine, Department of Pathology, San Diego, CA 92093.
The t(1;19) translocation of pediatric pre-B cell leukemia forms the E2A-Pbx1 chimeric transcription factor gene and induces the constitutive expression of the sequence-specific factor, E2A-Pbx1. Expression of E2A-Pbx1 in NIH3T3 fibroblasts induces transformation. When E2A-Pbx1 is introduced into normal mouse marrow progenitors, its constitutive expression results in myeloid leukemias, many of which are factor-dependent. Based on this observation, we investigated whether expression of E2A-Pbx1 can block differentiation without affecting factor-dependence in the myeloid lineage. To assess such an activity, we infection of myeloid progenitors with an E2A-Pbx1 retrovirus and grew them in the presence of GM-CSF, predicting that a myeloblastic outgrowth might result. In the presence of GM-CSF, control marrow progenitors proliferated and differentiated into neutrophils and macrophages, and ceased to grow within 4 weeks. However, when marrow progenitors were infected with the E2A-Pbx1 retrovirus and grown in the presence of GM-CSF, myeloblastic populations of GM-CSF-dependent progenitors overgrew the cultures within 3 weeks. These cells have now been cultivated *in vitro* for 1 year. The myeloblastic outgrowths did not occur if marrow was infected with E2A-Pbx1 virus and grown in the absence of GM-CSF or if marrow was infected with helper virus and grown in the presence of GM-CSF. All cell lines arising from E2A-Pbx1 virus-infected marrow are clonal, express E2A-Pbx1 protein, and are strictly GM-CSF-dependent. IL3 does not substitute for their factor-dependence. 30 of 35 cell lines contain 2-20% neutrophil differentiation as assessed by nuclear morphology and 6 of 35 clones exhibit both myeloid and T-cell surface antigens. Based on this data, we suggest that the role of E2A-Pbx1 in pre-B leukemia is to block or retard B-cell differentiation.

B 1004 MOLECULAR CHARACTERIZATION OF DNA-BINDING PROTEIN ABNORMALLY DISTRIBUTED WITHIN THE CELLS OF PATIENTS WITH ATAXIA-TELANGIECTASIA,
Kum Kum Khanna, Duygu Findik, Lu Hong and Martin Lavin, Molecular Oncology Unit, Queensland Institute of Medical Research, Bancroft Centre, 300 Herston Road, Brisbane, Australia 4029

Ataxia-telangiectasia (A-T) is characterized by immunodeficiency, neurodegenerative changes, hypersensitivity to ionizing radiation and predisposition to cancer. We have recently described the appearance of a specific DNA binding protein in nuclei from human cells exposed to ionizing radiation (1). The DNA-binding protein was present in the cytoplasm of untreated cells, apparently being translocated to the nucleus in response to radiation exposure. This protein was originally identified by its ability to bind the 72bp distal respect of the SV40 enhancer and the protected motif was shown to be 5′ ACCCTAACTGACA 3′. A similar activity is constitutively present in unirradiated nuclei of cells from patients with the human genetic disorder Ataxia-telangiectasia. Activity was shown to be present in unirradiated nuclear extracts from 3 A-T cell lines, but was not detected in 3 controls. Purification of the binding activity from A-T nuclei and control cytoplasm by affinity chromatography gave rise to a set of 4 similar sized bands on SDS-PAGE. Southwestern and UV crosslinking experiments revealed that a 70 kDa polypeptide had the highest affinity binding for both cell types. We obtained N-terminal and internal sequence for the 70 kDa protein. Oligonucleotides were synthesized based on amino acid sequence and cDNAs were generated using PCR employing gene specific primers and an oligo-dT adaptor primer. The sequence of the full length cDNA clones will be presented. It seems evident that this protein plays an important role in cellular response to radiation damage.

Reference: Singh, S.P. and Lavin, M.F. (1990) Mol. Cell. Biol. 10, 5279-5285.

B 1005 **IDENTIFICATION OF *AML1-MTG8* FUSION TRANSCRIPTS GENERATED BY THE CHROMOSOMAL TRANSLOCATION t(8;21) IN HUMAN ACUTE MYELOID LEUKEMIA,**
Tomoko Kozu, Hiroyuki Miyoshi, Kimiko Shimizu,*Nobuo Maseki, †Yasuhiko Kaneko and Misao Ohki, Department of Immunology and Virology, Saitama Cancer Center Research Institute, *Hematology Clinic, and †Department of Laboratory Medicine, Saitama Cancer Center Hospital, Ina, Saitama 362, Japan.

The chromosomal translocation t(8;21) is found frequently in acute myeloid leukemia(AML) with maturation (FAB-M2). We have previously mapped the translocation breakpoint of t(8;21) within a gene named *AML1* on chromosome 21. *AML1* encodes a human homologue of "runt", a pair-rule gene of Drosophila that encodes a transcription factor. From several circumstantial evidence, the 5' part of *AML1* seems to play a crucial role in leukemogenesis. In this study, we cloned cDNAs synthesized from fusion transcripts of *AML1* with a novel gene named *MTG8* on chromosome 8. The *MTG8* probes detected 7.8 kb and 6.2 kb transcripts in 4 patients carrying t(8;21), but failed to detect any transcript in peripheral blood, bone marrow cells and leukemic cells without t(8;21). These results suggest that 7.8 kb and 6.2 kb RNA correspond to *AML1 -MTG8* fusion transcripts and are associated with t(8;21). The junction of *AML1-MTG8* fusion on the mRNA level revealed by PCR were identical in 7 patients of t(8;21) AML, indicating that their fusion points on the DNA level are localized at a unique region. Since the PCR detection of the *AML1-MTG8* mRNA fusion is highly sensitive, it can be used to develop a sensitive system for diagnosis and detection of minimal residual disease in t(8;21) leukemia.

B 1006 CHARACTERIZATION OF A DNA RECOGNITION SEQUENCE FOR THE ONCOGENIC HOMEODOMAIN PROTEIN, E2A-PBX1. Qiang Lu and Mark P. Kamps. University of California San Diego, School of Medicine, Department of Pathology, La Jolla, CA 92093.

E2A-Pbx1 has been identified as the chimeric gene product formed by the t(1;19)(q23;p13.3) chromosomal translocation, which occurs in 20% of pediatric pre-B cell acute lymphoblastic leukemias. E2A-Pbx1 contains the transactivation domain of E2A and the homeodomain of an uncharacterized DNA-binding protein, designated Pbx1. E2A-Pbx1 transforms NIH3T3 fibroblasts and produces myeloid leukemia in mice, presumably by altering transcription of genes normally regulated by Pbx1 or other members of the PBX gene family.

In preparation for determining the biochemical role of E2A-Pbx1 as a probable transcription factor, the sequence-specific binding of Pbx1 was examined. Recombinant Pbx1 protein strongly selected DNA sequences containing ATCAATCAA from populations of degenerate oligonucleotides. Both gel mobility shift analysis and DNA footprint analysis demonstrate that Pbx1 binds this recognition sequence specifically.

Current results using a CAT reporter gene driven by Pbx1-recognition sequences to assess the biochemical functions of Pbx1 and E2A-Pbx1 will be presented.

B 1007 Evi-1 zinc finger protein in murine and human myeloid leukemias: DNA binding and transcriptional regulation.
Kazuhiro Morishita*, Tetsunori Funabiki, Evan Parganas, and James N. Ihle. *Biol. Div., Natl.,Cancer Center Res. Inst. JAPAN, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis TN USA

The Evi-1(Ecotropic viral integration site 1) gene is a potential proto-oncogene in murine myeloid leukemias activated by retroviral insertions in Evi-1 locus(1), and in human myeloid leukemias activated by chromosomal rearrangements(2). The Evi-1 protein contains seven zinc finger repeats at N-terminal, three repeats and an acidic domain at C-terminal end. To determine the binding consensus sequence to the Evi-1 protein, we used random oligonucleotides based on the CASTing method(Cyclic Amplification and Selection of Targets), which used GST-fusion protein to detect binding sequences. We found a 15 and a 9 base consensus sequences, respectively. The first DNA binding domain bound to GACAAGATAAGATAA, but the second DNA binding sequences were GAAGATGAG. The GST-fusion protein to the DNA binding domain 1 did not bind to the second DNA consensus sequence, but GST-fusion protein to second DNA binding domain bound to first DNA binding sequence with low affinity. Furthermore, DNA binding affinity of these two binding consensus sequences to full length-Evi-1 protein represented that the first DNA binding sequence is little higher in affinity with full Evi-1 protein than that of second binding sequences.

In order to determine whether these two DNA binding consensus sequences are involved in transcriptional regulation, we inserted these consensus sequences in various combinations before the CAT TK promoter construct or others, and are currently investigating the transcriptional regulation of Evi-1 protein by using these constructs to transfect fibloblasts or hematopoietic cell lines.

(1)Morishita et al., Cell 54:833-840, 1988
(2)Morishita et al., Proc. Natl. Acad. Sci. 89:3937-3941, 1992

B 1008 THE REGULATION OF EXPRESSION OF THE RETINOBLASTOMA SUSCEPTIBILITY GENE. Nicole Osifchin[1], Toshiyuki Sakai[2], Douglas Schuerer[1], Seong-Jin Kim[3] and Paul D. Robbins[1], [1]Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, [2]Department of Preventative Medicine, Kyoto Prefectural University, Kyoto, 602, Japan and [3]Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892.

Inactivation of the retinoblastoma tumor suppressor gene (Rb) has been implicated in the pathogenesis of a variety of human tumors. Although most mutations in Rb associated with tumorigenesis involve the structural gene, a small subset of familial retinoblastomas contain mutations in the promoter region alone, demonstrating the importance of the Rb promoter in maintaining normal cell growth. Two Rb promoter point mutants derived from familial retinoblastoma patients have been described which are associated with reduced transcription and which lie in potential Sp1 and ATF consensus sites. In gel shift assays we have observed binding of Sp1 and a yet uncharacterized factor, RBF-1, to the first wild-type sequence and binding of members of the ATF family to the second wild-type sequence. We are currently examining the effects of the ATFs on transcription from the Rb promoter. Our results suggest that at least one member of the ATF family, ATF-2, can functionally regulate Rb promoter activity. We have previously demonstrated that Rb protein can positively regulate transcription mediated by Sp1 and ATF-2. Consistent with both this previous observation and the fact that Sp1 and ATF-2 can bind to the Rb promoter, we have demonstrated that Rb can positively autoregulate its own expression in CCL-64 cells. Analysis of the *cis*-acting element(s) and *trans*-acting factor(s) responsible for conferring positive regulation by Rb to the Rb promoter will be presented.

The p53 tumor suppressor protein is a transcriptional activator. Since potential targets for activation by p53 are genes that encode growth inhibitory factors such as Rb, we have examined the effect of p53 on Rb promoter activity. In transient transfection assays in CCL-64 cells we have observed that p53 can activate Rb promoter activity at low input doses of a human p53 expression plasmid. In contrast, p53 is able to repress Rb promoter activity at high input doses of the p53 expression plasmid. We have mapped the p53 positive regulatory element to a region 3' to the Sp1 and ATF-2 binding sites that contains a potential p53 consensus binding site. The region conferring negative regulation by p53 appears to lie within the basal transcriptional region of the promoter and studies are currently underway to identify specifically the *cis*-acting sequences responsible.

B 1009 THYMIC T-CELL LYMPHOMA IN oct-2 TRANSGENIC MICE, Xiaofeng Qin, HeikYung Suh, Jay Wayne, Ziva Misulovin, Thomas Gerster*, Robert G. Roeder+and Michel C. Nussenzweig, Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10021, *Biozentrum der Universitat Basel, CH-4058 Basel, Switzerland, +The Rockefeller University, New York, NY 10021

POU-homeo proteins have been implicated in the early developmental processes of lymphoid and neuronal cell lineages. Here, we report on the oncogenic potency of a POU-homeo domain transcription factor. Transgenic mice that express a POU domain polypeptide derived from the human oct-2 developed thymic lymphoma. 50% of the animals that carry the transgene developed lymphoma by four months of age. The lymphomas are composed of immature thymocytes that represent a number of developmental intermediates and can be maintained as stable cell lines. In addition to tumorgenesis, the transgene caused severe premalignant perturbation of T lymphocyte development in the thymus. There was progressive loss of the CD_4CD_8 double positive population and accumulation of early undifferentiated precursor cells. Although the transgene expressed at a similar level in mature peripheral T cells, the structure and function of the peripheral compartment appeared to be unaltered. These findings should help further our understanding of the role of POU-homeo proteins in regulating lymphocyte development.

B 1010 EXPRESSION OF THE wt1 WILMS' TUMOR GENE BY HUMAN MELANOMA CELLS, Ulrich Rodeck, Anna Bossler, Ullrich Graeven, Hans Menssen, & Csaba Kari, *The Wistar Institute of Anatomy and Biology, 3601 Spruce Street, Philadelphia, Pennsylvania, USA [U.R., A.B., C.K., U.G.]; Freie Universität Berlin, Berlin, Federal Republic of Germany [H.M.]*

We detected expression of the Wilms' tumor wt1 gene in cultured human melanoma cells at the mRNA and protein levels. Using RNA polymerase chain reaction analysis, wt1 transcripts were detected in 7 of 9 melanoma cell lines; by contrast, none of 5 normal melanocyte strains expressed wt1 at detectable levels. In Northern blot analysis, steady state wt1 mRNA levels were found in 2 of 4 melanoma lines investigated but not in normal melanocytes. WT1 protein was expressed by 5 of 5 melanoma cells, but was undetectable in normal melanocytes. In transient co-transfection assays of various melanoma cell lines, the wild-type WT1 protein invariably acted as a transcriptional repressor on the PDGF-A chain promoter which contains a WT1 binding site. RNA PCR analysis of the entire coding region of wt1 expressed by melanoma cell line WM 902-B revealed no gross alterations in the wt1 structure. This cell line expressed a splice variant described earlier in fetal kidney and Wilms' tumors (Splice I; Haber et al., PNAS (USA) 88:9618-9622, 1991). Sequence analysis of PCR products is under way. Our results indicate that wt1 gene expression is not restricted to malignant urogenital cells and may be associated with tumor progression in melanoma.

B 1011 THE HIV-1 TAT PROTEIN TRANSACTIVATES THE INTERLEUKIN-6 GENE, Giuseppe Scala, Maria R. Ruocco, Massimo Mallardo, Battista Squitieri, Francesca Baldassarre, Vincenzo Giordano, Salvatore Venuta*, and Ileana Quinto, Departement of Biochemistry and Medical Biothecnology, University of Naples "Federico II" Naples; Departement of Clinical and Experimental Medicine, University of Reggio Calabria, Catanzaro, Italy.

The human immunodeficiency virus 1(HIV1)-infection is associated with various clinical features, including severe psoriasis, B-cell lymphoma and Kaposi's sarcoma. For these diseases, a pathogenetic role for a deregulated production of interleukin-6 (IL6) has been proposed. The molecular mechanisms underlaying the abnormal IL6 secretion of HIV1-infected cells and patients are obscure and may require a transactivating function of HIV1 gene product on IL6 gene. This hypothesis was tested by utilising the pIL6Pr-CAT plasmid, a IL6 promoter-CAT construct, as a target for the transactivating function of the HIV1 TAT protein. This plasmid was obtained by inserting the BamH1-Xho1 5' upstream sequences of IL6 gene, excised from pGEM B672A plasmid, into compatible sites of pEMBL-CAT plasmid. By cotransfecting pIL6Pr-CAT and pSVT8 plasmid, a tat-expressing eukaryotic vector, in MC3 B-lymphoblastoid or in epithelial HeLa cells, we show that TAT transactivates the human IL6 promoter. The results were confirmed when pIL6Pr-CAT was transiently expressed in MC3 or HeLa cells costitutively expressing the tat gene in sense or antisense orientation. The biological relevance of the tat-induced IL6 expression was tested by generating bulk cultures of 7TD1 cells, an IL6-dependent mouse cell line, stably expressing the tat gene. These Tat-positive cells expressed the endogenous IL6 gene, secreted consistent amounts of murine IL6 and grew efficiently in absence of exogenous IL6. Moreover, these tat-positive 7TD1 cells were able to sustain the growth of parental 7TD1 cells and showed a dramatic increase in their tumorigenic potency. These results suggest that TAT proteins may play a role in the pathogenesis of some HIV1-associated diseases by regulating the expression of host cellular genes. This work was supported by grants from AIDS project, AIRC and CNR.

B 1012 INTERACTION OF HTLV-1 Tax1 WITH SRF CAUSES THE ABERRANT INDUCTION OF CELLULAR IMMEDIATE EARLY GENES THROUGH CArG BOXES, Motoharu Seiki, Haruo Tsuchiya, Tatsuya Chuhjo and Masahiro Fujii, Department of Molecular Virology and Oncology, Cancer Research Institute, Kanazawa University, 13-1, Takara-machi, Kanazawa, JAPAN

Tax1 of human T-cell leukemia virus type 1 (HTLV-1) is a transcriptional activator for viral gene expression and is also a transforming protein through inducing the expression of several cellular genes under the control of mitogenic signals. We identified the CArG boxes as a Tax1-responsive *cis*-element for the cellular immediate early genes, *c-fos*, *egr-1*, and *egr-2*. Using a chimeric protein consisting of the CArG-binding factor, $p67^{SRF}$, and the heterologous DNA-binding domain of a yeast transcription factor GAL4, we demonstrated that Tax1 activates the transcriptional activity of $p67^{SRF}$ through the GAL4 binding site. The C-terminal half of $p67^{SRF}$, which lacks domains for DNA-binding, dimerization, and ternary complex formation with $p62^{TCF}$, was sufficient for the activation by Tax1. Tax1 produced in *E, coli* bound $p67^{SRF}$ *in vitro*. The complex formation *in vivo* was also indicated by the evidence that the acidic activation domain of VP16, by fusing to $p67^{SRF}$, can complement the transcriptional activation function of a mutant Tax1 *in trans*. Thus, Tax1 activates the CArG-mediated transcription without mitogenic signals through interaction with a CArG-binding factor $p67^{SRF}$. This must be one of the primary steps by which Tax1 causes aberration in growth control of the infected cells.

Genes & Development, in press.

B 1013 c-ABL BINDS TO THE c-MYC PROMOTER, Kurt C. Sizer and Linda M. Boxer, Department of Medicine, Stanford University School of Medicine and Veterans Administration Medical Center, Stanford, CA 94305

c-abl has recently been reported to bind to a specific DNA sequence; the mutant abl protein, p210 bcr-abl, found in chronic myelogenous leukemia does not bind to DNA. By in vivo footprinting we have determined that a protein binds to a sequence in the c-myc promoter which resembles the c-abl consensus sequence. This binding site shares homology with the c-abl binding site in the hepatitis B virus enhancer. Gel shift analysis with the c-myc promoter binding site and Molt-4 T cell nuclear extract revealed a pattern similar to that seen on gel shift with c-abl and the c-abl binding site from the hepatits B virus enhancer. Further, these sequences were found to cross compete for protein binding. UV-crosslinking demonstrated that the protein which binds to the c-myc region has a molecular mass of 140 kd, which is the same as that of c-abl. An anti-abl antibody recognized the gel shift complex formed with the c-myc promoter fragment. Transient transfection experiments are being performed to assess the functional significance of the c-abl binding site and to determine what role c-abl plays in the transcriptional control of c-myc.

Late Abstracts

CHARACTERISATION OF A CELLULAR TRANSCRIPTION FACTOR WHICH CO-ORDINATES CELL CYCLE EVENTS WITH TRANSCRIPTION, Lasantha R. Bandara and Nicholas B. La Thangue, MRC National Institute for Medical Research, Mill Hill, London, NW7 1AA

The sequence-specific transcription factor, DRTF1, is down-regulated as F9 embryonal carcinoma stem cells differentiate to parietal endoderm cells. DRTF1, which binds to the E2F motif, associates with a number of other non-DNA binding proteins such as the retinoblastoma tumour suppressor gene product and the related p107 protein. This complex is dissociated by the transforming proteins of several DNA tumour viruses, for example, the adenovirus E1a and SV40 large T antigen, releasing the free form of this transcription factor, which predominates in stem cells. Moreover, mutant Rb alleles which have lost the capacity to regulate growth encode proteins that fail to bind to DRTF1.

It is known that cyclin A regulates at least two kinase subunits, $p34^{cdc2}$ and $p33^{cdk2}$ which are widely believed to be necessary for progression through mitosis and S phase respectively. Cyclin A also forms a stable complex with DRTF1 and is able recruit $p33^{cdk2}$, but not $p34^{cdc2}$, to DRTF1. The ability of cyclin A to activate and target a cell cycle kinase to this transcription factor will probably play an important role in regulating cell cycle progression and defines a mechanism of action for coupling cell cycle events to transcription.

EPITAXIAL GROWTH OF RNA POLYMERASE II CRYSTALS: A POTENTIALLY GENERAL APPROACH TO STRUCTURAL PROBLEMS . Edwards, A.M.*, Darst, S.A.#, Hussey, D., Li, Y. and Kornberg, R.D. *Department of Biochemistry, McMaster University, Hamilton, Ontario, #Rockefeller University, New York, NY and Department of Cell Biology, Stanford University, Stanford, CA.

Two-dimensional (2D) crystals of a wide variety of proteins can be formed on a lipid monolayer. These crystals are suitable for analysis by electron microscopy and image processing. Using these techniques, we have solved the structure of RNA polymerase II (pol II) to a resolution of 16Å. In the course of our studies on RNA polymerase, we discovered that, under certain conditions, polymerase crystallized in a multilayered form of that presumably grew in an epitaxial fashion from the 2D crystals. We explored the possibility that lipid layer 2D crystals in general might nucleate the growth of 3D crystals for X-ray diffraction experiments and observed that 2D crystals of both streptavidin and pol II, the only cases tested so far, effectively nucleated the growth of large 3D crystals. The details of these experiments will be described. The relative ease with which 2D crystals can be grown on lipid layers, the propensity of the crystals to grow epitaxially and our success with two unrelated proteins suggests a general and practical way to grow protein crystals for X-ray diffraction experiments.

ISOLATION OF cDNA CLONES ENCODING PROTEINS THAT SPECIFICALLY INTERACT WITH THYROID HORMONE RECEPTOR Jae Woon Lee and David D. Moore, Department of Molecular B[illegible]y, Massachusetts General Hospital, Boston, MA 02114

We have obtained cDNA clones encoding proteins that specifically interact with the thyroid hormone receptor by using a yeast genetic system developed in the laboratory of Dr. Roger Brent. This system, similar to an approach described by Fields and Song (Nature 340, 245-246, 1989), allows direct genetic selection for proteins capable of interacting with a desired protein. In short, a transcriptionally inactive lexA/TR chimera consisting of the intact lexA fused to the ligand binding domain of TR was expressed in yeast strain in which expression of the LEU2 gene is dependent on binding of an activator to upstream lexA operators. When an additional chimeric protein consisting of a potent transcriptional activation domain fused to a protein capable of interacting specifically with the lexA/TR chimera is introduced into cells, LEU2 gene expression is activated and leucine is not required for growth. A plasmid cDNA library in which the inserted sequences are fused to a transcriptional activation domain (B42) was introduced into these yeast cells with or without thyroid hormone T3. A number of leucine independent colonies that contained candidate TR-interacting cDNAs were obtained in both conditions. Characterization of these initial clones has identified at least 14 distinct cDNAs. Five have strong homology to proteins that function in the nucleus. The others have no apparent homology to known proteins in current databank. Remarkably, nearly all the cDNAs showed very strong dependence on hormone for interaction. The basic characterization of these new cDNA clones is under progress.

DUAL FUNCTIONALITY OF UPSTREAM REGULATOR OF HEPATITIS B VIRAL CORE GENE, Ling-Pai Ting and Chiou-Hwa Yuh, Graduate Institute of Microbiology and Immunology, National Yang-Ming Medical College, Shih-Pai, Taipei, Taiwan, ROC

Regulation of gene expression is determined by factors that bind to specific DNA sequences in the promoter and/or enhancer regions to stimulate or repress the basal transcription. We now reported that a 106-base pair (bp) regulatory element upstream of the basal core promoter (BCP) of hepatitis B virus (HBV) has a dual function: it stimulates the transcriptional activity of endogeous BCP in a position- and orientation-dependent manner, while activates the heterologous simian virus 40 early promoter (SVp) in a position- and orientation-independent manner. We believe, this is the first example that one gene's upstream activator is another gene's enhancer. Further analysis shows box-α, which is a indispensable part of a bipartite structure of the enhancer, could also serve as an upstream activator. Mutational analysis reveals the identical sequence requirement for both functions. The fact that not all basal transcription machinery is identical for all promoters may explain why BCP and SVp respond to the sequence, i.e. box-α, differently.

HIP-116: A MEMBER OF THE SNF2/SWI2 FAMILY OF DNA HELICASES THAT BINDS TO THE SV40 ENHANCER. P.L. Sheridan, M. Schorpp, W.L. Brown, and K. Jones, The Salk Institute, La Jolla, CA 92037.

The SNF2/SWI2 protein functions as a global activator of RNA pol II transcription in yeast and is a member of a newly-emerging family of a putative DNA helicases. We have isolated a cDNA clone encoding a human DNA-binding protein (HIP116) that is homologous to SNF2/SWI2 in a region that spans seven co-linearly distributed domains commonly found in DNA helicases and includes a variant type of D-E-A-D box, D-E-G-H. In addition, HIP116 contains a putative zinc finger or zinc cluster structure similar to that found in a distinct family of nuclear regulatory proteins, as well as a separate N-terminal DNA-binding domain. Recombinant HIP116, expressed and purified from bacteria, was found to bind specifically to the initiator region of the HIV-1 promoter and to an extensive region of the SV40 enhancer in DNase I footprint experiments. This HIP116 binding site on the SV40 enhancer overlaps the binding sites for the Oct-1 and TEF-1 transcription factors. Interestingly, polyclonal antiserum raised against HIP116 specifically and quantitatively shifted a protein-SV40 DNA complex in HeLa nuclear extracts that is identical in its DNA-binding specificity to that reported previously for the TEF-1 enhancer factor. By contrast, the anti-HIP antiserum did not affect complexes formed between the Oct-1 protein and the Sph motif, nor the binding of TEF-1 to its distinct site on the SV40 GT-IIC motif. These findings indicate that HIP116 binds directly to this domain of the SV40 enhancer, potentially in a complex with the TEF-1 transcription factor. We propose that the activity or local structure of the SV40 enhancer might be affected by a member of the SNF2/SWI2 subfamily of proposed DNA helicases that is capable of binding directly to the enhancer.