TRANSCRIPTIONAL CONTROL OF CELL GROWTH

Organizers: Kathryn Calame and Leonard Guarente

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Plenary Sessions Page

Poster Sessions

Transcriptional Control of Cell Growth

3-Dimensional Structure of Regulatory Proteins

E 001 YEAST RNA POLYMERASE II TRANSCRIPTION: STRUCTURE AND REGULATION, Roger D. Kornberg, Andrew R. Buchman, Daniel I. Chasman, Seth A. Darst, Aled M. Edwards, William J. Feaver, Peter M. Flanagan, Opher Gileadi, Raymond J. Kelleher III, Yahli Lorch, Neal F. Lue, and Michael Sayre, Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305.

Three aspects of yeast RNA polymerase II transcription will be described: (1) Purification and structure of enzyme active in initiation. Immunoaffinity-purified enzyme complemented the deficiency of a nuclear extract from a pol II^{ts} strain in accurate initiation from a pol II promoter. This purified enzyme formed two-dimensional crystals on lipid layers from which structural information could be derived. (2) Fractionation of a pol II transcription system. Five components are required in addition to pol II for accurate initiation at yeast and mammalian promoters. One of these components can be replaced by TFIID. Another component becomes dispensable as the remaining three are further purified. (3) Mechanism of transcriptional activation. An assay has been devised for a factor(s) that mediates transcriptional activation. Neither TFIID nor pol II is effective in this assay, but an active fraction has been derived from yeast nuclear extract.

E 002 HIGH-RESOLUTION STRUCTURE OF THE LAMBDA REPRESSOR-OPERATOR COMPLEX: IMPLICATIONS FOR PROTIEN-DNA RECOGNITION AND GENE REGULATION, Carl O. Pabo, Howard Hughes Medical Institute, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The structure of the λ repressor-operator complex has been solved and refined at 2.5 A resolution (Jordan and Pabo, 1988), and this talk will consider the implications this structure has for protein-DNA recognition and gene regulation. Other recent results that will be discussed include:
1) genetic and structural studies of repressor's N-terminal arm and
2) structural studies of the arc repressor from bacteriophage P22.

Jordan, S.R. and Pabo, C.O., (1988) Structure of the Lambda Complex at 2.5 A Resolution: Details of the Repressor-Operator Interactions. Science, 242, 893-899.

E 003 UNEXPECTED DETERMINANTS OF SPECIFIC PROTEIN NUCLEIC ACID INTERACTIONS, P.B. Sigler; Department of Molecular Biophysics and Biochemistry, and the Howard Hughes Medical Institute, Yale University, New Haven, Connecticut.

New data-collecting technology has extended the resolution of the *trp* repressor/operator complex from 2.4 A to 1.9 A. This has produced a more accurate chemical account of the interface between the repressor and operator. The most striking feature is the role played by fixed water molecules in augmenting specific sequence affinity. Our crystal structures show water molecules to be firmly hydogen-bonded to the naked protein and to act as extensions of the protein's reactive surface. In the repressor/operator complex these fixed water molecules mediate interactions with the phosphate backbone as well as the functional groups of the bases responsible for sequence specificity. These highly polarized hydrogen bonds also appear to focus the electrostatic forces that stabilize the complex. The first two hydrophobic residues (Ile79 and Ala80) of the so-called "recognition helix" do not make intimate van der Waals contact with the bases but help to shield the base-specific water-mediated interactions from competing interactions with ambient solvent. It has become evident recently that comparable specific protein/nucleic acid and protein/sugar complexes also use fixed water molecules to mediate specific contacts between the protein and its target.

Sequence-specific flexibility in the operator's structure also appears to contribute to *trp* operator's special affinity for *trp* repressor. Many of the backbone and base-specific contacts could not be made unless the DNA bends towards the major groove at the TA step between the fourth and fifth base pair from the center of the operator. Nuclease susceptibility and crystal structures of naked B-DNA indicate that such sequences allow DNA to bend easily in a direction required to make these contacts.

Chromatin Structure in Transcriptional Complexes

E 004 PROTEIN-DNA ARCHITECTURE AT THE 5' END OF THE HSP26 GENE OF DROSOPHILA, S.C.R. Elgin*, B.D. Allan*, T.J. Dietz*, D.S. Gilmour*, R.L. Glaser‡, Lu Qin*, G.H. Thomas*, J.R. Lis‡, *Department of Biology, Washington University, St. Louis, MO 63130, and ‡Section of Biochemistry, Molecular and Cellular Biology, Cornell University, Ithica, NY 14853.

Sequence level analysis of the chromatin structure upstream of hsp26 indicates a pattern of protein-DNA interactions that maintains the regulatory heat shock elements in an accessible conformation within the two DNase I hypersensitive sites prior to heat shock; after heat shock, a clear footprint demonstrates the binding of the heat shock transcription factor at these positions. Creation of the two DH sites appears to be the consequence of specific binding of nonhistone chromosomal proteins, including the TATA box binding protein, and the specific positioning of nucleosomes. Analysis of the chromatin structure of a series of 5' deletion mutants of hsp26 indicates that the TATA box and downstream elements alone are insufficient to generate the 5' DH sites. An adjacent (CT)n element, which has been shown to adopt an H-form (triple helix structure) in vitro, is required for full gene activation. Analysis using diethylpyrocarbonate modification fails to find evidence of the H-form in isolated nuclei, while DNase I footprinting shows the region to be protected before and after heat shock, suggesting protein binding. A protein of 66 kD which binds to linear (CT)n DNA has been purified from nuclear extracts; this protein may be the same or closely related to the GAGA transcription factor previously described by Biggin and Tjian (1). In vitro studies suggest a stabilizing intereaction between the (CT)n binding protein and the TATA box binding protein. Further analysis of mutant constructs indicates that both formation of appropriate DH sites and the capacity for inducible transcription requires the interaction of multiple elements, including (CT)n, with the TATA box. As is the case for hsp26, the (CT)n binding protein and TATA box binding protein are already associated with the inactive but inducible histone H3 promoter. The results suggest that in addition to being an important transcription factor, the (CT)n binding protein may play a major role in establishing the observed chromatin structures for this type of gene.

1. M.D. Biggin and R. Tjian (1988) Cell 53, 699-711.

E 005 REGULATION OF TRANSCRIPTION BY HISTONES IN THE YEAST, *Saccharomyces cerevisiae*. Michael Grunstein, Linda Durrin, Lianna Johnson and Paul Kayne. Molecular Biology Institute and Department of Biology, University of California, Los Angeles, CA. 90024.
Several examples will be presented to illustrate the effect of histones and nucleosomes on the regulation of transcription. We have recently found that nucleosome loss, caused by glucose repression of a *GAL1* promoter-histone H4 gene fusion, leads to a general activation of regulated genes. Also, histone H4 N-terminal deletions and point mutations, which are viable, specifically derepress the silent mating loci of yeast. Genetic evidence, involving second site suppressor analysis, suggests that an interaction between the silencer repressor protein, SIR3, and histone H4 is responsible for inactivating the silent mating loci. Finally, we have examined the effect of preventing acetylation of the H4 N-terminus on transcription. Evidence will be presented to show that the ability to acetylate histone H4 is necessary for efficient RNA synthesis.

Regulation of Transcription in Lower Eukaryotes

E 006 Mcm1 - A PROTEIN INVOLVED IN TRANSCRIPTION AND DNA REPLICATION IN YEAST. Bik K. Tye, Steve Passmore, Vicky Chang and Randy Eible. Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853.

Mcm1 is a protein of multiple functions. It is involved in the maintenance of minichromosomes in an *ARS*-specific manner, it is also involved in the transcriptional regulation of mating type specific genes[1]. Mcm1 carries out its multiple functions by acting synergistically with a number of cofactors. It acts as a transcriptional activator of α-specific genes by binding cooperatively with the α1 protein[2] to promoters of α-specific genes. Similarly, it acts as a transcriptional repressor of *a*-specific genes in α cells by binding cooperatively with the α2 protein to promoters of *a*-specific genes.

Mcm1 binds to the dyad symmetry element 5'-CCTAATTAGG and related sequences which we refer to as Mcm1 Control Elements (MCEs). MCEs are also found in *ARS*s, the presumed chromosomal replication origins in yeast. In fact, *ARS*s that are active in the *mcm1-1* mutant seem to contain multiple copies of MCEs while *ARS*s whose function is greatly affected in the mutant contain fewer and poorer MCE sites. We are able to demonstrate Mcm1 binding to these MCE sites found in *ARS*s. The role of Mcm1 in *ARS* function will be discussed.

MCM1 encodes a protein of 286 amino acids containing striking homology to two known regulatory proteins: the yeast ARG80 protein[1] and the human serum response factor (SRF)[3]. The homology between Mcm1 and SRF lies in the dimerization/DNA binding domain.

1. Passmore et al., J. Mol. Biol.204, 593-606 (1988).
2. Passmoe et al., Genes & Develop. 3, 921-935 (1989).
3. Norman et al., Cell. 55, 989-1003 (1988).

Basic Transcription Machinery in Microbes

E 007 FUNCTION OF THE *E. COLI* ENHANCER-RECEPTOR PROTEIN SIGMA-54, Jay D. Gralla, Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA 90024

The gln AP2 promoter of *E. coli* is activated from a distance in response to nitrogen starvation. Under all physiological conditions the transcription factor sigma-54 directs core RNA polymerase to form a closed complex at the promoter. Upon nitrogen starvation the activator protein NR1 becomes bound at a remote position and triggers the pre-bound promoter complex to open the DNA and begin transcription. This behavior contrasts with that of the usual *E. coli* sigma-70 promoter at which activation from long distances apparently cannot occur. The DNA sequence of the *E. coli* sigma-54 gene reveals elements that resemble those of eukaryotic transcription factors, consistent with its central role in mediating activation at a distance in *E. coli*. These include a highly acidic domain, a glutamine-rich domain, an overlapping domain containing periodically arrayed leucines, and a helix-turn-helix domain. The sigma-54 protein and a number of its mutants have been expressed from plasmids in cells lacking chromosomal sigma-54. These cells were then analyzed in two ways. First, the ability of the protein to restore sigma-54 function was assessed. Then *in vivo* footprinting was used to explore the interactions with the promoter. A comparison of the two results allows identification of certain critical protein domains and leads to proposals for the role of these domains in mediating transcriptional control in this system.

E 008 REGULATION OF A DEVELOPMENTAL SIGMA FACTOR GENE BY REARRANGEMENT, TRANSCRIPTION, AND PROCESSING OF ITS PRODUCT.

R. Losick*, B. Kunkel*, L. Kroos#, S. Cutting* and P. Stragier+. *Harvard University, Department of Cellular and Developmental Biology, 16 Divinity Avenue, Cambridge, MA 02138. #Michigan State University, Department of Biochemistry, East Lansing, MI 48824. +Institut de Biologie, Physico-Chimique, 13, Rue Pierre et Marie Curie, 75005 Paris, France.

Gene expression during endospore formation in *Bacillus subtilis* is compartmentalized in the mother-cell and forespore chambers of the sporangium. This differential gene expression is governed by compartment-specific RNA polymerase sigma factors named σ^G and σ^K. We show that the gene for the mother-cell sigma factor σ^K is controlled at three levels: chromosomal rearrangement, transcription, and processing of its product.

(i) **Chromosomal Rearrangement.** The structural gene *sigK* for σ^K is created by a compartment-specific chromosomal rearrangement. *sigK* is a composite of two truncated genes named *spoIVCB* and *spoIIIC*, which are separated by about 30 kb. During sporulation, *spoIVCB* and *spoIIIC* are brought together by site-specific, reciprocal recombination, which deletes intervening DNA. This recombination is catalyzed by the product of sporulation gene *spoIVCA*

(ii) **Transcription.** Activation of the promoter for *sigK* is controlled by the product of the mother-cell regulatory gene *spoIIID*, a small transcriptional control protein that binds to the transcription initiation region of the rearranged gene. *spoIIID* is itself preferentially expressed in the mother-cell and causes selective activation of the *sigK* promoter in the mother-cell.

(iii) **Processing of the *sigK* Product.** The primary product of *sigK* is a pro-protein containing 20 amino acids that precede the amino terminus of mature σ^K. It is inferred that pro-σ^K is inactive and that its conversion to the mature factor is controlled by the product of regulatory gene *spoIVF*.

Site-specific recombination, *spoIIID*-activated transcription and pro-σ^K processing ensure that σK-directed gene expression occurs at the correct time (late in development) and in the correct place (the mother-cell) during formation of the endospore.

Basic Transcription Machinery and Factors in Higher Eukaryotes

E 009 FUNCTIONAL ANALYSIS OF MAMMALIAN PROMOTER- AND ENHANCER- BINDING TRANSCRIPTION FACTORS. Stephen P. Jackson, Albert Courey, Frank Pugh, Naoko Tanese, Trevor Williams and Robert Tjian, HHMI, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720

In order to understand the mechanism and regulation of transcriptional initiation in eukaryotes, we have isolated and characterized a number of mammalian sequence specific transcription factors, including Sp1, CTF and AP-2. For Sp1, we have found that the two most potent transcriptional activation regions are rich in glutamine residues. In addition to Sp1, a wide variety of other sequence-specific transcription factors also contain glutamine-rich regions. We have demonstrated that one such region, from the Drosophila Antennapedia protein, can activate Sp1-responsive promoters when fused to the Sp1 DNA binding domain.

To explore the properties of the Sp1 glutamine-rich activation domains, we have varied the distance between the transcriptional start site and the Sp1 binding sites. We have found that the glutamine-rich domains can activate transcription even when anchored to the DNA 1.5 kb downstream from the transcriptional start site. Furthermore, the distally and proximally bound Sp1 molecules activate transcription synergistically. We have also examined an Sp1 deletion mutant from which the DNA binding domain has been removed but which contains the glutamine-rich regions. By itself, this mutant is inactive. However, in conjunction with wild-type Sp1, it synergistically activates transcription in a process we have termed superactivation. Superactivation is a specific process since Sp1 glutamine-rich domains do not superactivate the hybrid Sp1/Antennapedia protein. The two synergistic phenomena described above can be explained by postulating that molecules of Sp1 directly interact with one another. Glutaraldehyde crosslinking data show that such an interaction can indeed occur.

In other studies, we have mapped the transcriptional activation domains of the CTF and AP-2 proteins. Interestingly, these regions are not glutamine-rich, but instead are characterized by a high proline content. The apparent unrelatedness between the Sp1 and CTF/AP-2 activation regions suggests that these proteins operate through different molecular pathways. Experiments are currently in progress to test this possibility by investigating interactions between these transcription factors and components of the general transcriptional apparatus.

E 010 MULTIPROTEIN COMPLEXES DIRECT TRANSCRIPTION BY RNA POLYMERASE II. Phillip A. Sharp, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139

Initiation of transcription by RNA polymerase II is specified by four factors in addition to the polymerase. These four factors, TFIIA, B, D and E form a complex along with RNA polymerase II on promoter segments containing a TATA element. Addition of ATP alters this initiation complex probably by movement of E. This multiprotein complex can only be formed if all factors are present but intermediate complexes have been characterized which form if specific subgroups of factors are mixed with the promoter fragment. We hope to analyze how transcription factors that bind to enhancer elements stimulate the formation of this initiation complex. Multiprotein complexes have also been resolved in the study of the mechanism by which the Herpes Simplex Virus (HSV) transactivator protein TIF or VP16 interacts with promoter sequences. Purified TIF protein will form a sequence-specific complex with the cellular protein Oct-1 and another cellular protein C1. The two latter proteins are thought to contact the specific sequence, $^{TGCATGCTAATGATATTCTTT}_{ACGTACGATTACTATAAGAAA}$, but their simultaneous binding is dependent upon TIF. A third cellular protein, C2, also binds to this multiprotein complex.

E 011 EXPRESSION OF XENOPUS AND MOUSE RIBOSOMAL RNA GENES Barbara Sollner-Webb, Regina Porretta, Louise Pape, Ken Ryan, Susan Kass, Mark Paalman, Sheryl Henderson, and John Tower. Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

The genes encoding the 40-47S pre-rRNA are transcribed actively in the nucleolus by RNA polymerase I. This efficient expression is under the control of a bipartite promoter that exhibits very precise helical and spacing constraints. Transcription factor 'D' binds to the upstream half of the core promoter domain (residue -35 to -15) and evidently also to the domains surrounding residue -140. This complex then directs the binding of factor 'C', a subform of RNA polymerase I that is specifically activated to participate in accurate rDNA initiation. The data indicate that growth-state regulation of rDNA transcription is mediated by the varying abundance of this active polymerase subform. The action of the activated polymerase in normal growing, down-regulated, and up-regulated cells will be discussed. In contrast, the down-regulation of rDNA transcription in adenovirus infected cells is due to the sequestration of factor D, apparently by the replicated adenovirus DNA molecules, not to alterations in factor C availability.

Sequences upstream of the promoter also greatly augment rDNA transcription. First is a promoter-proximal terminator that serves to protect the transcription complex on the adjacent promoter from the disassembly which occurs when polymerases instead transcribe through the promoter. Upstream of this terminator in both mouse and frog are polymerase I-specific enhancers. These enhancers are orientation independent but exhibit a notable position dependence; the Xenopus enhancer works when 1 kb upstream from the start site but it becomes a transcription silencer when moved >1.5 kb upstream. The rDNA transcriptional level is also augmented by spacer promoters located further upstream in the rDNA. The great similarity between species as distant as Xenopus and mouse suggests that rRNA gene expression in other organisms may be similarly controlled.

Once formed, the primary mouse rRNA transcript is rapidly processed at residue +650. This processing occurs in a specific ribonucleoprotein complex which forms on the rRNA substrate. In collaboration with K. Tyc and J. Steitz, we have recently shown that the U3 nucleolar snRNP binds to the rRNA substrate and that it is required for the primary rRNA processing reaction.

Transcription Factors in Embryonic Development

E 012 GENETIC CONTROL OF NEURONAL DIFFERENTIATION IN *C. ELEGANS*. Martin Chalfie, Macy Au, Jeff Way, Ding Xue, Cathy Savage, and Shohei Mitani. Department of Biological Sciences, Columbia University, New York, NY 10027

We have identified 18 genes that are required at four different steps in a genetic pathway for the development of a single type of cell: the touch receptor neurons of the nematode *Caenorhabditis elegans*. Mutations in these genes affect either the generation of the cells, the specification of their differentiation, the maintenance of this differentiation, or cell function. The first three groups contain five genes (*lin-14*, *lin-32*, *unc-86*, *mec-3*, and *mec-17*) that appear to regulate touch cell development, and the last group (function) contains twelve genes (*mec-1, 2, 4 -10, 12, 14, 15* and *18*) that are likely targets of this regulation. None of the "regulatory" genes is solely expressed in the touch cells (with the possible exception of *mec-17*), although some of the "function" genes are. For example, *mec-3*, a homeobox gene, specifies touch cell differentiation, but is expressed as well in two other pairs of neurons (the PVD and FLP cells). It is also required for its own maintained expression. In contrast, the *mec-7* gene encodes a β-tubulin that is required for the fifteen-protofilament microtubules that are only found in the touch receptor neurons. By *in situ* hybridization and antibody staining, the *mec-7* product is only found in these cells. Presumably it is the combinatorial action of the non-cell-specific regulators that permits the differentiation of this single type of cell.

E 013 TRANSCRIPTIONAL REGULATION BY THE ANTENNAPEDIA HOMEOTIC GENE OF DROSOPHILA, Matthew P. Scott, Shigeo Hayashi, Gary M. Winslow, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309-0347.

The ectopic expression of the *Antennapedia* (*Antp*) protein in the head primordia of the fly, due to either a mutation or to the use of a heat shock promoter, leads to the development of legs in lieu of antennae. *Antp* normally controls the formation of thoracic structures in the fly. The presence of essentially a single protein in the head primordia is therefore capable of completely altering the fates of a large and complex array of cells. We have shown that the *Antp* protein is capable of sequence-specific DNA binding and that in cultured cells the protein is capable of activating transcription. We have studied the properties of *Antp* protein by modifying its structure and testing its ability to affect transcription in cultured cells. The protein contains a homeodomain which is sufficient for DNA binding. The homeodomain alone, without the rest of the protein, can compete with the intact protein and thus prevent transcriptional activation. In addition we have introduced modified proteins into the fly under the control of a heat shock promoter to assess their abilities to induce antenna to leg transformations. The sites upon which the protein acts have been altered to learn about the specificity of DNA binding and transcriptional activation by the protein. The protein has been partially purified from cultured cells and tested for DNA binding using band shifts and DNAse I footprinting. In vitro the protein binds to both a repeated TAA sequence and to the sequence TCAATTAAATGA with a dissociation constant of $< 10^{-8}$. We have also found that serine phosphorylation is important in controlling DNA binding in vitro.

Transcription Factors Induced By Growth and Differentiation Signals (joint)

E 014 TRANSIENT GENE EXPRESSION IN RESPONSE TO GROWTH FACTORS, TUMOR PROMOTERS AND AGENTS THAT INDUCE DIFFERENTIATION, Harvey R. Herschman, Department of Biological Chemistry, UCLA School of Medicine, Los Angeles CA 90024

We have used differential screening of a cDNA library prepared from 3T3 cells treated with the tumor promoter tetradecanoyl phorbol acetate (TPA) in the presence of cycloheximide to isolate a series of cDNAs for genes that are induced as primary responses to this agent. We refer to these as TPA induced sequences, or TIS genes. The TIS genes are rapidly (30-90 min) and transiently induced by TPA in 3T3 cells. They are also induced by other mitogens, such as epidermal and fibroblast growth factor. Co-administration and down-regulation experiments suggest that expression of these genes can be induced by several independent second messenger pathways. We find that the TIS genes can, in general, be induced in a wide variety of cells, by many ligands. However, specific cell types demonstrated "extinction" of the ability to express certain TIS genes, in response to any inducer. The TIS10 gene cannot be induced in PC12 pheochromocytoma cells by any inducer, while the TIS1, 7, 8, 11 and 21 genes can be induced by TPA, EGF, FGF or nerve growth factor. Similarly, while the other TIS genes can be induced by TPA or granulocyte-macrophage colony stimulating factor in myeloid cells, the TIS1 gene cannot be induced by either agent in these cells. It appears that discreet subsets of TIS genes can be induced, by a variety of ligands, in specific classes of cells. In situ hybridization data demonstrate that restricted expression of the TIS genes occurs within subpopulations of cells in the central nervous system. Sequence data demonstrate that the TIS genes include presumptive DNA binding proteins, transcription factors, autocrine/cytokine factors, and proteins whose sequence does not suggest any known function.

E 015 THE EGR FAMILY OF ZINC FINGER PROTEINS. Vikas P. Sukhatme.
Department of Medicine and Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637.
The Egr family of immediate early growth response genes is currently comprised of four members Egr-1, 2, 3, and 4. All contain three highly similar zinc finger structures of the Cys_2-His_2 type. Egr-1, 2, and 3 are all inducible by multiple mitogenic stimuli whereas Egr-4 is not. Egr-1 and Egr-2 are the best characterized members of this group. We will discuss how extracellular signals initiating growth or differentiation regulate induction of these genes and how c-Fos is involved in their negative regulation. We will present data on the characterization of the Egr-1 gene product and discuss approaches being used to define a target sequence and phenotype. Collectively, our data point to a broad role for these proteins as nuclear signal transducers in diverse biological processes.

E 016 STRUCTURE AND FUNCTION OF SRF, Richard Treisman, Susan John, Richard Marais and Roy Pollock, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, U.K.
SRF (Serum Response Factor) is a protein that binds to the Serum Response Element (SRE), a short regulatory sequence required for the rapid and transient transcriptional induction of cellular "immediate-early" genes by a vareity of growth factors and mitogens (1). SRE sequences also act as constitutive promoter elements in vivo, and purified SRF acts as a constitutive transcription factor in vitro. SRF expressed by in vitro translation of synthetic RNA or in insect cells using a baculovirus vector exhibits specific DNA binding activity and is active in in vitro transcription assays. SRF is phosphorylated in vivo (2): we have used the recombinant baculovirus SRF and in vitro synthesised SRF to investigate the nature and function of SRF phosphorylation. A variety of mutant forms of SRF have been constructed and expressed and these proteins have been tested for DNA binding, in vitro transcriptional activation activity, and for formation of ternary complexes at the c-fos SRE with accessory protein p62 (3). The results indicate that the central region of the molecule is required for DNA and p62 binding while activation requires the C terminal half. In order to investigate the properties of the protein in vivo we constructed expression plasmids encoding intact and mutant SRF, or chimeric proteins in which SRF sequences are joined to heterologous DNA binding domains. Results of experiments in which these plasmids were cotransfected with promoters containing SREs or the cognate operators will be presented.

1. Norman,C., et al. (1988) Cell 55, 989-1003.
2. Prywes,R., et al. (1988) Proc.Natl.Acad.Sci.USA. 85, 7206-7210.
3. Shaw,P.E. et al. (1989) Cell 56, 563-572.

Development of Hematopoietic Cell Lineages

E 017 PROTEINS WITH DIFFERENT PREDICTED DNA-BINDING AND DIMERIZATION MOTIFS ACTIVATE IMMUNOGLOBULIN HEAVY CHAIN TRANSCRIPTION, Kathryn Calame and Chris Roman, Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, N.Y. 10032

At least three transcriptional activator proteins bind to sites in both the immunoglobulin heavy chain (IgH) enhancer and the V_{H1} promoter: Oct-2, uEBP-E and uEBP-C2(uE3). Others have shown that Oct-2 binds to DNA through a homoebox domain, a motif it shares with regulator proteins from diverse species. We have purified uEBP-E and uEBP-C2 to homogeneity and cloned cDNAs which encode them.

Purified uEBP-E is a 44 kD protein which binds to the IgH enhancer and promoter sites with high affinity ($K_d=2x10^{-11}M$). Analysis of the predicted protein sequence derived from cDNA clones shows that uEBP-E contains a basic region immediately upstream of a heptad repeat of leucines, an arrangement shared by a family of DNA binding proteins including c-fos, c-jun, C/EBP, CREB and GCN4. By analogy to these proteins, the leucine repeats of uEBP-E are probably dimerization domains and the basic region interacts with DNA. We are currently studying which members of this family may form heterodimers with uEBP-E. The uEBP-E gene is single copy in the genome and expressed at moderate levels in all tissues examined.

Purified uEBP-C2 exists as either a dimer or a tetramer of 42-45 kD subunits. Amino acid sequence derived from cloned cDNAs shows that uEBP-C2 contains a basic region upstream of a "myc homology" region followed by a heptad repeat of leucines and finally by a proline rich region. "Myc homology regions" are shared by the myc proteins, Myo D, several drosophila proteins involved in neural development and transcription factor USF. Both the myc homology and leucine repeats are potential protein-protein interaction domains. We are testing the possibility that tetramers of uEBP-C2 could promote the formation of intrastrand loops between IgH promoters and the enhancer. The uEBP-C2 gene is single copy in the genome and is expressed at low levels in all tissues examined except brain, where its expression is strikingly elevated. We are exploring the possibility that this protein may also play an important role in neural development or function.

E 018 CONSTITUTIVE AND REGULATED CLASS II MHC GENE EXPRESSION, Laurie H. Glimcher, Department of Cancer Biology and Medicine, Harvard School of Public Health and Harvard Medical School, Boston, MA 02115. The class II major histocompatibility complex (MHC, Ia) antigens are a family of integral membrane proteins whose expression is strictly regulated. These molecules have a limited tissue distribution and their expression is regulated both developmentally and in response to external stimuli. Three highly conserved transcriptional motifs, W, X and Y, are essential for constitutive class II gene transcription. cDNAs for a family of leucine-zipper proteins that bind to the class II Aα X box motif have been isolated. Their binding characteristics suggest that the coordination of class II expression is not controlled through X box binding factors. It is possible that the X box binding proteins interact with some other common transcription factor(s) whose activity is responsible for differentiated or lymphokine-induced signals. One candidate is a recently identified DNA-binding protein complex (termed complex A) which binds upstream of the murine Eβ class II gene. Complex A binding activity is restricted to tissues which express class II, is developmentally regulated in cells of the B lineage in accordance with class II expression and is responsive to two different Ia-inducing lymphokines, interferon-γ (IFN-γ) and interleukin-4 (IL-4). The target sequence for complex A, which includes the 3 previously defined transcriptional motifs W, X and Y, is a cis-acting transcription element only in class II-positive cells. The loss of complex A binding activity in a human Ia mutant cell line, RJ2.2.5, correlates with functional loss of class II expression. Furthermore, this cell line, whose defect is complemented by a locus on mouse chromosome 16, re-expresses both class II and complex A upon transfection with mouse genomic DNA. It is likely that complex A plays a critical role on both constitutive and regulated class II MHC gene expression.

E 019 REGULATION OF THE HUMAN BETA-GLOBIN GENES F. Grosveld, M. Antoniou, P. Belhumeur, E. deBoer, P. Collis, N. Dillon, P. Fraser, D. Greaves, O. Hanscombe, M. Lindenbaum, S. Philipsen, S. Pruzina, D. Talbot and M. Vidal. Laboratory of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

We have used the dominant control region of the human beta-globin gene domain to drive the expression of a number of constructs in a copy number dependent fashion in transgenic mice. Using the human alpha- and beta^{S}-genes, we have derived transgenic mouse lines which show varying degrees of sickle cell anaemia. Using individual genes or combinations, we have determined the parameters that are important for the correct developmental regulation of the human alpha- and beta-globin genes. Lastly, the dominant control region has been dissected into individual elements and at least some of the factors, important in the functioning of this region, will be discussed.

Transcription Factors Involved in Tissue Determination and Tissue-Specific Expression (joint)

E 020 STUDIES ON THE CELL-TYPE SPECIFICITY OF C/EBP FUNCTION, Steven McKnight, Robert Umek and Alan Friedman, Carnegie Institution, Department of Embryology, Baltimore, MD 21210.

CCAAT/enhancer binding protein (C/EBP) is a sequence-specific DNA binding protein that was first purified from rat liver nuclei. Aside from liver, C/EBP is also expressed at high levels in adipose and placental tissues. C/EBP expression is limited to terminally differentiated cells. For example, hepatocytes are the only cells of the liver that express C/EBP. Likewise, C/EBP is not expressed in cultured adipocytes until they are induced to assume the terminally differentiated state. Finally, we have noticed that C/EBP concentrations are dramatically reduced in cells derived from either human or rodent hepatomas. Given the restricted tissue and cell-type distribution of C/EBP, we have considered the possibility that it plays a role in executing the terminally differentiated state, and that its loss might be a necessary step during neoplastic transformation. In order to investigate these ideas, we have attempted to define target genes that are either induced or repressed by C/EBP. Evidence will be presented showing that the serum albumin gene is a target for trans-activation by C/EBP. An expression vector capable of encoding C/EBP potently activates transcription from the albumin promoter when tested in cultured liver cells. Intriguingly, the same vector is only capable of weak stimulation of the albumin promoter in cultured fibroblasts. Attempts are underway to identify determinants of the C/EBP polypeptide that cause it to function in a cell-type specific manner.

Regulated Transcription Factors

E 021 MOLECULAR MECHANISMS OF TRANSCRIPTIONAL REGULATION IN YEAST, Struhl K, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115.

The yeast GCN4 protein binds to upstream promoter sequences of 30-100 genes involved in amino acid biosynthesis and coordinately induces their transcription in response to amino acid starvation. GCN4 binds as a dimer to a 9-bp region, with optimal binding to the palindrome ATGACTCAT. The 60 C-terminal amino acids of GCN4 are sufficient for specific DNA binding and also for dimerization. The GCN4 DNA-binding domain is similar in sequence to the jun oncoprotein, the oncogenic version of the vertebrate AP-1 transcription factor. Moreover, GCN4 and jun bind the same DNA sequences, and jun efficiently activates transcription in yeast cells indicating a basic similarity in the molecular mechanism of eukaryotic transcriptional activation. The GCN4 DNA-binding domain contains a "leucine zipper" region that is sufficient for dimerization; however, the leucine residues are not critical.

In addition to the DNA-binding domain, transcriptional activation by GCN4 requires a short acidic region in the center of the protein. Acidic regions of 35-40 amino acids are sufficient for full activation when fused directly to the DNA-binding domain. The activation region is a repeated structure composed of small units that act additively which presumably interacts with other proteins of the transcriptional machinery.

The *his3* promoter contains two functionally distinct TATA elements, T_R and T_C, but only T_R can activate transcription in combination with GCN4 or GAL4. The sequence TATAAA is sufficient for T_R to activate transcription, but almost all single bp substitutions abolish function. Interestingly, the TATTTA double mutant is functional and 3 mutations activate transcription in combination with GCN4 but not GAL4. Finally, a wide variety of sequences completely unrelated to TATAAA can serve as the T_R element. These observations suggest that multiple proteins can perform the TATA function for transcriptional activation.

Hormone Receptors in Transcription (joint)

E 022 NUCLEAR RECEPTORS AS INDUCIBLE ENHANCER FACTORS, Pierre Chambon, LGME/CNRS and U.184/INSERM, Faculté de Médecine, 67085-Strasbourg Cédex, France.

Steroid/thyroid hormone and retinoic acid receptors are ligand-inducible transcriptional enhancer factors which bind to specific cis-acting responsive DNA elements. In vivo and in vitro structure/function studies using in vitro engineered mutants and chimeric receptors expressed in animal and yeast cells have revealed the existence of several functional domains responsible for ligand binding, nuclear localization, dimerization, DNA binding and activation of transcription. I will discuss the work currently performed in our laboratory and present a model aimed at explaining how nuclear receptors may function.

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Late Addition

E 023 HOW TRANSCRIPTION FACTORS CONTROL WHERE AND WHEN CELLS DIVIDE DURING EMBRYOGENESIS, Patrick H. O'Farrell, Bruce A. Edgar and Christian F. Lehner, Department of Biochemistry, University of California, San Francisco CA 94143

The timing of embryonic cell divisions in Drosophila is precisely controlled. The pattern of the division 14 has been particularly well characterized. All cells start cell cycle 14 at the same time after 13 rapid and synchronous divisions but cells in different positions in the embryo differ in the length of cycle 14. The lengths of cycle 14 are regulated by evolutionarily conserved regulators that govern entry into mitosis to produce a precise spatio-temporal pattern. One of these regulators, the products of the string gene, is limiting. The time of string gene transcription is regulated in an intricate pattern that predicts the time of mitosis (1). Induced premature expression of string induces premature mitoses. The string gene is homologous to and can substitute for the S. pombe cell division cycle gene, cdc25. The time of string transcription, and thus the time of cell division, is regulated by combinations of known transcriptional regulators that control pattern formation in the embryo.

Cyclin A and cyclin B, two homologous proteins were previously thought to be the best candidates for the regulation of the timing of cell division because of the striking change in their levels during the cell cycle. In many species these proteins accumulate throughout the cell cycle and are abruptly degraded at mitosis. We have shown that the levels of cyclins do not determine the time of mitosis 14 (2).

During development the regulation of the cell cycle changes. At different times different regulators are limiting so that the pivotal regulator changes (3).

1) Edgar B.A. and O'Farrell, P.H. (1989) Cell 57, 177.
2) Lehner, C.F. and O'Farrell, P.H. (1989) Cell 56, 957.
3) O'Farrell et al (1989) Science 246, 635.

Structural Aspects of Transcription Machinery

E 100 **IDENTIFICATION OF A CARTILAGE-SPECIFIC PROMOTER WITHIN INTRON 2 OF THE CHICK α2(I) COLLAGEN GENE,** **Sherrill L. Adams and Vickie D. Bennett, Department of Anatomy and Histology, University of Pennsylvania School of Dental Medicine, Philadelphia, Pennsylvania 19104-6003.**
Prechondrogenic mesenchymal cells produce an extracellular matrix containing type I collagen. During chondrogenesis (the differentiation of mesenchymal cells into cartilage-producing chondrocytes), the cells stop producing type I collagen and initiate synthesis of a large amount of the cartilage-specific collagen, type II. However, chondrocytes continue to transcribe the gene encoding the α2 subunit of type I collagen [α2(I)] at a high rate, and they accumulate a substantial amount of α2(I) collagen mRNA which is associated with 3-4 ribosomes. We have characterized the cartilage α2(I) collagen mRNA, to determine the molecular basis for the lack of α2(I) collagen synthesis in cartilage. We have found that the 5' end of the α2(I) collagen mRNA in cartilage differs from the 5' end of the mRNA in cells and tissues that actively synthesize α2(I) collagen (for example, bone and tendon). This difference in mRNA structure results from the use of a cartilage-specific transcription start site within intron 2 of the α2(I) collagen gene. The use of the cartilage transcription start site replaces exons 1 and 2 with a 96 base exon contained within intron 2. The resulting transcripts contain several small open reading frames, all of which are out of frame with the collagen coding sequence. The cartilage form of the mRNA no longer encodes α2(I) collagen, thus explaining the absence of α2(I) collagen in cartilage. In prechondrogenic limb mesenchyme, transcription of the α2(I) collagen gene initiates at the previously described (bone/tendon) promoter. Thus the cessation of α2(I) collagen synthesis that occurs during differentiation of prechondrogenic mesenchymal cells into chondrocytes apparently results from the switch in promoter utilization, from the bone/tendon promoter to the cartilage promoter.

E 101 **TRANSCRIPTIONAL ACTIVATION MECHANISMS MEDIATED BY SV40 EARLY PROTEINS.**
James C. Alwine, Gwen Gilinger, Maryann Gruda and Joe Manuppello. Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6076; FAX (215) 898-9557.
The SV40 early proteins, T/t antigen, have been shown to mediate the transcriptional activation of many viral and cellular promoters. The SV40 late promoter is specifically activated by large T antigen; evidence suggests that the mechanism of transcriptional activation does not require T antigen to interact with DNA. Activation is mediated through modification or induction of cellular factors. To determine the factors and mechanisms involved we characterized two simian cell nuclear factors which bind to late promoter sequences known to be needed for T antigen-mediated activation. These factors have been designated A and B. The A factor binds to sequences containing the AP-1 and Octamer (Oct 1) binding sites within the 72 bp repeat region. The B factor binds to adjacent sequences within SV40 nucleotides 225-270. Both of these factors are significantly altered in T antigen containing simian cells (COS or infected CV-1) as compared to uninfected CV-1 cells. This indicates that these are target factors through which T antigen functions. Specifically, the Band A and B factors from T antigen containing monkey cells: 1) bind more stably; 2) are charged differently; and 3) have microheterogeneous modified forms as indicated by both factor binding and sizing experiments. The band A factors are relatively small in size (20-25Kd) but footprint over a large region of DNA which corresponds very well to the Oct 1 binding regions. Our data suggest that multiple factors bind, possibly cooperatively. Overall we feel that the modified forms of the A factor found in T antigen containing cells facilitate the formation of transcription complexes on the late promoter element in the AP-1/Oct 1 binding region. In agreement with this model, mutations within the AP-1/Oct 1 binding site both abrogate binding and diminish transcriptional activation of the late promoter in the presence of T antigen.

E 102 PURIFICATION OF ACTIVE RECOMBINANT HIV-1 TRANSACTIVATOR (TAT), Christophe Ampe, Steve C. Schultz, Kevin M. Weeks, Donald M. Crothers and Thomas A. Steitz, Departments of Molecular Biophysics and Biochemistry, Chemistry and HHMI at Yale University, New Haven, CT 06511
Problems encountered in purifying the HIV trans activator (tat) from *E. coli* are poor expression levels, low solubility and rapid oxidation of the protein. Previous purification procedures employed denaturing agents[1] which probably yielded non-native protein which aggregated readily and demonstrated no or low specific affinity for the tat recognition site (tar) *in vitro* (unpublished results). We overcame these problems by constructing a fusion gene under the control of a T7 promotor. The gene encodes for a fusion protein consisting of the cAMP-binding domain of catabolite gene activator protein, a linker including a recognition site for the blood coagulation factor Xa and either full length tat (tat86) or the first exon (tat72). The fusion protein, which is expressed at high levels, is purified by affinity chromatography over a cAMP-agarose column. Gel retardation assays demonstrated that the fusion proteins have moderate affinity for tar; digestion with factor Xa generates native tat86 or tat72 which bind the tar RNA with high affinity and form distinct complexes. The method will be used to produce large quantities of active tat for structural studies of the transactivator and its complex with the recognition site.
1 Frankel, A.D., Bredt, D.S. and Pabo, C.O. (1988) Science 240, 70-73.

E 103 PHYSIOLOGICAL STIMULI INDUCE CHANGES IN MITOCHONDRIAL DNA STRUCTURE AND TRANSCRIPT ABUNDANCE IN STRIATED MYOCYTES. Brian H. Annex and R. S. Williams, Departments of Medicine and Microbiology. Duke University. Durham, N.C 27710.

Both transcription and replication of the mammalian mitochondrial (mt) genome originate from within a discrete region that is capable of forming a triplex DNA structure known as the displacement (D) loop. In cultured, non-contracting mouse C2 myogenic cells only about 5% of the mtDNA molecules exhibit the triplex (D-loop) structure, while the remaining mtDNA is present as exclusively duplex DNA. However, in continuously contracting mouse cardiomyocytes, approximately 70% of the mtDNA assumes the D-loop structure. A similar difference in the proportion of D-loop forms is observed when mtDNA is isolated from the hearts of hibernating (10% D-loop) as compared to free-flying (50% D-loop) bats. Moreover, as assessed by primer extension and S1 nuclease analyses, the length and initiation site of the 7S mtDNA within the D-loop triplex also varies under these different physiological conditions. We also observed a 10 fold higher abundance of cytochrome b mt mRNA relative to 12S mt rRNA in cells that contain a high proportion of D-loop forms. These results indicate that synthesis or stability of the 7S mtDNA strand within the mt D-loop region is subject to physiological regulation. In addition, physiologically mediated differences in mtDNA structure are accompanied by differences in the relative abundance of mt mRNA versus rRNA transcripts, suggesting that formation of the triplex mtDNA structure may influence the frequency of transcriptional termination following mt rRNA synthesis.

E 104 Photoaffinity mapping of subunits of yeast transcription factor IIIC to various sites in the SUP4 tRNA gene, Blaine Bartholomew, Burkhard R. Braun, George A. Kassavetis, and E. Peter Geiduschek, Department of Biology and Center for Molecular Genetics, University of California - San Diego, La Jolla, CA 92093.

A photoreactive crosslinker, connected to the pyrimdine ring by approximately a 10 Å tether, was incorporated at specific sites in the *S. cerevisiae* SUP4 tRNA gene. The TTP analog, 5-[N-(p-azidobenzoyl)-3-aminoallyl]-deoxyuridine triphosphate, was incorporated into DNA using T4 DNA polymerase, specific oligonucleotide primers, and single stranded M13 DNA template containing the SUP4 gene. The site containing the modified nucleotide was radioactively labeled by incorporating adjacent [α-^{32}P] dNTP to the modified nucleotide. The number of aryl azido dUTP residues incorporated into DNA was specifically limited by withholding one or two dNTPs. Synthesis of the DNA strand was then completed in the presence of excess unlabeled and unmodified dNTPs.

Purified factor IIIC was bound to DNA in the absence of light and then covalently crosslinked by UV irradiation. Crosslinked proteins were identified by extensive nuclease digestion, SDS-PAGE analysis, and autoradiography. A single 145 K polypeptide was photoaffinity labeled at a site proximal to the *box B* promoter element and a 95 K polypeptide was photoaffinity labeled at a site inside the *box A* promoter element. Additional proteins of 55 and 135 K were detected when the crosslinking sites were immediately adjacent to *box A* and in the intervening sequence, respectively. Template competition experiments and the effects of promoter mutations define all four of these polypeptides as components of IIIC or specifically associated with IIIC.

E 105 SPECIFICITY OF TRANSACTIVATION OF GENE EXPRESSION BY VARICELLA ZOSTER VIRUS GENE 62 PROTEIN, Joan L. Betz[1,2], Davol G. Tedder[2], Susan Wydowski[2] and Lewis I. Pizer[2], [1]Department of Biochemistry, Biophysics and Genetics and [2]Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, CO 80220.

The study of viral trans-acting proteins has been invaluable in deciphering mechanisms of action of eukaryotic transcription factors. The trans-acting q4 protein of Herpes simplex virus, essential for expression of viral early and late genes and for repression of immediate early genes, binds to specific DNA sequences from which a consensus (q4 binding site) has been derived. Although the q4 binding site sequence is not present in all HSV promoters that q4 trans-activates, introduction of this sequence into heterologous promoters increases their ability to be trans-activated. We have been studying the homologous trans-activating gene 62 protein (ORF62) of the herpesvirus Varicella Zoster virus, which has not yet been shown to bind specific DNA sequences. Using transfection assays and a chloramphenicol transacetylase (CAT) reporter gene, we have examined the functioning of ORF62 in terms of the sequence specificity of its trans-activation. Using a series of HSV-1 promoters deleted for q4 binding sequence or containing additional copies, we have shown that the trans-activation of gene expression by ORF62 was increased by the presence of the q4 binding sequence, similar to results we have obtained with q4. We are also examining the regulation of expression from ORF62 to determine whether the presence of q4 binding sequences in that promoter is correlated with negative regulation of that gene, analogous to results with q4 autoregulation.

E 106 HTLV-1 TAX_1 INTERACTS WITH A CELLULAR TRANSCRIPTION FACTOR WHICH BINDS TO A TAX_1-RESPONSIVE ELEMENT, John N. Brady, Paul F. Lindholm, Mike F. Radonovich and Susan J. Marriott, Laboratory of Molecular Virology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Transcription of the Human T-Cell Leukemia/Lymphoma Virus (HTLV-1) is controlled through the interaction of viral and cellular proteins with regulatory elements located in the viral long terminal repeat (LTR). The viral regulatory protein, Tax_1, has been shown to activate transcription of the LTR through at least two independent elements, the 21 base pair repeats (TRE-1) and an element located from -117 to -163 (TRE-2). Although Tax_1 does not bind DNA directly, we have previously demonstrated that a cellular protein can mediate the indirect interaction of Tax, with TRE-2. We have recently purified a 36 kD nuclear protein from HeLa cells which binds to TRE-2 and allows the indirect interaction of Tax_1 with this element. This protein is thus referred to as "Tax_1 interaction factor 1" (TIF-1). We have also shown that TIF-1 is able to activate transcription from the viral LTR in vitro. Both Tax_1 and TIF-1 interact with a zinc affinity chromatography column, suggesting the possibility that the interaction between the two proteins is mediated by zinc or that the interaction of TIF-1 with TRE-2 requires zinc. We have analyzed the efficiency of DNA binding by the TIF-1 apoprotein in the presence and absence of zinc. No detectable difference was observed. The requirement for zinc in Tax_1 - TIF-1 heterodimer formation is currently under investigation. The contribution of protein-protein interactions to Tax_1-mediated transactivation of the HLTV-1 LTR will be discussed.

E 107 MAPPING THE TRANSCRIPTIONAL TRANSACTIVATION FUNCTION OF SV40 LARGE T ANTIGEN. Charles Cole, Jiyue Zhu and Philip Rice, Department of Biochemistry and Molecular Genetics Center, Dartmouth Medical School, Hanover, NH 03756.

To map and study the activities of SV40 large T antigen, a large set of deletion and linker insertion mutants were examined for their ability to transactivate transcription from a number of promoters. Mutant dlA2420 produces an N-terminal 138 amino acid fragment of T antigen. This mutant was able to transactivate the SV40 late (SVL) promoter. It lacks the DNA binding domain of large T. Mutant dlA2831 is missing amino acids 5-35 of T antigen and was also able to transactivate the SVL promoter. Although the DNA binding domain of T antigen was not required for transactivation of the SVL promoter, linker insertion and small in-frame deletion mutants in the DNA binding domain totally abolished the transactivation function.

Some mutants unable to transactivate the SV40 late promoter were able to transactivate the Rous sarcoma virus LTR promoter. Mutations at several sites in the middle of the T antigen coding region reduced the ability of T antigen to transactivate the RSV promoter. Therefore, transactivation of the RSV promoter was more sensitive to alterations in the structure of T antigen than was transactivation of the SVL promoter. This could indicate that T antigen transactivates different promoters by different mechanisms. The transcactivation levels were quite sensitive to the ratio of plasmid encoding the T antigen to the reporter plasmid.

There was no correlation between the ability to transactivate these promoters and the ability to transform or immortalize rodent cells, suggesting that transactivation does not play a critical role in the process of malignant tranformation. The ability of these mutants to transactivate the c-fos promoter is being examined as is the possible contribution of small t antigen to transactivation.

E 108 A NON-OVERLAPPING REPRESENTATION OF REGULATORY REGIONS ALLOWS TO SEARCH FOR COMMON RULES OF DIFFERENT TRANSCRIPTION UNITS. Collado-Vides J. Department of Biology Room 56-438. Massachusetts Institute of Technology. Cambridge, Mass. 02139.

A strictly successive representation of molecular categories within transcription units (TU's) is obtained by the Markedness Principle (MP). Based on diverse types of evidences available in the literature, operator (Op) and activator (I) categories are grouped under a common ITRM category - initiation-of-transcription regulatory mechanism. Assignement of either a negative or positive feature to ITRM by the MP specifies it as Op or I respectively, and, distinguishes non-marked and marked cases.

The non-overlapping representation allows us to search for common rules of the type X --> Y, read "rewrite X as Y", for the description of different TU's. Intermediate categories between the initial TU symbol and molecular categories are shown to be useful in derivations of TU's, for including regulatory and organizational information restricting the possible rules. In the analysis of the genetic switch of phage lambda, we show that mutational information but not affinity differences can be used for restricting the rules, if the goal is to find common rules for the description of different TU's.

E 109 THREE-DIMENSIONAL STRUCTURE OF PROKARYOTIC AND EUKARYOTIC RNA POLYMERASES BY ELECTRON CRYSTALLOGRAPHY OF TWO-DIMENSIONAL CRYSTALS, Seth A. Darst, Elizabeth W. Kubalek, Aled M. Edwards, and Roger D. Kornberg, Department of Cell Biology, Beckman Laboratories, Fairchild Center, Stanford University, Stanford, CA 94305

Prokaryotic and eukaryotic RNA polymerases, including *Escherichia coli* RNA polymerase holoenzyme and yeast RNA polymerases I and II, form two-dimensional crystals on layers of positively charged lipids. The three-dimensional structure of the *E. coli* enzyme, determined to 27 Å resolution, reveals a deep cleft on the surface that is likely to be the DNA binding and active site region. The cleft is the appropriate shape and dimensions to bind duplex DNA and also shows structural similarity with the DNA-binding region of *E. coli* DNA polymerase I. Subunit-specific monoclonal antibodies are being used to identify the subunits in the *E. coli* RNA polymerase structure. Crystals of an anti-α IgG antibody/*E. coli* RNA polymerase complex diffract to higher resolution than the native polymerase crystals. These crystals are being analyzed to identify one of the α subunits and to improve the resolution of the *E. coli* RNA polymerase holoenzyme structure to around 15 Å. Because of extensive amino-acid sequence homology of the two largest subunits of the *E. coli* enzyme with the largest subunits of eukaryotic RNA polymerases, it seems likely that essential features of the three-dimensional structure are also conserved. Electron microscopy of single molecules of yeast RNA polymerase II reveals a large cleft in the molecule, as is found in the *E. coli* RNA polymerase. Two-dimensional crystals of yeast RNA polymerase II, which diffract to 25-30 Å resolution, are being analyzed to reveal the structural similarities and relationships with the *E. coli* enzyme.

E 110 HISTONE VARIANTS H2AVD AND H3.3 OF DROSOPHILA MELANOGASTER. S.C.R. Elgin*, A. van Daal*, S. Fretzin*, E.M. White‡, and M.A. Gorovsky‡. *Department of Biology, Washington University, St. Louis, MO 63130 and ‡Department of Biology, University of Rochester, Rochester, NY 14627.

We have cloned and characterized the sequences for two Drosophila histone variants, H2AvD and H3.3. Such variants are expressed at a basal level (rather than being S-phase regulated) as polyA+ mRNAs. Screening of cDNA libraries with homologous probes has identified the appropriate cDNA clones; genomic clones have been recovered for H2AvD. The gene encoding H2AvD is a single copy gene located in the interval 97D1-5, on a different chromosome from the repetitious histone gene cluster at 39D2-E2. The protein obtained by conceptual translation is 98% homologous (excluding the C-terminal tail) to the H2A.Z variant of mammals, while only 59% homologous to the Drosophila H2A gene at 39D2-E2. An analysis of 6 H2A variant and 11 H2A S-phase regulated genes using maximum parsimony indicates that the variant gene was established prior to the separation of yeasts and Tetrahymena from other eukaryotes. H2AvD is expressed at high levels maternally and in the early embryo. An antibody specific to this protein shows widespread, but not general, staining of the polytene chromosomes. The cDNA sequence of the H3.3 gene also shows high conservation of the variant. The amino acid sequence derived differs in five amino acids from the H3 protein encoded at 39D2-E2, but is identical to the mammalian H3.3. In contrast, comparison of the DNA sequences of the two types of H3 genes of Drosophila shows only 77% homology; numerous neutral substitutions are observed. The high degree of conservation of the variant histones suggests that they play an essential role, perhaps generating alternative nucleosome structures pertinent to biological function.

E 111 **THE SECOND EPSTEIN BARR-VIRUS DETERMINED NUCLEAR ANTIGEN, EBNA2, REGULATES THE TRANSCRIPTION OF THE EBV LATENT MEMBRANE PROTEIN, LMP, BY ATAGONIZING THE SUPPRESSION OF THE TRANSCRIPTION.** **Robin Fåhraeus, Anne Ricksten**, Ann Olsson, Ann Jansson, **Lars Rymo. Dep. of Tumor Biology Karolinska Institutet S-**104 01 Stockholm and Dep. of med. Biochemistry Gothenburg University S-400 33 Sweden. EBV immortalizes B-cells after in vitro infection. EBNA2, a nuclear protein encoded by the BamHI YW fragment of the viral genome is known to be required for viral immortalization. The membrane antigen LMP, is encoded by the BamHI N fragment of the viral DNA. It has been shown to transform rodent fibroblast. We have used different DNA fragments from the 5' end of the LMP gene coupled to the CAT-gene in a plasmid construction. A 673bp fragment was transfected to a EBV negative Burkitt lymphoma line, DG75. No CAT activity was observed. The same construct induced CAT ativity in EBNA2 positve DG75 cells. A similar effect was observed when the BamHI YW deleted, EBNA2 negative P3HR-1 cell was cotransfected with an EBNA2 gene and the 673bp fragment construct. When a 90bp fragment of LMPs 5'end was used, CAT activity was detected both in EBNA2 negative and positive cells. These results indicates that EBNA2 activates transcription of the normally suppressed LMP gene.

E 112 CHROMOSOMAL ORGANIZATION OF THE GLOBIN GENE DOMAIN, Donald Fleenor and Russel Kaufman, Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

The beta-globin gene domain contains multiple regulatory elements required for the correct developmental and tissue specific expression of globin genes. Transgenes are expressed at normal levels only when surrounded by segments of DNA from the globin gene domain that produce strong DNase supersensitive sites. We have characterized the DNase supersensitive site located 20 kb 3′ to the beta globin gene. Primary DNA sequence reveals that the region is extremely A/T rich (80%) over a 900 bp region. The region contains topoisomerase I and II recognition sites based on their homology to the vertebrate consensus sequence. We have identified 8 potential nuclear scaffold attachment sites and have confirmed that the segment is attached to the nuclear matrix in erythroid cells. In addition, we find a short segment that has enhancer function in erythroid cells. Finally, we identify a DNA sequence motif that is repeated in the DNase supersensitive sites located 5′ to the globin gene complex. We hypothesize that this segment of DNA which confers strong DNase supersensitivity to chromatin in erythroid precursor cells may serve as a nuclear chromatin organizer of the globin gene domain. DNA protein interactions in the region may provide tissue specific interactions and allow for management of DNA torsional stress during replication or transcription. We conclude that the unique organization of the short region of DNA identified by the DNase supersensitivity site may organize the globin gene domain and allow high level, developmentally correct expression of globin genes.

E 113 **Evidence that a repressor gene located on mouse chromosome 7 is involved in the regulation of a group of genes induced by growth arrest signals and DNA-damaging agents. Albert J. Fornace, Jr., Nikki J. Holbrook†, Daniel D. Petersen*, M. Christine Hollander, and Daniel W. Nebert*, N.C.I. and *N.I.C.H.H.D., N.I.H., Bethesda, MD, and †N.I.A., N.I.H., Baltimore, MD.**

In both bacteria and eukaryotes, one effect of DNA damage is the transient inhibition of DNA synthesis and cell growth; such delays can have a protective effect since mutants lacking growth arrest responses are hypersensitive to certain DNA-damaging agents. Recently, more than 20 cDNA clones encoding different DNA-damage-inducible (DDI) transcripts have been isolated from rodent cells[1]. We have now found that 5 of these clones encode genes that are coordinately induced either by DNA-damaging agents or by other cell treatments that induce growth arrest such as serum reduction, medium depletion, or contact inhibition; these genes have been designated *gadd* (growth arrest- and DNA damage-inducible). Marked overexpression of the *gadd* genes was found in the homozygous deletion c^{14CoS}/c^{14CoS} mouse that is missing about a 1 cM portion of chromosome 7 near the *c* (albino locus. Overexpression of the *Nmo-1* [NAD(P)H:menadione oxidoreductase, EC 1.6.99.2] and other Phase II genes of the [*Ah*] battery have also been found in this mutant[2]. The *Nmo-1* gene is believed to play a central role in protection against oxidative stress, and we have recently found that it is also DDI. Our findings indicate that a *trans*-acting negative effector(s) encoded by a gene in the deleted portion of chromosome 7 is involved in the regulation of these stress response genes located on different chromosomes. Two of the *gadd* genes, A45 and A153, have been cloned and sequenced. Studies with the promoter of the A45 *gadd* gene are underway and will be presented with emphasis on its role in the negative control of cell growth.

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E 114 ***IN VIVO* FOOTPRINTING OF A YEAST HEAT SHOCK GENE PROMOTER REVEALS MARKED HELICAL DISTORTION AND CONSTITUTIVE OCCUPANCY OF HEAT SHOCK AND TATA ELEMENTS,** David S. Gross, Karen E. English, Kerry W. Collins, and Seewoo Lee, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, LA 71130. We describe here for the first time successful application of the hydroxyl radical technique for *in vivo* footprinting. In combination with two complementary techniques, DNase I footprinting and dimethyl sulfate (DMS) methylation protection, we have obtained a high resolution map of the promoter region of the yeast *HSP82* heat shock gene. We find that irrespective of transcriptional state, basal or induced, the promoter-proximal heat shock element, HSE1, and the TATA box are tightly bound by proteins, presumably heat shock factor (HSF) and TFIID, respectively. Whereas "HSF" binds tightly within the major groove of HSE1, as discerned by protection of guanines from DMS methylation, "TFIID" appears to bind principally to the sugar-phosphate backbone, as revealed by strong protection from hydroxyl radical cleavage. In addition, while HSE1 is strongly footprinted by DNase I, the TATA box is only weakly footprinted. Strikingly, both elements are associated with marked distortion of the DNA double helix *in vivo*. "HSF" binding to HSE appears to cause a non-B-form structure, based on a local 12 bp periodicity of hydroxyl radical protection and the presence of multiple DNase I hypersensitive nucleotides flanking HSE1, whose pattern changes following heat shock. Similarly, helix distortion is evident in the vicinity of the TATA box, since hydroxyl radical detects a microhypersensitive site within an adjacent polypurine tract. Finally, in the upstream region extending from -140 to at least -290, the DNA is cleaved by hydroxyl radical in a highly modulated, non-random fashion. As DNase I cleavage exhibits no such periodicity, we suggest that this region is coiled *in vivo*, but is not packaged into a sequence-positioned nucleosome. We are currently introducing mutations into the *HSP82* promoter by gene transplacement, and assessing their structural and functional consequences.

E 115 TOPOISOMERASE ACTIVITY AND THE EXPRESSION OF DISEASE RESISTANCE RESPONSE GENES (DRRG) OF PEAS, L. A. Hadwiger, Chin C. Chiang and Andrew Pettinger, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Pea endocarp tissue responds to the challenge of plant pathogenic fungi by overexpressing several major DRRG genes. A 17 Kd product(s) homologous with the gene designated DRRG49 constitutes up to 25% of the label of total proteins in fungal-challenged legumes denoting the importance of one of these unknown functions. Tissue becomes susceptible to fungal attack if one prevents the expression of this group of genes. The promoter sequence and 3' sequence of DRRG49 contain clusters of topoisomerase II consensus sites along with an SV40-like promoter sequence, 2 AP-1 sites, inverted repeats, Z-DNA forming potential, TATA box, etc. If these topoisomerase II consensus regions denote scaffold attachment sites the structural gene exists on a <1 Kb chromosomal loop. Yields of partially purified topoisomerases I and II activity in pea tissue are dramatically enhanced within 3 hours following fungal challenge corresponding to the enhanced accumulation of DRRG49 homologous RNA. This mRNA accumulation is also enhanced by: Compounds which limit gyrase activity, the cationic elicitor compound, chitosan, which has the potential to alter DNA conformation, trioxsalen 4'aminomethyl-HCl, a psoralen which, with UV 360 light, forms monoadducts to pyrimidine bases after intercalation and actinomycin D, a DNA intercalating molecule (intercalations occur an average of one molecule/10,000 bp pea DNA). The DRRG49 promoter activity is transferable to transgenic tobacco tissue and is inducible by fungal challenge. A model for the proposed transcriptional control of this gene based on helical alteration of a chromosomal loop will be presented.

E 116 THE INITIATION OF TRANSCRIPTION FROM SV40 MINICHROMOSOMES IN VITRO. Ulla Hansen, Susan Batson, Catherine Heath, Mark Samuels, and Rebecca Sundseth. Dana-Farber Cancer Inst., Boston, MA.

An in vitro system has been developed for studying initiation of transcription from an in vivo constructed chromatin template, in order to investigate the role of chromatin in gene expression. Partially purified SV40 minichromosomes isolated from infected CV-1 cells are transcribed in a reconstituted transcription system in the presence of alpha-amanitin, to inhibit elongation of endogenous RNA polymerase II, and an alpha-amanitin resistant RNA polymerase II. All resulting transcripts are therefore newly initiated in vitro. The pattern of transcription from both the early and late SV40 promoters on the minichromosome templates is very similar to the pattern of mRNA observed in vivo at 48 hours post-infection in SV40 infected cells, but distinct from the pattern of transcripts initiated on deproteinized SV40 DNA templates in vitro. The pattern of transcription from the minichromosomes is differentially stimulated by the addition of the SV40 DNA binding transcription factors, Sp1 or LSF. Transcription is as efficient as from deproteinized DNA templates.

Whereas enhancers stimulate transcription when positioned large distances from the promoter in vivo, in vitro experiments on DNA templates have shown stimulation of transcription by enhancers only when positioned up to 400 bp upstream of the initiation site. Thus, chromatin might be required for position-independent enhancer stimulation of transcription. Our strategy to investigate this problem involved generating SV40 virus stocks from mutants in which the SV40 enhancer has either been deleted or moved 3' or 5' to the SV40 promoter at various distances. All virus stocks, including the deleted enhancer mutant stocks have been obtained. Transcription from the wild-type and mutant minichromosomes is compared to transcription from their deproteinized counterparts. In initial experiments, the enhancer stimulated transcription to a much greater extent on the chromatin template than on the deproteinized DNA. The effects of moving the enhancer 3' or 5' of the promoter will be discussed.

E 117 Inactivation of a translation initiation factor inhibits RNA polymerase II transcription in mammalian cells. Randal J. Kaufman*, Monique V. Davies*, Barbara Wu^, Jerry Pelletier#, and Nahum Sonenberg+. * Genetics Institute, Cambridge MA; ^Department of Biochemistry, Northwestern University, Evanston IL; +Department of Biochemistry, McGill University, Montreal CA, and #Center for Cancer Research. Mass Inst Tech., Cambridge, MA..

Poliovirus infection of cells results is a rapid shut-off of host protein synthesis. Concordant with shutoff, cleavage of the p220 subunit of the cap-binding protein complex is induced by the poliovirus protease p2A. p220 proteolysis is thought to inhibit cap-dependent translation of host mRNAs. Expression of p2A in transfected COS-1 cells independently induces p220 cleavage. The effect of p2A expression on expression from a co-transfected marker gene was studied. Cleavage of p220 did not result in an inhibition of mRNA translation from the co-transfected gene. However, co-transfection of p2A did result in reduced mRNA accumulation from the co-transfected gene. Nuclear run-off experiments suggest that the major cause for the reduction is primarily due to a reduction of active transcription complexes in the p2A transfected cells. The results suggest that p220 may play a role in RNA polymerase II transcription.

E 118 THE TAX1 PROTEIN OF HTLV-1 TRANSACTIVATES THE AP-1 ENHANCER ELEMENT AND STIMULATES AP-1 COMPLEX FORMATION, David J. Kelvin, Sheldon B. Grove, and William L. Farrar. Biological Carcinogenesis Development Program, LMI, BRMP, NCI, Frederick, MD, 21701. HTLV-1 codes for tax1, a transactivating protein that stimulates transcription by activating cis regulatory elements. Since the AP-1 regulatory element is found in several cytokine and cytokine receptor genes we decided to examine the effect tax1 has on AP-1 dependent transcription. Transient cotransfection assays were performed using a plasmid expressing tax1 and a plasmid construct containing 3 AP-1 sites and a CAT gene. tax1 had the capacity to induce a 12 fold increase in CAT expression from the 3 AP-1 plasmid. Stable Jurkat cell lines constitutively expressing tax1 were examined for AP-1 dependent CAT expression. We found the AP-1 CAT construct was constitutively expressed at a level four times higher in tax1 cell lines than in the parental Jurkat cell line. We also examined the formation of AP-1 complexes in tax1 cell lines using gel mobility shift assays. When a probe containing the AP-1 sequence was assayed with tax1 Jurkat cell line nuclear extracts a specific band could be generated. This band was greatly diminished in Jurkat extracts, however, an identical band of the same intensity could be generated in extracts from Jurkat cells treated with PMA for a period of three hours. We hypothesize that tax1 preempts signal transduction pathways that normally activate DNA binding protein complexes that are involved in the regulation of growth and proliferation, such as AP-1 and NF-kB.

E 119 REGULATION OF A NOVEL MAMMALIAN STRESS RESPONSE GENE WHOSE EXPRESSION IS INDUCED BY DNA DAMAGE AND GROWTH ARREST, **Jennifer D. Luethy, Jong S. Park, Joseph Fargnoli, Albert J. Fornace, Jr.*, and Nikki J. Holbrook, National Institute on Aging, Baltimore, MD 21224, and *NCI, NIH, Bethesda, MD 20892**

In bacteria, treatments that damage DNA or inhibit replication induce a series of genes which constitute the SOS response. Likewise, in mammalian cells DNA damage results in increased transcription of a number of genes, only a few of which have been identified. We have been examining the structure of a gene Gadd 153, which like bacterial SOS response genes is highly induced by growth arrest as well as by DNA damage. The rodent gene spans a distance of about 6kb and contains 4 exons. It is transcribed into a 0.9 kb mRNA and encodes a protein of 168 amino acids. About 1300 bp of the promoter region have been sequenced. A TATA-like sequence ATAAAA is present 32 bp 5' of the cap site, and an inverted CCAAT box is located at -76. The 5' region is relatively GC rich and contains 7 Sp1 binding sites within the region from -60 to -400. A fragment from -767 to +21 has been shown to drive expression of the CAT reporter gene in an orientation dependent fashion. It is active basally in a variety of cell types and is enhanced by treatment with the DNA alkylating agent methyl methanesulfonate (MMS). This fragment also enhances the expression of a heterologous promoter in cells treated with MMS. Despite the presence of an AP-1 binding site at -245, the fragment is only minimally responsive to the phorbol ester TPA. Deletion analysis indicates that the MMS responsive element lies within the region from -232 to -36 relative to the transcription start site. Current experiments are aimed at more precisely delineating the DNA damage response site and identifying the transactivating factors which interact with this element. In addition, we are examining the mechanism by which Gadd 153 expression is regulated by growth arrest.

E 120 ELONGATION BY RNA POLYMERASE II ON NUCLEOSOMAL TEMPLATES, Donal S. Luse and Michael G. Izban, Dept. of Mol. Genetics, Biochemistry and Microbiology, Univ. of Cincinnati Col. of Medicine, Cincinnati, OH 45267-0524 We are studying the ability of RNA polymerase II to elongate nascent RNA chains on nucleosomal templates. RNA polymerases are initiated at the Ad 2 major late promoter and allowed to elongate to about +15 in the presence of limiting NTPs. These elongation complexes are then partially purified and reconstituted into chromatin using Xenopus oocyte extracts. When excess NTPs are added the paused RNA polymerases do not elongate efficiently on the nucleosomal templates; most transcripts are <500 bases. However, control elongations on mock reconstituted templates give RNAs >1000 bases. At normal salt concentrations (up to 150mM K^+ or Na^+) three major pauses, each about 150 bases apart, and a number of minor pauses are seen during elongation on the nucleosomal templates. All of the pauses are relieved during elongation in salt (1M) or Sarkosyl (0.5%) levels which displace nucleosomes from DNA. If, however, the complexes are rinsed in salt up to 0.67M and then returned to low salt before elongation the pauses are unaffected. These results are consistent with pausing by the RNA polymerase before each of three positioned nucleosomes.

E 121 THE ROLE OF THE CELLULAR PROTEIN EBP1 / NF-κB IN TRANSCRIPTIONAL ACTIVATION OF HIV, **James R. Matthews, Lilian Clark and Ronald T. Hay, Department of Biochemistry and Microbiology, University of St.Andrews, U.K.** The long terminal repeats (LTR's) of the integrated proviral form of human immunodeficiency virus (HIV) contain two repeats of a sequence previously identified from the SV40 viral enhancer as a binding site for the HeLa cell protein EBP1 - these sites act as HIV's inducible transcriptional enhancer. Purification of EBP1 from HeLa cell nuclear extracts by two rounds of DEAE-Sepharose chromatography followed by three rounds of recognition site affinity chromatography revealed a 57kD protein. Mobility shift experiments using Jurkat T-cell extracts suggested that that EBP1 was similar or identical to the activated form of the inducible transcription factor NF-κB. Recent studies using mobility shift assays to monitor complex formation between EBP1 and an HIV binding site oligonucleotide have shown that binding of EBP1 is stimulated by the presence of GTP, and dramatically so by the polyamine spermidine. Since spermidine can nfluence DNA conformation, it may be an altered DNA conformation upon binding of EBP1. Experiments with restriction fragments bearing circular permutations of an EBP1 binding site in mobility shift experiments suggest that EBP1 induces a bend at its binding site.

The EBP1 binding site has a string of four purines on the bottom strand followed by six purines on the top strand which all interfere with EBP1 binding when methylated. Dimethyl sulphate protection experiments have shown that binding of EBP1 protects the two guanines at either end of the binding site from methylation, although some internal bases show enhanced levels of modification after binding EBP1. In particular a cytosine which is methylated at a position involved in base-pair hydrogen bonding - possibly implying a breakdown in base pairing. Molecular graphics studies have shown that EBP1, in addition to making base and backbone contacts over a full turn of the double helix, makes virtually all of these contacts on one face of the double helix. These studies suggest that EBP1 may employ a unique mechanism to recognise its binding site - quite distinct from other well-characterised DNA-binding motifs.

E 122 EVIDENCE THAT DNA REPAIR IS COUPLED TO TRANSCRIPTION, I. Mellon, Dept. of Pathology, University of Kentucky, Lexington, KY 40536-0093

We previously reported that UV-induced pyrimidine dimers are selectively removed from the transcribed DNA strand of the dihydrofolate reductase gene in hamster and human cells. Since transcription is blocked by pyrimidine dimers in template DNA, a DNA repair mechanism that specifically detects transcription-blocking lesions should be important for cell survival after UV-irradiation. The process of nucleotide excision repair removes various types of DNA damage including the pyrimidine dimer, but the molecular details of this process are not well understood in mammalian cells. In contrast a detailed description has been achieved in *E. coli*; most if not all of the genes involved have been cloned and their products studied *in vitro*. To improve our understanding of the relationship between transcription and repair we have examined repair in the separate DNA strands of the lactose operon of UV-irradiated *E. coli*. We find a dramatic difference in the repair of the two strands only when transcription is induced. Most dimers are removed from the transcribed strand of the induced operon within 5 minutes after UV-irradiation. In the nontranscribed strand, repair is significantly slower and resembles that found in both strands of the uninduced operon. These results suggest that there is a mechanism that couples DNA repair and transcription. We are currently studying the nature of this link using the biochemical and genetic tools available in *E. coli*.

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Mellon, I. and Hanawalt, P. C. (1989) Nature (in press).

E 123 SV40 T-ANTIGEN BINDING SITE I AND THE REGULATION OF THE SV40 NUCLEOSOME-FREE PROMOTER, Barry Milavetz and Dianne Kube, Department of Biochemistry and Molecular Biology, University of North Dakota School of Medicine, Grand Forks, ND 58202

The nucleosome-free promotor region of Simian Virus 40 [SV40] is characterized by three major features. First, the region is present in only a subpopulation of SV40 chromosomes. Second, the boundaries of the region occur at multiple sites. Third, sites within the nucleosome-free region are frequently occupied by proteins. Since the virally encoded T-antigen is a critical regulatory protein of the virus and T-antigen binding site I is located near one of the boundaries of the nucleosome-free region on the early side of the promotor, we have investigated the role of T-antigen interactions at this site on the regulation of these three features of the SV40 nucleosome-free region. By analyzing mutants which do not have normal interactions at site I by restriction endonucleases digestion and Bal 31 digestion, we have found that T-antigen binding to site I is not required for generating a nucleosome-free region, for setting the early boundaries, or for determining if internal sequences are occupied. However, T-antigen binding at this site is one of the factors responsible for determining the proportion of chromosomes which contain a nucleosome-free region. Based upon their homology to known regulatory elements, we have identified copies of the OCT, TC, and GT SV40 enhancer regulatory elements surrounding T-antigen binding site I. The role of these elements in the regulation of the nucleosome-free region and their interaction with cellular protein factors is presently under investigation.

E 124 TRANSCRIPTIONAL REGULATION OF THE 'LEAKY-LATE GENES OF HERPES SIMPLEX VIRUS TYPE 1
Robert L. Millette, Ronald Wobig, Shin Chen, Patricia Perry, and Robyn Hauser, Department of Biology, Portland State University and Department of Microbiology and Immunology, Oregon Health Sciences University, Portland, OR 97207
The β^{2} or 'leaky-late' (LL) genes of herpes simplex virus type 1 (HSV-1) are typified by the gene for the major capsid protein, VP5 or ICP5. These genes are characterized by their requiring prior expression of several immediate-early (IE) genes and viral DNA replication for maximum levels of transcription. To understand how this class of HSV genes is transcriptionally regulated, we have carried out gel mobility shift, DNase I protection, and transient expression assays using reporter gene (CAT) constructs. Using mobility shift assays we have identified two major protein-DNA complexes that are formed with the VP5 promoter. The complexes appear to involve cellular proteins since they were observed with both uninfected and infected cell nuclear extracts. Binding competition studies with various heterologous promoter DNAs have shown that the major complexes are unique to the LL genes of HSV. DNase I protection experiments have mapped one of the complexes to a promoter sequence (GGGCCATCTTGAAT) located -67 to -80 relative to the VP5 cap site. To determine the role of this sequence in the transcriptional regulation of the VP5 gene, we have constructed deletions of this region and tested them in transient expression assays with and without viral transactivation. Preliminary results from these studies suggest that the above sequence is not involved directly in viral transactivation, but that it appears to play a role in repressing VP5 transcription in the absence of viral immediate-early proteins.

E 125 EVIDENCE FOR HIGH MANNOSE-SPECIFIC INTERACTIONS IN DNA-PROTEIN COMPLEXES,B.B.Mishra, J.Decker, A.V. Muchmore, Metabolism Branch, National Cancer Institute, NIH, Bethesda Maryland 20892. O-linked glycosylation may be important for the activation of transcription by the RNA polymerase II activator SP1.We have evidence that high mannose oligosacharides which have a wide range of biological actions on peripheral blood mononuclear cells, activates the synthesis of interleukin 1 and prostaglandin E2, as well as inducing a temporal increase in c-fos messenger RNA. To investigate the role for N-linked branched high mannose oligosaccharides in transcription, we tested the effect of highly purified N-linked oligosaccharides (Man 5, Man 6 and Man 9) on the interaction between SV 40 enhancer sequences and their binding proteins. In gel-shift assays, Man5 but not Man9 blocked most of the protein binding to AP1, and AP3, consensus binding sites. These same oligosacharides did not however interfere with the binding of either Oct 2 , or AP2 to their consensus binding sites. This effect of Man5 appeared to be concentration dependent, disappearing at concentrations of about 0.5 e-4M . Neither high concentrations of a panel of monosacharides and aminosugars nor the lectins concanavalin A, wheat germ agglutinin and phytohaemagglutinin could mimic the effect of Man5. The evidence presented is consistent with an induced or allosteric effect of the oligosacharides on nuclear proteins, leading to alterations in DNA-protein or protein-protein interactions in the nuclear-transcriptional complex.

E 126 **REGULATION OF THE PROLIFERATING CELL NUCLEAR ANTIGEN BY ADENOVIRUS E1 PRODUCTS,** G.F. Morris and M.B. Mathews, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724. The proliferating cell nuclear antigen (PCNA), also known as cyclin, functions in replication of DNA by stimulating the processivity of DNA polymerase δ. This link to DNA metabolism, as well as the correlation of PCNA expression with cell proliferation, prompted us to clone the promoter for PCNA from human placental DNA so that its activity during periods of cell growth could be examined in detail. The cloned promoter directs the synthesis of a PCNA mRNA with the correct size for a properly initiated transcript in nuclear extracts from HeLa and 293 cells and potentiates the synthesis of a reporter mRNA (CAT) in transfected BRK, HeLa and 293 cells. Like a number of promoters for "housekeeping" genes, the PCNA promoter is G-C rich and does not possess an identifiable TATA element. Deletion analyses indicate that sequences greater than 50 nucleotides upstream from the cap site are required to direct synthesis of CAT. The adenovirus E1 genes transactivate PCNA-CAT fusion constructs upon co-transfection into HeLa cells. We are currently delineating the gene products of the adenovirus E1 region that are required for maximal transactivation of the PCNA promoter in HeLa cells by producing individual E1 gene products from a constitutive promoter. Cis-acting elements of the PCNA promoter responsive to co-transfection by individual or combinations of E1 gene products will also be discussed.

E 127 A GENETIC ASSAY FOR IDENTIFYING COMPONENTS OF THE TRANSCRIPTIONAL MACHINERY OF DROSOPHILA, Mark A. Mortin, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138. Recessive-lethal mutations in the two largest subunits of RNA polymerase II (polII), which display genetic characteristics different than deficiencies for their respective loci, are likely to result in the structural alteration of the proteins they encode. Second-site suppressors, i.e. compensatory mutations, of this recessive lethality will probably identify genes that encode proteins that contact the two largest subunits of polII. I have mutagenized flies mutant for one of five different recessive-lethal polII mutations and screened more than 11 million of their progeny for suppression of the lethal phenotype; 41 second-site suppressors were recovered. Twelve of the twenty suppressors that have been mapped are extragenic. Six extragenic suppressor mutations define four different loci capable of mutating to a suppression phenotype. All ten extragenic suppressors that have been tested display allele specificity. The four loci defined by this method will likely identify components of the transcriptional machinery and accessory factors. This has been confirmed for two suppressor mutations, which are alleles of the second-largest subunit of polII. They suppress only one mutation in the largest subunit suggesting that the lesions responsible for the suppression and recessive-lethal phenotypes may identified physically interacting domains within the two largest subunits.

E 128 THE INDUCTION OF HIGH AFFINITY PHOSPHOPROTEIN BINDING ACTIVITY SPECIFIC TO THE 3' REGION OF RUBELLA VIRUS GENOMIC RNA Hira L. Nakhasi*; Tracey A. Rouault**; Teh-Yung Liu*; Richard D. Klausner** Division of Biochemistry and Biophysics, CBER, FDA* and Cell Biology and Metabolism, NICHHD, NIH**, Bethesda, MD 20892.
Rubella virus virion consists of a 40S single-stranded polyadenylated genomic RNA of (+) polarity encapsidated by a capsid protein. The viral envelope consists of two virus coded proteins, E1, E2 and portions of the host cell membrane. RNA replication is initiated at the 3' - end of the virion RNA and is catalyzed by RNA-dependent RNA polymerase. Structural analysis of rubella virus RNA revealed that there is a 11 nucleotide inverted repeat located 59 nucleotides 5' to the poly A tail. This RNA sequence is capable of forming a stem-loop structure and can be stable under physiological conditions. We have observed that in the virus infected and uninfected cells, there is a protein(s) species that binds specifically to the 3' stem-loop structure of the viral RNA using gel retardation assay. However, the protein(s) from the infected cells has a significantly high affinity for the stem-loop. Treatment of the virus infected cell lysate with alkaline phosphatase abrogated the affinity of the binding protein(s) suggesting that phosphorylation could be the cause of the enhanced binding affinity for the protein(s). SDS-PAGE analysis of UV induced cross-linked RNA-protein complexes showed that there are two species of proteins with M_r of 63kDa and 68kDa respectively. The appearance of the high affinity binding protein correlated with the negative RNA synthesis.

E 129 IMMEDIATE-EARLY GENE REGULATION OF THE PROMOTER OF HERPES SIMPLEX VIRUS UL42 ENCODING THE DNA POLYMERASE ACCESSORY PROTEIN. Deborah S. Parris[1,2] and Leo Goodrich[2], Department of Medical Microbiology and Immunology[1] and Program in Molecular, Cellular, and Developmental Biology[2], Ohio State University, Columbus, Ohio 43210. The product of the herpes simplex virus type 1 (HSV-1) gene UL42 encodes a double-stranded DNA-binding protein which is essential for replication in vivo and serves to stimulate the activity of the viral encoded DNA polymerase (pol) in vitro. Both pol and UL42 are expressed as early genes and are dependent upon the prior expression of one or more of the 5 immediate-early genes. In order to begin to understand the regulation of these genes, we have defined and dissected the promoter of UL42 and linked it to a chloramphenicol acetyl transferase (CAT) expression cassette. Our results have defined those regions of the UL42 promoter responsive to immediate-early gene regulation. Interesting, the promoter, unlike most early promoters, is not highly responsive to ICP4. It is most responsive to ICP0 which stimulates the full-length promoter up to 60-fold. Together, ICP4 and 0 act synergistically increasing promoter activity up to 150-fold. By contrast, another immediate early gene, ICP27, fails to stimulate the promoter activity alone and actually down-regulates the UL42 promoter when present with ICP0 and ICP4. The sites on the UL42 promoter to which these gene products bind currently is being studied.

E 130 TRANSCRIPTIONAL ARREST OF C-MYC RNA SYNTHESIS BY A PROMOTER-SEQUENCE-SPECIFIC OLIGONUCLEOTIDE. Edith H. Postel, S.J. Flint and M.H. Hogan. Department of Biology, Princeton University, Princeton, NJ 08544-1014, and The Baylor Center for Biotechnology, The Woodlands, TX 77381.

The -115 bp region of the human c-myc promoter plays an important role in transcription of c-myc both in vivo and in vitro (Hay et al., 1987; Cooney et al., 1988, Postel et al., 1989). This region of the c-myc DNA is composed of pur/pyr sequences that exhibit nuclease-hypersensitivity (Siebenlist et. al., 1984, Boles and Hogan, 1987), bind the transcription factor PuF (Postel et al., 1989) and are involved in triple helix formation (Cooney et. al., 1988). This latter observation, showing colinear triplex formation in vitro between a site-specific oligonucleotide and the c-myc duplex DNA at the -115-bp region of the c-myc promoter, as well as the demonstration that the same oligonucleotide repressed transcription from c-myc in an in vitro system suggested that c-myc transcription may be regulated at the level of template structure. We now show evidence that this site specific oligonucleotide can also regulate c-myc transcription in vivo: micromolar amounts of the oligonucleotide added to HeLa cells in culture enter the nucleus and are able to inhibit c-myc RNA synthesis in a sequence-specific and gene-specific manner.

E 131 STRUCTURAL STUDIES ON THE TRANSCRIPTIONAL ACTIVATOR GAL4.

J.F. Povey, G.P. Diakun* and E.D. Laue, Department of Biochemistry, Tennis Court Road, Cambridge, CB2 1QW, England. *SERC Daresbury Laboratory, Daresbury, Warrington, WA4 4AD England.

The N-terminal DNA binding domain of GAL4 contains the sequence: Cys-X_2-Cys-X_6-Cys-X_6-Cys-X_2-Cys-X_6-Cys which is highly conserved amongst other fungal gene regulatory proteins. Recent work on a protein consisting of the the N terminal 147 amino acids of GAL 4, GAL4 (1-147), showed 1-1.5 molZn^{2+}/mol protein.^{113}Cd NMR spectroscopy on a sample where the zinc had been removed and replaced with cadmium suggested that the two cadmium ions contained within the protein were each coordinated by three or four sulphur atoms.

We present here the results of Extended X Ray Absorption Fine Structure (EXAFS) studies which suggest that the structure of GAL4 is somewhat different to the conventional zinc finger.

E 132 STRUCTURE OF THE 434 PHAGE REPRESSOR COMPLEXED WITH THE OR3 OPERATOR. David W. Rodgers, Marie Drottar and Stephen C. Harrison. Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

Bacteriophage 434 repressor binds as a dimer to six different viral operators, which are 16 base pairs long and have approximate twofold symmetry. The regulatory function of this protein depends on its differing (up to 40-fold) affinity for the six operators. The structure of the N-terminal domain of repressor, which contains a helix-turn-helix binding element, complexed with the OR1 operator detailed an intricate set of contacts to bases and backbone phosphates that can be made only if the conformation of the DNA deviates significantly from ideal B-DNA. The structure of the N-terminal domain bound to another operator sequence, OR3, has now been solved and refined against data that extend to 2.5 Å resolution. One half-site of the OR3 operator has the same sequence as the corresponding half-site of OR1, and the conformation of the DNA and bound monomer are essentially unchanged. The sequence of the other half-site differs from OR1 at three consecutive base pairs. Here there are conformational differences in the DNA and protein monomer that alter some of the specific binding interactions. Thus the exact nature of the binding can vary with the operator sequence.

E 133 DEVELOPMENTAL REGULATION OF THE MURINE CD4 GENE, John F. Sands and Per A. Peterson, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

We have isolated three overlapping cosmids containing the murine CD4 gene. These cosmids have been extensively restriction mapped. About 90% of the cosmids have been subcloned. Using these subclones, we have probed 75 kb of the chromatin associated with the CD4 gene for DNaseI hypersensitivity sites. Thirteen sites have been identified. Six of the DNaseI hypersensitivity sites correlate with CD4 expression in both thymocytes and thymoma cell lines. These six hypersensitivity sites are not present in fibroblast, B cell or macrophage chromatin. Our results indicate that the CD4 gene's transcriptional regulatory *cis*-acting elements are not clustered at the immediate 5' end of the CD4 coding region but are spread over a region of many kilobases both 5', 3' and within the gene. This information is being used to create transgenic mice expressing genes in the same manner as the murine CD4 gene.

E 134 3 Å RESOLUTION X-RAY CRYSTALLOGRAPHIC ANALYSIS OF CAP COMPLEXED WITH A 30 BASE-PAIR DNA SEQUENCE INDICATES THAT THE DNA IS SEVERLY BENT, Steve C. Schultz, George C. Shields & Thomas A. Steitz, Department of Molecular Biophysics and Biochemistry and HHMI at Yale University, New Haven, CT 06511

To determine the precise nature of interactions between E. coli catabolite gene activator protein (CAP) and its operator DNA, we have crystallized CAP complexed with a 30 base-pair DNA segment and are determining its structure at 3 Å resolution. We have used both molecular replacement and isomorphous replacement techniques for phase determination. Rotation and translation function searches were used to orient the known CAP-cAMP structure (1) in the CAP-DNA complex crystal. Rigid body refinement of the two CAP domains suggests that the complex is symmetric about a dyad axis. The positions of Br atoms in a CAP-DNA crystal containing DNA substituted at its ends by BrdU were located by difference Patterson and Fourier methods. The positions of these bromine atoms indicate that the DNA is severely bent. Indeed, they agree quite well with the model of Warwicker et al. (2) in which a DNA bend of 150° is proposed. At present electron density maps phased from the CAP structure alone show the DNA poorly; however, we anticipate that averaging and molecular replacement procedures as well as use of iodinated DNA will yield a precise structure of the CAP-DNA complex.

1. McKay, D.B., Weber, I.T. & Steitz, T.A. (1982) J. Biol. Chem. 257, 9518-9524.
2. Warwicker, J., Engelman, B.P. & Steitz, T.A. (1987) Proteins: Structure, Function, and Genetics 2, 283-289.

E 135 AN ADDITIONAL SP1 TRANSCRIPTION FACTOR BINDING SITE IN A SIMPLE PROMOTER INCREASES E1A DEPENDANT TRANSCRIPTION AND OVERCOMES SPACING CONSTRAINTS AND TATA ELEMENT DEPENDANCE, Robert Segal[1] and Arnold J Berk[2], Department of Medicine[1] and Department of Microbiology, Molecular Biology Institute[2], University of California at Los Angeles, Los Angeles, Ca 90024

In the simple adenovirus 2 Early 1B (E1B) promoter, moving the single Sp1 transcription factor binding site (GC-box) progressively futher upstream from the proximal TATA-box, progressively reduces transcription - inserting an additional 22 bases between the GC-box and TATA-box (30 base pair total separation), reduced transcription to the same degree as deleting the distal element (Wu and Berk, 1988). Such rigid spacing constraints are not observed in other more complex promoters which tolerate insertion of sequence between the several distal regulatory elements and the TATA-box. In order to understand the spatial constraints that effect the transcriptional regulation from these class II promoters in vivo, we examined the effect of placing two GC-boxes, 11 base pairs apart (i.e. in phase), at strategic distances from the TATA-box, in reconstructed mutant viral E1B promoters. Placing the two GC-boxes eight base pairs from TATA, resulted in increased transcription compared to the wild type promoter with a single GC-box at this position. The influence of the 2 GC-boxes fell off rapidly at first without a noticible phasic effect and remained constant until a 70 base pair separation. Comparison of selected mutants demonstated E1A responsiveness including a two GC-box-TATA-box minus mutant, suggesting an alternate path for E1A transactivation when the TATA-box is absent. Thus, we conclude that two Sp1 transcription factor binding sites increase transcription in a simple promoter, overcoming the spacing constraints of a single SP1 binding site and can be transactivated by E1A even in the absence of a TATA-box.

E 136 UNUSUAL DNA STRUCTURES IN THE METHYLATION OF DNA, Steven S. Smith, City of Hope, Shapiro Res. Bldg. Rm. S101, 1500 E. Duarte Rd., Duarte, CA 91010. Active genes are often unmethylated at certain CG sites. However, transfection experiments have shown that methylation of the majority of CG sites in a gene has no obvious effect on its activity. This coupled with the lack of general effect of DNA methylation on the binding of eukaryotic transcription factors, and the virtual absence of methylation from organisms like *Drosophila* and *Caenorhabditis*, suggests that any link between gene expression and DNA methylation is indirect. *De novo* methylation may be a consequence of the maintenance of the transcriptionally inactive state in some organisms. Compacted chromatin states may promote the formation of unusual DNA structures in inactive gene sequences. Data will be presented showing that the human DNA methyltransferase prefers substrates that adopt unusual DNA structures. The enzyme recognizes foldback structures, gapped structures and structures containing G4-DNA (putative chromosome pairing intermediates formed at G-rich motifs). These unusual DNA structures are methylated at *de novo* reaction rates that can be nearly forty-fold higher than sequences in normal B-DNA conformations. This important property of the enzyme is consistent with proposals for its mechanism of action. It suggests that the potential for the formation of unusual DNA structures during the chromosome cycle and the sexual cycle is linked to the evolution of DNA methylation and DNA methyltransferase specificity.

E 137 INHIBITION OF AP1 BY ADENOVIRUS E1A: A NOVEL FUNCTION FOR CR 1. H.Th.M. Timmers, R. Offringa, H. van Dam, A. Zantema, J.L. Bos, P. Herrlich[1] and A.J. van der Eb, Laboratory for Molecular Carcinogenesis, University of Leiden, P.O. Box 9503, 2300 RA Leiden, The Netherlands; [1]Kernforschungszentrum Karlsruhe, Institut fur Genetik und Toxikologie, P.O. Box 3640, D-7500 Karlsruhe, F.R.G.

Cells transformed by adenovirus E1A exhibit a reduced transcription of several mitogen-inducible genes (c-*myc*, JE, stromelysin, collagenase). Detailed analysis of the collagenase promoter shows that E1A repression is mediated via the AP1 binding site. Analysis of AP1 in E1A-transformed cells indicates that: (i) synthesis of AP1 constituents is not inhibited (c-*jun*, *jun*B and *fos*B are even activated), (ii) E1A represses trans-activation by FOS and by JUN proteins overexpressed from heterologous promoters, (iii) DNA binding activity of AP1 is not affected. Together these data suggest that E1A inhibits the trans-activating function of AP1. Since we cannot detect association between E1A and AP1 proteins, we are now focussing on differences in post-translational modification.
Mutation analysis of E1A shows that conserved region 1 (CR1) is essential for repression of these cellular genes. The fact, that CR1 is also required for transformation, strengthens the notion that E1A transforms cells by redirecting transcription of cellular genes.

E 138 A DISTANT ENHANCER IN THE REGULATION OF HUMAN β-LIKE GLOBIN GENES DURING ERYTHROPOIESIS, Dorothy Tuan, Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139 The human β-like globin gene family is arranged on the chromosome in the transcriptional orientation of 5' embryonic (ε) gene - fetal (Gr and Ar) genes adult (δ and β) genes 3'. The expression of these genes is normally restricted to cells belonging to the erythroid lineage and undergoes a programed switching mechanism according to the developmental stages of the erythroid cell. By DNase I hypersensitivity mapping and the CAT assays, we have identified an erythroid specific enhancer element at -11 Kb 5' of the ε gene and thus at -54 Kb 5' of the adult β gene. It is capable of enhancing the expression of a cis-linked CAT gene by up to 300-fold and appears to act in a developmental-stage independent fashion. This apparent developmental independence, coupled with DNA deletion mappings in γδβ-thalassemic patients suggests that this enhancer element may be the master switch for transcriptional activation of the whole β-like globin gene locus. How the enhancer element exerts its regulatory activity over 54 Kb of DNA and across 5 β-like globin genes is a question we attempt to address. By constructing recombinant clones containing the enhancer element located at various distances to a cis-linked test gene and studying their in vivo RNA transcription, we have obtained results which suggest that the distant enhancer element may interact with the cis-linked promoter by a tracking mechanism of the transcriptional apparatus in addition to the possibility of loop formation.

E 139 INVESTIGATIONS INTO YEAST α2 INTERACTIONS WITH MCM1 AND a1 PROTEINS , Andrew K. Vershon and Alexander D. Johnson, University of Calfornia-San Francisco

The yeast α2 repressor works in combination with the MCM1 and a1 proteins to repress transcription of a-specific and haploid-specific genes, respectively. Both α2 and a1 have homology to the homeodomain, while MCM1 has strong homology in the DNA binding domain to the mammalian transcription factor SRF. All three proteins can bind DNA alone, but interact cooperatively to bind to their respective operators. We have purified intact and several truncated fragments of the α2 protein to examine which regions of the protein interact with MCM1 and a1. We are currently isolating point mutations in α2 that fail to repress one form of transcription but not the other and will examine their ability to bind cooperatively with MCM1 and a1 to their respective operators *in vitro*. These mutations should define the regions of interaction between α2 and these other proteins.

E 140 HIV-1 TRANSACTIVATOR PROTEIN AND TRANS-ACTIVATION RESPONSIVE RNA COMPLEX: SPECIFICITY, STOICHIOMETRY AND MACROMOLECULAR CONTACT, Kevin M. Weeks, Christophe Ampe, Steve C. Shultz, Thomas A. Steitz and Donald M. Crothers, Departments of Chemistry and Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

HIV-1 replication requires expression of the transactivator (tat) protein; genetic studies indicate that tat acts via the trans-activation responsive region (tar), a cis-acting sequence located between residues +1 and +58 in the 5' viral long terminal repeat. By employing a novel protein purification scheme which preserves the native conformation of tat and by extending quantitative gel retardation methods to protein-RNA interactions, we show that the transactivator interacts specifically with tar RNA. Native tat exhibits strong preference for tar RNA over tar DNA and binds unrelated RNA molecules with moderate affinity. In the absence of metal ions, tat binds as a monomer; at higher concentrations of protein, two tat monomers bind independently to tar. Data from studies chemically probing intermolecular contacts will also be presented.

E 141 DELETION MAPPING OF THE DNA BINDING DOMAIN IN A TRANSACTIVATING PROTEIN ENCODED BY HERPES SIMPLEX VIRUS, Kent W. Wilcox and Chin Lee Wu, Department of Microbiology, Medical College of Wisconsin, Milwaukee, WI 53226. The HSV-1 immediate early protein ICP4 represses transcription by binding to specific sites in the promoters of at least two immediate early viral genes. The DNA binding capability of ICP4 is also required for activation of transcription from promoters associated with early and late viral genes. We have expressed a series of ICP4 deletion mutants in E. coli as trpE fusion proteins. Cell extracts containing these trpE:ICP4 fusion proteins have been tested for DNA binding activity by gel mobility shift assays and DNase I protection experiments. The results indicate that codons 262 to 490 are sufficient to encode a DNA binding protein with the same sequence specificity as native ICP4. Expression of recombinant genes containing codons 275 to 490 or 262 to 464 yielded polypeptides with greatly reduced DNA binding capability.

E 142 A NEW METHOD FOR DETECTING DNA LOOPING *in vivo*, Hai-Young Wu, Hyeon-Sook Koo and Leroy F. Liu, Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205. DNA loop formation by transcription factors has been suggested to be a principal mechanism for transcription regulation. We have developed an *in vivo* method to detect DNA looping induced by protein binding. This method is based on predictions from the recently proposed twin-supercoiled domain model of RNA transcription. Dimeric plasmid DNA, which contains two protein binding sites, can separate the DNA into two equal sized topological loops due to protein-protein interactions. The transcription within each loop on a dimeric plasmid causes enhanced accumulation of positive supercoils ahead of and negative supercoils behind the RNA polymerase relative to that on a monomeric plasmid DNA. Therefore, when positive supercoils on a plasmid DNA are assayed for in bacteria by the inactivation of DNA gyrase, dimer molecules are seen to have a greater accumulation of positive supercoils than monomer molecules. This "dimer effect" was observed on plasmid DNAs containing a 21 bp essential lac repressor binding sequence in bacterial cells expressing the lac repressor. The analysis of the "dimer effect" on plasmid DNA supercoiling is expected to be generally applicable to aid studies of DNA looping *in vivo*.

Transcription in Microbes and Lower Eukaryotes; The Basic Features of Eukaryotic Transcription-I

E 200 THE REGULATION OF EXPRESSION OF YEAST ACID PHOSPHATASE GENES, Shi-Zhou Ao, Shanghai Institute of Biochemistry, Academia Sinica, Shanghai 200031, China.

PHO3, PHO5 and PHO11 genes of the yeast acid phosphatase family were cloned and analyzed by DNA sequencing. Homologous regions were found in the 5'-flanking sequence of these genes. Expression of these genes with respect to the concentration of inorganic phosphate (Pi) is differential. PHO5 and PHO11 genes are inducible by low Pi, whereas PHO3 gene remains essentially unaffected. The 5'-flanking regions of PHO5 and PHO11 genes were studied by deletions. The results showed that upstream activation sites (UAS) of these genes share high homology in nucleotide sequence. A series of oligonucleotides corresponding to these UASs has been synthesized and tested to control PHO5 gene expression. A 50-bp oligonucleotide can compete with UAS in promoting gene expression by low Pi. The interaction between UASs of PHO5 or PHO11 genes and protein factors was analyzed. A specific protein binding to UASs of these genes was detected in yeast cell extracts by gel retardation assay. The effects of the concentration of Pi on the UAS binding activity were studied. The pattern of protection from DNase I by this factor was determined. We are developing a yeast RNA polymerase II transcription system in vitro to study the interaction between protein and protein, protein and DNA in the transcription initiation at acid phosphatase genes.

E 201 **PURIFICATION AND TRANSCRIPTIONAL STUDIES OF HAP1 A YEAST TRANSCRIPTIONAL ACTIVATOR**, Olivia Bermingham-McDonogh and Leonard Guarente, Department of Biology, Massachusetts Institute of Technology, Cambridge, Ma 02139

HAP1 a 1482 amino acid protein is a transcriptional activator of both CYC1 and CYC7. Previous studies have shown that the protein can be divided into an N-terminal DNA binding domain, a heme responsive domain and a C-terminal acidic activation domain. HAP1 binds with similar affinity to two dissimilar sequences *in vitro*: a site in the UAS of CYC7 and UAS1 of CYC1. The transcriptional activity at the CYC1 site *in vivo* is much greater than at CYC7. In addition a mutation of HAP1 which changes a single amino acid in the DNA binding domain called HAP1-18 increases the level of transcription from the CYC7 gene. We are purifying both HAP1 and HAP1-18 in order to study the activation properties of these proteins *in vitro*. Our approach to the purfication is as follows: the proteins can be overproduced in yeast approximately 1000x, using these extracts as starting material the proteins are purified over heparin-agarose and a DNA affinity column. In order to increse our yields of protein we are also utilizing the T7 system for production of these proteins in *E.coli*.

E 202 CLONING OF HUMAN TRANSCRIPTION FACTORS BY COMPLEMENTATION IN YEAST, Daniel M. Becker and Leonard Guarente, Department of Biology, MIT, Cambridge, MA 02139. The astonishing degree of functional conservation in the transcriptional machinery of eukaryotes should allow the identification of genes encoding human transcription factors by their ability, when expressed in yeast, to complement mutations in the corresponding yeast proteins. We have constructed a cDNA library from HeLa cells in a new high efficiency yeast expression vector. We are transforming this library into yeast strains carrying mutations in components of the yeast CCAAT-binding complex, HAP2/3/4, and will screen for human clones that can correct the mutant phenotype. These clones will encode the subunits of the heteromeric human transcriptional activator CP1. We are also screening the library for clones that can complement the spt15 mutation in the yeast TATA factor TFIID.

E 203 GRF2, AN ABUNDANT YEAST PROTEIN WHICH BINDS TO SEQUENCES IN UAS'S, CENTROMERES, AND TELOMERES HAS INTRINSIC UAS FUNCTION. Daniel I. Chasman, Neal F. Lue, Yahli Lorch, Andrew R. Buchman, Janice W. LaPointe, and Roger D. Kornberg. Department of Cell Biology, Beckman Laboritories for Structural Biology, Stanford University School of Medicine, Stanford CA 94305. Previous studies of the organization of the chromatin of the GAL1-GAL10 intergenic region in S. cerevisiae, identified the importance of the protein GRF2 (for General Regulatory Factor 2, formerly factor Y) binding to the DNA in this region for the establishment of discrete positioning of nucleosomes. By deriving a consensus binding sequence for GRF2 and searching the yeast data base for homologous sequences, we have found additional binding sites for GRF2 throughout the yeast genome, in the UAS's of a number of polymerase II transcribed genes, in the ribosomal RNA enhancer and promoter, in telomeres, and in centromeres. GRF2, an abundant DNA binding protein, has been purified and found to have a molecular weight of 127kD by denaturing gel electrophoresis. GRF2 binding sites have moderate stimulatory effects on transcription in vivo when positioned upstream of a CYC1 promoter-LacZ fusion gene. When combined with a polythymidine UAS sequence, GRF2 binding sites have large, synergistic, stimulatory effects on transcription in vivo.

E 204 MOLECULAR ANALYSIS OF A DOMINANT CONSTITUTIVE ALLELE OF THE YEAST PHO4 GENE, Caretha L. Creasy and Lawrence W. Bergman, Department of Chemistry, Ohio University, Athens, OH 45701
The product of the PHO4 gene is involved in the transcriptional derepression of the phosphate-repressible acid phosphatase gene (PHO5). Furthermore, it has been proposed that the interaction of the PHO4 gene product and the PHO80 gene product is responsible for the repression of acid phosphatase transcription. To investigate this interaction, we have randomly mutagenized a centromeric plasmid containing the PHO4 gene and subsequently transformed a yeast strain containing a disruption of the genomic PHO4 gene and a plasmid where the expression of the HIS3 gene is directed by the PHO5 promoter. This has allowed the selection of mutants which constitutively express acid phosphatase. Subsequent experiments have demonstrated that the mutation is dominant and linked to the plasmid-borne PHO4 gene. Molecular analysis of the PHO4 gene will be reported.

E 205 THE ROLES OF DNA REPLICATION AND DNA SUPERCOILING IN GENE ACTIVATION, M.E. Cullen, M.W. Leonard, A.C. Wilson, A.C.Brewer and R.K. Patient, Department of Biophysics, King's College, University of London, LONDON WC2B 5RL, U.K.

When transiently transfected into a number of different mammalian cells lines, we find that enhancement of various vertebrate and viral genes is stimulated by DNA replication. The effect is not merely the consequence of increased template copy number nor of gross chromatin assembly. We are attempting to relate these results to the observed preferential replication early in S-phase of active genes and the reprogramming of viral gene expression after template replication. We are currently transfecting synchronised cell populations to determine if the magnitude of the stimulation, which varies between transfection methods, is related to the point in the cell cycle when transfected templates can enter the cell nucleus. Nuclear extracts from synchronised cells are being used to determine if critical transcription factors vary in a cell cycle-dependent manner. In this way, we hope to test the hypothesis that replication may act by disassembling inactive transcription complexes, thereby allowing reprogramming of gene expression.

DNA supercoiling facilitates strand separation and thereby transcription, both in vitro and in vivo. Although the bulk of DNA in chromatin does not appear to be under torsional stress, the existence of localised, possibly transient, supercoiling is harder to test. To address this question, we have made use of the extreme sensitivity to superhelical stress of the $(A\text{-}T)_n$ tract upstream of the Xenopus β globin gene, which adopts cruciform geometry when supercoiled. For templates transfected into HeLa cells or microinjected into Xenopus oocyte nuclei, we find that the active gene is indeed under torsional stress, as demonstrated by $(A\text{-}T)_n$ cruciform extrusion, whereas no extrusion is detected for the inactive gene. This is not merely a consequence of transcriptional elongation, because blockage of RNA polymerase translocation by coinjection of α-amanitin into oocytes does not prevent formation of the supercoil-dependent cruciform. Furthermore, in HeLa cells cruciform extrusion can be promoted by the SV40 enhancer in cis, even if transcription is blocked. Thus, it appears that the transcriptionally poised but non-transcribing template is under torsional stress. We will discuss possible sources of such supercoiling and the implications these have for the mechanism of enhancement.

E 206 IDENTIFICATION OF A MUTATION WHICH ACTIVATES TRANSCRIPTION OF PHEROMONE-RESPONSIVE GENES IN *SACCHAROMYCES CEREVISIAE*, Jennifer L. Davis and Jeremy Thorner, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720. Transcription of genes encoding mating functions is induced by exposure of cells of the yeast *Saccharomyces cerevisiae* to peptide pheromones. Induction is mediated through a *cis*-acting DNA sequence, termed the pheromone-response element (PRE), found upstream of all pheromone-inducible genes. Proteins involved in this regulation are likely to include factors that bind specifically to the PRE. Two such factors have been identified: the product of the *STE*12 gene (Dolan et al., PNAS 86: 5703-5707, 1989) and, a distinct protein called PRE-binding factor (PBF) (F. Gimble and J.Thorner, unpub.). The mechanism by which each of these proteins contributes to the control of expression of pheromone-responsive genes is not known. To understand the role of these factors, and their interaction with each other and with components of the general transcription apparatus, we employed a genetic screen to identify mutants constitutive for transcription of pheromone-responsive genes. A *ste*12Δ strain carrying a plasmid in which PREs were inserted in place of the UAS in a *CYC*1 promoter-*lacZ* fusion was mutagenized with EMS and survivors were screened for blue color on X-gal plates in the absence of pheromone. In this way, a mutation (*mot*1, for modifier of transcription) was obtained. Experiments employing various UAS-promoter-*lacZ* fusions, *SUC*2 gene fusions, a-factor and α-factor halo assays, and hybridization analysis of poly(A)+ RNA indicate that in *mot*1 cells pheromone-responsive genes are activated, but expression of other unrelated genes is reduced. The TATA box appears to be important for the activation observed in *mot*1 cells, but may not be the sole determinant of this effect. The *mot*1 mutation is recessive and not allelic to any of the *SIT*genes (Arndt et al., Cell 56: 527-537, 1989). Because *mot*1 cells display slow growth at 30°C and are inviable at 37°C, we are attempting to clone the *MOT*1 gene by complementation of these growth phenotypes.

E 207 MOLECULAR CHARACTERIZATION OF A SUPPRESSOR OF THERMOSENSITIVE MUTATIONS IN THE LARGEST SUBUNIT OF RNA POLYMERASE I AND II, M.A. Drebot[1], H. Himmelfarb[2] , M. Nomura[3], and J.D. Friesen[1], [1]Research Institute, Hospital for Sick Children, Toronto, Canada. [2]Dept. of Biochemistry and Molecular Genetics, Harvard University Cambridge, MA. [3]Department of Biological Chemistry, California College of Medicine, University of California, Irvine, CA. As part of a genetic analysis of Saccharomyces cerevisiae RNA polymerase II we report the cloning of a DNA fragment able to suppress the thermosensitive phenotype of mutations in the largest subunit of RNA polymerase I (RPA190) and II (RP021). Both temperature-sensitive mutations (rpo21-1 and rpa190-1) reside within a putative zinc-finger domain that is shared between the two large subunits. The suppressing fragment complements the rpo21-1 and rpa190-1 phenotypes when present on a single copy plasmid but is unable to suppress other temperature-sensitive rpo21 mutants. The complementing portion of this fragment contains a truncated open reading frame corresponding to the 3' end of a gene we have named SUP131. We have isolated the full-length SUP131 gene and have shown that the amino-terminal end not present in the suppressor fragment has a glutamine rich region similar to that present in HAP1. Further characterization of the cross-suppression phenotype of Δ SUP131 will be presented.

E 208 INTERFERENCE BETWEEN ADJACENT POL II AND POL III PROMOTERS IN *SACCHAROMYCES CEREVISIAE*, David Engelke*, Melissa Hull*, Mark Johnston+ and James Erickson+, *Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109-0606, +Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110

Promoter interference between pol II and repetitive pol III transcription units has been implicated in the regulation of a number of genes in higher eukaryotes. By juxtaposing tRNA genes with different upstream activator sequences (UASs) in *S. cerevisiae*, we have found that the tRNA promoter eliminates function of the UAS when inserted upstream as much as 120 base pairs. Interference is not dependent on the orientation of the tRNA gene and is seen with combinations of two different tRNA genes (SUP4 and SUP53) and two UASs (UAS_G and UAS_{CUP1}). Point mutations in either tRNA internal promoter eliminate repression of the UAS, confirming that the tRNA promoter itself is responsible. Chromosomal footprinting shows that this phenomenon is unlikely to be caused by direct steric interference between tRNA transcription complexes and UAS binding proteins, however. Investigations of the mechanism of this indirect interference and a discussion of its possible role in transcriptional regulation are presented.

E 209 GENETIC ANALYSIS OF THE UNUSUAL TRANSCRIPTION REGULATORY REGION OF A Ty2 ELEMENT OF YEAST, Philip J. Farabaugh, Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21228

Regulation of transcription initiation of members of the Ty2 family of retroviral-like transposons of the yeast *Saccharomyces cerevisiae* is unusual in several respects. The terminal repeat region of a Ty2 element includes a UAS which is unlike canonical UASs in that it is not sufficient to stimulate transcription. It is however required in concert with sites located within the transcribed region to stimulate maximal transcription. The downstream sites are unusual in yeast first in their location; UASs have been shown not to function within the transcribed region, making the Ty sites unlike a canonical UAS. The downstream sites are complex and occupy a region of approximately 400 bp. The downstream regulatory sites include sites having either a positive or a negative effect on transcription from the Ty2 promoter. The positively acting and negatively acting regions are like higher eukaryotic enhancers and silencers in that they function both within and without the transcription unit. The enhancer site acts when present in either orientation. We have been unable to demonstrate orientation independence for the silencerlike site, suggesting that it may be either an unusual silencer or an example of a novel type of site. In addition the silencerlike site confers a highly unusual phenotype; expression of constructions including the silencer is not proportional to plasmid copy-number. This means that the concentration of template is not rate-limiting *in vivo* for these constructions. The implication of this observation for the mechanism of action of the site will be discussed.

E 210 FRACTIONATION OF A YEAST NUCLEAR EXTRACT THAT SUPPORTS ACCURATE INITIATION BY RNA POLYMERASE II. Peter M. Flanagan, William J. Feaver, Raymond J. Kelleher III, Neal F. Lue, Michael H. Sayre, Opher Gileadi, Janice W. LaPointe and Roger D. Kornberg, Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305.

A yeast nuclear extract has been resolved into five fractions that are required in addition to RNA polymerase II for accurate initiation of transcription. One of these fractions can be replaced by purified yeast TFIID. Another fraction becomes dispensible when the remaining ones are further purified. The remaining fractions can be derived from a yeast whole cell extract, providing a convenient source of material for their purification to homogeneity. Properties of the reconstituted transcription system will be presented.

E 27 GFI AND GFII ARE ABUNDANT MULTI-FUNCTIONAL DNA-BINDING PROTEINS THAT MAY MEDIATE GENERALIZED GROWTH CONTROL OF MITOCHONDRIAL BIOGENESIS IN YEAST. L.A. Grivell, J.C. D W.C. van Heeswijk and

J.H. de Winde, Section for Molecular Biology, Department of Molecular Cell Biology, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands.

GFI and GFII are two abundant DNA-binding proteins in *S. cerevisiae* (1,2). These factors bind to the 5'-flanks of many nuclear genes coding for mitochondrial proteins, to many other genes involved in processes essential to cell growth and to genetic elements important for cell division (ARS, CEN). The factors, or proteins closely resembling them, have been independently observed by others and have been variously named SBF-B, ABF1, TAF and SUF (GFI) and CP1/CBP1 (GFII). GFI, like another abundant DNA-binding protein, designated RAP1, TUF or GRF1 can act as an activator or repressor of transcription. As yet, little is known of the effects of GFII on transcription, although a role as activator seems likely. Both GFI and GFII may form part of a regulatory circuit that links the rate of cell growth to a number of essential cellular processes, including mitochondrial biogenesis, ribosome biosynthesis and DNA replication.

We have determined dissociation rates for several complexes between GFI and various binding sites in the yeast genome and found them to vary over a 70-fold range. Strong binding sites for GFI are present in the upstream activating sequences of the COR2 gene encoding the 40 kDa subunit II of the QH2:cytochrome c reductase, the gene encoding ribosomal protein S33 and in the intron of the actin gene. The binding site in the ARS1-TRP1 region is of intermediate strength. All strong binding sites conform to the sequence 5'-RTCRYYYNNNACG-3'. Modification interference experiments and studies with mutant binding sites indicate that critical bases for GFI recognition are within the two elements of the consensus DNA recognition sequence.

To get insight into the roles played by GFI and GFII in mitochondrial biogenesis, we have constructed a mutant form of the COR8 gene, lacking the (overlapping) binding sites for both factors, and have studied the transcriptional activity of this in relation to growth on different carbon sources. The mutant construct still responds to carbon source control, but displays a reduced level of transcription, consistent with the idea that GFI and GFII act synergistically with other regulatory factors to control gene expression.

References

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The *Autographa californica* nuclear polyhedrosis virus (AcNPV) gene, IE-N has been functionally mapped in the viral genome by transient expression assays in Spodoptera frugiperda cell culture. By its temporal expression during the viral life cycle and its transient expression in the absence of viral protein translation, IE-N is an immediate early gene. IE-N mRNA is highly expressed 0-3 hpi and undetectable after 6-8 hpi. To further study the regulation and function of IE-N, the nucleotide sequence of 2 kb of the PstI-N AcMNPV fragment encoding IE-N was determined and the sequence similarity to other AcMNPV viral genes was calculated to identify possible viral regulatory motifs. Subsequently, IE-N-CAT (NCAT) constructs were analyzed by transient expression assays to determine both cis- and trans-acting viral factors influencing IE-N expression. We show that the viral enhancer hr1 cis linked to NCAT stimulates expression in the presence of host cellular factors. Functional IE-N further stimulates the hr1-NCAT construct in trans as well as NCAT alone. This suggests that IE-N auto-regulation is mediated by promoter sequences rather than the hr1 enhancer. Down regulation by another immediate early AcMNPV gene, IE-1, is shown to be mediated only by hr1 sequences. Thus, transient expression assays have been utilized to determine viral factors which may be involved in gene regulation of the AcMNPV gene, IE-N, during early viral infection.

E 214 CHARACTERIZATION OF A NEGATIVE UPSTREAM REGULATORY ELEMENT WHICH MODULATES REPRESSION OF TRANSCRIPTION OF THE YEAST ENOLASE GENE *ENO1*, Michael Holland, Andrew Carmen, and Alan Pepper, Dept. of Biological Chemistry, University of California, Davis, CA 95616. Transcription of the yeast enolase gene *ENO1* is repressed approximately 20-fold in cells grown on a medium containing glucose as carbon source. Sequences which modulate repression of transcription (URS element) were mapped between positions -212 and -143 relative to the *ENO1* transcriptional initiation site (+1). Deletion mutations which remove all or portions of this URS element abolish repression of *ENO1* transcription. A factor (URS binding protein) binds specifically to a site near the 5' terminus of the URS element and makes specific contacts with nucleotides extending over the entire URS element. This factor has been partially purified and characterized. Insertion of the URS element between the upstream activation sites and TATAAA element of the enolase gene *ENO2* does not cause repression of *ENO2* transcription. When one of the two upstream activation sites (UAS elements) in *ENO2* is deleted, however, expression of an *ENO2* gene containing a URS element is repressed 20-fold in cells grown in a medium containing glucose. These data suggest that URS activity is highly dependent on the nature of the UAS element that activates transcription of *ENO2*. A novel dominant selection was developed for isolating mutants defective in URS activity. The isolated mutants were placed into three complementation groups, designated *snr1*, *snr2*, and *snr3* (suppressor of negative regulation). The basal level of transcription of the *ENO1* and *SSA1* genes was elevated 5 to 20-fold in strains carrying the *snr* mutations. The *SSA1* gene (encodes the major yeast hsp70 protein) contains a URS element which is structurally very similar to the *ENO1* URS element. We suggest that the URS element is a general regulatory element which modulates repression of transcription of many yeast genes.

E 215 **IDENTIFICATION OF A DNA ELEMENT REQUIRED FOR BASAL LEVEL (GCN4-INDEPENDENT) TRANSCRIPTION OF THE ILV1 GENE OF YEAST.** S.Holmberg, J.Remacle*, M.C.Kielland-Brandt*, J.G.L.Petersen* and T.Nilsson-Tillgren, Inst. of Genetics, University of Copenhagen and *Dept. of Yeast Genetics, Carlsberg Laboratory, DK-2500 Copenhagen, Denmark. The ILV1 gene of Saccharomyces cerevisiae encodes threonine deaminase cata-lyzing the first step in isoleucine biosynthesis. The only known regulation of ILV1 is by general control. The promoter contains 5 sequences related to the GCN4 protein binding element TGACTC, one of which binds GCN4 protein tightly in vitro. In a yeast strain carrying a deletion of GCN4 the basal level of ILV1 transcription is largely unaffected. A GC-rich element (between -192 and -172 relative to the ATG) essential for basal level expression has been identified by functional analysis of deletion mutants made in vitro. When this "basal element" is deleted, a 30-fold reduction in expression is seen, even in a $GCN4^{+}$ strain. Gel mobility shift assays using a crude nuclear extract have identified a protein which specifically interacts with the ILV1 "basal element". The element was tested for UAS activity by fusing 1, 2 or 3 copies to the downstream element of the CYC1 promoter. In no case was any activation of the CYC1 promoter segment obtained. As a functional TATA-like sequence is lacking in the promoter we hypothesize that the ILV1 "basal element" is involved in the binding of a rather general transcription factor like a TATA-binding factor. The latter possibility is presently being tested by placing the element in TATA-probe chimeric promoters.

E 216 DICTYOSTELIUM DISCOIDEUM ACTIN EXPRESSION: *CIS* ELEMENTS AND INTERACTING *TRANS* FACTOR(S). Roderick T. Hori, and Richard A. Firtel, Department of Biology, Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093.

The Actin 6 and Actin 15 genes are both expressed in vegetative cells and are induced to maximum expression 2-4 hours after the onset of development. 5' deletion analysis has shown that a common *cis* element ($AAA^{A}/_{T}TGGG^{A}/_{T}TT$), designated ARE, is required for the vegetative expression of both genes (Cohen, et. al. EMBO J., 5, 3361. Nellen, et. al. EMBO J, 5, 3367.). Extracts from vegetative cells contain an activity that binds to an Actin 15 fragment containing the ARE. Competition experiments suggest the ARE is involved in the interaction. Upstream fragments from several vegetatively expressed genes, including Actin 6, compete against the Actin 15 fragment for this binding activity. However upstream regions from two genes expressed at later developmental times fail to compete. We are attempting to delineate the DNA binding site(s) and compare the levels of this binding activity at different developmental times. In addition, we are identifying the *cis* elements responsible for mediating the developmental induction of expression.

E 217 MUTATIONAL ANALYSIS OF THE BACULOVIRUS, ACMNPV, TRANSCRIPTIONAL REGULATOR, IE1: ANALYSIS OF *TRANS*-DOMINANT MUTANTS WHICH SUPPRESS *TRANS*-ACTIVATION OF DELAYED EARLY GENES, Gerald R. Kovacs[1], Linda A. Guarino[2], Max D. Summers[1,2], Departments of Biochemistry[1] and Entomology[2], Texas A & M University, College Station, TX 77843.
A major transcriptional activator of the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcMNPV), is the immediate early gene product IE1. IE1 is required for the expression of several delayed early and late AcMNPV genes in transient expression assays (enhancer-independent activity). IE1 is also essential for the *trans*-activation of genes *cis*-linked to the AcMNPV hr enhancer elements (enhancer-dependent activity). We have recently found an IE1-specific hr enhancer binding activity in *Spodoptera frugiperda* cells transfected with an IE1-expressing plasmid. In order to further our studies on the molecular basis of IE1 activity, we have chosen methods of in vitro mutagenesis to delete terminal and internal regions of the IE1 open reading frame. IE1 mutant genes were tested in transient cotransfection assays that measured the *trans*-activation of the delayed early reporter construct 39CAT; in addition, they were tested for IE1-specific hr enhancer binding activity. Our data show that a a 25 amino acid domain at the IE1 C-terminus is required for enhancer-independent and -dependent *trans*-activation of 39CAT, and is also essential for IE1-specific hr enhancer binding activity. A second domain, N-terminal to the *trans*-activating domain, was found to interfere with native IE1 *trans*-activation activity. That is, expression of the C-terminally deleted IE1 abrogated the ability of native IE1 from *trans*-activating the 39CAT reporter gene.

E 218 INTERACTION OF POSITIVE AND NEGATIVE REGULATORY PROTEINS WITH THE PROMOTER OF THE INO1 GENE OF Saccharomyces cerevisiae: EFFECTS OF RNA Pol II MUTATIONS, John M. Lopes and Susan A. Henry, Dept. of Biological Sciences, Carnegie Mellon Univ., Pittsburgh, PA 15213, and Charles Scafe and Richard A. Young, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142.
In yeast, the initial step in the synthesis of the membrane lipid phosphatidyl inositol is catalyzed by the product of the INO1 gene. Transcription of this gene is significantly reduced when cells are grown in the presence of the soluble precursors, inositol and choline. Mutations have been isolated that cause either constitutive derepressed expression (opi1) or absence of expression (ino2 and ino4). Sequence analysis of the INO1 promoter, and other genes encoding phospholipid biosynthetic enzymes, revealed the presence of a highly conserved and repeated 9 basepair element. Mobility shift and footprint assays suggest that the INO2 and INO4 gene products interact with the promoter as a heteromeric complex. The OPI1 product also interacts with the promoter but requires the formation of a 9-mer specific complex involving a genetically unidentified factor. Deletions analysis of the INO1 promoter demonstrated the existence of regions required for repression and activation of transcription upstream of the TATA box. In addition, these deletions identified a "constitutive promoter" element located **downstream** of the TATA box. The effects of RNA Pol II large subunit C-terminal deletions on transcription from the INO1 promoter deletions suggests an interaction between the C-terminus of this RNA Pol II subunit and specific regulatory proteins that bind upstream of the INO1 TATA box.

E 219 THE REGULATION OF MALTOSE FERMENTATION IN SACCHAROMYCES: ANALYSIS OF THE CRYPTIC NATURE OF THE MAL ACTIVATOR ENCODED BY MAL64, Corinne A. Michels, Lori A. Young, Departments of Biology and Biochemistry, Queens College and the Graduate School of CUNY, Flushing, NY 11367. The MAL6 locus is a cluster of four genes encoding maltose permease (the MAL61 gene), maltase (the MAL62 gene), the MAL activator required for maltose inducible transcription of the MAL61 and MAL62 genes (the MAL63 gene), and a cryptic MAL activator (the MAL64 gene). The MAL64 gene was demonstrated to exist when maltose fermenting revertants of mal63 non-inducible mutants were shown to carry mutations in a gene linked to, but distinct from, the MAL63 gene. Interestingly, these revertants constitutively expressed maltose permease and maltase. The MAL64 gene is 85% sequence homologous to MAL63 within the coding region yet the wild-type MAL64 gene is unable to complement mal63 mutations. In this report we explore the basis of the cryptic nature of MAL64 by constructing a series of MAL63/MAL64 hybrid genes. The results of these experiments in conjunction with the sequence of the MAL64-constitutive alleles allow us to conclude that the C-terminal region of the MAL64 protein functions as an inactivation domain incapable of responding to maltose induction.

E 220 TRANS-REGULATORY PROTEINS OF VARICELLA ZOSTER VIRUS (VZV) AND THEIR INTERACTION WITH c-jun AND c-fos. Sunil Nagpal & Jeffrey M. Ostrove. Medical Virology Section, LCI,NIAID,NIH, Bethesda, MD 20892. VZV open reading frames (ORFs) 4 and 62 are putative regulatory immediate-early (IE) genes that encode trans-activating proteins. In co-transfection assays using CAT reporter gene, ORF4 and ORF62 stimulated the expression of viral IE, early (E) and late (L) promoters. They act synergistically at the level of transcription. We have also mapped a VZV "trans-repressor" to ORF61. This is the HSV-1 ICPO (a trans-activator) homolog, as defined by gene location and the sharing of a cystein-rich putative zinc binding finger in the amino-terminal region. ORF61 inhibits the ORF4 and ORF62-meditated trans-activation of VZV IE, E and L promoters. ORF61 functions as a "transcriptional repressor" in inhibiting the increase in the steady-state RNA induced by trans-activators. ORF61 trans-repression appears to be specific for VZV, since it had no effect on SV-40 and Rous sarcoma virus promoters and did not inhibit the trans-activation of HTLV-1 LTR and HIV-LTR by tax and tat genes, respectively. We constructed a series of ORF61 mutants and found that all those that interrupt the ORF (both insertions and deletions) no longer possess the trans-repressor ability. This was demonstrated at the protein level as well as at the level of steady-state RNA synthesis. Further, co-transfection experiments showed that the oncogenes c-jun and c-fos also inhibit VZV ORF4 and ORF62-induced trans-activation, but they had no effect on tat activation of HIV-LTR. ORF61, c-jun and c-fos share the property of containing a hydrophobic domain. To elucidate the mechanism of trans-repression, studies using mutants in ORF61, c-jun and c-fos as well as ORF4 are being conducted.

E 221 ISOLATION AND MOLECULAR ANALYSIS OF *INO2*, A POSITIVE REGULATOR OF PHOSPHOLIPID BIOSYNTHESIS, D. Michele Nikoloff and Susan A. Henry, Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213 and Deborah K. Hoshizaki, Department of Biological Chemistry, College of Medicine, University of Illinois at Chicago, Chicago, Illinois 60612

In the yeast *Saccharomyces cerevisiae*, the biogenesis of membranes is a highly controlled process. In wildtype yeast cells, cytoplasmic and membrane-associated phospholipid biosynthetic enzymes are coordinately regulated in response to the soluble precursors, inositol and choline. Two regulatory genes implicated in this process have recently been cloned, namely *INO4* and *INO2*. Sequence analysis of the *INO4* gene reveals a significant similarity with the helix-loop-helix region of the Myc family of proteins. Molecular and genetic analyses suggest that *INO2* and *INO4* may associate in a heteromeric complex to activate transcription of the structural genes encoding the phospholipid biosynthetic enzymes. In this study, we also report the cloning, molecular characterization and sequence analysis of the positive regulatory gene, *INO2*. Gene replacement with a disrupted *INO2::TRP1* allele indicates that *INO2* is not essential for viability. Strains carrying an *INO2* gene disruption give rise to an inositol auxotrophy but show no cold or heat sensitivity. We have undertaken a genetic and molecular approach to study the interaction between the regulatory gene products which govern the synthesis and assembly of phospholipids. To this end, we have isolated suppressors of an *ino2* mutant strain which restore wildtype regulation upon the *INO1* structural gene.

E 222 MECHANISM OF MATING DEFECT OF THE YEAST SACCHAROMYCES CEREVISIAE CAUSED BY gal11 MUTATION, Masafumi Nishizawa, Yuriko Suzuki, Kunihiro Matsumoto* and Toshio Fukasawa, Department of Microbiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan, and *DNAX, Palo Alto, CA 94304.

GAL11 was first identified as a gene required for full expression of GAL1, GAL7 and GAL10 genes of the yeast Saccharomyces cerevisiae and was thought to function downstream of GAL4 and GAL80. With △gal11 mutant, we found that loss of normal GAL11 function not only reduces expression level of GAL1, GAL7, and GAL10 genes but also causes defect in sporulation, mating and growth on nonfermentable carbon sources. We studied mechanism underlying mating defect and found that the defect is specific to α cells. In gal11 cells, expression level of a-specific gene (STE2) was similar to that in wild type cells whereas expression of α-specific genes (STE3, MFα1) was greatly reduced. Expression level of MATα locus was also low, suggesting that deficiency in a positive factor (MATα1) is responsible for reduced expression of α-specific genes and therefore renders α-specific sterility to gal11 cells. Effect of gal11 mutation on expression of other genes is now under study to clarify the possibility that GAL11 is a general regulator of transcription in yeast.

E 223 IDENTIFICATION AND FUNCTION OF YEAST PROTEINS THAT BIND TO THE **POLYOMA** ENHANCER. John F. Pulitzer, Maria Ciaramella, Vincenzo Rocco, Aurora Storlazzi and Alessandra Pollice. I.I.G.B., Naples 80125, Italy. We have shown that in the yeast **Saccharomyces cerevisiae** the **polyoma** virus enhancer potentiates transcription by productive interaction with autoctonous yeast promoter elements. We have now identified in DNA binding assays and deletion mapping studies at least three yeast proteins that interact with oligonucleotide subdomains in enhancers from either wild-type **polyoma** or host-range mutants. Two of these proteins appear to belong to the yAP1/GCN4 family and bind to the enhancer A-domain, while one is an abundant yeast protein that binds to the enhancer B-domain. The A-domain contains sequences essential for transcription in mouse fibroblasts and yeast cells, sequences of the B-domain have an auxillary role and augment the function of the A-domain. We have devised techniques to isolate in yeast mutants affecting A- and B-enhancer function and will describe the properties of some of these mutants.

E 224 CAPPING OF U6 SMALL NUCLEAR RNA IS DIRECTED BY A CONSERVED BIPARTITE RNA MOTIF: STEM-LOOP AND AUAUAC SEQUENCE, Ravinder Singh, Shashi Gupta and Ram Reddy, Department of Pharmacology, Baylor College of Medicine, Houston, TX 77030 U6 small nuclear RNA(snRNA), a component of eukaryotic spliceosomes, is required for splicing of nuclear pre-mRNAs. Whereas trimethylguanosine cap-containing U snRNAs are transcribed by RNA polymerase II, the U6 RNA, shown to contain a nonnucleotide cap, is transcribed by RNA polymerase III. We recently characterized the cap structure of human U6 snRNA and showed that 5' end of U6 RNA is blocked by γ-monomethyl phosphate (Singh and Reddy, 1989, Proc. Natl. Acad. Sci., USA, in press). This cap structure is distinct from other known RNA cap structures Furthermore, the information for capping of the U6 RNA resides within initial 25 nucleotides of the U6 snRNA. The capping determinant in mammalian U6 snRNA is a bipartite element - a phylogenetically conserved stem-loop structure and an AUAUAC sequence following this stem-loop. Wild type capping efficiency requires AUAUAC motif immediately after the stem-loop and the γ-phosphate of the initiation nucleotide in close proximity of the capping determinant. Incorporation of a synthetic stem-loop followed by an AUAUAC sequence is sufficient to convert a non-capped heterologous transcript into a capped transcript. Transcripts containing initial thirty two nucleotides of Saccharomyces cerevisiae U6 snRNA are accurately capped in HeLa cell extract indicating that the capping machinery is conserved among evolutionarily distant eukaryotes. U6 snRNA-specific capping is distinct in that capping is RNA sequence-dependent while the capping of mRNAs and other U-snRNAs is tightly coupled to transcription and independent of the RNA sequence.

E 225 THE YEAST SIN3/SDI1 GENE REGULATES DNA-BINDING ACTIVITIES VIA PROTEIN-PROTEIN INTERACTIONS, David J. Stillman and Huaming Wang, Dept. of Cellular, Viral and Molecular Biology, Univ. Utah Med. Center, Salt Lake City, UT 84132 The SIN3 (SDI1) gene was identified as a negative transcriptional regulator of the yeast HO gene, bypassing the requirement for the SWI5 activator (Cell48:579). A DNA-binding protein which binds to the HO promoter at a site adjacent to the SWI5 binding was identified. This DNA binding protein is absent in extracts prepared from SIN3 mutants, and we refer to this possible repressor protein as "R." We have now demonstrated that SIN3 does not encode R, and determined that SIN3 regulates R activity via an intermediate protein. An inhibitor "I" has been identified which prevents R from binding to DNA. We believe that the R activity is not detected in extracts prepared from SIN3 mutants because R is sequestered by I. A proteolytic activity is not detected in fractions containing I, and I does not inhibit a number of other yeast DNA-binding activities. When SIN3 protein is added to in vitro DNA-binding reactions containing R and I, the DNA-binding activity of R is restored. Neither the inhibition of DNA-binding by I, nor the stimulation by SIN3 appear to be catalytic. We propose that SIN3 binds to I, and that this this I-SIN3 complex prevents I from binding to R. SIN3 thus acts as an indirect stimulator of the in vitro DNA-binding activity of R. The proposed model is similar to that proposed for the inhibitor of NF-κB (Bauerle and Baltimore, Cell 53:211). The analogy with NF-κB can be extended since the protein dissociating agent formamide interferes with the inhibition of R activity by I, as was observed for NF-κB and its inhibitor.

E 226 THE FISSION YEAST *pap1*$^+$ GENE ENCODES AN AP-1-LIKE FACTOR THAT FUNCTIONALLY INTERACTS WITH A NOVEL PROTEIN KINASE, Takasi Toda, Mizuki Shimanuki and Mitsuhiro Yanagida, Departemnt of Biophysics, Faculty of Science, Kyoto University, Kyoto, 606, Japan.
Staurosporine, an alkaloid produced in *Streptomyces*, is known to have a very potent inhibitory effect on protein kinase C *in vitro*. We have chosen to use the fission yeast *S. pombe* as a model system to identify cellular target molecules which interact with the drug. During the course of these studies, we isolated two genes that confer drug resistance, when carried on a multicopy plasmid. Nucleotide sequence determination indicates that one gene codes for a novel protein kinase, designated *spk1*$^+$ (*s*taurosporine-related *p*rotein *k*inase) and the other gene, for an AP-1-like factor, designated *pap1*$^+$ (*p*ombe *AP*-1-like factor). Functional interactions between *pap1*$^+$ and *spk1*$^+$ are discussed in term of eukaryotic cell cycle regulation.

E 227 REB1: A DNA BINDING PROTEIN FOR ALL SEASONS,
Jonathan R. Warner, Bernice E. Morrow and Qida Ju, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461
We have identified a protein, termed REB1, that which binds to the sequence ---CCGGGTA--- that is present in both the enhancer and the promotor regions of the repeated ribosomal RNA genes of *Saccharomyces cerevisiae*. We have purified REB1 to homogeneity; it is a protein of 120kD. REB1 binds elsewhere in the genome of yeast, e.g., in front of several genes involved in mating type switching, (D. Stillman, pers. comm.) and within the *GAL1-10* UAS region. From competition experiments we conclude that REB1 is identical to the protein termed Y, that can play some role in the organization of nucleosomes.(Fedor et al,JMB,*204*,109,1988) We have cloned the gene for REB1 by the specific binding of oligonucleotides to the product of a λgt-11 construct. Partial sequence analysis revealed that REB1 has sequences similar to the DNA binding domain of the oncogene, *myb*. Gene disruption established that *REB1* is an essential gene. Polyclonal antibodies raised against the LacZ-REB1 fusion protein purified from the λ clone have identified a cross-reacting protein in both mouse and human cell lines. The function of REB1 is being explored by use of conditional expression and gene modification experiments.
Supported by NIGMS.

E 228 *E. COLI* RNA POLYMERASE HAS A SPECIFIC BINDING SITE FOR THE 3'-OH TERMINAL 33-BASE RNA, A-Young M. Woody, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523.
To understand the mechanistic aspects of the involvement of the stem-loop structure followed by a string of Us for rho-independent termination, we have examined the interaction between *E. coli* RNA polymerase and a 33-base RNA possessing all the features of the 3'-OH terminal transcript (33 W) and a 33-base mutant RNA with no possibility of stem-loop formation (33 M1). The interactions were analyzed by nitrocellulose filtration and confirmed by gel retardation. The concentration of RNA was varied from 10 nM to 500 nM at an enzyme concentration of 100 nM in 10 mM Tris-HCl, pH 8.0/10 mM $MgCl_2$/1 mM DTT/50 mM KCl. The data were analyzed under the assumption that the *E. coli* RNA polymerase interaction with the RNAs is a non-cooperative single-step association process. From the Scatchard plot of data corrected for the 50% retention efficiency in filtration, Kd for RNA polymerase, 33 W was ca. 28 nM and the number of ligands per bound enzyme about 1.0. Thus *E. coli* RNA polymerase has a specific binding site for the 33 W. For the mutant RNA, 33 M1, Kd was ca. 640 nM, and n>1 which suggests multiple binding. Direct UV photocrosslinking between *E. coli* RNA polymerase and ^{32}p-labeled 33 W shows that the ß' subunit is the sole target. Work is in progress on two other 33-base RNA mutants, one rich in pyrimidines and lacking the stem-loop possibility and one which can form a stem-loop configuration at the 5' end but has no string of Us at the 3'-OH terminus. Transcription termination on templates containing these sequences will be studied. Possible roles of the specific binding site for the 3'-OH terminal RNA configurations in rho-independent termination will be discussed. (Supported by USPH GM-23697)

E 229 EXPRESSION AND LARGE SCALE PURIFICATION OF BACTERIAL EXPRESSED YEAST TFIID.

Tohru Yamamoto, Masami Horikoshi and Robert G. Roeder. Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021

Biochemical analyses of eukaryotic transcription initiation has been restricted by the difficulty in preparing large amounts of homogeneous factors. Recent success in cloning of the TFIID (TATA box-binding protein) gene from Saccharomyces cerevisiae has made it possible to overcome this problem for the first time. Using the E.coli bacteriophage T7 expression system we have overexpressed the yeast TFIID in a transcriptionally active form. In addition milligram amounts of this protein were purified to near homogeneity using a series of conventional column chromatographies. Additional biochemical studies with this bacterially-expressed yeast TFIID will be presented.

E 300 REGULATION OF rDNA TRANSCRIPTION BY THE PROTEIN KINASE CKII IN MAMMALIAN CELLS, F. Amalric, V. Baldin, P. Belenguer, G. Bouche and M. Caizergues-Ferrer, Centre de Recherche de Biochimie et de Génétique Cellulaires du C.N.R.S., 118 route de Narbonne, 31062 Toulouse France. Subcellular distribution of protein kinase CKII is directly related to cell growth. In growing primary culture of endothelial cells (ABAE), the protein kinase was distributed throughout the cytoplasm and the nucleus and was found more particularly accumulated into the nucleolus. In confluent cells, a redistribution of the protein kinase CKII was observed by immunocytochemistry. These variation in nuclear CKII amount were confirmed by "in vitro" labeling of nucleolin a major nucleolar protein, used as substrate by CKII. In nuclei prepared from confluent cells, nucleolin was unlabelled by ATP γ^{32}P while in growing cells nucleolin was one of the major phosphorylated protein. Concomitantly, the "in vitro" transcription of rDNA in the corresponding nuclei was highly reduced by 85%. Addition of exogeneous CKII that has been purified from nuclei of growing cells, to nuclei prepared from confluent cells induced a strong increase in the phosphorylation of specific proteins and among them, nucleolin. Simultaneously, the transcription of rDNA was increased by a factor of 5. These data suggest that in confluent cells, factors necessary for rDNA transcription were present in the nucleolus but at least one of them was in an inactive unphosphorylated form.

E 301 POLY ADP-RIBOSYLATION OF CHROMOSOMAL PROTEINS PARTICIPATES IN THE MODULATION OF GENE EXPRESSION BY CLASTOGENIC CARCINOGENS, Paul Amstad, Girish Shah, Georg Krupitza, and Peter Cerutti, Department of Carcinogenesis, Swiss Institute for Experimental Cancer Research, 1066 Epalinges/Lausanne, Switzerland. Poly ADP-ribosylation of chromosomal proteins represents a post-translational modification which is triggered by clastogenic carcinogens. It establishes a unique link between "genotoxic" DNA damage leading to structural changes of DNA and transitory modulation of gene expression.

Following exposure to the clastogenic carcinogens active oxygen and N-methyl-N-nitro-N-nitrosoguanidine(MNNG) ADPR-transferase served as major poly ADPR-acceptor in mouse epidermal cells. Inactivation of ADPR-transferase may prevent excessive poly ADP-ribosylation. Topoisomerase I served as minor acceptor. Its inactivation might retard DNA replication and allow more time for DNA repair. Poly ADP-ribosylation also occurred at histones and, therefore, may modulate local chromatin conformation.

Active oxygen and MNNG induce the immediate early genes c-fos and c-myc. The participation of poly ADP-ribosylation in the induction mechanism is suggested since the inhibition of ADPR-transferase by benzamid suppressed the transcriptional induction of c-fos and the consecutive increase in FOS-protein. Poly ADP-ribosylation of FOS and other transcription factors may alter protein/protein and DNA/protein interactions which are regulating the expression of immediate early genes.

E 302 PURIFICATION OF TWO NUCLEAR PORCINE HISTONE ACETYLTRANSFERASES. L. Attisano and P. N. Lewis. Dept. of Biochemistry, University of Toronto, Toronto, Ontario, Canada, M5S 1A8

Histone hyperacetylation has been shown repeatedly to be a characteristic of transcriptionally competent chromatin. As a prelude to a functional evaluation of the role of histone acetyltransferases in the process of transcriptional activation we report here the purification to apparent homogeneity of two forms of porcine liver histone acetyltransferases (HAT I and HAT II). Histone acetyltransferase activity extracted from liver nuclei was subjected to sequential chromatography on a variety of supports. During chromatographic separations two differentially eluting enzyme activities were observed and subsequently purified. Both forms have a molecular weight of 110,000 (as determined by gel filtration) and a pI of 6.9. The amino acid composition, heat inactivation profiles and Michaelis constants with respect to both acetyl CoA and histones were indistinguishable. However, the apparent molecular weights of the HAT I and HAT II polypeptides differed by SDS-PAGE, affinity purified polyclonal antibodies to both forms of the enzyme did not cross-react, peptide maps were different and the turnover numbers differed by 15 fold. Probably, two distinct forms of nuclear histone acetyltransferase exist. On the basis of our recoveries we estimate that there is one enzyme molecule for every 500 nucleosomes.

E 303 TRANSCRIPTION OF A HUMAN 7S L RNA GENE IS UNDER CONTROL OF A GENE-INTERNAL AND A GENE-EXTERNAL PROMOTER ELEMENT. Bernd-J. Benecke, Sebastian Bredow and Hartmut Kleinert. Dept. of Biochemistry NC-6, Ruhr-University, D-463 Bochum, Fed.Rep.of Germany.

Class III genes (gene transcribed by RNA polymerase III) were originally defined as being under the control of gene-internal promoters. Recently, however, a human 7S K RNA gene as well as various U6 snRNA genes were found to be under the exclusive control of a gene-external promoter. The human 7S L RNA promoter analyzed here represents an intermediate promoter structure with one part of the control region being located inside the gene at the 5'end of the coding region. This element partly overlaps the A-box homologue (located at +5 to +14) but clearly is not identical with it, since essential parts of the A-box can be substituted without losing promoter activity. The gene-external part of the promoter (around -45) contains an ATF-binding site, well known from cAMP-responsive class II genes. This ATF-site is functional with respect to the activity of the 7S L transcription in-vivo and in-vitro. Transcription factors recognizing the two parts of the promoter and required for accurate and efficient 7S L transcription are different from those of other class III genes.

E 304 THE CHIMERIC ACTIVATOR GAL4-VP16 HAS DRAMATIC EFFECTS ON TRANSCRIPTION IN YEAST NUCLEAR EXTRACTS. Shelley Berger[1], Steven Triezenberg[2], Andrea Cress[2], Steven Hahn[3], and Leonard Guarente[1]; [1]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; [2]Department of Biochemistry, Michigan State University, East Lansing, MI 48824; [3]Fred Hutchinson Cancer Research Institute, Seattle, WA 98104

GAL4-VP16 is a chimeric transcriptional activator containing the yeast GAL4 DNA binding domain fused to the herpes virus VP16 activation domain. *E. coli*-expressed GAL4-VP16 strongly activates transcription in yeast nuclear extracts from a DNA template containing GAL4 binding sites upstream of the yeast CYC1 promoter. Increasing the amount of GAL4-VP16 activator in the reaction results in a peak of transcription followed by inhibition (first observed in Roger Kornberg's laboratory). Similar inhibition of transcription mediated by GAL4-VP16 occurs from a template containing a polyA-polyT upstream activator sequence (UAS) from the yeast DED1 promoter, even though GAL4-VP16 does not bind to this UAS and, thus, does not activate transcription. We are studying these effects on transcription *in vitro*, as well as the relationship of the *in vitro* inhibition to *in vivo* squelching by authentic yeast GAL4, as observed by others.

E 305 v-src GENE PRODUCTS AND SERUM REGULATE TGF-β1 mRNA TRANSCRIPTION IN THE 32-D MYELOID CELL LINES. M.C. Birchenall-Roberts[1], J. Kasper[2], and F. W. Ruscetti[3]. [1,3] LMI, BRMP-NCI, [2] BCDP, PRI-NCI, Frederick, Maryland 21701

TGF-β_1 inhibits the proliferation of a variety of cell lines that also produce TGF-β_1 mRNA and protein during growth, suggesting feedback autocrine growth inhibition. Growth factor-independent 32-D-src and 32-D-abl cell lines sensitive to TGF-β_1 inhibition were established by infecting IL-3-dependent myeloid precursor cells with retroviruses containing the src or abl oncogene. Using Northern blot analysis, we have studied the regulation by serum of TGF-β_1 mRNA transcription in these cell lines. In IL-3-dependent and 32-D-abl cell lines deprived of serum, serum treatment stimulated higher levels of TGF-β_1 mRNA. Conversely, in the 32-D-src cell line, serum deprivation did not affect the constitutively high TGF-β_1 mRNA levels. v-src's role in transcription was recently supported by the studies of Dutta et al., who showed that a nuclear DNA binding factor induced by serum is also directly induced by v-src. These results suggest that a serum-responsive element is involved in the regulation of TGF-β_1 mRNA expression and that v-src gene products function like serum to maintain the high levels of TGF-β_1 mRNA, as in the IL-3-dependent and 32-D-abl cell lines.

[1]Dutta, A., Hamaguchi, M., Hanafusa, H. v-src Induces the level of a serum-responsive nuclear CCAAT binding factor that drives transcription from the Rous sarcoma virus LTR. Fifth Annual Meeting on Oncogenes, Frederick, MD, 1989.

E 306 THE RSV LTR ENHANCER FACTOR III: IS IT THE AVIAN HOMOLOGUE OF THE SERUM RESPONSE FACTOR?, Amy Boulden and Linda Sealy, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232. We have recently discovered a third protein factor (EFIII) which binds to a specific 26bp region (-110 to -89) of the RSV LTR in vitro. Through competition experiments, we have verified that this protein factor is distinct from the two previously reported trans-acting factors that bind to the RSV LTR. Sequence analysis of the binding site for this protein factor revealed a striking similarity to the serum response element previously reported [Treisman(1985) Cell 42,889-902, and Mohun et al.,(1987) EMBO J. 6, 667-673] with 10 of 12bp homology in the central core region. Transversion mutations within this central region of dyad symmetry, which had been reported to eliminate binding of the serum response factor to the SRE, similarly disrupted the enhancer factor III-DNA interaction. By methylation interference and protection assays, the sites of protein-DNA contact are identical to that of the SRF. In vivo analysis of the RSV LTR indicates that it is, indeed, serum responsive. The role of the EFIII binding site in mediating this effect is currently under investigation as are studies to determine whether the LTR is responsive to other growth control signals. The results of such regulatory studies will be presented during the poster session of this meeting.

E 307 MECHANISM OF INHIBITION OF TRANSCRIPTION BY METHYLATION, Joan Boyes and Adrian Bird, Institute of Molecular Pathology, Dr. Bohr Gasse 7, 1030 Vienna, Austria.

Many examples of the inhibitory effect of methylation on transcription have been described. The mechanism of this inhibition may be envisaged to occur by two potential mechanisms:

1) Directly: Transcription factors may no longer be able to recognise and bind to a methylated CpG in their recognition site.

2) Indirectly: A factor (eg a methylated DNA binding protein and/or chromatin) may bind to methylated CpGs and thus prevent transcription factors from binding.

To try to distinguish between these two models, transcription of variously methylated forms of the human alpha globin was compared in an *in vitro* extract and upon transient transfection into Hela cells. Inhibition was seen upon transfection into Hela cells but not when the same methylated constructs were transcribed in an *in vitro* extract. This, and other observations, are compatible with the indirect mechanism. A methylated DNA binding protein has recently been isolated within the laboratory. This protein, either alone or in the presence of chromatin, has the potential to confer the indirect inhibition of transcription by methylation. Attempts to reconstitute the inhibition seen *in vivo* in the *in vitro* system using the methylated DNA binding protein in the presence or absence of chromatin will be reported.

E 308 A HIGHLY REGULATED EXPRESSION SYSTEN OF FOREIGN GENES IN MAMMALIAN CELLS. Ulrike Burkard, Ulrich Deuschle, Fubao Wang and Hermann Bujard. Universität Heidelberg, Zentrum für Molekulare Biologie Im Neuenheimer Feld 282 6900 Heidelberg FRG.

A eukaryotic expression system based on selective transcription of genes of interest by T3 RNA polymerase under the control of E.coli lac repressor has been developed. The genes for T3 RNA polymerase and for lac repressor have been inserted into the genome of mammalian cell lines and the corresponding proteins regulate the transcription of the gene for luciferase under the control of a T3 promoter/lac operator fusion. The T3 RNA polymerase transcripts are not capped however efficient translation can be achieved by superinfection with vaccinia virus. As an alternative we have fused the 5' non-translated leader sequences of picorna viruses in front of the gene for luciferase to increase translation of the RNA transcripts. Applications of the system in the study of gene function will be discussed.

E 309 PROMOTER ANALYSIS OF THE HUMAN FERREDOXIN GENE

Bon-chu Chung and Chi-Yao Chang, Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan, Republic of China, 11529

Ferredoxin is an electron transport intermediate for mitochondrial cytochromes P450. It is expressed in adrenal, testis, placenta, liver and kidney. The 5'-flanking region of the human ferredoxin gene we cloned contains a TATA box and two GC boxes at 30, 60, and 100 bp upstream from the transcription initiation site. We have constructed various deletion clones connecting different regions of the 5'-flanking sequence to the bacterial chloramphenicol acetyltransferase (CAT) gene. After transfection of these plasmids into recipient cells and assaying CAT activity, promoter function was detected in a DNA fragment covering sequence at -94/+55. This DNA fragment, including a TATA and a GC box, is active in Y1, JEG-3, COS-1, and HeLa cells. DNase I footprinting and methylation protection analysis showed that both GC boxes were protected by proteins in HeLa and Y1 extracts. Since nuclear factor Sp1 is known to bind GC boxes, it may be the factor responsible for binding and constitutive expression of the ferredoxin gene.

E 310 OCT-2, THE LYMPOID CELL-RESTRICTED ACTIVATOR OF IMMUNOGLOBULIN GENE TRANSCRIPTION

Lynn M. Corcoran and David Baltimore, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142

There is a growing family of nuclear proteins that share the feature that they bind to a sequence motif called the octamer element. This sequence (ATGCAAAT) occurs in a wide variety of cellular and viral gene promoters, and in certain viral origins of replication. Clearly, these promoters are not all coordinately regulated by a single protein. They may, however, manage to achieve specificity by binding different octamer-binding proteins. One well known system of tissue-specific gene expression is the lymphocyte-specific expression of immunoglobulin (Ig) genes. Much work has focussed attention on the octamer element found just upstream of the TATA box in variable gene promoters, in the heavy chain enhancer, and perhaps also in the newly discovered enhancer 3' of the kappa constant region gene. This work culminated in the discovery of oct-2, an octamer-binding protein that is primarily expressed in lymphoid cells, and in the eventual cloning of its cDNA. The tissue-specificity of oct-2 expression occurs at the level of transcription, as oct-2 protein and mRNA are coincident in all the cells tested. These include all cells of the B cell lineage, some T cells and a small number of myeloid cell lines.

Oct-2 has been shown to be capable of stimulating transcription from a simple promoter (octa + TATA elements) in non-lymphoid cells by transient co-transfection. We have used this approach to perform structure/function analysis on the oct-2 protein, identifying domains essential for DNA binding and transcriptional activation. Studies to determine the effect of ectopic oct-2 expression on the activity of endogenous targets, the immunoglobulin genes, are also in progress and will be reported.

E 311 MUTUALLY EXCLUSIVE BINDING OF NUCLEAR FACTOR-I AND ACTIVATOR PROTEIN-2 TO OVERLAPPING PROMOTER SEQUENCES OF THE HUMAN GROWTH HORMONE GENE. Stéphane J. Courtois, Dominique A. Lafontaine, Frédéric P. Lemaigre, Serge M. Durviaux and Guy G. Rousseau, Hormone and Metabolic Research Unit, Louvain University Medical School and International Institute of Cellular and Molecular Pathology, B-1200 Brussels, Belgium.

Transcription of the human (h) growth hormone (GH) gene and its hormonal regulation is controlled by tissue-specific and ubiquitous trans-acting factors. The binding of these factors to promoter sequences can be visualized by DNase I footprinting. Among the footprints localized within 500 bp of the hGH gene promoter, only one (-267 to -289) had not yet been ascribed to a particular factor. By footprinting, band shift and methylation interference assays, we found that this factor belongs to the Nuclear Factor I (NF-I)/CCAAT Transcription Factor (CTF) family and that it occurs not only in Hela cells, which do not produce GH, but also in GH-producing pituitary (GC) cells. When NF-I/CTF was prevented from binding to the hGH gene promoter by a competing oligonucleotide, the Activator Protein 2 (AP-2) bound to the same region of this promoter as NF-I/CTF. AP-2 was present not only in Hela cell extracts, but also in GC cell extracts albeit at a much lower concentration. The methylation interference pattern for these two trans- acting proteins on the hGH gene promoter was confined to their respective, overlapping, consensus sequences. Also, the methylation interference pattern for NF-I/CTF on its hGH gene site was similar to that described on the origin of replication of adenovirus, despite differences in nucleotide sequence. This suggests that replication and transcriptional activation by this factor involve similar DNA-binding mechanisms. Work is in progress to determine the functional significance of the mutually exclusive binding of NF-I/CTF and AP-2 to the hGH gene promoter. Supported in part by Incentive Program in Life Sciences Grant n° 20, Belgium.

E 312 COPPER AND ACE1 REVERSIBLY INDUCE YEAST METALLOTHIONEIN GENE TRANSCRIPTION IN A MOUSE EXTRACT, Valeria Culotta and Dean Hamer, Laboratory of Biochemistry, National Cancer Institute, Bethesda, Md 20892.

Metal-regulated transcription of the yeast metallothionein (MT) gene can be achieved in vitro using general polymerase II factors from mouse cells, the yeast ACE1 regulatory factor and Cu(I) ions. Copper induces a conformational change in the N-terminal DNA-binding domain of the regulatory factor, facilitating its binding to the upstream activator sequence (UAS) of the yeast gene. Efficient in vitro transcription also requires the highly acidic C-terminal domain of the ACE1 protein; however low, but significant levels of RNA synthesis are obtained with the DNA binding domain alone, suggesting that more than one protein domain interacts with the polymerase II transcription machinery. ACE1 binding to the promoter facilitates the formation of a sarkosyl resistant transcription complex. By releasing the factor from this complex by Cu-chelating agents, we found that transcriptional activation by ACE1 is reversible and that the regulatory factor is needed for the maintenence as well as formation of the stable complex.

E 313 TRANSACTIVATION OF TRANSCRIPTION IN PARVOVIRUSES: A VIRAL PROTEIN SYNTHESIZED IN A WHEAT GERM EXTRACT STIMULATES TRANSCRIPTION *IN VITRO*

Christian Doerig*, Peter Beard, Francine Beutler and Bernhard Hirt

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We are interested in the regulation of transcription in the autonomous parvoviruses Minute Virus of Mice (MVM) and B19, a human pathogen. We showed that during a productive infection in EL-4 cells by MVM the non-stuctural protein NS-1 transactivates both viral promoters, i.e. the promoter of the capsid proteins gene and its own promoter, controlling transcription of the NS proteins genes. No direct interaction between NS-1 and the DNA was detected, but we found evidence for protein-protein interactions involving NS-1 and cellular factors.

B19 does not grown in tissue culture, due to its narrow tissue-specificity. By using cloned DNA, we showed that its genomic organization is similar to that of MVM. The NS proteins genes promoter was activated in trans by a viral gene product in transfected cells, and we identified the transactivating protein as NS1: NS1 synthesized in a wheat germ extract specifically stimulates transcription from this promoter. On the other hand, the capsid gene promoter of B19 was not activated by NS1 in non permissive cells, suggesting that regulation of transcription from this promoter could be involved in the tissue specificity of this virus.

E 314 RNA POLYMERASE B(II) GENERAL TRANSCRIPTION FACTORS, J.M. Egly, B. Cavallini, X.M. Zheng, I. Faus, L. Fischer, M. Gerard, H. Higgs, V. Moncollin and P. Chambon. Institut de Chimie Biologique, Faculté de Médecine, 11, rue Humann, 67085 Strasbourg Cédex, France.

We have previously reported that four HeLa cell factors, STF, BTF1, BTF2 and BTF3 are required in addition to RNA polymerase B(II) for accurate initiation of transcription in vitro (1). STF and BTF1 (the TATA-box recognizing factor) are required for the formation of a stable preinitiation complex with the DNA template (2). We have shown that the yeast Saccharomyces cerevisiae contains a factor BTF1Y that can substitute for the HeLa cell BTF1 (3). We have also reported that BTF2 and BTF3 which do not bind specifically to DNA form a stable ternary complex with RNA polymerase B (4). Addition of this ternary complex to the preinitiation complex leads to initiation of transcription in the presence of nucleoside triphosphates. We have now cloned the yeast BTF1Y gene (5) and expressed it and will report the cloning and expression of human BTF3 cDNA. The results of experiments aimed at reconstituting the preinitiation and initiation complexes using these cloned factors and purified STF, BTF2 and RNA polymerase B will be presented.

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E 315 PROPERTIES OF PROTEIN BINDING DOMAINS OF THE HUMAN β-POLYMERASE PROMOTER, Ella W. Englander, Steven G. Widen and Samuel H. Wilson, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

The core promoter for human DNA polymerase β contains discrete binding sites for mammalian nuclear proteins, two sites correspond to sequences identical with Spl factor binding element, a third site includes an eighth residue palindromic sequence, TGACGTCA, known as the CRE element. Nuclear extracts from a variety of tissues and cells representing different levels of mRNA expression were examined; these included rat liver and testes and cultured cells of human and hamster origin. The DNaseI footprint is strong over and around the palindromic element for each of the extracts, footprinting over the Spl binding sites is observed and two potential tissue specific binding sites, present in liver but not in testes are detected. Controlled proteolysis with trypsin was used to study structural properties of proteins forming the mobility shift bands. Following digestion with trypsin most of the palindrome binding activity of each extract corresponded to a sharp faster migrating band, potentially representing a DNA binding domain of the palindrome binding protein. The pattern of protein binding to the palindromic element was shown to be modified by phosphatase treatment in vitro; this modification was reversed by kinase A mediated phosphorylation. The palindrome element binding protein purified from bovine testes, was phosphorylated in vitro by protein kinase A. Two dimensional tryptic peptide mapping revealed that kinase A mediates the phosphorylation of a single tryptic peptide at the serine moiety.

E 316 ALTERNATIVE CIS-REGULATORY SEQUENCES IN INTRACISTERNAL A-PARTICLE (IAP) LTRs ARE CORRELATED WITH TRANSCRIPTIONAL ACTIVITY. Miriam Falzon and Edward L. Kuff, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20892

The LTR of a mouse IAP proviral element, MIAI4, contains two domains with sequence homology to the SV40 enhancer core. The more 3' domain, designated Enh2, is an important determinant of promoter activity in vitro. Using affinity chromatography on an oligonucleotide corresponding to Enh2, we have purified from 293 and MOPC-315 cells a protein fraction containing two major components of 75 kDa and 85 kDa (EBP-80). EBP-80 binds preferentially to the enhancer core sequence (represented by oligonucleotides from the MIAI4 and MSV LTRs, SV40 and polyoma virus). MIAI4 is a randomly cloned provirus, not known to be active in mouse cells. The Enh2 domain of a recently transposed IAP element, rc-mos, differs from that of MIAI4; in particular it lacks the central enhancer core motif itself. Nevertheless, the rc-mos "Enh2" sequence binds EBP-80 with 5-10 fold greater affinity than does the Enh2 sequence of MIAI4. The rc-mos LTR is also 8-10 times more active as a promoter both in vivo (transient transfection) and in an in vitro transcription system, compared to the LTR from MIAI4. "Enh2 swapping" experiments indicate that the Enh2 domain contributes to the difference in the in vivo promoter activity between MIAI4 and rc-mos. In vitro transcription from both the rc-mos and MIAI4 LTRs is enhanced by EBP-80. The rc-mos variant of the "Enh2" sequence is conserved in most of the LTRs of transposed/expressed elements. EBP-80 is present in higher concentrations in transformed cells. We propose that the preferential expression of IAP elements containing this version of the Enh2 domain reflects the prevalence of the EBP-80 transcription factor(s) in transformed cells.

E 317 DHFR PROMOTER ELEMENTS ARE DOWNSTREAM OF THE TRANSCRIPTION INITIATION SITE. Peggy J. Farnham and Anna L. Means, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706.

The murine dihydrofolate reductase gene is regulated by a bidirectional promoter that lacks a TATA box. To identify the DNA sequences required for dihydrofolate reductase transcription, the activities of various templates were determined by in vitro transcription analysis. Our data indicate that sequences both upstream and downstream of the transcription initiation site modulate the activity of the dihydrofolate reductase promoter. We have focused on two regions downstream of the transcription initiation site that are important in determining the overall efficiency of the promoter. Region 1, which includes exon1 and part of intron1, can stimulate transcription in either orientation in the normal downstream position and when inserted in either orientation upstream of the transcription start site. This region can also stimulate transcription in trans when the enhancer is physically separate from the promoter. Protein binds to two sites in region 1, both of which contain an inverted CCAAT box, suggesting that a member of the CTF gene family is responsible for the downstream enhancer activity. Deletion of region 2, spanning 46 nucleotides of the 5' untranslated region, reduces transcriptional activity by 5 fold. The protein binding site in the 5' untranslated region has extensive homology to binding sites in promoters that both lack (SV40 late) and contain (Adenovirus 2 major late promoter, c-myc) TATA boxes.

E 318 EVIDENCE THAT REGULATORY SEQUENCES FOUND IN RIBOSOMAL PROTEIN GENES ARE ALSO INVOLVED IN TRANSCRIPTIONAL REGULATION OF THE snRNP E PROTEIN GENE, M. P. Fautsch and E. D. Wieben, Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN 55905.

snRNP E protein is one of several proteins associated with the U snRNAs. Sequence analysis of the 5' upstream region has identified a potential Sp1 binding site, a sequence found in U1 snRNA (U1 B), and two sequences found to be important in transcriptional regulation of vertebrate ribosomal protein genes; the G+C block and the CTTCCG motif. The promoter activity of different segments of the 5' upstream region of the E protein gene was assessed by transfection of kidney 293 cells with E upstream sequences linked to the bacterial gene for chloramphenicol acetyl transferase (CAT). Cells transfected with constructs ranging from .15 kb to 3 kb contained similar levels of CAT activity. However, a 3-fold reduction was seen in cells transfected with constructs that contained only 57 bp of 5' upstream sequence. Site directed mutagenesis within the potential Sp1 binding site and the vertebrate ribosomal protein regulatory elements, found within 50 bp of the transcription start site, caused marked reductions in CAT activity. This suggests that these elements play an important role in regulating transcription of the snRNP E protein gene. More extensive mutational analysis of this region along with data from mobility shift assays and DNA footprinting experiments will further characterize the role that these sequences play in transcriptional regulation of the snRNP E protein gene.

E 319 THE 5'-FLANKING REGION AND FIRST INTRON OF THE HUMAN ORNITHINE DECARBOXYLASE GENE CONTAIN MULTIPLE GROWTH-REGULATORY ELEMENTS. Flanagan, M.A., Fitzgerald, M.C. and Wagner, R., Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati OH 45215. Ornithine decarboxylase (ODC) is a rate-limiting enzyme in the synthesis of polyamines, ubiquitous cellular components that are essential for protein biosynthesis and DNA replication. To understand how the increase in ODC activity that occurs in response to growth-stimulus is controlled at the level of transcription and translation, we have cloned and sequenced a 9.5 kb genomic fragment containing the human ODC gene and 1.5 kb of 5' flanking sequence. The gene is divided into 12 exons, with the first exon separated from the remainder of the gene by an intron of 2849 bp. The unusual arrangement of the first exon in relation to the remainder of the gene has prompted us to examine the role of the first intron, as well as the 5' flanking sequence, in the regulation of the ODC gene. A 996 bp region centered over the first exon has been identified which has a G+C content of 80%, contains 308 CpG dinucleotides (potential methylation sites) and 14 Sp1 binding sites, as well as two potential Z-DNA forming regions. The first exon is predicted to have extensive secondary structure which may be involved in the regulation of translational efficiency by growth-stimulation. A portion of this region, -85 to -374, has a G+C content of 87% and contains 8 of the GC boxes. The 996 bp region is also rich in putative growth-regulatory elements. It contains a cAMP responsive element and 6 AP-2 binding sites, 4 in the first intron. In the first intron there are also 3 AP-1 binding sites. The presence of these putative elements is consistent with the induction of ODC gene expression by both cAMP and protein kinase C-mediated signaling pathways.

E 320 cDNA CLONING OF LEUCINE ZIPPER CONTAINING PROTEINS THAT BIND TO THE TRANSACTIVATOR (p40tax)-DEPENDENT ENHANCER SEQUENCE OF HTLV-1 LTR, Jun-ichi Fujisawa, Tadashi Yoshimura and Mitsuaki Yoshida, Department of Viral Oncology, Cancer Institute, Toshima-ku, Tokyo 170, JAPAN.
Trans-activator (p40tax) of human T cell leukemia virus type 1 (HTLV-1) is a transcriptional factor that activates the long terminal repeat (LTR) of HTLV-1 and various cellular genes including that for interleukin-2 receptor. A sequence responsible for tax-mediated trans-activation of the LTR has been mapped into the transcriptional enhancer consisting of three direct repeats of 21 bp in the U3 region and twelve nucleotides in the 21 bp repeat was shown to be essential for the tax-dependent enhancer activity. Although the twelve nucleotides sequence contains a 5 bp consensus of cAMP response element (CRE), functional analysis indicated that the mechanism of tax-mediated transactivation is different from that of cAMP-mediated regulation. To identify the cellular protein that is involved in the trans-activation by p40tax we have screened a λgt11 cDNA library constructed from HUT102 mRNA and obtained three different clones that bind specifically to the 21 bp enhancer sequence. Nucleotide sequence analysis revealed that these cDNAs encode 28.7k, 29.2k and 54.5k dalton proteins, respectively, and that all three proteins contain the leucine zipper motifs found in other enhancer binding proteins. Involvement of these proteins in the trans-activation and/or cellular signal transduction will be discussed.

The Basic Features of Eukaryotic Transcription-II

E 321 IDENTIFICATION AND CHARACTERIZATION OF AN Ad2 MLP CAP SEQUENCE DNA-BINDING PROTEIN, Susan Garfinkel, John A. Thompson, Will Jacob, Toby Silverman, Roger Cohen and Brian Safer, NHLBI, NIH, Bethesda, Md. 20892. DNase I footprint analysis of the Ad2 MLP reveals distinct patterns of protection corresponding to the assembly of transcription components during transcriptional initiation. It has been suggested that the protection over the TATA box and CAP sequences is attributed to the binding of a single factor, TFIID. We have determined, however, that protection of the CAP region results from the binding of a novel factor, designated CBF, which is chromatographically and functionally distinct from TFIID.

DNase I footprint and gel electrophoresis mobility shift competition assays confirm that distinct polypeptides bind to the Ad2 MLP UPS, TATA box and CAP sequences. When templates are mutated within the CAP sequence, transcriptional activity from the Ad2 MLP is reduced both in vitro and in vivo, and correlates with CBF binding activity. Depletion of CBF from nuclear extracts also inhibits transcriptional activity from the Ad2 MLP. Addition of DNA-affinity purified CBF, free of TFIID or MLTF, is able to restore transcription to control levels.

E 322 FETAL RECRUITMENT OF γ-GLOBIN GENE EXPRESSION: DISPARATE PROTEIN BINDING TO SIMIAN AND PROSIMIAN γ-GLOBIN PROMOTERS. T. Gray[1], D. Gumucio[1], D. Tagle[2], H. Heilstedt[1], M. Goodman[2], and F. Collins[1], [1] Department of Human Genetics, University of Michigan, Ann Arbor; [2] Department of Molecular Biology and Genetics, Wayne State University, Detroit, MI

In humans, and all simians, γ-globin is expressed from early fetal life until birth. In contrast, the γ genes in rabbit, mouse and prosimian primates are expressed embryonically. To explore the possible mechanisms of this delay in developmental expression of γ-globin in simian primates, we are analyzing differences in the binding of nuclear proteins to promoter regions of simian and prosimian γ-globin genes.

The human γ-globin promoter contains CCAAT boxes at -88 and -115. CP1 binds both of these sequences in gel shift experiments. The proximal CCAAT box region of the prosimian galago γ-globin gene also binds CP1, as well as several additional proteins. Competition profiles suggest one of these may be related or identical to C/EBP.

The -140 region of the human γ-globin genes contains a CACCC sequence which binds Sp1 with high affinity. This element also binds an unrelated protein, -140B. -140B specifically binds the human γ-globin promoter, not the galago γ-globin or human β-globin CACCC.

The -195 to -175 region of the human γ-globin promoter contains binding sites for two proteins: the ubiquitous octamer binding protein, oct-1, and an erythroid specific protein, GF-1. The galago promoter retains the GF-1 sites, but does not bind oct-1.

In summary, we have detected multiple differences in the pattern of proteins which bind to simian and prosimian γ-globin promoters. We are attempting to determine whether the differential binding of these factors may account for the phenomenon of fetal recruitment.

E 323 Sp1 BINDS TO THE HUMAN γ-GLOBIN PROMOTER AND STIMULATES EXPRESSION. D. Gumucio[1], K. Rood[1], T. Gray[1], A. Courey[2], A. Saulino[1], K. Blanchard[1] and F. Collins[1]. [1]Department of Internal Medicine, University of Michigan, Ann Arbor 48109; [2] Department of Biochemistry, University of California, Berkeley 94720

We have used gel retardation and footprinting assays to identify several proteins which bind to the human γ-globin promoter. The CCAAT box binding protein, CP1, the ubiquitous octamer binding protein, Oct-1, and an erythroid-specific binding protein, GF-1, all bind with high affinity to this promoter. In addition, we have detected three binding sites for Sp1. The sites at -200 and -50 bind Sp1 weakly but specifically in gel shift experiments using both crude extracts and purified Sp1. A third site at -140 binds Sp1 with 5-6 fold higher affinity. Purified Sp1 protein produces a clear DNaseI footprint at -140 but not at -200 or -50. To assess the function of these sites, Drosophila SL2 cells, which lack Sp1, were co-transfected with an Sp1 expression plasmid and γ-globin promoter-CAT constructs. CAT expression from a wild type γ-globin promoter was 5-7 fold stronger in the presence than in the absence of the Sp1 expression plasmid. For a γ construct containing a point mutation at -144 (CACCC → AACCC), stimulation by Sp1 was only 2-3 fold. In gel shift experiments, the same mutation reduced the binding of Sp1 by 10 fold; in transient expression assays in human erythroleukemia cells, the -144 mutation reduced the relative strength of the γ-globin promoter by 3-4 fold. The CACCC sequence at -140 is known to be an important cis-acting regulatory element in γ-globin expression. These experiments suggest that the binding of Sp1 to this site may be an important determinant of the strength of the γ-globin promoter. An additional protein also binds the γ-globin CACCC sequence.

E 324 TRANSCRIPTIONAL CONTROL OF AN IMMUNOGLOBULIN KAPPA GENE, Stephen Hardy, André Darveau and Rudolf Grosschedl, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143. Starting with a whole kappa gene plus 1kb of upstream sequence, we have identified DNA sequences which affect *in vitro* transcription of the kappa-41 gene in lymphoid extracts. As expected a single base change in the octamer site reduced transcription ten fold. Curiously, the octamer mutation also reduced transcription in HeLa cell extracts. Apart from the octamer, we have detected several new sequences which are necessary for normal levels of transcription. Changes in these regions decreased transcription by amounts equal to or greater than the decrease resulting from the octamer mutation. Interestingly, all of these new regulatory sequences are located 3' to the mRNA cap site. In addition to sequences which act positively on kappa transcription, we have also found a region which appears to repress kappa expression. This region is also within the gene.

E 325 THE ATF TRANSCRIPTION FACTOR, Helen C. Hurst, ICRF Oncology Group, LONDON W12 OHS, U.K.

The ubiquitous transcription factor, ATF, was initially identified by its ability to interact with most promoters which can be activated by the adenovirus E1a protein. It is possible, therefore, that ATF can mediate the activation by E1a; indeed synthetic promoters consisting solely of a TATA box plus concatanated ATF binding sites are efficiently stimulated by E1a. The core binding site for ATF is indistinguishable from that of another factor, CREB, which is believed to mediate cAMP induction of certain promoters. As both ATF and CREB are 43-47 kd phosphoproteins which can be kinased in vitro by cAMP dependent protein kinase, it seemed that both stimulatory activities may be contained within one factor.

We have purified ATF to homogeneity from HeLa cells and raised polyclonal antibodies in mice. These antibodies interact with ATF generated gel retardation complexes and can be used to distinguish ATF and CREB proteins. We therefore conclude that ATF and CREB must be distinct proteins although they apparently interact with the same sites. Further studies will be required to understand the different roles of these proteins. To this end expression libraries have been screened using both ATF antibodies and oligonucleotide binding sites to search for an ATF clone. Our progress will be presented.

E 326 REGULATION OF 5S RNA TRANSCRIPTION IN ACANTHAMOEBA CASTELLANII, Martin A. Imboden, Michael G. Zwick, Brent D. Toland and Marvin R. Paule, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523

When transferred to a starvation medium, the protist *Acanthamoeba castellanii* undergoes cellular differentiation into a dormant cyst. During this process transcription of rRNA genes is completely turned off, whereas the 5S RNA genes are down-regulated to a level of about 15%. We recently have shown that the down-regulation of the rRNA gene expression is mediated by a modification of the RNA polymerase I. In order to investigate, whether 5S RNA gene transcription is down-regulated by the same mechanism, we started to investigate the cis elements and the transacting factors involved in 5S RNA gene transcription in *A. castellanii*.

Although 5S RNA gene promoters are primarily intragenic, recent reports stress that additional upstream sequences are required for efficient transcription of certain 5S RNA genes which have 5' flanking sequence homologies to our cloned 5S RNA gene from *A. castellanii*. Therefore, the effect of upstream sequences on the efficiency and accuracy of 5S RNA gene transcription was tested in an in vitro transcription system, and the results thereof are presented.

Transcription of the *Acanthamoeba* 5S RNA gene in a nuclear extract prepared from encysted cells is reduced to about 15% of the transcription activity obtained in a nuclear extract from vegetative cells. This down-regulation perfectly mimics the down-regulation observed in vivo. Unlike the rRNA genes, the 5S RNA genes are not down-regulated via RNA polymerase modification. The presented data rather show that a common as well as a gene specific pol III transcription factor is involved in the down-regulation of the 5S RNA gene expression in *A. castellanii*.

E 327 IDENTIFICATION AND CHARACTERIZATION OF A NOVEL TRANSCRIPTION FACTOR REQUIRED FOR EXPRESSION OF eIF-2α, W.F. Jacob, T.A. Silverman, R.B. Cohen, and B. Safer, LMH, NHLBI, NIH, Bethesda, Maryland. eIF-2 catalyzes binding of met-tRNA$_i$ to native 43S ribosomal subunits. Considerable information is available concerning posttranslational regulation of eIF-2 activity by specific phosphorylation-dephosphorylation of its α subunit. However, little is known about regulation of eIF-2 expression. We have recently cloned and characterized the promoter region of eIF-2α. The promoter contains four upstream DNase I hypersensitive (HS) sites extending from -650 to -40 bp, and a fifth near the first intron-exon junction. In vitro DNase I footprint analysis showed 8 distinct DNA-protein interactions organized into clusters which correspond well with the distribution of the five HS sites. None of the protected regions, however, shares obvious sequence homology with the binding sites of known regulatory factors. To initiate our analysis of factors required for eIF-2α expression, we selected a CAP-proximal element shown by in vivo methylation protection analysis to bind a potential regulatory factor. A striking feature of this element is its palindrome sequences within eight bp direct repeats. We have purified to apparent homogeneity a 66-68 kDa protein which we designate α-PAL. The α-PAL binding site extends from -10 to -74. By methylation protection analysis and mobility shift assay, the α-PAL binding site is actually composed of two adjacent sites, one with high and one with low affinity, which bind α-PAL in a non-cooperative manner. When the high affinity binding site is cloned upstream of the Ad2 basal promoter (TATA/CAP), in vitro transcription is stimulated 2-3 fold. When linked to a CAT reporter gene, activity of the eIF-2α promoter shows 2-fold dependence on the α-PAL element.

E 328 TRANSCRIPTIONAL REGULATION OF HIV-1, Shaw-Yi Kao, Victoria Lim, B. Matija Peterlin, Howard Hughes Medical Institute, Univ. of Calif., San Francisco, CA 94143

Human immunodeficiency virus-1 (HIV-1) encodes a *trans* activator *tat*. The interaction of *tat* and its target sequence (TAR) increases the efficiency of transcription. Nuclear run on experiments have shown that full-engths transcripts across the HIV-LTR can be detected in the presence of *tat*. However, in the absence of *tat*, the majority of the transcripts are much shorter than full- length. The short transcripts have been mapped to positions +57-+59. These results suggest that there is a repression of transcriptional elongation in the absence of *tat*. Nuclear run on experiments in the presence of .5% sarkosyl gave similar results, indicating that the effects of *tat* must be at the level of elongation instead of initiation of transcription. *In vivo* competition experiments reveal that sequences responsible for transcriptional repression are in the promoter region, since repression can be relieved by titrating with specific HIV LTR DNA sequences. We are also studying how *tat* interacts with TAR RNA by filter retention analysis. The binding affinity of *tat* to wild type TAR RNA is much stronger than to mutated TAR RNAs. Filter binding assays indicate that the binding sites for *tat* are at the top of the stem-loop structure of TAR RNA. These results suggest that TAR functions as the *tat* utilization site, in order to modify blocked transcription complexes and facilitate transcriptional elongation.

E 329 ACTIVATOR INTERFERENCE *IN VITRO*, Raymond J. Kelleher III, Peter M. Flanagan, and Roger D. Kornberg, Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305.
In an effort to identify the factors and interactions involved in the stimulation of RNA polymerase II transcription by eukaryotic gene activator proteins, we have analyzed the effects of the hybrid yeast-herpes virus activator GAL4-VP16 on transcription in yeast. When expressed in yeast cells from a single copy centromeric vector, GAL4-VP16 stimulated expression of a CYC1-lacZ reporter gene bearing a GAL4-binding site, but interfered with transcriptional activation conferred by a thymidine-rich upstream activating sequence derived from the yeast DED1 gene. This activator interference can be reproduced in a yeast in vitro transcription system. Addition of purified GAL4-VP16 protein to the in vitro system resulted in an inhibition of transcription from a template bearing the DED1 thymidine-rich element. An equivalent concentration of GAL4-VP16 activated transcription in vitro from a template bearing a GAL4-binding site. These results suggest the sequestration by GAL4-VP16 of a common factor(s) required for activation of transcription. The observed interference was relieved in vitro by a partially purified fraction derived from yeast nuclear extract, affording a functional assay for a factor(s) mediating transcriptional activation. Interference was not relieved by purified yeast TATA-binding protein (TFIID) or by purified yeast RNA polymerase II, suggesting that neither of these basic factors directly mediates transcriptional activation.

E 330 TRANSCRIPTION REGULATION OF A HUMAN 7S K RNA GENE IN-VITRO AND IN-VIVO. Hartmut Kleinert, Sebastian Bredow and Bernd-J.Benecke. Dept. of Biochemistry NC-6, Ruhr-University, D-463 Bochum, Fed.Rep.of Germany.
In contrast to other class III genes (genes transcribed by RNA polymerase III), the 7S K promoter is exclusively located upstream of the transcription start site. In-vitro transcription of the 7S K gene depends on the presence of a "core" promoter up to position -67. Accurate and efficient transcription in vivo, however, requires the presence of an additional 5'flanking region. Though these sequences include two octamer-motifs, this region does not act as a classical enhancer. By analyzing 5'deletion mutants, we identified a distal sequence element (DSE) essential for transcription in-vivo, which is located between positions -207 and -242. This DSE includes several putative transcription factor binding sites, known from the class II transcription system. The functional significance of these sites for efficient in-vivo transcription of human 7S K RNA was analyzed with mutants obtained by site-directed mutagenesis and was verified in DNase I footprinting experiments.

E 331 MECHANISM OF TFIIIA-INDUCED SUPERCOILING: A NEW ROLE FOR TRANS-ACTING FACTORS?, Eric B. Kmiec, JoAnn M. Sekiguchi, Allyson M. Cole, Wen-Man Liu, Dept. of Biochemistry/Biophysics, University of California, Davis, Davis, CA 95616. In addition to its fundamental role of nucleating the formation of stable transcription complexes, the *Xenopus laevis* 5S RNA specific transcription factor, TFIIIA, promotes a variety of DNA-associated metabolic reactions. We report that TFIIIA can induce a DNA supercoiling catalyzed by both the *Xenopus laevis* S-150 cell-free extract and a fractionated extract on plasmids containing a single copy of the Xenopus 5S RNA gene (somatic-type). Stimulated supercoiling occurs in the presence of high ATP to $MgCl_2$ ratios and at a factor to DNA ratio of 1 through a mechanism most likely involving type I topoisomerase. The highest level of stimulated supercoiling occurs when TFIIIA is incubated with DNA prior to the addition of the S-150 extract. Under these optimized conditions, the proteolytic sensitivity of TFIIIA is altered. Taken together, these experiments outline and establish a reliable and seminal system in which TFIIIA-induced DNA supercoiling can be observed reproducibly.

E 332 SARKOSYL INHIBITION OF TRANSCRIPTION BY RNA POLYMERASE III: STEPS IN PREINITIATION COMPLEX FORMATION AND REGULATION BY THE ADENOVIRUS E1A GENE PRODUCT, Robert Kovelman and Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021. We have used Sarkosyl to analyze steps along the pathway of transcription initiation by RNA polymerase III. 0.015% Sarkosyl inhibited transcription when present prior to incubation of RNA polymerase III, TFIIIB, AND TFIIIC with the VA_I gene, while it had no detectable effect upon initiation or reinitiation of transcription when added subsequently. The formation of the corresponding 0.015% Sarkosyl-resistant complex required the presence of both TFIIIB and TFIIIC but not nucleoside triphosphates. Thus, this complex differs from the stable complex of TFIIIC and the VA_I gene, and kinetic studies show similarities between this complex and a previously characterized rate-limiting complex formed on the 5S RNA gene. The addition of 0.05% Sarkosyl after this early step selectively inhibited a later step in the preinitiation pathway, allowing a single round of transcription upon nucleoside triphosphate addition but blocking subsequent rounds of initiation. This step occurred prior to initiation since nucleoside triphosphates were not required for the formation of the corresponding 0.05% Sarkosyl-resistant complex. These observations provided a means to distinguish effects of regulatory factors on different steps in promoter activation and function. Using 0.05% Sarkosyl to limit reinitiation, we determined that the E1A-mediated stimulation of transcription by RNA polymerase III resulted from an increase in the number of active transcription complexes.

E 333 LOCALIZATION OF TOPOISOMERASE I AND II CLEAVAGE SITES ON THE *DROSOPHILA* HSP70 HEAT SHOCK GENE, Paul E. Kroeger and Thomas C. Rowe, Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL 32610.

The position of topoisomerase I and II cleavage sites on transcriptionally active and inactive HSP70 chromatin have been compared in *Drosophila* Kc cells using the drugs camptothecin (topo I-specific) and VM-26 (topo II-specific). These drugs stabilize a cleavable complex between these enzymes and DNA which results in site-specific DNA breaks upon detergent lysis of cells. Topoisomerase I cleavage sites are localized within the transcribed region of the gene and are present only when the gene is transcriptionally active. A majority of the enzyme-mediated single-strand breaks occurred on the non-transcribed strand of the Hsp70 gene. Inhibition of heat-induced Hsp70 transcription by either DRB (5,6-dichloro-1-ß-D-ribofuranosylbenzimidazole) or Actinomycin D abolished topoisomerase I cleavage. Loss of topoisomeras I cleavage also paralleled the normal attenuation of Hsp70 transcription during recovery of cells from a 37°C heat shock. These results suggest that topoisomerase I performs an essential function in removing topological stress which arises during Hsp70 transcription.

In contrast, topoisomerase II cleavage sites occurred only at the ends of the transcribed region of the gene. Prior to transcriptional activation, topoisomerase II cleavage was primarily localized to the 5'-end of the gene with little cleavage at the 3'-end. However, following a 37°C heat-shock, topoisomerase II cleavage rapidly shifted from the 5'- to the 3'-end of the HSP70 gene. During recovery of the cells from a 37°C heat-shock there was a gradual reappearance of topoisomerase II cleavage at the 5'-end of the gene which temporally correlated with the attenuation of HSP70 transcription. The major topoisomerase II site at the 5'-end has been sequenced and occurs within a highly conserved element present in the leader sequences of the different *Drosophila* heat shock genes. Evidence suggests that this region may be involved in the negative regulation of Hsp70 gene expression.

E 334 BIDIRECTIONAL TRANSCRIPTION FROM THE N-myc PROMOTER RESULTS IN OVERLAPPING COMPLEMENTARY TRANSCRIPTS WHICH FORM AN RNA:RNA DUPLEX, Geoffrey Krystal & Barbara Armstrong, Medical College of Virginia and the McGuire Veterans Affairs Medical Center, Richmond Va. 23249. Nuclear runoff transcription studies reveal proportionate sense and antisense transcription across exon 1 of the N-myc locus in all small cell lung cancer and neuroblastoma cell lines studied, independent of gene amplification. The antisense transcripts, which initiate at multiple sites in intron 1, form an RNA duplex with approximately 5% of the sense transcripts. This RNA duplex has been detected by using an RNase protection technique designed to detect RNase-resistant RNA and by directionally cloning each strand of the RNase-resistant duplex. Duplex formation occurs with only a subset of the nearly two dozen sense transcripts derived from multiple initiation sites, with a critical determining factor being the precise structure at the 5' end of the sense RNA. Evidence suggests that duplex formation may serve to preserve a population of N-myc mRNA's which retain intron 1. In addition, the half-life of the duplexed RNA's appears longer than that of the bulk of the N-myc RNA, suggesting that duplex formation may alter the turnover of the RNA's involved. We hypothesize that bidirectional transcription, with subsequent duplex formation, may be a mechanism by which genes which lack TATA boxes and have multiple initiation sites may direct specific transcripts down alternative processing pathways.

E 335 **Regulation of Ribosomal RNA Transcription During Encystment of *Acanthamoeba castellanii*: Identification of a Structural Modification of RNA Polymerase I.** William Kubaska, Peggy Risi, Jack Williamson, Alan Lofquist and Marvin R. Paule. Department of Biochemistry & The Cell and Molecular Biology Program, Colorado State University, Fort Collins, CO 80523.

The mechanism of rRNA transcription initiation in the the eukaryote *Acanthamoeba*, involves binding of a *trans*-acting factor (**TIF**) to the region between ~-67 and -17 of the template. This **preinitiation complex** is recognized by polymerase I (**pol I**), which protects the promoter from DNase I in a region bounded by -16 and +18, to form the **initiation complex**. Footprinting of a mutant series shows the polymerase makes no sequence-specific contacts with the DNA, but is directed to the promoter solely by protein contact with TIF. When starved, *Acanthamoeba* ceases growth and transcription of rRNA stops. Direct modification of Pol I mediates this regulation. Pol I isolated from cysts is functional in elongation, but is unable to initiate at the promoter. This inactivity is a consequence of cyst pol I not binding to the promoter. Since binding is mediated by protein contact with TIF, the modified site may be involved in interaction with the factor.

Structural comparisons of cyst and vegetative pol I by SDS-PAGE shows no change in the subunits present. PAGE analysis in the presence of urea under basic conditions demonstrates that there are no changes in the 13.3, 15.5 or 17.5 kd subunits. Non equilibrium pH gradient gel electrophoresis reveals that there are changes in the charge of one of the subunits common to polymerases I and III. The 39 kd subunit becomes more acidic and lower in apparent molecular weight. Monoclonal antibodies raised against the 39 kd subunit were used to demonstrate that the additional polypeptides are indeed modified forms of the 39 kd subunit. Further, Western blot analysis at various stages of encystment show that the appearance of the modified form(s) of the 39 kd subunit correlate temporally with shutdown of transcription.

E 336 HISTONE H2A.X, A BASAL H2A VARIANT, IS ENCODED BY TWO mRNA FORMS WITH DIFFERENT 3′ ENDS IN HUMAN PROLIFERATING CELLS.

Cecilia Mannironi,William M.Bonner, and Christopher L.Hatch.Laboratory of Molecular Pharmacology,Division of Cancer Treatment,National Cancer Institute,National Institute of Health,Bethesda,Maryland 20892.

The H2A histone family in mammals contains four described members, named H2A.1,H2A.2,H2A.X and H2A.Z. The former two proteins with almost identical sequences,are synthesized in concert with chromatin replication, while the latter two, so called basal variants, are synthesized throughout the cell cycle. A cDNA of a human H2A.X has been isolated and sequenced. The H2A.X cDNA is a 1583 bases long followed by a poly A tail,with 73 nucleotides in the 5′ UTR, 432 in the coding region and 1078 in the 3′ UTR. The H2A.X 3′UTR is unusual in that it contains not only poly A addition motifs and poly A tail, shown to be involved in replication-independent type histone mRNA regulation, but also the dyad simmetry and the U7 binding consensus sequences involved in the processing and stability of replication-type histone mRNAs. Two forms of H2A.X mRNA were found in human proliferating cell cultures. The longer polyadenylated mRNA is 1600 bases long and behaves as a basal type histone messenger. The shorter, 550 bases long, behaves as a replication type histone mRNA: it is not polyadenylated and decreases after inhibition of DNA synthesis. Our results suggest that the two transcripts can be under different control with respect to cell cycle regulation. The sequencing and characterization of an H2A.1 cDNA isolated from the same basal library suggests that the coexistence of replication and basal type processing signals is not a peculiarity of the H2A.X mRNA and alternative 3′ end formation of histone RNA may be involved in the cell cycle control of histone synthesis.

E 337 PROPERTIES OF RAP30/74, A GENERAL INITIATION FACTOR THAT BINDS TO RNA POLYMERASE II, Susan McCracken, Mary Sopta, Marie Killeen and Jack Greenblatt Banting and Best Dept. of Medical Research and Dept. of Medical Genetics, University of Toronto, Toronto, CANADA M5G 1L6

RAP30/74 is a heteromeric general initiation factor whose RAP30 subunit binds to RNA polymerase II. RAP74 is highly phosphorylated on serines and threonines. One third to one half of the RAP30 in HeLa or HL60 cells is phosphorylated on two serines near the C-terminus. Full length cDNAs encoding RAP30 have been cloned and sequenced and the RAP30 polypeptide has been expressed in E.coli and in S.cerevisiae. The amino acid sequence of RAP30 contains a region of limited homology with the E.coli initiation factor sigma 70. A DNA helicase activity is associated with RAP30/74. The properties of the DNA helicase suggest that it is responsible for the transition from a closed complex to an open complex at the promoter and for the ATP hydrolysis requirement for initiation by RNA polymerase II. Other properties of the initiation helicase will be described.

E 338 BINDING OF HIP1 PROTEIN DETERMINES THE SITE OF TRANSCRIPTION INITIATION FOR THE DIHYDROFOLATE REDUCTASE GENE, Anna L. Means and Peggy J. Farnham, McArdle Laboratory, University of Wisconsin-Madison, Madison, WI 53706.
The dihydrofolate reductase (DHFR) gene is characteristic of many housekeeping genes; it is expressed at low levels in all growing cells, exhibits growth-related regulation of expression, and has a GC rich promoter with no apparent binding site for the transcription factor TFIID. We have been investigating the mechanism by which RNA polymerase II is positioned to begin transcription on promoters that lack binding sites for the transcirption factor TFIID. We have found that a protein, HIP1, is responsible for positioning the site of transcription initiation for the DHFR gene. HIP1 binds at the site of initiation to the sequence ATTTCGCGCCA rather than to upstream sequences, distinguishing it from TFIID. Transcription from the DHFR promoter always initiates within the HIP1 sequence, regardless of its distance from upstream sequences. One binding site for the transcription factor Sp1 and one HIP1 site cloned into the pUC19 vector are sufficient to initiate transcription. We are purifying HIP1 to test its role in transcription of other housekeeping promoters. We are also performing saturation mutagenesis of the HIP1 binding site to determine sequence requirements for HIP1 binding and function.

E 339 IDENTIFICATION OF SEQUENCE ELEMENTS THAT DETERMINE WHETHER AN EXCISABLE INTRON IS REQUIRED FOR mRNA FORMATION, Janet E. Mertz, Wang-Shick Ryu, and Chung-Yih Wang, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706
Whereas transcripts of many intron-containing genes (e.g., SV40 late and β-globin) fail to accumulate in the cytoplasm in the absence of excisable introns, transcripts of at least some intronless protein-coding genes [e.g., herpes simplex virus thymidine kinase (HSV-TK)] accumulate efficiently in the cytoplasm. To identify sequence elements which determine whether or not an excisable intervening sequence is necessary for efficient cytoplasmic accumulation of mRNA, we constructed HSV-TK/human β-globin hybrid genes. Quantitative S1 nuclease mapping was used to determine the amounts of the RNAs accumulated in the nucleus and cytoplasm of monkey cell transfected with the hybrid genes and cDNA versions of them. We found that the HSV-TK gene contains at least two different types of sequence elements that can enable efficient cytoplasmic accumulation of HSV-TK and human β-globin mRNA in the absence of excisable introns: one, acting transcriptionally, maps within its promoter; another, acting posttranscriptionally, maps within its transcribed region. On the other hand, the human β-globin gene contains an element necessary for the efficient cytoplasmic accumulation of mRNA only within its second intron. We conclude that numerous different pre-mRNA processing pathways exist in mammalian cells in which transcription, 5'- and 3'-end formation, splicing, nuclear export, and translation are coupled, with specific sequence elements contained within genes determining to which pathways their transcripts are directed.

E 340 TWO DIFFERENT RNA POLYMERASE II INITIATION COMPLEXES CAN ASSEMBLE ON THE CKB PROMOTER
Mark T. Mitchell and Pamela A Benfield, Central Research and Development Department, E.I. Du Pont de Nemours & Co., Inc., P.O. Box 80328, Wilmington, DE 19880-0328
The rat brain creatine kinase (CKB) gene has a structurally complex promoter. Although it contains a consensus RNA polymerase II TATA box, this is located at -66 relative to the main transcription start site. *In vitro* transcription experiments reveal that two different preinitition complexes can assemble on the CKB promoter. Extracts from brain form exclusively a complex which initiates at the exact same start site seen in normal adult rat brain. However, extracts from liver (a tissue in which the CKB message is barely detectable) favor formation of an upstream initiating complex but formation of such a complex can be blocked if brain extract is added first.

E 341 INITIATION AND PREMATURE TERMINATION OF TRANSCRIPTION BY AN α-TUBULIN GENE IN *XENOPUS* OOCYTES, Garry T. Morgan and Kim M. Middleton, Department of Genetics, University of Nottingham, Queens Medical Centre, Nottingham NG7 2UH, U.K.

The *X. laevis* α-tubulin gene XαT14 is expressed in oocytes and in several tissue culture lines at a high level and at much lower levels in other cell types. When injected into oocytes the gene shows accurate and efficient initiation of transcription over a wide range of template concentrations. The region between -200 and -60 is required for initiation in oocytes and contains consensus sequences for binding of the transcription factors ATF,USF,CTF (x3) and HSTF. When injected at low DNA concentration (10 pg/nucleus) only correctly-initiated and elongated transcripts are produced,but as more DNA is injected premature termination of transcription occurs in the 5′ leader 45-72 nucleotides downstream of initiation,near a potential stem/loop. The extent of premature termination increases progressively with template dose even at levels at which the initiation of transcription is not yet at its peak. At high template doses (30-50ng) almost all transcripts are prematurely terminated. In addition prematurely-terminated transcripts become more prevalent with increased incubation times. We think premature termination of transcription is induced experimentally because of the perturbation of a cellular antitermination activity that operates at a crucial time during transcription initiation and that is somehow dependent on the amount of potential template available rather than on the number of transcribing polymerases. We are currently investigating the mechanism of termination and antitermination in nuclei isolated from injected oocytes.

E 342 CHARACTERISATION OF CELLULAR PROTEINS WHICH INTERACT WITH THE NEGATIVE REGULATORY ELEMENT OF THE HIV-1 LTR.

Kim Orchard[1,2], Neil Perkins[1], Caroline Chapman[1,3], Julian Harris[1,3], Vincent Emery[2], Graham Goodwin[1], David Latchman[3], Mary Collins[1].

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Expression from the HIV-1 LTR is known to be regulated by host cell responses such as T-lymphocyte activation, lymphokine action and macrophage differentiation. These events can potentially influence disease progression in infected individuals. A number of cellular proteins capable of binding to the HIV-1 LTR have been identified, one of these, NFkB, binds to the enhancer region of the LTR and increases viral transcription in response to several stimuli. However, the region of the LTR upstream of -278 bp from the start of transciption has been identified as a negative regulatory element, removal of which allows an increase in LTR driven expression in the T-lymphocyte line Jurkat , and may therefore play a role in the maintenance of HIV latency. We have defined two major protein binding sites within this region, which bind previously undescribed human T-cell proteins. Further structural and functional characterisation of these proteins will be presented.

E 343 KINETIC CONTROL OF 5S RNA GENE TRANSCRIPTION

Lawrence J. Peck, Department of Biology, Sinsheimer Labs, University of California, Santa Cruz, CA 95064

The 5S RNA genes in the frog *Xenopus laevis* are actively transcribed during oogenesis. During early embryonic development the oocyte-type family of 5S RNA genes becomes transcriptionally repressed. In contrast, the somatic-type family of 5S RNA genes, with 50-fold fewer copies, remains transcriptionally active. An *in vitro* transcription system from a whole oocyte extract mimics this transcriptional bias. In this extract, somatic-type 5S RNA genes are transcribed 100-fold more efficiently than oocyte-type 5S RNA genes. We have recently determined that this transcriptional bias is a result of vastly different rates of stable complex assembly between the two types of genes. Stable complexes are detected using a template exclusion assay. Preincubation of one type of gene in the extract, under conditions of limiting transcription factors, will block transcription of a second gene added later. Only the gene added first will be transcribed upon subsequent addition of labeled nucleotide triphosphates. In this extract, stable complexes are formed on both somatic and oocyte-type 5S RNA genes and either type of gene will block transcription of the other if added first. We have measured the rate of stable complex assembly on both types of 5S RNA genes by varying the preincubation time and the concentration of 5S RNA genes. Somatic-type 5S RNA genes form stable complexes much more rapidly than oocyte-type 5S RNA genes. In this *in vitro* transcription system, transcription factor TFIIIA is present in excess over the amounts of added 5S RNA genes. For this reason and since the measured affinities of TFIIIA for the two types of 5S RNA genes are similar, it is unlikely that TFIIIA binding contributes to the observed difference in the rate of stable complex assembly. Once stable complexes are formed, roughly equivalent numbers of transcripts are produced from the two types of genes. Thus under conditions where transcription factors are limiting, stable complexes will form predominantly on the somatic-type genes with the result that the oocyte-type genes will be transcriptionally inactive. This mechanism, of limiting transcription factors combined with kinetic differences in transcription complex assembly, may be involved in the repression of the oocyte-type 5S RNA genes during the early development of *Xenopus laevis* and in other systems of regulated gene expression where identical transcription factors are utilized.

E 344 TRANSCRIPTIONAL CONTROL IN VSV: SINGLE-SITE MUTATION IN THE TEMPLATE-ASSOCIATED CAPSID PROTEIN MODIFIES ATP REQUIREMENTS AND READTHROUGH SYNTHESIS, Jacques Perrault, Joseph L. Chuang, Lisa C. Childers, and R. Lynn Jackson, Biology Department and Molecular Biology Institute, San Diego State University, San Diego, CA 92182

Transcription of the vesicular stomatitis virus RNA genome shares several important but little understood features with that of eukaryotic cellular genes, namely, transcriptional activation via an acidic activating domain, phosphorylation-dependent activity, and complex ATP requirements. We report here that a single charge mutation (Arg to His) in the template-associated nucleocapsid protein (N) of the VSV polR mutants relaxes ATP requirements, promotes readthrough synthesis at the leader-N gene junction in vitro (antitermination at this junction is required to switch from transcription to replication), and leads to restricted growth in mouse L vs BHK cells in vivo. We have isolated six phenotypic revertants which now grow on L cells. The extent of reversion to wild-type (partial or complete) for readthrough synthesis and for both types of ATP requirements (β, γ bond hydrolysis and high concentration requirements for mRNA synthesis) shows that all of these functions are very tightly linked. Sequence analysis of the revertant N genes reveals that five of the six revertants are due to second-site suppressor mutations while one is a true revertant. These results suggest that binding of the polymerase complex to the N-RNA template alters the conformation of a putative ATP binding and/or utilization site(s) in the L protein and that such modulation plays an important role in regulating transcription and replication activities.

E 345 SEQUENCE, SECONDARY STRUCTURE, AND PROTEIN BINDING TO THE SHORT RIBOSOMAL DNA LENGTH VARIANT IN PEA, Kenneth J. Piller, Scott R. Baerson and Lon S. Kaufman, Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois at Chicago, Chicago, Il 60680.

The genes for 18S, 5.8S, and 25S ribosomal RNAs (rRNAs) are highly reiterated and arranged in tandem arrays in both plants and animals. Located between the 3' end of the 25S rRNA and the 5' end of the 18S rRNA is the intergenic region (IGR), believed to contain all of the *cis*-acting sequences required for rDNA expression and regulation. There are two major rDNA length variants in *Pisum sativum* cv. Alaska. Published data suggest that the shorter length variant (S) is expressed only during specific stages of development whereas the longer length variant (L) is expressed constitutively. Expression of the S variant correlates with cell division as opposed to cell expansion or plastid development. The relationship between rDNA expression and cell division is being explored. We have determined the nucleotide sequence of the 2859 bp IGR of the S variant, as well as the putative site of transcript initiation and a major processing site. Sequence analysis of the IGR reveals nine tandem repeats (subrepeats), approximately 180 bp in length. Subrepeats are present in the IGR of many organisms, including *Xenopus* and *Drosophila*, where they have been shown to possess enhancer activity. Sequence analysis of the pea IGR also reveals a thermodynamically stable stem-loop structure and palindrome near the site of initiation and 5' 18S border, respectively. The sequence immediately surrounding the site of initiation shows homology to analogous regions in radish, maize, and wheat. We have also identified those DNA fragments, derived from regions surrounding the site of initiation, which test positive in mobility shift assays. We are currently determining the precise location of those sequences to which proteins bind using various DNA footprinting techniques. Supported by USDA grant # 88-37261-3734 to LSK.

E 346 A COMMON TRANS-ACTING FACTOR REGULATES EXPRESSION OF BOTH RAT GROWTH HORMONE (rGH) AND MOUSE p12 SECRETORY PROTEASE INHIBITOR GENES, François Pothier, Sylvain L. Guérin, Suzanne Robidoux and Fernand Labrie, Molecular Endocrinology Laboratory, CHUL Research Center, Laval University Medical Center, Québec, Canada, G1V 4G2

The gene encoding the mouse secretory protease inhibitor p12 represents an interesting example of a cell-type specifically regulated gene. While p12 steroid-dependent expression is restricted to the ventral prostate, the coagulating gland and the seminal vesicule, a constitutive low level of expression is detectable only in the pancreas. To investigate the mechanisms and factors involved in the p12 cell-specific expression, we cloned fragments containing various lenghts of the p12 promoter upstream of the CAT gene from the reporter plasmid pOCAT1. No CAT activity was detected following transient transfections of p12-promoter/CAT plasmid constructs in a variety of non-prostatic cell-lines, which might suggest that most of the tissue-specific regulatory sequences involved in that mechanism are contained on a 843bp fragment. Experiments to test that hypothesis are actually underway. A 5'-end labeled DNA fragment containing the sequence from +40 to -484 was used in DNase1 footprinting assays to look at the trans-acting proteins interacting with the p12 proximal promoter region. A clear 22 bp protected region (p12.A) was detected between the TATA and the CAAT-boxes (position -45 to -66), using nuclear extracts prepared from a variety of cell-lines including the rat pituitary GH4C1 and the human prostatic DU145 cells. G residues protected from methylation in DMS footprinting assay identified a 10 bp contact region (5'-GTGGGTGGAG-3') probably of critical importance for recognition by the trans-acting protein binding to p12.A. Detailed examination of the protected sequence revealed a very good match (9 out of 10 bp) with the rat growth hormone(rGH) binding site for the transcription factor GC2. In addition, a double-stranded unlabeled oligonucleotide containing the rGH GC2 binding site clearly competed for the protein binding to the p12.A site in DNase1 assays, suggesting that the same (or a very similar) trans-acting factor(s) (which has been shown to be very close to but probably different from the transcription factor Sp1) regulates expression of both rGH and p12 genes.

E 347 THE DROSOPHILA RNA POLYMERASE II ELONGATION FACTOR DmS-II, David H. Price, Tricia K. Marshall, Hongliang Gou, Biochemistry Department, University of Iowa, Iowa City, IA 52242; Ann E. Sluder, and Arno L. Greenleaf Biochemistry Department, Duke University Medical Center, Durham NC 27710. **A 36 kDa protein which has an effect on the elongation properties of RNA polymerase II has been purified from nuclear extracts of Drosophila Kc cells. The factor, DmS-II, stimulates RNA polymerase II during the transcription of double-stranded DNA templates when the nonphysiological divalent cation manganese is present. In the presence of physiological mono- and divalent cations the factor reduces the tendency of RNA polymerase II to pause at specific sites along a dC-tailed template including the major pause encountered after 14 nucleotides have been incorporated. Dilution experiments indicate that DmS-II is not stably bound to the elongation complex. We have used a completely defined system and show that the properties of DmS-II are intrinsic to the factor and not mediated through other factors. Antibodies have been produced against the purified protein and are currently being used to screen a lambda gt11 expression library.**

E 348 C-MYC PROTEIN EXHIBITS INTRINSIC PROTEIN KINASE AND PHOSPHATASE ACTIVITIES. Håkan Randahl, Marie Henriksson and George Klein. Dept. of Tumor Biology, Karolinska Institutet, S-104 01 Stockholm Sweden.

The product of the c-myc oncogene has been implicated in different regulatory functions. It has been suggested that the c-myc protein participates in regulation of replication and that it may influence transcription both in positive and negative ways. We have purified the c-myc protein to apparent homogeneity by immunoaffinity chromatography using two different monoclonal antibodies. Whether one or both immunoaffinity columns were used alone or in succesion the eluate contained three major proteins of 66, 64 and 45 kDa. By immunoblot analysis it was confirmed that the 66 and 64 kDa proteins were c-myc. The 45 kDa protein has either homologous sequences to c-myc or was very firmly associated to c-myc. Fractions from the column contained phosphatase activity that dephosphorylated casein and a protein kinase activity that phosphorylated serine and to a lesser extent threonin in an autophosphorylation mode. In the absence of substrate the enzyme also hydrolyses ATP. All activities were inhibited by a third, polyclonal antibody against c-myc. Enzyme kinetics showed that the protein kinase had a Km for histone H1 and for ATP of 25 and 20 μM, respectively. Km for ATP in the ATP hydrolysing reaction was 65 μM. However, when histone H1 was included in the reaction Km decreased to 15 μM for ATP and Vmax decreased four times.

We conclude that the ATP binding site for the kinase/phosphatase reaction resides in the same protein molecule, c-myc.

E 349 MODULATION OF A CONSTITUTIVE TRANSCRIPTIONAL BLOCK AT EXON I CONTROLS EXPRESSION OF THE HUMAN C-MYC PROTO-ONCOGENE, Gian G. Re, G.R. Antoun and T.F. Zipf, Division of Experimental Pediatrics, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030.

To elucidate the transcriptional control mechanism of the human c-myc proto-oncogene we have investigated c-myc mRNA synthesis in HL60 cells both under proliferation and induced differentiation conditions. By gel electrophoresis of total cellular RNA followed by RNA blot hybridization, using various c-myc gene specific DNA probes we indentified a novel c-myc transcript of approximately 550 bases in length. By size and sequence specificity this RNA qualified as the prematurely terminated transcription product of the c-myc gene which contains exon I sequences only as observed by others in slot hybridization of nuclear run off products. Without precedent, this RNA was present along with c-myc 2.4 kb mRNA in normally proliferating HL60 cells. In cells that were induced to differentiate by DMSO the level of this small transcript increased as that of the 2.4 kb mRNA disappeared. In cells regrown for 24 hours in the absence of DMSO both the small transcript and the c-myc mRNA returned to their original levels. This observation supports the notion that in proliferating HL60 cells a constitutive partial transcriptional block at the end of exon I of the c-myc gene is in effect. This partial block causes the synthesis of a small amount of prematurely terminated transcript along with the predominant 2.4 kb mRNA. Upon induction with DMSO the block becomes complete and totally impedes transcription leading to the synthesis of the small RNA segment only.

E 350 DIFFERENTIAL EXPRESSION OF XENOPUS CLASS III GENES IN VITRO IS ATTRIBUTABLE TO TFIIIC. Wanda F. Reynolds, La Jolla Cancer Research Foundation, 10901 N. Torrey Pines Road, La Jolla, CA 92037. Certain Xenopus class III genes, including OAX, oocyte-type 5S RNA genes, and perhaps tDNAmet, are expressed specifically in immature oocytes. This stage specific pattern of expression is also apparent in vitro: in immature oocyte extracts, OAX and tDNAmet are expressed at high levels relative to the somatic 5S gene. Conversely, in mature oocyte extracts, OAX and tDNAmet are relatively inactive. In a reconstituted system using partially purified factors, OAX and tDNAmet are transcriptionally active in the presence of TFIIIC isolated from immature, but not mature, oocytes. This suggests that stage-specific differences in TFIIIC result in selective gene expression.

E 351 A NOVEL *ama-1* ALLELE THAT DECREASES ALPHA-AMANITIN SENSITIVITY OF *Caenorhabditis elegans* RNA POLYMERASE II BY 40,000-FOLD. T.M. Rogalski, M. Golomb and Donald L. Riddle, Division of Biological Sciences, University of Missouri, Columbia, MO 65211
The *ama-1* gene encodes the largest subunit of RNA polymerase II (RNAP II) in the nematode *C. elegans*. Rare, dominant mutations in *ama-1* result in an RNAP II that is 150-fold less sensitive to alpha-amanitin than the wild-type enzyme (Sanford, Golomb and Riddle, 1983, J. Biol. Chem. 12804-12809; Rogalski, Bullerjahn and Riddle, 1988, Genetics 423-434), but they do not completely eliminate sensitivity of the enzyme to the toxin. To determine whether it was possible to obtain a completely resistant RNAP II, we devised a selection for a super-resistant derivative of the resistant *ama-1(m118)* parent. One mutant was found among $4x10^6$ F1 progeny of EMS-mutagenized worms. In contrast to the resistant (class 1) parent, which exhibits near normal growth and fertility, the super-resistant (class 2) mutant develops slowly, has fewer viable progeny at 20°C, and is sterile at 25°C. The genetic analysis indicated that this new mutation, *m526*, was within the *ama-1* gene. Fine-structure mapping experiments were able to separate the parental *m118* mutation from *m526* at a very low frequency, suggesting that these two mutations are physically close, within a few hundred base pairs in this 9 kb gene.
Preliminary results indicated that RNAP II from the class 2 mutant was unstable *in vitro*, so we measured the amanitin sensitivity of this enzyme using a nuclear run-on assay. RNA made in the presence of various concentrations of amanitin was hybridized to DNA from genes transcribed by RNAP I, II, and III, respectively. The class 2 mutant enzyme was 50% inhibited by 200 μg/ml amanitin, a concentration that inhibits RNAP III by 90%. By comparison, the class 1 resistant parent was 50% inhibited by the toxin at only 0.5 μg/ml. Using an *ama-1(m118m526)* strain, we have recently isolated several mutants (class 3) that exhibit increased amanitin resistance *in vivo* in comparison with the class 2 parent. These strains will be analyzed to determine whether any carry RNAP III mutations.

E 352 CHARACTERIZATION OF THE FACTORS THAT DIRECT THE TRANSCRIPTION OF RAT RIBOSOMAL DNA, Larry Rothblum, David Smith, Daniel O'Mahony and WenQin Xie, Weis Center for Research, Geisinger Clinic, North Academy Avenue, Danville, PA 17822 The protein components that direct and activate accurate transcription by rat RNA polymerase 1 have been studied in extracts of Novikoff hepatoma ascites cells. A minimum of at least two components, besides RNA polymerase I, have been identified and are necessary for the efficient utilization of templates. The first factor, rat SL-1, is a) sufficient to direct transcription by pure RNA polymerase 1 and b) is required for the species-specific recognition of the rat RNA polymerase I promoter. Rat SL-1 directed the transcription of templates deleted to -31, the 5'boundary of the core promoter element (+1 being the transcription initiation site). The second factor, rUBF increased the efficiency of template utilization. Transcription of deletion mutants indicated that the 5' boundary of the domain required for rUBF lay between -137 and-127. Experiments using block substitution mutants confirmed and extended these observations. Transcription experiments using those mutants demonstrated that two regions within the upstream promoter element were required for optimal levels of transcription in vitro. The first region was centered on nucleotides -129/-124. The 5' boundary of the second domain mapped to between nucleotides -106/-101. DNAase footprint experiments using highly purified rUBF indicated that rUBF bound between -130 and -50. However, mutating nucleotides -129/-124 did not affect the rUBF footprint. These results indicate that basal levels of transcription by RNA polymerase I may only require SL-1 and the core promoter element. However, higher transcription levels are mediated by additional interactions of rUBF, and possibly SL-1, bound to distal promoter elements.

E 353 POSSIBLE ROLE FOR 7SL/ALU GENE FAMILY IN NEGATIVE REGULATION OF CELL GROWTH, Kazuichi Sakamoto, C. Michael Fordis, Christopher D. Corsico, Tazuko H. Howard, Tony Giordano and Bruce H. Howard, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

We have been interested in the involvement of interspersed repetitive sequences in the regulation of mammalian cell growth. To test this possibility we introduced Alu or 7SL RNA genes into HeLa cells with a cell surface marker plasmid (pHSV-IL2R). Transiently transfected cells expressing the plasmid-encoded Tac subunit of the IL2 receptor were subjected to magnetic affinity cell sorting and analyzed with respect to ^{3}H-thymidine incorporation. In this protocol, DEAE-dextran mediated transfection with these genes caused suppression of ^{3}H-thymidine incorporation in recipient cells. This inhibitory activity was dependent on the presence of two sequence motifs: the B box of the RNA polymerase III promoter and an undecameric sequence, GAGGCNGAGGC, which is homologous to the T antigen binding site of SV40 DNA replication origin. We isolated a HeLa nuclear protein fraction which binds to the GAGGCNGAGGC and several other Alu-specific sequences. The involvement of this Alu-specific DNA-(or RNA-) binding factors in transcription of these pol III genes and in DNA replication is currently under study.

E 354 ACTIVATION AND REPRESSION OF TRANSCRIPTION BY THE SAME TRANSCRIPTION FACTORS: FOS AND JUN

Axel Schönthal*, James Feramisco*, Harald Konig, Stephan Gebel, Bernd Stein, Marita Büscher, Hans Jobst Rahmsdorf, Helmut Ponta, Peter Herrlich, Institute for Genetics and Toxicology, Nuclear Research Center, 7500 Karlsruhe, W. Germany *UCSD Cancer Center, La Jolla, CA 92103.

The human collagenase gene is activated by serum growth factors, TPA (12-O-Tetradecanoyl-Phorbol-13-Acetate), and oncoproteins (eg. ras, src, mos) via the AP-1 binding site, a nonanucleotide sequence within the promoter. We show that the nuclear Fos and Jun oncoproteins cooperate in formation of AP-1, the transcription factor which binds to this promoter element. Blocking of either one of these two proteins by anti-sense expression vectors strongly reduces the AP-1 mediated transcriptional activation by all of the above inducers. This shows that Fos and Jun proteins are nuclear components of signal transduction pathways and act as inducers of gene transcription.

Furthermore, both proteins are able to suppress transcriptional activity of the human c-fos promoter. Transfection of Fos or Jun expression vectors suppress basal and serum induced activity of a cotransfected fos promoter/CAT construct as well as of the endogenous c-fos gene. In support of this result the activity of a fos promoter/CAT construct is increased by cotransfection of anti-sense fos or jun expression vectors. Interestingly this repression of fos promoter activity does not require the AP-1 binding site of fos: the dyad symmetry element (DSE) alone is the major target of repression; the AP-1 binding site plays a role in regulation of basal promoter activity. These results show that in addition of being transcriptional activators the Fos and Jun Proteins are also able to suppress promoter activity.

E 355 DOSAGE COMPENSATION OF THE X-LINKED PGD GENE IN DROSOPHILA, Max J. Scott and John C. Lucchesi, Department of Biology, The University of North Carolina at Chapel Hill, Chapel Hill, N.C., 27599-3280.

In the fruit fly Drosophila melanogaster males(XY;2A) compensate for the lower dose of X chromsomes relative to females(2X;2A) by a 2-fold increase in the transcription rate of X-linked genes. It has been previously shown that the X-linked 6 Phosphogluconate dehydrogenase (Pgd+) gene, as part of a 7.4kb fragment, is properly dosage compensated when translocated to autosomal sites via P-element transposition. To determine the location of the dosage compensation elements we have constructed 5', 3' and internal deletions of the Pgd+ gene as well as a Pgd promoter-lac Z fusion. At least 4 transformed lines for each construct were assayed for dosage compensation, the results of which will be presented.

E 356 **Transcriptional Regulatory Sequences Within the Mouse LINE-1 Retrotransposon**
Diana M. Severynse, Clyde Hutchison III and Marshall H. Edgell, Department of Microbiology and Immunology Univ. of N.C. at Chapel Hill, Chapel Hill, N.C. 27599

LINE-1 (L1) is a long interspersed repetitive sequence found in all mammals. Full length L1 elements have an A-rich 3' end, are flanked by short direct repeats and contain two open reading frames, one of which shares homology to a variety of reverse transcriptases. These structural features, as well as the presence of allelic polymorphisms resulting from L1 insertions, suggest that L1 is a retrotransposon. Therefore, transcription of L1 is necessary for its proliferation and movement in the genome. The absence of LTRs flanking L1 suggests that novel features may exist at the 5' end of this retrotransposon in order to preserve the transcriptional regulatory sequences during transpostion. We are in the process of characterizing the sequences involved in the transcriptional regulation of the mouse L1 element.

Two different tandemly repeated monomer units can be found at the 5' end of a full length mouse L1. These monomers (designated A and F) are approximately 200 bps in length and are G-C rich. We have identified promoter activity within the 5' end of the A-type L1 element, using the bacterial chloramphenicol acetyltransferase assay. A single A-monomer can increase CAT activity up to 25 fold over the vector alone in a transient expression assay in mouse L cells. CAT activity is only observed when the monomer is oriented in the same direction to the CAT gene as it is to the open reading frames within L1.

In order to better characterize the sequences within the 208 base pair monomer that may regulate the activity of the mouse L1 promoter, a series of deletional clones have been generated using PCR. Preliminary results have shown that deletion of 41 bps from the 5' end of the A-monomer results in an 80% decrease in CAT activity. These results indicate that sequences at the 5' end of the A-monomer may be important in the regulation of the mouse L1 promoter activity. Continued analysis of deletional clones is in progress.

E 357 INTERACTIONS OF CELLULAR DNA BINDING PROTEINS WITH A SEQUENCE MOTIF INVOLVED IN E1A TRANSACTIVATION OF AAV P5 PROMOTER,
Yang Shi, Long-Sheng Chang and Thomas Shenk, Howard Hughes Medical Institute, Department of Biology, Princeton University, Princeton, NJ 08544

Transcription of the adeno-associated virus P5 promoter is induced by adenovirus E1A gene products. Two adjacent cis-elements involved in E1A transactivation have previously been identified: a binding site for the major late transcription factor and a tandemly repeated 10bp sequence. Several cellular proteins bind to this 10bp repeat sequence. Upon incubation with nuclear extracts prepared from Hela cells with an oligonucleotide that contains the 10bp repeat sequence, two major sequence-specific DNA-protein complexes were detected, I and II. With mobility-shift assays, we failed to detect any qualitative or quantitative difference in the ability of extracts prepared from uninfected or wild-type adenovirus-infected Hela cells to form complex II. However, chromatographic fractions that contain complex II-forming activity from infected cells manifested at least 5-10 fold higher UV crosslink efficiency to the DNA containing the repeat sequence as compared to the uninfected extract. This suggests that the cellular protein that forms complex II may be modified by adenovirus gene products. A DNA with two-bp substitutions in the 10bp repeat sequence failed to form complex II. This same mutant DNA, when placed upstream of SV40 promoter, also failed to respond to E1A gene products in a transfection-based transcription assay. Therefore, the formation of complex II is correlated with the ability of the 10bp repeat element to mediate E1A-induced transcription. Work is in progress to delineate the molecular basis underlying the observed difference in UV crosslinking efficiency of factors from uninfected as compared to infected Hela nuclear extracts.

E 358 ANALYSIS OF cJUN/cFOS INTERACTIONS IN VIVO AND IN VITRO. Tod Smeal, Peter Angel, Jennifer Meek, and Michael Karin. Department of Biology, Department of Pharmacology, School of Medicine, M-036, UCSD, La Jolla, CA 92093. Landshultz et al. have suggested that the dimerization of cJun and cFos is mediated by the interdigitation of an orderly repeat of leucine residues forming a "leucine Zipper". We have analyzed the interaction of cJun's leucine repeat with itself and with cFos. Binding to the AP-1 site requires dimerization of these proteins. Although cFos itself does not seem to dimerize and bind the AP-1 site, Jun:Fos heterodimers have a higher stability than Jun homodimers, which accounts for the heterodimers increased DNA-binding activity. Mutational analysis indicates that at least three of the repeated leucines are important for homodimer formation. However, these residues can be mutated without affecting formation and activity of Jun:Fos heterodimers. Furthermore, mutations between the leucines of cJun affects cJun's ability to form homodimers with itself and heterodimers with cFos. These mutations suggest that the specificity of the dimerization domain is not limited to the leucine residues. These findings support the proposal (by O'Shea et al.) that these proteins dimerize via formation of a coiled coil. Interestingly, an insertion mutant that displaces the zipper domain by half an helical turn away from the DNA binding domain can still dimerize, but fails to bind DNA and acts as a transdominant inhibitor of both cJun and cFos. Due to the specificity the dimerization domain cJun, cFos and other zipper containing proteins, it should be possible to design specific inhibitory proteins that will help elucidate the roles of this family of transcription factors in cellular processes ranging from differentiation to transformation.

E 359 EXPRESSION OF THE *DROSOPHILA* TROPOMYOSIN I GENE IS CONTROLLED BY 5' PROMOTER AND INTRON ENHANCER SEQUENCES THAT BIND TISSUE-SPECIFIC FACTORS, Robert V. Storti, Joshua Schultz, Jay Lichter, and Terese Tansey. Department of Biochemistry, University of Illinois College of Medicine, Chicago, Il. 60612. Transcriptional control of the *Drosophila* tropomyosin I (TmI) gene expression has been investigated by P-element transformation and rescue of the TmI gene *Ifm(3)3* flightless tropomyosin mutant. The mutation effects splicing of the TmI gene in indirect flight muscle. We have shown previously [Tansey et. al.(1987) EMBO J. 6:1375] that the TmI gene can transform and rescue *Ifm(3)3* flightless flies. In order to determine the cis-acting DNA sequences that control TmI gene expression, deletion mutants of the TmI gene constructed *in vitro* have been analyzed for expression by transformation and rescue of flightless mutant flies and by measuring TmI mRNA levels by primer extension analysis in the transformants. The results indicate that there are at least two regions that control expression. One is located within 800 bps 5' of the transcriptional start site. A second control region is located within the first intron of the gene and has some properties characteristic of an enhancer element. The enhancer-like element can drive expression of a heterologous hsp70 promoter/TmI chimeric gene in indirect flight muscle and rescue *Ifm(3)3* flightless flies. Gel shift analysis of proteins from nuclear extracts of early (myoblast) and late stage (myotube) embryos indicate the present of binding factors that are common to both extracts as well as factors that are presence in only late stage embryos when the TmI gene is normally expressed.

E 360 THE ACIDIC ACTIVATION DOMAIN OF HSV VP16 BINDS THE TATA-BOX FACTOR TFIID. Keith F. Stringer, C. James Ingles and Jack Greenblatt. Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6.

The HSV encoded protein VP16 has a C-terminal transcriptional activation domain rich in acidic amino acids. To understand molecular mechanisms of transcriptional activation, we have expressed a protein A-VP16 activation domain fusion in E. coli and used the purified chimeric protein for affinity chromatography experiments with Hela cell transcription extracts. Columns of both pA-VP16 agarose and pA-agarose bound about 0.1% of the total applied protein. The pA-VP16-agarose, but not pA-agarose, depleted nuclear extracts of a factor required for Ad2MLP transcription. The VP16 bound general initiation factor could be eluted with 0.5M KCl. Four lines of evidence suggest that the initiation factor depleted on the VP16 column was TFIID. The eluted activity stimulated transcription of AdMLP by Hela cell nuclear extracts. It restored transcriptional activity to extracts heated to 47°. The VP16 bound factor also provided TFIID activity in template commitment experiments; transcription of a second added template required addition of the VP16 column eluate. Finally, this activity eluted from DEAE-cellulose with 0.17 M KCl as does human TFIID. The binding of TFIID by the acidic activation domain of VP16 may be direct or indirect; experiments with cloned S. cerevisiae TFIID expressed in E. coli should distinguish between these possibilities.

E 361 ROLE OF SV40 SPECIFIC TRANSCRIPTION FACTORS IN RNA POLYMERASE II INITIATION COMPLEX FORMATION, Rebecca Sundseth and Ulla Hansen, Laboratory of Eukaryotic Transcription, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

The Simian Virus 40 (SV40) early and late gene promoters are localized within a 300 bp segment of the viral genome consisting of multiple adjacent and overlapping transcriptional control elements. Cellular proteins have been shown to specifically interact with many of these sites, and in particular, the HeLa cell protein, LSF, binds within two different elements (-45 and -265 relative to the late start site, L325)(Huang, Sundseth and Hansen, manuscript submitted). LSF specifically stimulates initiation of transcription at L325 when added to a reconstituted transcription reaction containing the general transcription factors (TF) TFIIA, IIB, IIE, IID and RNA polymerase II. The assembly of general factors onto a promoter appears to be an ordered process resulting in a functional complex which initiates transcription in the presence of NTP's. Thus, the mechanism of LSF stimulated initiation might include its interaction with one or a combination of the general factors to enhance or stabilize DNA-binding or association with the rest of the general transcription machinery. The interaction of LSF with the general transcription factors and its effect on their function during initiation complex formation will be presented. In addition, the potential involvement of other SV40 specific factors which bind near LSF(e.g. Sp1, AP1, AP4) in LSF mediated stimulation of transcription will be addressed.

E 362 CRITICAL STRUCTURAL ELEMENTS OF THE TRANSCRIPTIONAL ACTIVATION DOMAIN OF VP16. Steven J. Triezenberg and W. Doug Cress, Department of Biochemistry, Michigan State University, East Lansing, MI 48824.

Expression of HSV-1 immediate-early genes is specifically and potently activated by a virion protein termed VP16 or Vmw65. The transcriptional activation domain of VP16 is rich in acidic amino acids. Secondary structure predictions suggest that this region forms an amphipathic alpha helix. We have constructed a set of missense mutants to test the hypotheses that negative charge and/or amphipathic secondary structure are critical attributes of this domain. The function of mutant proteins has been scored in transient transfection assays. Overall, we find a correlation between the number of acidic amino acids and the strength of the activation domain, implying that negative charge is a critical feature. However, some mutants with identical numbers of acidic residues differ significantly in their ability to trans-activate, suggesting that secondary structure or charge distribution are also important. We are currently testing mutants with altered secondary structure but unaltered charge to explicitly test this hypothesis. Mutants designed to test the amphipathic character of this region have been inconclusive so far. To support our mutational analysis, we are now developing spectroscopic assays for direct analysis of helical structure in these proteins.

E 363 PUTATIVE IDENTIFICATION OF A REPRESSOR OF ß-INTERFERON EXPRESSION WHICH BINDS OVER THE TATA-BOX, Kanna V. Visvanathan and Stephen Goodbourn, Gene Expression Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK. Induction of ß-interferon gene transcription by dsRNA and virus requires regulatory elements which act both positively and negatively. We have identifed constitutive and inducible factors for one such element and shown that a mutation which selectively impairs binding of the inducible factor, NF-κB, abolishes induction (Visvanathan and Goodbourn, EMBO (1989) **8**, 1129). To identify negatively acting factors, we have used the gel retardation assay to detect sequence-specific activities which are decreased in extracts from induced cells. One such activity binds over the TATA box. A down-mutation for this binding activity shows increased basal levels of mRNA and, conversely, a mutation in the TATA box which decreases expression increases its binding activity. We therefore refer to this binding activity as TATA box Occlusion Factor, TOF. In Namalwa cells, in which dsRNA alone does not induce ß-interferon expression despite the presence of high levels of the positively acting factor NF-kB, dsRNA fails to reduce TOF activity, consistent with the possibility that inactivation of TOF is one of the steps required for derepression of the ß-interferon gene.

E 364 THE SPACING BETWEEN TWO CIS PROMOTER ELEMENTS IS A MAJOR FACTOR DETERMINING THE RNA POLYMERASE SPECIFICITY DURING TRANSCRIPTION OF U-snRNA GENES IN ARABIDOPSIS THALIANA. Franz Waibel, Pierre Vankan and Witold Filipowicz. Friedrich Miescher-Institut, P.O.box 2543, 4002 Basel, Switzerland

We have cloned several active U-snRNA genes of the higher plant Arabidopsis thaliana and characterized their promoter structure. The U2, U4 and U5 genes are transcribed by RNA polymerase II (pol II), the U6 genes by pol III. Despite being transcribed by different polymerases, the promoters of the pol II (U2,U4,U5) and of the pol III (U6) genes are almost identical. Pol II and pol III genes require only two cis upstream elements, the TATA-like box at position -30 and the UsnRNA gene-specific Upstream Sequence Element (USE: RTCCCACATCG). Both elements are interchangable between the two classes of genes. However, the spacing between the USE and the TATA-like box differs, being four DNA helical turns in the pol II genes and three helical turns in the pol III genes. We have demonstrated that the insertion of 10 bp of DNA between the elements of a pol III-specific promoter converts it to a pol II promoter and, vice versa, the deletion of 10 bp between the elements of a pol II promoter converts it to a pol III promoter.

These results provide strong support for a common evolutionary origin of RNA polymerases and give also an example how, during evolution, a diversification of eucaryotic promoters could have taken place. In addition they show, that the promoter structure of UsnRNA genes and the elements, which define the polymerase specificity are completely different in plant and vertebrate cells.

E 365 TRANSACTIVATION OF FIBRONECTIN BY THE ADENOVIRUS PROTEIN E1A IS MEDIATED THROUGH MULTIPLE ELEMENTS IN THE FIBRONECTIN GENE, Weintraub, S.J. and Douglas Dean, Department of Respiratory and Critical Care Medicine, Washington University School of Medicine, 660 South Euclid, Box 8052, Saint Louis, MO 63110. The fibronectin (FN) gene is transactivated by the adenovirus E1A (13s) protein in transfection assays. Serial 5' deletion mutations of the FN promoter were used in transfections to determine the site through which this transactivation is mediated. Deletion of the 5' flanking region to position -122 has little effect on E1A responsiveness. This is notable since this deletion removes the promoter's cyclic AMP responsive element (CRE) and CRE's are known to be important for E1A's transactivating activity in other genes. The first 122 bp of the FN promoter contain multiple Sp1 sites and a TATA box. Deletion to -37 removes the Sp1 sites. This results in a marked but incomplete loss of the response to E1A. However, mutation of the TATA box in the -37 construct results in complete loss of E1A responsiveness. When an Sp1 site is added to the inactive mutant TATA box, E1A responsivity is restored. Responsiveness is also restored when a CRE is added to this construct. Therefore, three different elements in the FN promoter are E1A responsive, an Sp1 site, a CRE, and the TATA box.

E 366 THE ROLE OF CIS-ACTING ELEMENTS IN TRANSCRIPTIONAL REGULATION OF THE MURINE CLASS II MHC LOCUS Aa, Maryann Z. Whitley, Mark R. Boothby, Robert J. Sisk and Laurie H. Glimcher, Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115. Class II Major Histocompatibility Complex (MHC) molecules are expressed in a tissue specific manner in B lymphocytes, macrophages, and thymic epithelium. This expression is regulated both by developmental signals and external stimuli. Two closely spaced consensus sequences, X and Y, are located at approximately -135, upstream of the initiation codon. In transcriptional assays employing the reporter gene chloramphenicol acetyl transferase (CAT), the Aa X and Y boxes alone can attain only a fraction of the activity observed with a larger 1.3kb upstream fragment. A second element further upstream of X and Y, termed BRE-1, is capable of binding a nuclear factor which is regulated in B cells by IL-4 and by differentiation. Serial deletions from the 5'end of the 1.3kb fragment have identified yet a third positive region, located around -965. Constructs have been made in which each of these elements, X, Y, BRE-1, and the -965 region, has been deleted or otherwise mutated, leaving the others intact. Analsis of these constructs has lead to a model of transcription at the Aa gene in which all the elements must act cooperatively to maintain appropriate expression.

E 367 A TRANSCRIPTION FACTOR THAT RECOGNIZES THE CCAAT ELEMENT OF THE HUMANP70 PROMOTER. B. Wu, Department of Biochemistry, Molecular Biology & Cell Biology, Northwestern University, Evanston, IL 60208. The activity of the serum stimulated and adenovirus E1a inducible promoter of the human HSP70 gene is governed by the CCAAT element at position -67 relative to the transcriptional initiation site. A synthetic oligonucleotide corresponding to this CCAAT element detects a 100-120 kD polypeptide by southwestern assay. As expected, a synthetic oligonucleotide corresponding to a linker-scan mutation in the CCAAT element, previously shown to be defective for binding *in vitro* and severely reduced in activity *in vivo*, binds very weakly in the southwestern assay. Using the binding assay, a cDNA clone was isolated from a WI-38 library constructed in lambda-gt11. Overlapping clones were isolated by DNA:DNA hybridization and the resultant composite contains an open reading frame sufficient to encode a 110kD polypeptide which will be referred to as CCAAT binding factor CBF. The deduced amino acid sequence of CBF shows no homology to either of the previously identified CCAAT factors, CTF and CBP. In co-transfection assays, stimulation provided by CBF in *trans* is both promoter selective and dependent on the presence of a CCAAT element. The HSP70 promoter is stimulated by CBF, but neither the SV40 nor the RSV promoters are responsive. Furthermore, a linker-scan mutation in the CCAAT element of the HSP70 promoter abolishes responsiveness to CBF stimulation.

E 368 EFFECTS OF MUTATIONS IN THE B BLOCK ELEMENT REGION ON PROTEIN-DNA INTERACTIONS DURING TRANSCRIPTION OF THE VARNA1 GENE, Guang-Jer Wu and Bandea, C.I., Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322.
Transcription initiation of eukaryotic genes is controlled by cis-acting regulatory elements and their interactions with transcription factors and RNA polymerases. Two essential and several important cis-acting regulatory elements are defined by transcription analyses of linker-scanning mutants with clustered substitutions in the control region of the VARNA1 gene whose transcription is mediated by RNA polymerase III. Functions of these cis-acting elements in regulation of transcription of the VARNA1 gene are suggested from genetic studies. Further studies on effects of point mutations in these elements on transcription have defined the function of each base pair. Taking advantage of this large resource of genetic mutants, we have employed the gel mobility shift method and the DNaseI-footprinting assay to study effects of mutations on protein-DNA interactions during formation of transcription complexes. From the results of effects of various mutations on the interaction of the partially purified TFIIIB and TFIIIC with the gene, we concluded that mutations in the B block element affect the interaction of the TFIIIC with this element, but not the TFIIIB. Furthermore, the mechanism of interaction of the TFIIIC with the two halves of the B block palindrome is more complex than what is expected, suggesting sequences surrounding the B block palindrome and sequences in the A block vicinity may affect its interaction.

E 369 **Studies on the binding of transcription factors to the promoter and non transcribed spacer of eukaryotic ribosomal RNA genes.** Qin Yang, Han Li, Laura Hoffman and Marvin R. Paule. Department of Biochemistry, Colorado State University, Fort Collins, CO 80523.
Ribosomal RNA transcription in the eukaryote *Acanthamoeba* is directed by a single transcription factor, **TIF**. TIF binds between -17 and -67 of the promoter, directing RNA polymerase I to bind by protein-protein contact. Following initiation, TIF remains bound and repetitively directs additional polymerases to initiate. Once a rRNA gene is transcriptionally activated in this way, it functions until the next round of DNA replication. The details of binding of TIF to the promoter are important to understand, and mechanisms involving the presence of additional TIF binding sites upstream of the gene promoter are being considered as methods of enhancing the assembly of potentially active complexes on the promoter. We are examining the mechanism of binding of TIF to the promoter using gel shift assays to evaluate the binding constant, determination of the sequence of the spacer to look for additional TIF binding sites, and determining the effect of upstream sequences on the rate of transcription from the gene promoter. Studies *in vitro* have failed to demonstrate any sequences upstream of -47 which are required for optimal transcription by polymerase I. However, the spacer DNA in *trans* can act as a specific competitive inhibitor of transcription *in vitro*. This result suggests that there are binding sites for TIF within the spacer, and further suggests that *Acanthamoeba* has a series of enhancer elements within its spacer similar to those in *Xenopus*.
We have begun studies of the binding of TIF to the core promoter. Surprisingly, TIF binds with a biphasic profile indicative of two binding sites **on TIF**. Both strong (K_d =3.4 x 10^{-11} M) and weak (K_d = 6.3 x 10^{-9} M) components are well resolved. The possible significance of this finding will be discussed.

E 370 PHOSHORYLATION DEPENDENT BINDING OF A 138 KDal MYC INTRON FACTOR (MIF) TO A REGULATORY ELEMENT IN THE FIRST INTRON OF THE *c-myc* GENE,
Maria Zajac-Kaye[1] and David Levens[2]. [1]Medicine Branch and [2]Laboratory of Pathology, National Cancer Institute, NIH, Bethesda, Maryland 20892.
We have previously identified a 20 base pair (bp) region in the first intron of the human *c-myc* gene as a binding site for a nuclear protein and have shown that binding was abolished by a point mutation present in a corresponding region in Burkitt lymphoma (BL) *c-myc* DNA (Zajac-Kaye et al. Science 240; 1776-1780 1988). This 20bp region is mutated in 6 out of 8 BL sequenced to date. To study the role of this 20bp intron sequence in the regulation of the *c-myc* gene we have purified a nuclear protein which binds to this element. Using south-western blot analysis we identified a 138 KDal protein which binds to the wild type 20bp *c-myc* sequence but does not bind to the BL sequence containing point mutation in its binding region. To confirm that the 138 KDal protein is MIF, the retarded protein:DNA band was exised from a DNA mobility shift gel and subjected to SDS-PAGE gel electrophoresis. A 138 KDal protein was visualised with gold stain only when the retarded band was generated with the *c-myc* specific wild type intron I oligonucleotide probe and was absent when a nonspecific oligonucleotide or no oligonucleotide was used. In addition we showed that the 138 KDal MIF is a phosphoprotein and that treatment of the purified MIF with potato acid phosphatase abolished binding to the 20bp *c-myc* specific oligonucleotide probe, while the binding activity was protected by the inclusion of phosphatase inhibitors. We conclude that phosphorylation is required for the specific DNA:protein interaction and that the phosphorylation state of MIF may determine its active form required for the control of *c-myc* expression. The MIF represents an important example of a nuclear protein for which its cognate sequence is found to be frequently mutated in neoplastic cells.

E 371 THE 43-KDa PROTEIN EXPRESSED *IN VITRO* SHOWS SPECIFIC DNA BINDING ACTIVITY TOWARDS CRE, Alex Zhu and Jack E. Dixon, Department of Biochemistry, Purdue University, West Lafayette, IN 47907

A 43-KDa DNA binding protein which recognizes the cAMP-responsible elemert (CRE) of the rat somatostatin promoter has been purified from rat brain by DEAE-Sepharose and specific DNA affinity chromatography. The purified protein has shown specific DNA binding activity as indicated by Southwestern blotting, renaturation and DNase I footprinting studies. The requirement of this protein for somatostatin gene transcription has been confirmed with an *in vitro* transcription system. Here we report the cloning of this 43 KDa protein from total RNA derived from the CA-77 cell line by the polymerase chain reaction. Sequencing analyses of seven different clones indicate that the 43 KDa protein cDNA from CA-77 cells is very similar but not identical to the cDNA isolated from human placenta and PC-12 cells. Deduced amino acid sequence demonstrates a leucine zip adjacent to a charge-rich region, characteristic for a DNA binding protein. The cDNA clone driven by the T7 promoter has been *in vitro* transcribed and translated in the rabbit reticulocyte lysate system. The protein thus expressed binds specifically to the CRE DNA fragment, but not to a CRE mutated fragment by a gel retardation experiment. Further studies will be focused on identification of different domain structures of the protein synthesized *in vitro*.

Transcription Factors in Cell Differentiation

E 400 CHARACTERIZATION OF AN ADDITIONAL FACTOR REQUIRED FOR EFFICIENTASSOCIATION OF FOS AND JUN WITH THE AP-1 BINDING SITE. Cory Abate, Daniel Luk, Reiner Gentz and Tom Curran, Dept. of Molecular Oncology & Virology, Roche Institute of Molecular Biology, Nutley, NJ 07110

The protein products of the proto-oncogenes c-*fos* and c-*jun* (Fos and Jun, respectively) form a heterodimeric complex that interacts with the DNA regulatory element known as the AP-1 binding site. Mutagenesis studies have suggested that Fos and Jun associate via a parallel interaction of their leucine zipper domains which results in appropriate juxtapositioning of adjacent DNA-binding domains. To characterize these properties, the leucine zipper and DNA-binding domains of Fos and Jun were expressed in *E. coli* and highly purified preparations were obtained. Purified Fos and Jun rapidly formed a protein complex *in vitro*; however, the heterodimeric complex had a low apparent affinity for DNA. The affinity for DNA was increased approximately 100 fold by the addition of a factor that is rather ubiquitous in eukaryotic cells. This factor stabilizes the association of Fos and Jun with DNA but does not appear to interact directly with DNA. Further characterization of this factor will be presented and its potential role in regulating the transcriptional responses mediated by Fos and Jun will be addressed.

E 401 ISOLATION AND CHARACTERIZATION OF cDNA CLONES ENCODING PROTEINS THAT BIND SPECIFICALLY TO REGULATORY SEQUENCES OF *DROSOPHILA ADH* GENES., Ted Abel and Tom Maniatis, Harvard University, Cambridge, MA. The *Drosophila mulleri Adh-1* gene is expressed in the larval fat body and in three other larval tissues. Three regulatory elements are necessary for the correct expression of *Adh-1*: two enhancer elements--one located 5' (BOX B, -269 to -181) and the other 3' (BOX C) to the gene--and an upstream promoter element (BOX A, -91 to -60) (Fischer and Maniatis, 1988, Cell,53:451). Both of the enhancer elements behave as fat-body specific enhancers in association with a "neutral" promoter. However, the expression of *Adh-1* in multiple cell types, is the result of synergistic interactions between the fat-body specific enhancer elements and the BOX A promoter element. To investigate the molecular mechanisms by which this array of cis-acting elements interacts to yield the complex stage and tissue-specific expression of *Adh*, we screened bacteriophage λ expression cDNA libraries for cDNA clones encoding proteins that bind specifically to BOX A or BOX B. We isolated two cDNA clones (BBF-1 and BBF-2) and one cDNA clone (ABF-1) that encode proteins that bind to BOX B and BOX A, respectively. The proteins encoded by each of these cDNA clones were expressed in bacteria and shown to bind specifically to their respective regulatory elements. ABF-1 binds to a sequence between -59 and -72 of the *Adh-1* promoter. Significantly, ABF-1 also binds specfically to the proximal promoter of the *D. melanogaster Adh* gene, a promoter that is also expessed in larvae. The protein encoded by BBF-2 binds to a sequence between -242 and -269 of the *Adh-1* promoter in the region of two linker scanning mutations (LS7 and LS8) which reduce expression in vivo. Indeed, BBF-2 binds at least 25-fold less well to these mutants relative to the wild-type sequence. Sequence analysis of BBF-2 has shown that it contains a heptad repeat of leucine residues, but no other known DNA binding domains. Work is in progress to identify the DNA-binding domain(s) of these proteins

their role

E 402 REGULATION OF ORNITHINE DECARBOXYLASE DURING TPA INDUCED DIFFERENTIATION OF HL-60 CELLS. Yvonne Alexander, Keith Vass, George Birnie. Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD, Scotland. Ornithine decarboxylase (ODC. EC.4.1.1.17) catalyses the decarboxylation of ornithine to putrescine and its expression is widely viewed as an important correlate of cell growth. Cloned ODC cDNA was used to investigate the mechanisms which mediate the induction of mammalian ODC in TPA treated HL-60 cells. A peak in enzyme activity is observed 6hr after TPA treatment and this can be correlated with an increase in ODC mRNA. Data from nuclear run-on transcription analysis indicate that an increase in transcription can account for the induction of ODC mRNA. Work is currently underway with the murine ODC gene to characterise DNA sequences that are responsible for mediating the effects of TPA on its transcriptional regulation by constructing chimeric genes containing variable regions around the 5' end of the murine ODC gene fused to the bacterial chloramphenicol acetyl transferase (CAT) gene.

E 403 ACTIVITY OF THE IG KAPPA 3' ENHANCER IS DEVELOPMENTAL STAGE SPECIFIC AND COMPENSATES FOR LOSS OF INTRON ENHANCER FUNCTION, Michael L. Atchison and Jagan M. R. Pongubala, Dept. of Animal Biology, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA 19104.

The activity of the immunoglubulin kappa gene intron enhancer is controlled by factor NF-κB. In pre-B cells, which lack functional NF-κB, the intron enhancer is inactive. At later developmental stages (B cell and plasma cell) functional NF-κB appears and the kappa locus becomes constitutively active. One plasmacytoma cell line, S107, lacks NF-κB and therefore does not express transfected genes controlled by the kappa intron enhancer. However, the endogenous kappa genes in S107 cells remain active. We show here that the recently identified kappa 3' enhancer [EMBO J. 8:1959 (1989)] can compensate for loss of intron enhancer function in S107 cells. However, this 3' enhancer cannot compensate for lack of NF-κB at the pre-B cell stage. Our results demonstrate that the kappa 3' enhancer is a stage specific enhancer, active at the B cell and plasma cell stage, but inactive at the pre-B cell stage.

E 404 RETROVIRAL INSERTIONS IN THE CB-1/FIM-3 LOCUS ACTIVATE TRANSCRIPTION FROM THE EVI-1 PROMOTER, Christopher Bartholomew and James N. Ihle, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38105.

Retroviral insertions in the Evi-1 common site of integration in myeloid leukemias have been shown to activate the expression of a putative transcription factor of the zinc finger family of proteins. Consistent with this, antisera against bacterially expressed Evi-1 protein uniquely detects a 145 kDa nuclear, DNA binding protein in myeloid cells in which the gene is activated. We have previously shown (Bartholomew, et al., Oncogene 4:529-534, 1989) that the retroviral insertions in the CB-1/Fim-3 locus also result in the expression of the Evi-1 gene. To determine the mechanisms of activation, we have established the physical linkage between the two loci and determined the promoter usage in cells with proviruses in the CB-1/Fim-3 locus. By chromosomal walking, we found that the CB-1/Fim-3 locus is 90 kb 5' to the Evi-1 site of viral integrations and the most 5' exon identified to date. All the viral insertions are in the opposite orientation relative to the gene suggesting that activation is not due to a promoter insertion mechanism. Primer extension and cDNA cloning were used to show that the 5' end of the transcripts were derived from an additional 620 bp of the 5' exon. S1 nuclear protection studies with genomic probes from this region reveal multiple transcription initiation sites. The same pattern of transcription initiation sites are seen with RNA from kidney and ovary where the Evi-1 gene is normally expressed. These results demonstrate that retroviral insertions 90 kb from the Evi-1 gene activate expression from the normal promoter and support a model in which the proviruses act as enhancer elements.

E 405 **A COMMON REGULATORY ELEMENT SHARED BY THE MUSCLE AND BRAIN CREATINE KINASE GENES,** Pamela A. Benfield, Robert A. Horlick, James Patterson, Mark T. Mitchell and Grace M. Hobson, Central Research & Development Department, E.I. Du Pont de Nemours & Co., Inc., P.O. Box 80328, Wilmington, DE 19880-0328.

The two cytoplasmic isoforms of creatine kinase are expressed differentially in different tissues. The muscle isoform (CKM) is expressed in skeletal muscle whereas the brain isoform is expressed in a wide variety of tissues including smooth (but not skeletal) muscle. Both isoforms are coexpressed in cardiac tissue. We have purified and characterized a factor, TARP-1 (TA rich recognition protein) isolated from HeLa cell nuclei and which binds to a positive cis-acting regulatory element in the CKB promoter. TARP-1 also binds to two sequences in the 5' muscle-specific enhancer of the CKM gene and thus may represent a factor important for regulation of both brain and muscle creatine kinase genes.

E 406 IDENTIFICATION OF THE Egr-1 GENE PRODUCT, A ZINC FINGER PROTEIN INDUCED BY GROWTH FACTORS AND DIFFERENTIATION SIGNALS. Xinmin Cao[1], Raymond A. Koski[2], Martin McKiernan[2], Andrea Gashler[1], Robert Gaffney[1], Charles F. Morris[2], Rick V. Hay[3], and Vikas P. Sukhatme[1]. Department of Medicine[1], Molecular Genetics and Cell Biology[1], and Pathology[3], Howard Hughes Medical Institute[1], University of Chicago[1,3], Chicago, IL 60637 and Molecular and Cellular Biology Department, Amgen, Inc.[2], Thousand Oaks, CA 91320.

The mouse Egr-1 gene is an immediate-early response gene induced by diverse signals that initiate growth and differentiation. Its cDNA sequence predicts a protein with zinc fingers. We have generated a rabbit antiserum directed against an Egr-1 fusion protein. A 80kd protein has been identified in serum-stimulated mouse fibroblasts by using either the unpurified or affinity column purified rabbit antiserum. This rabbit antiserum also specifically recognizes the Egr-1 gene product in cells transfected with Egr-1 cDNA and gene constructs. Egr-1 protein is serum-inducible in murine fibroblasts with peak levels at 1-2 hours following stimulation. The rat homologue has also been identified in rat PC12 cells. In addition, we show by cell fractionation and immunocytochemistry that the Egr-1 protein is located in the nucleus. We also demonstrate that the protein is phosphorylated and binds to DNA.

E 407 A C/EBP-LIKE TRANSCRIPTIONAL FACTOR THAT BINDS TO THE UPSTREAM SEQUENCES OF MOUSE α1-ACID GLYCOPROTEIN GENE, Ching-Jin Chang and Sheng-Chung Lee, Institute of Biological Chemistry, Academia Sinica, P.O. Box 23-106, Taipei, Taiwan

For study the regulation of expression of mouse α1-acid glycoprotein (α1-AGP) gene, we have characterized the DNA binding factors that interact with the regulatory region of this gene. cDNA clones corresponding to one of the DNA binding factors, AGP/EBP, have been isolated. It shares high degree of homology in the DNA binding domain and the "leucine zipper" region with C/EBP. From the result of in vitro transcription assay, we conclude that this protein is a positive transcriptional factor. The relative binding affinity of AGP/EBP with sequences derived from a number of liver genes (including the motif recognized by C/EBP) together with the structure relationship between AGP/EBP and C/EBP in the DNA binding domain, suggest that a family of C/EBP-like DNA binding transcription factors exists.

E 408 ENFORCED EXPRESSION OF c-myc IN MURINE MYELOID CELLS REGULATES CELL CYCLE AND THE TRANSCRIPTION OF THE ORNITHINE DECARBOXYLASE GENE, John L. Cleveland, Brenda C. Simmons and David S. Askew, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38105.

We have investigated the regulation and function of the c-myc proto-oncogene in Interleukin-3 (IL-3) mediated growth and signal transduction. In murine myeloid cells IL-3 regulates the transcription of several genes, some rapidly such as c-myc, c-pim, c-fos and jun B, whereas others such as ornithine decarboxylase (ODC) are delayed and require protein synthesis. We and others have previously demonstrated that two of these genes, c-myc and ODC, are absolutely required for growth of these cells. In this study we report that one of the functions c-myc may provide to promote cell growth is by regulating the transcription of ODC and concomitant DNA synthesis. Introduction of a retroviral vector expressing c-myc into two different myeloid cell lines replaces the need for IL-3 for ODC mRNA expression. Since ODC mRNA half life remains unaltered in c-myc virus infected clones the regulation of ODC by exogenous c-myc appears to be at the level of transcription. This level of control is being confirmed using nuclear-run assays and by testing ODC promoter CAT constructs. Exogenous c-myc also has dramatic effects upon cell cycling: In the absence of IL-3 control cultures rapidly become blocked in G1 whereas c-myc clones progress through S phase and then exit the cell cycle. These results then suggest that c-myc functions in the cell cycle by controlling transcription of a set of genes required for the cell to enter S phase.

E 409 HNF-1 SHARES SEQUENCE MOTIFS WITH THE POU DOMAIN CONTAINING TRANSCRIPTION FACTORS. Pamela B. Conley, S. Baumheuter, Dirk Mendel, C.Kuo, C. Turk, M.Graves, G.Courtois, Cynthia Edwards* and G. Crabtree, Howard Hughes Medical Institute, Beckman Center/CMGM, Stanford University School of Medicine, Stanford, CA 94305 and *GeneLabs Inc., Redwood City, CA.

Hepatocyte Specific Nuclear Factor 1 (HNF-1) is a transcriptional activator important for the transcription of over 12 liver-specific genes, and is postulated to play a role in establishing the hepatocyte phenotype. Using degenerate oligonucleotides derived from partial amino acid sequences from affinity purified HNF-1, both partial and full-length cDNAs encoding rat HNF-1 were isolated by screening a λ gt10 library with a 132bp probe generated by polymerase chain reaction of poly A(+) rat liver RNA. Sequence analysis of these cDNAs indicate that HNF-1 is a member of the homeobox protein family and shares two short sequence motifs (the acidic and basic regions) with the POU domain proteins Oct-1, Oct-2, Pit-1 and *unc* 86. The highest degree of sequence homology is centered around the WFANRR motif in the third helix of HNF-1, which differs by a single amino acid from the analogous highly conserved region (WFCNRR) in other POU proteins. In addition, where most POU-specific domains contain and A and B box, HNF-1 is lacking the B box. Analyses of the pattern of expression of HNF-1 in adult rat tissues indicate that HNF-1 mRNA is abundant in liver and kidney and is 4- and 10-fold less abundant in intestine and spleen, respectively. No HNF-1 mRNA could be detected by RNase protection analysis in the C2 dedifferentiated hepatoma cell line nor in somatic hybrids where liver-specific gene expression is extinguished. At present we are attempting to demonstrate HNF-1 binding activity in nuclear extracts and/or transactivation of a cotransfected plasmid containing an HNF-dependent promoter in COS cells by expressing HNF-1 using a T-antigen dependent expression vector.

E 410 DEVELOPMENTAL GENE PRODUCTS AS SEQUENCE SPECIFIC DNA-BINDING PROTEINS

Claude Desplan, Pierre Gönczy, Malini Vashishtha and Jessica Treisman. Howard Hughes Medical Institute, The Rockefeller University, Box 151, 1230 York Avenue, New York, N.Y. 10021, Tel:(212) 570 7965.

Early *Drosophila* development is directed by maternal genes, which define the polarity of the embryo, and a hierarchy of zygotic genes which sets up the segmented pattern. The first zygotic genes to be expressed are the gap genes. Their role is to read and interpret coarse positional information deposited in the egg by the mother and to refine this information by cross-regulatory interactions. The three gap genes which have been analyzed molecularly all contain zinc finger motifs. This homology suggests that they might act as regulators of transcription. We have analyzed the Krüppel (Kr) and Hunchback (Hb) gap gene products and obtained results consistent with this hypothesis. Kr protein produced in *E. coli* is able to bind to the sequence AAGGGGTTAA, while Hb recognizes the consensus sequence ACNCAAAAAANTA. Synthetic repeats of these consensus sequences are also recognized, showing that they are sufficient for binding. We have identified two Kr binding sites which could mediate the repression by *Kr* of the proximal promoter of the *hb* gene. *In vivo*, this promoter drives *hb* expression in an anterior stripe and in the absence of *Kr*, the posterior border of this domain expands into the *Kr* domain. We also show that binding sites for Hb are present upstream of *both hb* promoters. *In vivo* these sites may allow *hb* to influence its own expression.

Over 20 genes involved in development encode homeodomain (HD) containing proteins. The HD includes a domain similar to the helix-turn-helix motif present in many prokaryotic DNA-binding proteins. The HD proteins can be classified on the basis of the sequence of their presumed recognition helix. We have been able to define a very simple rule which determines the specificity of the several classes of homeodomains. The last amino acid of the recognition helix appears to be the necessary and sufficient determinant of the specificity of a homeodomain protein. In particular, we have changed the specificity of Paired to that of another pair-rule gene product, Fushi-tarazu, by replacing a serine by a glutamine at position 9 of the recognition helix. We have also changed its specificity to that of Bicoid, by replacing the same serine by a lysine. Other site-directed mutations in the helix-turn-helix do not further alter the specificity. We have also defined a second binding activity in Paired. This activity is independent of the recognition helix since major disruption of this motif leaves the binding unaffected.

E 411 THE THYROID SPECIFIC TRANSCRIPTION FACTOR TTF-1 CONTAINS AN HOMEO-DOMAIN, Roberto Di Lauro, Stefania Guazzi, Donato Civitareale, Melanie Price, Rainer Frank, Stacy Wiedenmann, European Molecular Biology Laboratory, 6900 Heidelberg, FRG.

The thyroid specific expression of the thyroglobulin promoter correlates with the exclusive presence in thyroid cells of the transcription factor TTF-1. TTF-1 binds at three sites in the thyroglobulin promoter. Mutations at two of the three sites abolish both binding of TTF-1 and promoter activity suggesting that TTF-1 is essential for thyroglobulin promoter function. We have purified TTF-1 from calf thyroids and sequenced a tryptic peptide of the purified protein. A degenerate oligonucleotide , derived from the peptide sequence, has been used to isolate, from a calf thyroid cDNA library, a cDNA clone coding for the amino acids sequence of the tryptic peptide. A rat thyroid cDNA clone, isolated by hybridization with tye calf cDNA insert has been sequenced and shown to code for a protein containing an homeodomain. The cDNA insert hybridizes to a thyroid specific mRNA and codes for a protein that displays the same binding properties of the protein purified from the thyroid.

E 412 TRANSCRIPTIONAL REGULATION OF THE ADULT *ADH* GENES, Dean A. Falb and Tom Maniatis, Harvard University, Cambridge, MA 02138.

The promoter of the Drosophila mulleri *Adh-2* gene and the distal promoter of the Drosophila melanogaster *Adh* gene are both activated in a tissue specific manner in the adult fly. We have localized tissue specific enhancer elements in the 5' flanking regions of each of these genes through P-element transformation experiments. Both of these enhancers behave as fat body specific enhancers in association with an *hsp70* promoter / *lacZ* gene fusion. Nuclear extracts prepared from adult nurse cells and whole adult flies contain a factor, AEF-1, which binds to a common sequence element within these enhancers. AEF-1 also binds to sequences in the Drosophila *Yolk Protein* gene fat body enhancer. Mutation of the AEF-1 binding site prevents the binding of AEF-1 *in vitro* and results in a reduced level of transcription *in vivo*. To further investigate the role that AEF-1 plays in the tissue specific and temporal expression of *Adh* genes, we screened a bacteriophage λ expression cDNA library with a multimerized AEF-1 binding site probe. We isolated a cDNA which encodes a protein containing 4 zinc fingers. When protein from this cDNA is produced in bacteria, it gives the same methylation interference pattern on the *Adh* adult enhancer as AEF-1 from nuclear extracts.

E 413 THE EFFECT OF DUPLICATIONS IN THE A ENHANCER DOMAIN OF POLYOMA VIRUS IN TISSUE CULTURE AND IN THE MOUSE. Michele M. Fluck, Ming Shu Chen and Andrea Amalfitano Microbiology, Michigan State University, East Lansing, Mi. 48824-1101.

We have been studying hr-t mutants of polyoma virus which lack a functional middle T-antigen. All strains studied so far display a rearranged enhancer. The rearrangement patterns are varied, but with a common feature, that is the duplication (1-3 times) of the A enhancer domain. This is sometimes accompanied by deletions of adjacent regions (in the B enhancer domain) which are presumed to be compensatory. In 10 of 11 cases studied, the rearrangement of the enhancer confers a cis-acting growth advantage. This is best seen in mixed infections in which the mutants outcompete a wild type strain containing the A2 enhancer (one copy of the A and one copy of the B enhancer domains), leading to a decrease in viral DNA replication in permissive mouse cells or inhibition of transformation in nonpermissive rat cells. The enhancer mutations are responsible for the strong competition observed with these strains, since competition is reduced when middle T-antigen defective mutants without rearranged enhancers are used in competition with the A2 wild type, or when hr-t mutants are used with a wild type which itself contains a rearranged enhancer. Thus, the inhibition may be caused by competition for a limited factor. Given that all mutants carried through the same isolation protocol have evolved with similar rearrangements, we believe that these rearrangements represent a compensatory mutation for the absence of middle T-antigen. Since middle T activates AP1 mediated transcription and the reiterated A domain contains an AP1 binding site, it appears that AP1 is a likely candidate for the effect. These altered enhancers have been introduced in wild type strains and used to infect mice. The pattern of viral replication and persistence are altered and will be reported.

E 414 ANALYSIS OF THE ACTIVITY OF THE HUMAN GM-CSF GENE PROMOTER IN HUMAN LEUKEMIA CELL LINES. John K Fraser, Judith C. Gasson, Juan J. Guerra, Chi Y. Nguyen, and Stephen D. Nimer. Division of Hematology-Oncology, Dept of Medicine, UCLA School of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

Human Granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the proliferation and maturation of myeloid progenitor cells and enhances the functional activity of mature neutrophils, macrophages, and eosinophils. Expression of GM-CSF is tightly regulated and is restricted to activated T-lymphocytes and certain cytokine-activated fibroblast, endothelial, and macrophage cell types. We have previously demonstrated that inducible enhancement of gene expression in T-lymphoblast cell lines is mediated by a promoter region immediately 5' of the cap site and that this region is the site of DNA:protein interactions in both stimulated and unstimulated cells.

In the present study we have examined the ability of the human GM-CSF gene promoter region to drive the expression of the reporter gene chloramphenicol acetyl transferase (CAT) in human leukemia cell lines by electroporation with GM-CSF promoter/CAT constructs and assay for CAT activity at 24 hours. Our results show that the T-lymphoblast cell line Jurkat and the chronic myeloid leukemia cell line K562 exhibit no constitutive promoter activity using a construct extending to 626bp upstream of the cap site. By contrast, the B-lymphoblast cell line 729 and the AML cell line KG-1 show significant CAT production with this construct. Deletion of sequences from the 5' end of this plasmid increased CAT expression in all cell types indicating the presence of elements capable of suppressing promoter activity. The nature of these negative regulatory elements is currently being examined by site-directed mutagenesis.

E 415 A POTENTIAL METAL-BINDING REGION UPSTREAM OF A HOMEODOMAIN IN THE *C. ELEGANS* CELL LINEAGE GENE *lin-11*, Freyd, G., Kim S., and Horvitz, R., Dept. Biology, MIT, Camb., MA 02139

Mutations in the gene *lin-11* cause certain cells involved in the development of the vulva of the nematode *Caenorhabditis elegans* to divide symmetrically instead of asymmetrically. This gene functions to allow a particular blast cell type to produce daughter cells of two different types rather than of the same type. We cloned the gene by identifying a transposon-insertion allele of *lin-11*. The transposon was shown to map to *lin-11* and to cause the loss of *lin-11* function (two spontaneous revertants lost the transposon insertion).

A putative *lin-11* cDNA has been sequenced and found to encode a homeodomain that is very similar to that of *mec-3*, a gene involved in the development of the *C. elegans* touch neurons (Way and Chalfie, *Cell* **54**, 5, 1988). Upstream of the homeodomain in *lin-11* are two repeats of a cysteine- and histidine-rich motif of the sequence $CxxCx_{17\text{-}19}HxxCxxCxxCx_{16\text{-}20}C$ (where x represents an amino acid residue). Two repeats of this motif are also found in the genes *mec-3* and Isl-1, the latter of which encodes a protein from rat that binds the insulin enhancer region and has a homeodomain most similar to that of *lin-11* (T. Edlund, personal communication). In addition, one copy of this motif is found in a cysteine-rich intestinal protein (CRIP) cloned from rat (Birekenmeier and Gordon, *PNAS* **83**, 2516, 1986).

The cys/his region defines a novel protein motif. It is reminiscent of known metal-binding regions that contain somewhat similar configurations of cysteine and histidine residues. Interestingly, so far this motif has been found in association with only a particular type of homeodomain. Like other motifs associated with homeodomains, such as POU and the paired box, the cys/his region seems likely to function either in protein/protein interactions or in protein/DNA interactions.

E 416 MYOGENIN EXIBITS DISTINCT FEATURES OF MYOGENIC ACTIVITY FROM MYOD1, Atsuko Fujisawa-Sehara, Yoko Hosoda, Yoko Nabeshima, Yo-ich Nabeshima, Division of Molecular Genetics, National Institute of Neuroscience, NCNP, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187, Japan.

Using a synthetic oligonucleotide corresponding to the myc homology region of MyoD1 as a probe, we isolated MyoD1 and a related cDNA. Although the latter was found to be mouse myogenin, carboxyl terminal 70 amino acids predicted from the cDNA was different from those reported previously (Wright et al.,1989; Edmondson et al.,1989). A conserved domain was found in this region among mouse myogenin predicted from our cDNA, MyoD1 and human Myf5. In order to compare the myogenic conversion caused by the expression of myogenin and MyoD1, the transformants which express exogenous myogenin or MyoD1 constitutively were isolated. We show the evidence that myogenin exhibits distinct features of myogenic activity from MyoD1. Myogenin was found to convert 10T1/2 cells to "immature" myoblasts.

E 417 c-fos EXPRESSION AND PROLIFERATION INDUCED BY ras REQUIRE C-KINASE ACTIVATION AND A SERUM RESPONSE ELEMENT PATHWAY, Cécile Gauthier-Rouvière, Anne Fernandez and Ned J.C. Lamb,Cell Biology,CRBM-CNRS,INSERM, B.P.5051, 34033 Montpellier Cedex France
It is likely that the ras oncoproteins are regulatory proteins controlling a critical aspect of cell proliferation and we are investigating the molecular mecanisms by which ras oncogene expression causes the malignant transformation of cells. Microinjection of the ras oncogene protein into quiescents fibroblasts induces the rapid expression of c-fos and transiently stimulates DNA synthesis and cell proliferation. It is believed that the 5' region of the c-fos gene contains, among promotor regions, the serum response element (SRE), a short sequence of dyad symmetry which bind a protein factor, SRF, that is required for induction of c-fos transcription by serum, mitogens and activators of protein kinase C. In order to assess if c-fos induction by ras microinjection also required the activation of SRE, double-stranded DNA oligonucleotides corresponding to SRE or mutated SRE were microinjected together with the ras oncogene protein into quiescent cells. We found that cells injected with the ras protein plus SRE showed a very low background level of nuclear fos immunofluorescence similar to that observed in uninjected cells whilst coinjection of mutated SRE did not alter the level of fos expression induced after ras injection. This result indicates that SRE can inhibit c-fos expression induced by ras. In addition, coinjection of the ras protein with SRE into quiescent cells inhibited the incorporation of 5-Br-deoxyuridine normally observed in cells injected with ras alone. Moreover, we demonstrate that such inductive effects of ras are dependent on activation of functional calcium/phospholipid-dependent protein kinase C (C-kinase). By microinjection of an inhibitory peptide which specifically inhibits C-kinase, or anti-C-kinase antiserea, both ras induced c-fos expression and DNA synthesis can be inhibited. These results emphasize the importance of ras protein in the control of proliferation through generation of second(s) messenger(s) that ultimately act within the nucleus to promote changes in gene expression. Indeed, it seems clear that a connection existes between ras activity within cells and c-fos expression through SRE/SRF.

E 418 STRUCTURE AND EXPRESSION OF MURINE GERM-LINE IMMUNOGLOBULIN ε HEAVY CHAIN TRANSCRIPTS INDUCED BY INTERLEUKIN 4, Steve Gerondakis, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, 3050.
The murine lymphokine, interleukin 4 [IL4] is able to specifically promote isotype switching to IgG1 and IgE in cultures of mitogen stimulated B cells. Emerging evidence suggests that germ-line immunoglobulin heavy chain gene transcription may direct switching by modulating switch region accessibility to a recombinase. In this study, cloned cDNA copies of the germ-line ε transcript have been used to determine the genomic organisation of this transcription unit. The 5' end of these transcripts are derived from an exon, denoted Iε, located 2 kb 5' of the Cε switch region. Nucleotide sequence analysis reveals that this RNA does not encode a protein, as the Iε exon contains termination codons in all reading frames. Germ-line ε transcripts can be detected in cultures of normal splenic B cells treated with IL4 within 24 hours, and this expression correlates with subsequent switching to Cε. Consistent with the IL4 inducibility of this RNA is the identification of a motif upstream from the site of transcription initiation that closely resembles a transcription element implicated in the IL4 regulation of the gene encoding the murine class II histocompatibility antigen, $A\alpha^k$. These data lend support to the accessibility model of isotype switching and implicate IL4 in the transcriptional activation of the Cε locus.

E 419 c-fos REPRESSES IMMEDIATE EARLY GENE EXPRESSION VIA CArG ELEMENTS. David Gius, Xinmin Cao, and Vikas P. Sukhatme. Department of Molecular Genetics and Cell Biology, Department of Medicine, and the Howard Hughes Medical Institute,University of Chicago, Chicago, IL 60637.

Several genes including c-fos, c-jun, Egr-1 and Egr-2 are rapidly induced and quickly repressed following mitogenic stimulation. Little is known about the mechanism by which these immediate early genes are down-regulated. In transient assays, we show that c-fos regulates expression of Egr-1, Egr-2 and c-fos. Using deletion mutants of the Egr-1 promoter region, we demonstrate that the target sequence for this negative regulation is a CArG box (sequence of the form $CC(A/T)_6GG$). Finally, using synthetic oligomers, we have determined that CArG sequences derived from Egr-1 and c-fos confer both serum inducibility and fos-directed repression to a heterologous promoter. Our data indicates that c-fos plays a key role in the negative feedback of immediate early gene expression and that this function may be independent of interaction with the c-jun gene product.

E 420 INCREASED EXPRESSION OF SP1 AND NFκB TRANSCRIPTIONAL ENHANCER ELEMENTS IN K562 CELLS TRANSFECTED WITH THE MYELOID-SPECIFIC c-*fes* TYROSINE KINASE GENE, Robert I. Glazer, Flavia Borellini, Angelo Aquino, Gang Yu, and Steven Josephs[1], Laboratory of Biological Chemistry and Laboratory of Tumor Cell Biology[1], National Cancer Institute, Bethesda, MD 20892

When the immature myeloblast cell line K562 is transfected with the human genomic c-*fes* gene,they acquire characteristics of more mature granulocytic cells and the ability to undergo differentiation. To explore the role of the c-*fes* tyrosine kinase in the differentiation process, we prepared nuclear extracts from K562 cells stably transfected with the 13.2 kbp genomic c-*fes* sequence (clone WS-1). Southwestern blotting was performed using a 640 bp 5'-LTR region of HIV-1 containing binding sequences for the transcription factors SP1 and NFκB. Nuclear extracts from both wild type and transfected cells revealed the presence of a 100 kDa DNA binding protein, which was significantly more abundant in clone WS-1. Mobility shift experiments indicated that the DNA binding activity was completely competed by a synthetic oligodeoxynucleotide coding for an SP1 binding consensus sequence. DNase footprinting revealed protection of two of the three SP1 binding sites in the HIV-1 LTR, and additional enhanced protection of the NFκB binding sequences in WS-1 cells, even though NFκB binding proteins were not detectable in Southwestern blots. Therefore, it appears that the response evoked by the c-*fes* tyrosine kinase leads to enhanced expression of transcriptional regulatory factors associated with the appearance of the mature myeloid phenotype.

E 421 TRANS-ACTIVATION OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR AND THE INTERLEUKIN-2 RECEPTOR IN TRANSGENIC MICE CARRYING THE HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 TAX GENE, Jeffrey E. Green,[1] C. Glenn Begley,[2] David K. Wagner,[2,3] Thomas A. Waldmann,[2] and Gilbert Jay,[4] Laboratory of Molecular Virology[1] and Metabolism Branch[2], National Cancer Institute, Bethesda, Maryland 20892; Westside Veterans Administration Medical Center, Chicago, Illinois 60612[3], and Laboratory of Virology, Jerome H. Holland Laboratory, American Red Cross, Rockville, Maryland 20855[4]

Three lines of transgenic mice carrying the human T-cell lymphotropic virus type 1 tax gene have previously been reported to develop neurofibromas composed of perineural fibroblasts. Tumors from these mice and tumor cell lines derived from them expressed high levels of tax RNA and protein. They also expressed high levels of the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene as measured by proliferative responses and by Northern (RNA) blot analysis. Although other tissues, in the transgenic mice also expressed high levels of tax, they did not express the gene for GM-CSF. This indicates that tissue-specific cellular factors, in addition to tax, are required for GM-CSF gene expression. Systemic effects of excessive GM-CSF production were demonstrated. The interleukin-2 (IL-2) receptor was also found to be expressed by the tumors and tumor cell lines as measured by IL-2-binding and cross-linking studies. This is the first demonstration that the IL-2 receptor can be activated by tax in a nonlymphoid cell type. These in vivo findings are consistent with other reports which have demonstrated in vitro cis-regulatory elements within the 5'-flanking regions of the genes for GM-CSF and the IL-2 receptor which are responsive to trans-activation by the tax gene.

E 422 CELLULAR TRANSCRIPTIONAL ACTIVATORS MEDIATING THE T-LYMPHOMAGENICITY OF A MURINE RETROVIRUS, Bengt Hallberg, Anders Thornell, Pia Nilsson, Brit Corneliussen and Thomas Grundström, Department of Applied Cell and Molecular Biology, University of Umeå, S-901 87 Umeå, Sweden.

We have identified the binding sequences on the DNA for proteins which together mediate most of the transcriptional activity of a murine leukemia virus, SL3-3, in vivo in T cells where the enhancer is preferentially active. The SL3-3 enhancer factor 1 (SEF1) proteins where found to recognize two different DNA sequences within the enhancer. Mutation in either site resulted in decreased transcriptional activity *in vivo* preferentially in T cells. The nucleotide specificity for SEF1 binding and results of SEF1 purification will be presented.

SL3-3 enhancer factor 2 (SEF2) proteins were found to interact specifically with another sequence of the enhancer. Mutant analysis separated the SEF2 proteins into two groups which show different nucleotide sequence requirements for DNA binding. cDNA cloning of different SEF2 proteins shows that they belong to the E-box family of proteins. Characterisation of the cDNA clones and SEF2 proteins produced in *E. coli* will be presented.

SL3-3 enhancer factor 3 (SEF3) and Nuclear factor I (NFI) site binding proteins were also found to bind different sequences in the enhancer and to show different degrees of importance for its activity in different cell types. Histone H1 was shown to be an NF1 site binding protein whose interaction with the DNA was modulated by its phosphorylations.

E 423 CREB-MX AND c-JUN FORM A COMPLEX WHICH BINDS TO THE CYCLIC-AMP, BUT NOT TO THE TPA RESPONSE ELEMENT, Lionel B. Ivashkiv, Hsiou-Chi Liou, Catherine J. Kara, William W. Lamph, Inder M. Verma and Laurie H. Glimcher, Department of Cancer Biology, Harvard School of Public Health, Boson, MA 02115 and Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, CA 92138. Proto-oncogene products c-Fos and c-Jun form a complex which binds with high affinity to the TPA-response DNA element (TRE) and stimulates transcription of phorbol ester inducible genes. We have identified, by screening a λgt11 expression library, protein CREB-MX, which contains a leucine zipper domain and binds to the cyclic-AMP response DNA element (CRE). Immunoprecipitation and glutaraldehyde crosslinking studies show that CREB-MX forms a complex with c-Jun. Complex formation is dependent upon intact leucine zipper domains in both proteins. CREB-MX-c-Jun complexes can co-exist with c-Fos-c-Jun complexes and bind with high affinity to CRE, but not to TRE sequences. These results suggest that changes in expression of CREB-MX, c-Fos, and c-Jun which alter the ratio of CREB-MX-c-Jun to c-Fos-c-Jun complexes would affect the relative expression of c-AMP and phorbol ester inducible genes.

E 424 EGF AND TPA MEDIATED INDUCTION OF UROKINASE PLASMINOGEN ACTIVATOR mRNA SYNTHESIS IN GROWING MURINE KERATINOCYTES

Morten Johnsen, Pernille Rørth and Francesco Blasi, Institute of Microbiology, University of Copenhagen, DENMARK.

In vivo, keratinocytes exhibit continous and controlled growth. We have investigated the synthesis of urokinase plasminogen activator mRNA in exponentially growing cultures of primary murine keratinocytes and keratinocyte cell lines BALB/MK and 308. The tumor promotor 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) induced urokinase mRNA synthesis. We made a series of progressive 5' deletions as well as internal deletions of murine uPA gene upstream DNA. These were joined with *lacZ* and *cat* reporter genes, and used to map the TPA responsive region of the promoter. We found the TPA-responsive sequence to reside within a 90 base pair Hae III fragment. The fragment is located 2.4 kb. upstream of the mRNA cap site and conferred TPA inducibility on reporter gene expression independent of its distance and orientation to the transcription initiation site. The same fragment was also necessary and sufficient for induction of uPA mRNA synthesis after EGF addition to the cells. We sequenced the DNA, and did not find any AP-1 consensus sequence in the responsive fragment. Analysis in cells with high expression of the *ras*-oncogene showed slightly elevated reporter enzyme levels from uPA-*cat* constructs, but no change in the induction after TPA addition.

E 425 MYRISTYLATION OF FBR v-FOS PROTEIN IS RESPONSIBLE FOR ITS DEFECTIVE TRANSCRIPTIONAL REGULATION AND DNA BINDING ACTIVITY, Nobuyuki Kamata, Robert Jotte and Jeffrey T Holt, Cell Biology Department, Vanderbilt University, Nashville, TN 37232

Although both cellular and molecular functions of c-fos protein are well studied, functions of v-fos proteins are not understood. c-fos expression is known to repress the c-fos promoter/CAT activity (negative autoregulation) and to activate TRE/ CAT when cotransfected with c-jun. In vitro translated c-fos protein shows strong DNA binding activity to TRE sequence when mixed with equal amounts of c-jun protein. However, we found that FBR v-fos protein showed decreased transcriptional activity and exhibited much less DNA binding activity than that of c-fos protein, despite equivalent heterodimerization with c-jun protein. Furthermore, this decreased activity of FBR protein was transdominant because it inhibited Fos-Jun DNA binding and transcriptional effects of c-fos protein. Mutational analysis of FBR protein revealed that the N-terminal gag region is responsible for decreased DNA binding activity: 1) Deletion of this region restored DNA binding activity and 2) transferring gag to c-fos protein inhibited binding activity. Finally, we demonstrated that FBR protein is myristylated at the N-terminal glycine (the usual site for myristylated proteins). A mutant FBR protein (alanine instead of N-terminal glycine) was no longer myristylated, and regained not only DNA binding activity in vitro but also repression of the c-fos promoter in transient assays. These results suggest that FBR protein is functionally defective and that protein myristylation contributes to its cellular and molecular functions.

E 426 THE BREAKPOINT IN (1;19)(q23;p13) TRANSLOCATIONS OF PRE-B CELL LEUKEMIAS FUSES THE DNA-BINDING DOMAIN OF A NEW HOMEOTIC GENE TO THE 5' HALF OF THE KE2 GENE.

Mark Kamps, Cornelius Murre, and David Baltimore. Whitehead Institute for Biomedical Research, Cambridge, MA 02142

The breakpoint of the (1;19)(q23;p13) transversion that typifies 30% of pre-B cell ALL has been shown to occur within the coding sequence of the KE2 gene, which encodes a transcription factor that binds to the KE2 site of the immunoglobulin kappa chain enhancer. From 697 cells, which contain the t(1;19)(q23;p13), we have cloned a transcript that is the apparent product of the transcriptional unit at the breakpoint. The transcript contains 5' sequences that encode the aminoterminal portion of the E2A protein until the sequences that encode the basic/helix-turn-helix DNA-binding portion are reached. At this point, the RNA sequence diverges from that of KE2 and rather encodes approximately 250 amino acids of a unrelated protein that contains a homeobox. The homeotic sequence is distinct from any that has been reported and is most closely related to the yeast A1 gene product. These data suggest that the protein encoded by the fusion transcript will have the transcriptional activity of the E2A protein and the DNA-binding specificity of the homeotic protein. Work is now underway to determine the biological activity of the protein encoded by the fusion transcript.

E 427 A cDNA FOR A HUMAN CRE-BINDING PROTEIN WHICH IS DISTINCT FROM CREB AND EXPRESSED PREFERENTIALLY IN BRAIN, Catherine J. Kara, Hsiou-Chi Liou, Lionel B. Ivashkiv and Laurie H. Glimcher, Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115

The cyclic AMP response element (CRE) is found in many cellular genes regulated by cAMP, and similar elements are present in the early genes of adenovirus which are activated by E1A. We have isolated a cDNA encoding a human DNA-binding protein which recognizes this motif in cellular and viral promoters. This cDNA is distinct from that of the transcription factor CREB, which also recognizes this motif, thereby demonstrating that the CRE is the target of more than a single DNA binding protein. The protein encoded by this cDNA, HB16, contains a highly basic, putative DNA binding domain, and a leucine zipper structure thought to be involved in dimerization. Deletional analysis of HB16 demonstrates that the leucine zipper is required for its interaction with DNA. Despite its structural similarity to the TRE-binding proteins c-Jun and c-Fos, HB16 has approximately a 10-fold lower affinity for the related TRE sequence than for the CRE sequence. Although HB16 and CREB both recognize the CRE motif, an extensive binding analysis of HB16 reveals differences in the fine specificity of binding of the two proteins. HB16 mRNA is found at varying levels in many human tissues, but is most abundant in brain, where its expression is widespread. Genomic Southern analysis demonstrates that the HB16 gene is strongly conserved throughout evolution. The existence of more than one CRE binding protein suggests the CRE motif could serve multiple regulatory functions.

E 428 **Multiple Doses of Diacylglycerol and Calcium Ionophore are Necessary to Activate AP-1 Enhancer Activity and Induce Macrophage Differentiation** Andrew S. Kraft*, Fred Wagner*, Michael Karin†, and Felicia William*, Division of Hematology/Oncology*, University of Alabama in Birmingham, Birmingham, Alabama35294, and the Department of Pharmacology†, University of California at San Diego, La Jolla, California 92093 Diacylgycerols are important intracellular second messengers. Increases in diacylglycerols and intracellular calcium induced by the binding of polypeptide growth factors activate protein kinase C and stimulate the transcription of specific gene products. Like diacylglcyerols, phorbol esters bind to the regulatory portion of protein kinase C. Regulation of gene transcription by phorbol esters has been shown recently to involve the induction of c-*jun* and c-*fos* proto-oncogenes, the dimerization of Jun and Fos proteins, and the binding of this dimer to the AP-1 enhancer sequence, 5'-TGAGTCA-3'. which then stimulates gene transcription. To examine whether diacylglycerols also modulate gene transcription through this mechanism, we compared the effects of diacylglcyerols and phorbol esters on U937 human monoblastic leukemia cells which are known to be induced to differentiate by these agents. We found that although a single dose of diacylglycerol (DiC8) induces elevations in both c-*jun* and c-*fos* RNA, unlike phorbol esters, it neither activates the AP-1 enhancer to stimulate transcription nor induces the differentiation of these cells. In contrast, multiple doses of DiC8 given at two-hourly intervals stimulate (1) repeated elevations in the messenger RNA levels for these proto-oncogenes, (2) increases in Jun protein, (3) activation of transcription from the AP-1 enhancer element and (4) the induction of markers of differentiation. This suggests that activation of this transcriptional response element may play a key role in hematopoietic differentiation.

E 429 PHENOTYPIC DOMINANCE AND EXTINCTION OF GENE EXPRESSION IN MURINE MYELOMAxFIBROBLAST SOMATIC CELL HYBRIDS. Laskov, R[1]., Sharir, H[1]., Streich, B[1]., Ber, R[2]., Zaritsky, A[3] and Bergman, Y[1]. [1]Hebrew University-Hadassah Medical School, Jerusalem, 91010. [2]The Rappaport Institute for Research, Haifa, 31096. [3]Ben Gurion University, Beer Sheva, 84105. We have studied the mechanism of gene extinction and activation in a series of somatic cell hybrids between mouse myeloma and fibroblasts. All the hybrids were adherent and have a fibroblast like phenotype. Molecular analyses revealed that plasma cell specific genes like the productively rearranged Ig genes, the mouse CD20 and the J chain genes were extinguished in the hybrids. In contrast, fibroblast specific genes like fibronectin and α_2(I) collagen were expressed. Extinction was not due to chromosomal loss or lack of the relevant genes. The dominance of the fibroblast specific traits was confirmed by analysis of the pattern of the secretory proteins and by 2D gel analyses of cytoplasmic proteins of parental and hybrid cells. To learn about the mechanism(s) of this phenomenon we have looked for the presence of B-cell specific transcription factors in our hybrids by DNA gel shift assays. It was found that the NF-kB that binds to the B region of the k-gene enhancer, was present in variable amounts in the nuclear extracts of the hybrids. In contrast, the NFA2/OTF2A factor, known to bind to the octamer motif in the promoters of the Ig genes, was absent in all the hybrids tested. Northern blot analysis using a cDNA probe of the oct-2 gene which codes for the NFA2/OTF2A showed that oct-2 mRNA was absent in the hybrids. Our results indicate that extinction of the Ig genes and probably of other B-specific genes is mediated through an indirect mechanism by extinguishing of a positive B-cell transcription factor.

E 430 DROSOPHILA KRUPPEL IS A TRANSCRIPTIONAL REPRESSOR, Jonathan D. Licht, Martha J. Grossel, James Figge and Ulla M. Hansen, Laboratory of Eukaryotic Transcription, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.
Kruppel (*Kr*), one of the zygotically active segmentation genes controlling *Drosophila* embryonic development is expressed in a restricted domain during the blastoderm stage of *Drosophila* embryogenesis and controls development of the thoracic and abdominal segments as well as the Malphigian tubules of the fly. *Kruppel* encodes a polypeptide with five potential DNA-binding zinc finger structures, disruption of which yields *Kr* mutants. In order to determine whether *kruppel* protein is a transcription factor, the native protein as well as *lac* repressor-*kruppel* fusion proteins were expressed in HeLa cells. Native and chimeric *kruppel* repressed transcription from a herpes simplex virus thymidine kinase promoter-CAT reporter gene containing *kruppel* binding sites >120 bp upstream from the start site of transcription. *Lac* repressor-*kruppel* fusion proteins also repressed transcription from *lac* operator containing reporters. Gel mobility shift assays demonstrated that *lac-kruppel* fusion proteins were produced in human cells and could bind to a synthetic lac operator. Fusions including the zinc finger region of *kruppel* could bind to a radiolabeled duplex *kruppel* binding site. The DNA binding and repression functions of *kruppel* were dissociable. A fusion protein including amino acids 26-213 of *kruppel*, excluding the zinc fingers of *kruppel* repressed transcription from a *lac* operator containing reporter. Extracts from cells expressing this fusion protein bound to the *lac* operator but not to the *kruppel* binding site. A fusion protein including amino acids 213-466 of *kruppel* was able to bind to *kruppel* binding sites but did not repress CAT activity from *lac* operator or *kruppel* binding site containing reporter genes. The N-terminal region of *kruppel* responsible for repression is hydrophobic and rich in alanine. This data is consistant with genetic evidence of repression of adjacent gap gene expression by *kruppel* and suggests that the *kruppel* gene product may be a negative regulator of transcription in *Drosophila*.

E 431 EVIDENCE FOR A STEM CELL-SPECIFIC REPRESSOR OF MOLONEY MURINE LEUKEMIA VIRUS EXPRESSION IN EMBRYONAL CARCINOMA CELLS, Tatjana Loh, Laura Sievert, Richard Scott, E.I. DuPont de Nemours Co, Inc. Central Research and Development, Wilmington, DE 19880. A negative regulatory element (NRE) spans the tRNA primer binding site (tRNA PBS) of Moloney murine leukemia virus (M-MuLV) and represses M-MuLV expression specifically in embryonal carcinoma (EC) cells. By base-pair mutagenesis, we precisely defined the NRE as the 18 bp of the tRNA PBS. Since expression from the M-MuLV promoter is restricted independent of the position and orientation of the tRNA PBS element, we characterize it as a silencer sequence that is preferentially active in mouse EC cells. A DNA binding activity specific for the M-MuLV tRNA PBS sequence is seen in Exonuclease III protection assays using crude nuclear extracts. Binding is strongly correlated with repression in EC cells: mutations within the tRNA PBS that relieve repression also disrupt the binding activity, and nuclear extracts from permissive, differentiated EC cell cultures show reduced binding for the tRNA PBS sequence. These results indicate the presence of a stem cell-specific repressor that extinguishes retroviral expression via the NRE.

E 432 SEQUENCES DOWNSTREAM FROM THE HUMAN FETAL GLOBIN GENE ARE IMPORTANT FOR ITS DEVELOPMENTALLY SPECIFIC EXPRESSION, Nadya L. Lumelsky and Bernard G. Forget, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06510. The human ε-globin gene is expressed in the yolk sac, two γ-globin genes (Aγ and Gγ) are expressed in fetal erythroid cells, and the β-globin gene is expressed mainly in adult erythroid cells. The selective expression of individual genes in the β-globin gene cluster during development appears to be regulated primarily on the level of gene transcription. In this work we undertook the analysis of the human Aγ-globin gene in search of cis-acting regulatory DNA sequences which modulate its tissue and developmental specific expression. Various segments of the Aγ-globin gene coding and 3' flanking sequences were positioned upstream of the γ-globin gene promoter which drives a firefly luciferase (reporter) gene. The constructs containing γ-gene globin sequences were introduced into the erythroid cell line K562, and the expression of the reporter gene in transient and stable transfectants was measured. K562 cells synthesize globin chains of embryonic and fetal origin, but no adult β-globin is observed. Treatment of these cells with hemin results in an increase of the rate of γ-globin transcription (and an increase in fetal hemoglobin production), a process which mimics erythroid differentiation. We were particulary interested in identifying the cis-acting regulatory sequences important for hemin induction of γ-globin gene expression. Our results indicate that the region downstream of the γ-globin gene polyadenylation site plays a role in this process.

E 433 NERVE GROWTH FACTOR INDUCTION OF A HOMEODOMAIN-LIKE PROTEIN IN RAT PC12 CELLS, Susan J. Marriott, Sharon C. Doll, Gordon Guroff* and John N. Brady, Laboratory of Molecular Virology, NCI, NIH, Bethesda, MD 20892, *Section on Growth Factors, NICHHD

Rat PC12 cells offer a model system for studying processes associated with neuronal differentiation. Nerve growth factor (NGF) treatment of these cells induces a differentiation pathway which is reversible upon removal of NGF. A primary site of NGF action on these cells is at the transcription level and involves induction of various genes including c-fos and c-myc. Expression of homeobox-containing genes in mammalian tissues appears to play an important role in regulation of cellular differentiation. It has been suggested that homeodomain-containing proteins mediate their effect on cell differentiation by acting as transcriptional regulators. Our goal was to determine whether homeodomain-containing proteins play a role in neuronal differentiation observed upon NGF treatment of PC12 cells. We synthesized three overlapping peptides containing amino acid sequences conserved within the homeodomain. Polyclonal rabbit antiserum was produced against each peptide and tested for authentic homeobox reactivity against purified Ultrabithorax protein. This antiserum was used to detect the presence of homeodomain-like proteins in nuclear extracts of NGF treated and untreated PC12 cells. Low level expression of a 52 kD protein was detected in nuclear extracts from untreated PC12 cells. Expression of this protein increased to a level 6 times that observed in untreated cells following one day of NGF treatment. The level remained steady through three days of treatment and declined to a level seen in untreated cells by 5 days of NGF treatment. Expression of c-raf, which has previously been shown to be unaffected by NGF treatment, remained constant throughout the course of NGF treatment. We are currently investigating the ability of extracts from treated and untreated PC12 cells to activate transcription in vitro.

E 434 EXPRESSION OF GHF-1 IN PITUITARY DERIVED CELL LINES. Alison McCormick, Helen Brady and Michael Karin. Department of Pharmacology M-036, UCSD, La Jolla, CA, 92093. Expression of growth hormone is largely due to the pituitary specific trans-acting factor GHF-1. Recently cloned and sequenced, GHF-1 has been identified as a homeobox containing protein with sequence homology to human B-cell specific oct-2, yeast mating factor a1 and several drosophila homeobox proteins such as Eve. Prd and IAB-7. Noting the importance of temporal and spatial expression on homeobox containing proteins in determining developmental pattern and cell type specificity, it is of interest to examine what regulates GHF1 expression in the anterior pituitary. The full length GHF1 cDNA was used to screen a rat genomic library, and subsequent positives were rescreened with the 5' end of the cDNA in order to isolate the GHF1 promoter. The promoter region was confirmed with both primer extension and RNase protection assays, sequenced and subcloned in front of the CAT reporter gene. Tissue specificity of the promoter region was analyzed by in vivo transfection and in vitro transcription, and deletion analysis has identified the minimal elements required for maintaining tissue specificity. Footprint analysis of this promoter region clearly shows differences between expressing and non expressing cell lines. Site directed mutagenesis of pituitary specific footprint regions is being used to clearly define cis elements and trans-acting factors involved in the tissue specific expression of GHF1.

E 435 INTRINSIC GROWTH FACTOR EXPRESSION IN NORMAL AND SIMIAN SARCOMA VIRUS TRANSFORMED RAT KIDNEY FIBROBLASTS, Peter G. Milner, Yue-Sheng Li, Charles Kodner, Ruth Hoffman, Melissa Chu, Benton Tong and Thomas F. Deuel, Division of Hematology/Oncology, Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110

The v-sis oncogene of the simian sarcoma virus (SSV) encodes a mitogenic polypeptide with structural and functional near identity to the B-chain of PDGF. When transfected into normal rat kidney (NRK) fibroblasts, the v-sis oncogene induces anchorage-independent growth, soft agar colony formation, and other features of malignant transformation. The expression of other growth factors in SSV-transformed cells has not been analysed and may contribute to the malignant phenotype. Large quantities of NRK and SSV-NRK cells were grown to confluence and cell lysates prepared. The lysates of $4x10^9$ NRK, and SSV-NRK cells were passed over a 40ml heparin-sepharose column and the proteins eluted by applying a 0-2M NaCl gradient. Growth factor activity was assayed by measuring ^{3}H-Thymidine incorporation in NRK cell mitogen assays. The 0.5-1M NaCl fraction of the SSV-NRK cells contained v-sis by Western blot analysis. While there was a two fold change in the growth factor activity in this fraction following transformation, the most significant change was an 18 fold increase in the activity eluting in the 1-2M NaCl range. The only known growth factors which elute in this range are the fibroblast growth factors (FGFs). We have demonstrated expression of basic FGF in SSV-NRK cells by Northern blot analyses, and solid phase ELISA. In addition, at least one other FGF-like growth factor is present in the 1-2M NaCl fraction. These results suggest a potential role for growth factors other than v-sis in transformation by SSV.

E 436 EFFECT OF EVI-1 GENE ON THE DIFFERENTIATION OF THE MYELOID CELL LINE 32DCL3 BY G-CSF, Kazuhiro Morishita, Evan Parganas, Tak Matsugi, and James N. Ihle, Department of Biochemistry, St.Jude Children's Research Hospital, TN 38101

The Evi-1 gene was identified in murine myeloid leukemias by retroviral insertions in Evi-1 or Fim-3/CB-1 common viral integration sites. The sequence of the Evi-1 gene predicts a 120 kd protein with ten repeats of the zinc finger DNA binding domain. Antisera against Evi-1 detect a 145 kd nuclear protein with DNA binding activity. To study the effect of Evi-1 gene expression on myeloid differentiation, we infected a myeloid cell line, 32Dcl3, with a retrovirus containing the Evi-1 gene. 32Dcl3 are dependent in IL-3 for growth and will differentiate into granulocytes in IL-3 free media with G-CSF. The Evi-1 infected cells continued to require IL-3 for growth, and are morphologically and phenotypically identical to control cells. However, these cells did not differentiate into granulocytes in G-CSF, and lost their viability in comparison to parental cells in the absence of IL-3. In addition, Evi-1 infected cells did not express transcripts of the myeloperoxidase (MPO) gene when transfered to G-CSF. In contrast, the control cells express high MPO transcripts after one day in G-CSF. These experiments demonstrate that the aberrant expression of the Evi-1 gene in myeloid cells can interrupt their normal differentiation program.

E 437 SERUM RESPONSE FACTOR (SRF) IS GLYCOSYLATED IN CULTURED FIBROBLASTS AND MOUSE EMBRYOS, **Sun-Yu Ng, Shu-Hui Liu and Bi-Hung Peng, Institute of Molecular Biology, Academia Sinica, Nankang 11529, Taiwan, Republic of China**

SRF is the 67kD protein that interacts specifically with the serum response elements (SREs) found in the promoters of the proto-oncogene c-fos, β- and γ-actin, and other early response genes. The transcriptional stimulation of these genes in response to serum occurs rapidly and is not blocked by protein synthesis inhibitors. Furthermore, SRF is present in quiescent cells and can bind SRE sequences in vitro. Thus the early response gene activation mediated by SRF may involve SRF modification(s). We have investigated the possible modification of SRF with N-acetylglucosamine residues using wheat germ agglutinin affinity chromatography. We found that SRF in proliferating HeLa cells is predominantly glycosylated. Similarly, SRFs from quiescent NIH/3T3 and CHO fibroblasts are also glycosylated. Therefore glycosylation of SRF may not be the primary regulatory mechanism involved in the transcriptional induction of early response genes although possible transient and/or partial deglycosylation has not been ruled out. In contrast, of the multiple SRE-binding complexes found in mouse embryos, only the slowest migrating complex contains glycosylated SRE-binding protein(s) whereas the fast migrating complexes contain unglycosylated SRE-binding proteins.

E 438 ISOLATION OF GENES WHICH ARE INDUCED LATE BY A TUMOR PROMOTER OR EGF IN A MOUSE OSTEOBLATSIC CELL LINE, Kiyoshi Nose, Hiroyuki Saitoh and Toshio Kuroki, Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Minatoku, Tokyo 108, Japan

Growth factors and a tumor promoting phorbol ester, TPA, induce many types of "early response" genes, most of which encode transcriptional factors. To identify possible target genes for these early response gene products, we tried to isolate cDNA clones which are induced late by TPA. cDNA library was constructed from mouse osteoblast cells (MC3T3) treated with TPA for 4 hr, and screened by differential hybridization. Seven independent clones were isolated, and all of them were maximally induced 4 to 8 hr after TPA addition. From sequencing analysis, three of them turned out to be metallothionein II, one was identical to osteopontin and others (OTS-2, -7, and -8) were found to be novel genes. OTS-2, -7, and -8 genes were induced by TPA or EGF, but not by serum or diacylglycerol, and the induction was suppressed by cycloheximide. Among mouse tissues, OTS-2 was expressed in all the tissues examined, OTS-7 was highly expressed in kidney and expressed in many other tissues, while OTS-8 was expressed exclusively in lung. Open reading frame of OTS-8 gene and its transcription/translation product suggested that this gene encode 27 kDa protein which has hydrophobic regions both in N- and C-terminal parts.

E 439 REGULATION OF XENOPUS HISTONE H2B GENE TRANSCRIPTION IN OOGENESIS AND EARLY EMBRYOGENESIS, Michael Perry and Craig S. Hinkley, Department of Biochemistry and Molecular Biology, University of Texas/M.D. Anderson Cancer Center, Houston, TX 77030

Histone gene expression is cell-cycle independent during oogenesis and very early embryogenesis in amphibians; however, it is tightly coupled to DNA synthesis later in embryogenesis and in most somatic cells. To investigate the mechanism of the switch in histone gene transcriptional regulation, we have identified specific regulatory sequences necessary for histone H2B gene transcription in injected Xenopus oocytes and gastrula-stage embryos. Sequence motifs commonly required for H2B transcription in both oocytes and embryos correspond to binding sites for known transcription factors including TFIID, CCAAT binding protein, and ATF/CREB. An octamer-like sequence was not required for H2B transcription in oocytes but was required for maximal transcription in embryos. DNA binding assays revealed the presence of an octamer-binding protein in extracts from oocytes and embryos. These results indicate that interaction of the oocyte octamer binding protein with the H2B promoter is insufficient for transcriptional activation and suggest that subsequent octamer binding protein modifications or interactions are necessary for maximal transcription of H2B in Xenopus embryos. To explore these possibilities, we isolated sequences that encode octamer binding proteins from Xenopus oocyte and embryo cDNA libraries. In vitro expression of a full-length Xenopus oct-1 homologue results in the synthesis of a protein with binding properties that are indistinguishable from the oocyte-derived factor. Antisera are being prepared to determine the distribution of this factor in oocytes and embryos and examine for developmental stage-specific modifications and interactions of this maternal protein.

E 440 FOS-JUN INTERACTION: A PARADIGM FOR TRANSCRIPTIONAL REGULATION, Lynn J. Ransone, Jane Visvader, V.J. Dwarki, Kim Morley, Penny Wamsley and Inder M. Verma, Molecular Biology & Virology Laboratory, The Salk Institute, La Jolla, CA 92037.

Gene expression is modulated by the specific interaction of nuclear proteins with unique regulatory sequences in the genome. Nuclear oncoproteins fos and jun (AP-1) cooperate in forming a very stable heterodimeric complex that binds to the AP-1 site with increased affinity. The "leucine zipper" domain of both fos and jun is necessary for the formation of this heterodimer. We have undertaken a mutational analysis of (1) individual leucine residues, (2) neighboring amino acids within the "leucine zipper" domain, and (3) residues in the highly basic domain of both the fos and jun proteins, to examine the contribution of these amino acids to the formation of a stable fos/jun heterodimer, the formation of jun homodimers and the binding potential of the heterodimeric complex to the AP-1 site. Mutations of single residues within the "leucine zipper" domain had no effect on protein complex formation. However, mutagenesis of the first leucine of the heptad repeat in either fos or jun drastically reduced binding of the complex to DNA. Mutations within the fos and jun basic regions and alteration of the spacing between the basic and "leucine zipper" domains indicate that the basic region of fos has a crucial role in determining the DNA binding affinity of the transcriptional complex. This is further substantiated by making site specific mutations in the basic amino acids in fos protein. We have also generated fos-jun chimeras to access the role of "leucine zipper" in the formation of homodimer. Finally, we will also discuss the role of fos/jun complex in transcriptional transactivation.

E 441 TRANSCRIPTIONAL ACTIVATION AND REPRESSION BY THE C-FOS SERUM RESPONSE ELEMENT

Victor M. Rivera, Ravi Misra, Morgan Sheng, Janet Wang and Michael E. Greenberg.
Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston MA 02115

Serum stimulation of quiescent fibroblasts results in a dramatic increase in c-fos transcription that peaks by 15 minutes and is then rapidly repressed to basal levels within 60 minutes. Using a nuclear run-on assay to follow directly the kinetics of transcription of mutant c-fos constructs, we demonstrate that the 20 bp serum response element (SRE) is the site of regulation of both the induction and repression events. Mutagenesis of this element reveals that the 14 bp inner core binds the serum response factor (SRF) and is by itself sufficient to mediate both the induction and shut-off of serum-stimulated transcription. The palindromic outer arms of the SRE stabilize the binding of SRF and thereby enhance the transcriptional response to serum. These results, coupled with binding studies, suggest that autoregulation by the c-fos gene product is not effected by a direct interaction of Fos/Jun complexes with the c-fos promoter but is likely to be mediated by either a novel function of the Fos protein or by an effect of Fos on the expression of another gene.

In order to study the mechanism by which the SRE mediates transcriptional induction and repression we have raised antibodies against the DNA binding/dimerization domain of SRF. Immunoprecipitation and peptide mapping experiments using HeLa and PC12 cells demonstrate the existence of several distinct SRF-related phosphoproteins. Data regarding the modifications and DNA binding properties of these SRF family members and their roles in c-fos transcriptional regulation will be presented.

E 442 THE ACTION OF C/EBP AND AP-1 SITE BINDING IN GENE EXPRESSION DURING ADIPOCYTE DIFFERENTIATION, Gregory S. Robinson, Roman Herrera, Hyo Sung Ro, Kleanthis G. Xanthopoulos Bruce M. Spiegelman, Department of Cellular and Molecular Biology, Dana Farber Cancer Insti Boston, MA 02115

Adipocyte differentiation is a complex event involving the transcriptional activation of ma new genes, several of which are involved in lipid metabolism. Analysis of the differentiation-specific gene adipocyte P2 (aP2), an intracellular lipid-binding protein, reveals that at least two distinct sequence elements in the aP2 promoter contribute to the expression ot the chlormaphenicol acetyltransferase (CAT) gene in chimeric constructions transfected into adipose cells. An AP-1 site at -120, shown earlier to bind Jun- and Fos-1 proteins, serves as a positive regulator of CAT gene expression in adipocytes but is specifically silenced by adjacent upstream sequences in preadipocytes. Sequences upstream the AP-1 site at -140 (i.e., AE-1 site) can function as an enhancer in both cell types when linked to a viral promoter but can stimulate expression only in fat cells in the context of intact aP2 promoter. The AE-1 sequence binds an adipocyte protein identical or very closel related to a CAAT box/ enhancer-binding protein (C/EBP) that has been implicated in the regulation of several hepatocyte-specific genes. A functional role for C/EBP in the regula of th aP2 promoter is indicated by the fact that C/EBP mRNA is induced during adipocyte differentiation and the aP2 promoter is transactivated by cotransfection of a C/EBP express vector into preadipose cells. These results indicate that sequences which bind C/EBP and t Fos-Jun complex play major roles in the expression of the aP2 gene during adipocyte differentiation and demonstrate that C/EBP can directly regulate cellular gene expression.

E 443 OCT-3, A NEW POU-HOMEOBOX GENE EXPRESSED IN PLURIPOTENT EMBRYONIC STEM CELLS, **Mitchell H. Rosner, M. Alessandra Vigano, Patricia Fast, Anup Dey, Keiko Ozato and Louis M. Staudt, Metabolism Branch, NCI,and NICHD, National Institututes of Health, Bethesda, MD 20892**

Embryonal carcinoma (EC) cell lines have been used as a model system for early mammalian development and have been shown to express cell type specific proteins that bind specifically to the octamer DNA motif (ATGCAAAT). We screened a murine EC cell cDNA library with DNA probes derived from Oct-2, an octamer binding protein expressed in lymphoid cells. The hybridizing cDNAs defined a novel gene, Oct-3, which has both homeobox and POUbox homologies. Oct-3 most likely encodes the EC cell-specific octamer binding protein, NF-A3, since in vitro transcription and translation of the Oct-3 cDNA yielded a protein with biochemical and DNA binding characteristics indistinguishable form NF-A3. Oct-3 mRNA was detected in both mouse and human undifferentiated EC cells and in pluripotent embryonal stem cells. In contrast with other homeobox genes, Oct-3 mRNA was down-regulated upon differentiation of EC cells. Southern and Northern blot analyses suggested that Oct-3 belongs to a multigene family that expresses distinct mRNA species at different times during embryonic development.

E 444 FOS/JUN DNA-BINDING IS DECREASED BY C-TERMINAL DELETION OF THE FOS PROTEIN. Kevin E. Salhany, Nobuyuki Kamata and Jeffrey T. Holt, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Fos protein functions in regulation of transcription through interactions with Jun and possibly other components of AP-1. To identify and characterize new regions of Fos protein (besides the basic region and the leucine repeat) which may be important to its function in regulation of transcription, we created a series of mutant Fos proteins through linker insertional mutagenesis which created various C-terminal deletions of Fos protein (by introduction of termination codons) or insertions of proline residues within the full length protein. The sites of mutation were confirmed by restriction mapping, and protein mobility on SDS/PAGE. In vitro DNA-binding activity of 12 Fos mutants (5 with deletions, 7 with proline insertions) was studied by gel mobility shift assays. Truncation of Fos just past the leucine repeat region (Fos198) resulted in decreased DNA-binding activity, suggesting that portions of the deleted C-terminal region may be important for Fos/Jun DNA binding. A proline insertion at amino acid 198 does not alter DNA binding, suggesting that our result is due to loss of C-terminal sequences and not a simple disruption of this site. Termination of the Fos protein between the basic DNA binding region and the leucine motif confirms other studies which suggest the importance of the leucine repeat region in DNA binding. Although altered DNA binding of Fos198 suggests that other domains in the C-terminal region may play a role in DNA-binding, examination of 6 proline insertion mutants within the C-terminal domain has not yet identified specific regions involved in DNA binding.

E 445 NRF-1: A TRANS-ACTIVATOR OF NUCLEAR RESPIRATORY GENES IN ANIMAL CELLS, Richard C. Scarpulla and Mark J. Evans, Department of Molecular Biology, Northwestern Medical School, Chicago, IL 60611

The assembly of the respiratory apparatus requires the coordinate expression of a large number of genes from both nuclear and mitochondrial genetic systems. A novel activator protein, designated as nuclear respiratory factor 1 (NRF-1), was defined by mutational and DNA binding analysis of the somatic cytochrome c promoter. Functional NRF-1 sites are also observed in recently isolated nuclear genes encoding cytochrome c oxidase subunit VIc (COXVIc) and the RNA subunit of a ribonucleoprotein endonuclease (MRP RNA) involved in mitochondrial DNA replication. Synthetic oligomers of these sites competitively displace NRF-1 binding to the cytochrome c promoter and function independently to stimulate transcription in promoter reconstruction experiments. These results suggest a mechanism for coordinating the activities of nuclear and mitochondrial genomes through the concerted modulation of nuclear genes.

E 446 DIFFERENTIAL EXPRESSION OF EGR-1 IN THE MURINE B LYMPHOCYTE RESPONSE TO SURFACE IMMUNOGLOBULIN SIGNALING MAY BE RELATED TO THE MATURATIONAL STATE OF THE CELL. Seyfert, V.L.*, Sukhatme, V.P.#, Cao, X.#, and Monroe, J.G.* *Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA.

We have been studying the differential regulation and role of an immediate early gene, Egr-1, in the B lymphocyte response to signaling via surface immunoglobulin (sIg). In contrast to mature B lymphocytes, we have shown that Egr-1 is not inducible by either anti-immunoglobulin antibodies (anti-u) or PMA in the immature B lymphocyte line, WEHI-231. Here, we show that the inability to induce Egr-1 in WEHI-231 is not due to a lack of trans-activating factors in these cells, since the Egr-1 promoter is functional when used to drive transcription of the reporter CAT gene. Rather, we postulate that hypermethylation of Egr-1 in WEHI-231 accounts for the lack of its expression in these cells, and have evidence demonstrating that Egr-1 is more methylated in WEHI-231 than in cells capable of expressing this gene. Furthermore, demethylation of Egr-1 with 5'-azacytidine facilitates sIg-mediated induction, whereas methylase induced methylation of the Egr-1 promoter blocks sIg signal-induced expression. We believe that hypermethylation of Egr-1 in WEHI-231 is associated with the relative developmental immaturity of these cells. The correlation between B lymphocyte maturity and the expression of Egr-1 is further supported by our finding that WEHI-231 cells pretreated with lipopolysaccharide (LPS), an agent previously shown to induce maturation of immature B lymphocytes, express Egr-1 following anti-u or PMA stimulation with kinetics similar to that of mature B lymphocytes. Improtantly, pretreatment of WEHI-231 cells with LPS also blocks the anti-Ig induced growth inhibition of these cells suggesting a correlation between Egr-1 expression and a positive growth response to sIg signaling.

E 447 RESTRICTED EXPRESSION AND DIFFERENTIAL SPLICING OF HOMEOBOX CONTAINING GENES IN MOUSE AND MAN. Wei-Fang Shen, Kristina Detmer, Patricia Lowney, Teresa A. Simonitch-Eason, Javier C. Corral, Craig Hauser, Frank M. Hack, H. Jeffrey Lawrence, and Corey Largman. Martinez VA Medical Center, Martinez, CA, Department of Internal Medicine, University of California, Davis and Scrips Institute, LaJolla, CA

Homeobox proteins are widely believed to be a family of transcription factors which play a major role in development. We investigated the expression of homeobox-containing genes in human hematopoiesis and in murine embryonic and adult tissues. We have demonstrated 1) lineage-restricted expression of 5 homeobox genes in erythroid and monocytic cell lines 2) expression of additional homeobox genes in other cell lineages (HL60 and lymphoid cells); and 3) modulation of expression during differentation. The Hox 2.2 gene is expresed as multiple transcripts in mouse and man. Two classes of transcripts have been identified: unspliced "intron" containing and spliced "non-intron" containing. The ratio of spliced to unspliced transcript varies with tissue and species, suggesting that Hox 2.2 gene function may be regulated by splicing events that occur in a cell or tissue specific manner.

E 448 MULTIPLICITY IN THE HLH, κE2-BINDING PROTEINS, Sun, X-H, Murre, C., Kamps,M, and Baltimore. D. Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

The E2 sequence binding proteins of the immunoglobulin κ light chain gene enhancer contain amphipathic helix-loop-helix (HLH) structures, which have also been found in a number of proteins controlling differentiation and development. The HLH structure has been shown in vitro to bind to the κE2 sequence, and form homodimers and also heterodimers with other proteins containing HLH motifs. A gene encoding some of the κE2 binding proteins, designated E2A, and several cDNAs have been cloned and characterized. We found several alternative splicing events which allow the mRNAs to encode different proteins with the HLH structure. More interestingly, the gene contains two alternative exons which code for two similar but not identical HLH structures, E12 and E47. These two HLH structures have previously been shown to bind to κE2 with different affinities using in vitro translated proteins. We have overproduced polypeptides containing the two HLH structures in *E. coli* and purified them to homogeniety. With these purified proteins, we showed that E12 binds to κE2 sequence several thousand-fold more weakly than does E47. Kinetic studies of the HLH structures in DNA binding and dimerization among themselves are in progress. The DNA sequence binding specificies of the HLH structures are also being evaluated.

E 449 DEVELOPMENTAL REGULATION OF CLASS II TRANSCRIPTION FACTORS IN *XENOPUS LAEVIS*, Sherrie R. Tafuri and Alan P. Wolffe, Laboratory of Molecular Biology, NIDDK , NIH, Bethesda, MD 20892.

Oocytes synthesize and sequester over 10,000 times the normal nuclear amount of several general class II transcription factors. Simple and efficient *in vitro* transcription extracts derived from oocytes and eggs demonstrate the factors to be functional. In order to characterize the biological regulation of these factors, we have initially cloned and characterized several members of a gene family homologous to the inverted CCAAT box binding transcription factor, NF-Y. Sequence data reveals that the gene products of the isolated clones are not related to the previously isolated CCAAT box binding factors CEBP and CTF/NFI. Preliminary northern blot analysis suggests that one variant is found in a number of tissues whereas the other is more cell type specific. Further information on the developmental regulation and the tissue specificity of *Xenopus* NF-Y is described.

E 450 MULTIPLE GENES ENCODING ZINC (II) FINGER DOMAINS ARE EXPRESSED IN HUMAN HAEMATOPOETIC CELLS, Hans-Jürgen Thiesen, Basel Institute for Immunology, CH-4005 Basel

Multiple cDNAs encoding zinc (II) finger structures were isolated from human cell lines of T cell origin. Initial restriction analysis, genomic southern blotting and partial sequence comparisons revealed at least 30 non-overlapping cDNAs designated cKox (1-30) encoding zinc finger motifs. Based on our screening results we estimate that about 70 zinc finger genes are expressed in human T cells. Zinc finger motifs are probably present in a large family of proteins with quite diverse and distinct functions. Analysis of cKox 1 demonstrated that Kox1 is a single copy gene which is differentially expressed within various cell lines representing diverse haematopoetic and non-haematopoetic cell lines. The 11 zinc finger of recombinant cKox1 were shown to coordinate zinc and to bind DNA . Further analysis of the predicted amino acid sequence revealed a putative leucine zipper structure N-terminal to the finger region which suggests a potential domain for homo- or heterodimer formation. However, comparisons of individual fingers in cKox1 with finger regions of cKox2-cKox30 demonstrate that some zinc fingers are highly conserved in their putative alpha helical DNA binding region supporting the notion of a zinc finger specific DNA recognition code.

E 451 POU-DOMAIN REGULATORY GENES ARE EXPRESSED IN THE DEVELOPING CENTRAL NERVOUS SYSTEM OF DROSOPHILA, **Maurice N. Treacy, Xi He, Charles S. Zuker, and Michael G. Rosenfeld. UCSD School of Medicine M-013, La Jolla,CA 92093.** Embryogenesis in *Drosophila melanogaster* is controlled by a network of regulatory factors, many of which share a 60 amino-acid region of homology referred to as the homeodomain (1,2). Investigation of organ-specific gene transcription in mammals and neural lineage determination in *C. elegans* led to the discovery of regulatory proteins containing a variant homeodomain and a second highly conserved 76-78 amino acid domain, together referred to as the POU-domain (3). Recently a large family of POU-domain genes were identified in mammals, all of which were shown to be expressed during neural development (4). The expression of POU-domain proteins in such phylogenetically distinct species led us to examine their potential expression in *Drosophila*. We report the isolation and characterization of two novel POU-domain genes from *Drosophila*, referred to as dPou-1 and dPou-2. Despite a screening strategy unbiased for tissue origin, both dPou-1 and dPou-2 are found in neural tissue, and exhibit extraordinary homology to two classes of mammalian POU-domain proteins. dPou-1 maps to the X chromosome at position 13C1-3, and dPou-2 to region 65D1-3 on the left arm of the third chromosome. *In situ* hybridization to whole embryos indicates that dPou-1 is expressed initially in the third hour of embryogenesis and is restricted to the mesoderm and the developing neuroblasts. Following germband shortening, dPou-1 transcripts are restricted to the supraesophageal ganglion and ventral cord. dPou-2 shows a similar pattern of expression with higher levels of hybridization signal, and additional expression seen in the spiracle sensory organ. The observation that the first two identified *Drosophila* genes encoding POU-domain proteins exhibit expression largely restricted to the developing central nervous system, suggests a role for the POU-domain gene family in neurogenesis in all metazoans.

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E 452 ACTIVATION OF AN NF-κB-LIKE TRANSCRIPTION FACTOR VIA THE TYPE B TNF RECEPTOR. MODULATION OF RECEPTOR FUNCTION BY PROTEIN KINASE C. Adolphus P.G.M. van Loon, M. Brockhaus, Hans-Ruedi Loetscher, Roland Remy, Michael Manneberg and Hans-Peter Hohmann, Central Research Units, F. Hoffmann-La Roche & Co AG, CH-4002 BASEL Switzerland.

Tumor necrosis factors (TNF) α and β activate an NF-κB-like protein by a protein kinase independent mechanism. We studied NF-κB activation by TNFα and TNFβ in two different cell lines, HL60 cells and HEp2 cells, which differ in their cell surface receptors for TNF. Both, the A and B type of TNF-receptors bind TNFα and TNFβ. In both cells, maximal activation of NF-κB required binding of TNFα to less than 15% of all cellular TNF receptors. A monoclonal antibody specific for type B TNF receptor activated NF-κB, suggesting that neither TNF itself, nor its internalization were necessary for activation of NF-κB via the receptor. After pretreatment of the cells with the protein kinase C activator PMA, a decreased degree of activation of NF-κB by TNFα and TNFβ was observed and also a concomittant disappearance of TNF binding sites. Both effects were abolished by pretreatment of the cells with the protein kinase inhibitor staurosporin, suggesting a possible involvement of a phosphorylation event. Phosphorylation of the TNF receptor itself was studied, using SW480 cells, which over-produce the type A TNF receptor. We observed, however, constitutive phosphorylation of the receptor at serin residues and also peptide mapping did not show any difference in the phosphorylation pattern of the type A TNF receptor from PMA-treated and untreated SW480 cells. Our results show, that at least the type B TNF receptor directly mediates activation of NF-κB by TNF and that phosphorylation of the type A receptor itself does not mediate its PMA-induced downregulation. An additional factor may exist, whose phosphorylation leads to the rapid down-regulation of the TNF receptors.

E 453 REGULATED MHC CLASS I EXPRESSION IN NEUROBLASTOMA.
Laura J. Van 't Veer, Anil Rustgi, Paula Lutz & Rene Bernards. Department of Molecular Genetics, MGH Cancer Center, Charlestown MA 02129.
In neuroblastoma the expression of MHC Class I is inversely correlated with the N-*myc* oncogene expression. We have previously shown that N-*myc* suppresses MHC Class I gene expression at the level of transcription. More specifically, our data indicate that N-*myc* acts to suppress the activity of a nuclear factor, H2TF1, that binds to the MHC class I gene enhancer. To investigate how N-*myc* supresses the activity of the H2TF1 factor, we have cloned two factors from human cDNA libraries that bind the MHC Class I gene enhancer, ACF1 and RC4. The putative DNA binding domains of these proteins share a zinc finger motif similar to each other and similar to another factor previously described to bind the MHC Class I gene enhancer. Data on the characterization of these factors will be shown.

E 454 IDENTIFICATION AND CHARACTERISATION OF THE *egr-1* GENE PRODUCT AS AN INDUCIBLE, DNA-BINDING , NUCLEAR PHOSPHOPROTEIN, Catherine. M. Waters, David. C. Hancock and Gerard. I. Evan Imperial Cancer Research Fund Laboratories Dominion House, 59 Bartholomew Close, London EC1A 7BE. UK. The *egr-1* gene (also termed *NGFI-A*, *zif*/268 and *Krox*-24) is predicted to encode a protein with three 'zinc finger' domains. Analagous DNA-binding motifs are present in several putative transcriptional regulatory proteins. The mRNA of *egr-1* is rapidly and transiently induced by a variety of growth factors in diverse cell types. We have used a synthetic peptide corresponding to the C-terminus of the predicted *egr-1* protein to generate mouse and rabbit polyclonal antibodies. These antibodies have been used to identify the *egr-1* protein, designated $p75^{egr-1}$, by immunoprecipitation, immunoblotting and immunocytochemical analyses. $p75^{egr-1}$ is induced within 1-2hrs of mitogenic stimulation and has a half-life of under 2 hours. The protein is phosphorylated and has been localised to the nucleus by both immunocytochemical analysis and biochemical fractionation. The *egr-1* protein is released from nuclei under isotonic or hypertonic salt conditions or by treatment with DNAase and binds double-stranded DNA *in vitro*. *Egr-1* protein is abundantly expressed in normal rat brain. Together these data support the emerging hypothesis that the *egr-1* protein may be a transcriptional regulator

E 455 PHORBOL ESTER INDUCTION OF RIBONUCLEOTIDE REDUCTASE,
Bob K. Choy, Grant A. McClarty and Jim A. Wright, Department of Biochemistry, Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Canada R3E 0V9.
Ribonucleotide reductase is a rate limiting enzyme in the synthesis of deoxyribonucleotide precursors of DNA. Its catalytic activity requires both its large (M1) and small (M2) subunits which are non-coordinately expressed. While the M1 subunit is constituitively expressed by proliferating cells, the M2 subunit expression is cell-cycle dependant with a tight correlation to S-phase. The phorbol ester tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), has been shown to lead to altered expression of specific genes such as the proto-oncogenes *c-fos*, *c-myb* and *c-myc* as well as ornithine decarboxylase (*ODC*). We show for the first time that TPA also strongly induces rapid accumulation of the M2 mRNA of ribonucleotide reductase mRNA and consequent elevated enzyme activity in proliferating BALB/c 3T3 fibroblasts. The enzyme activity is transiently elevated within ½ hour of treatment with 0.1μM of TPA and returns to near normal levels within 24-48 hours. TPA responsive elements and their associated transcription factors may be one of the transcriptional regulators of the cell cycle dependant M2 gene. The demonstration that TPA can cause rapid and transient alterations in ribonucleotide reductase suggests that the enzyme may also play an important role in the critical events involved in the process of tumor promotion. This work is supported by the National Cancer Institute of Canada.

E 456 TRANS-ACTING FACTORS IN THE REGULATION OF 2',5' OLIGOADENYLATE SYNTHETASE (2,'5' OAS) GENE EXPRESSION BY INTERFERON-α/β (IFN-α/β), Cong Yan and Igor Tamm, The Rockefeller University, New York, NY 10021

The 2',5' OAS gene enhancer region AB forms six complexes with protein factors in murine BALB/c 3T3 cells as shown by the gel retardation assay. These protein factors show differential protein-DNA binding activities at different pH values. One of these is a constitutive factor (CF) which displays element A specificity. Its role appears to be to stabilize the binding of other factors to DNA. Another protein factor is the IFN-α/β-response factor (IRF) which shows element B specificity. It preexists in the cytoplasm and is a phosphoprotein. IRF appears to be complexed to an inhibitor in the cytoplasm and to dissociate from the inhibitor and to translocate into the nucleus upon treatment of cells with IFN-α/β. We propose that IFN-α/β treatment of BALB/c 3T3 can trigger at least two events: 1) loosening of a tight inhibitor-IRF complex with the release of free IRF; this may be mediated via phosphorylation of IRF by an enzyme that is neither protein kinase C nor protein kinase A; 2) translocation of IRF into the nucleus and binding to the enhancer element B, which results in the activation of 2',5' OAS gene expression.

E 457 REGULATION OF THE EXPRESSION OF MOUSE MHC CLASS I GENES BY ADANOVIRUS EIA.

Kazushige Yokoyama[1], Alain Israel[2], Philipe Kourilsky[2], and Gabriel Gachelin[1,2]. RIKEN[1](The Institute of Physical and Chemical Research)Tsukuba,Ibaraki,Japan.Institut Pasteur[2],Paris Cedex,France.

The promoter of mouse major histocompatibility complex class 1 gene has already been dissected in datail and various transcriptinal factors responsible for basal or induced activity have been characterized. The CRE/ICS(Class 1 regulatory element/Interferon concensus sequences) regions were occupied by the factors of CREB,Ap2,KBF1,KBF2,(NFkB) and several IFNFs. Previous studies have demonstrated that primary rodent cells transformed by the oncogenic Ad12 virus express reduced amounts of class 1 antigens as opposed to cells transformed by the non-oncogenic Ad5 virus. During lytic infection of mouse embryonal cells by Ad12 virus, the expression of class 1 gene is increased and the E1A gene products are also proposed to cause this increase. The mechanism of transcriptional regulation of the H-2K^{bm1} class 1 gene by adenovirus12-E1A was studied in transfected rat embryonal fibroblasts. Results of long-term expression of the chloramphenicol acetyltransferase(CAT) gene placed under the control of the 5'-flanking region of the mouse H-2K^{bm1} gene and the results of nuclear run-on transcription of H-2K^{bm1} by E1A gene product. Deletion studies in the H-2K^{bm1} promoter region revealed that a proximal 58 bp upstream sequences(-194 to -136) and a distal 316 bp sequences(-1837 to -1521) respectively contribute to positive and negative(for the first time) regulations mediated by E1A . Both regulatory elements of MHC class 1 gene promoter region are responsible for the differential expression of the H-2K^{bm1} gene in Ad12 transformed cells. By the competition experiment with oligonucleotides, we identified 57 bp(-1652 to -1595) which are responsible for the negative regulations of H-2K^{bm1} gene.The sequences contain three indirect symmetrical repeats(GAAGGAA,GGTGTTT,TGGTGTT) and (GAA) repeats. A nuclear factor binding to the nagative element has been detected only extracts derived from cells expressing Ad12-E1A. The immunoselection and PCR amplification methods showed the E1A-(or via E1A associated molecules) molecules can interact with this negative regulatory factor which bind to 57 bp. A gene cloning of this regulatory factor is now proceeding.

E 458 TRANSCRIPTIONAL REGULATION BY DROSOPHILA HOMEODOMAIN-CONTAINING PROTEINS, James B. Jaynes and Patrick H. O'Farrell, Dept. of Biochemistry & Biophysics, Univ. of California, San Francisco, CA 94143.

Drosophila developmental regulators that contain homeodomains can regulate transcription either positively or negatively in cultured cells. The negatively acting homeoproteins can repress genes activated by either the yeast Gal4 protein or the rat glucocorticoid receptor. The homeoprotein repressors can function at a distance, repressing artificial reporter genes that contain homeodomain binding sites upstream of activator binding sites. Simple binding of proteins to the homeodomain binding sites is not sufficient for repression, suggesting that a specific repression function is required. In addition, competition for binding sites between homeoprotein activators and repressors that bind to the same sites can produce a sharp off/on response of a target gene to increasing activator concentration. Such regulatory characteristics are likely to be important in development for combinatorial control of transcription and for selecting between alternative developmental pathways.

Tissue-Specific Regulated Transcription Factors-I

E 500 **A REGULATORY GENE RESPONSIBLE FOR IL-6 EXPRESSION BELONGS TO A C/EBP FAMILY, S. Akira, H. Isshiki, O. Tanabe, T. Nakajima, S. Kinoshita, T. Simamoto, Y. Nishio, T. Hirano and T. Kishimoto, Institute for Molecular and Cellular Biology, Osaka University, 1-3, Yamada-oka, Suita, Osaka 565, Japan.**

Interleukin 6 is a cytokine with many biological activity such as stimulation of immunoglobulin synthesis, enhancement of myeloma and hybridoma growth, regulation of acute phase protein synthesis by hepatocytes, proliferation of multipotential hemopoitic stem cells. The multiple action of IL-6 suggests that it plays an important role in a complex cytokine network excerted in inflammation, immunological reaction, and tissue injury. Recently it has been proposed that the dysregulation of IL-6 expression is actually involved in the pathogenesis of autoimmune diseases and certain lymphoid malignacies, especially multiple myelomas. In oder to know mechanisms of such abnormal expression of IL-6 it is necessary to clarify the mechanism of IL-6 gene regulation. In this study we clarified cis-acting regulatory elements and trans-acting factors responsible for IL-1 induced IL-6 gene expression. Studies on 5' deletion mutants demonstrated that the IL-1 responsive element was mapped within the IL-6 promoter region from -180 to -122. Two types of nuclear factors that bind to this region, one constitutive and one inducible were identified by gel retardation assay. These two factors (NF-IL6) recognized a 14bp dyad symmetry, ACATTGCACAATCT. Direct screening of a λ gt 11 library using the NF-IL6 binding sequence as a probe isolated a single recombinant bacteriophage that specifically binded to NF-IL6 binding motif. This cloned NF-IL6 showed the high degree of homology with C/EBP (CCAAT/ enhancer binding protein purified from rat liver).

E 501 TWO INSULIN-SENSITIVE DNA BINDING MOTIFS INTERACT WITH FACTORS SPECIFICALLY EXPRESSED IN LIPOGENIC TISSUES, M. Alexander-Bridges, Rachel Galli, M. Denaro, Lynne Giere, and N. Nasrin, Massachusetts General Hospital, Howard Hughes Medical Insutitute Boston, MA 02114

The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA is increased ten-fold in cultured 3T3L1 adipocytes, in the fat and liver of rats fasted and refed a high carbohydrate, low fat diet and in the fat of diabetic rats treated with insulin. These effects are **not** seen in muscle, an insulin sensitive tissue which expresses sufficient receptors to mount a hormone response and are **not** seen in cultured 3T3L1 fibroblasts, where insulin regulates the Egr-1 gene despite the fact that there are only 7,000 receptors per cell.

We have previously shown that a human GAPDH-CAT fusion gene which is induced three-fold by insulin in stably transfected 3T3 adipocyte and four- to eight-fold in stable H35 hepatoma cell lines contains two independent elements, IRE-A and IRE-B, which work together to mediate the inductive effect of insulin.

The binding of the IRE-A to a nuclear protein is increased three to four-fold in insulin-stimulated 3T3 adipocytes or extracts made from insulin treated diabetic rats and ten-fold in nuclear extracts from fasted-refed rat liver nuclei. Binding activity is abolished by phosphatase treatment. The IRE-B binds a protein which is induced four- to eight-fold in 3T3 adipocytes exposed to insulin.and is present only in differentiated 3T3 adipocytes. Extraction efficiency was controlled by measuring the activity of the constituitively active SRF, the transcription factor which likely mediates the effect of insulin on Egr-1 gene expression.

The tissue specific expression of these factors, IRE-A and IRE-B apparently accounts for the tissue specificity of the response of the GAPDH gene to insulin.

E 502 DNA/PROTEIN INTERACTIONS WITHIN THE MURINE AND HUMAN PROXIMAL *LCK* PROMOTERS, James M. Allen and Roger M. Perlmutter, Howard Hughes Medical Institute, Department of Immunology, University of Washington, SL-15, Seattle, Washington 98195. The *lck* gene encodes a protein tyrosine kinase that is expressed almost exclusively in T-lymphocytes, presumably due to the binding of specific transcription factors to *lck* regulatory sequences. The *lck* gene has two promoters yielding transcripts that encode identical proteins. A 584 basepair fragment derived from the proximal *lck* promoter is sufficient for lymphocyte-specific expression of reporter genes in transgenic mice. These constructs were also active when transfected into Jurkat cells (a human T-cell line), a result consistent with the hypothesis that the transcription factors responsible for regulating *lck* gene expression are conserved between mouse and human. The presence of several distinct regions of significant homology between the murine and human DNA sequences within the 584 basepair fragment described above supports this hypothesis. We have demonstrated, by gel shift analysis, that factors present in nuclear extracts derived from various tissues bind to several of these regions. At least two regions appear to bind factors present only in lymphocytes. These cis-linked sequences should permit the identification of novel transcription factors which regulate *lck* gene expression.

E 503 REGULATION OF HLA CLASS II GENES, Göran Andersson, Sue Tsang, CharlesVoliva, and Matija Peterlin. Howard Hughes Medical Institute, University of California San Francisco, San Francisco, CA 94143.

Major histocompatibility complex (MHC) class II represent a coordinately regulated multigene family consisting of up to fifteen genes present in the HLA-D region of the human MHC. Class II genes are expressed at high levels in mature B lymphocytes, not expressed in pre-B or B-cell blasts, and their expression can be induced by several stimuli (including IFNγ) in most somatic cells. We have been interested in the cell-type specific and inducible expression of the DRA, DOB, DQA2, and DQB2 genes. These genes share regulatory motifs, i.e. transcriptional enhancer and promoter elements, with other class II genes. In the DRA promoter, the conserved upstream sequences are denoted Z-, X-, and Y-boxes. Systematic 5' deletions, 3' insertions, and clustered point mutations mapped B cell-specifc and IFNγ-inducible regulation to the Z- and X-boxes. Biochemical analyses revealed that Z- and X-boxes interact with ubiquitous and tissue-specific *trans*-acting factors. Since they contain shared regulatory motifs, Z- and X-boxes might represent duplicated enhansons. We have isolated three genes that code for X-box-specific-*trans*-acting factors from an expression cDNA library made from B cell mRNA. These cDNA clones have been partially characterized and expressed.

E 504 **11 bp SEQUENCE, WHICH IS UNIQUE TO HUMAN IL-4, IS ESSENTIAL FOR RESPONDING ANTIGEN STIMULATION IN T CELLS,** Naoko Arai, Shoichiro Miyatake, Ken-ichi Arai, and Etsuki Abe, DNAX Research Institute, Department of Molecular Biology, 901 California Avenue, Palo Alto, California 94304. The production of IL-4 is restricted to T cells. In mouse cell lines, IL-4 is produced only by T_H2 cells but not by T_H1 cells, which instead produce IL-2 and IFN-γ. Luciferase fusion gene with various lengths of 5'-upstream sequence of huIL-4 were transfected into Jurkat cells. The huIL-4 gene containing the 176 bp sequence upstream from the CAP site responds to $p40^{tax}$ and E2, viral trans-activators, as well as PMA/A23187. This observation was also confirmed using the B21 cell line, an human T cell clone, as well as an HTLV-1 transformed T cell clone. further analysis identified a 11 bp sequence (P-region: -79 to -69 of huIL-4[1]) as the minimum seuquence confering PMA/A23187 responsiveness to the basic promoter. The P-region does not share any homology with the IL-2 promoter, even though surrounding sequences share 84% homology between IL-2 and IL-4 (IL-2: position -91 to -66, IL-4: positions -91 to -80, and -67 to -53). Site-A (position -89 to -73 of hIL-2), recognized by NFIL-2A protein(s)[2], was identified as one of the essential regions for the IL-2 gene expression. The analysis and characterization of binding proteins which recognize P-region of hIL-4 gene will be discussed.

[1]Arai, et al., (1989) J. Immunol 142:274
[2]Durand, e.a., (1988) Mol. Cell Biol. 8:1745

E 505 TRANSCRIPTIONAL DEREGULATION OF C-MYC IN SPONTANEOUSLY ARISING t(6;7) RAT IMMUNOCYTOMAS

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We have previously shown that the t(6:7) chromosomal translocation in spontaneously arising rat immunocytomas (RIC) moves the c-myc gene to the IgH locus on chromosome 6. In 10 out of 14 tumors the c-myc breakpoints are clustered in a 1.5 kb region 5' of exon 1. The IgH target was frequently found to be Sε. Both human and mouse c-myc have been shown to be regulated at level of transcriptional attenuation. We preformed nuclear run-on analysis and showed that this type of regulation is present also in rat c-myc. However, this attenuation is completely lost in three RICs investigated to date. In many Burkitt's lymphomas, a low level of attenuation has been correlated to structural changes within exon 1. Sequence analysis of the corresponding sequences in RICs show no structural changes in this region.
In most rat tissues, the more proximal of the two promotors (P2) is preferred but in RIC, a considerable part of the transcription is initiated from P1 and from a novel cryptic promotor located approximately 85 bp downstream of P1. It has been suggested that the attenuation is dependent of the site of initiation. Further analysis will demonstrate how the changed pattern of initiation influences the attenuation in RICs.

E 506 DNA-BINDING OF NF-κB LIKE PROTEINS TO THE IL-2 PROMOTER IS INHIBITED BY CsA AND FK506

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Activation of T lymphocytes by antigens plays a key role in the immune response. The immunosuppressive drugs Cyclosporin A (CsA) and FK506, which are structurally unrelated, both interfere with this process mainly by blocking the transcription of the T cell growth factor gene Interleukin 2 (IL-2). The transcriptional activation of this gene involves the induction of transcription factors that bind specifically to the κB-enhancer element (-204/-193) in the IL-2 promoter. Here we demonstrate the inhibition of mitogen-dependent DNA-binding of NF-κB like proteins to this region by both immunosuppressive drugs. The inhibition of DNA/protein complex formation by CsA and FK506 correlates with the inhibition of IL-2 mRNA synthesis. The activity of FK506, when compared to CsA is 10 to 100 x more potent in its ability to inhibit sequence specific nucleoprotein complexes and IL-2 mRNA synthesis.

E 507 REGULATION OF HEME BIOSYNTHESIS: STUDIES OF THE LIVER AND ERYTHROID GENES FOR HUMAN 5-AMINOLEVULINATE SYNTHASE, Michael J. Bawden, Timothy C. Cox, Helen M. Healy, and Brian K. May, Department of Biochemistry, University of Adelaide, Adelaide 5001, Australia. The mitochondrial enzyme 5-aminolevulinate synthase (ALA-synthase) is the first and rate-limiting enzyme in the hepatic heme biosynthetic pathway. About 85% of the heme synthesised in the body occurs in erythroid tissue for hemoglobin formation. Most of the remainder is synthesised by the liver for cytochrome P450 proteins involved in oxidation of foreign compounds. All aerobic cells obligatorily synthesise heme for respiratory cytochromes. The liver and erythroid forms of ALA-synthase are encoded by separate genes which poses the important question of how tissue-specific expression of these genes is regulated during growth and development. Using a cDNA probe for hepatic ALA-synthase we have established in rats that this mRNA is expressed in all tissues. Levels of this mRNA are repressed by heme in all tissues studied but induced by drugs only in the liver and kidney. Nuclear run-on experiments have confirmed that heme and drugs modulate hepatic ALA-synthase mRNA levels by regulating gene transcription (1). Studies in mouse erythroleukaemic (MEL) cells show that regulation of the erythroid gene is different from that in liver - gene transcription is increased during cell differentiation but is not heme repressed (2). We have now isolated cDNA clones for the human hepatic and erythroid forms of ALA-synthase. The genes have been localised to chromosome 3 and the X chromosome respectively. Genomic clones for these isozymes have been characterized and studies are in progress to determine the DNA elements and regulatory proteins involved in the control of these genes.

1. Srivastava, G., Borthwick, I.A., Maguire, D.J., Elferink, C.J., Bawden, M.J., Mercer, J.F.B. and May, B.K. (1988) J. Biol Chem. *263*, 5202-5209.
2. Elferink, C.J., Sassa, S. and May, B.K. (1988) J. Biol Chem. *263*, 13012-13016.

E 508 Isolation and characterization of a cDNA encoding a protein binding both the mouse αA-crystallin gene and the CRE enhancer of MHC class I genes.

K. Becker, T. Nakamura, J. Flanagan, H. Westphal, J. Piatigorsky and Keiko Ozato.
NEI and NICHD, National Institutes of Health, Bethesda, MD, 20892.

MHC class I gene expression is controlled by a number of regulatory regions upstream of the transcription initiation site including the CRE and ICS. Similarly, the lens A-crystalline promoter contains cis-acting elements necessary for transcriptional control. By screening a lambda gt11 mouse lens cDNA library with an αA-crystalline upstream probe, a clone, pYTN8.1, was obtained which binds the αA4-crystalline control element. Comparative screening showed that this protein also binds the mouse MHC class I region I cis-acting element with high affinity. Southern analysis using pYTN8.1 as a probe shows a single copy gene. Northern analysis shows a message size of 10kb and expression in most adult tissues. This cDNA is being expressed by baculovirus vectors in Sf9 cells for isolation and purification of the protein product and for antibody production. Immunological characterization of the protein will be presented.

E 509 RABBIT AND HUMAN ARYLAMINE N-ACETYLTRANSFERASE GENES ARE EXPRESSED IN A TISSUE-SPECIFIC MANNER AND CONSIST OF UPSTREAM UNTRANSLATED AND SINGLE CODING EXONS, Martin Blum, Denis M. Grant, Cathrine Saner, Markus Heim, Anne Demierre, Markus Beer and Urs A. Meyer, Dept. of Pharmacology, Biocenter of the University, CH-4056 Basel, Switzerland.

Two N-acetyltransferase isozymes (NAT-A and NAT-B) have been identified and characterized in rabbit and man by protein purification as well as by cloning and functional expression of the respective genes. NAT-B is the target of a clinically and toxicologically important genetic polymorphism affecting more than 50% of Caucasian populations. Biochemical data suggest that NAT-B is markedly reduced in slow acetylators. NAT-A and NAT-B differ in their affinity for arylamine and hydrazine substrates and in their tissue specificity. NAT-A was characterized by high affinity for simple aromatic arylamine substrates (e. g. p-aminobenzoic acid) and was present at low levels in all tissues in the rabbit. NAT-B was found only in rabbit liver and duodenum and preferentially metabolized arylamine substrates with bulky side groups (e. g. sulfamethazine).

Analysis of the NAT genes in rabbit and man revealed small untranslated upstream exons whereas the complete coding sequence was contained on a single large exon of 870 bp. We are presently analyzing a) the promotors of the different genes to identify sequences involved in tissue-specificity, b) how the 5' untranslated exons are involved in the regulation of the genes, and c) what molecular mechanism leads to the acetylation defect in man.

E 510 MYOGENIN IS A MUSCLE-SPECIFIC REGULATORY FACTOR THAT BINDS TO THE MUSCLE CREATINE KINASE ENHANCER, Thomas J. Brennan and Eric N. Olson, Department of Biochemistry and Molecular Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

Establishment of the muscle lineage is accompanied by the expression of a family of muscle-specific regulatory factors, that includes myoD, myogenin and myf-5, which share a high degree of homology within a region that contains a basic and helix-loop-helix domain. Transfection of many non-muscle cell types with any one of these genes results in activation of the entire myogenic program. To explore the mechanism through which myogenin regulates myogenesis, we have prepared affinity purified antibodies against peptides corresponding to regions of myogenin that do not share homology with myoD or other myogenic regulatory factors. Antibodies against myogenin detect a protein of 32 kD in nuclear extracts from C2 myotubes but not in nuclear extracts from C2 myoblasts or 10T1/2 cells. Immunoflourescence analysis using the affinity purified antibodies has revealed that myogenin is localized primarily to the nuclei of differentiated myocytes. Myogenin is also detected in nuclei from proliferating 10T1/2 cells which have been transfected with an expression vector encoding myogenin, despite the fact that muscle specific genes are not expressed in these cells until after mitogens are removed from the culture medium. These results indicate that mitogenic signals block the actions of myogenin. Myogenin has been shown to activate the muscle creatine kinase (MCK) enhancer in transient transfection assays. During myogenesis, two MCK enhancer-binding factors are expressed coordinately with myogenin and bind to the core of the MCK enhancer which has been localized to the region between -1204 and -1137 bp upstream from the transcription initiation site. Immunoprecipitation of in vitro translated myogenin with anti-myogenin antibody coprecipitates the immunoglobulin enhancer binding factor E12 indicating that myogenin forms a heterodimer with this ubiquitous factor. The in vitro translated myogenin - E12 heterodimer binds specifically to a sequence in the MCK enhancer but not to heterologous DNA fragments. The fact that DNA binding of myogenin is dependent on heterodimerization suggests that the susceptibility of various cell types to the actions of myogenin is influenced by the factors with which it may interact. (Supported by ACSCD343-A and HD39849 to E.N.O.)

E 511 DISTINCT ENHANCERS IN THE HUMAN α AND δ TCR GENES WITH HIGHER ACTIVITY IN THEIR RESPECTIVE T-CELL SUBSETS, H. Elizabeth Broome, Donna Lineman Williams, and Louise C. Showe. Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.

T-cell receptor (TCR) molecules are heterodimers of either α and β chains or γ and δ chains. The TCR-δ gene is nested between the TCR-α variable and Joining gene segment regions. The co-expression of α and δ chains has never been demonstrated, and in most cases, α expressing cells have deleted δ from both alleles. The mechanisms which determine the expression of α versus δ chain genes are likely to play a role at the point in T-cell development where commitment to either an α/β or a γ/δ T-cell occurs. Using transient transfection assays and the luciferase reporter system with an SV40 promoter, we have identified and sequenced two T-cell specific enhancer elements associated with the human TCR α and δ chain genes . One enhancer (Eδ) is located 5' of the TCR-δ constant region. The second enhancer (Eα) is located 3' of the constant region of the TCR-α gene, consistent with what has been reported for the mouse. In the α/β expressing Jurkat T cell line, Eα is 6 to 7 times more active than Eδ. The converse is true in the γ/δ expressing Peer T cell line where enhancement by Eδ is 3 to 5 times greater than by Eα.

E 512 A NUCLEAR MATRIX ATTACHMENT REGION IN THE AVIAN β-GLOBIN GENE ENHANCER IS COVALENTLY BOUND TO A NON-TOPOISOMERASE PROTEIN. T.W. Brotherton, D. Zenk, J. Bennett, and G. Ginder. Dept. of Int. Med., Univ. of Iowa and VA Medical Center, Iowa City, IA. The nuclear matrix is a complex of proteins, insoluble in high salt, that provides shape and organization to the nucleus. DNA is bound to the nuclear matrix at sites called matrix attachment regions (MARs). Although recent evidence has shown that both replication and transcription take place in close association with the nuclear matrix, the role of this structure in those processes is unclear. We have examined the avian β-globin gene domain for the presence of MARs. Our results demonstrate that one MAR is located in the 480 bp HhaI fragment of the adult β-globin gene that contains the 3′-enhancer and DNaseI-hypersensitive sites. DNA from this region is bound to protein in vivo in a complex that is resistant to dissociation by SDS, phenol, mercaptoethanol and boiling. Incubation of erythroid nuclear matrices with a 480 bp fragment of cloned DNA that covers the β-globin enhancer results in covalent linkage to protein, without the introduction of single or double strand DNA cuts. Cold competitor DNA cannot displace endogenous DNA that is matrix bound, or the 480 bp cloned fragment after protein binding has occurred. Although the β-globin enhancer DNA is neither matrix attached or protein bound in avian brain cells, brain nuclear matrices will bind, covalently, the 480 bp enhancer element DNA fragment. In avian thymus tissue nuclei, β-globin gene enhancer DNA is covalently bound to protein, but not matrix associated in physiologic salt buffers. These results suggest that: 1) DNA becomes covalently linked to protein at MARs; 2) that excess binding protein is present in nuclear matrices; 3) that this protein is not topoisomerase I or II; and, 4) that matrix association of the β-globin gene enhancer may correlate with active transcription.

E 513 AT-RICH NUCLEAR PROTEIN BINDING SITES IN THE PROMOTER AND AN UPSTREAM REGULATORY REGION OF THE HUMAN ALBUMIN GENE, Jeannie Chan, Hidekazu Nakabayashi and Taiki Tamaoki, Department of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada.

The 5' flanking region of the human albumin gene was analyzed for transcriptional regulatory elements by transfecting chimeric CAT plasmids into two hepatoma cell lines HuH-7 and huH-1. An upstream element, UE1, located between -1.5 and -1.7 kb could stimulate the albumin promoter in transient transfection assays. Nuclear proteins that interact with UE1 are present only in hepatoma nuclear extracts and recognize the sequence GTTACTAATTGAC. A similar sequence GTTAATAATCTAC is present in the albumin promoter. The nuclear protein that binds to the AT-rich sequence of UE1 also binds to the AT-rich sequence of the albumin promoter as shown by competition gel mobility shift assays. Hence one feature of the transcriptional elements of the human albumin gene is the tandem arrangement of an AT-rich motif in the promoter and a distal regulatory region. Interestingly, similar AT-rich motifs are also present in the promoter and enhancer of the evolutionally-related alpha-fetoprotein gene.

E 514 EVIDENCE FOR REGION-SPECIFIC REGULATION AND CORTISONE INDUCTION OF SIALYLTRANSFERASE mRNA EXPRESSION IN THE DEVELOPING INTESTINE. Shu-heh W. Chu, Boris V. Zemelman, C. Keith Ozaki and W. Allan Walker, Department of Pediatrics, Harvard Medical School and The Children's Hospital, Boston, MA 02115

The immature intestinal epithelium is characterized by increased sialyltransferase (ST) activity that controls the sialylation of glycoproteins and glycolipids. Cell-surface sialic acid with specific glycosidic linkages may serve as a receptor determinant for certain viruses, bacteria and their toxins, and thereby may increase the host susceptibility in neonates. Further study of the rat small intestine reveals that developmental changes in ST activity occurred primarily in the distal (ileal), but not in the proximal (jejunal) region. Furthermore, the Galβ1,4GlcNAc α2,6-ST activity, but not the Galβ1,4GlcNAc α2,3-ST activity, was the major ST activity under developmental regulation. Northern blots of total RNA prepared from the proximal and distal small intestine of neonatal and adult rats were probed with a cDNA clone encoding the rat liver α2,6-ST (a gift of Dr. James C. Paulson, UCLA). The data show that α2,6-ST mRNA expression was age-dependent and regionally specific, with the highest level of mRNA expressed in the immature distal gut. Cortisone, a known transciption modulator, when injected into suckling rats, induced expression of ST mRNA only in the distal intestine. A parallel induction of distal ST activity by cortisone was also noted. This study indicates that the levels of ST activity are well correlated with the levels of ST mRNA and suggests that developmental variation in the intestinal ST activity is mainly caused by the control of the steady state levels of ST mRNA.

E 515 T CELL-SPECIFIC REGULATION OF THE CD3-ϵ GENE, Hans C. Clevers, Nils Lonberg, Elizabeth Lacy and Cox Terhorst. Dept. Clinical Immunology, Academic Hospital Utrecht, P.O.box 85500, Utrecht, Holland. The gene encoding the CD3-ϵ chain of the T cell receptor (TCR)/CD3 complex is uniquely transcribed in all T lineage cells. The human CD3-ϵ gene, when introduced in the mouse germ line, was expressed in correct tissue-specific fashion. The gene was then screened for T lymphocyte-specific cis-acting elements in transient CAT assays. The promoter functioned irrespective of cell type. An enhancer with strict T cell-specificity was found in a DNAse I-hypersensitive site downstream from the last exon, 12 kb from the promoter. The DNAse I-hypersensitive site was found in T cells only. The CD3-ϵ enhancer was unusual in that it coincided with a CpG-island. This CpG-island was hypomethylated independent of tissue-type.

E 516 PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF HEPATOCYTE NUCLEAR FACTOR 2 (HNF 2) INVOLVED IN LIVER-SPECIFIC TRANSCRIPTION OF THE HUMAN ALPHA-1-ANTITRYPSIN GENE, Gokul C. Das AND Vangipuram S. Rangan, Department of Molecular Biology, The University of Texas Health Center at Tyler, P.O. Box 2003, Tyler, Texas 75710.
Specific interactions between functionally defined cis-acting DNA sequences and the cellular transacting factors result in the preferential expression of certain gene in a tissue-specific manner. The upstream region of the human alpha-1-antitrypsin (AAT) gene binds proteins from liver cells. Two of these proteins, hepatocyte nuclear factors 1 and 2 (HNF 1 and HNF 2), that bind within 100 bp upstream of the TATA box are involved in liver-specific transcription of this gene. We report here, for the first time, the purification of HNF 2 from rat liver nuclei to apparent homogeneity by DNA sequence-specific affinity-chromatography. The purified protein showed a single polypeptide band of molecular weight 68 KD on SDS-polyacrylamide gel electrophoresis. Denaturation and subsequent renaturation of the polypeptide after extraction from the gel matrix exhibited specific binding to the recognition sequences and protected the same region of the promoter against DNase-I digestion. Studies elucidating the specific role of this purified protein on in vitro transcription of the alpha-1-antitrypsin gene are underway.

E 517 A FETAL BONE MARROW-SPECIFIC NUCLEAR PROTEIN BINDS TO SEQUENCES IN THE $^{A}\gamma$-GLOBIN GENE PROMOTER AND ENHANCER, Joseph DeSimone, Donald E. Lavelle, and Eva M. Eves, Department of Medicine, University of Illinois and VA West Side Medical Center, Chicago, Illinois 60612. The mechanism responsible for the fetal to adult hemoglobin switch during human and simian primate gestation is unknown. The 745bp EcoRI-Hind III fragment located 3' to the human $^{A}\gamma$-globin gene has been suggested to have enhancer activity and may affect fetal globin gene expression. Nuclear extracts from Hela, MEL (an adult-globin expressing cell line), K562 (a fetal-globin expressing cell line), pre-switch fetal baboon bone marrow and adult baboon bone marrow erythroid cells were tested for binding activity against a battery of fragments from the $^{A}\gamma$ globin enhancer (+1959 to +2704) and the $^{A}\gamma$ globin promoter (-299,+36). A DNA binding protein was found that appears to be both erythroid and fetal-specific and binds to two sites in the $^{A}\gamma$ enhancer and to one site in the $^{A}\gamma$ promoter. The consensus sequence for these three binding sites is AATGGAA, which is similar to core sequences of several other developmentally controlled genes. The redundancy of these binding sites suggests a DNA looping mechanism for functional interaction between the $^{A}\gamma$ promoter and enhancer elements.

E 518 Muscle-Specific Regulation of the Myosin Light Chain 1/3 Gene.

M. Donoghue*, B. Wentworth*, J. Kornhauser#, J. Merlie# and N. Rosenthal*. *Department of Biochemistry, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118. #Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

The rat myosin light chain 1/3 (MLC 1/3) gene locus is under the control of a strong, muscle-specific enhancer located downstream of the coding region, over 24 Kb away from the MLC1 transcription start site. When linked to an MLC1 promotor-CAT transcription unit in transgenic mice, the MLC enhancer is sufficient to induce high levels of developmentally regulated CAT gene expression exclusively in skeletal muscle cells. To investigate the molecular basis of MLC enhancer activity we have cotransfected nonmuscle cells with an MLC enhancer-driven expression vector and vectors coding for myogenic factors. One of these myogenic factors, MyoD, has been shown to bind to a number of sites within both the rat and human MLC enhancers. Together, these results indicate that MyoD plays an important role in the function of the enhancer. These experiments offer information regarding the interaction between a muscle-specific enhancer and factors involved in muscle differentiation.

E 519 CLONING AND CHARACTERIZATION OF A FACTOR WHICH BINDS THE INTERFERON CONSENSUS SEQUENCE OF CLASS I MAJOR HISTOCOMPATIBILITY COMPLEX GENES, Paul H. Driggers, Shannon A. Gleason, David L. Ennist, Wai-Han Mak, Ettore Apella, and Keiko Ozato, Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda Md. 20892. Genes of the Class I Major Histocompatibility Complex exhibit both tissue-specific and developmental regulation. Transcription of these genes can be induced by treatment of cells with type I (alpha/beta) and type II (gamma) interferons. Our laboratory has previously used an in vivo expression assay (CAT assay) and the electrophoretic mobility shift assay to define upstream regulatory elements controlling transcription of the murine class I H-2L^d gene. The class I regulatory element (CRE) binds nuclear factors which are involved in the enhancement of class I gene transcription in cells which express these genes at high levels. The interferon concensus sequence (ICS) controls transcriptional induction by interferons, and binding of nuclear factors to this element have been extensively characterized. We have screened lambda phage expression libraries with a concatenated ICS probe to isolate clones encoding ICS binding factors. One such clone (ICSBP) was isolated from a cDNA library derived from stimulated macrophages. DNA sequence analysis of ICSBP indicated that the clone is related to the regulatory factors mIRF-1 and mIRF-2 described by Fujita et al. Northern analysis revealed mRNA species of 3kb and 1.8 kb sizes. Significant levels of expression were observed only in lymphoid cells and cells of lymphocyte/macrophage lineages. In macrophage cell lines mRNA expression was inducible to high levels by treatment with gamma but not alpha/beta interferons. Southwestern analysis indicated specific binding of bacterially expressed ICSBP protein to the ICS and related sequences from other interferon inducible genes. Ongoing investigations of this clone are centered on elucidation of the functional role of ICSBP in the regulation of interferon-inducible genes.

E 520 TRANSCRIPTIONAL CONTROL SEQUENCES IN THE 5' REGION OF THE HUMAN ALCOHOL DEHYDROGENASE GENE *ADH2*. Howard J. Edenberg and Lucinda G. Carr. Departments of Biochemistry, Medicine, and Medical Genetics, Indiana University School of Medicine, Indianapolis, IN 46223, U.S.A.

The alcohol dehydrogenase (*ADH*) genes are expressed at high levels in adult mammalian liver. We have identified two sequence elements in the human *ADH2*[3] gene required for its expression. Gel retardation detects at least five complexes between nuclear proteins from mouse liver or rat hepatoma cells and the proximal promoter region. Two sites of strong interaction were localized to regions homologous to the mouse *Adh-1* gene, and the nucleotides contacted were identified by methylation interference. One maps between nt -94 and -84; the other is from nt -72 through -64. Oligonucleotides in which these contacts have been mutated do not compete for binding of the nuclear factors. This 5' region of the human *ADH2* gene is capable of directing accurate *in vitro* transcription in nuclear extracts from hepatoma cells. Deletion analysis indicates that the smallest portion of the proximal promoter region capable of directing significant transcription extends to nt -93, which contains both of the identified contact regions. Both of the cis-acting sequences identified here are important for initiation of transcription from the *ADH2* promoter, as demonstrated by the reduction of *in vitro* transcription by the presence of oligonucleotides that compete for binding of nuclear factors, and the lack of reduction when the non-binding mutated oligonucleotides are added.

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E 521 ANALYSIS OF THE MURINE OCT-2:A LOCUS ENCODING A DEVELOPMENTALLY REGULATED TRANSCRIPTION FACTOR. Andrew L. Feldhaus, Christopher Klug, Cheryl Miller, and Harinder Singh, Howard Hughes Medical Institute, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637. B-cell-specific transcription of immunoglobulin genes has been shown to be dependent upon Oct-2. In addition, Oct-2 has been detected in the murine CNS and brain suggesting that Oct-2 may play a role in neurogenesis. We have isolated two types of murine Oct-2 cDNAs which, unlike their human Oct-2 counterparts, contain numerous nucleotide differences in overlapping regions. The differences between the cDNAs are also seen at the genomic level. Amplification of the POU domain from murine genomic DNA revealed two distinct POU domains which correspond to the two types of murine Oct-2 cDNAs. These observations suggest that these Oct-2 mRNAs do not arise by alternative splicing but are encoded by different genes.

E 522 INTERFERON-γ REGULATES BINDING OF TWO NUCLEAR PROTEIN COMPLEXES IN A MACROPHAGE CELL LINE, Patricia W. Finn, Catherine J. Kara, John Douhan III, Tu Tran Van, Virginia Folsom and Laurie H. Glimcher, Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115

Macrophages are sensitive targets for the actions of interferon-γ (IFN-γ). A 2 to 5 fold IFN-γ mediated increase in class II gene expression has been reported and we find that the cell surface expression of class II MHC antigens in P388D1, a murine macrophage cell line, is induced after twenty-four hours of treatment with as little as 1 unit per ml of recombinant IFN-γ . To identify the upstream sequences responsible for the transcriptional effects of IFN-γ, P388D1 cells were transfected with plasmid constructs containing 5' upstream sequences of the mouse Eg gene fused to the CAT gene. Progressive deletions at the 5' end of the Eg CAT constructs have allowed us to define an upstream region required for IFN-γ inducibility. This 126 base pair sequence extends from position -192 to -66 relative to the start site of transcription, and includes three conserved motifs, W, X, and Y. X and Y are two conserved elements located in the same positions in all class II genes sequenced to date. W is a conserved motif located just upstream of the X motif in all class II beta chain genes. Using W, X and Y as a target sequence in mobility shift assays, we find that IFN-γ treatment of P388D1 cells results in increased binding of one protein in nuclear extracts, and decreased binding of a second, faster migrating protein. Both IFN-γ induced Eg gene transcription and the formation of these IFN-γ modulated complexes requires the presence of the three conserved motifs, W, X and Y.

E 523 NEGATIVE REGULATION OF MHC CLASS II GENE EXPRESSION IN TROPHOBLASTS. William L. Fodor and Richard A. Flavell. Department of Immunobiology, Yale University School of Medicine. New Haven, CT. 06510. Trophoblastic cells of embryonic origin repress MHC class II gene expression throughout fetal development and are unresponsive to IFNγ treatment, with respect to inducing class II gene expression. Analysis of this phenomenon is being investigated at the level of protein-DNA interactions that exist for the human DRα promoter in placental trophoblasts as well as in two choriocarcinoma cell lines, JEG-3 and Jar trophoblastic cells. Preliminary analysis of protein-DNA interactions utilizing gel mobility shift DNA-binding assays with protein extracts from term placenta (see Figure 1) and Jar cells has identified a protein-DNA complex specific for the Z-box domain of the DRα promoter. The specificity of this protein-DNA interaction was determined by chemical DNase footprint and mutational analyses of a Z-box oligonucleotide. The specific binding domain was localized to a GCAAGAA heptanucleotide and resembles the glucocorticoid-receptor-hormone binding domain. The biological significance of this interaction was assayed in transient transfection experiments. Jar cells were transfected with 5' serial deletions of the DRα promoter that were cloned upstream of the human growth hormone reporter gene (DRαpr-hGH). In all of the DRα deletion constructs that contained the Z-box domain hGH expression was not detected, however deletion of the Z-box resulted in a 2-3 fold increase in hGH expression above background levels. Currently, the Z box oligonucleotide is being cloned into the human chorion gonadotropin hormone alpha gene promoter (a constitutively expressed gene in trophoblasts) and will then be assayed for its ability to repress the expression of the human growth hormone reporter gene. Figure 1; gel-shift assay with placental extracts incubated with the Z-box oligonucleotide (A) no competitor, (B) 100X Z-box competitor, (C) 100X non-specific competitor. The arrow indicates the specific complex.

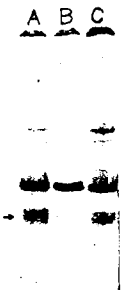

FIGURE 1

E 524 ERYTHROID-SPECIFIC ACTIVATION AND DEREPRESSION OF THE CHICK β-GLOBIN PROMOTER IN VITRO. T. C. Fong and B. M. Emerson, Department of Regulatory Biology, The Salk Institute, San Diego, California, 92037. Transcriptionally active extracts were prepared from chick red blood cells isolated at different stages of development. The template activity of cloned β-globin genes is highest in extracts from definitive red cells, where the endogenous gene is normally expressed, and lowest in extracts from primitive or nonerythroid tissues. This system has been used to identify regulatory elements and assign functions to the proteins that bind within the promoter region of the β-globin gene. Regulation of β-globin expression is achieved, in part, by factors whose composition changes during red cell development. Three proteins which bind to the promoter region have been purified and their effect on transcription of the β-globin gene have been determined in vitro. A chick red cell specific TATAA binding protein, which is present in primitive and definitive red cells, is required for basal level transcription. Two other proteins, PAL and CON, bind at adjacent sites in the promoter region but have opposite effects on transcription in vitro. Levels of PAL, a potent repressor, are highest in mature red cells while those of CON, an activator, are highest in actively transcribing cells. The effect of PAL can be overcome by blocking its binding site with a protein having similar recognition sequence but a dissimilar function.

E 525 TISSUE SPECIFIC NUCLEAR FACTORS MEDIATE EXPRESSION OF THE CD3δ GENE DURING T CELL DEVELOPMENT. K. Georgopoulos*, D. Galson# and C. Terhorst*. *Laboratory of Molecular Immunology, Dana Farber Cancer Institute, Harvard Medical School. #Department of Biology and Center for Cancer Research, M. I.T.

An obligatory step towards T cell maturation is expression of the CD3 gene products which occurs very early during thymic differentiation and may even precede development of the thymus. Delineation of the transcriptional mechanisms that determine expression of the CD3 complex in immature and mature T cells will help us understand the molecular events that govern T cell development. We have previously reported that a 400 bp region 3' of the CD3δ gene functions as a transcriptional enhancer with strong specificity for T cells. We have identified two elements in the CD3δ enhancer which mediate its T cell restricted function. Element δA can function as an independent enhancer and its activity correlates with the presence of a tissue specific nucleoprotein complex. Element δB has no independent function but augments the activity of element δA. A nucleoprotein complex found in mature T cells has been identified which correlates with activity of this element. Together, δA and δB are sufficient to reconstitute the activity of the CD3δ enhancer. Since these sequences are also found in the regulatory domains of other members of the TCR/CD3 complex, elements δA and δB and their cognate nuclear factors may play an important role in T cell development. We are currently engaged in isolating the genes that code for these nuclear factors which will provide us with tools to study T cell development.

E 526 LIVER-SPECIFIC TRANSCRIPTION OF TYROSINE AMINOTRANSFERASE (TAT) IS RELATED TO THE BINDING OF AT LEAST ONE LIVER SPECIFIC TRANSCRIPTION FACTOR TO THE PROMOTER.
Groyer A., Groyer-Schweizer G.[2], Cadepond F.[2], Grange T., Baulieu E.E.[2] & Pictet R. CNRS Institut Jacques Monod, 75005 PARIS & [2]INSERM U33, 94275 Bicêtre France.

The rat TAT gene's proximal promoter (-350 to -1 bp; pTAT) was inserted into the p(C2AT)19 vector. Its transcriptional activity was tested in an *in-vitro* assay using either TAT expressing (liver) or non-expressing (spleen) nuclear extracts (NEs). Liver NEs (2.4 mg/ml protein) promoted the transcription from pTAT as well as from an ubiquitous promoter (Adenovirus Major Late, pAdML), with a maximum for 30 µg/ml template. In contrast, **with spleen NEs, transcription of pTAT was almost undetectable** (≤6 % of that supported by pAdML at 30 µg/ml). Comparative DNAse I footprinting experiments showed that the TAT promoteur was almost entirely protected between nt -1 to -200 with **stronger protections** being elicited by spleen NEs particularly between nt -116 and -130 and **by liver NEs between nt 147 and -199.** Gel retardation patterns generated by liver and spleen NEs were identical for the ds-oligos spanning nt -65 to -85 (II), -84 to -109 (III) and -110 to -136 (IV) and liver-specific for ds-oligos spanning nt -140 to -163 (V) and -163 to -200 (VI). Cross-competition experiments led us to conclude that II binds the ubiquitous NF-Y, and that the liver-specific factor(s) which binds to V and VI was probably C/EBP or a related thereof. Functional involvement of these factors was demonstrated by the inhibition that ds-oligos II, V and VI exerted on pTAT driven transcription (40-60%) when added in excess to liver NEs.

E 527 REGULATORY SEQUENCES FOR A NEW GLUCOCORTICOID INDUCIBLE HUMAN CYTOCHROME P-450 GENE INCLUDE A GLUCOCORTICOID RESPONSIVE ENHANCER, P.S. Guzelian, J.D. Schuetz, D.T. Molowa, and E.G. Schuetz , Depts. Medicine/Pathology, MCV, Richmond, VA 23298. We have recently cloned and sequenced a human cDNA, HLp-2, related to the glucocorticoid (GC) inducible cytochrome P-450, HLp. Northern blot analysis confirmed that this gene is induced by dexamethasone (dex) in cultured human hepatocytes. To evaluate the DNA regulatory domains conferring dex responsiveness we transfected into a human hepatoma cell line (Hep G2) a 5'-flanking sequence of the HLp-2 gene fused upstream of the promoter in the Herpes-simplex Thymidine kinase chloramphenicol acetlytransferase vector (TK-CAT) . Expression of TK-CAT was inhibited regardless of the orientation of the HLp-2 gene fragment This block was reversed, partially, in a dose-dependent fashion by the addition of dex to the culture medium. Systematic deletions of the HLp-2 gene revealed a negative regulatory domain which upon removal permitted unimpaired basal expression of TK-CAT and also permitted the HLp-2 cellular promoter to drive CAT expression. Subcloning into TK-CAT (in either orientation) of an isolated 220 bp 5' HLp-2 fragment lacking the negative regulatory domain gave dex responsive CAT expression. The dex response was increased greatly by cotransfection of this vector with (pRShGRα) a human GC receptor expression vector. However, dose-response analyses indicated that the HLp-2 gene fragment which lacks the full consensus DNA sequence for GC response is 50-fold less responsive to dex when compared to (MMTV-CAT), a gene that contains this element. We conclude that control of HLp-2 gene expression involves both a negative inhibitory element and a positive, glucocorticoid responsive element distinct from the traditional GC response.

E 528 IDENTIFICATION OF DNA-BINDING PROTEINS REGULATING AN IMMUNOGLOBULIN GENE THROUGH *IN VITRO* TRANSCRIPTION AND OLIGONUCLEOTIDE COMPETITION, David G. Johnson and Philip W. Tucker, Department of Microbiology, Southwestern Medical Center at Dallas, Dallas, TX 75235 By adding duplex oligonucleotides with potential DNA-binding motifs to an *in vitro* transcription assay, DNA-binding proteins involved in transcriptional regulation can be identified and functionally analyzed. Using an immunoglobulin heavy chain gene (IgH) promoter and B or pre-B cell nuclear extract we have analyzed the role of several transcription factors in IgH transcription. An oligonucleotide containing the octamer sequence, ATGCAAAT, specifically inhibited *in vitro* transcription of the immunoglobulin gene while another oligonucleotide, containing a mutated form of the octamer did not. The ability of these two oligos to inhibit *in vitro* transcription corresponds to their ability to compete for octamer-binding proteins in a gel retardation assay. At least two other proteins, TFIID (the TATA-box binding factor) and μEBP-E (an IgH enhancer-binding factor) have been shown to play a role in transcription of the IgH gene using this technique. These last two proteins also reveal the sensitivity of this technique since neither protein can be shown to bind the IgH promoter in a gel retardation assay when crude nuclear extract is used. These results demonstrate that this technique is not only more sensitive than other techniques such as gel retardation or DNA footprinting in detecting DNA-binding proteins but also has the advantage of giving functional as well as DNA-binding data.

E 529 INVOLVEMENT OF κB-TYPE ENHANCERS IN THE TRANSCRIPTIONAL ACTIVATION OF THE MOUSE TNF-α GENE IN PRIMARY MACROPHAGES. [1]C.V. Jongeneel, [1]C. Drouet, [2]S.A. Nedospasov, [3]M. Collart, and [1,2]A.N. Shakhov. [1]Ludwig Inst. Canc. Res., 1066 Epalinges, Switzerland; [2]Engelhardt Inst. Mol. Biol., Moscow 117984, USSR; [3]Dept. of Pathology, U. of Geneva Med. School, Switzerland.

Tumor necrosis factor (TNF-α) is a macrophage product whose pleiotropic effects are associated with the development of an inflammatory response. Overproduction of TNF-α can lead to serious systemic injury and death. Production of TNF-α is tightly regulated at both transcriptional and post-transcriptional levels.

We have characterized cis-acting elements in the TNF-α promoter by transfecting primary bone marrow-derived macrophages with plasmids containing presumed control elements linked to the bacterial CAT gene. Constructs containing 1 kb of the TNF-α promoter were >20-fold inducible by LPS. 5' deletion mapping localized two regions (-655 to -451 and -301 to -242) whose loss caused a drop in LPS inducibility. The same regions also bound nuclear proteins induced by LPS. The first region contains a strong κB enhancer consensus at nt -510. The second region (nt -301 to -242) contains only a $(CA)_{13}$ repeat and a MHC class II Y box.

Insertion of two or more copies of the TNF-α κB enhancer upstream of an enhancer-less HSV TK promoter conferred LPS inducibility upon the hybrid promoter. Therefore, this element is sufficient in itself for LPS inducibility. Additional evidence for the role of κB enhancers came from the demonstration that a plasmid expressing the HTLV-1 Tax gene trans-activated the TNF-α promoter and the TNF κB/TK hybrids in macrophages. As seen for LPS induction, two or more copies of the enhancer were required. However, in contrast to LPS induction, the Tax effect could be inhibited by IFN-γ.

A more careful examination of the TNF-α upstream region revealed the presence of a total of four κB-type enhancers, three of which were shown to bind nuclear proteins induced by LPS. We speculate that multiple κB-type enhancers are involved in LPS-mediated activation of the TNF-α promoter, and that the Y box can additionally modulate its level of expression.

E 530 DEREPRESSION OF THE IgH ENHANCER IN NON-B CELLS UPON OVER-EXPRESSION OF THE POSITIVE TRANSCRIPTION FACTOR ITF-1, Tom Kadesch, Diane Ruezinsky, Holger Beckmann and Paula Henthorn, Howard Hughes Medical Institute and Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6145

Activity of the immunoglobulin heavy chain (IgH) enhancer is restricted primarily to cells of the B lymphoid lineage. This activity is facilitated through several sites that bind both ubiquitous and cell type-specific proteins. We describe the identification of a negative element that represses the otherwise ubiquitous activity of the μE3 motif of the enhancer specifically in non-lymphoid cells (NIH/3T3). This negative motif is coincident with the μE5 site, which acts to augment enhancer activity in B cells. We have recently isolate two cDNAs from a B cell library that encode positive-acting transcription factors, denoted TFE3 and ITF-1, which bind to the μE3 and μE5 sites of the IgH enhancer, respectively. Transfection of NIH/3T3 cells with the cDNA that encodes TFE3 is not sufficient to overcome the repression mediated by the μE5 site. However, when ITF-1 is over-expressed in NIH/3T3 cells by transfection, the repressive action of the μE5 site is reversed. Moreover, over-expression of ITF-1 allows further activation of the IgH enhancer in non-B cells upon co-transfection of the cDNA that encodes TFE3. Hence, cell type-specific activity of the IgH enhancer can be mediated through the relative balance of positive- and negative-acting transcription factors. However, we propose that negative control constitutes only one of perhaps several different layers of regulation that act together to restrict activity of the IgH enhancer to B lymphocytes.

E 531 REGULATION OF LYMPHOKINE GENE EXPRESSION: ABROGATION OF LYMPHOKINE GENE EXPRESSION IN EXTRATHYMICALLY DEVELOPED T-CELL TOLERANCE, Kang, Sang-Mo, Bart Beverly, Ronald Schwartz, and Michael J. Lenardo, Laboratory of Immunology, NIAID, National Institutes of Health, Bethesda, MD 20892. Evidence is now accumulating in both in-vivo and in-vitro systems that mature T cells can be "tolerized" to antigenic stimuli if the T cells are presented with antigen/MHC in the absence of a second "costimulatory signal". T-cells which have been anergized in such a manner show an inability to proliferate when rechallenged with a "normal" stimulus i.e. antigen plus untreated antigen presenting cells. In addition, such T-cells show a markedly diminished lymphokine response. We have been working with a normal murine T-cell clone, A.E7, which is of the TH1 phenotype. This clone in the anergized state shows a complete block in the production of IL-2 and a partial block in the production of gamma-interferon, IL-3, and lymphotoxin(LT), despite normal inositol turnover and diacyl glycerol activation. We have been studying the co-regulation of these genes in the anergized state. Two important inducible nuclear factors that regulate lymphokine gene expression are NF-kB and NFAT. Specifically, NF-kB appears to regulate the promoters of IL-2, LT, and possibly gamma-interferon-- the same lymphokines whose induction is suppressed in T-cell anergy. NFAT is known to regulate IL-2 gene expression in tumor cell lines. We have found that the regulation of NF-kB is aberrant in A.E7 clones. This has important implications since, unlike tumor cell lines, A.E7 cells depend on antigenic stimulation and subsequent IL-2 production for proliferation. We have also shown the induction of NFAT by antigenic stimulation in our normal T-cell clones. We are further exploring the role of these and other nuclear factors in the development of T-cell anergy. In addition, the functional role of the 5' untranslated regions of these lymphokine genes in the development and maintenance of anergy are being studied using transient transfection techniques.

E 532 CHARACTERIZATION OF ASF-1: A FACTOR WHICH IS INVOLVED IN ROOT EXPRESSION OF THE CAULIFLOWER MOSAIC VIRUS 35S PROMOTER, Fumiaki Katagiri, Eric Lam and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY 10021. The 35S promoter of the cauliflower mosaic virus (CaMV) is a strong and constitutive promoter in plants. We have analyzed cis-acting elements of the 35S promoter in transgenic tobacco. When the sequence upstream of the -90 region is deleted, this promoter shows preferential expression in root. The as-1 element in the -75 region is a positive regulator which is responsible for this preferential expression in root. For example, when the element is inserted into a green-tissue specific promoter, it confers root expression on the promoter. Tobacco nuclear extracts contain a DNA binding factor, designated ASF-1, which binds to as-1 in vitro. as-1 has a tandem repeat of TGACG motifs. Mutations in the TGACG motifs affect the in vivo function of as-1 as well as ASF-1 binding in vitro. This correlation strongly suggests that ASF-1 is a factor responsible for as-1 function. To further characterize the factor, we isolated tobacco cDNA clones encoding DNA binding proteins for the TGACG motif. Five positive clones were obtained, and they were divided into two groups according to the DNA sequence homology of their inserts. The proteins encoded by the two groups were named TGA1a and TGA1b. TGA1a and TGA1b produced by lysogens show different binding specificities to different TGACG-containing binding elements. TGA1a has the same binding specificity as ASF-1. Moreover, the mRNA level of TGA1a is very high in root, compared with that in leaf. These results suggest that TGA1a corresponds to ASF-1. TGA1a and TGA1b have a basic region and a leucine-zipper that are highly homologous to the DNA binding domains of CREB, GCN4, and c-Jun.

E 533 LOCUS-SPECIFIC CIS-ACTING TRANSCRIPTIONAL REGULATION OF K^d, D^d AND L^d CLASS I GENES IN THE BALB/C S49 LYMPHOMA SUBLINES, Jill B. Keeney and Ted H. Hansen, Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110.
The S49 tumor sublines are variants isolated from a single parent BALB/c tumor which demonstrate locus-specific shut-off of their K^d, D^d and L^d genes. Four phenotypically different sublines were characterized at the DNA and RNA level. Southern blot analysis indicated that no major chromosomal deletions have occurred, and treatment of the sublines with 5-azacytidine had no effect on Class I expression. Thus, methylation differences between loci are unlikely. None of the repressed Class I antigens could be induced with interferon even though the expressed antigens were fully inducible. Northern blot analysis revealed message only for the expressed antigens, showing that the repression mechanism is acting at the transcriptional level. Rnase protection analysis confirmed this result and demonstrated that the transcriptional repression is exquisitely specific for the K^d, D^d and L^d genes as other "class I-like" messages are present in all the cell lines. Fusion of the S49 lines with Class I positive tumors resulted in hybrid cell lines expressing Class I antigens from both fusion partners, but the negative Class I antigens originating from the S49 partner were not expressed. These findings are best explained by the occurrance of multiple independent mutations among the Class I genes in their cis-acting elements of transcriptional regulation.

E 534 FUNCTIONAL CHARACTERIZATION OF THE OCT-1 AND OCT-2 PROTEIN, Iris Kemler and Walter Schaffner, Institut für Molekularbiologie II der Universität Zürich, Hönggerberg, 8093 Zürich, Switzerland.
Immunoglobulin (Ig) genes are expressed in cells of the B-cell lineage but not in non-B cells. An important component of this B-cell specificity is the conserved octamer sequence ATGCAAAT, present in the promoter and the enhancer. The same element occurs also in non-tissue-specific promoters such as the histone H2B gene. The octamer sequence is recognized by the two B-cell specific factors Oct-2A and Oct-2B and the ubiquitous Oct-1 protein. Why then does the Oct-1 protein not activate a B-cell specific promoter in non-B cells? We are addressing this question by performing experiments with the cDNAs for the Oct-2 protein (Müller et al., Nature, 336, 544-551, 1988) and the Oct-1 protein (kindly provided by Winship Herr; Sturm et al., Genes & Dev. 2, 1582-1599, 1988). Recently it was shown that a promoter consisting of an octamer element and a TATA box can be activated in non-B cells by coexpressing the Oct-2A cDNA. Surprisingly, preliminary results show that the same promoter can also be activated by cotransfection of the Oct-1 cDNA, though to a lesser extent. This agrees with in vitro transcription experiments where the same observation was made (LeBowitz et al., Genes & Dev. 2, 1227-1237 1988). These results have left open the question if the regulation of Ig gene expression is achieved by quantitative or qualitative differences between the Oct-1 and Oct-2 proteins. For a more refined analysis we have initiated transfection experiments with Oct-1 deletion mutants and of domain swap experiments between the Oct-1 and Oct-2.

E 535 EVIDENCE FOR POSITIVE AND NEGATIVE REGULATORY ELEMENTS IN THE 5'-FLANKING SEQUENCE OF THE VON WILLEBRAND FACTOR GENE, Danièle Kerbiriou-Nabias, Zahra Assouline, Valérie Ferreira, Edith Destremau, Bruce R. Bahnak and Dominique Meyer. INSERM U.143, Hôpital de Bicêtre, 94270 Le Kremlin-Bicêtre, France.
Von Willebrand factor (vWF) is synthesized in a cell specific manner by endothelial cells and megakarocytes. We have sequenced a 2,158 bp fragment upstream of the transcription initiation site and the first three intron-exon junctions of the human vWF gene. The first exon of 250 bp contains the entire 5'-untranslated region and has two open reading frames with inframe stop codons. The second exon starts with the ATG translation initiation codon. We have investigated the role of 5'-flanking DNA sequences in regulating the expression of the gene in endothelial cells and cell lines that do not express vWF. Varying lengths of flanking sequences extending from 2,123 bp 5' to 19 bp 3' of the transcription initiation site were linked to the bacterial chloramphenicol transacetylase gene in the pSB1 vector. The constructs were tested in transient transfection assays in bovine aortic endothelial, COS and HeLa cells. A plasmid containing the ß-galactosidase gene was used as a transfection control. Sequences from -89 upstream to +19 bp downstream of the cap site showed functional promotor activity in all three cell lines. An AT-rich region resembling a TATA box is found 32 bp upstream of the transcription initiation site; however, no consensus sequences for other ubiquitous upstream promotor elements have been identified. The promotor function was confirmed by the fact that a plasmid construction containing the -89 to +19 bp region of DNA in inverse orientation loses promotor activity. Longer sequences up to -1,243 completely suppressed promotor activity in all cell types. Constructs including 2,123 bp flanking DNA restored promotor activity only in the endothelial cells. This activity was lost if the first 89 bp sequence upstream of the cap site was deleted from the construction. These results suggest that negative and positive regulatory elements normally interact to regulate the endothelial cell specific expression of the vWF gene.

E 536 REGULATION OF EXPRESSION OF THE Vδ1 T CELL RECEPTOR GENE SEGMENT, Gary Kikuchi, Gregory P. Einhorn, and John E. Coligan, Biological Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

The murine γδ T cell receptor is expressed earlier in thymic ontogeny (about day 14 of embryogenesis) than the αβ T cell receptor, which is expressed at about day 17. Vδ1 is one of the first gene segments within the α/δ T cell receptor locus to be expressed during fetal thymic ontogeny. In adult mice, Vδ1 is expressed primarily by dendritic epidermal T cells in the skin.

We have isolated a genomic lambda clone from the rearranged Vδ1 gene expressed by a dendritic epidermal T cell hybridoma. Restriction map and nucleotide sequence analysis of this clone confirmed that it contained 5′ flanking sequences as well as the leader, VDJ, and the first of four exons of the constant region. The transcriptional start site was mapped by primer extension and RNase protection assays. Subclones of the original lambda clone containing only 5′ flanking sequences of the Vδ1 gene were constructed. Transient transfection assays using the reporter gene chloramphenicol acetyltransferase have confirmed that these 5′ flanking sequences promote transcription in T cells. Gel mobility shift analyses have identified several binding sites for nuclear proteins from T cells that may act as transcriptional regulatory factors. Experiments are in progress to evaluate the apparent tissue specificity of this promoter.

E 537 A DNA BINDING PROTEIN SPECIFIC TO THE NERVOUS SYSTEM THAT RECOGNIZES A MYELIN GENE PROMOTER, Jin G. Kim and Lynn D. Hudson, NINDS, National Institutes of Health, Bethesda, MD 20892

The differentiation of a progenitor cell to an oligodendrocyte in the central nervous system is accompanied by activation of genes encoding myelin proteins. These proteins are unique to the myelin sheath, an extension of the plasma membrane that envelops axons. To examine the signals controlling myelin gene expression, we have isolated genes encoding proteins that bind regulatory regions of the most highly expressed myelin gene, proteolipid protein (PLP). First, sites in the PLP promoter that bind nuclear brain proteins were identified by gel mobility shift and footprinting assays. Then a human brain λgt11 library was probed with multimers of these binding sites. One probe containing the recognition site GGAGCCC detected a cDNA clone encoding a novel protein with a highly charged region (named PBP1 for PLP binding protein). The 2:1 ratio of basic to acidic residues in this domain is reminiscent of other DNA binding domains. On Northern blots, a discrete 4 kb transcript encoding PBP1 was found only in brain and sciatic nerve. The tissue restricted expression of PBP1 and the presence of the GGAGCCC motif in two other myelin specific genes recommends PBP1 as a key modulator of myelination, a function that can be tested in vitro and in transfected glial cells.

E 538 ANALYSIS OF A TISSUE-SPECIFIC ENHANCER OF THE RAT CALCITONIN/CGRP GENE IN NEURAL AND ENDOCRINE CELL TYPES, Elliott S. Klein, Lana Stolarsky-Freidmann, and Michael G. Rosenfeld*, Eukaryotic Regulatory Biology Program and *Howard Hughes Medical Institute, School of Medicine M-013, UCSD, La Jolla, California 92093.

The rat calcitonin/CGRP gene is expressed in a tissue-specific manner which is restricted to both the endocrine and nervous systems. Expression of this gene in the brain and peripheral nervous system results in the synthesis of a neuropeptide (CGRP) in contrast to the production of a hormone (calcitonin) in the C cells of the thyroid. We have identified an enhancer located within the 5'-flanking region of the calcitonin/CGRP gene. The function of this enhancer is restricted to cells of neuronal or C cell origin by transient transfection analyses. Specifically, while the calcitonin/CGRP enhancer is functional in a neuronal cell line (B103) and a medullary thyroid carcinoma derived cell line (CA), both of which express their endogenous calcitonin/CGRP genes, a variety of other cell lines tested (HeLa,G/C,AtT-20,HIT,CV-1) were not competent for enhancer function. Data will be presented in support of similar transacting factors being involved in the tissue restricted expression of this gene within endocrine and neuronal cell types.

E 539 MOLECULAR CLONING OF A PU-BOX DNA BINDING PROTEIN EXPRESSED IN B CELLS AND MACROPHAGES, Michael J. Klemsz, Antonio Celada, Scott R. McKercher and Richard A. Maki, LaJolla Cancer Research Foundation, LaJolla, CA. 92037.
A purine-rich sequence (PU box, 5'-GAGGAA-3'), has been shown to be an important cis acting element for expression of SV-40 variants in lymphoid cells and IL-2 gene expression in T cells. Nuclear factors that bind the PU box have been found in B and T cells but not in fibroblasts. We have cloned a cDNA for a murine macrophage PU box DNA binding protein. The PU-1 gene is expressed in macrophages and B cells, but not T cells, suggesting that PU-1 belongs to a family of PU box binding proteins. Deletion analysis of the cDNA shows that the binding domain is located in the highly basic carboxyl terminal portion of the protein. The ability of PU-1 to trans-activate appropriate expression vectors will be discussed.

E 540 TISSUE SPECIFIC REGULATION OF THE RAT GROWTH HORMONE PROMOTER, Kristin E. Konzak, Sylvain Guerin and David D. Moore, Wellman 9, Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.
The transcriptional activity of the rat growth hormone (rGH) promoter is controlled on many levels, including a particularly potent cell type specific regulation. Two kinds of transcription factors bind the 5' flanking sequence of the rGH promoter and are involved in determining the activity of this promoter in various kinds of transiently transfected cell types. One factor negatively regulates rGH promoter activity in non-pituitary cells and binds two cis-acting silencer elements, located approximately 300 and 500 base pairs upstream of the RNA start site. The more proximal element causes a 5-10 fold decrease in promoter activity when transfected into non-pituitary cells, while the distal element may have a larger negative effect. The second is the pituitary-specific positive regulatory factor Pit-1/GHF-1, which binds to two sites near the TATA box in the rGH promoter. To determine the relationship between the silencers and Pit-1/GHF-1 in regulating rGH promoter activity, three distinct splice site variants of Pit-1 have been isolated from a pituitary cDNA library. Non-pituitary cell lines were cotransfected with various combinations of Pit-1 clones and non-silencer containing rGH promoter constructs to determine the activity of each cDNA type. The three cDNAs differ in their ability to transactivate rGH in this assay. Additional cotransfection experiments are underway to establish the respective activities of the silencer binding repressor (SBP) and Pit-1 activator when both factors are present in the same cell. These experiments should discriminate between various models for regulation of pituitary development.

E 541 MULTIPLE PROTEIN BINDING SITES WITHIN A MUSCLE-SPECIFIC ENHANCER FROM THE HUMAN MYOGLOBIN GENE. William E. Kraus, Franklin C. Wefald, Ivor J. Benjamin, and R. Sanders Williams. Departments of Medicine and Microbiology, Duke University, Durham, NC 27710.

Previously we identified an upstream region of the human myoglobin gene that functions as a muscle-specific enhancer (JBC 264:13896, 1989). A polypyrimidine motif (CCTCCCCTT) within the enhancer binds nuclear proteins of at least two types: a 100 kD protein (MbBF1) expressed in both muscle and non-muscle tissues; and a second factor (MbBF2) found only in cardiomyocytes and skeletal muscles that express high concentrations of myoglobin mRNA. Another protein binding site within the enhancer differs by 3 bases (GGTGCCCTT) and binds MbBF1 with higher affinity. When linked to myoglobin core promoter sequences, deletion or mutation of either of these sites has little effect on enhancer function if the remaining site remains intact. Constructs bearing the myoglobin upstream enhancer are *trans*-activated by co-transfection with the MyoD gene driven by a constitutive promoter. In preliminary experiments, a recombinant MyoD fusion protein (gift of Andrew Lassar) binds only weakly to myoglobin enhancer sequences under conditions in which strong binding to M-creatine kinase enhancer sequences is observed. Other experiments are underway to determine whether MyoD is a component of heterologous protein complexes that bind to the myoglobin enhancer, or *trans*-activates through mechanisms that do not involve direct DNA binding.

E 542 FUNCTIONAL ANALYSIS OF THE HRAS1 VARIABLE TANDEM REPEAT. Theodore Krontiris, Marie Green and William Trepicchio, Tufts-New England Medical Center, Boston, MA 02111.
The tandem repetition of short nucleotide sequences (14-100bp) is an important source of human genetic polymorphism. Such variable tandem repeats (VTRs, minisatellites) are often found in the vicinity of genes; positions immediately upstream (insulin), downstream (HRAS1, apoB) and within introns (IL-1, Rb-1) have been described. To determine whether minisatellites represent genetic regulatory elements, we have conducted studies with the HRAS1 VTR. Mobility shift assays with crude nuclear extracts from a panel of human and murine cell lines revealed a species-specific factor present in all human cell lines examined (EJ, HeLa, Jurkat, CEM, BL-1, RPMI 7951). No binding was evident in a murine pre-B line (PD31) or fibroblast line (NIH3T3). Considerable variation in the level of this factor was observed, with the highest activity occurring in a bladder carcinoma line (EJ) and the lowest in two lymphocyte lines (CEM, BL-1) and a melanoma line (RPMI 7951). DNA footprinting detected a minimum binding site of 15 nucleotides, GGGACGCCACACTC. Comparison with previously described regulatory sequences revealed the HRAS1 site to be structurally quite similar to the consecutive array of AP2 and AP3 binding sites present in the 5' terminus of the SV40 72bp enhancer. We also showed that placement of the HRAS1 VTR downstream of an enhancerless CAT gene resulted in 10- to 20-fold increased expression of this reporter gene in transfected EJ cells. This activity was absent in NIH3T3 and reduced (to 5-fold) in CEM. Thus, the enhancer-like activity of the VTR corresponded roughly to the level of binding activity in crude nuclear extracts. In summary, (1) the HRAS1 VTR is recognized by specific nuclear factor(s); (2) its organization of binding sites (and perhaps their identity) is similar to the SV40 enhancer; and (3) it displays species and cell-type specific regulation of transcription.

E 543 DIFFERENTIAL ACTIVITY OF MODIFIED ELASTASE I ENHANCERS IN TRANSFECTED CELLS AND TRANSGENIC MICE. Fred Kruse, Galvin H. Swift, Robert E. Hammer and Raymond J. MacDonald, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235.
The 134-bp elastase I (EI) enhancer, which directs pancreatic acinar cell-specific transcription, contains three essential domains. Mutations within any single domain inactivate the enhancer in transfected pancreatic acinar tumor cells, but have little or no effect on pancreatic enhancer activity in transgenic mice. Only when any two of the three domains are mutated is the enhancer inactivated in transgenic mice, thus demonstrating a domain redundancy not detectable in transfected pancreatic cells. The absence of domain redundancy in cultured cells was not due to the particular acinar cell line used, the reporter gene, or whether the reporter gene was expressed transiently or stably integrated. We conclude that a transcription factor or modification of a factor(s) present in pancreatic cells of an animal is absent in pancreatic acinar cell lines. The loss of such a factor may occur during the transition of terminally differentiated pancreatic cells in situ to less differentiated cells in culture. To investigate this possibility, we have generated transgenic mice containing an EI enhancer domain mutant fused to the human growth hormone (hGH) reporter gene and the transcriptional regulatory sequences of trypsin I fused to SV40 large T antigen (TI-TAg). The TI-TAg transgene induces pancreatic acinar cell tumors in which cells are less differentiated than normal pancreatic tissue. Preliminary results indicate that hGH message levels drop dramatically in many of the less differentiated tumor nodules, but remain unaffected in the surrounding nontumorigenic tissue. These results are consistent with coincident dedifferentiation and loss of factors affecting elastase I enhancer function.

E 544 T CELL-SPECIFIC EXPRESSION OF THE IL-2 RECEPTOR GENE, Kuang, Anna, Sang-Mo Kang, and Michael Lenardo. Molecular Development of the Immune System Unit, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892. NF-kB is known to be a pleiotropic mediator of activation signals. Since NF-kB is found in a number of genes with differing cell-specificties, it is of importance to identify regions of these genes which confer cell-type specificity. One such example is the regulation of expression of the IL-2 receptor α gene (IL-2R) during the activation of T cells. Recent evidence implicates NF-κB as part of a T cell signal to turn on the IL-2R gene. The IL-2R gene promoter contains a site similar though not identical to the motif in the Ig κ enhancer that binds NF-κB with the same affinity and mediates its regulatory effect. However, it is not known why the IL-2R is not turned on in non-T cells demonstrating high levels of NF-κB activity.

We have studied in detail a short oligonucleotide of the IL-2 receptor promoter that contains the NF-κB binding site and its adjacent downstream region. When compared in transient transfection studies to a similar region from the Ig κ gene enhancer, the IL-2R oligonucleotide responded to NF-κB only in T cells; in contrast the κ enhancer site responded to NF-κB in a variety of cell types. Thus the downstream region immediately adjacent to the NF-κB site in the IL-2R promoter appears to repress expression mediated by the NF-κB site in non-T cells and enhance expression in T-cells. In addition the immediate downstream region without the NF-kB site appears to have T cell -specific enhancer activity. Thus, NF-κB and other nuclear factors binding this downstream flanking region appear to work together in conferring T cell specificity. By conducting mutagenesis studies and attempting to identify the important nuclear factors, we hope to understand how NF-κB and other nuclear factors that bind the downstream region interact and contribute to the cell type specificity of the IL-2 receptor α gene.

E 545 CHARACTERIZATION OF THE PROXIMAL PROMOTER REGION OF THE RAT CARBAMYL PHOSPHATE SYNTHETASE I GENE "IN VITRO" AND "IN VIVO". Monique Lagacé, and Gordon C. Shore, Department of Biochemistry, McGill University, Montreal, Canada, H3G 1Y6.
Carbamyl phosphate synthetase I (CPSI) is a mitochondrial matrix enzyme that is encoded by a nuclear gene. The gene is expressed only in hepatocytes and epithelial cells of the small intestine. We have analyzed the 5' flanking sequence of the CPSI gene by transient expression in HepG2 and Reuber H35 cells using chloramphenicol acetyl transferase as marker gene, as well as by "in vitro" transcription using liver nuclear extracts. Both analyses reveal that the CPSI proximal promoter lies within 530 bp upstream of the start of transcription. Previous footprint analysis of the region extending to position -161 revealed the existence of two cis-acting regulatory elements at position -35 to -69 and -104 to -124. The later contains a functional C/EBP binding site, GTTGCAAC, which is requisite but not sufficient to activate CPSI transcription. At least one more element located between position -165 to -530 is reguired for CPSI promoter activity. We are currently identifying such element(s) by footprint and band shift analysis, as well as by functional analysis "in vivo" and "in vitro".

E 546 IDENTIFICATION OF A NEW preB LYMPHOID-SPECIFIC ENHANCER ELEMENT IN THE IMMUNOGLOBULIN HEAVY CHAIN ENHANCER, Towia A. Libermann and David Baltimore, Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA.

We have identified a new enhancer element in the IgH enhancer between the µE2 and the µE3 sites. In addition to previously observed DNAse footprints we detected a new lymphoid-specific protected region. To test the possibility that this DNA motif is a lymphoid-specific enhancer element we inserted an oligonucleotide coding for this site into a c-fos promoter/CAT plasmid and assayed transcriptional activity of the constructs after transient expression. Surprisingly, this element acts as an extremely potent enhancer in all preB lymphoid cell lines tested, but is inactive in mature B cells and only slightly active in plasma cells, mature T cells, macrophages and mammary cells. We have shown by gel mobility shift and DNAse footprinting assays that different nuclear factors bind to this site in lymphoid and non-lymphoid cells. However, no difference was detected between preB and mature B cells or plasma cells suggesting that the difference between preB and mature B cells might be due to either posttranslational modification or interaction with a second preB-specific protein. Because LPS is able to induce some degree of maturation in preB cells, we checked whether LPS would down-regulate activity. Indeed, LPS treatment of preB cells transfected with the enhancer/CAT plasmid reduced transcription drastically. We suggest that this enhancer element plays an important role as a preB-specific enhancer element in the regulation of lymphoid-specific genes during B cell differentiation.

E 547 CELLULAR UPTAKE OF THE HTLV-I TRANS-ACTIVATOR PROTEIN, TAX_1. Paul F. Lindholm, Susan J. Marriott, Scott D. Gitlin and John N. Brady, Laboratory of Molecular Virology, NCI, NIH, Bethesda, Maryland 20892
The HTLV-I Tax_1 protein is a transcriptional *trans*-activator of the HTLV-I LTR and several cellular genes including IL-2, IL-2 receptor, and GM-CSF. During a series of experiments designed to test the biological activity of purified Tax_1 from *E. coli*, we observed that Tax_1 is readily taken up by tissue culture cells. Addition of purified Tax_1 to the growth media results in rapid cellular uptake of the protein by lymphoid and epithelial cells. Cellular fractionation studies indicated that approximately 80 percent of the incorporated Tax_1 is present in the nuclear fraction. Tax_1 was detectable as early as one hour after introduction of Tax_1 into the tissue culture media. Cellular incorporation of Tax_1 increased rapidly over the first 8 hours. Ten to 15ng of Tax_1 were incorporated in $2x10^5$ cells indicating an average uptake of approximately 10^3 molecules per cell. The addition of the lysosomotrophic agent chloroquine, did not increase the level of Tax_1 protein uptake, suggesting that cellular uptake of Tax_1 occurs by a mechanism different from the uptake of the HIV *trans*-activator, Tat. Cellular uptake and nuclear localization of Tax_1 was observed with Jurkat, CV-1 and HeLa cells. We are presently studying the mechanism of Tax_1 endocytosis.

E 548 **A New Member of the Leucine Zipper Class of Proteins Binds to the HLA DRα Promoter,** Hsiou-Chi Liou, Mark R. Boothby, Patricia W. Finn, Nasrin Nabavi, Roger Eddy, Thomas Shows, Laurie H. Glimcher, Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115; Department of Medicine, Harvard Medical School, Boston, MA 02115; Department of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York, 14263.

Several mutants derived from transformed human B cell lines have defects in a positive transacting function for major histocompatibility complex (MHC) class II gene expression. The lack of HLA-D expression in at least one such mutant line mapped to the MHC class II X box, a conserved transcriptional element in the promoter region. The cloning of a λgt11 complementary DNA encoding a DNA binding protein (human X-box binding protein, hXBP-1) whose target is the human DRα X box is reported here. This cDNA detects genes that map both to human chromosomes 5 and 22, and encodes a protein with structural similarities to the c-jun proto oncogene product. The level of transcripts detected by the hXBP-1 cDNA are reduced in the class II deficient mutant B cell line 6.1.6. These studies point to a role for hXBP-1 in regulating class II expression.

E 549 REGULATION OF CLASS II GENE EXPRESSION IN THE MOUSE, Richard A. Maki, Antonio Celada, Michael J. Klemsz and Scott R. McKercher, La Jolla Cancer Research Foundation, La Jolla, CA 92037

The regulation of Class II gene expression requires a number of cis-acting regulatory elements, which include the W, X and Y boxes. We have demonstrated that nuclear proteins specifically interact with each of these elements. These proteins are currently being characterized. The protein that binds to the Y box of the I-Aβ gene has been purified from the B cell lymphoma A20-2J and shown to consist of two components. Component A has a molecular weight of 34 kDa and has a low affinity for binding to the DNA. Component B has a molecular weight in the range of 42-46 kDa and has little or no affinity for DNA. When components A and B are combined, the affinity of the complex for DNA is increased dramatically.

The protein that binds to the promoter proximal X box of the I-Aβ gene has also been purified from A20-2J cells and shown to have a molecular weight of about 43kDa. A second X box, the promoter distal X box, is located about 1500 bp upstream and although the sequence of this X box is similar to the sequence of the promoter proximal X box, oligonucleotides containing the sequence of this X box do not compete for the protein binding to the promoter proximal X box as measured by a gel retardation assay. Based on this observation, it is likely that different proteins bind to the promoter proximal and promoter distal X boxes of the I-Aβ gene.

E 550 BIOCHEMICAL AND IMMUNOLOGICAL ANALYSIS OF H2-RIIBP, A PROTEIN THAT BINDS TO THE CRE ENHANCER OF MHC CLASS I GENES, Mickey Marks, Ben-Zion Levi, Steven Hirschfeld, Toshi Nagata, Ettore Appella, and Keiko Ozato, NICHD-LDMI, National Institutes of Health, Bethesda, MD 20892. The expression of MHC class I genes is controlled by a number of regulatory regions upstream of the transcription initiation site. One of these sequences, the class I regulatory element (CRE), can be divided into two separate enhancer-like elements, region I and region II. By screening a mouse liver cDNA library in lambda gt11 with region II probes, we have cloned a factor, H2-RIIBP, which binds to region II of the CRE as well as to the estrogen response element (ERE) of the vitellogenin gene. The factor, which bears strong homology to steroid receptors, was subsequently expressed from a recombinant Baculovirus vector in SF9 insect cells, and rabbit antisera were prepared against a partially purified protein product. This protein retains the ability to bind to both CRE region II and the ERE in gel mobility shift experiments. Data is presented regarding the structural characterization of recombinant H2-RIIBP and the intracellular distribution of the endogenous product in cells that do or do not express MHC class I antigens.

E 551 myb-HOMOLOGOUS GENES IN ANTIRRHINUM FLOWERS, Cathie Martin, Franscisco Culianez-Marcia, David Jackson, Departments of Genetics and Cell Biology, John Innes Institute, Colney Lane, Norwich NR4 7UH, UK

The C1 gene of maize controls the expression of anthocyanin biosynthetic genes in a tissue-specific manner. It has been shown to contain an N-terminal domain that is highly homologous to the myb proto oncogenes of human and Drosophila[1]. The protein structure and mutational data from C1 suggest that this is a transcriptional activator of the anthocyanin biosynthetic genes.

We have been interested to determine whether genes controlling the spatial pattern of floral pigmentation in Antirrhinum are homologous to C1 and of the myb proto oncogene type. We have isolated cDNAs to 6 myb-like proteins from flowers. The DNA binding domain is highly conserved in each. Two of the genes are expressed only in flowers and most highly expressed in older flowers. Two genes are expressed in flowers and roots but are not detected in leaves or fruiting bodies. Two other genes appear to be constitutive. One of these shows altered transcript sizes in different plant organs suggesting that alternate transcript processing may be involved in expression of this gene.

It would appear that myb-homologous proteins represent a large class of transcriptional activators in plants. We are attempting to discover if these are involved in the control of cell growth as in animal systems or whether nature has modified a pre-existing system to serve rather different control functions in plants.

[1] Paz-Ares et al. EMBO J. **6**:3553-3558

E 552 CHARACTERIZATION OF TRANSCRIPTION FACTOR NF-GM3 OF MOUSE GM-CSF GENE FROM HUMAN T CELL LEUKEMIA LINE JURKAT, Shoichiro Miyatake[1], Joseph Shlomai[1], Kenji Sugimoto[1], Akio Tsuboi[1], Kyoko Yokoto[1], Ken-ichi Arai, Naoko Arai[1],[1]DNAX Research Institute, Department of Molecular Biology, 901 California Avenue, Palo Alto, California 94304.

Battery of lymphokine genes are induced coordinately in T cells stimulated by antigens or infected by HTLV-I. The stimulation by antigen leads to the activation of two signals, the activation of protein kinase C and the increase of CA^{++} influx. $p40^{tax}$, transactivator encoded by HTLV-I genome, seems to activate various lymphokine genes. Both stimuli utilizes the common DNA motif to activate GM-CSF gene. We have identified several DNA binding proteins that interact GM-CSF promoter region in the nuclear extract of human T cell leukemia line Jurkat. Transfection experiment showed that the region between upstream regulatory sequence and TATA box (-32 to -54) is also required for efficient transcription of GM-CSF promoter. The mutations in this region also decrease the level of transcription in vitro. Single band designated as NF-GM3 is detected by gel shift assay using this region as a probe. The transcription activity of the extract is reconstituted by the flow through and 0.6M fractions of phosphocellulose collumn and NF-GM3 is eluted in the 0.6M fraction. The NF-GM3 in this fraction was further purified by DNA affinity collumn. The transcription activity was reconstituted by the flow through of phosphocellulose, flow through and 1.0M fraction of DNA affinity collumn. This strongly suggests that NF-GM3 is required for the transcription of the GM-CSF promoter. The apparent molecular weight of this protein is about 300K by gel filtration. Whether this protein is induced by the activation signal and further purification and characterization of this protein is underway.

E 553 IMMEDIATE-EARLY GENE EXPRESSION IN HEPATOCYTES, Kenneth L. Mohn, Thomas M. Laz, Anna E. Melby, Jui-Chou Hsu, Hung Q. Nguyen and Rebecca A. Taub, Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 During cellular proliferation, prior to any *de novo* protein synthesis, immediate-early genes are expressed which play an important regulatory role in mitogenesis. This group of genes includes several known and postulated DNA-binding proteins which presumably regulate mitogenesis by altering the transcription of genes involved in later aspects of this response. Comprehensive studies identifying and cataloging immediate-early genes have been conducted in fibroblasts and lymphocytes but, to date, no such study has been conducted in cells of epithealial origin, the most common cell type involved in human cancer. In an effort to identify epithealial cell-specific transcription factors involved in the regulation of proliferation, we have undertaken such a study utilizing two different systems: rat hepatocytes stimulated to proliferate by 70% partial hepatectomy and quiescent, serum-starved Reuber H35 rat hepatoma cells stimulated with insulin, a potent mitogen in this cell line. cDNA libraries have been constructed from both cell types three hours post mitogenic stimulation in the presence of cycloheximide. To date, we have differentially screened approximately 35,000 recombinate phage and have identified 310 clones which demonstrate increased mRNA abundance in proliferating hepatocytes. After further characterization, 170 of these induced clones have been shown to represent 40 different genes. Through hybridization to panels of known genes and sequence analysis, we have identified seven of these induced genes as c-fos, jun a, erg-1, IP-10, ubiquitin, actin and histone H-3. The remaining 33 induced genes require further characterization but initial sequence analysis suggests that they are novel. Three of the induced genes are not expressed in fibroblasts and, thus, may represent hepatic/epithealial cell specific factors. We are continuing to differentially screen these libraries and characterize the identified clones according to tissue specificity and time course of expression.

E 554 EVIDENCE THAT NF-κB REPRESENTS A FAMILY OF INDUCIBLE DNA BINDING PROTEINS EXPRESSED IN A BIPHASIC MANNER. Jerry A. Molitor, Dean W. Ballard, Warner C. Greene, Howard Hughes Medical Institute, Departments of Medicine and Microbiology and Immunology, P.O. Box 3037, Duke University Medical Center, Durham, North Carolina 27710. Transcriptional activation of various cellular (κ-Ig, interleukin-2, interleukin-2 receptor-α) and viral (HIV-1) genes involves nuclear protein binding events at related κB enhancer elements. Recent studies have revealed that NF-κB is expressed in unstimulated cells but its biologic function is negated by complex formation with a cytoplasmic inhibitor termed IκB (Bauerle, P. A & Baltimore, D. L. (1988) Science 242, 540-546). However, following appropriate induction, this factor-inhibitor complex is disrupted allowing rapid translocation of NF-κB to the nucleus. Using UV-crosslinking, we have detected a biphasic pattern for the activation and nuclear translocation of NF-κB activity that involves four discrete proteins. Specifically, phorbol ester stimulation of Jurkat human T-cells results in the nuclear appearance of three major κB specific protein species (75 kD, 55 kD and 51 kD) within 20 minutes. These three proteins are constitutively expressed, induced in the absence of de novo protein synthesis and form cytoplasmic complexes with IκB. In contrast, we have also detected a prominent fourth κB specific protein, approximately 86 kD in size, appearing 16-24 hours after induction. In contrast to the "early" κB specific proteins, the "late" expression of this 86 kD factor is dependent upon de novo protein synthesis. These findings suggest that NF-κB corresponds to a family of inducible DNA binding proteins that are expressed in a biphasic manner in human T cells. It is possible that these different proteins may mediate different functional responses within the cell via the same cis-regulatory sequence.

E 555 CELL-SPECIFIC RESPONSES OF THE ORNITHINE DECARBOXYLASE GENE TO ACTIVATION OF SECOND MESSENGER PATHWAYS, David R. Morris and Mitchell Abrahamsen, Department of Biochemistry, University of Washington, Seattle, WA 98195

The ornithine decarboxylase (ODC) gene shows an immediate-early response to mitogenic activation. In both T-lymphocytes, activated through the antigen receptor, and Swiss 3T3 fibroblasts, activated by serum mitogens, ODC mRNA begins to be elevated above the resting level by 30 min and is maximally induced 10- to 20-fold by several hours after activation. Activation of protein kinase C is necessary and sufficient for elevation of ODC expression in both cell systems. Transcription of the ODC gene is undetectable in runon assays with nuclei from resting fibroblasts and is induced upon mitogenic activation of these cells. In contrast, the ODC gene is strongly transcribed in nuclei from resting T-cells and there is no change in transcription rate after stimulation by mitogen. In neither cell type is there evidence for involvement of transcriptional pausing or stabilization of mature ODC mRNA in the regulation of this gene. We suggest that regulation of the ODC gene in mitogen-activated T-cells involves intranuclear stabilization of pre-mRNA molecules.

Response of ODC expression to elevated cAMP is also quite different in the two cell types. In lymphoid cells, elevation of intracellular cAMP results in down-regulation of ODC expression. On the other hand, activation of protein kinase A in fibroblasts (and in an adrenal cell line) stimulates ODC transcription ca. 10-fold. The molecular basis of this cell-specific positive or negative regulation is being investigated using chimeric expression constructs.

E 556 TRANSCRIPTION OF THE *gfa* GENE IS REGULATED BY BOTH AN UPSTREAM AND A DOWNSTREAM INITIATOR WHICH INTERACT COOPERATIVELY, Y. Nakatani, M. Brenner, and E. Freese, NINDS, NIH, Bethesda, MD 20892

The *gfa* gene encodes glial fibrillary acidic protein (GFAP), an intermediate-filament protein expressed primarily in glial cells. We have used in vitro transcription studies to show that the basal level of *gfa* transcription is controlled by two distinct initiators, i.e., promoter elements that direct transcription from a specific start site. One initiator is located around 25 bp upstream from the transcription start site, contains a TATA box and apparently acts together with a sequence found around the transcription start site. The other initiator is located between +11 and +50 bp downstream from the transcription start site and contains a sequence having homology with the box A sequence common to promoters of RNA polymerase III. Most of this second region overlaps with the protein-encoding sequence which starts at bp +17. The sensitivity of transcription to α-amanitin indicates that both initiators are used by RNA polymerase II.

E 557 IDENTIFICATION OF A NOVEL INDUCIBLE DNA-PROTEIN INTERACTION IN THE KAPPA IMMUNOGLOBULIN ENHANCER, Keats Nelms and Brian Van Ness, Institute of Human Genetics and Department of Biochemistry, University of Minnesota, Minneapolis, MN 55455. Control of kappa immunoglobulin light-chain gene expression requires the interaction of tissue specific and developmentally regulated DNA-binding proteins with the kappa gene promoter and enhancer. Deletion of enhancer sequences upstream from the NF-κB binding site in the mouse enhancer has been shown to impair enhancer function, implying additional proteins may interact with these sequences. In surveying this region for sites of protein binding in the mouse light chain enhancer, a novel DNA-protein interaction, designated κBF-A, was detected approximately 50 bp upstream from the NF-κB binding site. κBF-A binding activity is high in LPS-stimulated pre-B cells but undetectable in unstimulated pre-B cells and kappa producing plasmacytomas, appears concomitantly with NF-A2 binding activity in activated pre-B cells, and correlates with high level induction of kappa transcription in these cells. In pre-B cells, this site was shown to contribute to the function of the kappa enhancer as an inducible, cis-acting element. The homologous region of the human light chain enhancer was found to display binding properties indistinguishable from the octamer binding motif found in kappa light chain and heavy chain promoters and the heavy chain enhancer.

E 558 NUCLEAR PROTEINS INVOLVED IN CARDIAC SPECIFIC AND DEVELOPMENTAL CONTROL OF ANF GENE TRANSCRIPTION. Mona Nemer, Ali Ardati, Mona Greenbaum, Stefania Argentin, Jacques Drouin. Laboratoire de génetique moléculaire, Institut de recherches cliniques de Montreal, Montreal (Quebec) CANADA, H2W 1R7.

During cardiac cell growth, expression of the atrial natriuretic factor (ANF) gene is under differential control in heart atria and ventricles. In the fetal and newborn heart, the ANF gene is expressed at similar levels in both heart compartments. However soon after birth, and in parallel to the decrease in the mitotic index of the heart, ANF mRNA levels are progressively decreased in the ventricles and increased in the atria. A similar change occurs during cardiac hypertrophy, a process thought to mimic the molecular events involved in normal cardiac cell growth. In order to define the molecular mechanisms involved in tissue specific and developmental control of the ANF gene in the heart, we have used DNA-mediated gene transfer to define the DNA elements required for cardiac specific expression. Rat ANF sequences extending to 1.6 Kb upstream of transcription initiation are necessary for maximal promoter activity in primary cardiocyte cultures. This region of the promoter contains two clusters (a proximal and a distal one) of DNA elements which bind nuclear proteins present in cardiac tissues. At least one of these elements which shows homology to C EBP binding sites binds a nuclear protein which appears to be specific to cardiac tissues. Nuclear extracts prepared from adult atria are enriched for this protein as compared to extracts prepared from adult ventricles. Thus, the relative amount of this protein appears to correlate with the differential expression of the ANF gene in adult atria and ventricles. Further characterization of both tissue specific and ubiquitous nuclear proteins that bind the ANF promoter during various stages of cardiac cell growth are currently underway.

E 559 ISL-1, AN ISLET SPECIFIC INSULIN ENHANCER BINDING PROTEIN, BELONGS TO A NEW CLASS OF HOMEODOMAIN CONTAINING PROTEINS. Torbjörn Norberg,Stefan Thor,Olof Karlsson Helena Ohlsson and Thomas Edlund,Dept of Microbiology,University of Umeå,S-90187 Umeå, Sweden. **The selective expression of the rat insulin 1 gene is mainly dependent on a β-cell specific enhancer located at positions -103 to -332 bp in the 5'-flanking region of the gene. By mutational analysis most if not all of the cis-acting elements of importance for the β-cell specific transcriptional activity of the insulin gene have been defined. Five distinct regions within the insulin enhancer have been identified as binding sites for nuclear factors. All of these protein-binding DNA elements correspond to the cis-acting elements which are of importance for β-cell specific activity of the enhancer. By screening a cDNA expression library, from a rat insulinoma cell line (RIN), with a dsDNA probe containing two of the above mentioned protein binding sites a cDNA encoding a protein capable of binding to the -222 to -211 region of the insulin enhancer has been isolated.In the adult rat the corresponding RNA has only been detected in the islets of Langerhans.The encoded protein, named Isl-1 contains a homeodomain which is most closely related to that of the recently isolated *lin-11* gene of C. elegans (G. Freyd and R. Horvitz pers.comm.). In addition, Isl-1 shares a novel Cys/His domain with *lin-11* and also with *mec-3*, another C. elegans homeodomain containing protein (M. Chalfie pers.comm.). The overall structure of these three proteins is very similar and together they define a new class of homeodomain containing proteins. The known functions of *mec-3* and *lin-11* in cell type and cell lineage specification and the specific expression pattern of Isl-1 implies a function for Isl-1 in the development of pancreatic endocrine cells.**

E 560 AN ASSAY FOR THE LYMPHOID RESTRICTED TRANSCRIPTION FACTOR NF-AT IN INDIVIDUAL VIABLE CELLS **Jeffrey Northrop*, Steve Flering+, Gerald Crabtree*, and Leonard Herzenberg+ Departments of Pathology* and Genetics+ and the Howard Hughes Medical Institute Beckman Center, Stanford University, Stanford CA. 94305.**

The induction of Interleukin-2 (IL-2) gene expression during the process of T lymphocyte activation requires signals provided through both antigen receptor stimulation and secreted products of macrophages. Through a largely unknown pathway, these signals are ultimately integrated at the level of the IL-2 enhancer, a region from -52 to -326 bp from the start of transcription. Within this enhancer are contained binding sites for multiple transcription factors. One such factor, NF-AT, appears only after T cell activation in response to signals from the antigen receptor. In order to further characterize the function of NF-AT and therefore the process of T cell activation, we have developed a single cell assay for this transcription factor. Stable transformants of the human T cell line, Jurkat, were derived using a construct composed of 3 copies of the NF-AT binding site linked through the basal IL-2 promoter to the E. coli *lacZ* gene. Using the FACS/GAL assay for B-gal, we find that these cells will transcribe *lacZ* in response to signals that activate the endogenous IL-2 gene. Stimulation of clonal cell lines reveals distinct cell to cell variability of expression of B-gal within the clone. FACS sorting of stimulated cells into B-gal+ and B-gal- populations followed by mini-nuclear extract preparation and gel shift analysis, indicates that B-gal+ cells are enriched 4-fold in NF-AT binding over the level present in B-gal- cells. This indicates that variability of B-gal expression reflects variability in NF-AT levels. That B-gal- cells do contain low levels of NF-AT may reflect a requirement for all 3 binding sites to be occupied for transcriptional enhancement much as multiple factors are simultaneously required for IL-2 enhancer activity. We have in addition, been able to select cells which somewhat over express NF-AT. This method should prove generally useful in the study of a wide variety of transcription factors.

E 561 TRANS-ACTING DNA BINDING FACTORS OF MAIZE ADH1 AND ADH2, Anna-Lisa Paul and Robert J. Ferl, Department of Vegetable Crops, University of Florida, Gainesville, FL 32611.

The positions of DNA binding factors in close association with the 5' flanking region of maize alcohol dehydrogenase-1 (Adh1) and alcohol dehydrogenase-2 (Adh2) were determined with genomic sequencing and in vivo dimethyl sulfate (DMS) footprinting. The intensities of the DMS footprints in the 5' regions of Adh1 and Adh2 were quantitated over an eight hour hypoxia induction time course and compared with mRNA levels from each time point.

In Adh1, one set of factors over positions -130 and -110 is constitutively present, whereas other factors centered over positions -180 and -100 are evident only after Adh1 has been induced. Removing hypoxic stress for 2 hours restored the DMS footprint associated with the 5' region in uninduced cells.

All of the factors bound to the 5' region of Adh2 were constitutively present, centered around positions -210, -160 and -80. Removing cells from hypoxic stress did not affect factor binding in the Adh2 promoter.

In vitro gel retardation and footprinting experiments have shown that a multi-protein complex is associated with the Adh1 promoter. Some of the component proteins have been partially purified and characterized.

E 562 Regulation of Retinoic Acid Receptor Gene Expression in Skin-derived Fibroblasts Monica Peacocke and Hui Tsou, Department of Dermatology, Boston University School of Medicine, Boston MA 02118 Vitamin A and its derivatives have profound effects on skin. As well as playing a role in development, retinoids effect growth and differentiation of epithelial and mesenchymal cells. In order for retinoids to have an effect, the presence of a high affinity receptor is required. Three genes for distinct retinoic acid receptors have recently been cloned (RAR alpha, RAR beta and RAR gamma) and all are members of a large multi-gene family which act as ligand inducible transcriptional enhancer factors. We studied the expression of RAR alpha and beta in skin-derived fibroblasts. Quiescent fibroblasts express high constitutive levels of RAR alpha (2 mRNA species of 2.8 and 3.8 kb) and the expression of these mRNAs does not change with stimulation by retinoic acid (RA). In contrast, fibroblasts do not express RAR beta, but stimulation with RA induces the expression of the 2 RAR beta mRNAs (3.1 kb and 3.6 kb) as early as 2 hours after stimulation, with 30-fold induction evident at 4 hours, with levels remaining high as long as 24 hours after with a single dose of RA. The protein synthesis inhibitor cycloheximide (10 ug/ml) had no effect on the induction of RAR beta, however, RA + cycloheximide induced 20-fold more RAR beta at 6 hours after stimulation than RA alone. Ligands such as thyroid hormone and dexamethasone did not induce RAR beta. These data suggest that RA specifically induces RAR beta but not alpha in skin-derived fibroblasts. That cycloheximide superinduces RAR beta in the presence of RA but not alone suggests that this gene may be regulated at the level of mRNA stability. Thus, we believe that regulation of these two RAR genes is clearly different in skin-derived cells suggesting different physiological roles for these molecules in mediating the varied effects of retinoids in the skin.

Abstract Withdrawn

E 564 THE AP1 COMPLEX WHICH FORMS ON A VIRAL ENHANCER SHOWS TISSUE VARIATION. John P. Quinn, A.R. Farina, K. Gardner and David Levens. The Gibbon Ape Leukemia Virus enhancer element contains an AP1 consensus sequence which is the major determinant of enhancer activity in both HeLa and MLA144 cells. Whereas the major DNA binding complex in HeLa cells is composed of c-fos/c-jun ,the complex in MLA144 cells is distinct from either c-fos and c-jun. The specific complex which forms in MLA144 cells is composed of at least a heterodimeric complex whose formation appears to driven by mass action. The MLA144 cell is a primate T cell line; however it would appear that a similar complex can occur in the human T cell line Jurkat. Interestingly one of the factors appears to be constitutively expressed in this cell line whereas the other is expressed in response to T cell activation; both factors are required for complex formation. In addition to this abundant complex there is in MLA144 a minor complex in which one of the components is related to fos (FRA). We have undertaken the biochemical purification and molecular characterization of the major DNA binding complex in MLA144 which we will present.

E 565 RAF GENE EXPRESSION AND CHARACTERIZATION OF THE HUMAN A-RAF AND C-RAF PROMOTER REGIONS. U. Rapp,[1] U. Brennscheidt,[1] T. Beck,[2] J. Lee, G. Sithanandam, S. Storm,[1] and J. Cleveland,[3]. [1]Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD 21701, [2]Program Resources Inc. Frederick, MD 21701, and [3]St. Jude's Children's Hospital, Memphis, TN 38101. There are three active raf proto-oncogenes in man, c-raf, A-raf, and B-raf. Northern hybridisations to RNA from 36 adult and fetal mouse tissues indicate that (1) c-raf is expressed in all tissues, although steady-state levels vary 5-fold between tissues, (2) B-raf is expressed at highest levels in cerebrum, (3) A-raf is expressed preferentially in tissues which are regulated by steroid hormones. In order to delineate the structures responsible for the differential expression, we have begun to characterize the promoters for the individual raf genes. Nucleotide sequencing of genomic DNA clones, primer extension, and S1 nuclease have been used to identify the 5' ends of the A-raf and c-raf-1 RNA's. Consistent with its ubiquitous expression, the c-raf promoter region has features of a house-keeping gene in that it is GC-rich, lacks a TATA box, contains heterogeneous RNA start sites and three SP1 sites. Moreover, an octamer binding motif (ATTTCAT), is located at -500 bp. The A-raf promoter displays features of a regulated eukaryotic promoter in that it contains a TFIID binding site (TATA-box), sequences identical to the binding sites of transcription factors found in adeno-virus early gene promoters, and a consensus glucocorti-coid response element (TGTTCT) is located within exon 1. Both A-raf and c-raf promoters direct 60-90% of reporter gene expression relative to the SV40 early gene promoter in COS cells. Sequentially deleted sequences flanking exon 1 of both genes show positive and negative effects on the level of reporter gene expression.

E 566 PROSTATE-SPECIFIC ANTIGEN: GENE STRUCTURE AND REGULATION OF EXPRESSION.
P.H.J. Riegman[1], R.J. Vlietstra[1], J.A.G.M. van der Korput[1], J.C. Romijn[2] and J. Trapman[1]. Departments of Pathology[1] and Urology[2], Erasmus University, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands.
Prostate-specific Antigen (PA) and human Glandular Kallikrein (hGK) are kallikrein-like serine proteases, which are exclusively synthesized and subsequently secreted by the epithelial cells of the prostate. PA is widely used as a prostate-specific tumor marker. The PA and hGK gene are tandemly located on chromosome 19. The PA gene was isolated and completely sequenced. It is composed of five exons and has a size of about 6 kb. The promoter region showed a high percentage of homology with the corresponding region of the hGK gene. In addition to a TATA-box, several other cis-acting regulatory elements were recognized. Analysis of the promoter region of the PA gene by DNaseI footprinting, using nuclear extracts from an androgen-dependent prostate tumor cell line revealed the protection of a GC-box and several other so far not yet described sequence motifs. Expression of PA and hGK mRNA was determined in different prostate tumors and prostate tumor cell lines. Various different transcripts of both genes can be detected as a result of alternative splicing. High levels could be detected in the androgen-dependent, but not in the androgen-independent lines. The PA and hGK expression levels in an androgen-dependent prostate tumor cell line grown in steroid-depleted medium could be induced by the synthetic androgen R1881.

E 567 IDENTIFICATION OF CIS-ACTING REGULATORY ELEMENTS IN THE CHICKEN βB1-CRYSTALLIN GENE.
H. John Roth and Joram Piatigorsky, NEI, NIH, Bethesda, MD 20892.
The β-crystallins of the chicken eye lens are composed of at least six different polypeptides, each of which exhibits a characteristic temporal and spatial pattern of expression in the developing lens. The βB1-crystallin polypeptide is unique in that its mRNA accumulates only in the equatorial epithelial and fiber cells, but not in the central epithelial cells of the chicken lens. We have used transient expression assays, DNase I footprint analysis, and gel retardation analysis to identify sequence elements which regulate βB1-crystallin gene expression. Sequences from positions -152 to +30 of the βB1-crystallin gene fused to the chloramphenicol acetyl transferase (CAT) gene directed high levels of CAT expression when transfected into cultured lens cells. Deletion of sequences from positions -152 to -110 inactivated the promoter. Consistent with this result, lens nuclear extract protected two distinct regions (positions -120 to -115 and -85 to -60) from DNase I digestion in footprinting assays. These putative βB1-crystallin gene regulatory regions contained sequences similar to cis-acting regulatory sequences previously identified in a number of different genes. The sequences from positions -88 to -76 were similar to the PEA 2 binding site of the polyoma virus enhancer. Two distinct sequence elements similar to the octamer binding motif (positions -125 to -118 and -74 to -67) were also present. Results of site-directed mutagenesis and gel retardation experiments indicating that these same sequences also regulate chicken βB1-crystallin gene expression are presented. Thus, the lens-specific expression of the βB1-crystallin gene apparently requires regulatory elements used by other genes in non-lens tissues.

E 568 IDENTIFICATION OF TWO DIFFERENT PROTEINS BINDING TO EACH RAT GROWTH HORMONE (rGH) SILENCER SEQUENCES I AND II,
René Roy, Sylvain L. Guérin and David D. Moore, Molecular Endocrinology Laboratory, CHUL Research Center, Laval University Medical center, Québec, Canada, G1V 4G2
The gene encoding rat growth hormone (rGH) is significantly expressed only in the anterior lobe of the pituitary gland, its expression being maintained completely silent in all the other tissues examined. We demonstrated that such a strong cell-specific repression of rGH gene expression in these cells is essentially determined by the combined action of both positive and negative regulatory mechanisms. This negative action is determined by the specifc interaction of a trans-acting repressor (SBP-I) with a cis-acting silencer site located 300bp upstream of the rGH cap site. Detailed examination of the rGH upstream regulatory sequence permitted us to identify another functional silencer site (silencer II) highly homologous to silencer I and centered 200bp further upstream from it. Gel shift experiments using oligonucleotides containing either the silencer I or II sequence revealed two specifically retarded DNA/protein complexes (A and B), both competed by an unlabeled silencer I-containing oligo but not by a similar fragment containing point-mutations for those nucleotides critical for recognition by SBP-I. Since the specifically retarded complexes detected are identical whether we used silencer I or II as labeled probe, and since they can be cross-competed using either silencer-containing oligos, we conclude that the same protein (or protein complex) binds to both silencers. Detailed DMS-methylation interference footprinting performed on each of the retarded complexes (A and B) revealed striking differences in the methylation patterns that could not be detected in DNaseI footprinting, providing evidences for the binding of two different proteins at the rGH silencer I site. Examination of the DNA sequence recognized by these proteins also revealed significant homologies with the DNA binding site described for the well known transcription factor NF-1. Although preliminary UV-crosslinking experiments using silencer I as labeled probe identified two proteins of 52Kd and 59Kd, experiments are actually underway to test whether these rGH silencer-binding proteins belong to the NF-1 family of transcription factors.

E 569 cDNA CLONING AND ANALYSIS OF OCT-2B, A VERY RARE, LYMPHOID SPECIFIC OCTAMER BINDING PROTEIN Edgar Schreiber, Jacek Blaszynski, Patrick Matthias, Michael M. Müller-Immerglück, Adriano Fontana and Walter Schaffner, Institut für Molekularbiologie II der Universität Zürich, CH-8093 Zürich, Switzerland
The octamer sequence ATGCAAAT, present in the promoter and enhancer of the immunoglobulin heavy chain (IgH) genes, is a major determinant of the cell type specific expression of IgH genes in B-lymphocytes. However, the octamer sequence is also a promoter or enhancer element in some housekeeping genes. The differential usage of this regulatory sequence is mediated by different species of octamer binding proteins. One species of 100 kDa (oct-1) is present in all cell types and accounts for the ubiquitous type of transcription. The lymphoid cell specific protein of 60 kDa (oct-2A) specifically stimulates immunoglobulin promoters which consist essentially of a TATA-box and an octamer sequence upstream of it. In addition to oct-2A, another lymphoid-specific octamer binding protein of 75 kDa (oct-2B) exists in B-cells. Proteolytic clipping bandshift assay (PCBA) of isolated proteins revealed that the 75 kDa oct-2B species is closely related to the oct-2A protein. We have isolated a cDNA clone encoding the oct-2B protein: alternative splicing of oct-2 gene transcripts yields a protein which is identical to the oct-2A protein but extends at the COOH-terminus for 75 amino acids. The additional sequence dramatically affects the stability of the oct-2B transcript. Several lines of (circumstantial) evidences suggest a role for the oct-2B factor in the long range activation by the IgH enhancer. We are currently testing the function of the oct-2B protein in enhancer and promoter activation by ectopic expression in B-lymphocytes and non-B cells.

E 570 MULTIPLE PROTEIN FACTORS BIND TO THE EFII REGION OF THE ROUS SARCOMA VIRUS LTR ENHANCER. Rosalie C. Sears and *Linda Sealy, Dept. of Cell Biology and *Dept. of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232. The trans-acting enhancer factor EFII, identified in high-salt nuclear extracts from quail fibroblasts and chick embryos, was shown using *in vitro* footprinting to bind a 38 nucleotide sequence (-229 to -192) in the U3 region of the Rous sarcoma virus LTR (MCB 7:787, 1987). This cis-element contains a tandem repeat, although gel mobility assays using mutant oligonucleotides demonstrate that only one of the repeats is required for EFII binding. It is now clear, through the use of specific competitor oligonucleotides in the gel mobility assay with EFII, and through direct-binding mobility shifts, that EFII is not the same as either the trans-acting factor C/EBP (MCB 9:1155, 1989), or IBF (NAR 15:9841, 1987), two other protein factors shown to bind to the same 38 nucleotide region of U3. EFII is a tissue restricted nuclear factor which is also expressed in chick embryo fibroblasts and a transformed avian B-cell line, but is absent in liver cells. In the B-cell extract, an additional binding factor (ABFI) is observed to form a slower migrating complex. ABFI appears to bind, and cause stabilization of the EFII-DNA complex, as observed by the shift of bound oligonucleotide from the lower band to the upper band with increasing amounts of extract. There are two GCAAPy motifs in the EFII DNA sequence located at the 3' end of each repeat. Competitor oligonucleotides that contain the sequence GCAAPy eliminate the ABFI shift, yet have no effect on the EFII shift. The results suggest that ABFI requires a non-specific protein-protein interaction, as well as recognition of the DNA sequence (GCAAPy), for specific binding. Methylation protection and methylation interference assays are being performed on the EFII and ABFI complexes to determine the specific protein-DNA interactions which exist. The functional significance of these proteins for LTR enhancer activity *in vivo* is being investigated, and purification by sequence-specific affinity chromatography is underway.

E 571 IDENTIFICATION OF A NOVEL TRANSCRIPTION FACTOR INVOLVED IN TNF AND IL-1 INDUCTION OF G-CSF GENE EXPRESSION, M. Frances Shannon, Filomena S. Occhiodoro, Elizabeth S. Kuczek, Linda M. Pell and Mathew A. Vadas, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia.
Haemopoietic growth factors (HGF's) are generally expressed in a tissue specific and inducible manner. For example subsets of these genes are expressed in antigen or mitogen-activated T cells and in TNF and IL-1 treated endothelial cells or fibroblasts. Transcriptional activation, mRNA stability and translational control have been shown to be important in controlling the final levels of the gene products.

We have previously identified several novel transcription factors which bind to specific sequences in the promoter regions of a number of these HGF genes. One of these proteins, NF-GMa, binds to a sequence termed CK-1 (5'GAGATTCCAC3') which is highly conserved in all HGF genes so far identified. The CK-1 sequences from a number of HGF genes (including GM-CSF, G-CSF, IL-3 and IL-5) show a wide range of affinity for NF-GMa. This protein has a broad cellular distribution but appears to be functional only in endothelial cells and fibroblasts. In these cell types TNF- and IL-1 treatment increases levels of NF-GMa binding as well as activating transcription from the NF-GMa binding site in the G-CSF gene. This effect appears to be specific since phorbol ester has no effect on CK-1 controlled transcription. Although T cell lines contain high levels of NF-GMa, no TNF, IL-1 or phorbol ester induction of CK-1 directed transcription can be detected. The mechanism of TNF induction is currently being investigated. NF-GMa has been purified by DNA-affinity chromatography and is presently being cloned.

E 572 ISOLATION OF A cDNA ENCODING A SPECIFIC DNA BINDING PROTEIN FOR THE NEGATIVE ELEMENT OF THE GASTRIN PROMOTOR, Babette Simon, Timothy C. Wang and Steven J. Brand, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114
Gastrin is a growth factor transiently expressed in fetal islets during a period of rapid growth and development. Gastrin gene transcription is also commonly derepressed in islet cell tumors and the gastrin secreted by these islet tumors acts as an autocrine growth factor. The gastrin gene promoter is comprised of tandem positive and negative cis-regulatory elements. Binding of a trans-acting repressor to the gastrin negative element (GASNE) lying between -106 and -82 may constitute a molecular switch important for repressing islet-specific expression of the gastrin gene during development. We have isolated a cDNA encoding a GASNE DNA binding protein by screening an islet cell cDNA lambda GT 11 library with a ^{32}P labeled DNA probe containing concatamerized copies of the GASNE. One plaque was isolated after screening 10^6 plaques which specifically bound to the GASNE probe. Gel mobility shift assays demonstrated expression of a beta galactosidase fusion protein which specifically binds to the GASNE element, but not to other regulatory elements in the gastrin promotor or a mutated GASNE. The cDNA comprises 3.1 kb with an ORF of 1.9 kb following the beta galactosidase sequence. Preliminary data indicate that this DNA binding protein is similar to other transcriptional repressors which regulate a number of genes controlling cell proliferation.

E 573 A NOVEL SILENCER REGULATES CLASS I GENE EXPRESSION IN VIVO. Dinah S. Singer, Jean E. Maguire, and Jocelyn Weissman, Expt'l Immunology Branch, NIH, Bethesda Md. 20892.
Class I MHC genes encoding transplantation antigens are expressed at various levels in all somatic tissues. The molecular mechanisms regulating these quantitative tissue specific differences have been investigated. A novel silencer has been found between -700 and -750 bp ustream of the class I gene, PD1; this silencer requires the presence of a downstream enhancer to function. To examine the role of this silencer element in establishing in vivo patterns of class I gene expression, promoter constructs with and without the silencer, ligated to CAT, were introduced into transgenic mice. All transgenic mice expressed CAT. Removal of the silencer element resulted in increased expression from the downstream promoter in all tissues; however, the magnitude of the increase varied among tissues, suggesting that the silencer regulates tissue specific levels of class I gene expression.
In vivo competition studies in transfected cells demonstrate that the activity of the silencer is mediated through trans acting factors. Direct gel shift assays have identified nuclear factors which bind to the 50 bp silencer domain, giving rise to two discrete complexes. Inhibition of protein synthesis leads to the rapid loss of these silencer complexes, with a concomitant increase in class I RNA suggesting that tissue specific levels of class I expression are determined by the silencer and specific labile nuclear factors. Support for this model comes from analysis of silencer factors in various tissues where the levels of silencer factors from a given tissue are inversely related to the level of class I expression. The nature of these factors and their specific recognition sites will be discussed.

Tissue-Specific Regulated Transcription Factors-II; Hormonal Regulation of Transcription

E 574 A NOVEL ARRANGEMENT OF BINDING SITES FOR RAT LIVER NUCLEAR PROTEIN C/EBP LOCATED 5' AND 3' TO THE TATA PROMOTER ELEMENT OF THE HUMAN CLASS I ALCOHOL DEHYDROGENASE GENE, Mark J. Stewart and Gregg L. Duester, Department of Biochemistry, Colorado State University, Ft. Collins, Co 80523.
Human class I alcohol dehydrogenase (ADH) is composed of three isozymes (α, β, and γ) encoded by three genes displaying differential patterns of expression during liver development. Mammalian ADH catalyzes the oxidation of numerous alcohols (including ethanol) primarily for detoxification. In human liver, α ADH is expressed during the first trimester of fetal development with the additional expression of β during the second trimester, and γ several months after birth. Transient transfection studies of human hepatoma cell lines with various ADH-chloramphenicol acetyltransferase (CAT) fusion plasmids indicated that the α ADH gene is much more transcriptionally active than the β and γ ADH genes in HepG2, but that both the α and γ ADH genes are equally transcribed in Hep3B. The promoter regions have been subjected to DNase I footprint analysis using nuclear extracts derived from adult rat liver. Protection occured in several locations including one, between positions -51 and -10 in β and γ (-55 and -10 in α), which shares homology with known binding sites for the rat liver nuclear protein C/EBP. Purified C/EBP was shown by footprint analysis to bind at two distinct sites in the β and γ ADH promoters located at positions -51 to -31 and -21 to -10. β had a greater affinity for C/EBP than γ, and the α ADH promoter bound C/EBP very weekly at -21 to -10. The TATA-box promoter element at -30 to -22 was not protected by C/EBP suggesting that the flanking C/EBP molecules may create a novel binding pocket for TFIID, the TATA-binding general transcription factor. The different affinities for C/EBP displayed by the α, β and γ ADH promoters may be involved in their differential expression during liver development.

E 575 EVIDENCE FOR TWO DISTINCT PATHWAYS OF IL-2 RECEPTOR α CHAIN GENE EXPRESSION: ROLES OF THE κB, SRE, NF-IDR1 AND SP1 ELEMENTS, Michel B. Toledano, Dragos G. Roman, Nancy F. Halden and Warren J. Leonard, Cell Biology and Metabolism Branch, NICHD, NIH, Bethesda, 20892. The IL-2Rα promoter contains a κB site and binding sites for additional factors within a fifty base-pair region extending from -290 to -240. These sites include one similar to the c-*fos* serum response element (SRE) which binds a factor related if not identical to serum response factor, a potential SP1 site, and a sequence overlapping with one of two imperfect direct repeat which binds a novel factor NF-IDR1. In mutagenesis experiments, whereas alteration of the κB site decreases activity in HTLV-1 transformed MT-2 T cell line but not in PMA-induced Jurkat T cells, mutation of the NF-IDR1 site decreases activity in PMA-induced Jurkat but increases activity in MT-2 cells. Mutation of the SRE-like site decreases activity in both cell types but the effect in Jurkat is more pronounced. Mutation of the potential SP1 site increases activity in both cell lines, suggesting possible negative regulatory function associated with this site. The spatial organization of these elements in a short fragment of the promoter define an original transcriptional unit. The differences observed between MT-2 and PMA-induced Jurkat of the functional effects of mutation of these sites suggest the existence of two distinct pathways of activation of the IL-2Rα chain gene.

E 576 PHORBOL ESTER STIMULATION OF THE CATHEPSIN L PROMOTER IS MEDIATED BY ELEMENTS DOWNSTREAM OF THE TRANSCRIPTION INITIATION SITE, Bruce R. Troen, Shyam S. Chauhan, and Michael M. Gottesman, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109 and Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892

The major excreted protein (MEP) of mouse fibroblasts is the precursor to a lysosomal acid protease (cathepsin L) induced by malignant transformation, growth factors, tumor promoters, and cAMP. Cathepsin L plays a major role in intracellular protein catabolism and in immune function and has been implicated in myofibrillar, myocardial, and renal pathology. We have cloned a fully functional gene for MEP from NIH 3T3 cells and have constructed a series of transcriptionally active MEP–CAT vectors that contain various regions of the MEP gene and MEP promoter region upstream of the CAT reporter gene. As few as 300 base pairs immediately upstream of the transcription initiation site are sufficient for promoter activity. The MEP–CAT constructs that contain only regions upstream of the promoter are not stimulated by TPA treatment, but are induced by treatment with a cAMP analog. Primer extension, using RNA from cells transiently transfected with MEP–CAT fusion plasmids, demonstrates that phorbol ester treatment increases the amount of the transcript from constructs containing the promoter and downstream sequences, but not from constructs containing only the 5'–flanking region of the MEP gene. Nuclear run–off experiments confirm that the increase in enogenous MEP mRNA is mediated by increased initiation of transcription and not via transcriptional attenuation. The sequence of the MEP promoter contains three potential binding sites for the AP-2 transcriptional factor, which has been shown to mediate both phorbol ester and cAMP responsiveness of other promoters. These results suggest that the MEP gene is regulated in a complex manner by these upstream sequences in concert with additional downstream elements in either the first or second intron. Gel retardation studies will be an initial step in searching for novel factors that possibly bind to downstream motifs in the MEP gene that are TPA responsive.

E 577 COOPERATION OF TWO SEQUENCE ELEMENTS CONFERS MAXIMUM TISSUE SPECIFICITY TO THE RAT INSULIN II GENE, Ming-Jer Tsai, Yi-Zhong Gu and Young-Ping Hwung, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

The insulin gene is expressed exclusively in the pancreatic β cells and its expression is regulated mainly at the transcriptional level. Using linker scanning mutations, we have defined at least 7 elements which are important for the promoter/enhancer activity. In order to determine which of these elements are important for the tissue-specificity, we inserted oligonucleotides corresponding to these elements in front of a minimal promoter construction which contains only the ovalbumin gene TATA box. Two of these elements, RIPE3a (-110 to -86) and RIPE3b (-126 to -100) were able to induce the expression of the minimal promoter in insulin producing cells (HIT cells) but not in nonspecific cells (BHK, 3T3 and CV1 cells). In constrast, constructions containing mutated RIPE3b oligonucleotide were not functional. These results indicate that both RIPE3a and RIPE3b elements are important for the tissue specificity of the insulin II gene. However, these two regions independently displayed low level of activity as compared to the whole enhancer/promoter. To examine whether these two elements cooperate with each other to reach the maximum tissue-specificity, we inserted an oligonucleotide containing both regions (-126 to -86) into the same vector. The resulting constructions had 10X higher activity than the activity of either element alone. Therefore, these two elements cooperate to confer maximum tissue-specificity.

E 578 TRANSCRIPTION FACTORS REGULATING THE EXPRESSION OF GM-CSF GENE IN JURKAT CELLS, Akio Tsuboi, Kenji Sugimoto, Shoichiro Miyatake, Ken-ichi Arai and Naoko Arai, DNAX Research Institute, Department of Molecular Biology, 901 California Avenue, Palo Alto, California 94304. Helper T cells produce a number of lymphokines including GM-CSF when stimulated by antigens. This activation can be mimicked not only by lectins or anti-T cell receptor antibodies, but also by the combination of phorbor ester and Ca ionophore. A region between -96 and -72 upstream of the mouse GM-CSF gene (CLE2/GC box) is responsible for transcription activity induced by both phorbol ester and Ca ionophore in vivo[1]. In this report, we first prepared transcriptionally active nuclear extracts from stimulated as well as non-stimulated Jurkat cells and attempted to identify DNA binding proteins recognized this sequence. Gel retardation assay revealed that this region contains at least two DNA binding motifs: one is NF-GM2 binding sequence, GGTAGTTCCCC, positions between -91 and -81, and the other is GC-box sequence like positions between -83 and -77. NF-GM2 binding activity which can be competed with NF-kB binding sequence, GGAAAGTCCCC, was detected only in the nuclear extract from stimulated cells, whereas GC-rich sequence can be recognized by non-inducible factors constitutively. Furthermore, it was demonstrated that inducible as well as non-inducible binding proteins are essential components for efficient transcription in vitro. We are in the process of purifying the inducible factor(s) NF-GM2 on the basis of both DNA binding and in vitro transcription activity to elucidate the last event of signal transduction pathway regulating the activation of lymphokine genes. Characterization of NF-GM2 protein is underway.

1) Miyatake, et al. (1988) MCB 8:5581

E 579 TESTIS-SPECIFIC EXPRESSION OF THE RAT C-MOS PROTO-ONCOGENE: A POSSIBLE ROLE IN MEIOSIS, Frans A. van der Hoorn, Heide A. Tarnasky, Judith E. Spiegel[1] and Steven K. Nordeen[1], Dept. Med. Biochemistry, University of Calgary Health Sciences Centre, Calgary, Alta. T2N 4N1 and 1) Dept. Pathology, University of Colorado Health Sciences Center, Denver, CO 80262. We detected c-mos RNA in gradient purified rat male germ cells. Primary spermatocytes at the pachytene stage, early spermatids and late spermatids all express three c-mos RNA species of 5, 3.6 and 1.7 kb at a very low level. In contrast, only one c-mos RNA species of 1.7 kb has been detected in mouse early spermatids: mouse pachytene spermatocytes do not appear to express c-mos. Although we did not rule out the possibility that multiple c-mos promoters are active in the same cell, our preliminary RNA mapping data suggest that splicing accounts for the observed RNA species. In agreement with the RNA data we detected c-mos protein in premeiotic and post-meiotic rat male germ cells. It has an apparent molecular weight of 44 kD as determined by SDS-PAGE and Western blotting. We are investigating the origin of the size difference between the observed protein and the predicted size of 36 kD of the mos protein. Our data together with those obtained by us and other groups studying c-mos expression during oogenesis suggest an important role for c-mos during meiosis. We are currently addressing questions concerned with the regulation of c-mos expression in rat male germ cells. Our previous results had demonstrated that an enhancer is present in the c-mos locus. We recently reported binding of Nuclear Factor 1 or a closely related molecule to the enhancer: however, site-directed mutagenesis of the NF-1 site eliminated NF-1 binding but not enhancer activity in fibroblasts. We are using testis-specific in vitro transcription to study the role of NF-1 and other factors in the regulation of c-mos and other testis-specific genes which we recently isolated.

E 580 MUTATIONS WHICH DISRUPT DNA BINDING AND DIMER FORMATION IN THE E47 HLH PROTEIN MAP TO DISTINCT DOMAINS, Anna Voronova and David Baltimore, Whitehead Institute for Biomedical Research, Cambridge, MA 02142

E47 is a sequence-specific DNA binding protein that is involved in the expression of the immunoglobulin κ light chain. The E47 cDNA was isolated by screening a λgt11 expression library with an oligonucleotide probe identical to the κE2 sequence GGCAGGTGG located in the immunoglobulin kappa chain enhancer.

The region of the E47 polypeptide required for specific recognition of DNA is related in amino acid sequence to a number of other regulatory proteins, including the *myc* family proteins, proteins which regulate muscle differentiation, and proteins important for Drosophila development. These proteins all contain a basic amino acid region followed by two short amphipathic helices separated by an intervening loop, the HLH motif, which presumably is a dimerization domain. In addition to homodimers, the HLH proteins can also form heterodimers that bind specifically to a common DNA sequence.

To define the region required for the specific contact with the DNA and for the dimer formation, as well as to determine whether the two can be functionally separated, site-directed mutagenesis of the E47 gene was performed. Mutations in the basic domain of the protein abolished DNA binding. However these mutants were able to form dimers with the E47 wild type protein. No DNA binding was observed when a double mutation in the helix region of E47 disrupted protein dimerization. The data suggests that the E47 protein dimerizes via the helix-loop-helix domain which contains highly conserved hydrophobic residues. Dimerization is required for DNA binding, which in turn is determined by a specific sequence of basic amino acids positioned upstream of the HLH motif. However in the absence of the DNA binding dimerization still occurs.

E 581 A cDNA FROM PANCREATIC ß-CELLS ENCODING A PUTATIVE TRANSCRIPTION FACTOR OF THE INSULIN GENE. Michael D. Walker, Cheol Won Park, Ada Rosen and Ami Aronheim, Dept. of Biochemistry, Weizmann Institute, Rehovot 76100, Israel.

Cell specific expression of the insulin gene is achieved through transcriptional mechanisms operating on multiple DNA sequence elements located in the 5' flanking region of the gene. Of particular importance in the rat insulin I gene are two closely similar 9 bp sequences IEB1 and IEB2 (GCCATCTGC/G) : mutation of either of these leads to dramatic (5-10 fold) reduction in transcriptional activity. We have screened an expression cDNA library (λgt11) derived from mouse pancreatic endocrine ß-cells with a radioactive DNA probe containing multiple copies of the IEB1 sequence. A cDNA clone isolated by this procedure shows efficient, reproducible binding to the probe but much weaker binding to either an unrelated DNA probe or to a probe bearing a single base pair insertion within the recognition sequence. Gel mobility shift assay using a series of wild type and mutant DNA sequences shows that the ability of each sequence to bind protein correlates well with its ability to function in vivo in the context of the insulin gene 5' flanking sequences. We conclude that the isolated cDNA may encode a transcription factor that participates in control of insulin gene transcription.

E 582 REGULATION OF THE GASTRIN PROMOTER IN ISLET CELLS BY ADJACENT POSITIVE AND NEGATIVE DNA ELEMENTS. Timothy C. Wang, Babette Simon, and Stephen J. Brand, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

Gastrin is expressed in fetal pancreatic islets, but is repressed at birth as islets terminally differentiate. DNA transfection and in vivo competition studies have identified adjacent negative and positive elements between -108 and -76 in the gastric promoter which control gastrin transcription in islet cells. The negative element (-108 to -82) contains the sequence ATTCCTCT which is also found in the negative element of the B-interferon promoter. Gel mobility shift and DNAase I footprinting studies have shown specific binding of islet nuclear extracts to the gastrin negative element. Immediately downstream of the negative element lies a positive element (-82 CATATGG -76) which activates gastrin transcription in islet cells. The sequence of the positive element resembles the islet specific enhancer elements of the insulin gene (CATCTGG/C). Gel mobility shift assays and in vivo competition studies indicate that this positive element activates the gastrin promoter by binding the same islet cell transcription factor(s) which binds enhancer elements in the rat insulin gene. The tandem organization of negative and positive elements suggests a switch for controlling the transient transcription of the gastrin gene during fetal islet development.

E 583 TCDD RECEPTOR-STIMULATED *IN VITRO* TRANSCRIPTION FROM THE CYTOCHROME P_1-450 GENE PROMOTER IN MOUSE HEPATOMA CELL NUCLEAR EXTRACT, Long-ping Wen and James P. Whitlock, Department of Pharmacology, Stanford University, Stanford, CA 94305

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a potentially toxic environmental contaminant, activates the rate of transcription of the cytochrome P_1-450 gene. This activation of transcription is accomplished by the formation of TCDD-receptor complex in the cytosol, followed by its "translocation" to the nucleus and subsequent binding to the specific DNA sequences called DREs. An *in vitro* transcription system, which mimics this *in vivo* induction, has been developed. Nuclear extracts, prepared from TCDD-treated mouse hepatoma cells and after fractionation on a phosphocellulose column, direct transcription from P_1-450 promoter at a much higher rate than their counterparts prepared from non-treated cells. Moreover, the partially-purified rat liver TCDD receptor appears to be capable of stimulating transcription from P_1-450 promoter in this reconstituted *in vitro* system.

E 584 Regulation of Gene Expression by the Myosin Light Chain 1/3 Gene Enhancer.

Bruce Wentworth, Erick Berglund, James Engert, Maria Donoghue and Nadia Rosenthal. Department of Biochemistry, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118.

The skeletal myosin light chain 1/3 locus is an attractive system to study the developmental genetics of myogenesis. The two myosin light chains, MLC1 and MLC3, appear sequentially in late fetal and early postnatal skeletal muscle development, respectively, as a result of differential transcription from two promoters. MLC 1/3 gene transcription is regulated by an enhancer element located 25 kb downstream of the MLC1 promoter, 3 kb beyond the polyadenylation signal sequence. The size of the original 0.9 kb DNA isolate has been reduced to a 180 bp subfragment possessing full enhancer activity. DNA hybridization experiments have been used to demonstrate that the human MLC locus contains a sequence that is highly similar to the rat enhancer in both structure and position. In collaboration with Dr. H. Arnold, we have identified a 280 bp enhancer element downstream of the human MLC gene with over 80% homology to the rat counterpart. The rat and human MLC enhancers are bound by nuclear proteins which are important to the developmental regulation of gene expression in skeletal muscle. Three MyoD1 binding sites have been identified in the rat enhancer suggesting that this protein regulates gene expression via multimer formation on the MLC enhancer. In addition, a CArG motif located immediately 3' of a MyoD1 site binds to the MAPF proteins involved in the muscle specific expression of the chicken skeletal actin gene. Other non-muscle nuclear proteins bind to sequences within the rat MLC enhancer, including an A/T rich motif located in the 3' portion of the enhancer, and to a novel binding domain found 5' of the MyoD1/CArG sites that is conserved between rats and humans. Mutational analysis is providing insight into the contribution of each binding protein to enhancer function. The data suggest that the MLC enhancer is a complex molecular switch regulating muscle specific gene expression via an interplay of many nuclear regulatory proteins.

E 585 TISSUE-SPECIFIC REGULATION OF THE NEURONAL TYROSINE HYDROXYLASE GENE, Sungok Yoon and Dona M. Chikaraishi, Departments of Molecular Biology and Neurosciences, Tufts University Medical School, Boston, MA 02111
Tyrosine hydroxylase (TH) is a rate-limiting enzyme in catecholamine biosynthesis and is regulated in a tissue-specific manner at the transcriptional level. From transfection studies using pheochromocytoma (Pc) cell line, the tissue-specific element was identified between -212 and -187 bp upstream of the TH gene (F. Cambi, B. Fung and D.M. Chikaraishi, to be published in J. Neurochemistry). In this study, the putative tissue-specific segment was analyzed for factor binding in vitro. In gel-retardation assays using crude nuclear extract, the segment forms a tissue-specific complex, and in Southwestern experiments, the segment identifies 3 tissue-specific proteins of 48Kd, 40Kd and 38Kd in size. The DNase I footprinting experiments using a longer fragment, however, revealed an hierarchical pattern of protection. The hierarchy, in ascending order, was POU box (-174/-162), AP-I site (-208/-198), TE-II, a putative tissue-specific element (-198/-189), and TE-I, another putative tissue-specific element (-185/-174). This result may indicate that the tissue-specific element of the TH gene works in cooperation with the other well-known elements.

E 586 PHOTOPRECEPTOR-SPECIFIC EXPRESSION OF ß-GALACTOSIDASE IN THE RETINAS OF TRANSGENIC MICE CARRYING A RHODOPSIN PROMOTER/LACZ CONSTRUCT. Donald Zack, Jean Bennett, Yanshu Wang, Carol Davenport, John Gearhart, and Jeremy Nathans, Departments of Molecular Biology and Genetics and Physiology, Johns Hopkins Medical School, Baltimore, MD 21205. Rhodopsin, the rod visual pigment, is expressed in a tissue-specific and developmentally-regulated manner. Despite recent advances in the understanding of the structure, physical properties, and biology of rhodopsin, little is known about the mechanisms which regulate its expression. To define the cis-acting regulatory DNA sequences required for its correct tissue-specific expression, transgenic mice were generated with a construct containing 2200 bp of DNA 5' of the bovine rhodopsin gene fused to a modified lacZ gene. Three independent lines carrying the transgene were obtained. All showed photoreceptor-specific expression of ß-galactosidase activity. Enzyme activity was undetectible in brain, liver, and tail. These results demonstrate that the 2200 bp fragment contains sufficient regulatory information to direct the correct tissue-specific expression of rhodopsin and that the signals have been conserved between the bovine and murine species. The 2200 bp fragment has been sequenced. Areas of homology with upstream sequences from other species are suggestive of possible regulatory elements. Transgenic mice with deletions of the 2200 bp fragment are being generated to define the minimal sequence required for photoreceptor-specific expression.

E 600 HIGH RESOLUTION ELECTRON MICROSCOPY LOCALIZES GLUCOCORTICOID RECEPTOR EXPRESSION WITHIN SPECIFIC CELLS OF THE PITUITARY AND LIVER. Tony Antakly, Mohammed Badr, Mona Nemer, Xudong Zhang,Department of Anatomy, McGill University, Montreal, Quebec H3A 2B2 and Clinical Research Institute of Montreal.

Although most tissues contain glucocorticoid receptors, expression of the receptor within heterogenous tissues such as the pituitary remains largely unknown. Since glucocorticoid regulation of target genes is often cell-specific it becomes crucial to define the specific cell types which express the glucocorticoid receptor. We have now developed an electron microscope technique for the localization of the glucocorticoid receptor and its mRNA in pituitary and liver tissues. An immunocytochemical technique using 10 nm gold particles was used for the subcellular localization of glucocorticoid receptor in thin sections of tissues embedded at low temperature (-30°C) in a low viscosity resin: lowicryl K4M. This method was shown to preserve antigeneicity while providing adequate ultrastructural details. Glucocorticoid receptor mRNA localization was achieved by *in situ* hybridization prior to embedding the tissue blocks using ^{35}S-labeled probes. A combination of the immunogold technique and *in situ* hybridization allowed us to visualize and quantitate both the receptor and its mRNA within individual cells. In the pituitary, a clear cellular heterogeneity was observed within corticotropes, mamotropes and somatotropes while gonadotropes were very weakly labeled. Melanotropes were devoid of immunocytochemical staining as expected (Antakly et al. Science 1985) but mRNA labeling was observed. Hepatocytes were labeled but not Kupffer cells. This high resolution procedure can be applied to study other trans-acting factors and monitor their intracellular dynamics and their regulation during development and differentiation.

E 601 NATIVE PROGESTERONE RECEPTOR MEDIATED CELL-FREE TRANSCRIPTION OF A TARGET PROMOTER, Milan K. Bagchi, Ludger Klein-Hitpass, Sophia Y. Tsai, Nancy L. Weigel, Ming-Jer Tsai and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

The biological effects of female sex steroid progesterone are mediated by intracellular progesterone receptors (PR). PR regulates in vivo transcription of target promoters containing progesterone response elements (PRE). We have now devised a cell-free system in which highly purified native PR, when added to HeLa nuclear extracts, can effect remarkable enhancement of transcription from a PRE-containing promoter. PR does not activate transcription from promoters lacking PRE. The transcriptional enhancement can be eliminated by the addition of oligonucleotides containing PRE, confirming that PR is the transactivator. Both A and B forms of chicken progesterone receptor can stimulate transcription in vitro. Under optimal assay conditions, PR can induce synthesis of at least one transcript for every ten promoters during a 45 min reaction. Hormone-free PR activated by salt treatment also enhances transcription indicating that purified PR does not require bound hormone to activate transcription in vitro. Preliminary kinetic studies suggest that PR forms a stable and functional preinitiation complex with the promoter and the factors present in HeLa nuclear extract. This complex can rapidly commence RNA synthesis when presented with nucleotides. We plan to use this in vitro transcription system to gain further insight into the mechanism of gene activation by steroid receptors.

E 602 ERB A BINDING SITE IS AN ESSENTIAL MODULE OF A CHICKEN LYSOZYME SILENCER ELEMENT, Aria Baniahmad, Christof Steiner, Anja C. Köhne and Rainer Renkawitz
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Expression of the lysozyme gene is a marker for the differentiation of macrophages, lysozyme transcription being gradually increased during maturation. In addition to enhancer elements, which increase their activity upon macrophage differentiation, we found two cell specific silencer elements, which loose their repressing activity during maturation [1]. Several cell types without lysozyme gene transcription show strong silencer activity. Silencer elements placed upstream or downstream of a heterologous promoter-gene unit repress transcription independent of their orientation and position, although their repressing activities 3′ of the gene are reduced. We found that all of these silencer elements have a modular stucture quite similar to enhancer elements. Their modules (nuclear factor binding sites) are specific for silencers, not found in enhancer elements. One exception is a strong negative module, which can be bound either by the product of the oncogene v-erbA or by the thyroid hormone receptor (the c-erbA proto-oncogene), thereby turning into an enhancing module upon the addition of thyroid hormone.

[1] Baniahmad, A., Muller, M., Steiner, Ch. and Renkawitz, R.: *EMBO J.* **6**, 2297-2303 (1987)

E 603 SPECIFIC DOMAINS OF THE GLUCOCORTICOID RECEPTOR ARE INVOLVED IN SYNERGISM WITH OTHER TRANSCRIPTION FACTORS

Claudia Baniahmad, Marc Muller, Christian Kaltschmidt and Rainer Renkawitz
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We have previously shown that several transcription factor binding sites within a steroid responsive unit can synergistically increase the steroid response (1). Here we have examined the ability of deletion mutants of the human glucocorticoid receptor to function cooperatively. We have identified a number of domains which contribute to the synergism shown with several transcription factors. Synergism with different transcription factors needs different receptor domains. In contrast to cooperativity between glucocorticoid receptor binding sites, functional cooperativity between receptor and other transcription factors cannot be explained by increased DNA-binding affinities.

(1) Schüle, R., Muller, M., Kaltschmidt, C. and Renkawitz, R.: Science 242, 1418-1420(1988).

E 604 DNA ELEMENTS INVOLVED IN THE ESTRADIOL-INDUCED AND LIVER-SPECIFIC EXPRESSION OF THE CHICKEN APO VERY LOW DENSITY LIPOPROTEIN II GENE. Johanna M. Beekman, Jan Wijnholds, Ingrid J. Schippers and Geert AB. Department of Biochemistry, University of Groningen, The Netherlands.

The apo VLDL II gene is only expressed in the liver of the laying hen. The expression is strictly controlled by estradiol. The regulation is primarily on the level of transcription.

To define the 5' flanking regions important for expression of the gene, we constructed several series of deletion mutants. Transient expression in chicken embryonic hepatocytes showed that the Estrogen Responsive Element at position -177 to -164 is important for estradiol induction. Similar results were obtained after transfection of the estrogen-responsive MCF-7 cell line.

Using the genomic sequencing technique we studied the protein-DNA interactions as they occur in vivo in the 5' flanking region (-1300 to +200) of the VLDL gene. We found several in vivo protein-DNA interactions. For instance on the ERE at position -177 to -164 and on an element resembling the COUP element found originally in the chicken ovalbumin gene. These interactions are only present in the tissue where the gene is transcribed. In vitro DNase I footprinting confirmed these in vivo interactions.

E 605 MUTANT ANALYSIS OF THE DNA BINDING DOMAIN OF THE v-*erb* A PROTEIN FROM AVIAN ERYTHROBLASTOSIS VIRUS. Beverly G. Bonde and Martin L. Privalsky. Depts. of Genetics and Microbiology, University of California, Davis, CA 95616

Avian Erythroblastosis Virus causes cancers in susceptible chickens and transforms cells in culture. One of two oncogenes carried in its genome is designated v-*erb* A. This gene encodes a mutant version of a thyroid hormone receptor, a transcriptional modulator which binds specific enhancer-like sequences termed TRE's (thyroid hormone responsive elements) and either activates or supresses transcription from the associated gene. The v-*erb* A mutant receptor binds DNA in a sequence-specific fashion but has been reported recently to supress transcription from a TRE which is activated by a normal receptor. This is an important clue in determining how the mutant receptor participates in neoplastic development and how its structural differences lead to functional differences from its cellular counterpart, c-*erb* A, which is necessary for normal growth and development.

Previous studies in our lab of insertional mutations in the v-*erb* A gene showed that any mutation in the DNA binding domain abolished DNA binding ability and biological functioning. Mutations in the remnants of the hormone binding domain, which no longer has the ability to bind hormone in the viral protein, had varying effects on DNA binding and biological function depending on their location. This domain, by analogy to the related steroid receptors, could be important for heterodimeric and homodimeric protein-protein interactions. DNA binding, therefore, appears to be critical for receptor functioning so we set out to inverstigate these findings more closely.

Initially, we developed a sequence-specific DNA binding assay using a genomic clone of the rat growth hormone receptor as the target DNA. This gene is known to have multiple sites for thyroid hormone receptor binding. Subsequently, we have made amino acid substitutions at critical points in the DNA binding domain of v-*erb* A which differ from c-*erb* A to study their affect on DNA binding specificity. We have also created truncations at specific points in the hormone binding domain to study this domains role in DNA binding and biological functioning. Here we present the results of this work.

E 606 COOPERATIVE INTERACTION OF STEROID RECEPTORS, M. Suzanne Bradshaw, Sophia Y. Tsai, Bert W. O'Malley and Ming-Jer Tsai, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.
Fundamentally important to understanding how steroid receptors induce gene expression is to determine how receptors cooperate with each other and with other transcription factors. We have previously shown that a glucocorticoid/progesterone response element (GRE/PRE) functioned in the absence of a distal promoter element and that two GRE/PREs acted synergistically. When a single estrogen response element (ERE) was inserted 5' to a single GRE/PRE, we found that an ERE can functionally interact with a GRE/PRE. The interaction between two heterologous SREs was less synergistic than between two GRE/PREs. Our lab has previously shown that cooperative binding of the progesterone receptor contributes to functional synergism bewteen two GRE/PREs. However, an ERE and a GRE/PRE demonstrate no cooperative binding. Using progesterone receptor mutants, we have delineated two regions of the receptor required for cooperativity between both heterologous and homologous receptors. These two regions are the transactivation domains of the receptor, and are sufficient for partial cooperativity between two GRE/PREs. However, a construction containing the two activation domains is not sufficient for cooperativity between heterologous SREs. The amino-terminus is also required. Thus, three regions of the progesterone receptor, two activation domains and the amino-terminus, are required for cooperative interaction of heterologous steroid receptors.

E 607 GLUCOCORTICOID RECEPTOR-DEPENDENT DISPLACEMENT OF A SPECIFIC NUCLEOSOME ON THE MOUSE MAMMARY TUMOR VIRUS PROMOTER IS NECESSARY FOR TRANSCRIPTIONAL ACTIVATION, Emery H. Bresnick, Sam John, Diana Berard, Philippe LeFebvre, Charles Rories, Gordon L. Hager, Lab of Experimental Carcinogenesis, National Cancer Institute, Bethesda, MD 20892. Our laboratory has shown that an array of phased nucleosomes are positioned on the mouse mammary tumor virus (MMTV) LTR in episomes and as single integrated copies. The hormone-activated glucocorticoid receptor (GR) binds to DNA regulatory elements on one of these nucleosomes (nuc B), leading to the displacement of nuc B, and the formation of a preinitiation complex that consists of at least two factors, NF1 and TFIID. Thus, nuc B represses the MMTV promoter by restricting access of transcription factors. Butyrate, an inhibitor of histone deacetylation, prevents GR-dependent displacement of nuc B. Inhibition of nuc B displacement is not due to inactivation of the GR, as saturation binding experiments show that the amount of GR and the affinity for hormone is unaffected by butyrate. Furthermore, the GR can activate transcription of an LTR-luciferase construct equivalently in untreated and butyrate treated cells in a transient expression assay. These results are consistent with the hypothesis that butyrate induces a modification of chromatin that prevents the GR from binding to nuc B, or alternatively, that nuc B is frozen in a state that is incompetent to undergo displacement. To distinguish between these two mechanisms, a synthetic nuc B fragment was reconstituted with core particles from untreated and butyrate treated cells, and the ability of the GR to interact with these molecules is being assessed.

E 608 HUMAN ESTROGEN RECEPTOR OVER-EXPRESSED IN INSECT CELLS FORMS MULTIPLE PROTEIN-DNA COMPLEXES. Myles Brown and Phillip A. Sharp, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139
To explore the steps by which estrogen activates its receptor, we have employed a baculovirus expression system to over-produce the human estrogen receptor in insect cells. The estrogen receptor made in this system is full length, is recognized by a monoclonal antibody to the human estrogen receptor, and binds estrogen specifically. Using the gel mobility shift assay, we have been able to show specific binding of the recombinant estrogen receptor to the estrogen response element (ERE). The recombinant estrogen receptor binds the ERE both in the absence and presence of estrogen if the binding is carried out in the absence of Mg^{2+}. When Mg^{2+} is added to the binding reaction, the estrogen receptor binds the ERE only in the presence of hormone. This effect is more pronounced at higher temperatures. Tamoxifen, a non-steroidal anti-estrogen, is able to stimulate ERE binding to the same extent and under the same conditions as estradiol. Estradiol stimulates formation of an estrogen receptor-ERE complex with increased mobility compared to the complex formed without hormone or with tamoxifen. These results show that specific DNA binding of the estrogen receptor is not absolutely dependent on the presence of hormone and that estradiol but not tamoxifen is able to induce a change in the estrogen receptor that may be important in understanding its ability to differentially activate target genes. We propose a model in which the estrogen receptor-ERE complex can exist in at least three distinct states and that these are responsible for the differences in biological activity of estrogen, estrogen withdrawal, and tamoxifen.

E 609 ISOLATION AND CHARACTERIZATION OF THE PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1) FROM RAT HTC HEPATOMA CELLS: SEQUENCES INVOLVED IN REGULATION BY GLUCOCORTICOIDS AND cAMP

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Glucocorticoids and cyclic nucleotides regulate tissue-type plasminogen activator (tPA) activity in HTC rat hepatoma cells primarily by modulating plasminogen activator-inhibitor (PAI-1) gene expression. In contrast, regulation of tPA activity in reponse to these agents in normal rat hepatocytes is primarily modulated by direct effects on tPA message and protein. To investigate the molecular mechanisms underlying this regulation, we have cloned the rat PAI-1 gene from an HTC genomic library. Two positive clones were isolated, one of which contains the entire PAI-1 gene with approximately 10 kb of flanking DNA. The gene is 10.5 kb in size, it has 9 exons and 8 introns, and has a high degree of structural similarity to the human PAI-1 gene. Computer assisted DNA sequence analysis of 2.5 kb of 5' flanking DNA as well as 2 kb of the 3' flanking region has identified multiple sites in the 5' flanking region which agree with consensus sequences for cAMP response elements (CREs), glucocorticoid response elements (GREs) and binding sites for AP2. This region also contains several stretches of putative Z-DNA. We are currently assessing these sequences for protein binding using gel retardation assays and DNAse footprinting and for functional activity by construction of hybrid genes and analyses of their hormonal regulation in transfected HTC hepatoma cells and primary hepatocytes. Comparison of the results in these two cell types should identify differences in the mechanism of regulation between hepatocytes and hepatoma cells.

E 610 TRANSCRIPTIONAL INHIBITORY REGIONS WITHIN THE LIGAND BINDING DOMAIN OF THE HUMAN GLUCOCORTICOSTEROID RECEPTOR ARE INVOLVED IN THE 8S HETEROOLIGOMER FORMATION.

Françoise CADEPOND, Nicole JIBARD, Ghislaine SCHWEIZER-GROYER, Ingrid SEGARD-MAUREL, Stanley HOLLENBERG, Vincent GIGUERE, Ron EVANS and Etienne-Emile BAULIEU. INSERM U33, Labhormones, 94275 Bicêtre, France and the Howard Hughes Medical Institute, Gene Expression Laboratory, The Salk Institute, San Diego, California 92138, USA.

Following sedimentation analysis of transiently expressed human glucocorticosteroid receptor (hGR) deletion mutants, two apparently unrelated sequences within the ligand binding domain (LBD) appear to be involved in the formation of the non-DNA binding 8S heterooligomeric complex. Each region (the first one located between amino-acids 550 and 599 and the second present in the COOH terminal part of the GR molecule between amino-acids 696 and 777) independently contributes to the formation of the 8S GR complex. These two LBD subregions include those previously shown to exhibit a repressive activity on the constitutive truncated hGR (0/559) (Hollenberg et al., Cancer Research, 1989, Suppl. 49, 2292s-2294s). These results extend the previously observed correlation between lack of transcriptional activity and the heterooligomeric form of GR (Pratt et al.,1988, J Biol. Chem. 263, 267-273). These findings support the hypothesis according a physiological role for hsp90 in the masking of the DNA binding domain of the receptor. The corresponding interacting regions of the hsp90 are under current investigation.

E 611 **AN NF1-RELATED ACTIVATOR SEQUENCE MEDIATES TRANSCRIPTION FROM THE ESTROGEN REGULATED VITELLOGENIN PROMOTER.** Tsu-Chung Chang and David J. Shapiro. Department of Biochemistry, University of Illinois, Urbana IL 61801.

In order to identify the DNA sequences essential for estrogen-regulated and liver-specific vitellogenin (vit.) gene expression, we have employed site-specific mutagenesis to create a variety of mutations in the 5'-flanking region of vit. B1 gene. The effect of these constructs on transcription were studied by using a homologous cotransfection system with cell lines derived from *Xenopus* hepatocytes and fibroblasts. Mutation of the CAAT box or deletion of a conserved nematode box or the region between -301/-121 had no significant effects upon vit. promoter activity. However, in addition to the estrogen response elements (EREs) at -334/-302, an eight bases DNA element with weak sequence homology to the NF1 element is essential for efficient transcription from vit. promoter. Deletion or mutation of this vit. activator (VA) sequence reduces promoter activity by at least an order of magnitude. Reinsertion of this sequence into the VA-deleted construct restores the promoter activity in a position dependent manner. Gel mobility shift assays have been used to identify a protein in liver cell and oocyte extracts which binds to this DNA sequence with high specificity. Competition studies indicates that this protein is an NF1-like factor. The VA region linked to a TATA box can function as a more powerful independent transcription activator than the NF1 consensus sequence in a similar construct. These data indicate that the VA binding factor is related to, but probably not identical to NF1. Although a VA element linked to a TATA box is an efficient transcription activator, the VA element at a similar location in the vit. promoter is unable to activate transcription unless estradiol-estrogen receptor complex is bound to the EREs. The extraordinary transcriptional activity of vit. promoter is therefore regulated by the interaction of the NF1-like factor and VA sequence as well as the EREs and estrogen-estrogen receptor complex. (Supported by NIH grant # 16720)

E 612 THE TRANS-ACTING ESTROGEN RECEPTOR IS PHOSPHORYLATED IN RESPONSE TO ESTRADIOL, R. Rex Denton and A. C. Notides, Environmental Health Sciences Center, University of Rochester School of Medicine and Dentistry, Rochester N. Y. 14642. Steroid hormone receptors, like many other trans-acting factors, have been characterized as phosphoproteins. The characteristics of estrogen receptor phosphorylation were examined by immunoprecipitation of the receptor from [^{32}P]-labeled MCF-7 cell lysates using a highly specific polyclonal antibody. Analysis of the immunoprecipitated estrogen receptor by SDS-polyacrylamide gel electrophoresis indicated the human estrogen receptor is a phosphoprotein with molecular weight 67kD. Two-dimensional gel electrophoresis and western blot analysis showed that the estrogen receptor had charge heterogeneity typical of covalently modified proteins. The [^{32}P]-labeled estrogen receptor in MCF-7 cells was found to contain [^{32}P]-serine as the only modified amino acid. Importantly, cells treated with estradiol prior to cell lysis demonstrated that the estrogen receptor underwent a hormonally-dependent phosphorylation within minutes of estradiol treatment. Two-dimensional analysis of immunoprecipitated estrogen receptor demonstrated that a more acidic form of the estrogen receptor is found only in nuclei isolated from MCF-7 cells. Furthermore, tryptic mapping of the estrogen receptor has been performed to determine the location of estrogen receptor phosphorylation. These results demonstrate that estrogen receptor phosphorylation occurs under *in vivo* conditions and that it may be involved in modulation of the receptor's transcriptional activity.

E 613 L-TRIIODOTHYRONINE (T3) REGULATES BINDING OF NUCLEAR FACTOR(S) TO THE CAAT-MOTIF OF THE S14 GENE. Benoit J. Deschamps and Norman C.W. Wong, Department of Medical Biochemistry and Medicine, U of Calgary, AB, Canada T2N-1N4.
The rapid induction of the rat hepatic mRNA-S14 by T3 makes it an ideal model for studying the hormonal regulation of gene expression. Recent studies (D.B. Jump (1989) JBC 264, 4698) demonstrated the presence of 5 DNase I hypersensitive sites (HS 1-5) at the 5' end of the gene in rat liver. The HS-1 is located immediately adjacent to the transcription initiation site and found only in tissues where the S14 gene is abundantly expressed and T3 regulated. To examine the binding of nuclear proteins to the HS-1 site we have isolated a DNA fragment (USS-1, -278 to +71) containing a portion of the HS-1 site. DNase I footprinting results demonstrated that hepatonuclear extracts contained an activity which protected nucleotides -63 to -48 (PS-1, TTGGCGTCCTGTCAAT) from digestion by DNase I. A DNA duplex identical to the protected nucleotides was synthesized and used in the gel retardation assay to examine the effect of T3 on PS-1 binding activity. Results demonstrated that PS-1 binding activities in eu- and hyperthyroid extracts were increased 1.8 and 4-fold, relative to the hypothyroid rats, thus correlating with the thyroid state and mRNA-S14 in the animals. To examine the role of the PS-1 binding proteins in S14 gene transcription a plasmid containing a S14 DNA fragment (-441 to -2) was inserted infront of a 'G' free cassette (GFC, gift from R. Roeder) and used it in an *in vitro* transcriptional assay. Incubation of the S14-GFC plasmid with hepatonuclear extracts and ^{32}P-UTP resulted in an RNA product of 377 bases. The rate of S14-GFC transcription was highest in hepatonuclear extracts derived from hyperthyroid animals. Displacement of the PS-1 binding protein from the S14-GFC diminished the rate of transcription. In summary, the PS-1 binding activity appears to be dependent on the thyroid state of the animal and probably plays an important role in the transcription of the S14 gene *in vitro*.

E 614 MOLECULAR ANALYSIS OF THE *nti* GLUCOCORTICOID RECEPTOR MUTANT, Ellen S. Dieken, Eckart Meese and Roger Miesfeld, Department of Biochemistry, University of Arizona, Tucson, AZ 85724. The observed cellular response to dexamethasone (dex) treatment in S49 cells is an irreversible induction of programmed cell death (apoptosis). We are studying two independently isolated GR mutants (*nti*) that are unable to induce apoptosis. Extensive DNA sequence analysis of over 30 *nti* GR cDNAs revealed that altered mRNA splicing results in the synthesis of a truncated GR protein. In addition, multiple exon 1 coding sequences were observed in both wildtype and mutant receptor cDNAs. The deleted amino terminal 407 residues include the previously defined "modulatory" domain of GR; no changes were found in the other functional domains of the *nti* GR. Moreover, this new transcript contains a novel in-frame start codon located in exon 1, resulting in the addition of 41 amino acids. Functional studies have shown that the *nti* GR is capable of inducing transcription of an MMTV-CAT reporter plasmid 10-fold in co-transfected CV1 cells (transcriptional induction of the same reporter plasmid by wildtype GR is 20 times greater), demonstrating that dex-induced S49 cell death requires GR sequences in addition to those sufficient for low level transcriptional regulatory activity. We are currently investigating the molecular defect in *nti* GR splicing by using both PFGE analyses to detect genomic rearrangments, and PCR to sequence RNA splice sites. Preliminary PFGE experiments have indicated that genomic rearrangements might in fact, exist in one of the *nti* mutants.

E 615 MECHANISMS OF GLUCOCORTICOID REPRESSION OF PRO-OPIOMELANOCORTIN GENE TRANSCRIPTION, Jacques Drouin, Yu Lin Sun, Marc Therrien, Alaka Mullick and Mona Nemer. Laboratoire de génétique moléculaire, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA, H2W 1R7.

The pro-opiomelanocortin (POMC) gene is specifically expressed in two cell types of the pituitary gland which process POMC into different secretory products. Glucocorticoids are synthesized in the adrenal gland in response to one of these products, ACTH, and they exert a negative feedback on anterior pituitary ACTH secretion and POMC gene transcription. By using DNA-mediated gene transfer into culture cells and in transgenic mice, we have defined a 543 bp fragment of the POMC gene which is sufficient for pituitary-specific expression and glucocorticoid repression in the anterior pituitary. Within this 5'-flanking fragment of the gene, multiple regulatory elements contribute to tissue-specific expression; in particular, a putative corticotroph-specific transcription factor has been identified and shown to bind to a unique sequence of the POMC promoter. A" negative glucocorticoid response element" (nGRE) was also localized at position -63 bp. This nGRE was shown to be devoid of any GRE activity. The nGRE binds the purified glucocorticoid receptor (GR) and GR:nGRE protein:DNA complexes were shown to differ from GR:GRE complexes. Formation of this unique GR:nGRE complex was correlated with repression by the use of point mutations. The nGRE was also shown to bind the COUP transcription factor, suggesting that mutually exclusive binding of COUP and GR may be involved in the mechanism of repression. In conclusion, the mechanisms of glucocorticoid repression at the POMC nGRE may involve 1) the lack of intrinsic GRE activity, 2) the formation of a unique GR:nGRE complex which differs from GR:GRE complexes, and 3) the displacement of COUP transcription factor by GR.

E 616 **CONTROL OF FUNCTION, GROWTH AND DIFFERENTIATION EXPRESSION IN THE DOG AND HUMAN THYROID CELL : A COMPLEX REGULATORY NETWORK AND ITS ELEMENTS -** Dumont, J.E., Institute of Interdisciplinary Research, University of Brussels, Brussels, Belgium. - The function of the dog and human thyroid cells is controlled by the TSH-adenylate cyclase-cyclic AMP-protein kinase, and the TSH-acetylcholine-phospholipase C-diacylglycerol-iP_3-Ca^{++} cascades. These two cascades have both similar and antagonistic effects on the iodine metabolism in the cell. The Pi cascade modulates the cyclic AMP system by diacylglycerol activation of the TSH and inhibition of the prostaglandin response; its calcium also activates the catabolism of cyclic AMP by Ca^{++} calmodulin dependent phosphodiesterase. Differentiation expression of thyroid cells in culture is evaluated by the induction by TSH through cyclic AMP of iodide transport and of thyroglobulin and thyroperoxidase gene expressions (effects on transcription). While thyroperoxidase gene promoter involves a cyclic AMP regulatory element and fits well with present concepts of cyclic AMP regulated genes, thyroglobulin gene activation is delayed and requires prior de novo protein synthesis and involves quite a different mechanism. Epidermal growth factor and phorbol esters shut off differentiation expression. Proliferation of the thyroid cell is positively regulated by TSH through cyclic AMP, by epidermal growth factor through tyrosine protein kinase and by phorbol esters through protein kinase C. These pathways are largely distinct but converge on cMyc and cFos gene expression and cyclin synthesis. Elements of these regulatory networks have been cloned and sequenced : five G protein modulating receptors including the 1 adrenergic receptor, "calcyphosine" which binds Ca^{++} and is phosphorylated in intact cells by cyclic AMP dependent protein kinases and the thyrotropin receptor.

E 617 MOLECULAR ANALYSIS OF HUMAN ß-MYOSIN HEAVY CHAIN PROMOTER. John G. Edwards, Joseph E. Bahl, Choong-Chin Liew and Eugene Morkin, University of Toronto, Toronto, Canada M5G 1L5 and University of Arizona College of Medicine, Tucson, AZ 85724

The ß-myosin heavy chain (ß-MHC) gene in the rat is known to be negatively regulated by 3,5,3'-triiodo-L-thyronine (T_3). Because of conservation of sequences in the 5' flanking region between the rat and human ß-MHC genes, T_3 regulation has been studied in rat fetal heart cells using deletion mutants of the human promoter region fused to the CAT gene. In heart cells, T_3 inhibited the expression of human ß-MHC constructs with an EC_{50} of 5×10^{-9}M. The time course of inhibition was similar to that of endogenous ß-MHC mRNA, reaching about 40% of control values after 72 hrs. Three cis-acting elements were identified: 1) a basal promoter containing CAAT and TATAA sequences; 2) a proximal T_3 response element (TRE); 3) a strong positive element that was constitutive expressed and not regulated by T_3; 4) additional upstream positive and negative elements, possibly including another TRE. Activation of combinations of these cis-acting elements may be sufficient to explain the pattern of expression observed in vivo with variations in thyroid status.

E 618 CHARACTERIZATION OF THYROID HORMONE RECEPTOR CIS-ACTING ELEMENTS OF THE HUMAN α-MYOSIN HEAVY CHAIN GENE PROMOTER, Irwin L. Flink, and Eugene Morkin, Departments of Internal Medicine, Pharmacology, and Physiology, and the University Heart Center, University of Arizona College of Medicine, Tucson, AZ 85724

The human α-myosin heavy chain (α-MHC) gene is under positive regulation by 3,5,3'-triiodo-L-thyronine (T_3), however, the mechanism by which T_3 modulates its transcription is not clearly understood. We have used two kinds of techniques, an avidin-biotin-complex-DNA binding assay and dimethylsulfate interference, to characterize the interaction of T_3 receptors to target sequences located in the 5'-flanking region of the α-MHC gene. Liver T_3 receptors bind with high affinity (K_d=0.82 nM) to a site located at -140/-156 base pairs (TRE_1) upstream from the CAP site, and with considerably lower affinity (K_d=23 nM) to a second site (TRE_2) at -112/-123. Methylation interference experiments demonstrate that T_3 receptors react with guanosines in TRE_1 on the antisense strand which are located within two heptameric imperfect direct repeats (underlined) 5'-TCTGGAGGTGACAGGAGGACA-3'. In contrast, the T_3 binding element in TRE_2 is comprised of an octamer (underlined, 5'-GGAGGAGGAGCC-3') of one purine-rich half-site on the sense strand, and two additional guanosines located immediately downstream on the antisense strand. These results suggest that the pattern of guanosines involved in T_3-receptor-DNA complex formation may underlie the differences observed in the binding affinities of a strong (TRE_1) and weak (TRE_2) TRE.

E 619 DIFFERENTIAL EXPRESSION OF DISTINCT THYROID HORMONE RECEPTORS IN CHICKEN DEVELOPMENT, Douglas Forrest, Maria Sjöberg and Björn Vennström, Dept of Molecular Biology CMB, Karolinska Institute, Stockholm S-10401, Sweden. A variety of c-*erbA* cDNAs in different species have been shown to encode thyroid hormone receptors (TRs) which are related in structure, and may be grouped as α and β types which derive from distinct genes. It is uncertain why different forms of TR are produced: we tested the hypothesis that TRs may differ in developmental functions by comparing expression of distinct TRs in chicken development. We have isolated a cDNA coding for a β type of chicken TR, which binds thyroid hormone (T3) with a Kd = 0.4nM, in the same order as the previously studied chicken TRα. At the amino acid level TRβ is 95% related to TRα in the N-terminal/DNA-binding domain (although the β N-terminus is 36 residues shorter) and 90% in the C-terminal T3-binding region. The genomic restriction maps corresponding to TRα and β differ indicating that each derives from a distinct gene. Marked differences in expression of TRα and β in embryonic development were revealed by Northern blot, PCR and RNA protection analyses: α mRNA was detected in all tissues from earliest stages (day 4), although with some variation in levels. In contrast, β mRNA displayed restricted expression (mainly brain, eye and yolk sac) at low levels which, however, increased at later developmental stages. This suggests that distinct TRs differ in developmental functions, perhaps differing in target gene specificity or in interaction with tissue-specific transcription factors. The TRα and β *trans*-activating capacities will be investigated by co-transfection assays with T3-responsive reporter constructs.

E 620 MODULATION OF DEXAMETHASONE MEDIATED INHIBITION OF EGF-STIMULATED CELL GROWTH BY AMPLIFICATION OF EGF-RECEPTORS IN NIH 3T3 CELLS, Brad Foster and Ted W. Reid, Department of Ophthalmology and Visual Science, University of California Davis Medical Center, Sacramento, CA 95816

The synthetic glucocorticoid dexamethasone (dex) effects transcription levels of target genes via the interactions of dex-activated glucocorticoid receptors with glucocorticoid response elements. Depending on the nature of these response elements, transcription may be enhanced or repressed. When quiescent NIH 3T3 fibroblasts were treated with epidermal growth factor (EGF) in the presence of dex, the EGF-induced stimulation of DNA synthesis was ablated. In contrast, the stimulatory effects of basic fibroblast growth factor (bFGF) were unaffected by dex. The differential effects of dex in EGF and bFGF stimulated cells could represent a qualitative difference between the mechanism of action of EGF and bFGF receptors, or a quantitative difference in receptor numbers or efficiency. In an attempt to resolve this question we compared the effects of dex in normal NIH 3T3 fibroblasts and NIH 3T3 fibroblasts which had been infected with a retroviral vector containing human EGF-receptor which resulted in the over expression of the receptor in these cells. We found that the dex-mediated inhibition of DNA synthesis in EGF-stimulated cells can be modulated by increasing the number of EGF receptors on the cell.

E 621 PHOSPHORYLATION OF P75 gag-v-erbA IS REQUIRED FOR FULL BIOLOGICAL ACTIVITY OF THE v-erbA ONCOGENE OF AVIAN ERYTHROBLASTOSIS VIRUS. Jacques Ghysdael[1], Martin Zenke[2], Hartmut Beug[2], and Corinne Glineur[1] (1) INSERM U186/CNRS URA 1160 - Institut Pasteur, Lille, France, (2) Research Institute of Molecular Pathology, Vienna, Austria.

The 75Kd protein (P75 gag-v-erbA) encoded by the v-erbA oncogene of Avian Erythroblastosis Virus (AEV) is a mutated, ligand-independent version of the cc-erbA α – encoded nuclear receptor for the thyroid hormone 3,5,3' triiodothyronine (T3). Expression of v-erbA inhibits the temperature-induced differentiation of chick erythroblasts transformed by temperature-sensitive versions of oncogenic protein tyrosine kinases including v-erbB and v-sea. This differentiation block is mediated at least in part by suppression of transcription by P75 gag-v-erbA of erythrocyte-specific genes. The 46 Kd T3 receptor was previously found to be phosphorylated on two distinct sites (Goldberg et al., 1988, EMBO J., 7, 2425-2433). Only one of these sites is retained in P75 gag-v-erbA and consists of two adjacent serine residues (S16 and S17 in P75 gag-v-erbA) located 21 amino-acids upstream from the DNA binding domain. As a first step to investigate the role of these phosphorylation events in erb-A protein function, mutants were generated in which S^{16}/S^{17} were converted into either alanine or threonine residues. Conversion of S^{16}/S^{17} into alanine residues both abolished phosphorylation of P75 gag-v-erbA in its v-erbA-encoded domain and drastically impaired its ability to inhibit the temperature-induced differentiation of chick erythroblasts.Conversion of S^{16}/S^{17} into threonine residues resulted in the restoration of both phosphorylation of the v-erbA domain of P75 gag-v-erbA, and the ability of P75 gag-v-erbA to inhibit erythroblast differentiation. Futhermore, treatment of wild-type AEV-transformed erythroblasts with the protein kinase inhibitor H7 was found to result in a dose-dependent inhibition of P75 gag-v-erbA phosphorylation and in the induction of terminal differentiation. These results indicate that phosphorylation of P75 gag-v-erbA is required for full biological activity of v-erbA and support the notion of an essential role for v-erbA protein phosphorylation in its activity as transcriptional repressor of erythocyte-specific genes.

E 622 A GC-RICH ELEMENT CONFERS EGF AND PHORBOL ESTER RESPONSIVENESS TO THE GASTRIN PROMOTER BUT NOT CYCLIC AMP RESPONSIVENESS, Juanita M. Godley, Stephen J. Brand, Gastrointestinal Unit, Massachusetts General Hospital, Boston,MA 02114

We have previously demonstrated that EGF, VIP and TRH are all potent regulators of gastrin gene transcription. VIP and TRH are neuropeptides released from nerves that likely represent neurocrine regulation of the antral G cell. However, EGF is not found in large quantities on the gastric mucosa. Instead, TGF-α, a homologue of EGF that binds the EGF receptor is quite abundant in the fundic and antral mucosa. Therefore, EGF regulation of gastrin gene transcription may be equivalent to an autocrine or paracrine effect of TGF-α on the antral G cell. We have used a clonal pituitary cell line, GH_4, to study the effects of these three peptides and their intracellular mediators. EGF, TRH and VIP all increase gastrin promoter activity 3- to 5-fold. Phorbol esters (TPA) and cAMP also increase gastrin promoter activity 3-fold and 5-fold respectively. The cis-acting DNA sequence conferring EGF, TRH, VIP, TPA and cAMP responsiveness to the gastrin gene lies within a 42 bp segment of DNA -40 to -82 bp upstream from the transcription initiation site. A GC-rich 14 bp sequence at -56 to -69 [GGGGCGGGGTGGGG] conferred both EGF and TPA responses to the gastrin promoter, but not VIP and cAMP responsiveness. One copy of this GC rich motif reconstituted the EGF, TRH and TPA response. DNAase I footprinting analysis confirmed that this GC-rich sequence is the predominant site of specific nuclear protein binding. The VIP and cAMP responses were not conferred by this 14 bp GC rich sequence alone. Moreover, this entire 42 bp upstream segment does not contain any sequence homology to known cyclic AMP response elements. Thus, it appears that the VIP/cAMP element for the gastrin promoter is a novel cyclic AMP regulatory element and may also represent synergistic effects between multiple response elements binding different trans-acting factors.

E 623 RECEPTOR SELECTIVITY OF HORMONE RESPONSE ELEMENTS: A NOVEL THYROID HORMONE RESPONSE ELEMENT (TRE) FOR THYROID HORMONE BUT NOT FOR RETINOIC ACID RECEPTOR. Gerhart Graupner, Thomas Hermann, Xiao-kun Zhang and Magnus Pfahl, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Thyroid hormone receptors (TR) and retinoic acid receptors (RAR) belong to a sub-family of nuclear receptors that bind to DNA sequences which are related to the vitellogenin gene estrogen responsive element (ERE). We have reported recently that TRs can act as transcriptional activators and repressors and can thereby regulate RAR activity from specific thyroid hormone response elements (TRE) (Graupner et al., Nature 340, 653, 1989). For a more detailed analysis of the sequence-specific recognition by members of the thyroid/retinoic acid receptor sub-family, several growth hormone TRE related sequences were inserted upstream of the TK promoter of a CAT construct and tested in transient cotransfection assays. A distinct nucleotide sequence could be identified that acts as an efficient TRE but not as RAR responsive element. Gel retardation assays demonstrated that the receptor mediated induction of a CAT reporter gene in vivo corresponds well to the receptor-DNA binding in vitro. Dimerization of the TRE was necessary to obtain optimal binding in vitro and strong induction by TRs in the transient transfection assay. Our results suggest that there are two distinct types of TREs: those that allow activation by a larger group of receptors sharing high homology in the DNA binding domain, and those that allow activation by thyroid hormone receptors only. We, therefore, propose an additional level of regulatory control by TREs that separate RAR response from TR response.

E 624 INTERFERON-INDUCIBLE TRANSCRIPTION FACTORS: PURIFICATION AND ACTIVITY, Matthew J. Guille, John Parrington, George R. Stark and Ian M. Kerr, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, U.K.
Transcription of the 6-16 gene is activated transiently by treating cells with type I (α/β) interferons. This activation requires an interferon-stimulable response element (5'GGGAAAATGAAACT3') within the 6-16 promoter. This element, but not one including, for example, the mutation 5'GGGAAAATGACACT3', is capable of conferring interferon responsiveness on marker genes. Specific complexes are formed between this element and a pre-existing cytoplasmic factor(s) whose DNA-binding activity and nuclear translocation are activated rapidly by interferon. In order to investigate further the mechanism by which binding of interferon to its receptor activates transcription of the 6-16 gene, we are purifying this and related factors and testing for factor-dependent transcription regulated by the ISRE and 6-16 promoter in vitro.

E 625 HIGH LEVEL EXPRESSION OF HUMAN ALPHA AND BETA-GLOBIN CHAINS IN MURINE RED CELLS DIRECTED BY THE BETA-GLOBIN DOMINANT CONTROL REGION (DCR), O. Hanscombe, D.R. Greaves, M. Vidal, P. Fraser, D. Whyatt and F. Grosveld, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.
We have reported the use of sequences flanking the human beta-globin locus to direct high level expression of the human beta-globin gene (1) and the human alpha-globin gene (2) in the fetal liver of transgenic mice. Using a cosmid construct containing both the human alpha-globin and beta-globin genes with the beta-globin DCR we have obtained transgenic mouse lines which express human haemoglobin (HbA) in the erythrocytes of adult mice at levels equal to or higher than that of endogenous mouse haemoglobin. We have used these lines to investigate the developmental regulation of the human alpha and beta-globin genes and by using a human beta-globin gene with the codon 6 mutation Glu-Val we have obtained a transgenic mouse model of sickle cell anaemia.
1) Grosveld et al. (1987) Cell, 51, 975-985.
2) Hanscombe et al. (1989) Genes and Development, in press.

E 626 ANDROGEN REGULATION OF HBGF1/aFGF AND TR3/nur77 mRNA ACCUMULATION AND TRANSCRIPTION IN THE HUMAN PROSTATE CARCINOMA CELL LINE, LNCAP-Adep, Harris, S.E., ‡Chui, I.-M., *Chang, C., Hall, J.A., Harris, M.A., and Rong, Z., W. Alton Jones Cell Science Center, Lake Placid, NY 12946, ‡Department of Internal Medicine, The Ohio State University, Columbus, OH 43210, *Department of Surgery, The University of Chicago, Chicago, IL 60637, The human prostate carcinoma cell line, LNCAP-Adep are growth stimulated (15X) by 10^{-9}M testosterone(T) in combination with HBGF1/aFGF or EGF. T also induces HBGF1/aFGF mRNA accumulation. The mechanism of androgen receptor (AR) mediated stimulation of growth and HBGF1 production are being analyzed by determining the transcription rate of the HBGF1/aFGF gene using nuclear run-on assays and by determining the HBGF1/aFGF mRNA degradation rates using Actinomycin D block techniques. The 5'-flanking regions of the LNCAP HBGF1/aFGF gene are also being pursued using LNCAP 5'-non-coding HBGF1/aFGF probes. Progress will be reported. TR3/nur77 gene encodes a transacting factor/DNA binding protein expressed in the G0→G1 of the cell cycle in a variety of cells. T and other growth factors induces TR3/nur77 mRNA accumulation in LNCAP-Adep cells. The role TR3/nur77 and AR plays in HBGF1/aFGF mRNA regulation will be analyzed by sequence analysis of HBGF1/aFGF gene and by co-transfection of HBGF1/aFGF 5'-flanking-CAT constructions with TR3/nur77 and AR expression vectors.

E 627 COOPERATIVE ACTION OF A VIRUS-INDUCIBLE DNA SEQUENCE IN INTERFERON TYPE 1 PROMOTERS AND THE INTERACTION WITH ITS DNA BINDING NUCLEAR FACTOR

H. Hauser, M. Nourbakhsh, C. Scharnhorst and K. Hoffmann; GBF, Genetics of Eukaryotes, D-3300 Braunschweig, FRG

The major target sequence for virus inducibility within the human Interferon-ß promoter is AAGTGA. This sequence is represented in a more or less related form in several copies within all known mammalian type 1 interferon promoters. We have investigated this DNA element for its ability to cooperate with other DNA elements of the human Interferon-ß promoter. Multimers of AAGTGA can confirm virus inducibility to heterologous promoters. However, these multimers do not stimulate a heterologous promoter from distal sites. Therefore, they do not act as a typical inducible enhancer ("donor"). An enhancer function can be reconstituted by insertion of short DNA elements from the Interferon-ß gene upstream sequences as "acceptors" in front of the heterologous promoter. Our results show that AAGTGA and related elements can function as acceptor sequences indicating the cooperation of identical elements within natural IFN type I promoters.

IRF-1 is a nuclear DNA binding protein which specifically recognizes AAGTGA. A low basic expression of the respective mRNA is induced by virus or interferon treatment of the cells. Overexpression of a cDNA clone encoding IRF-1 leads to cytotoxic effects in some cell types. Using specific mutagenesis we have defined regions responsible for DNA binding and cytotoxicity.

E 628 THE HUMAN erbA-T PROTEIN: A THYROID HORMONE RECEPTOR ISOFORM WITH NOVEL REGULATORY PROPERTIES AND MODIFIED AFFINITY TO DNA. Thomas Hermann, Ken Wills, Maty Tzukerman, Gerhart Graupner, Xiao-kun Zhang and Magnus Pfahl, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

The human erbA-T protein has been previously described as a new thyroid hormone receptor (TR) isoform (Benbrook, D. and Pfahl, M. Science 238, 788). It differs from TRα by its extended carboxyterminal sequence that results from alternative splicing. To investigate the gene regulatory properties of this TR isoform, erbA-T was compared to TRα and TRβ in transient cotransfection experiments. We find that erbA-T is not a T3-dependent transcriptional activator. Hybrid receptor constructs - containing either the DNA or the hormone binding domain of erbA-T and the corresponding domain of the estrogen receptor - were used to dissect the functional properties of erbA-T domains. Our results show that the DNA binding domain of erbA-T is functional while the hormone binding domain cannot be activated by T3. We have previously shown that thyroid hormone receptors act as TRE specific repressors in the absence of ligand (Graupner et al., Nature 340, 653). In contrast, erbA-T showed only weak repressing activities when compared with TRα. Gel retardation analyses indicate that the extended hormone binding domain of erbA-T reduces its affinity for various TREs, which is consistent with its weak repressing activities. Our results show that as a result of the extended carboxyterminus erbA-T is unable to act as a transcriptional activator and is only a weak transcriptional repressor of certain TREs. We suggest that the altered DNA specificity of erbA-T may allow transcriptional control via novel TREs or by an as yet unidentified regulatory mechanisms.

E 629 DELAYED SECONDARY RESPONSE: A SECONDARY GLUCOCORTICOID RESPONSE ELEMENT CONTAINS SPECIFIC GLUCOCORTICOID RECEPTOR BINDING SITES AND DELAYED ENHANCER FUNCTION, Patrick Hess*, Meenakshi, T.*, Guy Chiu-Kai Chan*, Jan Carlstedt-Duke+, Jan-Ake Gustafsson+, and Farhang Payvar*, *E.A. Doisy Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, MO 63104; and +Department of Medical Nutirition, F60 Novum, Huddinge University Hospital, S-141 86 Huddinge, Sweden. We have identified and characterized a region downstream from rat alpha$_{2U}$-globulin gene promoter, between ~+1800 and +2010, that specifically mediates delayed secondary response to glucocorticoids. Unlike previously characterized primary glucocorticoid response elements, this secondary glucocorticoid response element dictates an inductive process preceded by a time-lag of several hours and blocked by cycloheximide. Reminiscent of the primary glucocorticoid response elements, this secondary glucocorticoid response element confers hormonal regulation upon a linked heterologous promoter from a downstream position with respect to transcription start site and, remarkably, also interacts selectively with purified glucocorticoid receptor. These results imply that receptor binding to certain secondary glucocorticoid response element in vivo may participate in mediation of secondary responses to glucocorticoid hormones by "delayed" enhancement of transcription from preexisting initiation sites.

E 630 MULITPLE FACTORS INVOLVED IN THE TRANSCRIPTIONAL CONTROL OF THE MOUSE MAMMARY TUMOR VIRUS PROMOTER, Mike Huang, Mark Toohey, Jae Lee, Jennifer Pierce, Pat Moffitt, and David O. Peterson, Department of Biochemistry & Biophysics, Texas A&M University, TX 77843.

Transcription of the proviral genes of mouse mammary tumor virus (MMTV) is subject to control at several levels. *Cis*-acting DNA elements, distinct from the promoter itself, which regulate transcription include the steroid hormone response element (HRE) which mediates induction by several classes of steroid hormones, and a negative regulatory element (NRE), which mediates repression of basal promoter activity and thereby maintains transcriptional inactivity in the absence of a hormone inducer. These elements are binding sites for *trans*-acting proteins which modulate transcription by interaction with the basal promoter transcription apparatus. To understand the basic organization and function of the MMTV promoter, systematic linker-scanning and point mutations have been constructed. Gel electrophoresis mobility shift assays and other techniques were then employed to determine specific binding of factors to mutation-sensitive elements of the promoter. An interesting observation on the organization of the promoter is that adjacent binding sites for factors more or less lie on the same face of the DNA helix. In particular, spacing mutations between the adjacent binding sites for NF-1 and a protein which binds to an octamer-related sequence suggest a possible requirement for a specific alignment in the binding sites of the two transcription factors. More detailed investigation awaits the purification of each transcription factor and characterization of its binding and transcription activity in vitro.

E 631 MUTATIONAL ANALYSIS OF cis-ACTING ELEMENTS INVOLVED IN THE EXPRESSION OF THE RAT GROWTH HORMONE GENE. **Jeff Hubenthal-Voss, Bob McEvilly, Laura Wilson, Maurice Treacy, and Michael G. Rosenfeld. University of California San Diego and the Howard Hughes Medical Institute, La Jolla, CA 92093.**

The rat growth hormone (rGH) gene has served as a model system for studying tissue specific gene expression in vitro because of the availability of pituitary derived cell lines which are permissive or restricted for growth hormone expression. In this study, the effects of site specific mutations in the rGH enhancer on the level of expression of chimeric reporter genes has been measured. Mutations in consensus factor binding sites for ubiquitously expressed proteins (e.g. AP-2) and tissue specific factors (e.g. Pit-1) were made and data on the effects these mutations on the expression of rGH in permissive and non-permissive cell lines will be reported. Furthermore, data on the effects of mutations in sites not previously shown to bind proteins on the cell specific expression of rGH will be presented. The data indicate the potential role of protein-protein interactions on the level of expression of the rGH gene and that *cis*-acting sites which do not bind proteins but may organize the enhancer greatly influence the level of rGH expression.

E 632 MAPPING OF AN ESTROGEN RESPONSE ELEMENT WITHIN THE 5' FLANKING REGION OF THE MOUSE C-FOS GENE, S. M. Hyder, G. M. Stancel and D. S. Loose-Mitchell, Department of Pharmacology, University of Texas Medical School, P.O. Box 20708, Houston, Texas 77225.

The rapid induction of c-fos message (within 3h) following estrogen administration in rats indicates that the expression may be a direct result of interaction of estrogen receptor with the fos promoter. We have conducted transient transfection assays with defined upstream promoter regions of the mouse c-fos gene and the estrogen responsive GH4 cell line. The -351 to +44 fragment of the mouse c-fos promoter was ligated to a reporter plasmid expressing chloramphenicol acetyl transferase (CAT). This fragment responded to estradiol with 2-3 fold induction of CAT activity. Dose response analysis indicated that the maximum response occurred with 20nM estradiol. Chronic dose of estradiol (10-7M) suppressed this induction. The endogenous c-fos gene of GH4 cells was also stimulated 2-3 fold determined by northern blot analysis. The basal level of fos-cat expression was elevated in transfected cells. Northern blot analysis indicated constitutive expression of c-jun in the GH4 cells. Initial experiments indicate that removal of the serum response element and the adjacent AP1 binding region lowers the basal level of expression of the fos-cat construct. Surprisingly, phorbol esters do not stimulate the transfected gene, although transcription of the endogenous gene is stimulated several fold following phorbol ester treatment. This suggests that the phorbol ester responsive elements are different between the human and the mouse gene.

E 633 DIFFERENT DNA ELEMENTS REGULATE GLUCOCORTICOID-INDUCED TRANSCRIPTION OF THE PHOSPHOENOLPYRUVATE CARBOXYKINASE (PEPCK) GENE IN RENAL AND HEPATOMA CELLS.

Enyu IMAI, John MITCHELL and Daryl K. GRANNER, Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee, 37232.

Glucocorticoids increase the synthesis of hepatic and renal PEPCK, the rate-limiting enzyme in gluconeogenesis, by directly increasing transcription of the PEPCK gene. The purpose of this study was to identify the glucocorticoid response unit (GRU), the minimal DNA sequence required for glucocorticoid induction of the PEPCK gene, in H4IIE and LLC-PK1 cells, which are derived from rat hepatoma and porcine kidney, respectively. Purified glucocorticoid receptor (kindly supplied by J. A. Gustaffson) bound to four regions: A=-1178 to -1153, B=-395 to -349 , C=-132 to -102 and D=-52 to -37 relative to transcription start site of the PEPCK gene. Regions of functional importance were defined using chimeric genes composed of various segments of the 5' flanking region of the PEPCK gene fused to the chloramphenicol acetyltransferase (CAT) reporter gene. These were co-transfected with a glucocorticoid receptor expression vector (pSVGR1) into LLC-PK1 or H4IIE cells. Binding regions A and C had no apparent function in either cell type. The major GRU in H4IIE cells is composed of two contiguous receptor binding sites (region B; -395 to -349) and two accessory factor binding sites, AF1 (at -455 to -431) and AF2 (at -420 to -403). A second (proximal) GRU consists of binding region D plus an additional DNA sequence located between -99 to -52; this proximal GRU is 20% as active as the distal GRU in H4IIE cells. In contrast, maximal glucocorticoid induction of the PEPCK gene in the LLC-PK1 cells required just the two contiguous GREs in binding region B (-395 to -349); the accessory factor sites were not necessary. This response in LLC-PK1 cells did not occur in the presence of the sequence between -600 and -529, suggesting that this region acts as a suppressor of the glucocorticoid induction. These results suggest that different DNA elements are nessesory for glucocorticoid induction of the PEPCK gene in H4IIE cells as compared to LLC-PK1 cells. This represents a unique type of tissue specific regulation of hormone-induced gene transcription.

E 634 ACTIVATED RAS AND TPA BYPASS SYNERGISTICALLY THE IL-3 GROWTH STIMULUS OF A MAST CELL LINE, Roland Imber(1), Christoph Moroni(2) and Doriano Fabbro(1). University Clinic Medical School (1) and Institute for Medical Microbiology (2), CH-4031 Basel, Switzerland.

Expression of v-Ha-ras or of mutated c-Ha-ras (Val 12) leads to a strong reduction of the IL-3 growth requirement of a bone marrow-derived mast cell line. Upon injection into mice tumors develop which exhibit complete independence of exogenous IL-3. Expression of normal c-Ha-ras has no effect on the IL-3 requirement nor do the cells become tumorigenic. A temporary reduction of the IL-3 requirement of the parental PB-3c can also be obtained by treating the cells with TPA. When the lines expressing mutated ras are treated with TPA a more pronounced bypass of the IL-3 requirement of PB-3c can be observed compared to the effects on the IL-3 requirement caused by either ras or TPA alone. Cell lines exhibiting a reduced IL-3 requirement do not show measurable differences in protein kinase C activity but express constitutively high levels of c-fos.

E 635 PROTEIN KINASE C-INDEPENDENT ACTIVATION OF $NF_{\kappa}B$ BY TUMOR NECROSIS FACTOR, Martin Krönke, Albrecht Meichle, Gabriele Hensel, and Stefan Schütze, Clinical Research Group, Max-Planck-Society, D-3400 Göttingen, F.R. Germany

Tumor necrosis factor (TNF) is an inflammatory polypeptide that affects the growth differentiation and function of virtually every cell type. The ability of TNF to change cell behaviour is based on its gene regulatory properties. Recently it has been reported that TNF can activate two well known transcription factors, AP-1 and $NF_{\kappa}B$. Transcriptional responses to TNF are triggered by interaction of TNF with high affinity cell surface receptors; however, the pathways by which TNF receptors communicate TNF signals to the transcriptional apparatus of the nucleus remain largely unclear. Recently, we have obtained evidence that TNF can stimulate protein kinase C (PK-C), a known activator of $NF_{\kappa}B$. In this study, we have tested whether TNF-mediated activation of PK-C can be directly linked to the induction of $NF_{\kappa}B$. Using phorbol ester binding-studies and histone III-S phosphorylation assays, we first demonstrate with K562 and Jurkat cells, that TNF induces a transient activation and translocation of PK-C from the cytosol to the membranes. Gel retardation assays using a 30 bp oligonucleotide containing the κB-site of the HIV-1-LTR enhancer revealed that, following TNF-treatment, a κB-binding protein emerges with similar kinetics when compared to PK-C activation. However, TNF could induce $NF_{\kappa}B$-like factors in Jurkat cells treated with the protein kinase C inhibitor H7, which completely prevented PK-C activation by TNF. Since κB binding-protein was also induced by TNF in Jurkat cells depleted for PK-C by long-term exposure to high dose phorbol ester, we conclude that TNF can activate $NF_{\kappa}B$-like factors independent of PK-C. Moreover, TNF induction of $NF_{\kappa}B$ was not sensitive to H8, a protein kinase inhibitor that preferentially inactivates PK-A. Together, the data suggest a novel, PK-A- and PK-C-independent mechanism of TNF-mediated activation of κB-binding proteins.

E 636 MOLECULAR STRUCTURE AND EXPRESSION OF THE CHICKEN AVIDIN RELATED GENES, Markku S. Kulomaa, Riitta A. Keinänen, Mika J. Wallén, Tarja A. Kunnas, Tarja A. Toimela, Reino M. Soimasuo, Tuija A. Vasara and Timo K. Joensuu, Department of Biomedical Sciences, University of Tampere, SF-33101 Tampere, Finland.

Avidin is a host defense protein induced specifically by progesterone in the oviduct and by inflammation in most of the chicken tissues. Using the cDNA for egg-white avidin, multiple genes were detected from a chicken genomic library as well as in a hybridization analysis of the chromosomal DNA. None of the 5 genes sequenced, is however capable of encoding the known peptide chain of the egg-white avidin, and they are thus called "avidin related genes" or *avr1-avr5*. The putative four exons of the *avrs* would encode "avidin related proteins" or AVR1-AVR5 with an overall homology between 70 and 80% to the egg-white avidin. Hybridization analysis and amplification by PCR of the avidin mRNA from the oviduct and intestine are used to study, whether *avrs* are expressed and whether they could produce the inflammation-induced avidin. The upstream region of the *avrs* have been found to contain some interesting homologies to the known gene regulatory elements (e.g. HSE, AP-1, Fos/Jun, half palindromic GRE/PRE), and transfection studies are used to study whether they are functional in vitro.

E 637 DIRECT PHYSICAL DEMONSTRATION THAT THYROID HORMONE CAN INHIBIT AN ESTROGEN RESPONSE BY RECEPTOR COMPETITION FOR SITES ON DNA, Peter J. Kushner, Fred Schaufele, David W. Silversides, Frances M. DeNoto and Thomas N. Lavin, Metabolic Research Unit, University of California, San Francisco,CA 94143.

We find that a region of DNA from the Xenopus vitellogenin A2 gene that is known to bind and mediate transcriptional activation by the estrogen receptor is also bound by the thyroid hormone receptor at an overlapping but not identical site. Nonidentity is shown by the ability of oligonucleotides representing subregions to distinguish between the two receptors. When reporter genes bearing the vitellogenin sequences are introduced into HeLa cells along with expression vectors for the two receptors the estrogen response (which is 500 fold) is severely (96%) inhibited by thyroid hormone. The degree of inhibition is strongly dependent on the "dose" of thyroid hormone receptor, suggesting that inhibition may occur when the thyroid hormone receptor binds to its response element and displaces the estrogen receptor. To test this possibility we examined the ability of the thyroid hormone receptor to compete with the estrogen receptor for binding to the vitellogenin DNA in vitro (assayed by gel retardation) and have found that the two receptors do indeed compete with one another for binding.

E 638 MECHANISM OF ESTROGEN RECEPTOR BINDING TO THE ESTROGEN RESPONSIVE ELEMENT (ERE): INVOLVEMENT OF A NON-B DNA STRUCTURE, Deborah A. Lannigan and Angelo C. Notides, Department of Biophysics, Univ. of Rochester School of Medicine, NY14642

We have demonstrated that the estrogen receptor binds specifically and with high affinity to the "coding strand" of the ERE of the rat prolactin gene. Based on the anomalous gel mobility of the coding strand we postulated that the coding strand "folded" into a unique structure and the estrogen receptor recognizes this structure in addition to the actual sequence of the ERE (Lannigan, D.A. and Notides, A.C. (1989) Proc. Natl. Acad. Sci. USA 86, 863-867). To test this hypothesis we determined if the ERE possessed unusual DNA structure (non-B DNA) by probing the upstream region of the rat prolactin gene with S1 nuclease, which will cleave most non-B DNA structures. We found that specific S1 nuclease sensitive sites map to the coding strand of the ERE. No cleavage by S1 nuclease was detected in the non-coding strand. The differential sensitivity of the ERE strands to S1 nuclease suggests that the strands are in distinctive conformations. S1 nuclease cleavage was dependent on supercoiling. Additionally, when the ERE was moved into foreign DNA no S1 nuclease cleavage was detected. Thus the environment of the ERE is important in determining its conformation. This unusual conformation may contribute to the selectivity of the estrogen receptor for the ERE.

E 639 MULTIPARITITE STRUCTURE OF A NEGATIVE REGULATORY ELEMENT ASSOCIATED WITH A STEROID HORMONE-INDUCIBLE PROMOTER. Jae Lee and David O.Peterson, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843
We have identified a negative transcriptional regulatory element (NRE) within the MMTV LTR that mediates an 8- to 10-fold repression of the MMTV promoter (Morley, K. L., M. G. Toohey, and D. O. Peterson. Nucleic Acids Res. 17:6973-6989). A series of linker scanning and small internal deletion mutations within the 63 bp NRE sequence indicates that the NRE is composed of multiple sequence elements and that alteration of any of these multiple elements has an effect on NRE activity. Examination of the NRE sequence reveals a 7 bp element (CAAGPuAG) which is repeated 5 times (with at least a 5-out-of-7 match) within the 63 bp NRE, and our mutational analysis as well as DNaseI footprinting experiments are consistent with this element being important for NRE function. The multiparitite nature of the NRE is supported by experiments which demonstrate that a 26 bp oligonucleotide containing two copies of the CAAGPuAG element is able to mediate repression of the MMTV promoter when present in multiple copies but not when present as a single copy. Our results relate to those of Langer and Ostrowski (Mol. Cell. Biol. 8:3872-3881), who have identified sequences with many of the properties of the NRE embedded within the MMTV hormone response element (HRE/NRE) between about -170 and -140. Using a gel retardation assay, we have shown that at least one common protein interacts with both the NRE and the HRE/NRE.

E 640 PROMOTER ANALYSIS OF THREE MEMBERS OF A GENE FAMILY INDUCIBLE BY TYPE I AND TYPE II INTERFERONS, Andrew R. Lewin, Andrew M. Ackrill, Laurence E. Reid, George R. Stark and Ian M. Kerr, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, U.K.
A cosmid clone was isolated containing three members of the 1-8 gene family - 9-27, 1-8D and 1-8U. On transfection subclones containing the 9-27 and 1-8U genes are responsive to both types of interferon. Consistent with this, the 9-27 gene contains an interferon-stimulable response element (ISRE), GGAAATAGAAACT which can confer responsiveness to type I and type II interferons on marker genes. The 9-27 ISRE is highly homologous to ISREs present in a number of genes predominantly responsive to type I interferon, e.g. 6-16. Indeed, the same ISRE appears capable of conferring inducibility either predominantly by type I or by both type I and II interferons. A detailed comparison of the 9-27 and 6-16 genes and the factors interacting with them suggests that more than one mechanism may be involved in mediating a differential response. The 1-8D gene is expressed constitutively. It contains a mutated ISRE (GGAAGAGGAAACT) that does not confer responsiveness to interferon. The nature of the 1-8U gene promoter is under investigation.

E 641 **NOVEL c-abl mRNAS ARE EXPRESSED IN RAT PAROTID GLANDS DURING IN VIVO ISOPROTERENOL ADMINISTRATION,** Prema Mertz, Andre Bernards * and Eleni Kousvelari, CIPCB, NIDR, NIH, Bethesda, MD 20892 and *The MGH Cancer Center, Charleston, MA 02129.
The c-abl gene is transcribed into two major mRNAs of 6.5 and 5.3 kb. Nine days treatment of rats with the ß-adrenoreceptor (ß-AR) agonist isoproterenol (ISO) results in the appearance of 1.5 and 1.3 kb c-abl mRNAs in rat parotid glands (RPG). The purpose of this study was to: (a) investigate the levels and specificity of c-abl gene expression in RPG during in vivo ISO treatment ; and (b) define the 5' end (s) of the ISO inducible transcripts. ISO was administered by a mini osmotic pump for 0-9 days. RNA was isolated from RPG, submandibular gland and heart, and analyzed by Northern blot hybridization to (i) v-abl cDNA , (ii) c-abl clone IV or (iii) clone 4.4 of the type IV c-abl cDNA. S1 nuclease analysis was performed using cDNA fragments from the c-abl 4.4 clone and primer extension analysis was done with a 20-nucleotide primer and RPG RNA. The 1.5 and 1.3 kb c-abl mRNAs were detected in the RPG only with v-abl and c-abl 4.4 cDNA probes. In the other tissues only the major c-abl mRNAs were present. S1 nuclease and primer extension analyses showed the cap site to be 356 nucleotides upstream of the XhoI site of the type IV c-abl cDNA. The data suggest that chronic ISO administration results in two c-abl mRNAs of 1.5 and 1.3 kb which are parotid gland specific and have a start site different from the type IV c-abl mRNA.

E 642 INTERACTIONS OF INSULIN AND PHORBOL ESTERS ON ß-ACTIN GENE EXPRESSION. Joseph L. Messina and Ruth S. Weinstock, Depts. of Physiology and Medicine, SUNY Health Science Center and Veterans Administration Medical Center, Syracuse, NY, 13210.
Insulin (Ins) and phorbol esters can rapidly stimulate transcription of several genes in rat H4 hepatoma cells. We believe that a common step in the regulation of gene expression is shared by both Ins and phorbol esters. Here we show that Ins or the phorbol ester, phorbol 12-myristate 13-acetate (PMA), induced the transcription of the ß-actin (ßA) gene in H4 cells. Stimulation of ßA transcription occurred rapidly, with a maximum 15-fold stimulation observed following only 15 min of either Ins (5 nM) or PMA (1 g/ml) addition. The increase in ßA transcription was transitory, returning to baseline within 120 min. PMA is believed to activate cellular protein kinase C (PKC). Cells were pretreated with PMA for 24 hours to reduce functional PKC activity. When a second addition of PMA was added to these pretreated cells, there was no increase in ßA gene transcription. When Ins was added to PMA-pretreated cells, the Ins-induced increase in ßA gene expression was reduced by 60%. When cells were desensitized to Ins by pretreatment with Ins for 24 h, further Ins addition was unable to stimulate ßA transcription. PMA-induced stimulation of ßA transcription was decreased by 57% in Ins desensitized cells. In summary, Ins and PMA rapidly stimulated ßA transcription. Pretreatment of H4 cells with PMA impaired the ability of Ins to stimulate ßA transcription. Conversely, Ins desensitization resulted in a decreased ability of PMA to increase ßA gene transcription. These findings support our hypothesis that a common regulatory signal for the induction of gene expression is utilized by both Ins and phorbol esters.

E 643 STRUCTURAL TRANSITIONS IN THE REGULATORY REGION OF THE HUMAN TRANSFERRIN RECEPTOR (TR) GENE. W. Keith Miskimins and Qian Ouyang, Department of Biological Sciences, University of South Carolina, Columbia, SC 29208. Functional TR are expressed in a cell proliferation dependent manner, quiescent cells having low levels and mitogen activated cells having elevated levels. Elevated levels in mitogen stimulated cells requires transcriptional activation of the gene. This is a late response occurring just before the onset of DNA synthesis. Proliferation dependent expression is observed in transfection experiments utilizing constructs that contain only 114 bp of 5′ flanking sequence. We have observed that elements within this region are able to undergo supercoil-dependent structural transitions. Chemical modification assays were used to show that the region from -81 to -97, when stabilized by supercoiling, can exist in a non-B conformation. This region is composed largely of alternating purines and pyrimidines and the pattern of modified nucleotides is consistent with the formation of Z-DNA. This motif is juxtaposed immediately next to protein recognition sites suggesting that conformational transitions could influence the function of transacting factors. A similar transition occurs within the human DHFR promoter that retains an identical spacing relative to a consensus protein binding site. A similar circumstance can be predicted for the human TK gene, within a region known to be important for growth dependent expression. In the TR gene a second supercoil-dependent modification is observed between -104 and -109. The nature of this alteration is unclear but it resides within a protein recognition site. Using in vitro transcription assays we have seen that supercoiled templates are much more efficient than linear templates. The elements described above are implicated in this response.

E 644 THE ACCESSORY FACTOR REGION OF THE PEPCK GENE GLUCOCORTICOID RESPONSE UNIT ENHANCES THE RESPONSE OF THE MMTV GRE TO DEXAMETHASONE. John Mitchell, Enyu Imai and Daryl Granner, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232
Glucocorticoid-mediated transcription of the phosphoenolpyruvate carboxykinase (PEPCK) gene is mediated through a complex glucocorticoid response unit (GRU) which consists of two contiguous glucocorticoid receptor (GR) binding sites located immediately 3′ from two accessory factor binding sites (AF1 and AF2). In rat hepatoma H4IIE cells neither the receptor binding elements nor the AF elements are responsive to glucocorticoids by themselves. The purpose of this study was to test whether these AF elements function in association with a glucocorticoid response element (GRE) out of the context of the PEPCK gene promoter.

Oligonucleotides of the wild type AF region (-433 to -396), or various segments of this, were inserted into a vector upstream from the MMTV GRE-TK promoter-CAT reporter gene. The wild type AF insert increased the response to dexamethasone (Dex) of the MMTV GRE by 2.75 fold. An AF oligo with the region -411 to -407 mutated did not allow for this additional induction. Mutation of the region -401 to -397 further augmented the Dex response; it resulted in >5-fold induction over MMTV GRE alone, and a 2x increase over that afforded by the wt AF.

	fold increase (+Dex/-Dex)	ratio (insert/MMTV)
MMTV alone	20x	1
+wt AF	55x	2.75
+Δ -411/-407	25x	1.25
+wt Δ -401/-397	106x	5.3

The PEPCK AF enhances the Dex response of the MMTV GRE, and this complex region contains at least two regulatory elements.

E 645 SERUM-STIMULATION AND GLUCOCORTICOID REPRESSION OF PROLIFERIN GENE EXPRESSION. John C. Mordacq and D.I.H. Linzer, Department of Biochemistry, Molecular Biology & Cell Biology, Northwestern University, Evanston, IL 60208. Proliferin (PLF) is a member of the prolactin/growth hormone family in mouse. PLF mRNA and protein are synthesized in a growth-related manner in several mouse cell lines: PLF mRNA and protein are not detected in resting cell cultures, while cells stimulated with serum re-enter the cell cycle and synthesize high levels of both PLF mRNA and protein during the G1 phase. We have investigated the regulation of proliferin gene expression by analyzing a collection of promoter mutations in transfected mouse cells. A 31 bp sequence (-234 to -204) is sufficient for serum- and phorbol ester-stimulated expression; this region includes an AP-1 site located between -231 and -224 and a cluster of sites located between -223 and -205 that resemble the SV40 enhancer Sph I elements. In addition, a positive and a negative element immediately upstream of the AP-1 site appear to modulate the response to serum and phorbol esters. Glucocorticoids inhibit the induced expression of the endogenous PLF gene. This repression is also mediated by the -234 to -204 region, and is glucocorticoid receptor dependent. The receptor binds to the PLF promoter and footprints a region just upstream of the AP1 site.

E 646 INDUCTION OF TRANSCRIPTION FACTOR AP-1 BY ADENOVIRUS E1A PROTEIN AND cAMP, Ulrich Müller[1], Michael P. Roberts[1], Walter Doerfler[2] and Thomas Shenk[1],[1]Howard Hughes Medical Institute, Department of Biology ,Princeton University, Princeton, NJ 08544, [2]Institut for Genetics, University of Cologne, Cologne, West Germany.
Adeonovirus E1A proteins and cAMP activate the transcription of an overlapping set of viral and cellular genes. In mouse S49 cells E1A proteins and cAMP work in synergy to activate several of these genes, indicating that the E1A- and cAMP-dependent activation mechanism are somehow linked or interactive. In parallel to the induction of transcription the DNA binding activity of transcription factor AP-1 is modestly induced by cAMP in S49 cells and induced to significantly higher levels by cAMP in the presence of E1A proteins. The induction requires cAMP dependent protein kinase A since it does not occure in the presence of a chemical kinase blocker or in a kinase deficient subline of S49 cells. Cytoplasmic levels of c-fos and jun-B mRNAs are rapidly increased by cAMP and the induction is substantially stronger in the presence of E1A proteins. Immunological data indicate that both c-fos and jun-B are part of the AP-1 activity induced by E1A and cAMP. The AP-1 activity binds efficiently to both AP-1 and ATF/CREB binding sites present in E1A- and cAMP-inducible promoters. Transfection assays provide evidence for a role of the AP-1 activity in the E1A- and cAMP-dependent activation of adenovirus genes via ATF/CREB and AP-1 DNA-binding sites.

E 647 DEXAMETHASONE REVERSION OF BPV-1 TRANSFORMED PHENOTYPE WITHOUT INHIBITION OF VIRAL ONCOGENE mRNAs IMPLIES HORMONE-SENSITIVE STEPS IN GROWTH REGULATION BYPASSED BY SOME OTHER ONCOGENES, M. Kerry O'Banion, Richard L. Levenson and Donald A. Young, Departments of Medicine and Biochemistry, University of Rochester Medical Center, Rochester, NY 14642. In the presence of dexamethasone bovine papillomavirus (BPV)-transformed C127 mouse fibroblasts assume a flattened morphology and attain a saturation density of only one-half that observed without hormone. This "reversion of transformation" is dependent on the continued presence of dex and occurs with concentrations as low as 1 nM. The reversion is not due to an inhibition of the BPV-1 oncogene-specific mRNAs, as determined by Northern blot and primer extension analyses. Growth inhibition was also observed in cells transformed by polyoma middle-T antigen as well as in the parental line, but cells transformed by the v-H-*ras*, v-*mos*, or v-*fes* oncogenes all show significantly increased saturation densities in response to dex. This may be due to hormonal activation of the retroviral LTRs; however, the growth of cells transformed by LTR-driven E5 or E6/7 oncogenes of BPV-1 is inhibited, rather than enhanced. It may be that dex alters the transcription or activity of host factors involved in BPV and polyoma cell transformation that are bypassed by the other oncogenes. This possibility is being explored by quantification of oncogene RNA levels in cells harboring the LTR constructs. [Supported by: USPHS grants DK1677, CA47650 and DK07902; Tobacco Research U.S.A. grant 1774; and a grant from the Wilmot Foundation.]

E 648 REGULATION OF ALKALINE PHOSPHATASE mRNA EXPRESSION BY 1,25-DIHYDROXY VITAMIN D_3 AND DEXAMETHASONE. Robert C. Penhallow and Howard H. Sussman, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305. The steady state levels of mRNAs encoding alkaline phosphatase isoenzymes were examined in two human breast carcinoma cell lines. MDA-MB-157 cells expressed the phenotypic breast alkaline phosphatase and BT20 cells expressed the non-phenotypic placental alkaline phosphatase isoenzyme, frequently re-expressed in neoplasms. Dexamethasone (DEX), which elicits a general effect on phosphatase expression, and 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$), a promoter of cell differentiation that correspondingly effects embryonic phosphatase expression, were chosen as perturbing agents for these experiments. The expression of both the AP isoenzyme mRNA phenotypic of breast produced by MDA-MB-157 cells and the embryonic alkaline phosphatase isoenzyme (PLAP) mRNA produced by BT20 cells were increased by treatment with DEX. By comparison $1,25(OH)_2D_3$ caused an increase in the tissue unspecific AP mRNA in the MDA-MB-157 cells, but caused a decrease in PLAP mRNA levels in BT20 cells. The level of each isoenzyme mRNA species is altered by either hormone in a dose and time dependent manner in both cell lines. In BT20 cells treatment with cycloheximide showed that ongoing protein synthesis is not required to potentiate the PLAP mRNA response to DEX, but is required for the action of $1,25(OH)_2D_3$. However, protein synthesis is required for the action of both hormones in the MDA-MB-157 cells. These data demonstrate that the DEX and $1,25(OH)_2D_3$ regulated expression of both of these alkaline phosphatase isoenzymes occurs via a complex mechanism involving control of mRNA abundance, not translational control of constant message levels.

E 649 MECHANISMS OF DEVELOPMENTAL GLOBIN GENE SWITCHING, Susan P. Perrine, Paul Swerdlow, and Douglas V. Faller, Childrens' Hospital, Oakland, CA 94609

Butyrate prevents the normally fixed developmental fetal to adult globin gene switch in vivo and in vitro. To examine the mechanisms whereby butyrate influences the expression of the gamma globin gene, analogues of butyrate (differing in chain length or halide, amine or sulfate substitution) were studied for their actions on gamma globin transcription in cultures of human erythroid progenitors. In addition, the activities of the analogues in enhancing the expression of a transfected chimeric gene consisting of the 5' upstream region of the globin genes driving the reporter gene NEO in stable transfections of erythroid cells were compared to that of butyrate. Chimeric genes consisting of globin promoter sequences upstream of the hGH gene were also studied in transient transfection assays a transcriptional response to these inducers. Butyrate and those analogues found to be active in erythroid progenitor cultures increased expression of the gamma globin-NEO gene by 300% and gamma globin-hGH gene by 250%. The size and charge of the analogues appear to be important in their activity on gamma globin gene expression in these systems. Butyrate has been postulated to exert its effects on cells via inhibition of histone deacetylases or stimulation of adenylate cyclase. There was no correlation between the ability of the analogues to affect globin expression and their ability to alter cellular cAMP levels. However, the potency of the analogues on inhibition of globin switching did correlate with their degree of inhibition of histone deacetylases. These data suggest that butyrate may prevent developmental suppression of gamma globin gene expression by maintaining active chromatin structure and directly or indirectly affecting binding of transcriptional factors.

E 650 ISOLATION AND CHARACTERIZATION OF A SEQUENCE WHOSE EXPRESSION IS TISSUE SPECIFIC OR PROLIFERATION DEPENDENT

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To isolate sequences induced by interleukin 2, a cDNA library was prepared from poly(A)+ RNA isolated from IL2-stimulated cloned T lymphocytes in late G_1 phase of the cell cycle. The library was screened by differential hybridization, selecting those clones which had higher steady-state mRNA levels after IL2-stimulation. Of 40,000 clones screened, 90 were considered positive and represented 21 different genes. Partial DNA sequences were obtained and, when compared with sequences in the NIH and EMBL databases, the following sequences were identified: 6 glycolytic enzymes, 4 cytoskeletal proteins, 3 proteins involved in protein synthesis, 1 DNA binding protein, and 7 novel sequences. The seven novel sequences were tested for their expression in other tissues. A sequence designated F5 is expressed at high levels in brain and at low levels in thymus and possibly large intestine, but in no other adult tissues tested. F5 is expressed in several continuously proliferating cell lines derived from neural cells. It is also expressed in adult cerebral cortex, cerebellum, diencephalon, olfactory bulb, brainstem and eye, but not in spleen. Thus this novel sequence appears to be expressed in tissues with proliferating cells or in neural tissue.

E 651 TRANSCRIPTIONAL INDUCTION OF COLLAGENASE MRNA BY HORMONES IN RAT OSTEOSARCOMA CELLS. C.O. Quinn, D.K. Scott, K. Kopp, J.J. Jeffrey, and N.C. Partridge, St. Louis University and Washington University Schools of Medicine, St. Louis, MO 63110.

Our laboratory has demonstrated previously that bone-resorbing hormones such as parathyroid hormone (PTH) and $1,25(OH)_2$ vitamin D_3 [$1,25(OH)_2D_3$] stimulate the rat osteosarcoma cell line UMR 106-01 to secrete a neutral collagenase. To study the regulation of collagenase gene expression in these cells, we first isolated a clone from a UMR cDNA library using oligonucleotides complementary to sequence of the rat uterine collagenase protein as probes. Obtaining a partial clone, we performed Northern blot analysis of UMR RNA and determined that the collagenase mRNA is 2.9 kb in size with maximum expression at 4 h after PTH (bPTH 1-34, 10^{-8}M) treatment. The levels of collagenase mRNA increase about 100 fold over control levels 4 h after PTH treatment and fall to approximately 50% of the 4 h level by 24 h. Nuclear run-on experiments confirm that collagenase gene expression is transcriptionally regulated in these cells. Similarly, $1,25(OH)_2D_3$ causes a dramatic increase in collagenase mRNA levels 24 h after treatment. These data correlate well with our previously reported protein studies using immunohistochemical and ELISA assays and support the hypothesis that these agents act as stimulators of collagenase gene expression.

E 652 ALTERING THE PHOSPHORYLATION STATE OF TRANSCRIPTION FACTORS REGULATES IN VITRO TRANSCRIPTION BUT NOT BINDING TO THE PEPCK CRE. Patrick Quinn and Daryl Granner Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232 Nuclear extracts from rat liver were used to analyze in vitro transcription from the phosphoenolpyruvate carboxykinase (PEPCK) promoter and to analyze binding to the PEPCK cAMP regulatory element (CRE). The effects of increasing phosphorylation of transcription factors in crude nuclear extracts with the purified catalytic subunit of cAMP-dependent protein kinase and of decreasing phosphorylation with alkaline phosphatase were analyzed. Catalytic subunit increased transcription of the PEPCK promoter in a concentration-dependent manner and its effects were negated by coincubation with protein kinase inhibitor peptide, a specific inhibitor of phosphorylation by cAMP-dependent protein kinase. Incubation with phosphatase resulted in concentration-dependent decreases in transcriptional activity. Addition of catalytic subunit reversed the inhibitory effects of phosphatase. Analysis of PEPCK promoter mutants demonstrated that these effects were mediated, at least in part, through CRE-independent sequences centered around the TATA box. These results suggest that altering the phosphorylation state of one or more components of the basic transcriptional complex can modulate transcription directly and that both CRE-dependent and CRE-independent factors are involved. Nuclear extracts exhibited specific binding to the PEPCK CRE which was displaceable by wild type but not mutant PEPCK CRE sequences and by CRE sequences of the α glycoprotein hormone and somatostatin genes. Binding to the CRE was not altered by incubation of nuclear extracts with either catalytic subunit or phosphatase, at concentrations which affected in vitro transcription. Taken together, these results support a model in which factors are constitutively bound to the CRE and transcription is enhanced by modification of these factors.

E 653 THE ROLE OF PROTEIN KINASE C ACTIVATION IN INTERFERON-INDUCED GENE EXPRESSION. Nancy C. Reich and Lawrence M. Pfeffer†, Department of Pathology, SUNY at Stony Brook, Stony Brook, NY 11794 and Laboratory of Virology †, The Rockefeller University, New York, NY 10021. Type I interferons (IFNs) induce the rapid transcriptional induction of a specific set of IFN-stimulated genes (ISGs). We have investigated whether the response to IFN involves protein kinase C (PKC) activation in the human HeLa cell line. Pretreatment of HeLa cells with 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7), a potent inhibitor of PKC, was found to block cytoplasmic accumulation of ISG mRNAs, and impair transcriptional induction of the ISGs by IFN (measured by nuclear run-on transcription assays). In contrast, treatment with another isoquinolinesulfonamide derivative (HA1004) which preferentially inhibits cyclic nucleotide-dependent kinases, had little or no effect on ISG expression. In addition, H7, but not HA1004, blocked the induction of antiviral activity by IFN against vesticular stomatitis virus. Binding assays with [^{3}H]-phorbol 12-myristate 13-acetate (PMA) demonstrated a translocation of PKC to the plasma membrane within 15 minutes of IFN treatment. Although IFN activates PKC, PKC activation alone (by PMA treatment) does not induce the expression of the ISGs. Therefore PKC activation appears necessary, but not sufficient, to induce the expression of ISGs and antiviral activity mediated by IFN.

E 654 TRANSCRIPTIONAL REGULATORY ACTIVITY OF THE RAT ANDROGEN RECEPTOR
Stephen Rundlett, Xi-Ping Wu and Roger Miesfeld, Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721. The androgen receptor was one of the last members of the steroid receptor family to be cloned and characterized. Although the cloned receptor has a high homology to other members of the steroid receptor family and binds testosterone, it is still not known if it acts as a transcriptional regulator. Since we are interested in delineating the molecular genetics of cell death in the prostate, we isolated full-length androgen receptor cDNA from the rat ventral prostate and utilized it in transient cotransfection assays. We find that the encoded protein 1) activates MMTV transcription >20-fold in an androgen-dependent manner (dex-induction by GR on the same reporter plasmid is 15 times greater), 2) functions as a dex-regulated chimeric receptor when fused to the rat glucocorticoid receptor ligand binding domain, 3) distinguishes between the AREs present in the MMTV LTR and the GRE present in the TAT gene; indicating that GREs and AREs are not identical. 4) can constitutively activate the MMTV ARE if ~200 amino acids of the carboxy terminal domain are removed. These results are in agreement with the notion that androgen mediated control of prostate cell viability is primarily at the level of transcription.

E 655 MOLECULAR COOPERATION AND INTERFERENCE OCCURRING DURING TRANSCRIPTIONAL CONTROL BY THE GLUCOCORTICOID RECEPTOR.
Sandro Rusconi , Michael Schatt, Udo Döbbeling, Ebrahim Zandi and Stefan Wieland.
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We have studied the protein-protein and protein-DNA interactions occurring during transcriptional activation in model systems (in vivo and in vitro) in which wild type and mutated forms of the glucocorticoid receptor (GR) and its DNA target site (GRE) have been employed. We demonstrate that GRE clusters can serve as very efficient and totally GR-dependent promoters or enhancers and that GR-mediated transcriptional control does not require specific interactions with TATA box binding factor(s) or direct interactions between remote enhancers and the target promoter. Furthermore, we have data suggesting that the architecture of the GRE cluster determines the efficiency of cooperation among the bound transactivators and that a rough correlation may exists between spatial organization of the GREs and the molecular size of the transactivator. A surprising observation is that a minimal portion of the rat GR (aa. 407-556 including the zinc fingers) is sufficient for transcriptional activation from distance (e.g 1.8 kb). While testing the GR in combination with other cloned transactivators, we observed a strong interference with the activity of the lymphocyte-specific octamer binding factor. We speculate that this phenomenon could be linked to the immunosuppressive properties of glucocorticoids. Systematic mutagenesis of the first zinc finger and surrounding regions (e.g. residues 406 to 465, without changing essential cysteines) has yielded some remarkable null-or semi-permissive mutants. We are testing the possibility that some dramatic changes in the specificity of DNA recognition might be the reason of the observed loss-of function in vivo. Finally, a direct blotting technique has been developed, which allows for the visualization of strong protein-protein interactions involving the GR and other cellular components, like for instance the 90 kd heat shock protein.

E 656 ESTROGEN RECEPTOR CAN ACT AS A GENE ACTIVATOR IN THE PRESENCE OF ESTRADIOL AND AS A REPRESSOR IN THE ABSENCE OF HORMONE.
Maty Tzukerman, Xiao-kun Zhang, Thomas Hermann and Magnus Pfahl, La Jolla Cancer Research Foundation, La Jolla, CA 92037
The estrogen receptor (ER) belongs to a subfamily of nuclear receptors that also includes the thyroid hormone receptors (TR) and retinoic acid receptors (RAR). ER and TRs show a high homology in their DNA binding domain and in their responsive elements. We have recently reported that TRs can work as transcription repressors in the absence of ligand. The classical model of steroid hormone action postulates that only the ER-estrogen complex binds to DNA and thereby effects gene expression. Here, we report that ER , in the absence of hormone, has repressor activity. Gene regulation by ER was investigated in cotransfection experiments using a synthetic ERE linked to a CAT reporter gene. This reporter gene can be activated by ER in the presence of estradiol but also to some degree by TRs and RARs. When ER was co-transfected together with TRs or RAR, those receptors were no longer able to induce the ERE-CAT gene. The use of hybrid receptors has shown that the ER-DNA binding domain is essential for the ERE specific repression. Gel retardation analysis showed that ER binds to the monomeric and the dimeric ERE in the presence and in the absence of its ligand. We conclude that ER has a dual regulatory function, in the presence of hormone ER functions as an ERE specific activator of transcription and, in the absence of hormone, however, ERE functions as an ER specific repressor.

E 657 IDENTIFICATION OF TWO UNIQUE PROTEIN BINDING SITES IN THE UPSTREAM GLUCOCORTICOID REGULATORY REGION OF THE RAT ALPHA-1 ACID GLYCOPROTEIN GENE, Paul M. Williams, Diego DeLorenzo, Thomas Ratajczak, Gordon Ringold, Institute of Cancer and Developmental Biology, Syntex Research, Palo Alto, CA 94304. An efficient glucocorticoid induction of alpha-1 acid glycoprotein mRNA in rat hepatoma cells requires the activity of one or more pre-existing and labile proteins in addition to the activated glucocorticoid receptor. The DNA sequence -120 to -42 upstream of the start of transcription is capable of conferring glucocorticoid induction. A glucocorticoid regulatory element has been mapped to the region -120 to -105. We have utilized gel mobility shift assays and DNase fooprinting to examine the region -120 to -64. Our data indicates at least two unique proteins bind to the DNA in this region. One protein interacts with the sequence between -90 to -81. The other protein can bind to two sites from either the region -81 to -72 or -113 to -104. These two binding sites contain an eight out of ten base homology. Interestingly, the binding site -113 to -104 overlaps the glucocorticoid regulatory element. We have made mutant oligonucleotides which do not bind to the proteins and are currently utilizing these oligonucleotides to generate mutant promoters to analyze the function of these two proteins in transcription of the alpha-1 acid glycoprotein gene. We are attempting to purify both DNA binding proteins from rat liver nuclear extracts.

E 658 **GLUCOCORTICOID REGULATION OF THE HUMAN β ALCOHOL DEHYDROGENASE GENE,** Laurie A. Winter and Gregg Duester, Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523. The 5'-flanking region of the human β alcohol dehydrogenase (ADH) gene was shown by DNase I footprinting to contain three tandem binding sites for purified glucocorticoid receptor. The three binding sites lie very close together between positions -245 and -171 with respect to transcription initiation. Since human ADH is expressed primarily in liver we tested the ability of a DNA fragment containing the glucocorticoid receptor binding sites to behave as a glucocorticoid response element (GRE) in a liver cell line. When a β ADH promoter fragment containing -272 to +31 was fused upstream of the CAT gene and transfected into the HepG2 hepatoma cell line there was a weak induction of CAT activity in the presence of dexamethasone. Heterologous ADH-CAT fusions, in which the putative GRE region was fused to the adenovirus major late promoter, exhibited a substantial amount of glucocorticoid induction in HeLa and CV-1B cells when cotransfected with a glucocorticoid receptor expression vector. Glucocorticoid regulation in HeLa and CV-1B was observed when either all three glucocorticoid receptor binding sites (sites 0,I,II) or the two distal sites (sites 0,I) were present, but not when just the proximal site (site II) was present. DNase I footprinting of the β ADH 5'-flanking region using a rat liver nuclear extract indicated the presence of several footprints designated A through F located between positions -10 and -209. No footprinting was observed directly over the GRE sites in this crude extract. Interestingly, footprint F located at -209 to -191 partially overlaps both GRE sites I and II, suggesting that binding of factor F and the glucocorticoid receptor (to sites I and II) is mutually exclusive. It is proposed that in tissues such as liver which contain the factor which binds the F site, the efficiency of glucocorticoid induction of ADH may be reduced (relative to tissues lacking factor F) due to a physical blockage of glucocorticoid receptor binding to GRE sites I and II.

E 659 NUCLEAR LOCALIZATION SIGNALS OF ESTROGEN AND PROGESTERONE RECEPTORS, Timo Ylikomi, Marie-Thérèse Bocquel, Meera Berry, Vijay Kumar, Christiane Quirin-Stricker, Pierre Chambon and Hinrich Gronemeyer. Institut de Chimie Biologique, Faculté de Médecine, 11, rue Humann, 67085 Strasbourg Cédex, France.
We have determined the sequences required for nuclear localization of human estrogen (hER) and chicken progesterone (cPR) receptors by transiently expressing receptor mutants in HeLa cells. Receptors were detected by immunohistochemical staining using the avidin-biotin-peroxidase technique. Unliganded wild-type hER and cPR were confirmed to be nuclear proteins. Deletion analysis revealed sequence motifs distinct from the so-called "DNA-binding fingers" which triggered nuclear localization. In hER complex nuclear localization signal(s) (NLSs) were mapped between the DNA and hormone binding domains, while cPR contained only a "simple" NLS. Analysis of chimeric receptors bearing the NLSs of the human glucocorticoid receptor (hGR) demonstrated that hGR has a weaker or more extensively masked constitutive NLS than hER and cPR. This renders the unliganded hGR partially cytoplasmic, while hormone-binding results in nuclear accumulation.

E 660 NUCLEAR RECEPTORS AS TRANSCRIPTIONAL REPRESSORS AND ACTIVATORS. Xiao-kun Zhang, Ken Wills, Maty Tzukerman, Gerhart Graupner, Thomas Hermann and Magnus Pfahl, La Jolla Cancer Research Foundation, La Jolla, CA 92037

We have recently reported that thyroid hormone receptors can function as transcription repressors on specific thyroid hormone response elements. We now further investigate this repressor action by thyroid hormone receptors, retinoic acid receptor, and estrogen receptor. Our transient transfection assay showed that in the presence of their ligands these receptors can induce transcription from a specific hormone response element, while in the absence of its ligand one receptor may repress the transcription activity of the others. This negative effect was found to be a direct consequence of receptor-DNA interaction measured by gel retardation. Our studies also showed that the receptors bind to a specific DNA sequence with varying affinities due to the difference in their DNA-binding domain. The affinity for their DNA is also controlled by the hormone-binding domain as revealed by our studies on alternative spliced thyroid hormone receptor isoforms and a number of hybrid recptors whigh contain foreign hormone-binding domains. We also observed that each receptor can bind to the specific DNA sequence in the absence of its ligand although the addition of ligand may change its DNA binding affinity positively or negatively or its binding pattern. Our in vitro DNA binding studies together with the transient transfection assay suggest that the repressor capacity of a receptor is dependent on its DNA binding affinity relative to that of another activated receptor. Our data therefore led us to propose that in the absence of its ligand a receptor can occupy a specific hormone response sequence and thereby prevent or compete with other activated receptor from acting as transcription activator, whereas in the presence of its ligand the conformation of a bound receptor is altered in such a way that transcriptional activation is allowed.