TRANSCRIPTION REGULATION IN DEVELOPMENT, DIFFERENTIATION AND DISEASE

Organizers: Gerald Crabtree and Matthew Scott

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Transcriptional Control and Transposition

B 001 ANALYSIS OF TFIID, HISTONES, AND OTHER TRANSCRIPTION FACTORS OF YEAST, Fred Winston, Karen M. Arndt, Steven Brown, Chris D. Clark, David M. Eisenmann, Joel N. Hirschhorn, Elizabeth A. Malone, Gregory Prelich, Stephanie L. Ricupero, and Michele S. Swanson, Department of Genetics, Harvard Medical School, Boston, MA 02115.

Insertion mutations of the yeast retrotransposon Ty, or of the long terminal repeats of Ty elements in promoter regions can inhibit or otherwise alter transcription of adjacent genes. Selection for suppressors of these insertion mutations has resulted in identification of over 17 genes, designated *SPT* genes (SPT = suppressor of Ty). In addition to effects on insertion mutations, *spt* mutations confer a number of other mutant phenotypes. Some *spt* mutations affect normal expression of cell-type specific genes required for mating and sporulation. Other *spt* mutations affect the ability of cells to utilize particular sugars as carbon sources. The distinct mutant phenotypes caused by different sets of *spt* mutants indicates that SPT gene products are required for different aspects of transcription. This idea is supported by the finding that one class of *SPT* genes includes a gene, *SPT15*, that encodes the TATA-binding factor TFIID, a protein essential for transcription by RNA polymerase II. A second class of *SPT* genes includes two genes, *SPT11* and *SPT12*, that encode histone proteins H2A and H2B. Therefore, mutations in general transcription factors and in chromatin components can have differential effects on transcription *in vivo*. In addition to these previously identified functions, other *SPT* genes encode functions not previously identified or studied that are important for TFIID function or for chromatin function. For example, the *SPT3* gene encodes a protein that physically interacts with TFIID and which is proposed to be "specificity factor," a function that helps TFIID choose between different TATA sequences *in vivo*. Similarly, *SPT4*, *SPT5*, and *SPT6* encode proteins of unknown function that are required for normal chromatin function. These three gene products, which by sequence analysis are unrelated to histones or HMG proteins, form a complex in the nucleus that is essential for growth. These results indicate that factors believed to be generally required for transcription are also involved in differential transcription of particular sets of genes.

Transcriptional Regulation in Differentiation-I

B 002 DEVELOPMENTAL REGULATION OF ß-GLOBIN GENE TRANSCRIPTION AND CHROMATIN STRUCTURE IN VITRO, Timothy C. Fong, Michelle Craig Barton, Steven F. Bodovitz, and Beverly M. Emerson, The Salk Institute, La Jolla, CA 92037.

The chick ß-globin gene family provides a useful model in which to study the molecular mechanisms involved in the tissue-specific regulation of transcription. This family consists of four genes that are individually expressed at different stages of erythroid development. Regulated expression is achieved through modulations of specific chromatin structures and by multiple transcription factors whose composition changes during red cell maturation. In our previous work, we analyzed the transcription of the chick adult ß-globin gene, which is expressed only in definitive red cells beginning at day 9 of embryonic development. Protein extracts prepared from red cells isolated at different stages of erythroid development transcribe adult and embryonic ß-globin genes in a pattern similar to that found *in vivo*. Efficient ß-globin expression occurs through the combinatorial action of multiple activator proteins when both the promoter and 3' enhancer regions of the gene are in nuclease hypersensitive chromatin structures. As red cells mature to become transcriptionally quiescent, a potent repressor, PAL, increases in concentration, binds to the ß-globin promoter, and inactivates expression *in vitro*.

In recent studies, we have examined the mechanism by which the distal 3' ß-globin enhancer activates the 5' promoter. The erythroid-specific protein, cGATA-1, regulates the chick ß-globin gene through GATA sequences present at the canonical TATA location in the promoter as well as in the distal 3' enhancer. We have analyzed ß-globin transcription in transfected erythroid cells and in erythroid extracts to determine whether cGATA-1 binding at -30 regulates promoter or enhancer activity. The interaction of both cGATA-1 and TFIID at the -30 GATA site is required for efficient ß-globin expression *in vivo* and the GATA enhancer site can functionally replace the TATA element in the ß-globin promoter. TFIID can initiate transcription *in vitro* by interacting with the cGATA-1/-30 GATA complex. Mutations that abolish TFIID binding to the -30 GATA box inactivate the promoter whereas elimination of cGATA-1 binding to this site selectively diminishes enhancer-dependent transcription. We propose that interaction of cGATA-1 with the distal 3' enhancer and the specialized TATA box confers erythroid specificity to the initiation complex by mediating promoter-enhancer communication.

Our efforts have also been directed towards understanding how the ß-globin gene is regulated in the context of the large chromosomal locus in which it resides. To address this question, we have incorporated cosmids containing the 38 kb globin gene locus into chromatin using extracts from Xenopus eggs. Incorporation of ß-globin genes in the presence of erythroid proteins isolated at different stages of red cell development may approximate the chromatin structure of the locus as it exists at specific times *in vivo*. We have examined the template activity of ß-globin genes within the reconstituted locus by *in vitro* transcription. In this way, we can assess the role of distal control elements and long-range chromatin structure in the activation of individual genes within the entire ß-globin gene locus.

B 003 THE REL/NF-kB FAMILY OF TRANSCRIPTION FACTORS, Alain Israël, Volker Blank, Mary Lee McKichan, Odile Le Bail, Frederique Logeat and Philippe Kourilsky, Institut Pasteur, Paris, FRANCE

The NF-κB transcription factor is made of an heterodimer of a 50 kD (p50) and a 65 kD (p65) subunits. These two proteins show a strong homology with the c-rel protooncogene, whic can form heterodimers with p50 or p65 and is also involved in the complex "NF-κB" response. The p50 subunit is derived from a 105 kD (p105) cytoplasmic precursor. we will present recent data concerning :

- the fonction of the C-terminal part of the p105 precursor, and especially of the so-called ankyrin repeats, in the cytoplasmic localisation as well as lack of DNA-binding ability of this protein.
- a new fonction of the p105 precursor in the context of the rel family.
- the activating or inhibitory activity of various members of the rel family.
- the promoter of the gene coding for p105 which contains a binding site for NF-κB, indicating autoregulation of the expression of this protein.

B 004 NUCLEAR-CYTOPLASMIC PARTITIONING OF THE DORSAL MORPHOGEN REGULATES ITS ACTIVITY AS TRANSCRIPTION FACTOR IN EARLY DROSOPHILA DEVELOPMENT, Christine Rushlow[1], Tony Ip[2], and Michael Levine[2], [1]Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, NJ, [2]Department of Biological Sciences, University of California, San Diego, La Jolla, CA.

A protein concentration gradient of the maternal morphogen *dorsal* (*dl*) establishes dorsal-ventral (D-V) polarity in the early Drosophila embryo. The *dl* gradient is formed by the regulated nuclear transport of *dl* proteins, and only protein that enters nuclei is active in the D-V patterning process. Peak levels of nuclear protein are found in the ventral-most region, the presumptive mesoderm, while little of no protein is found in the nuclei of dorsal regions. Once in the nucleus, how does *dl* control DV cell fate? Previous genetic studies have identified at least four genes that are regulated by *dl*. High levels of *dl* protein are thought to activate the expression of *twist* and *snail*, which are regulatory genes that control the differentiation of the mesoderm and are expressed in ventral regions. *dl* is also thought to restrict the expression of genes that control the differentiation of dorsal structures to dorsal regions of the embryo, such as *zen* and *dpp*. In *dl*-derived embryos, there is a failure to activate *twist* and *snail* in ventral regions. Moreover, *zen* and *dpp* become expressed ectopically and is seen in all regions of the embryo. A simple model for these regulated patterns of expression is that *dl* protein binds to sites within their *cis*-regulatory DNAs to promote the activation or repression of transcription. To test this possibility we performed DNA binding assays using *dl* proteins made in bacteria and DNA fragments from the *zen* promoter. We have found that the *dl* protein recognizes a sequence motif similar to that of the mammalian transcriptional activator NF-kB, and that these sequences are important for normal expression *in vivo*. Eventually we would like to understand how *dl* can both activate and repress transcription of several target genes in a concentration dependent manner along the DV axis of the early embryo.

Transcriptional Regulation in Differentiation-II

B 005 INTRACELLULAR SIGNAL TRANSMISSION: A NOVEL ROLE FOR THE PROLYL ISOMERASES *W. Michael Flanagan, Katharine S. Ullman, Blaise Corthesy, Peter Kao, Gerald R. Crabtree*, Stanford University, Stanford CA 94305

The prolyl isomerases FKBP and cyclophilin (also called immunophilins) have been implicated in specific biologic processes as diverse as cell cycle progression in yeast, vision in Drosophila and immunosuppression in humans. We have been interested in their role in T lymphocytes where they are felt to mediate the immunosuppressive effects of FK-506 and cyclosporin A respectively. The most enticing feature of the actions of these drugs on T cells is that they inhibit two opposing cell fates determined by the antigen receptor: programmed cell death in immature T cells and immunologic activation of mature T cells.

During normal development, T lymphocytes capable of responding to nearly any antigen are produced in the thymus as a result of the processes of differentiation and positive- and negative-selection. After having undergone this developmental programming these cells migrate to the peripheral lymphoid organs and when exposed to specific antigen respond with the sequential activation of several hundred genes over a 2 week period, resulting in proliferation and immunologic function. This autonomous program of T cell differentiation coordinates the immune response by cell-cell interactions and the sequential production of cytokines necessary for cell fate decisions, differentiation, and proliferation of precursors of B cells, macrophages and other cells involved in the immune response. We have been attempting to understand the events that initiate T cell activation by studying the lines of communication between the antigen receptor and the early genes necessary for T cell differentiation.

The immuno-suppressive prolyl isomerase inhibitors, cyclosporin and FK-506, block T cell activation and transcription of early genes without affecting known cell membrane or cytoplasmic events involved in signal transduction, implying that they function at a novel step in signal transduction. These drugs both specifically and completely block transcription activated by a T cell specific transcription factor, NF-AT, but have only modest effects on other transcription factors. NF-AT is a heterodimeric protein made up of a constitutive subunit residing in the cytosol, which after activation translocates to the nucleus and combines with a nuclear subunit that is synthesized *de novo*. Using analogues of FK-506 we have demonstrated that the biologic actions of these drugs is not due to their ability to inhibit prolyl isomerase activity but rather to a inhibitory complex formed between the drug and the isomerase. Similar conclusions have been reached from studies of cyclosporin-resistant yeast mutants (Tropschug et al, Nature, 1989). In addition, the Drosophila homologue of cyclophilin, *ninA*, does not have detectable isomerase activity (Shieh B. et al, Nature, 1989; C.S. Zuker, personal communication). We find that Cyclosporin A and FK-506 block nuclear import of the cytosolic subunit of NF-AT but have no effect on nuclear import of NFkB. Since the drug-isomerase complex does not form a stable complex with NF-AT, but does form a stable complex with calcineurin (See abstract by Schreiber et al.), we feel that calcineurin most likely functions in the signal transmission cascade by dephosphorylating the cytosolic subunit of NF-AT, allowing it to enter the nucleus where it activates early genes. The coincidence that prolyl isomerase inhibitors of different chemical structure alter signal transduction by virtue of the formation of a complex suggest that the normal role of these molecules is to bind an endogenous ligand producing a biologically active complex that is independent of prolyl-isomerase activity.

B 006 CHROMATIN STRUCTURE AND THE DEVELOPMENTAL REGULATION OF GLOBIN GENES. G. Felsenfeld, D. Clark, T. Evans. H. Gould, R. Hannon, J. Knezetic, E. Lee*, M. Minie, M. Reitman, H. Westphal*. Laboratory of Molecular Biology, NIDDK, and *Laboratory of Molecular Genetics, NICHD, National Institutes of Health, Bethesda, MD 20892

We have used the chicken globin gene family as a model system in which to study developmentally modulated expression. The information governing stage-specific expression appears to be carried in the local promoter elements of each gene. In earlier studies we analyzed the promoter of the adult β-globin gene, and the two 'adult' α-globin genes, α^D and α^A. We have now completed a study of π, the embryonic α-globin gene. The π promoter contains the information necessary to specify expression only in primitive lineage cells. Detailed examination of the binding sites and factors involved shows that this behavior reflects a complex interaction among three components that differ in abundance in the two cell lineages. A similar analysis has been made of the ρ-globin gene, an embryonic member of the β family. Correct expression of the members of the globin gene family depends upon the action of erythroid-specific enhancers, and upon the presence of the locus control region (LCR). We have shown that the β/ϵ enhancer also has the properties of an LCR: In transgenic mice, it confers position-independent expression on the β-globin gene. The function of the LCR appears to be connected with chromatin structural properties. We have investigated the effects of the β/ϵ LCR on chromatin structure by making transgenic mice that carry the β-globin gene with both its promoter and the LCR, with the promoter only, or with the LCR only. The results suggest two models for the roles of the promoter and the LCR in the organization of chromatin.

Viral Gene Regulation in Pathogenesis

B 007 TRANSCRIPTIONAL REGULATION OF HIV, Matija Peterlin, Alicia Alonso, Subir Ghosh, and Xiaobin Lu, Howard Hughes Medical Institute, Departments of Medicine, Microbiology, and Immunology, University of California, San Francisco, San Francisco, CA 94143-0724.

The long terminal repeats (LTRs) of HIV-1 and 2 are transcriptionally activated following T cell or macrophage activation, growth, and proliferation. Cellular transacting factors that participate in this response include nuclear factors κB (NF-κB) and of activated T cells (NF-AT) in HIV-1 and NF-κB and AP-3 in HIV-2. Following activation, HIV LTRs are transactivated by their respective transactivators (Tats). Tat interacts with an RNA stem-loop 3' to the site of initiation of viral transcription called TAR. Tat modifies an unstable to a stable (i.e. elongation competent) trans-cription complex. Evidence for efficient loading of RNA polymerase II at the HIV promoter by upstream transcriptional activators and utilization of Tat at TAR will be presented. By fusing Tat to the MS2 coat protein, a prokaryotic RNA binding protein, and replacing TAR with the MS2 operator, we were able to map activation and RNA binding domains of Tat. By characterizing Tats of HIV-1, HIV-2, and EIAV, a minimal functional lentiviral Tat of only 25 amino acids was constructed. Also, the defect in rodent cells that leads to low levels of transactivation by Tat and that is complemented by human chromosome 12 was defined. While mixing and matching HIV LTR sequences reveal differences in activation and transactivation that might explain distinct clinical courses of HIV-1 and HIV-2 infections, studies on Tat represent a new dimension of transcriptional control in eukaryotic systems.

Cell **62:** 769-776 (1990)
Genes Dev **3**: 547-558 (1989)
J Immunol **142**: 702-707 (1989), **145**: 4384-4354 (1990)
J. Virol. **65**: 1758-1764 (1991)
Nature **330**: 489-493 (1987)
PNAS **83**: 9734-9738 (1986), **84**: 6845-6849 (1987), **85**: 8286-8290 (1988)

B 008 *Abstract Withdrawn*

Transcriptional Control of Early Development

B 009 HOMEOTIC GENES AND CELL MIGRATION IN C. ELEGANS, Cynthia J. Kenyon, Judith Austin, Deborah Cowing, Jeanne Harris, Michael Müller-Immerglück, Naomi Robinson, Steve Salser, Bruce Wang. University of California, San Francisco, CA 94143-0554.

Insects and vertebrates contain evolutionary clusters of Antennapedia-type homeobox genes (HOM-C genes). In flies, and apparently in vertebrates as well, these genes function to give specific body regions their identities. Like flies and vertebrates, *C. elegans* also contains a HOM-C. One of the genes in this cluster, *mab-5*, is known to give a posterior body region its identity. Recent work indicates that two HOM-C genes that flank *mab-5* in the genome are responsible for the development of the body regions that flank the *mab-5* domain in the animal. In this presentation, the regulation of HOM-C gene expression will be discussed, as well as the role of these genes in cell migration.

Maintenance of Determined Cell States

B 010 POSITIVE AND NEGATIVE REGULATORS OF MYOGENESIS, Helen M. Blau, Farzan Rastinejad, Lydia Pan, Dan Rohrer, Charlotte Peterson, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305.

Heterokaryon experiments have revealed that the differentiated state requires continual regulation and depends on a complex interplay of positive and negative regulators. Mutant myogenic cell lines have been produced to focus on specific regulators at key points in the muscle differentiation pathway. These mutants are being characterized at the level of Northern and Western analysis and by fusion in heterokaryons. Genetic complementation with cDNA expression libraries and assays based on function are being used to isolate gene encoding products that act directly and indirectly to control differentiation.

Peterson, C.A., Gordon, H., Hall, Z.M., Paterson, B.M., and Blau, H.M. (1990) Cell 62:493-502.

B 011 IMPRINTING A DETERMINED STATE INTO THE CHROMATIN, Renato Paro,Axel Franke, Sabine Messmer, Axel Möhrle, Daniele Zink and Valerio Orlando, Center of Molecular Biology, University of Heidelberg, Germany.

We use the homeotic genes of *Drosophila melanogaster* as a model system to unravel the molecular mechanisms responsible for maintaining a determined state of a cell through developmental time. The complex pattern of homeotic gene expression is established early in development by the maternal and segmentation gene hierarchies. The resulting differential expression of the homeotic genes of the ANT-C and BX-C along the anterior posterior axis defines the position and the identity of the various body structures. Two groups of genes, the trithorax-group (trx-G) and the Polycomb-group (Pc-G) are responsible for subsequently maintaining homeotic genes active respectively inactive in the appropriate cell clones through the rest of development. Mutations in the genes of the Pc-G result in a complete depression of homeotic genes in late embryogenesis,suggesting a failure in the transmission of the pattern, set by the early determinative events. The *Polycomb (Pc)* gene, a member of the Pc-G, was shown to express a nuclear protein. The Pc protein was found to share a similar domain (chromo domain), extending over approx. 40 aa, with the heterochromatin associated protein HP-1. HP-1 is encoded by the gene *Su(var)205*, a modifier of position effect variegation (PEV). Apart from the molecular relationship that we have found, the Pc-G and the modifiers of PEV show some striking similaries at the genetic and physiological level. This let us propose that the Pc-G genes "heterochromatinize" homeotic genes and thus keep them stably and permanently repressed through developmental time.

In order to define the funtional role of the highly conserved chromo domain we have constructed a fusion gene between *Pc* and *LacZ*. The resulting Pc protein tagged with β-gal binds on polytene chromosomes of transgenic flies to the same target genes as the endogenous Pc protein. Immunofluoressence to whole cells (using anti-β-gal antibodies) show a punctuated distribution of the fusionprotein in the nucleus. We interpret this pattern as resulting from large Pc protein complexes that are associated with their respective target genes. Fusionprotein with *in vitro* induced mutations of the chromo domain show a homogenous distribution in the nucleus. On polytene chromosomes only a reduced number of binding sites is left. This preliminary data suggest that the chromo domain might be involved in defining target gene specificity and/or be involved in a packaging mechanism forming large Pc protein complexes. The latter being a interesting possibility considering our hypothesis that Pc might be involved in a heterochromatin-like process. In collaboration with H. Brock (UBC, Canada) we have found that the protein product of the gene *polyhomeotic (ph)*, also a member of the Pc-G, forms part of the large Pc protein-complex. Using double-labelling immunofluorescence techniques we can show that Pc and ph have the same binding sites on polytene chromosomes. Furthermore we can immunoprecipitate from embryonic nuclear extracts ph protein with anti -Pc antibodies and vice-versa. Indicating that the two proteins might interact at the molecular level. By further analysing the interactions between different members of the Pc-G we should be able to dissect the molecular mechanisms used by this group of genes for the maintenance of the repressed state of homeotic genes.

Structural Features of Transciption Factors

B 012 MECHANISMS OF TRANSCRIPTIONAL REGULATION BY THE POU HOMEODOMAIN PROTEINS OCT-1 AND OCT-2, Winship Herr, Rajeev Aurora, Michele Cleary, William Clouston, Gokul Das, Seth Stern, and Masafumi Tanaka, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

Many genes that control development encode sequence-specific DNA binding proteins that regulate transcription, such as those of the homeodomain class. Frequently, homeodomain proteins display quite similar DNA binding specificities but effect very different developmental programs. To understand the mechanisms by which homeodomain proteins can bind to the same DNA sequence and yet differentially regulate transcription we study transcriptional activation by the mammalian octamer-motif (ATGCAAAT)-binding proteins Oct-1 and Oct-2. The ubiquitously expressed Oct-1 protein is implicated in regulation of small nuclear RNA genes (snRNA) transcribed by RNA polymerase II (e.g. U2 snRNA) and RNA polymerase III (e.g. U6 snRNA), whereas the lymphoid Oct-2 protein is implicated in cell-specific regulation of typical mRNA promoters such as the immunoglobulin gene promoters. Oct-1 and Oct-2 are closely related proteins that belong to the POU class of homeodomain proteins. POU homeodomain proteins are characterized by a large region (150 to 190 amino acids) of sequence similarity, called the POU domain; this domain contains two subdomains, an N-terminal POU-specific domain and a C-terminal homeodomain. Both of these regions are responsible for the full sequence-specific DNA binding activity of these proteins.

Our studies of the activation potentials of Oct-1 and Oct-2 have revealed that these two proteins carry different types of activation domains, which lie outside of the POU DNA-binding domain. The C-terminus of Oct-2 specifies activation of TATA box-containing mRNA promoters whereas the C-terminus of Oct-1 is unique because it fails to activate mRNA promoters but instead can activate the U2 snRNA promoter. Thus, here, differential transcriptional activation is specified by promoter-selective activation domains that lie outside of the DNA-binding domain. This result contrasts with the strategy by which herpes simplex virus (HSV) activator VP16 (Vmw 65) which differentially regulates transcription by Oct-1 and Oct-2 by discriminating between the very similar (87% conserved) Oct-1 and Oct-2 DNA-binding domains.

VP16 is a HSV virion protein that activates the HSV immediate early genes by associating with Oct-1 and a second cellular factor we refer to as HCF to form a multiprotein-DNA complex. VP16 serves as a transcriptional adaptor that, by virtue of a very strong acidic activation domain, converts Oct-1 into a potent activator of mRNA promoters. VP16 associates with Oct-1 but not Oct-2 by specifically recognizing the Oct-1 homeodomain. A mutational analysis of VP16 association with Oct-1 in the absence of HCF indicates that VP16 contains a small homeodomain-recognition subdomain located near the acidic activation domain. VP16 association with Oct-1 not only affects the activation potential of Oct-1 but also results in co-recruitment of Oct-1 and VP16 to certain binding sites called TAATGARAT that lack a good similarity to the octamer motif. This co-recruitment results in a unique class of cis-acting elements that are only active in the presence of both Oct-1 and VP16.

B 013 TRANSCRIPTIONAL REGULATORY PROTEINS INVOLVED IN HEPATOCYTE-SPECIFIC GENE EXPRESSION, Patrick Descombes, Eileen Falvey, Daniel J. Lavery, Edward E. Schmidt, Jian-Min Tian, Jérôme Wuarin, and Ueli Schibler, University of Geneva, Switzerland.

The regulation of tissue-specific gene expression involves transcriptional activator proteins that themselves accumulate preferentially in certain cell-types. The question thus arises of how the cell-type specificity of such transcription factors is controlled. The genes specifying three liver-enriched trans-activator proteins, hepatocyte nuclear factor 1 (HNF-1), D-binding protein (DBP) and liver activator protein (LAP), have been isolated by molecular cloning techniques and their expression has been studied. The HNF-1 gene, encoding a POU-homeobox related protein, is activated early during liver differentiation. HNF-1 mRNA accumulates to substantially higher levels in hepatocytes and kidney cells than in other tissues, suggesting that the spatial expression of HNF-1 is governed mainly by transcriptional mechanisms. Indeed, a cis-acting element has been identified within the HNF-1 promoter that directs cell-type-specific transcription both in transfected cells and cell-free extracts. The transcription factor mediating trans-activation through this element has been identified as hepatocyte nuclear factor 4, a member of the steroid hormone receptor family. Indeed, co-transfection of an HNF-4 expression vector (generously provided by F. Sladek and J. Darnell, Rockefeller University) with a CAT reporter gene carrying the HNF-1 promoter results in a strong increase of CAT activity in the extracts of transfected cells. DBP and LAP are both members of the basic region-leucine zipper protein family. In contrast to HNF-1, LAP and DBP reach maximal levels only during adulthood and their mRNAs can be detected in most tissues. Thus, the tissue-specific accumulation of these two regulatory proteins appears to be controlled at a translational or post-translational level. Surprisingly, DBP levels vary about a thousand-fold during the day, maximal and minimal levels being reached at 8 p.m. and 8 a.m., respectively. This circadian rhythm is transcriptionally controlled, probably by the diurnal oscillation of peripheral steroid hormones. Putative DBP target genes, whose activity is also subject to a circadian fluctuation, have been isolated and characterized. During liver differentiation, the activity of LAP is modulated by a trans-dominant negatively acting protein, LIP. LAP and LIP are translated from one and the same mRNA by a leaky ribosome scanning mechanism. The ratio of LAP and LIP changes about five-fold during post-natal liver development. In transfection experiments, small changes in this ratio appear to result in large differences in the transcriptional stimulation of putative LAP target genes. Therefore, the gradual increase in LAP/LIP during hepatocyte differentiation would be expected to result in a much more abrupt switch in the activity of LAP target genes. Translational and post-translational mechanisms possibly accounting for the change in the LAP/LIP ratio will be discussed.

Environmental Induction and Cell-Cell Signalling

B 014 NEUROENDOCRINE GENE EXPRESSION IN HYPOTHALAMIC AND PITUITARY CELL DIFFERENTIATION.

Pamela L. Mellon[1,2], David B. Whyte[1], Kerry M. Barnhart[1], Satish A. Eraly[1,2], Claudia Stauber[1], Denise Lew[1]. [1]The Salk Institute, La Jolla, CA 92037, [2]School of Medicine, University of California, San Diego, CA 92093

Cell lineage relationships in the differentiation of specific tissues have been difficult to approach in mammalian systems. By targeting expression of an oncogene in transgenic mice using regulatory domains of tissue-specific genes expressed at different stages of development, we have immortalized specific pituitary and hypothalamic cells which maintain differentiated functions which reflect sequential stages of development. For pituitary cells, SV40 T antigen (Tag) expression was driven by the regulatory regions of the α- or β-subunit genes of luteinizing hormone (LH). Cell lines derived using the α-Tag hybrid gene express the α-subunit and respond to gonadotropin-releasing hormone (GnRH), but do not express β-subunit genes. In contrast, targeting expression with the LH β-subunit gene immortalized a more differentiated cell type which continues to express both the LH α- and β-subunit genes and responds to GnRH. Since the α-subunit gene is expressed quite early in development of the anterior pituitary, while the β-subunit mRNAs appear much later, targeting Tag expression to the early developing anterior pituitary with the α-subunit promoter has immortalized an early developmental precursor of the mature gonadotrope, while targeting with the β-subunit has immortalized a more differentiated cell in the gonadotrope lineage. Thus, T antigen has immortalized cells at different stages of development concurrent with the activation of transcription of the respective promoters used to drive its expression. These results demonstrate the potential for immortalizing cells at sequential stages of differentiation by targeting the expression of an oncogene with promoters from genes expressed at different stages of development in a cell lineage during organogenesis and provide a series of immortal cell lines representing the developmental lineage of the gonadotrope.

Immortalization by targeted oncogene expression has been less successful in neuronal cells than in other tissues. Though this approach has proved difficult, targeting with a gene which is very tightly expressed in a small population of neurons prior to the transition to the postmitotic state may be successful. We have produced specific tumors of GnRH-secreting neurons by introduction of a hybrid gene composed of the GnRH promoter coupled to the coding region for T antigen into transgenic mice. Clonal cell lines derived from these tumors express GnRH mRNA and secrete GnRH in the natural pulsatile pattern. Thus, by targeting oncogenesis to a specific, small population of neurons using the regulatory region of a gene which is expressed late in differentiation of that cell lineage, we have succeeded in immortalizing hypothalamic GnRH neurons which maintain many differentiated functions in culture. Immortal cells derived from spontaneous tumors which maintain specific differentiated phenotypes in culture have been invaluable in the study of molecular and cellular biology; however, the power to target immortalization provides cultured cell models of cells not previously available.

B 015 REGULATION OF THE INTERFERON SYSTEM BY IRF-1 AND IRF-2, Tadatsugu Taniguchi[1], Hisashi Harada[1], Jun Sakakibara[1], Nobumasa Watanabe[1], Hitomi Yamamoto[1],Nobuyuki Tanaka[1], Takashi Fujita[1], Luiz F. L. Reis[2], Jan Vilcek[2], [1]Institute for Molecular and Cellular Biology, Osaka University, JAPAN, [2]Department of Microbiology, New York University, Medical Center, USA

In the process of analyzing the mechanism of the IFN-ß gene expression, we have identified specific DNA motifs in the promoter region of the gene. One of the motifs correspond to the ubiquitous NFκB site, and the other is more specific to the IFN genes characterized particularly by the presence of the hexamer AAGTGA motif. We have found specific nuclear factors interacting with this motif, IRF-1 and IRF-2 molecules.

The functional role of IRF-1 and IRF-2 is getting clearer. There is ample evidence on the involvement of these nuclear factors in the IFN-ß gene induction. In fact, evidence has been provided that, following viral induction, IRF-1 is synthesized and modified to act on the IFN-ß promoter. We detect in the nuclear extract of NDV-infected mouse L929 cells a factor complex which involves IRF-1 and shown a higher affinity to the IFN-ß promoter than IRF-1 or IRF-2 (monomer). Our findings suggest that the complex indeed interacts with the promoter involving a larger DNA sequence element than either IRF-1 or IRF-2. Studies are underway to characterize further the nature of the complex. The functional role of IRF-1 in the IFN-ß gene expression has been assessed further using IRF-1 anti-sense RNA expression. As expected, IRF-1 anti-sense RNA expression inhibited the NDV- or Poly(rI): Poly(rC)-mediated IFN-ß gene induction, and this effect was reversed by IRF-1 cDNA expression. In addition, we have provided clear evidence for the involvement of IRF-1 in the regulation of the IFN-inducible MHC class I gene, by using this experimental approach.

Furthermore, we will provide evidence for the potential role of IRFs in other aspects of cellular responses.

B 016 INDUCTION OF THE HEAT SHOCK RESPONSE, Carl Wu, Joachim Clos, Gisele Giorgi, Sridhar Rabindran, and J. Timothy Westwood, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20892

The transcriptional regulation of eukaryotic heat shock genes is mediated by the activator protein heat shock factor (HSF), which binds with high affinity to heat shock regulatory elements upon stress induction. The binding of HSF is dependent on its conversion from an inactive monomer or small oligomer to a multimeric form. Cloned Drosophila and human HSF expressed in bacteria at nonshock temperatures yield constitutively active, multimeric HSF proteins, while expression in the homologous cells results in proper assembly of the inactive form. The homologous systems apparently possess specific inhibitors of HSF multimerization absent in E. coli. All eukaryotic HSFs examined have a conserved, N-terminal DNA-binding domain and an adjacent oligomerization domain composed of three conserved hydrophobic heptad repeats (leucine zipper motifs). A fourth, C-terminal zipper motif present in the Drosophila and mammalian HSF sequences may be involved in the formation of inactive HSF. Hence, the switch from the inactive HSF monomer or small oligomer to the active multimer could be dependent on the relative thermostability of intramolecular or intermolecular zipper interactions. Both inactive and active forms of HSF are localized by immunostaining in the cell nucleus. On polytene chromosomes, HSF undergoes redistribution from a general, nonspecific localization to the heat shock puff loci upon heat stress. In addition, over chromosomal 150 loci also display HSF localization, including loci coincident with key developmental genes. This result suggests a new role for HSF in the direct repression of cellular gene activity during heat shock.

Pathogenic and Therapeutic Mechanisms Involving Transcription

B 017 REGULATION OF MHC CLASS II GENES: PROMOTER OCCUPANCY IN VIVO, Laurie H. Glimcher and Catherine J. Kara, Department of Cancer Biology, Harvard School of Public Health, and Department of Medicine, Harvard Medical School.

The expression of the class II antigens of the major histocompatibility complex is tissue specific, developmentally regulated in cells of the B lineage, and cytokine inducible. DNA sequences termed X1, X2, and Y, upstream of human and mouse class II genes have been identified through transfection studies and transgenic analysis which are critical for the transcription of the class II genes. Furthermore, a number of transcription factors which bind to these promoter motifs have recently been isolated. In recent studies (Science 252:709, 1991) we have shown by in vivo footprinting that these elements were occupied only in cells that express class II genes, in spite of the presence of promoter binding proteins. In vivo analysis of mutant cell lines that exhibit coordinate loss of all class II MHC expression, including several lines from patients with the Bare Lymphocyte Syndrome, revealed two in vivo phenotypes. In one phenotype, the absence of promoter interactions in vivo suggested that some of these mutations may affect promoter accessibility and thus may involve chromatin structure. In cell lines representing the developmental pathway of B cells the use of in vivo footprinting demonstrated a transition from unoccupied to occupied to once again unoccupied class II promoters. Differences in promoter occupancy among the four murine class II genes in certain B cells suggest that this transition is not completely coordinate. Furthermore, factor binding was necessary but not sufficient for mRNA expression, suggesting that additional modification of bound factors may be required. Interferon-γ treatment led to increased promoter occupancy of the DRα and DRβ promoters at the same sites that are constitutively bound in mature B cells. These results demonstrate that changes in the transcription of the class II genes are associated with changes in factor binding at the promoter in vivo. Given the ubiquity of class II promoter binding proteins, these results suggest that throughout B cell development and upon cytokine stimulation the accessibility of class II promoter DNA is subject to regulation.

B 018 A COMMON THREAD IN CYTOPLASMIC SIGNALING PATHWAYS, Stuart L. Schreiber, Department of Chemistry, Harvard University, Cambridge, MA 02138.

The immunosuppressants FK506, rapamycin, and cyclosporin A (CsA) have proved to be illuminating probes of the signal transduction pathways that lead to T cell activation. FK506 and CsA both inhibit the T cell receptor-initiated signal that leads to the transcription of lymphokine genes, and the cellular effects of the two drugs are almost indistinguishable; the most prominent distinction between the effects of FK506 and CsA is that those of FK506 are antagonized by rapamycin, but those of CsA are not. A variety of data suggests that the biologically active forms of these drugs are complexes with protein receptors, known collectively as immunophilins; these fall into two classes, the CsA-binding proteins, or cyclophilins, and the FK506- and rapamycin-binding proteins, or FKBPs, which differ not only in ligand selectivity but also in sequence. We have found that the behavior of the CsA-cyclophilin and FK506-FKBP complexes in certain systems is quite distinct from the behavior of the separate partners. These observations lead to a biochemical rationale for some of the common effects of CsA and FK506.

The Nuclear Oncogenes

B 019 SERUM RESPONSE FACTOR: RELATIVES AND FRIENDS, Stephen Dalton, Roy Pollock and Richard Treisman, Transcription Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, UK.

Serum Response Factor (SRF) is a 67 kD phosphoprotein that binds to the Serum Response Element (SRE) found in the promoters of the c-fos and several other immediate-early genes (1). Recent work indicates that SRF acts to recruit to the SRE an accessory protein, p62/TCF; although p62/TCF alone does not bind the SRE, in the ternary complex it makes DNA contacts at the SRE left side (2). In Balb/C3T3 cells p62/TCF binding appears to link the c-fos SRE to the protein kinase C dependent signal transduction pathway (3).

As part of a search for SRF related DNA binding proteins that bind the c-fos SRE, we have isolated a set of three cDNAs that contain a common SRF-related DNA binding domain. These RSRF (Related to SRF) proteins neither dimerise with SRF nor bind the SRE in vitro, and we used an oligo-nucleotide selection strategy to determine their binding specificity. The potential genetic targets of these transcription factors will be discussed.

A genetic screen using yeast for the isolation of mammalian cDNAs that encode proteins that can interact with SRF at the c-fos SRE will be presented. A reporter strain was constructed that contains a chromosomal lacZ indicator gene placed under control of a UAS consisting of a c-fos SRE derivative. Transformation of this strain with plasmids encoding SRF showed that full length SRF activated the indicator only 4 fold in the presence of galactose, while an SRF-VP16 fusion showed a galactose-dependent induction of more than 100-fold. A library of randomly primed cDNAs from HeLa cell mRNA was constructed in a vector that expresses the cDNAs as galatose-inducible rusions with the potent acidic transcription activation domain of the HSV VP16 protein. This library was transformed into the indicator strain and colonies that activated the reporter gene in the presence of galactose identified by β-galactosidase color assay. cDNAs were recovered that activated the reporter gene in both an SRF-dependent and -independent fashion. The structure and properties of the encoded proteins will be discussed.

1. Treisman,R. (1990) Seminars in Cancer Biology 1, 47.
2. Shaw,P. et al. (1989) Cell 56, 563.
3. Graham,R. and Gillman,M.Z. (1991) Science 251, 189.

Cell Type Specification and Tissue Type-Specific Transcription; Transcriptional Control and Transposition

B 100 A ROLE FOR CREB/ATF PROTEINS IN TISSUE-SPECIFIC GENE REGULATION IN DROSOPHILA. Ted Abel and Tom Maniatis, BMB, Harvard University, Cambridge MA 02138. Expression of the *Drosophila alcohol dehydrogenase gene* (*Adh*) in adults and larvae is regulated by fat body-specific enhancers. The *Drosophila mulleri* larval fat body enhancer, Box B, is a 90 bp regulatory element located about 200 bp upstream of the *Adh-1* gene. Box B drives expression of the *Adh-1* gene in larvae and it acts as a fat body-specific enhancer when linked to a heterologous promoter (Fischer and Maniatis, 1988, Cell, 53:451).

To investigate the molecular mechanisms responsible for the tissue specificity of Box B, we have identified and characterized factors that interact with this enhancer. By screening bacteriophage λ expression cDNA libraries for cDNA clones encoding proteins that bind specifically to Box B, we have cloned a transcription factor, Box B Binding Factor-2 (BBF-2), which binds specifically to Box B. Remarkably, BBF-2 also binds to three other *Drosophila* fat body-specific enhancers, and to sequences required for the liver-specific expression of the human *Adh* and rat tyrosine amino transferase genes. Thus, BBF-2 may play a role in the coordinate regulation of gene expression in the *Drosophila* fat body.

DNA sequence analysis of cDNA clones encoding BBF-2 indicates that this protein is a member of the CREB/ATF family of transcriptional regulatory proteins. Studies have shown that a BBF-2 binding site is essential for fat body expression in transgenic flies, and we have shown that BBF-2 is a transcription activator in *Drosophila* tissue culture cells. Previous studies have implicated a mammalian CREB/ATF protein in liver-specific expression in mammals. Thus, we propose that a role for CREB/ATF proteins in tissue-specific gene expression has been conserved between *Drosophila* and man. Work is in progress to explore further the role of BBF-2 in the temporal and tissue-specific regulation of *Drosophila Adh* genes.

B 101 CLONING OF TRANSCRIPTION FACTOR RκB, A NOVEL DNA BINDING PROTEIN WHICH RECOGNIZES THE IL-2 RECEPTOR α CHAIN ENHANCER κB SITE. B. Adams, K. Leung, and G. J. Nabel, Departments of Pediatrics and Internal Medicine, University of Michigan, Ann Arbor, MI 48109.

Proteins which bind to conserved *cis*-acting elements found in promoter and enhancer regions may regulate differential gene expression in several ways. Unique combinations of common sites, as well as cell-specific transcription factors which bind common sites, may direct differential gene expression. RNA processing events or post-translational modification may also alter the activity of a transcription factor in a cell-type specific manner. We have recently isolated a cDNA encoding a tissue-specific DNA-binding protein, designated RκB, which may use several of these mechanisms to regulate differential expression of the interleukin-2 receptor α chain (IL-2Rα). RκB is primarily expressed in lymphoid cells, brain, and testis. It preferentially recognizes a conserved element in the IL-2Rα enhancer which is a closely-related variant of the 11 base pair κB site found in regulatory regions of the immunoglobulin (Ig) κ light chain, the human immunodeficiency virus (HIV), and several other cellular and viral genes. Although RκB can bind to both IL-2Rα and Ig/HIV κB sites, transient transfection assays suggests that overexpression of RκB in Jurkat T leukemia cells specifically stimulates the IL-2Rα enhancer but not the HIV enhancer. Two clones encoding different carboxy-terminal domains have been isolated: RκB activation function may reside in a glutamine-rich region encoded by one of the two alternatively spliced RκB messages, while the predicted amino acid sequence of the other protein product has no recognizable activation domain at the carboxyl terminus. The pattern of terminal exon use in RκB corresponds to the cell-specific and developmentally-restricted pattern of IL-2Rα expression. These studies indicate that several regulatory mechanisms may contribute to gene-specific transcriptional activation by RκB.

B 102 STRUCTURE AND EXPRESSION OF THE fur GENE, **Torik A.Y. Ayoubi, John W.M. Creemers, Anton J.M. Roebroek and Wim J.M. Van de Ven, Laboratory for Molecular Oncology, Center for Human Genetics, University of Leuven, Herestraat 49, B-3000 Leuven, Belgium.**

Furin, the translational product of the recently discovered *fur* gene, appears to be a new member of the subtilisin family of serine proteases and the first known mammalian proprotein processing enzyme with cleavage selectivity for paired basic amino acid residues. Structurally and functionally, it resembles the prohormone processing enzyme KEX2 of yeast *Saccharomyces cerevisiae*. Furin is expressed in a variety of tissues, if not all, and is most likely involved in the processing of proproteins that are secreted via the constitutive secretory pathway.

The topography of the human *fur* gene is established and the gene consists of 16 exons. The coding sequences are dispersed over 15 exons, starting in exon 2 and ending in exon 16. The intron/exon distribution is of the same degree of complexity as observed in the trypsin family of serine proteases. A putative "TATA" box was found 32 nucleotides upstream of a potential cap site. Alignment of human and mouse *fur* cDNA sequences revealed remarkable differences in the 5' untranslated regions. Alignment of the 5' non-coding sequences of mouse *fur* with the human *fur* genomic sequence revealed that these corresponded to a region in intron 1. Furthermore, the 5' untranslated region of a *fur* cDNA clone isolated from rat liver appeared to align with yet another region in human intron 1. We have tentatively designated these alternative *fur* exon sequences in mouse and rat as exon 1A and exon 1B, respectively. The results point towards the presence of alternative promoters, although the possibility of a single promoter and alternative splicing can not yet be fully excluded. In this context, the reported strong elevation of *fur* gene expression in a significant percentage of non-small cell lung carcinomas is of special interest.

B 103 TISSUE-SPECIFIC, DEVELOPMENTAL, HORMONAL AND DIETARY REGULATION OF RAT PHOSPHOENOLPYRUVATE CARBOXYKINASE-HUMAN GROWTH HORMONE CHIMERIC GENES IN TRANSGENIC MICE, Elmus Beale, Mary Short, David Clouthier, Ida Schaefer, Mark Magnuson, and Robert Hammer, Dept. of Cell Biology and Anatomy, Texas Tech Univ. Health Sciences Center, Lubbock, TX, 79430; Dept. of Biochemistry and the Howard Hughes Medical Institute, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235; and Dept. of Molecular Physiology and Biophysics, Vanderbilt Univ. School of Medicine, Nashville, TN, 37232

The cytosolic phosphoenolpyruvate carboxykinase (PEPCK) gene is expressed in multiple tissues and it is regulated in a complex tissue-specific manner. To map the cis-acting DNA elements that direct this tissue-specific expression we made transgenic mice containing truncated PEPCK-human growth hormone (hGH) chimeric genes. The transgenes contained PEPCK promoter fragments with 5' endpoints at -2088, -888, -600, -402, and -207 bp, while the 3' endpoint was at +69 bp. Immunohistochemical analysis showed that the -2088 transgene was expressed in the correct cell types (hepatocytes, proximal tubular epithelium of the kidney, villar epithelium of the small intestine, epithelium of the colon, smooth muscle of the vagina and lungs, ductal epithelium of the sublingual gland, and white and brown adipocytes). Solution hybridization of hGH mRNA expressed from the transgenes indicated that white and brown fat-, skeletal muscle- and heart-specific elements are located distally (-2088 to -888 bp) and liver, gut and kidney specific elements are located proximally (-600 to +69 bp). However, elements outside of the region tested are necessary for the correct developmental pattern and level of PEPCK expression in kidney. Both the -2088 and -402 transgenes responded in a tissue-specific manner to dietary stimuli and the -2088 transgene responded to glucocorticoid stimuli. Thus different tissues utilize distinct cell-specific *cis*-acting elements to direct and regulate the PEPCK gene.

B 104 A TPA-INDUCIBLE, POU-DOMAIN CONTAINING TRANSCRIPTION FACTOR FROM T-CELLS.

Bhargava,A.K., Reddy,P.S*., Charles,J.F., and Weissman, S.M., Department of Genetics, Yale University School of Medicine, New Haven, CT 06510; *Centre for Cancer Research, Massachusetts Institute of Technology, MA 02139.

POU-domain containing transcription factors have been shown to regulate both ubiquitous and cell-type specific genes. We have identified a POU-domain containing transcription factor from the T cell-line, JURKAT, that is induced with TPA (12-O-Tetradecanoylphorbol 13-acetate). Northern blot analysis of Jurkat cell-line RNA has shown a transcript of about 5 kb induced after 12 hrs of TPA treatment. This transcript is also expressed constitutively in the B cell-line, JY. Sequence analysis of a 3.2 kb partial cDNA clone has revealed a glycine/alanine rich region amino terminal to the POU-domain.

TPA is used *in vitro* as a co-stimulus in the activation of T cells, resulting in the induction of the interleukin-2 (IL-2) gene as early as 45 minutes. Bacterially expressed POU-domain protein binds to the distal octamer sequence (ATGCAATTAA) of the IL-2 gene enhancer. We are presently evaluating the role of this POU-domain containing transcription factor in the regulation of the IL-2 gene.

B 105 INTERACTION CLONING: ISOLATION OF A cDNA ENCODING FIP, A BASIC-HLH-ZIP PROTEIN THAT INTERACTS WITH cFOS, Michael A. Blanar and William J. Rutter, Hormone Research Institute and Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0534

To facilitate the isolation of genes encoding proteins that interact with other proteins, we have developed a facile method for producing a polypeptide probe that can be used for detection. The protein probe consists of a modified polypeptide containing a short amino-terminal extension in which resides recognition sites for a monoclonal antibody, a specific endopeptidase, and a site-specific protein kinase. In this study, the protein probe also includes the basic-Zip dimerization motif of cFos (cFos 'core'). cDNA clones, encoding proteins capable of specific interaction with the cFos 'core' probe, were isolated and characterized. In addition to cDNAs encoding Jun family members, shown previously to dimerize with cFos, a cDNA clone encoding a novel member of the basic-HLH-Zip family of proteins was also isolated. This cDNA encodes a polypeptide, FIP (fos interacting protein), that exhibits specific binding to oligonucleotide probes containing various HLH protein DNA binding motifs. Deletion of the carboxy-terminal Zip region abolishes the ability of FIP to bind DNA. In contrast to the other members of this family, FIP is capable of forming stable heteromeric complexes with cFos. This interaction suggests a connection between a member of the bHLH-zip family with members of the AP1 family of transcription factors. Such interfamily interactions dramatically increase combinatorial regulatory possibilities.

B 106 HNF-1 AND C/EBP ARE REQUIRED FOR THE ACTIVITY OF THE HUMAN APOLIPOPROTEIN B GENE SECOND INTRON ENHANCER, Alan R. Brooks and Beatriz Levy-Wilson, Gladstone Foundation Laboratories for Cardiovascular Disease, Cardiovascular Research Institute, University of California, San Francisco, P.O. Box 40608, San Francisco, CA 94140-0608

The tissue-specific transcriptional enhancer of the human apolipoprotein B gene contains multiple protein binding sites spanning 718 base pairs. Most of the enhancer activity is found in a 443-bp fragment (+622 to +1064) that is located entirely within the second intron of the gene. Within this fragment, a 147-bp region (+806 to +952) containing a single 97-bp DNaseI footprint exhibits significant enhancer activity, and comprises the binding sites for at least eight distinct nuclear proteins. One of these proteins was identified as HNF-1, which binds with relatively low affinity to the 5' half of a 20-bp palindrome located at the 5' end of the large footprint. A binding site for C/EBP (or one of the related proteins that recognize similar sequences) was identified in the center of the 97-bp footprint. This binding site is coincident or overlaps with the binding sites for three other proteins, one of which is heat-stable. Binding sites for two other proteins, one of which is heat-stable, are located within the central 50 bp of the footprint, but are distinct from the C/EBP binding site. Finally, the 3'-most 15 bp of the footprinted sequence contain a binding site for another nuclear protein. Mutations that abolish the binding of either HNF-1 or the four proteins that recognize the C/EBP site severely reduce enhancer activity. However, deletion experiments demonstrated that neither the HNF-1 binding site alone, the C/EBP binding site alone, nor both of them in combination are sufficient for the enhancer activity. Rather, HNF-1 and C/EBP act synergistically with the three other DNA-binding proteins in this region to enhance transcription of the apolipoprotein B gene.

B 107 HUMAN ALCOHOL DEHYDROGENASE GENE EXPRESSION: TISSUE SPECIFICITY. Celeste J. Brown, Lu Zhang, Howard J. Edenberg. Dept. of Biochemistry & Molecular Biology, Indiana Univ. School of Medicine, Indianapolis, IN 46202-5122 USA

The three human class I ADH genes, *ADH1, ADH2* and *ADH3*, are expressed at high levels in adult liver, but at varying levels or not at all in other tissues. We are analyzing the transcriptional control of these closely related genes by ascertaining differences in the binding of *trans*-acting factors from various tissue and cell nuclear extracts. We have mapped, by DNase I footprinting, a complex set of *cis*-acting sequences extending about 350 to 450 nt upstream of the transcriptional start sites of these three genes. We have found interesting differences both between extracts and between the different genes in a given extract.

Liver and kidney extracts produce footprints in all three genes in the region corresponding to -206 to -182 of *ADH2*. There are, however, large differences in the sizes of the protected areas in the different genes. This region contains sequences similar to the Hepatocyte Nuclear Factor 1 (HNF1) binding sites of the rat α- and β-fibrinogen genes.

Two regions in *ADH2* and *ADH3* are footprinted by purified C/EBP, however only the proximal site in *ADH1* is footprinted. This proximal C/EBP site of *ADH1* differs from the site in *ADH3*, in that the *ADH1* site is bound by proteins in all tissue extracts, whereas the analogous site in *ADH3* is bound only by the liver extract. A CACGTG site has been shown to be important for transcription of *ADH2*; it closely matches the binding site of the Adenovirus USF/MLTF factor. The *ADH3* site matches exactly, but a single nucleotide difference in *ADH1* prevents the site from being footprinted by the liver, kidney and spleen extracts tested.

These data reveal interesting differences in the regulation of the closely related human ADH genes.

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B 108 IDENTIFICATION OF 5' ENHANCER ELEMENTS IN THE MURINE IL-4 GENE Melissa A. Brown, David Tara and Wendy Pesterfield. Departments of Medicine and Microbiology-Immunology, Oregon Health Sciences University, Portland, Oregon 97201

Interleukin 4 (IL-4) is a multipotent cytokine whose expression in murine cells is limited to a subset of activated T cells, IL-3 dependent mast cells and some transformed mast cell lines. Previous studies in our laboratory have demonstrated that -797 bp 5' of the IL-4 transcription initiation site is sufficient to promote transcription of a CAT reporter gene in PMA stimulated T cells but is not active in inducible or constitutive IL-4 producing mast cell lines. Transfection of 5' deletion CAT reporter gene constructs of -797 into stimulated EL-4 cells showed that -87 bp is sufficient to promote CAT expression. Suprisingly, deletions of -442, -305, -231, -115 and -87 were active in ionomycin stimulated CFTL 12 mast cells indicating the presence of mast cell specific inhibitor sequences in this region. Sequences between -6300 and -797 enhanced IL-4 promoter driven CAT expression by 5-10 fold in inducible cell lines. To better define the sequences in this 5' region which regulate IL-4 gene transcription, the effect of deletions between -6300 and -797 on IL-4 promoter driven CAT expression was determined in transiently transfected PMA stimulated EL-4 T cells and ionomycin stimulated CFTL 12 mast cells. Deletion of sequences between -3.8 kb and -2.4 kb significantly reduced CAT activity in PMA stimulated EL-4 cells. This deletion had no effect on CAT activity in transfected CFTL 12 cells stimulated with ionomycin. These data suggest that a sequence between -3.8 and -2.4 kb can enhance IL-4 gene transcription in EL-4 T cells but not CFTL 12 mast cells and confirm our previous conclusions that different *cis* acting elements regulate IL-4 gene expression in T and mast cells.

B 109 **EXTINCTION OF α1-ANTITRYPSIN GENE EXPRESSION IN SOMATIC CELL HYBRIDS: EVIDENCE FOR MULTIPLE CONTROL,** **Gary A. Bulla and R.E. Keith Fournier,** Department of Molecular Medicine, Fred Huthinson Cancer Research Center, Seattle, WA 98104

Extinction is defined as the loss of tissue specific gene expression when cells originating from different tissue are fused. As a model to study mechanisms directing this repression, we focused on the liver specific alpha-1 antitrypsin (α1-AT) gene. Expression of the α1-AT gene was extinguished in hepatoma X fibroblast hybrids. The wild-type promoter was active in hepatoma cells but 100-fold less active in fibroblasts or hybrid cells. Using a 5' promoter deletion panel as well as point mutations in the α1-AT promoter, we were unable to functionally separate sequences involved in extinction from those required for activation.

Trans-acting factors LF-A1 and LF-B1 are both involved in driving the α1-AT gene. We found that point mutations which preclude binding of LF-A1 have a modest (5-10 fold) effect on promoter activity in the rat hepatoma cells, but that LF-B1 binding mutants resulted in a 100-fold decrease in promoter strength. Gel shift assays and Northern analysis show that LF-B1 is undetectable in either fibroblast parents or the cell hybrids, consistent with a model whereby extinction is mediated by the loss of *trans*-acting factors. To test this hypothesis, we stably transfected an LF-B1 expression cassette into parental and hybrid cells and monitored expression of both transfected and endogenous α1-AT genes. Although expression of cloned LF-B1 activated expression of the transfected α1-AT promoter 40-60 fold, it neither prevented nor reversed extinction of the chromosomal α1-AT gene. We conclude that although α1-AT extinction is accompanied by loss of LF-B1 expression in the hybrid cells, other mechanisms play a dominant role in extinction of the α1-AT gene.

B 110 **LIVER-SPECIFIC EXPRESSION OF RABBIT CYTOCHROME P4502C SUBFAMILY GENES IS REGULATED BY AN HNF-4 LIKE FACTOR**

David Chen, Pratap Venepally and Byron Kemper, Dept. of Physiology, University of Illinois, Urbana, IL 61801

CytochromeP450 genes encode enzymes that are crucial for eliminating exogenous toxic compounds and metabolizing chemical carcinogens. Thirty one genes in CYP450 family 2 have been cloned. Almost all of these genes are either exclusively or predominantly expressed in liver, but the mechanisms of this tissue-specific regulation are poorly understood, due to the lack of suitable cell lines to express these genes. By using a low background firefly luciferase vector to transfect HepG2 cells (hepatic) and COS cells (non-hepatic), expression was for the first time obtained in HepG2 cells when the reporter gene was fused to various lengths of 5'-flanking regions from three rabbit P4502C subfamily genes, CYP4502C1, 2C2 and 2C3. The highest expression level in HepG2 cells was 15% of that of a RSV luciferase control, while compared to only 3-6% in COS cells. Deletion of regions from -135 to -63 of C2, -126 to -68 of C1 and -125 to -59 of C3 resulted in a 6 fold decrease in expression in HepG2 cells, but not in COS cells. The gel mobility shift assay was used to show that a nuclear protein from HepG2, but not COS cells bound to the deleted sequences. The binding affinity to C2 sequence was the highest and to C3 was the lowest, which was correlated well with the gene expression levels in HepG2 cells. The binding was abolished when an oligonucleotide with a sequence similar to the hepatocyte nuclear factor 4 (HNF4) binding site was used as a competitor. These results suggest that HNF4, by interacting with a conserved cis-acting element around -100, regulates the liver-specific expression of the CYP4502C1, 2C2 and 2C3 genes. By comparing the sequences, similar HNF-4 like elements were also found in more than 20 genes of subfamilies 2A, 2C and 2D, which implies that this factor may also mediates the liver-specific expression of these genes.

B 111 TISSUE AND DIFFERENTIATION SPECIFIC EXPRESSION OF MULTIPLE HUMAN aFGF TRANSCRIPTS CONTAINING DIFFERENT UPSTREAM NONTRANSLATED EXONS, Ing-Ming Chiu,[1] René L. Myers,[1] Robert A. Payson,[1] Wen-Pin Wang,[1] and Larry L. Deaven[2] Department of Internal Medicine,[1] The Ohio State University, Columbus, OH 43210 and Life Sciences Division,[2] Los Alamos National Laboratory, Los Alamos, New Mexico 87545

We have previously reported the isolation of cDNA clones coding for human acidic fibroblast growth factor (aFGF) containing alternative 5' nontranslated exons. Using RNase protection analyses with riboprobes derived from three aFGF cDNA clones isolated in our laboratory, we were able to demonstrate the presence of at least four upstream, nontranslated exons which are alternatively spliced to the first protein-coding exon. Expression of these transcripts is regulated in a tissue specific manner, as the major aFGF transcript in human brain differs from that in kidney. Furthermore, the pattern of aFGF transcripts in most glioblastoma cell lines tested is different from that in normal brain tissue, suggesting that differential transcription of the aFGF gene may be important in the etiology and/or progression of human glioblastomas. Overlapping genomic clones containing three of the upstream, nontranslated exons have been isolated. They are shown to be contiguous with our previously isolated genomic clones that contain the three aFGF coding exons. Together, 130 kbp of the aFGF locus were isolated. The exon boundaries of the most 5' exon have been mapped by comparing its nucleotide sequence with the cDNA sequence and using RNase protection analysis. The canonical CCAAT and TATA sequences are located at the proper distances from the transcription start site as determined by RNase protection analysis. Because the aFGF transcripts are distributed in a tissue-specific manner, there must be some functional significance for this alternative usage of upstream nontranslated exons. It is most likely that the different aFGF transcripts result from multiple initiation sites controlled by distinct promoter elements. However, we have not ruled out the possibility of a single promoter such that the expression of multiple transcripts is controlled by tissue-specific splicing. The transcripts detected in several glioblastoma cell lines are not detected in normal brain and kidney. It is therefore possible that there is differentiation or developmentally regulated expression of the aFGF gene. Development or differentiation specific transcription factors may be present in these transformed cells which are not expressed in the adult brain. Characterization of the aFGF promoters may lead to the elucidation of important processes involved in development, differentiation and oncogenesis.

B 112 CHROMATIN STRUCTURE AND REGULATION OF THE IL3/GM-CSF LOCUS, Peter Cockerill, Frances Shannon and Mathew Vadas, Immunology, IMVS, Adelaide, Australia 5000

Haemopoietic growth factors regulate the survival, proliferation, differentiation and function of haemopoietic cells. The genes for several of these factors are clustered on human chromosome 5. This study concerns the interleukin 3 (IL3) and granulocyte-macrophage colony stimulating factor (GM-CSF) genes which are separated by just 10 kb of DNA within this cluster. These two genes appear to be co-expressed upon activation of most human T cells and T cell lines suggesting that they may share some regulatory elements. Most other cell types, however, can be induced to express GM-CSF but not IL3 indicating that distinct regulatory pathways also exist. While the proximal promoters of these genes have been extensively studied, no distal acting regulatory elements have yet been found. To identify distal regulatory elements such as enhancers or locus control regions we have used the approach of first mapping DNaseI-hypersensitive sites as this approach has often provided the first evidence for the existence of such elements. To date we have mapped 3 sites upstream of IL3 and 6 sites between IL3 and GM-CSF in studies extending from 30 kb upstream of IL3 to 10 kb downstream of GM-CSF. The sites upstream of IL3 are constitutive but are largely restricted to T cells and one of these sites lies within the promoter. These sites may therefore be involved in the tissue-specific expression of IL3. A cluster of three constitutive sites detected in all cell types lies between the two genes just downstream of IL3. A constitutive site 4 kb upstream of the GM-CSF gene was detected only in monocytic cells , and so this site could be involved in the constitutive expression of this gene in this cell type. One inducible site 3 kb upstream of GM-CSF appeared in all cell types upon treatment with phorbol ester and calcium ionophore. These conditions also induce expression of the endogenous GM-CSF gene. Significantly, we have obtained preliminary evidence from transient transcription assays using the same activation conditions suggesting that an inducible enhancer element lies upstream of the GM-CSF promoter. The GM-CSF promoter also often appears as a hypersensitive site which in at least some cases can be induced by interleukin 1. Continuing studies aim to determine the functions of all of these DNaseI-hypersensitive sites.

B 113 LINEAGE AND STAGE SPECIFIC EXPRESSION OF HUMAN HOX 1 AND HOX 2 GENES IN THE HEMATOPOIETIC COMPARTMENT. Isabelle Vieille-Grosjean, Valérie Roullot and Gilles Courtois. Laboratoire d'hématologie, INSERM U 217, DBMS CEN G 85X, 38041 Grenoble Cedex, France.

The molecular basis of commitment and differentiation of hematopoietic cells remain poorly understood. Among putative candidates involved in these processes are homeoproteins, a large family of transcription factors which play a major role during development. Using a strategy based on the polymerase chain reaction (PCR) we have isolated nine different Antennapedia-like homeobox (HOX) genes from purified human hematopoietic precursors. Their expression patterns, analyzed with a panel of leukemia-derived cell lines representing various blood phenotypes, appears to be lineage-restricted and for several of them modulated during differentiation. Extending our study to all the known members of the HOX 1 and HOX 2 clusters we have demonstrated that at least ten HOX 1 and seven HOX 2 genes are differentially expressed in the hematopoietic compartment. Interestingly HOX 1 genes are predominantly detected within cells of myelomonocytic origin whereas HOX 2 genes transcripts are more abundant in erythro-megakaryocytic cell lines. These observations support the notion that homeoproteins could be regulators of lineage determination during hematopoiesis.

B 114 CORRELATION OF MHC CLASS II (I-A) PROMOTER ACTIVATION WITH AN ALTERED FORM OF THE CCAAT BOX TRANSCRIPTION FACTOR, NF-Y, R. Alexander Currie, Depts. of Medicine; Biochem., Biophys, & Genetics, University of Colorado Health Sciences Center, Denver, CO 80262

All mouse and human major histocompatibility complex (MHC) class II (Ia) gene promoters contain a highly conserved DNA element, the Y-box, ~55 bp 5' of their transcriptional start sites, which specifically binds the ubiquitous nuclear CCAAT box factor, NF-Y. NF-Y is known to play an important functional role in the transcriptional activation of Ia genes during B cell development, and in antigen presenting macrophages during stimulation by interferon-γ (INF-γ). The ion exchange properties of NF-Y have been re-examined as a function of mouse and human B cell development, mouse macrophage induction by INF-γ, in a series of human Bare Lymphocyte Syndrome (BLS) B cells, and in a mutant human Burkitts lymphoma, RJ225. NF-Y derived from non-lymphoid HeLa cells separates on cation (P-11, mono S), and anion exchange (mono Q) columns into 2 fractions which must be recombined to reconstitute specific DNA binding. Analysis of NF-Y from a mature mouse lymphoma, A20, and several human Burkitts lymphomas (Raji, Daudi) on mono Q and DEAE columns demonstrates that NF-Y binds as an intact heterodimer with high affinity for anion exchange matrices (NF-Y*). In contrast, NF-Y from a mouse plasmacytoma, MPC 11, a human BLS lymphoma, BL-2, and the mutant Raji cell line, RJ225, dissociate on mono Q into 2 subunits. Stimulation of the mouse monocyte, P338D1, with IFN-γ causes an ~5 fold increase in the level of NF-Y* binding to mono Q. These results suggest that: 1.) cell lines which actively transcribe Ia genes contain a highly acidic form of NF-Y which does not dissociate over anion exchange columns, 2.) a BLS Ia^- cell line (BL-2) contains a form of NF-Y which does not bind to mono Q, 3.) NF-Y from the Ia^- mutant Raji cell line, RJ225, has an identical mono Q elution pattern as BL-2. Together, these results suggest that NF-Y undergoes a post-translational modification which correlates with the activation of Ia gene expression during lymphocyte development, and macrophage stimulation by INF-γ. Several mutant human lymphocytes which do not actively transcribe Ia genes appear to contain a form of NF-Y which behaves in a manner identical to non-lymphoid HeLa cells. These results suggest that a defect in NF-Y itself, or in the signaling pathway(s) which normally modifies NF-Y, is defective in these mutant B cells, and that this alteration may contribute to the lack of Ia gene expression.

B 115 MHC CLASS I GENE EXPRESSION COINCIDES OCCUPATION OF UPSTREAM REGULATORY SITES *IN VIVO* ,Anup Dey, Angela Thornton, Mathew Lonergan,*John Chamberlain,+ Sherman Weissman and Keiko Ozato, Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, Bethesda, MD 20895,* The Hospital for Sick Children, Toranto, Ontario, Canada +Yale University, School of Medicine, New Haven, CT 06510.

We have addressed the role of MHC class I upstream sequences in transcriptional regulation by *in vivo* footprinting.Human HLAB7 transgene and endogenous $H\text{-}2K^b$ genes have been analysed.The results reveal that in the spleen, which expresses MHC class I genes at a high level, there are three major regions which show prominent constitutive protection both for the mouse and human gene.In contrast,no protection was detected in brain where MHC class I genes are not expressed.Data show a correlation between expression of MHC class I genes and occupancy of regulatory sites *in vivo*. The *in vivo* occupied sites in spleen are,a)Enhancer(NFkB like)sequence b)Interferon consensus sequence and c) a novel site called 'site α',located approximately at -110 in HLAB7 and -100 in $H\text{-}2K^b$.Electrophoretic mobility shift assay demonstrates that both spleen and brain nuclear factors form complex with site α.This complex is specifically competed by oligonucleotides containing AP1 site or Region II, which binds to a member of nuclear hormone receptor family H-2RIIBP.A monoclonal antibody raised against H-2RIIBP,specifically but weakly precipitates the complex between H-2RIIBP and site α oligonuceotide.Transfection results provide evidence for the role of site α in the regulation of MHC class I gene expression.

B 116 THE *suppressor of Hairy-wing* ZINC FINGER PROTEIN OF *DROSOPHILA MELANOGASTER* POTENTIATES UPSTREAM POLY(A) SITES AND INTERFERES WITH INTERACTIONS BETWEEN TRANSCRIPTION CONTROL ELEMENTS, Dale Dorsett, Christina Holdridge, Jaeseob Kim and Joseph Jack, Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Many mutations in *Drosophila* are insertions of the retroviral-like gypsy transposon. Phenotypes associated with most gypsy insertions are suppressed by mutations in *suppressor of Hairy-wing* [*su(Hw)*], which encodes a zinc finger protein (SUHW) that binds a DNA sequence repeated several times 0.2 kb downstream of the gypsy 5' LTR. SUHW and gypsy interfere with gene expression by at least two mechanisms. SUHW increases polyadenylation of transcripts in the gypsy 5' LTR when gypsy is in a transcribed region. SUHW also potentiates other poly(A) sites, and does not alter transcript levels in the absence of an upstream poly(A) site. We propose that it pauses transcription, giving more opportunity for a weak poly(A) site in a nascent transcript to be recognized and cleaved. Consistent with this idea, SUHW also potentiates the *CYC1* poly(A) site in yeast, even though it is very different from higher eukaryotic poly(A) sites. SUHW also interferes with interactions between transcription control elements when positioned between them. SUHW represses heat shock transcription from the *hsp70* promoter when bound between the sites (HSEs) binding heat shock transcription factor, or between the HSEs and transcription start site. It does not repress when bound just upstream of the HSEs. Furthermore, SUHW interferes with activation of *cut* gene expression by a wing margin specific transcription enhancer from several positions throughout the 80 kb region separating the enhancer and transcribed region. We propose that SUHW places constraints on DNA and/or chromatin structures, preventing conformations required to bring proteins bound to different control elements into contact with each other. We hypothesize that SUHW could protect gypsy from position effects, or a normal gene from the control elements or transcription of a neighboring gene.

B 117 ANALYSIS OF A CLASS I MAJORHISTOCOMPATIBILITY COMPLEX GENE PROMOTER IN AN IN VITRO TRANSCRIPTION SYSTEM, Paul H. Driggers, Brian A. Elenbaas, Insong J. Lee, and Keiko Ozato, Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, National Insititutes of Health, Bethesda, Md. 20892

Genes of the class I major histocompatibility complex (MHC) exhibit both tissue-specific and developmental regulation. Our laboratory has previously used an in vivo expression assay (CAT assay) and in vitro protein-DNA binding assays to define upstream regulatory elements controlling transcription of murine MHC class I genes. We have developed an in vitro transcription system which carries out accurate initiation of RNA sysnthesis from a class I promoter fused to the G-free cassette. With B cell nuclear extracts we have observed that the upstream class I regulatory complex (CRC) that had been previously shown to function as a constitutive enhancer in an in vivo assay also enhances transcription in vitro in an orientation-independent fashion.In addition, another element which confers the strongest transcriptional enhancement in the in vitro system has been identified. This element was localized to a 25 base pair sequence between the CRC and the basal promoter. Nuclear factors that bind to this element have been characterized in detail by electrophoretic mobility shift assays and methylation interference experiments. We present evidence that this binding activity may belong to the CTF/NF1 family of transcription factors.

B 118 PHYLOGENETIC SPECIFICITY OF PROLACTIN GENE EXPRESSION WITH CONSERVATION OF PIT-1 FUNCTION, Harry Elsholtz, Sonali Majumdar-Sonnylal, Fei Xiong, Zhi Yuan Gong and Choy Hew, Departments of Clinical Biochemistry and Biochemistry, University of Toronto, Toronto, Canada

In mammals, the pituitary POU homeodomain protein, Pit-1, binds to proximal and distal 5'-flanking sequences of the prolactin (PRL) gene that dictate tissue-specific expression. These sequences are highly conserved among mammals but are dramatically different from PRL 5' sequences in the teleost species, *Oncorhynchus tschawytscha* (Chinook salmon). To analyze the molecular basis for pituitary-specific gene expression in a distantly related vertebrate, we transfected CAT reporter gene constructs containing 2.4 kb of 5'-flanking sequence from the salmon PRL gene into various cell types. Expression of the salmon PRL gene was restricted to pituitary cells, but in *rat* pituitary GH4 cells levels of expression were 40 to 100 fold lower than those obtained with a rat PRL (-3 kb) construct. Conversely, in primary *teleost* pituitary cells, -2.4 sPRL/ CAT was expressed at levels 3 to 10 fold higher than -3 rPRL/CAT. To determine whether species-specific transactivation by Pit-1 was sufficient to explain these species differences in PRL gene expression, we isolated a cDNA clone encoding the salmon Pit-1 POU domain and constructed a rat Pit-1 expression vector that contained salmon Pit-1 POU domain sequences substituted in frame. The chimeric Pit-1 encoded 13 amino acids unique to salmon. Co-expression of rat Pit-1 with salmon or rat PRL/CAT in transfected HeLa cells resulted in specific and strikingly comparable levels of promoter activation. Moreover, the specificity and efficacy of the chimeric salmon/rat Pit-1 was similar to wild-type rat Pit-1 in activating salmon and rat PRL/CAT. Our data suggest that the structure and function of Pit-1 are highly conserved among vertebrate classes, and that Pit-1 is not the primary determinant of the marked species-dependent expression of PRL genes observed in mammalian and teleost lactotrophs. The dramatic divergence of 5'-flanking sequences of mammalian and teleost PRL genes further suggests that conservation of Pit-1 function is not strictly limited by evolutionary flexibility within genomic regulatory regions.

B 119 DEVELOPMENTAL AND TISSUE-SPECIFIC EXPRESSION OF HUMAN TRANSFERRIN IN TRANSGENIC MICE. Kathryn Fischbach, Yan Lu, Laura A. Cox, Barbara H. Bowman and Gwen S. Adrian. University of Texas Health Science Center, San Antonio TX 78284-7762.

Transferrin (TF), the major iron-transport protein in blood plasma and cerebrospinal fluid, is synthesized in liver and brain and is essential for the appropriate tissue-distribution of iron. Our studies in transgenic mice carrying chimeric human TF-chloramphenicol (CAT) genes have revealed distinctive tissue-specific and developmental expression of the transgenes. Analyses of expression in transgenic mice of TF-CAT genes containing different lengths of TF 5' regulatory regions demonstrate that sequences between -622 and -307 bp direct tissue-specific expression. DNA-protein binding studies indicate that this regulation may be conveyed by an element between -474 to -454 bp that has homology to an HNF-3 binding site. The human glioma cell line HTB-16 possesses characteristics of oligodendrocytes, a brain cell type that synthesizes TF. Transient transfections of HTB-16 and Hep3B with TF-CAT plasmids present evidence for both liver-specific and brain-specific elements between -4.0 and -3.3 kb of the TF gene. Sequence analysis of this region revealed the presence of multiple potential liver and brain regulatory elements, such as binding sites for HNF-1, HNF-3 and AP-2.

Using several homozygous founder lines of transgenic mice carrying 0.67 and 1.2 kb of the 5' regulatory region of TF, we demonstrate a different developmental onset of expression in brain than in liver. In brain, developmental expression of these human TF transgenes parallels expression of the mouse TF gene, indicating that sequences required for proper developmental expression in brain reside between -622 and +46 bp of the human TF gene. In contrast, liver studies reveal a different pattern when human TF is compared to the mouse TF gene. The human TF transgenes are turned on between day 10 and day 20 while the endogenous gene already shows considerable expression by gestation day 18. The species difference in liver developmental expression may be because the human gene is actually turned on at a later developmental stage than the mouse gene or may indicate the absence of some region in these transgenes that is required for fully appropriate developmental expression in liver. DNaseI footprinting using liver nuclear extracts from 7 day old mice, which do not express the transgene, or 27 day old mice, which do express the transgene, revealed protection of a single region, -474 to -454 bp, by the expressing extract only. This is the same region that appears to be involved in liver-specific expression of human TF. In summary, these studies have revealed regions of the human TF gene important for developmental expression and appropriate tissue-specific expression.

B 120 CELL TYPE AND TISSUE TYPE-SPECIFIC TRANSCRIPTIONAL REGULATION OF MTS1, Heide L. Ford, EugeneTulchinsky, Dimitri Kramerov, Sayeeda B. Zain, Dept. of Biochemistry, University of Rochester School of Medecine and Dentistry, Rochester, NY 14642
The gene mts1 has been isolated from both radiation induced rat thyroid tumors and a mouse mammary carcinoma cell line. Mts1 was found to code for an 101 amino acid protein belonging to the subfamily of S-100 related small Ca+2 binding proteins. The mRNA encoded by the mts1 gene is specifically expressed in metastatic tumors (IR6) and cell lines (CSML-100), but not in their nonmetastatic counterparts (IR4 and CSML-0). Mts1 also exhibits tissue type-specific transcription. Our lab is concerned with characterizing the DNA control elements of the mts1. CAT constructs using different regions of the mts1 gene demonstrate the existence of an enhancer element in the first intron of the mouse mts1 gene. The ability of these CAT constructs to express in CSML-0 indicates that there are other levels of transcriptional regulation of the mouse mts1 gene. In vitro and in vivo footprinting analysis of mts1have been conducted in both CSML-0 and CSML-100. In addition, the differences in the methylation pattern of mts1 in CSML-0, CSML-100, and various tissue types has been examined using Msp1/HpaII. Finally, the role of Ca+2 in the regulation of mts1 gene expression is being studied.

B 121 Characterization of A Cell Type-Specific Negative Regulatory Region of the Human GM-CSF Gene. John K. Fraser**, Juan J. Guerra*, Chi Y. Nguyen*,Stephen D. Nimer*, and Judith C. Gasson**. *Division of Hematology-Oncology,Department of Medicine and Jonsson Comprehensive Cancer Center and *Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024.

Human granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the proliferation and maturation of normal myeloid progenitor cells, and can also stimulate the growth of acute myelogenous leukemia (AML) blasts. GM-CSF is not normally produced by resting cells, but is expressed by activated T-lymphocytes, macrophages and certain cytokine-stimulated fibroblasts and endothelial cells. Production of GM-CSF by cultured AML cells has been demonstrated, raising the possibility that expression of this growth factor in vivo could be autostimulatory for certain myeloid leukemia cells. To investigate this possibility, we examined the regulatory activity of various regions of the GM-CSF promoter in leukemic cell lines by transient transfection. Our results demonstrate the presence of a strong constitutive promoter element contained within 53 bp upstream of the cap site that is active in myeloid leukemia blasts and other cells. This constitutive promoter activity requires a repeated CATT(A/T) motif. We also identify a cell type-specific negative regulatory element located immediately upstream of the positive regulatory element (within 69 bp of the cap site) that is active in AML cell lines but not T-cells. Taken together, these results suggest that a tissue-specific negative regulatory region silences the strong constitutive promoter activity of the GM-CSF gene in AML cell lines. Aberrant or induced expression of GM-CSF by AML cells could arise from perturbation of DNA-protein interactions in this negative regulatory region. We are currently employing gel mobility shift assays to test this hypothesis.

B 122 IDENTIFICATION OF AN ENHANCER THAT REGULATES MYOD1TRANSCRIPTION, David J. Goldhamer and Charles P. Emerson, Jr. Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pa. 19111. MyoD1 is a skeletal muscle-specific regulatory protein that is involved in the establishent and maintenance of myogenic lineages. In order to study cis-acting sequences that regulate MyoD1 expression, we have cloned the human MyoD1 gene along with approximately 24 kb of 5' sequence from a pWE15 human cosmid library. The MyoD1 promoter, contained within 2.8 kb of 5' flanking sequence, yielded low level transcriptional activity (approximately 10-fold above background) when placed upstream of the chloramphenicol acetyltransferase (cat) reporter gene and transiently transfected into 23A2 myoblasts. Because the endogenous MyoD1 gene is expressed at high levels in 23A2 myoblasts, we tested whether sequences further upstream could enhance activity of the basal promoter. Cat activity was elevated 10 to 50 fold when the entire 24 kb of 5' sequence was placed upstream of cat. Sequences responsible for this activity are 18 to 22 kb upstream of the transcriptional start site, are equally active in both orientations, and can elevate transcription in transiently transfected and in stably transfected 23A2 myoblasts. This enhancer was cloned upstream of the thymidine kinase (tk) promoter to test its cell specificity. The enhancer is silent in the human choriocarcinoma cell line, JEG3, and is slightly active (2-fold or less) in HepG2 human hepatocarcinoma cells. Interestingly, the enhancer, in combination with either the MyoD1 promoter or the tk promoter, shows equal activity in C3H10T1/2 fibroblasts, which do not express the endogenous MyoD1 gene, and 23A2 myoblasts. These data show that cell type-specific factors regulate the activity of this enhancer, that 10T1/2 cells produce all factors required to activate the enhancer, and that the observed enhancer activity is not autoregulatory in nature, since 10T1/2 cells do not express muscle-specific helix-loop-helix proteins. These data also suggest that a cis-acting negative control system, such as methylation, must exist to repress MyoD1 transcription in 10T1/2 cells.

B 123 REGULATION OF AS GENE EXPRESSION BY AMINO ACID STARVATION: Luisa Guerrini, Shi Gong and Claudio Basilico, Dept. of Microbiology, NYU Medical Center, 550 First Avenue, NY, NY 10016.

We have studied the regulation of the expression of the Asparagine Synthetase (AS) gene in ts11 cells, a mutant of BHK hamster cells which encodes a ts AS. Incubation of ts11 cells at the non permissive temperature leads to a dramatic increase of AS mRNA levels, that can be prevented by addition of exogenous Asparagine (Asn) to the culture media. Furthermore, we found that the expression of AS mRNA is regulated not only by the concentration of Asn, but can be elevated by starvation for any essential amino acid both in culture and in vivo.

Regulation of AS mRNA levels has transcriptional and post-transcriptional components. Post-transcriptional regulation affects mRNA stability and requires translation. Transcriptional regulation requires cis-acting elements present in the promoter and first (untranslated) exon of the AS gene. These elements are contained within a region spanning nucleotides -90 to +110 and are likely to affect transcription initiation or elongation. Gel shift experiments enabled us to identify a region of 30 nucleotides in the first exon which appears to bind two single-strand DNA binding proteins. Preliminary results indicate that these proteins may compete for partially overlapping binding sites, one protein being a repressor and the other an activator of AS gene expression.

B 124 INHIBITION OF TRANSACTIVATION FUNCTIONS OF MYOD AND MYOGENIN BY SODIUM BUTYRATE, Laura Johnston*, Stephen Tapscott, and Harvey Eisen, *Dep't. of Pathology, University of Washington, and Fred Hutchinson Cancer Research Center, Seattle, WA. 98104

Sodium butyrate inhibits the fusion and biochemical differentiation of muscle cells. We find that treatment of muscle cell lines with 5mM butyrate shuts off expression of the muscle determination genes MyoD and myogenin, as well as many muscle-specific structural genes. Transfection of C3H10T1/2 cells with a MyoD expression vector (LTR-MyoD), does not allow for auto-activation of the endogenous MyoD gene in the presence of butyrate. In contrast, myogenin is activated in butyrate-treated cells which have been stably transfected with the LTR-MyoD. Although both MyoD and myogenin from nuclear extracts of these cells form heterodimers with an E2A gene product and bind DNA in an *in-vitro* gel shift assay in the presence of butyrate, this does not appear to be sufficient for activation of muscle-specific gene expression *in-vivo*. Furthermore, when 10T1/2 cells are co-transfected with the LTR-MyoD and the reporter constructs 4R-TKCAT or 4L-TKCAT (containing 4 copies of either the Left or Right MyoD binding sites from the muscle creatine kinase (MCK) enhancer fused to a minimal TK promoter driving the CAT gene), transactivation by MyoD is inhibited by butyrate. In contrast, the transactivation of 4L-TKCAT by the E2A gene product E47 is not inhibited by butyrate. Our data suggests that butyrate inhibits muscle differentiation by interfering with the function of MyoD and myogenin. The activation of myogenin but not MyoD by the LTR-MyoD in the presence of butyrate shows that there are qualitative differences in their activation pathways. In addition, not all bHLH proteins are functionally inhibited by butyrate, indicating some specificity in its mechanism of action.

B 125 GENOMIC ORGANIZATION OF THE ICSBP GENE THAT ENCODES A TRANSCRIPTION FACTOR INVOLVED IN INTERFERON-MEDIATED GENE REGULATION

Yuka Kanno, Paul H. Driggers, David L. Ennist, Christine A. Kozak* and Keiko Ozato, Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development and *Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda MD 20892.

ICSBP is a protein that binds to the Interferon (IFN) consensus sequence (ICS) of many IFN-γ regulated genes including MHC class I genes. ICSBP belongs to the family of IFN response factors (IRF1 and IRF2) which are presumed to play a role in IFN and virus-mediated gene transcription. ICSBP message is expressed predominantly in lymphoid tissues, and is induced preferentially by IFN-γ. The mouse ICSBP gene is present as a single copy and located on Chromosome 8, distal to Es-2. To characterize genomic organization of ICSBP gene and its regulatory region, we have isolated three overlapping clones from a murine genomic library encompassing the entire ICSBP genomic sequence. The gene spans 21 kb and consists of 9 exons. The presumed DNA binding domain is split into 2 exons separated by a 3500 bp intron. The transcription start site has been assigned by primer extension and S1 nuclease protection analysis. The 5'flanking sequence of 0.7 kb contains a number of putative IFN response elements(GAAANN), GC boxes, and typical TATA/CAAT elements. Analysis of factor binding to these upstream sequences in vivo will be presented.

B 126 RETROTRANSPOSON EXPRESSION IN THE GRAVID UTERUS, J.W. Kasik, C.C. Yue and E.J. Rice, Dept. of Pediatrics, Case Western Reserve University Room R-249, 3395 Scranton Road, Cleveland, OH 44109.

In an attempt to identify genes that are upregulated in the uterus during late pregnancy, a differential screen was preformed on a CF-1 strain mouse cDNA library. This library was constructed from uterus collected 1 and 2 days prior to birth and was screened using radiolabeled cDNA probes reverse transcribed from late (1 & 2 days prior to birth) and early (5 & 6 days prior to birth) gestation uterus. Two clones demonstrated upregulation late in pregnancy on northern blotting of total RNA and were identified by partial sequencing as alpha fetoprotein and kidney androgen regulated protein. A third clone demonstrated upregulation on northern blotting of poly A+ RNA; this was identified by partial sequencing as the VL-30 retrotransposon. A forth clone (clone 1-8-3) demonstrated upregulation on northern blotting of both poly A+ and total RNA, yielding a 5.5 kb message. Partial sequencing did not demonstrate a match with previously cloned genes, but did demonstrate homology with a number of mouse viral sequences. We suspect this clone may be a previously unreported retrotransposon.

B 127 CHARACTERIZATION OF THE PROMOTER REGION OF A HUMAN NONMUSCLE MYOSIN HEAVY CHAIN GENE, Sachiyo Kawamoto, Laboratory of Molecular Cardiology, NHLBI, NIH, Bethesda, MD 20892

The myosins found in both muscle and nonmuscle cells comprise a family of proteins having similar native structures, subunit compositions and functional properties. Tissue and developmental differences in the structure of myosin heavy chains (MHCs) have been demonstrated. Sarcomeric MHCs are known to be encoded by a multigene family. We recently identified two gene products for nonmuscle MHCs and demonstrated the tissue and cell type-dependent expression for the two MHC mRNAs as well as changes in mRNA expression associated with cell growth and differentiation (J. Cell Biol. 112, 915-924, 1991).

To understand the mechanisms responsible for regulating the expression of nonmuscle MHC genes, we cloned and have begun to characterize the 5' portion of a nonmuscle MHC gene which includes the promoter region. A major and a minor transcriptional start site were identified by primer extension and RNase protection analyses. The region both upstream and downstream from the transcriptional start sites is rich in GC sequence and contains a number of potential binding sites for the transcriptional factor Sp1, but lacks an apparent TATA box. This is consistent with MHC genes belonging to the family of house-keeping genes. Promoter activity has been monitored using luciferase as a reporter. A fragment of approximately 700 bp (400 bp upstream and 300 bp downstream from the major transcriptional start site) was inserted 5' to the reporter gene. Following transfection into NIH-3T3 cells, the luciferase activity due to the MHC construct was found to be comparable to that generated when the RSV long terminal repeat promoter was used. This activity was approximately 20-fold higher than that found when the thymidine kinase promoter was used. Present experiments are directed toward defining the minimum sequence necessary for promoter activity and identifying cell-type specific regulatory elements.

B 128 FUNCTIONAL ISOFORMS OF PIT-1 GENERATED BY ALTERNATIVE mRNA SPLICING.

Kristin Konzak and David D. Moore. Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

Pit-1 is a pituitary specific transcription factor that activates expression of the growth hormone (GH) and prolactin (Prl) genes, as well as autoregulates its own expression. We have isolated a cDNA encoding a variant isoform of Pit-1, called Pit-1β, that contains an insertion of 26 amino acids in the transactivation domain. PCR data indicates this splice variant is due to utilization of an alternative 3' splice acceptor at the end of the first intron in both the rat and mouse genes. Like the previously described Pit-1α, Pit-1β transactivates both the GH and Prl promoters, but the larger β isoform acts as a more potent inducer of GH in transient transfections. Pit-1α and Pit-1β form heterodimers *in vitro*, and have an additive effect on transactivation of both the GH and Prl promoters when cotransfected in tissue culture cells. Cell-type specific silencer elements located upstream of the rat GH promoter decrease expression of the GH promoter in non-pituitary cells, but do not repress the transcriptional activation by either Pit-1 isoform.

B 129 THE ROLE OF PROMOTER POLYMORPHISM AND ISOMORPHISM IN HLA-DR*B* GENE TRANSCRIPTION.

Thijs Leen and Jack Gorski,Immunogenetics Research, The Blood Research Institute, The Blood Center of Southeastern Wisconsin, Milwaukee , WI 53233

HLA-D region genes constitute a multigene family. They encode transmembrane heterodimers that are expressed constitutively in some tissues and can be induced in others. Conserved upstream sequences to all HLA-D genes have been identified and have been shown to be important for the expression of these genes. A major characteristic of HLA-D genes is their high level of polymorphism. This polymorphism extends into the promoter regions. In addition to allelic polymorphism the various HLA-D genes display isomorphisms in their coding and promoter regions. The genes in the HLA-DR subregion play a major role in the regulation of immune responses. The HLA-DR subregion contains one DR*A* locus and in most individuals multiple DR*B* loci. These are referred to as DR*B*-isotypes. The upstream regions of some DR-isotypes show differences in the conserved upstream elements. The steady state mRNA levels of the different DR-isotypes are not the same and this is thought to correlate with isotypic differences. The role of polymorphisms in the promoter region is unknown. To study this, we have isolated and characterized the 350 bp upstream region of a number of isotypes and alleles of DR*B* genes. We have determined the transcriptional activity of each of the promoters by transient chloramphenicol acetyltransferase (CAT) expression after electroporation of promoter-CAT constructs into the B cell line, Raji. We find that the level of expression of the DR-isotypes of the two different DR types correlate with the mRNA levels and published cell surface expression of each of the isotypes. Allelic forms of the same promoter also influence transcriptional competency. By shuffling the isotypic and polymorphic differences in the upstream regions of different DR*B* promoters we are attempting to map promoter regions involved in the observed differences in transcriptional activity. In addition to Raji we have been utilizing an HLA-D transcription deficient cell line SJO. The data derived from the mutation analyses of the different DR promoters, studied in these different cell lines, will increase our understanding of the regulation of Class II gene expression.

B 130 FUNCTIONAL AND MUTATIONAL ANALYSIS OF THE DROSOPHILA KRÜPPEL TRANSCRIPTIONAL REPRESSOR, J. Licht[1], M. Ro[2], M. Grossel[2] and U.Hansen[2].

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We have shown that the *Drosophila Krüppel* (*Kr*) protein is a DNA-binding repressor of transcription in mammalian cells. (*Nature* 346: 76-9, 1990). The transcriptional repression and DNA-binding activities of the *Kr* protein can be dissociated and the repression function of *Kr* can be transfered to a heterologous DNA-binding protein, the *lac* repressor. Fusion genes between portions of the N-terminal region of the *Kr* protein and the *lac* repressor were assayed *in vivo* for their ability to repress transcription from a reporter gene containing *lac* operators upstream of the herpes thymidine kinase (*tk*) promoter. We thus localized a repression region between amino acids 60 and 90 of the *Kr* protein. This portion of the *Kr* protein is predicted to form an alpha-helix with several hydrophobic faces. A *Kr* mutant missing amino acids 28-213 was still able to repress transcription from a reporter gene containing *Kr* binding sites, suggesting the presence of a second repression region within the C-terminal portion of the protein. Supporting this hypothesis a *Kr* mutant deleted for both amino acids 28-213 and amino acids 345-466 was not able to repress transcription. In addition, a fusion between the *lac* repressor and amino acids 345-466 of *Kr* repressed transcription from a *lac* operator containing reporter gene. Thus like many transcriptional activators the *Kr* protein contains more than one effector region. *Kr* represses transcription when bound at kilobase distances upstream or downstream from the start site of *tk* transcription, suggesting function through protein-protein interactions with factors bound to the *tk* promoter. *Kr* does not appear to repress transcription by a "squelching" mechanism as repression requires *Kr* binding sites in the reporter gene. Low amounts of *Kr* co-transfected with a reporter gene do not lead to activation and a monotonic decrease in transcription from a *Kr* binding site containing reporter gene is observed when co-transfected with increasing amounts of *Kr* expression plasmid, ruling out the phenomenon known as "self-squelching". We are examining the ability of the *Kr* protein to repress transcription activated by different *Gal4* fusion activators. Initial results suggest that *Kr* represses transcription activated by some activators but not others. This suggests that *Kr* may function by direct interaction with an activation domain or with the molecular target of an activation domain rather than some component of the basal transcriptional machinery required for all transcriptional activation events.

B 131 THYMUS-INDEPENDENT EXPRESSION OF A TRUNCATED T CELL RECEPTOR α mRNA BY MOUSE RENAL CELLS: MOLECULAR CHARACTERIZATION AND CELL LINEAGE.

J. Madrenas, F. Pazderka, N.A. Parfrey, and P.F. Halloran. Depts. of Immunology, Medicine, and Pathology. University of Alberta, Edmonton, Alberta, Canada.

During studies on gene expression, we detected a truncated form of T Cell Receptor (TCR) α mRNA in normal mouse kidney, and less abundantly, in brain. This transcript is 1.3-1.4 kb long, the complete form expressed in the spleen being 1.7 kb. The specificity of the transcript was confirmed by oligonucleotide probing, amplification of the Cα region by PCR, and by sequencing and hybridization with the amplified products. The expression of this truncated TCR α mRNA is not associated with expression of other TCR genes. The truncated TCR α mRNA is correctly spliced for the 4 Cα exons, is polyadenylated, and is detected in the cytoplasmic kidney RNA preparation, suggesting that the transcript is potentially translatable. Preliminary evidence suggests that the expression of the truncated transcript can be superinduced with cycloheximide.

In situ hybridization of normal kidney sections showed positive cells for TCR Cα in the subepithelial space. After renal cell fractionation, the truncated TCR α mRNA was detected in a fraction containing large interstitial cells which did not express CD3 and Thy-1, indicating that the transcript is not produced by mature T cells. In this fraction, the presence of NK cells was discarded on the basis of lack of expression of an NK cell marker (NK1.1). Expression of truncated TCR α mRNA is radiosensitive, suggesting that is expressed by bone marrow derived cells. However, the transcript is not detected in bone marrow. In addition, expression of the transcript is not affected by the nude mutation, and does not require an intact recombinase activity as it is present in SCID mice.

In summary, normal mouse kidney contains a cell population which expresses a translatable truncated TCR α mRNA upon interaction with the renal microenviroment. The cells expressing this transcript may be closely related to the lymphoid lineage and may undergo extrathymic differentiation under certain circumstances.

B 132 CIS-ACTING ELEMENTS THAT AFFECT RNA POLYMERASE III-TRANSCRIPTION OF A B1-ALU GENE IN WAYS WHICH COULD MODULATE THEIR POTENTIAL FOR TRANSPOSITION, Maraia, R.J., Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20892

The Alu family of short interspersed elements (SINEs) represent a highly repetitive class of transposed sequences in mammalia. These elements are responsible for human heritable disorders by DNA and RNA-mediated events. Alu dispersion probably involved an intermediate generated by RNA polymerase III (pol III). Three features presumably facilitate SINE amplification by retroposition: 1) internal transcriptional promoters, 2) poly A tracts and 3) 3'-oligo U tracts generated by pol III termination. The current model of Alu retroposition suggests that the terminal U tract base pairs with poly A to self prime cDNA synthesis by reverse transcriptase. A transcriptionally active subset of pol III-dependent mouse B1 (Alu-equivalent) genes generate processed cytoplasmic RNAs by removal of the poly A and terminal U tracts by a nuclear ribonuclease thereby theoretically reducing their potential for transposition (Maraia 1991, submitted). This model predicts that poorly processed B1 transcripts would undergo retroposition more frequently than efficiently processed B1 RNA. Cis-acting elements involved in pol III transcription and post-transcriptional processing were studied in vitro and in injected Xenopus oocytes. Three different regions of a B1 gene were altered by site directed and deletional mutagenesis. B box internal promoter substitutions abolished transcription as expected, while a 5'-flanking region deletion had slight stimulatory effects. 3' substitutions had surprising and dramatic effects (>100 fold) on post-transcriptional processing in vitro and in vivo by mechanisms that involved mutations distant from the processing site. In addition, processing was uncoupled from transcription by these sequences. Certain base sequences led to a significant lag (~1 hr) in processing following RNA production in vivo as compared with others. Thus, it was demonstrated that B1-Alu RNA post-transcriptional processing can be modulated by 3' sequences which are not part of the B1 element. These observations suggest that the locus into which a retroposed B1 element integrates could influence the efficacy of post-transcriptional processing and therefore the potential for further retroposition.

B 133 IN VIVO FUNCTION OF THE CLASS I REGULATORY ELEMENT IN Q10 TRANSCRIPTIONAL ACTIVITY. Jeffrey B. Marine, Yumiko Shirakata, Jeffrey J. Hooley, Diane E. Handy and John E. Coligan. Biological Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

We have generated three different lines of transgenic mice (CBAxB6) with the MHC class I gene D^d driven by the 400 basepair promoter region of Q10, a non-polymorphic MHC class I gene expressed in the liver, kidney and fetal yolk sac. One transgene is composed of the wild type Q10 promoter. Two mutant constructs have either a single site-specific mutation (M1) or two base pair substitutions (M3) in region I of the Class I Regulatory Element (CRE). These mutant promoters reconstitute the inverted repeat or the entire region 1 found in other class I genes, respectively. The CRE region 1 has been previously described as a positive cis-acting regulatory element essential for class I expression. Transcriptional regulatory factors such as NF-κB, KBF1/H2TF1 and H2RIBP have been shown to specifically bind to region I oligonucleotides.

Mice containing the wild type Q10 promoter strongly express the D^d molecule in a membrane-bound fashion in the liver and kidney, similar to the tissue-limited expression pattern described for endogenous Q10. In mice containing the site-specific mutant constructs, D^d expression is present in both the liver and the thymus. Thymic expression appears limited to double positive (CD4+/CD8+) thymocytes. Unlike classical class I or endogenous Q10, low-level expression of the of the transgene has been observed in the brain of all three transgenic lines. Expression has not been found in the spleen lymph nodes or bone marrow of any of our animals. These results suggest that the mutations in region I alter the tissue-specific regulation of the Q10 promoter in vivo, though an intact inverted repeat does not restore a pattern of expression characteristic of classical class I genes.

B 134 STRUCTURE AND TRANSCRIPTION OF THE CHICKEN βA3/A1-CRYSTALLIN GENE, Joan B. McDermott and Joram Piatigorsky, Laboratory of Molecular and Developmental Biology, National Eye Institute, NIH, Bethesda, MD 20892

Spatial and temporal regulation of the crystallin genes is critical for the refractive properties of the lens. The βA1- and βA3-crystallins are major, related polypeptides in the lenses of vertebrates. Here we show that a single, polymorphic gene encodes the βA3 and βA1 proteins and establish its structure. In addition, Northern blot and transfection experiments indicate the lens-preference for βA3/A1 gene expression. An abundant 0.9 kbp transcript was present in the lenses of hatchling chickens, but no detectable transcripts were found in the rest of the eye, brain, heart, kidney, liver or skeletal muscle. The chicken βA3/A1-crystallin gene has the same structure as the human orthologue: six exons with standard splice sites and two alternative start codons from which the protein products are apparently translated. We have also compared the mouse βA3/A1-crystallin promoter with those of the chicken and human genes. The -141/-72 sequence of the chicken promoter is approximately 70% identical to the human and mouse promoter for the βA3/A1 gene; by contrast, there is only approximately 50% sequence similarity between exon 1 of the chicken gene and exon 1 of the mouse and human genes. This suggests conservation of putative regulatory sequences in the βA3/A1-crystallin gene. The mouse and human regions further upstream show a high degree of identity, whereas the chicken sequence 5' to -141 has diverged. The chicken promoter contains a TATA box at -30, a thymidine-rich element from -163 to -218 and an AP-1 consensus sequence at -264. A chicken promoter fragment from -382 to +22 is sufficient to drive expression of the bacterial CAT gene in transfected chicken primary lens cells but not in fibroblasts. Deletion experiments suggest that the -382/-238 sequence may be functionally significant. The roles of the AP-1, T-element and conserved promoter regions are under investigation.

B 135 NEURAL-SPECIFIC EXPRESSION OF THE SCG10 GENE IS CONTROLED BY A CELL TYPE-PREFERRED SILENCER ELEMENT

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SCG10 is a neural-specific, growth-associated protein that is broadly expressed in embryonic central and peripheral neurons. SCG10 is expressed also in mature chromaffin cells and PC12 cells at low level and is inducible by the nerve growth factor (NGF). As a way of studying neuronal differentiation and chromaffin cell plasticity at the molecular level, we have been examining the control mechanisms for the neural-specific and NGF-inducible regulation of the rat SCG10 gene. We have previously found by cell transfection and transgenic mice experiments that a 4 kb 5'-flanking sequence of the SCG10 gene is sufficient to promote CAT gene expression preferentially in neuronal cells. By means of a series of deletion analysis in this region, we were able to localize two major regulatory domains that control SCG10 expression: a constitutive enhancer-like element in the promoter-proximal region and a silencer located at 1.5 kb upstream of the transcriptional initiation sites. The proximal region contains sequences homologous to several known enhancer elements, as well as sequences that are homologous to elements found in other neuron-specific genes. The functional significance of this homology is, however, not clear at the moment. The distal region (-1564 to -1390) preferentially blocks the enhancer function in non-neuronal cells. The distal region is also able to confer such preferential suppression upon a heterologous promoter in an orientation-independent manner. These data suggest that the SCG10 silencer functions predominantly in non-neuronal cells. In other words, the specificity of SCG10 expression appears to be achieved through a selective derepression mechanism in neuronal cells. Deletion analysis and addition experiments have localized the SCG10 silencer to a 36-bp region. This region contains a 22-bp element that shows strong homology to elements present in the silencer regions of the type II sodium channel gene and the synapsin gene, which are also neuron specific. However, it is distinct from other silencers that function outside of the nervous system. We are trying, by mobility-shift assays, to identify sequence-specific, DNA-binding proteins which interact with this silencer element, using HeLa cell nuclear extracts.

B 136 MULTIPLE COMPLEXES FORMED BY A FAMILY OF NF-kB/rel-RELATED FACTORS: STRONG PROTEIN-INDUCED DNA BENDING AND IMPLICATION FOR THE ROLE IN TRANSCRIPTIONAL CONTROL. Sergei A. Nedospasov and Dmitry V. Kuprash. Engelhardt Inst. of Molec. Biol., USSR Acad. Sci., 117984 Moscow, Russia.

A family of NF-kB/rel-related proteins plays an essential role in transcriptional activation of various cellular and viral genes. We compared the ability of kB sites flanked by natural and artificial sequences to form in vitro various NF-kB complexes from the nuclear extracts. High resoluton band shift gels indicated the presence of three groups of complexes, presumably involving along with recently cloned p50 and p65 other members of NF-kB/rel family. The overall pattern was dependent on the sequence context of a given kB site and could be altered by changing the flanking sequences. Classical Ig kB site predominantly formed one complex from the group II, whereas the high affinity kB site from the mouse TNF locus formed another complex from the group II and the major complex from the group I. As indicated by permutation analysis, formation of all NF-kB/rel-related complexes resulted in significant protein-induced DNA bending, with the angles varying from 80 to 130 degrees. The extent of bending was specific for the type of the complex, and not necessarily correlated with the affinity. Our data imply that the complex type, apparently defined by its protein moiety, as well as the gross DNA configuration are dependent on the sequence context. We speculate that a pair of properly spaced kB sites could facilitate the assembly of transcription complex by bringing distal parts of control elements into close proximity. Some factors of NF-kB/rel family may also directly contribute to activation, while others, lacking the activating domains, play their role mostly by influencing the geometry of DNA in the transcription complex and require the presence of additional activating signals.

B 137 ICSBP OF THE IFN-REPONSE FACTOR FAMILY DOWN-REGULATES MHC CLASS I PROMOTER ACTIVITY.

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ICSBP is a member of the interferon response factor (IRF) that binds to the IFN-response elements of MHC class I and other IFN-regulated genes as well as the virus inducible elements of IFN genes (Driggers et al, 1990). To study a functional role of ICSBP, transient co-transfection experiments have been performed using an ICSBP expression plasmid and a MHC class I CAT reporter gene. Expression of plasmid specific ICSBP mRNA was detected in cells transfected with the ICSBP plasmid. Co-transfection of the ICSBP plasmid resulted in a significant reduction in IFN-induction of MHC class I promoter activity in undifferentiated embryonal carcinoma N-Tera2 cells. ICSBP also down-regulated both constitutive and IFN- induced promoter activity in N-Tera-2 cells that had undergone differentiation and begun to express MHC class I genes following retinoic acid treatment. Analysis of various deletion and mutant reporters indicated that this down-regulation is mediated by the IFN-response element. Further, the down regulation was dependent on the DNA binding domain of the ICSBP. These results suggest that ICSBP functions as a transcription factor to control expression of MHC class I and other IFN regulated genes. Experiments are in progress to determine the effect of ICSBP on other IFN regulated genes.

B 138 β-ENOLASE EXPRESSION IN MYOBLASTS DOES NOT REQUIRE THE MYOD FAMILY OF REGULATORS AND DEMONSTRATES AGE-DEPENDENT MYOBLAST HETEROGENEITY, Charlotte A. Peterson, Mildred Cho, Farzan Rastinejad and Helen M. Blau, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305

In this report, we analyze the novel pattern of expression of the gene encoding the muscle-specific enzyme β-enolase. Unlike the expression of most muscle-specific genes that are activated during differentiation under the control of the MyoD family of helix-loop-helix (HLH) proteins, the β-enolase gene is expressed in undifferentiated myoblasts, suggesting that β-enolase expression is not controlled by the MyoD family. Analysis of β-enolase expression in a mutant myogenic cell line, NQO-1 that does not accumulate any of the known members of the MyoD family, confirms that β-enolase expression is not dependent on this family of regulators. Furthermore, we show that β-enolase is differentially expressed in myoblasts from different developmental stages. Whereas myoblasts from adult tissues accumulate high levels of β-enolase mRNA, β-enolase mRNA is essentially undetectable in myoblasts from fetal tissues. Therefore, β-enolase is a molecular marker that distinguishes populations of myoblasts present at different stages of development. Our results suggest that as yet unidentified myogenic regulators may be responsible for generating temporal heterogeneity in myoblasts during muscle development.

B 139 IDENTIFICATION OF SEQUENCE ELEMENTS INVOLVED IN HUMAN CR2 GENE TRANSCRIPTIONAL ACTIVATION AND REGULATION. Pham, C., O'Connor, S., Dehoff, M., Jacobi, S., and Holers, V.M. HHMI at Washington University School of Medicine, St. Louis, MO, USA.

Complement Receptor 2 (CR2) is expressed during the late-pre and mature stages of B lymphocyte development. To begin to study regulation of its cell and stage specificities, we have made a series of deletion mutants of the human CR2 promoter region and analyzed their activity by CAT assay in transiently transfected Raji cells. An activation domain is found between -69 and -149, while addition of sequence more 5' (-150/-320) consistently down regulates CAT expression. There is evidence by both footprint and gel retardation analysis that Sp1 and/or Sp1-like proteins are utilized in the activation domain. In addition, footprint analysis demonstrates several unique DNA binding sites 5' to the TATA box (-38/-47 and -61/-67), in the negative regulatory domain, and further upstream in an A+T rich region (-650/-620). Gel retardation analysis confirms that these protected DNA sequences also form specific DNA binding protein complexes with Raji nuclear extract. Studies are underway to determine the cell and stage specific utilization of these unique DNA binding sites. To further develop models of CR2 gene regulation we have characterized cell lines for CR2 expression by the reverse transcriptase-PCR method. By this technique, several pre B (REH, NALL-1, NALM-6) as well as T (CEM), monocyte (U937, THP-1) and fetal fibroblast (IMR-90) cell lines have no detectable CR2 mRNA. Ig-secreting late B cell lines (SKW, DHL-4) have very low levels of mRNA. Mature B cell lines (Raji, EB19, Ramos) express high levels of CR2 mRNA.

B 140 MUTATIONS AT THREE LOCI IN *DROSOPHILA* SUPPRESSING *white*^{apricot} INCREASE COPIA TRANSCRIPT ACCUMULATION AND PRODUCE SYNTHETIC LETHALITY IN DOUBLE MUTANTS, Leonard Rabinow and Bokyoung Yun, Waksman Institute, Rutgers University, Piscataway, N.J. 08855-0759 Numerous studies have identified chromosomal regions with dosage-sensitive effects on the transcription of specific unlinked loci. We are characterizing three dosage-sensitive loci, *Doa*, *Rem* and *Msu*, affecting expression of w^a, a copia retrotransposon insertion allele in *Drosophila*. Mutants of these three loci are recessive lethal, although heteroallelic combinations produce homozyogtes at low frequency. Homozygotes have wild-type eye color and disrupted eye morphology. Mutant homozygotes at any of the three loci have several identical effects: Levels of wild-type *white* RNA are increased; accumulation of transcripts initiating in LTRs of the w^a-copia element is increased; fifty percent of RNA products initiating in the 5' w^a-copia LTR is shortened, probably due to an altered site of transcription initiation; and total copia RNA levels are increased. Finally, *Doa*, *Msu* and *Rem* mutations interact with each other. Crosses between alleles of each of the modifiers with alleles of the other two reveal three outcomes in trans-heterozygotes: 1) simple additivity, 2) synergistic effects on w^a: wild-type eye pigmentation, accompanied by disrupted eye morphology similar to that seen in homozygotes at any one locus, and 3) allele-specific synthetic lethality. We hypothesize these effects are due to coordinate control by these three loci of a number of essential host genes in addition to copia, and that all three are involved in the same process in transcriptional regulation.

B 141 DIVERGENT EFFECTS OF cAMP ON LIPOPROTEIN LIPASE GENE TRANSCRIPTION IN ADIPOCYTES AND MYOCYTES, Mary V. Raynolds and Robert H. Eckel. Division of Endocrinology, University of Colorado Health Sciences Center, Denver, CO 80262.
The enzyme lipoprotein lipase(LPL) is synthesized by several cell types, including adipocytes and myocytes, and mediates plasma-derived fatty acid uptake for storage (adipocytes) or oxidation (myocytes). LPL gene expression is regulated inversely by cAMP in adipocytes and myocytes. Previous work in this laboratory has demonstrated that cAMP-mediated decreases in LPL activity and mRNA in adipocytes is due, at least in part, to suppression of LPL gene transcription (Mol. Endo 4:1460, 1990). Addition of a non-metabolized cAMP analog (10^{-5}M 8(4-chlorophenylthio)cAMP) to skeletal myocytes has the opposite effect: LPL enzyme activity and mass increase 2-fold, as does steady-state LPL mRNA. To determine whether these changes are due to stimulation of LPL gene transcription, C2 skeletal muscle cells were transfected with an expression vector containing the human LPL promoter (-480to+118) linked to the firefly luciferase gene. Cells were treated with 10^{-5}M forskolin or 500μM isobutylmethylxanthine (IBMX) for 16 hours to increase intracellular cAMP, and then were assayed for luciferase activity. Luciferase activity is induced 2-fold (forskolin) to 3.5-fold(IBMX) in treated cells compared with untreated cells, suggesting that in myocytes cAMP does increase LPL gene transcription. Possible mechanisms for divergent transcriptional regulation of the LPL gene in adipocytes and myocytes include differences in nuclear factors interacting with the DNA element(s) conferring cAMP responsiveness, or different cAMP response elements being utilized in each cell type. Currently, we are testing the response of LPL gene transcription to forskolin and IBMX by using human LPL promoter-luciferase constructs containing deletions within the promoter in order to identify DNA elements which mediate the cAMP effect, and to determine if the same regions of DNA transduce the cAMP signal in adipocytes and myocytes.

B 142 DBP *TRANS*-ACTIVATES THE PHOSPHOENOLPYRUVATE CARBOXYKINASE GENE PROMOTER IN A MANNER DISTINCT FROM THAT OF C/EBP, William J. Roesler, Pamela McFie and Cheryl Dauvin, Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0
The expression of the gene encoding the cytosolic form of phosphoenol-pyruvate carboxykinase (PEPCK) is limited to the liver, adipose and kidney, with highest levels of expression occurring in liver. Additionally, the expression of the PEPCK gene is developmentally regulated, with little expression occurring until just prior to parturition. It has been previously demonstrated that the CCAAT/enhancer binding protein (C/EBP) *trans*-activates the PEPCK promoter, and binds to several sites along the promoter. The additional observations that C/EBP is expressed in liver and follows the same developmental profile as PEPCK suggested that C/EBP plays an important role in the regulation of PEPCK gene expression. However, since C/EBP is expressed at high levels in lung, a tissue where PEPCK is not expressed, it suggests that other mechanisms are involved to provide PEPCK with its liver-specific expression. We now show that DBP, a transcription factor whose expression is limited to the liver, is also able to *trans*-activate the PEPCK promoter and with more potency than C/EBP. Both DBP and C/EBP bind with highest affinity to regions located at -85 and -245 in the promoter, but have subtle differences in their binding properties at several other sites. One binding site, located at position +50 in the promoter, is bound only by C/EBP. Using expression vectors for both C/EBP and DBP, we found that with 5'-deletion mutants of the PEPCK promoter, both C/EBP and DBP exerted their effects through similar regions of the promoter. However, the use of internal deletion mutants of the promoter identified distinct differences in the mechanism of activation by C/EBP and DBP. In particular, a region of the promoter between -86 and -117 inhibited the activation by DBP but had no effect on C/EBP's ability to do the same. Additionally, a region lying between -117 and -200 was necessary for the synergistic activation of the promoter by DBP, although it required upstream sequences for this activity. These results demonstrate that DBP and C/EBP, while having similar DNA binding specificities, have distinct functional differences in the context of the PEPCK promoter. These differences, along with the developmental profiles of C/EBP and DBP, may provide a mechanistic explanation for the liver-specific as well as the developmental profile of PEPCK gene expression.

B 143 CHOP10, A LEUCINE-ZIPPER CONTAINING PROTEIN THAT IS A TRANSCRIPTIONAL INHIBITOR OF C/EBP AND LAP. David Ron and Joel F. Habener, Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston MA 02114, U.S.A.
Transcriptional activation of the rat angiotensinogen gene during the acute-phase response is mediated by a cis-acting DNA element, the Acute-Phase Response Element (APRE). The APRE is a binding site for a family of C/EBP-like proteins. The acute-phase response leads to an alteration in the profile of C/EBP-like DNA-binding proteins in rat liver nuclei and in adipocytes. Part of this change is due to alterations in the level of previously identified C/EBP-like proteins, such as C/EBP and LAP. To investigate further the nature of the changes that occur in the C/EBP-like complex that binds to the APRE we developed a strategy for identification of proteins that can heterodimerize with C/EBP and LAP. Bacterially-expressed chimeric proteins consisting of the DNA-binding (and dimerization) domain of C/EBP or LAP fused to the high-affinity protein-kinase A site of the transcription factor CREB were constructed. The purified proteins were labeled by in vitro phosphorylation with ^{32}P-γATP and purified catalytic subunit of protein-kinase A. The labeled protein was used as a probe to detect dimerizing proteins on a western "zipper-blot". Several proteins that specifically bound the probe were identified in nuclear extracts of liver and adipocytes. Screening an adipocyte cDNA expression library with the aforementioned probes led to the isolation of a novel clone, Chop10. The cDNA encodes a leucine-zipper-containing protein that is missing the DNA-binding basic region. Chop10 protein expressed in bacteria binds specifically to C/EBP and LAP but the heterodimer fails to bind DNA. In transfection experiments in hepatoma cells, overexpression of Chop10 attenuated the ability of C/EBP and LAP to activate the APRE. Therefore, Chop10 appears to be a C/EBP-family counterpart of ID, an inhibitor of the myoD transcription factor. Chop10 mRNA was increased in rat liver during the acute-phase response. These results suggest a role for Chop10 in the negative regulation of the activity of the C/EBP-like complex during the acute-phase response by inhibition of DNA-binding.

B 144 IDENTIFICATION OF CIS-ACTING SEQUENCES REGULATING THE EXPRESSION OF *yp3* IN *DROSOPHILA MELANOGASTER*.

Elaine Ronaldson and Mary Bownes, Institute of Cell and Molecular Biology, University of Edinburgh, Mayfield Road EH9 3JR UK.

There are three major yolk proteins (YPs) in *Drosophila* which provide a source of nutrition during embryonic development. The YPs are encoded by single copy genes (*yp1*, *yp2* and *yp3*) located on the X chromosome, all of which have been cloned and sequenced. *yp1* and *yp2* are divergently transcribed and separated by a 1.2kb intergenic region whereas *yp3* is located more than 1Mb downstream from *yp1*. Their expression is strictly regulated in a sex-, tissue- and developmentally-specific manner, synthesis occuring only in the fat body and ovarian follicle cells of the adult female. Consequently, they provide a unique system for the study of independent yet coordinate transcriptional control of gene expression in higher organisms.

We are investigating the influence of different regions both surrounding and within the *yp3* gene using the expression of a reporter gene, β–Galactosidase (*LacZ*) fused either to the *Drosophila hsp70* promoter, or the *yp3* promoter region itself. Following *P* element-mediated germ-line transformation, histochemical staining for β-Galactosidase activity allows the effects of all transcriptional regulatory elements present to be observed.

We have identified sequences upstream of *yp3* responsible for activation and enhanced levels of transcription in both the ovary and fat body of adult females. A detailed deletion analysis, along with DNA footprinting and mobility shift assays using ovarian nuclear extracts should lead to the further localisation of important *cis*-acting sequences necessary for the observed regulation of *yp3* transcription.

B 145 ANALYSIS OF THE SPATIAL ORGANIZATION OF FACTOR BINDING SITES WITHIN THE KAPPA IMMUNOGLOBULIN INTRON ENHANCER, Judith T. Schanke and Brian G. Van Ness, Institute of Human Genetics and Department of Biochemistry, University of Minnesota, Minneapolis, MN 55455

Kappa immunoglobulin intron enhancer activity is controlled by both tissue specific and developmentally regulated DNA-binding proteins. Mutation of individual sequence motifs within the enhancer has been shown to significantly impair enhancer function. A species comparison of the enhancer reveals a highly conserved spatial organization among sequence motifs, suggesting the spatial arrangement of the binding sites may be essential for enhancer function. We are examining the importance of the positions of individual sequence motifs within the enhancer. Enhancer driven expression vectors were constructed with mutations in individual factor binding sites, (kB, kE2 or kE3). 212 bp DdeI mutated and wild-type enhancer fragments were inserted upstream of a native Vk promoter and the CAT reporter gene. Oligonucleotides containing wild-type motifs were then inserted either 25 bp or 2.4 Kb downstream from the corresponding mutated enhancer. The relative enhancer activities of wild-type, mutated and replacement enhancers are being compared by transient transfection into inducible pre-B and mature B cell lines in order to evaluate the individual position requirements of sequence motifs for full enhancer function. Preliminary data indicate the relative position of the NFkB binding site is important for full enhancer function.

B 146 QUANTIFICATION OF CONTRIBUTIONS TO PROLACTIN GENE EXPRESSION. Kathleen M. Scully and Michael G. Rosenfeld. University of California, San Diego, and Howard Hughes Medical Institute, La Jolla, California 92093.

Development of the anterior pituitary gland results in the appearance of five cell types, each type distinguished by the trophic hormone that it produces. The pituitary-specific POU-domain transcription factor, Pit-1, is involved in the specification and/or maintenance of three of these cell types and in transcription of the prolactin and growth hormone genes. Data from transgenic and pituitary cell transfection experiments indicate that physiological levels of prolactin gene expression are achieved by synergistic interactions between Pit-1 activated enhancer and promoter elements. In non-pituitary cell types, however, complementation with Pit-1 does not lead to a similar pattern of prolactin expression. Furthermore, during development, maximum prolactin expression lags far behind maximum Pit-1 expression. Together, these data suggest that additional pituitary-specific factors may be involved in prolactin gene expression.

We report the results of mutational analysis of the prolactin enhancer and promoter elements. Most of the enhancer function maps to known Pit-1 binding sites and an estrogen-response element. A portion of the enhancer function also maps to a region of DNA containing a cluster of potential binding sites for a variety of nuclear receptors. We also report that enhancer function and synergism between the enhancer and promoter elements do not require Pit-1 binding to the promoter elements.

B 147 Transcriptional Regulation of the Human Aminopeptidase N Gene in Hematopoietic Cells, L.H. Shapiro, B.G. Jones, R.A. Ashmun, and A.T. Look, Dept of Hematology/Oncology and Tumor Cell Biology, St. Jude Children's Research Hosp., Memphis, TN 38101

Aminopeptidase N (EC 3.4.11.2), is a cell surface metallopeptidase expressed by human granulocyte-monocyte progenitors (CFU-GM) and their more differentiated progeny in the granulocytic and monocytic lineages, but not by lymphocytes or hematopoietic cells of other lineages. In non-hematopoietic tissues, aminopeptidase N is expressed on the surface of fibroblasts, hepatocytes, and the epithelial cells that form brush borders of the small intestine and kidney. Two independent promoters regulate aminopeptidase N expression in cells of different tissues, creating distinct transcripts with different 5'-untranslated regions. A 400 bp genomic fragment containing the myeloid transcription initiation site regulated tissue-appropriate expression in hematopoietic cell lines, and thus includes elements responsible for aminopeptidase N expression by myeloid but not lymphoid cells. Sequence analysis of this 400 bp myeloid promoter fragment revealed two identical consensus helix-loop-helix protein binding sites (CAGATG) separated by 5 bp between -258 and -274. Gel mobility shift assays using an oligonucleotide probe containing both sites showed that this region bound a protein present in nuclear lysates of myeloid cells but not in aminopeptidase N-negative lymphoid cell lysates. Competition studies showed that this DNA-protein interaction was sequence specific. In addition, cold competitor oligonucleotides containing single mutations in the HLH consensus regions did not interfere with protein binding, indicating that the HLH consensus region was involved in the DNA-protein interaction. Because aminopeptidase N is expressed very early in myeloid cell development, the molecular mechanisms which govern its regulation may be fundamental to myeloid cell differentiation.

B 148 TISSUE-SPECIFIC GENE REGULATION OF MHC CLASS I, Q10 GENE. Yumiko Shirakata, Jeffrey B. Marine, Keiko Ozato* and John E. Coligan, Biological Resources Branch, National Institute of Allergy and Infectious Diseases, National Institute of Child Health and Human Development*, National Institutes of Health, Bethesda 20892

The classical major histocompatibility complex (MHC) class I molecules (HLA-A, B, and C in human, H-2K, D and L in mouse) are constitutively expressed on all cells except the brain. Mouse non-polymorphic class I molecules encoded by Qa/Tla region genes are highly homologous to classical class I genes, but have restricted tissue expression patterns. Though the sequence of Q10 promoter region is also highly homologous to the other MHC class I genes, the expression of Q10 is limited to the liver, fetal yolk sac and recently a low level of expression has been observed in the kidney. We are investigating the mechanism of the tissue-specific regulation of the Q10 gene.

Using the gel retardation assay, we have found two liver specific DNA binding proteins for the Q10 promoter/enhancer region. One liver specific factor binds to the TATA box region. This factor appears similar to a TATA box binding factor (TAF, HNF-4) reported by David-Watine et al. (1990), Sladek et al. (1990). The other liver specific DNA binding protein binds to the enhancer B region and has not been identified yet. To understand the interaction between these liver specific factors and the cis-element in vivo, we have performed in vivo footprinting analyses. In vivo footprinting analyses of the enhancer B region revealed a liver specific protected site and a hypersensitive site, and also a splenic (negative for Q10 expression) DNA specific protected site. No protection was observed around the TATA region. In vivo footprinting analyses of splenic DNA revealed protected site in the ICS region that was absent in liver DNA. These results suggest that the Q10 gene is regulated by both positive and negative regulatory factors. We are currently conducting in vitro transcriptional assays to confirm these observations.

B 149 DIFFERENTIAL INTERACTIONS OF FACTORS WITH THE ENHANCERS AND PROXIMAL REGIONS OF DEVELOPMENTALLY-REGULATED U4 SMALL NUCLEAR RNA GENES, William E. Stumph, Jon H. Miyake, Ihab W. Botros, and Zulkeflie Zamrod, Department of Chemistry and Molecular Biology Institute, San Diego State University, San Diego, CA 92182.

In the chicken genome there are two closely-linked genes, U4B and U4X, that code for different sequence variants of U4 small nuclear RNA. These two genes differ at seven nucleotide positions within the U4 RNA coding region and have only limited similarities in their 5'-flanking DNA sequences. Although both genes are expressed with nearly equal efficiency in the early embryo, transcription of the U4X gene is specifically down-regulated relative to U4B as development proceeds from the embryo to the adult. We have been studying the factors responsible for the differential expression of the U4X and U4B RNA genes. The Oct-1 factor binds with similar affinities to octamer motifs present in the U4X and U4B enhancers; thus Oct-1 is unlikely to be directly involved in the differential regulation of the two U4 genes. However, a second snRNA enhancer-binding protein (SBF) has a 20-30 fold lower affinity for the U4X enhancer than for the SPH motif in the U4B enhancer. Thus, one mechanism to account for the differential expression of chicken U4 genes is that the SPH motif-binding factor (SBF) is not a limiting factor in the tissues of the early embryo, but becomes limiting in adult tissues, leading to the specific down-regulation of U4X gene enhancer activity. Furthermore, we have identified a novel factor, PPBF, that binds sequence-specifically to the proximal regulatory region of the U4X gene but not to the proximal region of the U4B gene. This factor therefore probably plays an important role in controlling U4X, but not U4B, gene transcription. Thus, two distinct mechanisms, one enhancer-based and one proximal-based, may cooperate to effect the differential expression of the U4B and U4X genes during chicken development.

B 150 **Transcriptional Regulation of the Mouse Adult Fast Myosin Heavy Chain (IIB) Gene**

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We have cloned the murine adult fast IIB myosin heavy chain (MHC) gene, which is expressed in fast-contracting, glycolytic muscle fibers. A series of deletions of 5'-flanking region was ligated with the pUC118-CAT vector and was transfected into mouse C2 cells and viral-transformed quail myoblasts-myotubes. None of the IIB MHC promoter fragments gives activity in the homologous mouse C2 myotubes, but these constructions have the activity in quail myotubes depending stage-specific manner. Promoter fragments containing 192bp and 1430bp have the highest activity in quail myotubes, while the constructions longer than 1430bp have very low activity possibly due to the presence of "cardiac specific sequences" that was designated for the repression of cardiac myosin light chain expression in skeletal muscle. 1430bp construction has a complete consensus for bHLH protein and this site can bind to myoD in vitro. The fragment containing this MyoD binding site cannot confer activity to muscle creatine kinase (MCK) promoter, and requires the proximal fragment (-224 - -133) containing A+T- rich sequences in order to activate this basal muscle promoter. -192bp construction has two A+T-rich sequences. Distal A+T-rich sequence (AT-2) has a complete consensus of MEF-2 and proximal A+T-rich sequence (AT-1) has a tandem repeat of POU-domain gene binding consensus. This region together with the part containing CArG box sequences (around -100) can confer stage specificity to the IIB MHC promoter, and the mutation in AT-1 would abolish transcriptional activity. In the gel shift assay, AT-2 binds to MEF2 and AT-1 can also bind to MEF2 and one more ubiquitous factor. Between the CArG box and TATA box of this promoter, we have identified a region which bind the thyroid hormone receptor(T3R), MyoD and c-jun, as determined by in vitro gel shift assays. Proximal promoter constructs containing this region, transfected into C2 myotubes, are activated by co-transfected c-jun and c-fos (AP-1) , by MyoD, but not by the T3R. This complexity can explain the positive and negative regulation of late onset muscle specific gene.

B 151 *PAX-6*, A MURINE PAIRED BOX GENE, IS EXPRESSED IN THE DEVELOPING CNS, THE DEVELOPING EYE AND THE NASAL EPITHELIUM AND IS AFFECTED IN THE *SMALL EYE* MUTANT,

Claudia Walther and Peter Gruss, Department of Cell Biology, Max Planck Institute of Biophysical Chemistry, 3400 Göttingen, Germany

A murine multigene family of paired-box-containing genes, of to date eight members has been isolated which encode DNA-binding factors. All Pax genes analyzed to date exhibit a temporally and spatially restricted expression pattern during embryogenesis, compatible with a regulatory role in vertebrate development. The developmental importance of Pax genes is further highlighted by the correlation of *Pax-1* with the developmental mutant *undulated*. A point mutation in the *Pax-1* gene in *undulated* leading to an amino acid exchange in the paired domain causes a reduction of DNA-binding affinity. *Pax-6* encodes a rather divergent paired domain and paired-type homeodomain. *Pax-6* is the earliest expressed Pax gene described so far and is in contrast to all other Pax genes not expressed in segmented structures during murine embryogenesis. The expression pattern of *Pax-6* during embryogenesis in the brain, the pituitary, the eye and the olfactory epithelium are suggestive of a regulatory role for *Pax-6* in the development of these structures. *Pax-6* is located on mouse chromosome 2 in a region where the mutant *Small eye* maps. *Pax-6* is expressed in all structures which are affected in the *Small eye* mutant. Moreover, the *Pax-6* gene is mutated in different alleles of *Small eye*. We are currently investigating the effects of the mutated form of *Pax-6* on the DNA-binding ability and the results will be presented at the conference.

B 152 BINDING OF A TOPOISOMERASE II-RELATED PROTEIN 5' OF AN IMMUNOGLOBULIN HEAVY CHAIN PROMOTER, **Carol F. Webb and Kenton L. Eneff, Immunobiology and Cancer, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104**

We previously showed that interleukin-5 (IL-5) + antigen increased immunoglobulin μ mRNA levels 3 to 6-fold. Further studies identified two sequences 5' of the S107 heavy chain promoter that bound to IL-5 + antigen inducible B cell-specific protein complexes. Both sequences were A+T rich and appeared to bind to similar, if not identical, proteins. The more 3' of the sequences was required for induction of immunoglobulin mRNA levels with IL-5 + antigen, while the more 5' of these sequences acted as a strong nuclear matrix attachment region. Topoisomerase II is a major component of the nuclear matrix and has also been suggested to play a role in transcription. Two topoisomerase II isoforms of differing molecular weights exist in the mouse and may be differentially regulated. In this study, we have used anti-topoisomerase II antibodies (provided by Fred Drake, King of Prussia, PA) to demonstrate by mobility shift assay that a topoisomerase II-like protein is a component of the IL-5 + antigen inducible protein complexes that bind to both sequences. Attempts to reconstitute the mobility-shifted complex with purified topoisomerase II were unsuccessful. Thus, unique activation events, or binding of other protein(s), may be necessary for formation of the mobility-shifted complex. Western blot analyses indicate that the mobility-shifted topoisomerase II-like protein is more closely related to the p180 isoform. These data suggest a role for topoisomerase II in the regulation of immunoglobulin transcription by interleukin-5 + antigen.

B 153 CHARACTERIZATION OF PROTEINS BINDING IL-3 PROMOTER SEQUENCES. M. Wolin, M. Kornuc, R. Lau, S-K. Shin, S. Nimer. UCLA School of Medicine, Los Angeles, CA

We have been studying the expression of Interleukin-3 (IL-3) in HTLV-infected and uninfected T-cells and non-T-cells. DNase I footprinting of the IL-3 promoter shows 2 regions of DNA-protein interactions using nuclear extracts from HTLV-uninfected cells. One region extends from bp -173 to bp -128 which contains a potential CREB and Octamer binding site. A second footprint extends from bp -69 to bp -49. Footprinting of sequences using extracts from HTLV-infected cells, which do not express IL-3, shows protection of sequences from bp -173 to bp -103 which extends into the lymphokine consensus sequences, CK-1 and CK-2. To characterize the proteins binding to the footprinted regions, electrophoretic mobility shift assays coupled with UV cross-linking were done using T-cell crude nuclear extracts. UV crosslinking studies demonstrated 5 protein species of 35, 56, 61, 100 and 135 kD molecular weight. In addition, a 31 kD protein was present in the HTLV-infected S-LB-1 cells and in HeLa cells but not MLA 144. Competition experiments demonstrated that these proteins are incapable of binding to a consensus cyclic AMP response element or to a consensus octamer recognition sequence. We are continuing to characterize these proteins to understand the molecular mechanisms controlling IL-3 expression.

B 154 TRANSCRIPTIONAL REGULATION OF THE HUMAN CD2 GENE, David Wotton, Richard A. Lake and Michael J. Owen, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The expression of the cell surface protein CD2 is regulated in a tissue specific manner. Human CD2 is found exclusively on T lymphocytes, it is detectable shortly after entry of progenitor cells into the thymus then on >95% of thymocytes and all peripheral T cells.

A strong lymphocyte-specific transcriptional enhancer was identified within a DNase I hypersensitive site 3' of the human CD2 gene. Full activity, in a transient assay in CD2 expressing T cells, resided within a 530bp region. Lower levels of activity were detected in CD2 non-expressing T and B cells, the enhancer did not function in non-lymphoid lines.

DNase I footprinting identified six *cis*-acting sequences to which proteins bound. Four were novel, one was a concensus cyclic AMP response element, the other had homology both with the binding site for Ets transcription factors and with the NF-AT sequence from the IL-2 gene. A concensus GATA-3 binding site was also identified outside the core enhancer.

Reporter constructs in which each of these motifs have been deleted, both individually and in pairs have identified the sites most important for enhancer activity. The effects of cotransfection of Ets-1 and 2, and GATA-3 expression vectors on enhancer activity in transient assays were tested.

Electrophoretic mobility shift assays (EMSA) are beind used to assess the tissue distribution of proteins binding to the enhancer, and to identify candidates for purification and cloning. Both T cell and lymphoid specific factors have been identified by EMSA.

Transcriptional Regulation in Differentiation-I & II

B 200 FUNCTIONAL SPECIFICITY OF MYOD AND E47

I.E. Akerblom and Charles P. Emerson, Jr. Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111. The muscle-specific regulatory protein MyoD and the ubiquitously-expressed transcription factor E2/5 (E47) are both members of the class of E-box binding transcription factors containing a helix-loop-helix (HLH) structural motif important for dimerization. These factors heterodimerize *in vitro* and *in vivo* and bind both an immunoglobulin E-box and the E-boxes present in many muscle-specific enhancers. The transcriptional activity of this complex *in vivo* on these various binding sites remains to be determined. We have investigated the interactions between these factors using transient transfection analysis in both C3H10T1/2 cells, a fibroblast cell line where myoD is not expressed, and differentiated 23A2 cells, an azamyoblast cell line which expresses myoD. Dose-response curves have been generated by varying the amount of EMSVmyoD or SVE2/5 expression vectors transfected both alone and in combination, and measuring subsequent transcriptional activity using either the muscle-specific reporter gene, Tnlcat, or an E2/5 (E47)-specific target, $(E2+E5)_4$TATAcat (Henthorn, et al. Science 247;467). Introduction of myoD in either cell type results in transactivation of Tnlcat but not the E2/5 target in the absence of added E2/5. This indicates that the productive complex capable of activation of the muscle-specific enhancer is not sufficient either in quality or quantity to transactivate the immunoglobulin-specific target. Conversely, transfection of SVE2/5 results in efficient transactivation of the immunoglobulin target but only a low level of activity on Tnlcat in the absence of myoD. Taken together, the results suggest that functional specificity exists *in vivo* for these targets. In addition, preliminary results of experiments where both expression vectors are cotransfected suggest that the relative amounts of myoD and E2/5 in the cell determines the transcriptional activity of the respective targets. The results imply that the ratio of these HLH factors must be tightly controlled *in vivo*.

B 201 POSITIVE SELECTION AS A POSSIBLE RESCUE SIGNAL FROM GLUCORTICOIDS INDUCED DEATH OF THYMOCYTES,

Nathalie Auphan, Anne Jézo-Brémond and Anne-Marie Schmitt-Verhulst, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906,13288 Marseille cedex 09, France.

In order to determine the T cell differentiation stages which are the target of positive selection in the thymus, we have injected mice transgenic for the T cell receptor (TcR) of a CTL clone with glucocorticoids which lead to the deletion of immature non-selected thymocytes. In the neutral haplotype where no positive selection takes place, all immature thymocytes including those which are transgenic TcR^+ were deleted, whereas in case of positive selection some immature thymocytes were rescued which beared the transgenic TcR.

We are now trying to establish an in vitro system to determine if the signal transmitted after the interaction between TcR/CD3 complexes and the positive selecting elements which allow the thymocyte to differentiate further is also involved in resistance to glucocorticoids through the inhibition of the apoptosis signal delivered by the hormone. This hypothesis is supported by the fact that activation of PKC by a phorbol ester inhibits the DNA fragmentation induced by a steroid. In non lymphoid cells, it has also been shown that c-jun and c-fos activation could inhibit the transcriptional signal mediated by the hormone receptor. All together, these results could lead to the hypothesis that activation of immature thymocytes following interaction between TcR and the positive selecting element could stimulate PKC inducing a signal that could antagonize those responsible for apoptosis.and also induce differentiation into mature T cells.

B 202 IN VITRO TRANSCRIPTION OF THE CHICK BETA GLOBIN GENE LOCUS IN CHROMATIN ASSEMBLED TEMPLATES,

Michelle C. Barton, Steven F. Bodovitz, Beverly M. Emerson, Regulatory Biology Laboratory, The Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA 92038

The adult β-type chick globin gene resides within a genomic locus of 25-30 kb of DNA, encompassing four developmentally-regulated β-type genes. In order to recapitulate transcriptional regulation as it occurs in vivo, we cloned the entire globin locus on a single cosmid clone. This 38 kb insert clone is the template employed in our in vitro transcription studies. We utilized a Xenopus egg extract as a source of histones and nucleosome assembly factors in the formation of chromatin-assembled transcription templates. Incubation of the globin cosmid clone in these chromatin-assembly extracts formed templates which are repressed for transcription. Preincubation of the globin template with nuclear extracts of β^A-globin expressing (11-day) chick embryonic red blood cells prior to assembly of nucleosomes allowed the formation of transcriptionally active DNA structures. Chromatin assembled transcription of β-globin templates was RNA polymerase II dependent, as well as tissue- and developmental stage-specific. Addition of 11-day red blood cell extracts after the assembly of nucleosomes on the globin DNA template resulted in no change in the transcriptionally repressed state of the β^A globin gene. We are employing this assay system to study aspects of developmental function and regulation, such as the function of distal elements, not previously demonstrated by in vitro transcription of free DNA.

B 203 MYOGENIN-MEDIATED ENHANCER ACTIVITY IS REGULATED BY INSULIN-LIKE GROWTH FACTOR 1.

Erick Berglund, James C. Engert and Nadia Rosenthal. Department of Biochemistry, Boston University School of Medicine, Boston, Mass. 02118.

Insulin-like growth factor 1 (IGF-1) is known to stimulate terminal differentiation of muscle cells. Here we present molecular evidence of the myogenic effects of IGF-1 on differentiating L6E9 myoblasts, a neonatal rat cell line. These cells do not express endogenous myosin light chains 1 or 3 (MLC1/3), nor do they activate transfected CAT expression vectors driven by regulatory elements from the MLC1/3 locus. Previous work by Florini's group with the L6 system has strongly suggested that IGF-1 induced differentiation occurs through a pathway that involves myogenin, the predominant muscle determination factor in L6 cells. In this study, we have demonstrated that myogenin mRNA levels are significantly increased in IGF-1 treated L6E9 cells, in the absence of endogenous MyoD activation. In addition, we have observed concomitant activation of the muscle specific MLC1/3 enhancer in response to IGF-1. Finally, we have shown that an MLC-CAT reporter construct is transactivated by myogenin in L6E9 cells. This ability of additional transfected myogenin to upregulate MLC1 implicates a threshold in myogenin concentration which leads to qualitative differences in patterns of gene expression. Our results suggest that the establishment of this threshold may be under the control of IGF-1.

B 204 PRELIMINARY CHARACTERIZATION OF THE MOUSE THROMBOMODULIN PROMOTER, Phillip Bird, Peter Niforas, Georgina Sanderson and Cathy Bird, Department of Medicine, Monash Medical School, Clive Ward Centre, Box Hill Hospital, Box Hill 3128, Australia.

Thrombomodulin (TM) is an endothelial cell receptor which is a cofactor in the thrombin-mediated activation of protein C. TM expression is decreased by inflammatory mediators such as endotoxin, interleukin-1 and tumor necrosis factor whereas it appears to increase in response to cyclic AMP analogues. TM expression can also be induced in mouse F9 teratocarcinoma cells differentiating in response to a combination of retinoic acid (RA) and dibutyryl cyclic AMP (dbcAMP), but it is not yet clear whether this results from de novo transcription (1). We have cloned the mouse TM gene and sequenced 1440 bp upstream of the initiation codon. Using RNAse protection and primer extension assays the transcriptional start point was mapped approximately 170 bp upstream of the initiation codon. CAT reporter constructs containing portions of the TM promoter region were transfected into F9 cells which were subsequently treated with RA and dbcAMP. Levels of CAT activity rose significantly in transfected, treated cells indicating that transcription of TM increases in differentiating F9 cells. The region controlling this response lies within 500 bp upstream of the site of transcriptional initiation.

(1) Imada et al, Dev. Biol. 141, 426-30, 1990.

B 205 **TRANSCRIPTIONAL REGULATION OF THE INOSITOL TRISPHOSPHATE RECEPTOR DURING HL-60 CELL DIFFERENTIATION**

Peter G. Bradford, Rose Wang, and Pei Hui, Department of Phamacology & Therapeutics, SUNY at Buffalo, Buffalo, NY 14214

The inositol trisphosphate ($InsP_3$) receptor is an important regulator of intracellular signal calcium during cell stimulation including chemoattractant and cytokine stimulation of neutrophils and differentiated human promyelocytic leukemic (HL-60) cells. Here, we examined the $InsP_3$ receptor and its encoding mRNA in HL-60 cells and in HL-60 cells treated with 1 μM retinoic acid to stimulate differentiation into mature granulocytes. Radioligand binding studies using membranes from control HL-60 cells and 1-to-5 day retinoic acid-treated cells showed that the B_{max} of $InsP_3$ receptor binding increased from 0.22 pmol/mg protein to a maximum of 0.69 pmol/mg protein after 5 days retinoic acid treatment with no change in K_D (19 nM). Northern blot analyses identified 10 kilobase $InsP_3$ receptor mRNA transcripts in both control and retinoic acid-treated HL-60 cells. The $InsP_3$ receptor mRNA was present at low levels in HL-60 cells but was significantly elevated after 24 hours treatment with retinoic acid and reached maximal levels of approximately 4-fold greater than untreated cells after 4 days treatment with retinoic acid. Nuclear run-off assays indicated that the elevated steady state level of $InsP_3$ receptor mRNA in retinoic acid-treated HL-60 cells was a direct result of enhanced transcription of the $InsP_3$ receptor gene.

These studies indicate that the increased expression of the $InsP_3$ receptor protein which accompanies granulocytic cell differentiation is a reflection of elevated $InsP_3$ receptor mRNA levels in these cells and that this elevation is a consequence of specific regulation of gene transcription. Supported in part by USPHS grant GM39588.

B 206 MECHANISM OF TRANSCRIPTION OF YEAST U6 GENE

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U6 RNA is a member of a class of small nuclear RNAs which plays an essential role in nuclear pre-mRNA splicing. The U6 gene (SNR6) is transcribed by RNA polymerase C(III) *in vitro* and *in vivo*. The sequence of the U6 gene contains motifs characteristic of both class B and class C genes: a TATA box at -25, and a B block, which is unusually located downstream of the termination signal. *In vitro* transcription of this gene requires two factors : TFIIIB, a general pol C factor, and TFIID, a general class B factor. The B block promoter element and TFIIIC, the factor binding to the B block, are not required for the accurate *in vitro* transcription of U6 gene. However, in competition experiments, the presence of TFIIIC favoured the transcription of the B block-containing U6 gene.This suggested that the binding of TFIIIC favours transcription complex assembly. In gel shift assays the binding of TFIIIC allows the formation of a heparin-resistant TFIIIB-DNA complex. Nuclease footprinting experiments identified the binding of TFIIIB and TFIID in the close proximity of the initiation site. Analysis of transcription complex assembly should shed light on the role of the general factor TFIID in polymerase selection.

B 207 **DUAL NFKB BINDING SITES WITHIN THE CONTEXT OF THE HUMAN KAPPA IMMUNOGLOBULIN INTRON ENHANCER DO NOT SIGNIFICANTLY INCREASE OVERALL ENHANCER FUNCTION.**

Susan L. Christian and Brian G. Van Ness, Dept. of Laboratory Medicine and Pathology and the Institute of Human Genetics, University of Minnesota, Minneapolis, MN 55455.

Although the murine kappa intron enhancer has been studied extensively, very little is known about the human equivalent. Comparison of the sequences between the mouse and human intron enhancers shows a high degree of sequence similarity between them with similarly spaced transcription factor binding sites. One significant difference between these enhancers is the presence of a near perfect duplication of the NFKB binding site within the human enhancer. This second potential NFKB binding site is located 3 b.p. 5' of the original site in an inverted orientation. However, mobility shift studies and methylation interference analysis of these two sites indicate that only the 3' site binds NFKB. Mutagenesis of the 5' binding site using a 1 b.p. insertion creates a perfect duplication of the binding site that is capable of binding NFKB. Previous reports have shown that duplication of the murine NFKB binding site by itself demonstrated a ~12-17 fold increase over a single copy alone (Pierce et. al., PNAS 85:1482-1485). To test the functional significance of this additional NFKB binding site within the context of the full enhancer, 248 b. p. fragments of the human kappa enhancer with and without this one b. p. insertion were ligated into a CAT expression vector containing a kappa promoter. Transient transfection into mature B cell lines and one inducible pre-B cell line resulted in a 2-3 fold level of enhancement of a dual NFKB site over a single site. These results suggest that duplication of a strong activator sequence motif within the context of a full enhancer sequence does not significantly increase overall enhancer function.

B 208 DO SINGLE-STRAND DNA BREAKS PLAY A ROLE IN SKELETAL MUSCLE REGENERATION?, G. Coulton, B. Rogers, P. Strutt. M. Skynner, J. Morgan & D. Watt. Charing Cross & Westminster Medical School, London, UK, W6 8RF.

The close relationship between DNA structure and activation of gene transcription was exemplified by Weintraub & Groudine (1976) who showed that transcriptionally active genes were preferentially sensitive to nuclease digestion. Using DNA pol I for *in situ* 'nick' translation, Dawson and Lough, (1988) showed that dividing myoblasts did not incorporate biotin-dUTP but up to 24% of recently fused myotube nuclei were labelled and concluded that differentiation depends upon transient induction and repair of single-stranded DNA breaks.

We used *in situ* DNA polymerisation to study differentiation in regenerating mdx mouse muscle, (homologue of Duchenne Muscular Dystrophy). An mdx muscle contains surviving peripherally-nucleated fibres and lesions with necrotic and regenerating muscle fibres. After reaction in a medium containing Klenow DNA polymerase and digoxigenin-11-dUTP, the nuclei of a population of mononuclear cells in the inflammatory infiltrate of mdx lesions were intensely labelled, whereas nuclei of myotubes and muscle fibres were negative (C57BL/10 controls also negative). Labelling was DNA polymerase-dependant, required a DNA template with single-stranded gaps but was not due to exogenous exonuclease activity as a range of DNA polymerases gave the same staining pattern. Labelling was protease insensitive, and was blocked by pre-incubation in a medium containing dideoxynucleotides.

X-irradiation of mdx muscle prevents myoblast proliferation but not necrosis. Even 70 days after irradiation there were virtually no positive cells within mdx lesions (unirradiated controls had many). Therefore, DNA polymerase-labelled cells are endogenous to muscle and are not replenished from elsewhere. Structurally similar cells whose nuclei contain immunohistochemically-localised myogenin were also seen within mdx lesions but we have not been able to combine these two methods.

These results strongly indicate a role for DNA single-strand breakage in regenerative lesions of mdx mice. We must identify the cells involved and characterise the nature of DNA breaks within candidate genes such as, muscle creatine kinase. Clearly, *in situ* DNA polymerisation will prove to be beneficial for the investigation any cell differentiation system for example, during embryogenesis or carcinogenesis.

Dawson B. A. & Lough J. (1988) Dev. Biol. 127, 362-367
Weintraub H. & Groudine M. (1976) Science 193, 848-856

B 209 ANALYSIS OF HUMAN GLOBIN GENE SWITCHING IN TRANSGENIC MICE.

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High level expression of the genes of the human β-globin locus is driven by the locus control region (LCR) which is located at the 5' boundary of the locus. Our data suggests that switching from γ-globin expression in the foetal liver to β-globin expression in adult bone marrow is achieved by a combination of two different mechanisms. While silencing of the γ gene in the adult stage is mediated entirely by sequences immediately flanking the gene, the evidence suggests that expression of the β gene in the foetal stage is prevented by a combination of stage specific factors acting on the gene itself and polar competition from the γ gene.

In order to further study this phenomenon, we have developed a technique for joining cosmid inserts which has allowed us to generate transgenic mice carrying a 70 kb fragment containing the complete human β-globin locus. Transgenic mice have also been generated carrying a γ gene which contains the mutation at -117 which is associated with Greek hereditary persistence of haemoglobin and data will be presented on the expression of the mutant gene during mouse development.

B 210 **DEVELOPMENTAL ESTABLISHMENT OF HUMAN LIVER ALCOHOL DEHYDROGENASE GENE EXPRESSION CORRELATES WITH PROMOTER ACTIVATION BY HNF1, C/EBP, LAP, AND DBP**

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The human class I alcohol dehydrogenase (ADH) gene family contains three closely-related members which undergo sequential activation during liver development. The *ADH1*, *ADH2*, and *ADH3* genes are activated in early fetal, late fetal, and postnatal liver, respectively. The steady increase of ADH activity throughout liver development correlates with the maturation of the liver in general, and this may be related to the role of ADH in the synthesis of retinoic acid needed for differentiation of epithelial cells in the liver. An analysis of the promoters controlling the three human class I ADH genes has revealed that they display differential activation by several factors previously known to control transcription in the liver. In cotransfection assays utilizing mammalian expression vectors for liver transcription factors the *ADH1* promoter, but not the *ADH2* or *ADH3* promoters, was shown to respond to the transcription factor HNF1 which has previously been shown to regulate transcription in early liver development. The *ADH2* promoter, but not the *ADH1* or *ADH3* promoters, was shown to respond to C/EBP which has previously been shown to be particularly active as a transcription factor during the period from late fetal liver development to early postnatal liver development. The *ADH1*, *ADH2*, and *ADH3* promoters all responded to the liver-specific transcription factors LAP and DBP which have previously been shown to regulate transcription primarily in mature adult liver. The activation by LAP or DBP was very high compared to HNF1 or C/EBP activation, and a significant synergism between LAP and C/EBP was noticed when both factors were introduced simultaneously by cotransfection. Mutational analysis allowed the identification of a conserved element located at about position -40 bp upstream of the transcription start site which is responsible for promoter regulation by LAP and DBP, and there was no requirement for a TATA box at position -30 bp to exert these effects.

B 211 CLONING OF THE CELLULAR FACTOR WHICH BINDS THE FAR UPSTREAM ELEMENT (FUSE) OF THE C-MYC GENE, Robert Duncan[1], Mark Avigan[2], Leonard Bazar[2], Henry Krutzsch[1], and David Levens[1], [1]Lab of Pathology, National Cancer Institute, NIH, Bethesda, MD 20892 and [2]Department of Pathology, Georgetown University School of Medicine, Washington D.C. 20007

FUSE binding activity is reduced in nuclear extracts from HL60 and U937 cells following differentiation corresponding with the loss of c-myc transcriptional initiation. A FUSE binding protein (FBP) was purified and sequence obtained. Oligonucleotide primers based on this sequence amplified DNA fragments from undifferentiated U937 RNA by the reverse transcriptase-polymerase chain reaction. One of these PCR fragments hybridized with 3 overlapping clones from an undifferentiated HL60 cDNA library. Sequencing these clones revealed 1.8 kb of cDNA containing 377 AA residues of open reading frame encoding 41 kd of protein. The amino acid sequence is not homologous to any known DNA binding protein or transcription factor and contains a novel structure repeated 3 times at amino acid numbers 50, 100 and 150. A bacterially expressed, glutathione S-transferase/FUSE factor fusion protein exhibits FUSE binding specificity identical to the purified FBP in southwestern blots and gel retardation assays. DNA fragments from within the cDNA clone hybrize to a unique 2.6 kb mRNA on northern blots of poly A^+ RNA from most cell types and tissues examined. The steady state level of the message is dramatically reduced in HL60 cells upon differentiation. Co-transfection of a plasmid construct containing a FUSE factor message in the sense and antisense orientation with an appropriate reporter plasmid are in progress. The DNA binding specificity of the recombinant protein and reduction of the FUSE factor mRNA with differentiation suggest that we have cloned a FUSE binding protein which may play a role in c-myc gene expression.

B 212 IDENTIFICATION OF A NOVEL ZINC-FINGER CONTAINING DNA-BINDING PROTEIN RELATED TO THE DROSOPHILA KRUPPEL PROTEIN, Caterina Fognani and Lee E. Babiss, Department of Molecular Cell Biology, The Rockefeller University, New York, N.Y., 10021

We have previously shown that E1A oncogene expression is negatively regulated in undifferentiated fetal rodent cell-types by a nuclear factor that binds to the E1A promoter region, located just upstream of the canonical enhancer element. To begin to understand mechanistically how such a factor can function to regulate E1A gene transcription, we have used an oligonucleotide probe containing a binding site for this protein, to clone the cellular gene which encodes this factor. DNA sequence analysis of the 2.4kb cDNA clone analyzed revealed the presence of at least 4 well conserved zinc-finger DNA binding motifs, that are highly related to those found in the drosophila Kruppel protein. Additional studies have revealed that the gene encoding this protein is not a member of a multigene family of proteins. Analysis of the tissue distribution of the mRNA (3Kb) suggests a wide distribution of expression, but at highly variable rates among the different tissues. The significance of this variation in gene dose per organ and structure-function analyses will be presented.

B 213 **ZEB : A REPRESSOR OF E-BOX - MEDIATED TRANSCRIPTION**

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The developmental and tissue-specific regulation of transcription has been shown in a number of systems to be mediated by both positive and negative acting factors. The enhancers of a number of well-studied genes, including the immunoglobulin heavy (IgH) and light chain genes, the insulin gene, and the muscle creatine kinase gene contain the consensus sequence CANNTG. This motif, termed the E-box, is capable, in all of these systems, of being bound by various members of the basic-helix-loop-helix family of DNA-binding proteins (e.g. MyoD, E12/E47/E2-5, etc.), and to play a critical role in the tissue-specific expression of these genes. We have demonstrated previously that an E-box in the IgH enhancer (μE5) acts to stimulate transcription from a minimal promoter in B cells but actively represses transcription in non-B cells. Over-expression of the μE5-binding transcriptional activator E2-5 (ITF-1) results in a de-repression of transcription to normal levels. Using an oligonucleotide probe containing the μE5 site, we have isolated from a HeLa cell cDNA library a novel E-box - binding protein with three zinc fingers that we have designated ZEB (Zinc finger E-box-Binding protein). Here, we present evidence that ZEB, which binds a subset of E-boxes, is responsible for the repression of bHLH-mediated transcription seen in vivo in the IgH enhancer.

B 214 BRAIN 3.0: A POU-DOMAIN REGULATORY GENE EXPRESSED DURING NEURONAL DEVELOPMENT AND IN MATURE RAT SENSORY NEURONS, Michelle Renee' Gerrero, Bob McEvilly, Eric Turner, Xi He, and Michael G. Rosenfeld. University of California, San Diego and the Howard Hughes Medical Institute, La Jolla, California 92093. One family of sequence-specific DNA-binding proteins involved in mammalian development is the POU-domain gene family. Recently, a large family of POU-domain genes were identified in mammals, all of which were shown to be expressed during neuronal development[1]. We report the isolation and characterization of one of these new POU-domain genes: Brain 3.0. Expression of Brain 3.0 is first detected on rat embryonic day 11 in the developing neural tube. By embryonic day 13, Brain 3.0 expession is restricted to the developing brainstem, spinal cord and sensory ganglia. In the adult rat, Brain 3.0 is expressed primarily in the sensory ganglia. Brain 3.0 specifies a sequence-specific DNA-binding protein which is likely to be involved in the regulation of neuronal gene expression. A member of the POU-IV class, Brain-3.0 is highly homologous to the C. elegans unc-86 which is expressed in certain neuronal cell linages and is necessary for the determination of neuronal cell identity[2,3]. The similarity between the protein sequence and the pattern of expression in such divergent species as nematodes and mammals suggests highly conserved roles for the two proteins. Brain 3.0 is also highly homologous to the Drosophila POU-proteins I-POU and tI-POU which are transcriptional regulators in the developing Drosophila nervous system[4].

1 He, X., et al. Nature 340:35-42, 1989.
2 Finney, M., et al. Cell 55:757-769, 1988.
3 Finney, M., et al. Cell 63:895-905, 1990.
4 Treacy, M. N., et al. Submitted.

B 215 ANALYSIS OF PROMOTER-ENHANCER INTERACTIONS IN THE MLC1/3 LOCUS, Xiaohua Gong and Nadia Rosenthal, Dept. of Biochemistry, Boston University School of Medicine, Boston, MA 02118

The mechanism of promoter activation by distal regulatory elements in eukaryotic genomes remains obscure. We have analyzed the MLC1/3 locus as a model of distal enhancer action. The rat myosin light chain 1 and 3 proteins are generated from a single gene by transcription from two different promoters, 10kb apart. Both promoters are regulated by a strong muscle specific enhancer (180bp) located 3' to the MLC1/3 gene. During myogenesis, expression of MLC1 precedes that of MLC3, suggesting that the internal MLC3 promoter is independently regulated in developing muscles. To elucidate the unique properties of the MLC3 promoter, we generated deletions in the 629bp preceding the MLC3 start site. When these deletions were used to drive a CAT reporter gene linked to either an SV40 or an MLC enhancer, and transfected into C2C12 and L6 cells, we found some regions in the MLC3 promoter that are important for SV40 enhancer activity but not for MLC enhancer activity, and vice versa. We are defining the relative role of each MLC3 promoter region in muscle-specific expression by testing MLC enhancer mutants in these constructs. To elucidate the potential interactions between the MLC3 promoter and MLC enhancer, we are studying factors binding to the three E boxes (CANNTG) in the MLC3 promoter and the four E boxes in the MLC enhancer. One of the enhancer motifs, CATGTG, does not interact with myogenic factors, but specifically binds a novel factor present in certain non-muscle cells. We have isolated a corresponding clone, BRG1, from a Hela cDNA expression library using an oligonucleotide screen. Northern analysis reveals at least two transcripts (6 and 7 kb) corresponding to BRG1, present in Hela and Jurkat cells but absent in muscle cell lines. These data suggest that both myogenic and non-myogenic factors are involved in enhancer-mediated activation of MLC promoters.

B 216 DEVELOPMENTAL REGULATION OF A MUSCLE - SPECIFIC TRANSGENE. Uta Grieshammer, David Sassoon, and Nadia Rosenthal. Department of Biochemistry, Boston University School of Medicine. Boston, MA 02118.

The myosin light chain (MLC) 1/3 enhancer is a muscle-specific regulatory element that is responsive to myogenic factors in tissue culture. In order to understand the function of this enhancer *in vivo* we analyzed transgenic mice carrying multiple copies of an MLC1 promoter-CAT construct driven by the MLC enhancer. Using *in situ* hybridization we found that the transgene is expressed - like the endogenous MLC1 gene - exclusively in skeletal muscle. However, the spatial transcriptional regulation of the endogenous gene and the transgene are not identical. In newborns and adults, MLC1 transcripts are present in a subset of muscles, and the transgene is activated in many but not all muscles expressing MLC1. As opposed to the uniform expression of MLC1 in the intercostal and intervertebral muscles, CAT mRNA appears in a gradient with decreasing amounts of signal in a caudal to rostral direction. In addition, transcripts of the transgene are not detectable in some head and neck muscles and in the diaphragm where the endogenous gene is expressed. Interestingly, these differential patterns of expression between the endogenous gene and the transgene are already established during embryonic development, appearing as soon as the first transcription of MLC1 in the somites is detectable. In a comparative analysis, no correlation could be established between the expression patterns of MLC1 or CAT and the presence of transcripts of fiber-specific structural genes or several regulatory genes including the myogenic factors. Using additional CAT constructs in transgenic mice and primary cell cultures of different transgenic muscle types, we are currently identifying cis- and trans-acting elements responsible for the complex regulation of the MLC 1/3 locus *in vivo*.

B 217 REGULATION OF EXPRESSION OF THE HUMAN BASAL HISTONE VARIANT H2A.Z GENE IN EMBRYONIC AND DIFFERENTIATED HUMAN EMBRYONAL CARCINOMA (N-TERA) CELLS, Christopher L. Hatch and William M. Bonner, Laboratory of Molecular Pharmacology, National Cancer Institute, Bethesda, MD. 20892.

Histone H2A.Z is unique among histones in that it has only 60% amino acid sequence homology with other members of the H2A family; in addition, its sequence has been more highly conserved throughout evolution than that of the other H2As. The amount of H2A.Z transcript is unlinked to DNA replication; however, it is greatly down-regulated in quiescent cultures, due in part to a decrease in the rate of transcription. In addition, the rate of transcription of this gene appears to be elevated in particular embryonic states. Unlike the DNA-replication-linked histone variant genes the gene encoding H2A.Z is interrupted by intervening sequences. Promoter sequences upstream from the H2A.Z gene have been delineated by assay of CAT gene expression in transfected human embryonal carcinoma (N-Tera) cells as well as other human cell types. The core promoter region has been localized to the 234 base pairs upstream from the transcription start site. This region includes two GC boxes and three CCAAT boxes as well as a properly positioned TATA box. The proximal upstream region of sequence has been found to repress transcription initiated by the H2A.Z core promoter region as well as transcription initiated by the heterologous SV40 early promoter in differentiated N-Tera cells but not in undifferentiated embryonic N-Tera cells. Cis-acting sequence elements critical to the modulation of the rate of transcription of this gene have been localized by DNase I footprinting and gel shift assays. We are investigating how the factors that bind these sequences may individually or in concert affect the rate of transcription of this gene.

B 218 INDUCTION OF MONOCYTIC DIFFERENTIATION AND NF-κB ACTIVITY BY HIV-1 INFECTION

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The effects of HIV-1 infection on myeloid differentiation, induction of NF-κB DNA binding proteins and NF-κB regulated cytokine expression were investigated in a new model of HIV-1 infection. PLB-985 cells represent a myelomonoblastic cell population capable of granulocytic or monocytic differentiation following induction with different agents. PLB-985 cells were infected with HIV-1 strain IIIB; selection of chronically infected PLB-IIIB cells generated a population with a more monocytic phenotype as determined by differential staining, expression of monocyte specific surface markers and increased transcription of the *c-fms* proto-oncogene.Chronic HIV-1 infection of PLB cells did not lead to constitutive cytokine production; however, stimulation of PLB-IIIB cells by PMA or TNF resulted in high levels of IL-1β and TNF-α RNA and protein. To investigate the molecular basis of monocytic differentiation and gene expression in HIV infected cells, regulation of NF-κB activity was examined. Multiple DNA binding forms of NF-κB were purified and analyzed for interaction with the HIV enhancer or the IFN-ß PRDII domain. Polypeptides of 47, 51 and 56 kD possessed a high affinity for the palindromic PRD II site while p65, p72 and p85 had a low DNA binding affinity. Nonetheless, p65, p72 and p85 proteins were capable of forming DNA binding heterodimers with the p51 subunit in co-renaturation experiments. When NF-κB proteins from PLB-IIIB cells were analyzed by UV cross-linking, constitutive expression of a novel p86-p98 NF-κB complex was detected; this complex had a higher affinity for the PRD II NF-κB site than for the HIV enhancer. These experiments demonstrate that HIV-1 infection of myeloblasts may select for a more differentiated monocytic phenotype and that distinct NF-κB subunit associations may contribute to differential NF-κB mediated gene expression.

B 219 SERUM GROWTH FACTOR INDUCTION OF NFκB AND IκB mRNAs, Caryn Y. Ito, Jane C. Azizkhan and Albert S. Baldwin Jr., Curriculum in Genetics and Lineberger Comprehensive Cancer Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

NFκB, a nuclear transcription factor is constitutively expressed in mature B cells and was first characterized as a κ-light chain enhancer binding protein. It has since then been reported to bind numerous enhancer sequences including the MHC class I and HIV LTR enhancer sequences. In other cells, NFκB is sequestered in the cytoplasm by IκB, the inhibitor of NFκB. *Invitro* studies show that phosphorylation of IκB releases NFκB from this complex. NFκB is then thought to move into the nucleus where it binds to the DNA sequences and activates transcription. One form of NFκB is made up of a p65kD and p50kD heterodimer. The p65kD subunit contains the IκB interaction domain as well as a DNA binding domain. The p50kD subunit also contains a DNA binding domain and can form a homodimer, KBF-1. The p65, p50 and IκB cDNAs have recently been identified. Since serum growth factors have been shown to induce NFκB DNA-binding activation, the effects of serum induction on messages encoding IκB, p65 and p50 were examined in 3T3 cells. The cells were starved in 0.5% serum for 24-36 hours, stimulated with 20% serum, and harvested at various times. Analysis of these messages by Northern blotting indicate that the IκB message dramatically increases as early as one hour after serum stimulation. The p50 message, however, peaks much later between one and six hours while the p65 message is not affected by the addition of serum. Our data indicate that IκB is an early-immediate gene and that p50 and p65 are not simultaneously regulated. Ongoing experiments are underway to determine which serum growth factors and which transcriptional activators are responsible for induction of IκB and NFκB mRNAs.

B 220 REGULATION OF CLASS II MHC GENES BY cAMP AND DOMINANT NEGATIVE MUTANTS OF TRANSCRIPTION FACTOR mXBP (CRE-BP, ATF-2), Lionel B. Ivashkiv, Mark D. Fleming, and Laurie H. Glimcher, Department of Cancer Biology, Harvard School of Public Health, and Department of Medicine, Harvard Medical School, Boston, MA 02115

Aberrant expression of class II major histocompatibility (MHC) genes occurs in several autoimmune and inflammatory diseases, such as rheumatoid arthritis or lupus nephritis. Understanding mechanisms for downregulating class II MHC expression would be useful in designing therapeutic approaches to treat inflammatory diseases. Cell surface expression of class II antigens is known to be suppressed by agents, such as E series prostaglandins, which activate the cAMP signal transduction pathway. We have undertaken an analysis of cAMP-dependent repression of class II MHC genes by mapping the promoter elements which mediate repression and by manipulating the activity of a transcription factor, mXBP (CRE-BP, ATF-2), which binds to a cAMP response element (CRE) in the murine class II MHC Aα gene promoter. Surprisingly, repression by cAMP is not mediated by the CRE, but instead is mediated by the S and X1 DNA elements conserved in all Class II MHC promoters. We have created a panel of DNA-binding domain mutants of mXBP and correlated binding to a CRE with the ability to activate a reporter gene. Several of these mutants can suppress DNA-binding and transcriptional activation by wild type mXBP. These mutants are being overexpressed in cultured cells and in the lymphoid organs of transgenic mice and the resulting phenotype will be described.

B 221 TIME COURSE OF DOWN-REGULATION OF OCT-2 AND NF-KB IN EARLY HYBRIDS BETWEEN HUMAN LYMPHOMA CELLS AND FIBROBLASTS, Steffen Junker, Martin Lamm, Viggo Nielsen, and Patrick Matthias, Institute of Human Genetics, University of Aarhus, DK-8000 Aarhus C, Denmark, and Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel, Switzerland

When immunoglobulin (Ig) producing B cells are fused with fibroblasts, expression of Ig genes is selectively blocked at the level of transcription. We have previously shown that both the kappa promoter and the IgH intron enhancer are targets for this suppression mechanism and that Ig gene suppression correlates with absence of the B cell-specific transcription factor Oct-2 and its transcripts. On the basis of nuclear run-on transcription assays we demonstrate here that Oct-2 gene transcription is blocked in such hybrids. Moreover, by using fluorescence activated cell sorting to isolate hybrids from within 6h after fusion, we show that the time course of down-regulation of Oct-2 transcripts correlates with that of Ig transcripts. Also NF-KB is rendered transcriptionally inactive in B cell x fibroblast hybrids. The time course of disappearance of nuclear NF-KB, monitored by band shift assays, is presented. The rapid down-regulation of Ig gene transcription as a result of fusing B cells with fibroblasts can be delayed, but ultimately not avoided, if the fibroblast partner is gamma-irradiated prior to fusion. We show that both NF-KB and Oct-2 contribute to that phenomenon.

B 222 Repression of Immunoglobulin Enhancers by the Helix-Loop-Helix Protein Id: Implications for B Lymphoid Development

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It has been proposed that the helix-loop-helix (HLH) protein Id serves as a general antagonist of cell differentiation by inhibiting bHLH proteins specifically required for developmental programs (such as MyoD). We show here that ectopic expression of Id represses in vivo activity of the bHLH protein E2-5 (encoded by the E2A gene) and of both the immunoglobulin heavy chain (IgH) and kappa light chain (Igκ) gene enhancers to which E2-5 binds. Id does not affect the activity of the bHLH-zip protein, TFE3, which also binds these enhancers. We examined a large panel of B cell lines that represent different stages of lymphoid development and found only two that express Id mRNA. Those cell lines, Ba/F3 and LyD9, have been categorized previously as early B lymphoid progenitors. Unlike their more mature B lymphoid counterparts, Ba/F3 and LyD9 cells do not express Iμ sterile transcripts, which are indicative of IgH enhancer activity. Moreover, Ba/F3-derived nuclear extracts lack E2-box binding activity—indicating the absence of free bHLH proteins—and transfected Ba/F3 cells fail to support activity of the IgH enhancer. Hence, expression of Id correlates inversely with bHLH protein activity and enhancer function in vivo. These results suggest that Id may play a role early in B lymphoid development to regulate transcription of the immunoglobulin heavy chain locus.

B 223 USING A YEAST TRANSCRIPTION ASSAY TO STUDY DNA RECOGNITION BY REL FAMILY PROTEINS AND TO ISOLATE GENES CODING FOR INTERACTING FACTORS, Joanne Kamens, Russell Finley and Roger Brent, Department of Genetics, Harvard Medical School, Boston, MA 02115

vRel, cRel, Dorsal and NF-κB, members of a larger family of DNA binding regulatory proteins, have been implicated in activation as well as repression of target gene expression. Rel proteins interact to form homo- and heterodimers, recognize specific sites on DNA, and are known to interact with unrelated cellular factors; it is likely that such protein-protein and protein-DNA interactions contribute to proper regulation of target gene expression by these proteins. In previous work we used a yeast transcription activation assay to localize transcription activation domains of LexA-Rel fusion proteins (Kamens, et al. (1990) Mol. Cell. Biol. 10:2840-2847). More recently, we have taken advantage of the fact that Rel proteins activate transcription in yeast to study the binding of three Rel family proteins to their native sites (Kamens and Brent (1991) The New Biol., in press). These results show that vRel and cRel recognize two known NF-κB binding sites; Dorsal does not recognize NF-κB sites, but does recognize related sites upstream of the *Drosophila zerknüllt* (*zen*) gene. Our experiments demonstrate that the members of this protein family recognize similar, but not identical, sites in the promoters of target genes and identify a particular nucleotide apparently involved in the DNA-protein interaction. We also exploit the properties of LexA fusion proteins to study the dimerization and DNA contacting domains of cRel. Our results suggest that cRel homodimerizes and that dimer formation may be required for cRel to bind DNA. Transcription activation by the Rel proteins is cooperative; such cooperativity may be important for correct temporal and spatial regulation of target gene expression.

In the *Drosophila* embryo, Dorsal is thought to activate expression of the *twist* and *snail* genes, but to repress expression of the *zen* and *decapentaplegic* genes. In our system, Dorsal activates expression of target genes that contain the DNA element from the *zen* gene that is required for repression by Dorsal. As in previous studies, we account for this difference by suggesting that *Drosophila* contain accessory factors required for repression of *zen* expression which are absent in yeast and we are taking advantage of this difference to isolate genes encoding *Drosohila* proteins necessary for Dorsal to repress expression of *zen*. We are constructing a cDNA library that will express *Drosophila* proteins in yeast and plan to screen this library for clones that cause Dorsal to repress transcription in our system. To this end we are also designing yeast selections which will allow us to select for yeast cells in which the target gene is being repressed. Current selections only allow for the selection of yeast cells in which a target gene is being activated.

B 224 Factors Regulating Murine CD8 Gene Expression, Deborah B. Landry, James D. Engel, and Ranjan Sen. Department of Biology, Brandeis University, Waltham, MA 02254; and Department of Biochemistry and Molecular and Cellular Biology, Northwestern University, Evanston, IL 60201.
During T cell development, there is regulated expression of the T cell receptor and the coreceptors CD4 and CD8. Using a DNAse I hypersensitivity assay, we have identified potential cis-acting elements involved in the transcriptional regulation of the murine CD8 (Lyt2) gene. Three hypersensitive sites (HSS) located at -4.0 kb, and -2.0 kb, and in the promoter region correlate with CD8 cell surface expression in several murine thymoma cell lines. A T cell-specific DNA binding protein interacts with two adjacent elements within the -4.0 kb HSS region. These elements have strong sequence homology to a GATA binding motif (originally identified in erythroid-specific genes) as determined by the methylation interference pattern. In vitro translated GATA-3, a known T cell-specific GATA-binding protein, binds both CD8 GATA elements specifically and comigrates with the endogenous DNA binding protein in a gel shift assay. In addition, a GATA-3 expression vector can transactivate a reporter construct containing CD8 GATA dimers or a 150 bp fragment from the -4.0 kb HSS when cotransfected into non-lymphoid HeLa cells. Together this data suggests that the -4.0 kb HSS and the GATA sequences contained therein may play a role in regulating the T cell-specific expression of the CD8 gene.

B 225 DIFFERENTIAL EXPRESSION OF HOMEOBOX-CONTAINING GENES IN HEMATOPOIETIC TISSUES, Katharine M. Lang and James D. Griffin, Division of Tumor Immunology, Dana-Farber Cancer Institute, Boston, MA 02115
One of the major goals in biology is to understand the processes of development and differentiation. The hematopoietic system is a particularly good model for the study of developmental processes for several reasons. Blood cells are continuously being renewed *in vivo* from pluripotent stem cells in the bone marrow, and both stem cells and differentiating cells are readily available. In addition, both normal and neoplastic hematopoietic cells can be cultured *in vitro*, and their differentiation can be induced by the addition of external factors. One of the most useful hematopoietic cell lines is the human HL-60 promyelocytic leukemia line, which is biphenotypic and can be induced to differentiate to either monocytes or neutrophils. Differentiation of various cell types is thought to be initiated by activation of tissue-specific 'master switch' genes, which regulate whole programs of differentiation. In certain non-hematopoietic tissues, members of the homeobox-containing gene family are known to control aspects of morphogenesis and tissue-specific differentiation. Preliminary studies demonstrated that hematopoietic cells express several homeobox genes. Such genes are likely candidates to control differentiation in hematopoietic cells. Using inverse PCR a series of homeobox-containing genes expressed in HL-60 cells after induction with retinoic acid have been cloned. Seven homeobox-containing genes have been identified so far, five known genes and two potentially novel genes. The expression of one of the known genes, Hox 1.1, is induced 3-5 fold following induction of differentiation by retinoic acid. In addition, Hox 1.1 appears to be differentially expressed among the hematopoietic cell lineages. It is expressed in other myeloid cell lines such as U937 and Josk and in B-cell lines such as Daudi and Ramos. However, Hox 1.1 is not expressed in the erythroid cell line K562 or in the T-cell lines HPB-ALL and HT. We are further characterizing these homeobox-containing genes and are continuing to clone and identify novel genes.

B 226 CHARACTERIZATION OF A NEW FAMILY OF DNA BINDING PROTEINS EXPRESSED DURING DIFFERENTIATION OF TERA 2 CELLS, T.R. LeBon, E. Hodge, T.H. Huang, T. Okada, B.W. Merrills, P.N. Gertson, and K. Itakura, Dept. of Mol. Genet., City of Hope Beckman Res. Inst., Duarte, CA 91010
The major immediate-early (MIE) gene of human cytomegalovirus (HCMV) has provided a probe to identify and clone cellular DNA-binding proteins which are associated with gene regulation during differentiation. The modulator of MIE, (-1140 to -725) functions as a suppressor in non-permissive cells but enhances gene transcription in permissive cells. Tera-2 cells, a human embryonal carcinoma celline, are non-permissive, however, retinoic acid treatment switches these cells to a permissive state as well as inducing cellular differentiation. Our immediate goal was the molecular cloning of HCMV-MIE modulator binding proteins that may mediate the cell-specific activity of this element. Several selected sequence motifs within the modulator region of the HCMV-MIE gene were used to probe a human fibroblast cDNA lambda gt11 expression library. We are characterizing two clones (MRF-1 and MRF-2) of particular interest. *In vitro* translated proteins from partial cDNAs of both clones demonstrate sequence-specific DNA binding properties with overlapping recognition properties. Sequence data has revealed an extensively conserved domain in the clones: a 63% homology of DNA sequence with a corresponding 72% homology of predicted amino acid sequence within a region of 115 amino acids. No significant homology to other genes/proteins was found by database searches. Northern analysis of mRNA and total RNA has shown that MRF-1 hybridizes to a 7.5 kb message in retinoic acid treated Tera-2 cells while untreated cells have undetectable levels. In contrast, MRF-2 hybridizes to a 2.3 kb species which is expressed constitutively in Tera-2 and is unaffected by retinoic acid treatment. The potential role of these genes in regulating viral expression and cellular differentiation is being examined.

B 227 USING IN-VITRO PHOSPHORYLATED NF-KB p50 PROTEIN TO STUDY ITS INTERACTIONS WITH OTHER PROTEINS. Kenneth P. LeClair, Michael A. Blanar*, Phillip A. Sharp, MIT Center for Cancer Research, Cambridge, MA 02139; *UCSF Dept of Biochemistry and Biophysics, San Francisco, CA 94143.
An emerging principle in eukaryotic transcription is that the control of gene expression is governed in large measure by a complex, coordinated interaction between protein transcription factors and DNA binding site sequence elements in gene enhancers and promoters. The NF-KB factor is one example of a transcription factor whose DNA recognition element is shared by many genes, especially those involved in induced immune responses. Recently several groups obtained cDNA clones for the p50, p65 and I-KB proteins of the NF-KB complex. The cloning of p50 and p65 revealed that they both contain a domain with striking homology to rel oncoproteins and to the Drosophila gene product, dorsal.

By modifying a human NF-KB p50 cDNA we generated a form of p50 which can be ^{32}P-radiolabeled by in vitro phosphorylation. A 15 amino acid fragment was appended onto the amino terminus of the p50 protein to introduce an antigenic epitope as well as a specific substrate for heart muscle protein kinase. This modified p50 protein was expressed in a T7 promoter-driven bacterial system, purified by column chromatography, and shown to specifically bind NF-KB and related DNA sequence elements in gel mobility shift assays. When used as a probe of Western blots, the radiolabeled NF-KB p50 protein formed homodimers and heterodimers with rel-related proteins as well as with several cellular proteins. When radiolabeled NF-KB p50 protein was used as a probe of a λgt11 expression library we obtained cDNA clones containing the rel-homology regions of the human c-rel protein and the NF-KB p105 protein that is the precursor of NF-KB p50.

Another library screen performed under less stringent conditions has led to the isolation of cDNAs that encode proteins that are not members of the rel family of proteins. Databases searches revealed that some of these cDNAs had been previously cloned and encode transcription factors that bind DNA sequences distinct from the NF-KB sequence. The specificity of the interactions between NF-KB p50 and these non-rel proteins has been demonstrated by Western blotting and confirmed by immune coprecipitation studies. We are investigating the interactions between these transcription factors in gel mobility shift analyses. Because several immunologically relevant promoters contain both of these DNA binding sites, it is likely that the interaction between these transcription factors in vivo has functional significance for controlling gene expression.

B 228 MOLECULAR CLONING AND EXPRESSION ANALYSIS OF THE MHC-NB FACTOR WHICH BINDS TO THE NEGATIVE REGULATORY ELEMENT OF THE MHC CLASS I GENE

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The negative regulatory element (NRE) of the MHC class I gene has been shown to be involved in the repression of transcription in F9 EC cells. Using an NRE oligonucleotide as a probe in a southwestern assay, we have isolated a cDNA clone (MHC-NB) which encodes a new member of a distinct DNA binding protein family. This family is characterized by a nearly identical DNA binding region and alternating clusters of positive and negative charged amino acids and includes the MHC class II Y box binding factor YB-1/DbpA and the Xenopus Y box binding factors FRG Y1 and FRG Y2. The DNA binding region is homologous to the E. coli cold shock protein which is induced in response to low temperature. Northern and S1 nuclease assays indicate the presence of two alternatively spliced forms of MHC-NB mRNA with the shorter mRNA lacking a 207 bp region adjacent to the putative DNA binding region. The ratio of the two mRNA forms is variable in different tissues and cell lines. In testes, as well as undifferentiated F9 and P19 EC cells, the longer mRNA predominates, where as in spleen, thymus and differentiated NIH3T3, BW5147 and EL4 cells, the shorter mRNA is more abundant. Studies are being performed to determine the functional role of MHC-NB and whether alternative splicing is involved in its function.

B 229 COORDINATE EXPRESSION OF THE *DROSOPHILA* TROPOMYOSIN GENES IS CONTROLLED BY MUSCLE-TYPE SPECIFIC CIS-ACTING ELEMENTS

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Drosophila contains two tropomyosin genes that are coordinately expressed with other muscle genes during myogensis. Transcriptional control of the two tropomyosin genes (TmI and TmII) has been investigated by P-element transformation of Tm/*lacZ* and hsp70/*lacZ* reporter genes expressed under the control of Tm gene cis-acting control elements. The *lacZ* encoded ß-galactosidase activity serves as a marker for tissue-specific expression. The results of this work have allowed us to identify distinct cis-acting regions within the first intron of both genes that control the temporal regulation, the tissue-specificity, and the levels of expression of the genes. The patterns of ß-galactosidase activity as determined by X-gal substrate staining for the different elements correlate with endogenous expression of the Tm genes as determined by *in situ* hybridization of Tm gene probes. A 355 bp fragment located in the first intron of the TmI has been dissected and shown to contain separate cis-elements that control expression in different muscle of the embryo/larva and adult. A similar fragment occurs in the first intron of the TmII gene. Furthermore, the control regions, as determined by deletion analysis, bind stage specific proteins obtained from nuclei of late stage embryos at the time when muscle cells form.

B 230 TISSUE-SPECIFIC REGULATION OF INSULIN-LIKE GROWTH FACTOR-II (IGFII) GENE EXPRESSION DURING DEVELOPMENT.

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IGFII is involved in the regulation of fetal growth. It may also contribute to the proliferation and differentiation of a variety of somatic cell types. In human liver, IGFII gene expression is submitted to clearcut developmental and tissue-specific expression. In fetal tissues (including liver) 4 mRNAs are present. They are initiated at 3 distinct promoters : P2, P3 and P4 (leader exons 4, 5 and 6, respectively). In adult tissues, the same transcripts are synthetized, except in the liver where a single, unique, mRNA initiated at promoter P1 (leader exon 1) is detected. To unravel the molecular mechanism leading to the expression of h-IGFII gene from promoter P1 during the neonatal period, we have searched for an experimental model in which the pattern of IGFII gene expression recalls that observed in humans. Northern and dot blot analysis of RNA isolated from fetal bovine liver and muscle at various developmental stages revealed that bovine IGFII gene was expressed as a multitranscript family (5.2, 3.8, 3.4, 2.8, 2.1, 1.6, 1.3 kb) when probed with h-IGFII cDNA. In adult muscle, the pattern of IGFII transcripts was identical to that observed in fetuses. In contrast, in adult liver a single, unique transcript of 4.4 kb was synthetized. This developmental pattern of expression does remind that observed in humans. Moreover, only subsets of RNA hybridized when the northern blots were probed with human exons : human exon 5 (P3) hybridized with the 5.2, 3.4, 2.8 and 2.1 kb RNAs, whereas human exon 6 (P4) hybridized with the 3.8, 1.6 and 1.1 transcripts. The adult, liver-specific 4.4 kb RNA hybridized with neither human exon probes. In man, the different transcripts arise from multiple promoter usage, alternative splicing and polyadenylation. Assuming similar processes are operative in beef, our results can be interpreted as follows: in fetal bovine tissues IGFII gene transcription arise from at least two promoters (homologous to human P3 and P4), whereas in adult liver it is initiated at a single, distinct liver-specific promoter. If so, bovine liver will provide a reliable experimental model to study *in vitro* the mechanism instrumental in the trigger of the human liver-specific promoter P1.

B 231 REGULATION OF TRANSCRIPTIONAL ACTIVATION OF THE RAT MYOSIN LIGHT CHAIN ENHANCER

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The rat myosin light chain enhancer is a muscle-specific activator of the MLC1/3 locus. In vitro analysis of the MLC enhancer has identified three sites, A, B, C, which are E boxes and are necessary for transcriptional activation of an enhancer/CAT construct. Although all three sites bind to purified MyoD and myogenin, only sites A and C bind MyoD and myogenin in vivo as indicated by double gel shifts with myogenic antibodies using nuclear extracts. Mutational analysis also demonstrates that site B is needed for activation if site A is deleted. The B site is a consensus binding site for the transcription factor AP-4, a protein containing helix-loop-helix and leucine zipper motifs. In vitro transcribed/translated AP-4 binds to the B site but not to A or C. In functional assays cotransfecting an AP-4 expression vector with various reporter constructs, we have noted that AP-4 down-regulates the MSV and SV40 enhancers, both of which contain an AP-4 consensus sequence. We are currently investigating the role of AP-4 and other transcription factors in activating MLC transcription by using cotransfection experiments and in vivo footprinting to correlate in vitro binding data with MLC activity in the intact cell.

B 232 I-POU AND TWIN OF I-POU: A TWO AMINO ACID DIFFERENCE IN THE HOMEODOMAIN DISTINGUISHES A DNA BINDING ACTIVATOR FROM A PROTEIN BINDING INHIBITOR OF TRANSCRIPTION, Lorna I. Neilson, Maurice N. Treacy, Eric E. Turner, Xi He and Michael G. Rosenfeld. University of California, San Diego and the Howard Hughs Medical Institute, LaJolla, CA 92093. I-POU is a structurally distinct POU-domain protein which lacks two conserved basic amino acids in the N-terminal region of the POU-homeodomain and is co-expressed in the developing *Drosophila* nervous system with a second POU-domain transcription factor, Cf1-a. I-POU does not bind to DNA but forms a POU domain-mediated, high affinity heterodimer with Cf1-a, inhibiting its ability to bind and activate the dopa decarboxylase gene. The regions critical for this highly specific interaction are the basic amino acid cluster at the N-terminus of the POU-homeodomain, and the helical and specific amino acid information in the first two helicies of the POU-homeodomain. The POU-specific region and the DNA recognition helix of the POU-homeodomain are not required. Additionally, we have identified a novel, alternatively spliced transcript of the I-POU gene that encodes a variant of I-POU referred to as twin of I-POU (tI-POU) in which the two basic amino acid residues lacking within the N-terminus of the POU-homeodomain are restored. tI-POU, is no longer capable of interacting with Cf1-a, nor does it interact with I-POU. However, tI-POU is capable of specific DNA binding and transactivates reporter genes containing cognate DNA binding elements that are distinct from the Cf1-a recognition sequence. The I-POU genomic locus, therefore, simultaneously generates both a specific activator and inhibitor of distinct programs of neuronal gene transcription, differing only by the presence or absence of two basic amino acid residues in the POU-homeodomain.

B 233 CHARACTERIZATION OF CIS-ACTING REGULATORY ELEMENTS OF THE MOUSE *HOX-2.2* GENE

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Murine homeobox genes are expressed in stage-, region-, and tissue-specific patterns during embryonic development of the mouse. In order to characterize *cis*-acting regulatory elements of the murine homeobox gene *Hox-2.2*, we generated transgenic mouse embryos containing the *LacZ* reporter gene under the control of different fragments from the presumptive *Hox-2.2* promoter. In total, we have analyzed 13.2kb of genomic DNA covering the entire *Hox-2.2* region.
Our analyses suggested the presence of four regulatory elements: a limb-specific element which directed gene expression in the developing limb buds and the mesenchyme of the ventrolateral body region, a CNS element wich directed LacZ expression into the ventral regions of the developing spinal cord and a nephrogenic region directing reporter gene expression into developing mesonephric tubules and *mesonephric* ducts.
Although we have analyzed the entire *Hox-2.2* genomic region we have not identified all control elements necessary to mimic the endogenous *Hox-2.2* expression pattern. The implications of these findings for the regulation of clustered homeobox genes will be discussed.

B 234 SELECTION AND CHARACTERIZATION OF MUTANTS IN A SIGNAL TRANSDUCTION/TRANSMISSION PATHWAY, Andrew T. Serafini*, Steve Fiering*, Leonard A. Herzenberg* and Gerald R. Crabtree*†. *Department of Genetics, †Howard Hughes Medical Institute, Stanford University Medical School, Stanford, CA 94305-5125

We have used a novel approach to select clones of human Jurkat T cells that have defects in the antigen receptor signal transduction pathway. Our method employs a Jurkat human T cell line (NZdip-1) stably transfected sequentially with one construct in which NF-AT (nuclear factor of activated T cells) directs transcription of *lacZ* and then a second construct in which NF-AT directs transcription of Diphtheria toxin A chain *(dipA)* gene integrated at another site in the DNA. The single cell reporter gene assay, FACS-Gal, is used to study the activity of NF-AT after the Jurkat cells are stimulated with the calcium ionophore ionomycin and PMA. After mutagenesis, the NZdip-1 cells were stimulated and rare survivors selected. *Cis* mutations of the *dipA* locus were eliminated by selecting from the rare survivors, those cells which do not express *lacZ* after stimulation. We have isolated six independently derived *trans* mutants that activate neither NF-AT-directed construct. Several calcium-regulated events are blocked in these mutants and they do not make IL-2; however, they do induce the IL-2 receptor normally. Thus, these clones appear to have a specific defect in signal transduction. Initial complementation analysis of the clones have shown the mutants to be either dominant or in a single complementation group. Other initial studies indicate that some of the mutants are unable to translocate the consitutive cytoplasmic component of the NF-AT multimer to the nucleus.

B 235 ANALYSIS OF CIS ELEMENTS REQUIRED FOR REGULATION OF THE HUMAN α2 Na,K-ATPase GENE DURING SKELETAL MUSCLE DEVELOPMENT, Olga I. Shamraj and Jerry B Lingrel, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267

The expression of the three α-subunit isoforms of Na,K-ATPase is regulated in a tissue specific, cell specific and developmental specific manner. While the expression of α1 is ubiquitous, the α2 isoform is expressed predominantly in brain, skeletal muscle and heart and α3 is expressed primarily in brain and heart. In skeletal muscle, the α2 mRNA level increases dramatically following birth to become the major α-isoform transcript. This increase is paralleled by a large increase in high affinity ouabain binding sites in the rat soleus muscle after birth indicating that the α2 protein (a ouabain sensitive isoform) is present. Developmentally regulated induction of α2 is also observed in the murine C2C12 myogenic cell line. While proliferating myoblasts express little α2, there is a 6- to 12-fold increase in α2 mRNA levels and in ouabain-sensitive Na,K-ATPase activity in differentiated myotubes. To identify the cis-acting DNA elements of the α2 gene that are responsible for upregulation during skeletal muscle development, 1.6 kb of the upstream region of the human α2 gene was fused to the reporter gene chloramphenicol acetyltransferase (CAT). This construct has been tested in both C2 myoblasts and differentiated myotubes as well as in HeLa cells (a nonmuscle cell line that does not express the α2 gene) using transient assays. This construct gives high levels of CAT activity in C2 cells but not in HeLa cells suggesting that 1.6 kb of 5' sequence is sufficient to give muscle specific expression. Deletion analysis and site-specific mutagenesis of this construct is being undertaken to identify elements involved in upregulation of the α2 gene during skeletal muscle development.

B 236 ETS-RELATED PU.1 MEDIATES TRANSCRIPTION OF THE IMMUNOGLOBULIN J CHAIN GENE

Myung K. Shin and Marian Elliott Koshland. Department of Molecular and Cell Biology, University of California-Berkeley, Berkeley, CA 94720.

In a primary immune response, signals from the T cell lymphokines, IL-2 and IL-5, trigger antigen activated B cells to differentiate into IgM secreting cells. A critical event during this developmental process is the activation of the immunoglobulin J chain gene encoding the essential joining component of pentameric IgM. Recently, a major J chain promoter element positioned between -56 and -45 has been identified. Mutations within this region decrease the J chain promoter activity eight to ten fold. Using standard chromatographic methods, a 42 KD nuclear factor, JB, recognizing this element was purified from mature B cell line, K46R. Footprint analysis revealed that JB binds to the 12-bp AG-rich sequence (AAAGCAGAAGCA) that closely resembles the target site for Ets-related B cell and macrophage specific transcription factor, PU.1. Although the JB site lacks the critical GGAA core reported to be essential for ETS-domain binding, subsequent experiments proved that JB is PU.1. Rabbit antisera against PU.1 supershifted JB protein-DNA complex in gel mobility assay. Moreover, in vitro synthesized PU.1 migrated as a 42 KD polypeptide and protected the same residues as JB in DMS interference footprinting. Finally, proteolytic clipping binding assays showed that JB and PU.1 have identical degradation patterns. The function of the JB (PU.1) protein was analyzed by transient transfection assays in J chain-silent and J chain-expressing cells. In each case, expression of the JB (PU.1) activated cotransfected reporter constructs containing the JB cis-element. These results indicate that JB (PU.1) functions in B cells as a transcription activator of the J chain gene and is a likely target of the IL-2 and IL-5 signals.

* Rabbit PU.1 antisera and PU.1 cDNA were generously provided by Dr. R. A. Maki.

B 237 **GLUCOCORTICOID RECEPTOR INTERFERES WITH CREB-MEDIATED TRANSCRIPTIONAL ACTIVATION OF THE α-SUBUNIT GENE OF THE GLYCOPROTEIN HORMONES.**

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Transcription of the human glycoprotein α-subunit gene in the human placental cell line JEG-3 is positively regulated by the cyclic AMP-responsive element CRE, the tissue-specific element TSE (trophoblast-specific element) and α-ACT, an element also involved in cAMP regulation. Glucocorticoid treatment results in a 5 to 10-fold repression of α-subunit gene transcription dependent on the cotransfected glucocorticoid receptor (GR). It has been previously shown that this negative effect on transcription is mediated by the DNA binding domain of GR. However, mutating all potential GR binding sites in the α-subunit promoter does not abolish transcriptional repression indicating that GR does not need to bind to the α-subunit promoter in order to inhibit transcription. Moreover, a GR mutant in which the binding specificity has been altered into an ERE binding receptor by a substitution of only 3 amino acids within the first zinc finger, can repress α-subunit gene transcription. On the other hand, a mutant GR which has the second zinc finger replaced by the homologous finger of the thyroid receptor, exhibits no inhibitory activity, whereas if the first finger is exchanged, full repression can be measured. Interestingly, repression of the α-subunit promoter is dependent on its activation by CREB and CREB can also interfere with GR-mediated transcriptional activation independent of its ability to transactivate. Thus, repression of α-subunit gene transcription does not require DNA binding of GR to the identified binding sequences, but might be based on mutual cross-interference between GR and CREB resulting in inhibition of transactivation.

B 238 HOMEOTIC GENES OF THE Aα MATING TYPE LOCUS OF SCHIZOPHYLLUM ENCODE SELF/NON-SELF RECOGNITION PROTEINS WITH PROPERTIES OF TRANSCRIPTION FACTORS,

R.C. Ullrich[1], H. Wang[1], C.A. Specht[1], M.S. Stankis[2], G-P. Shen[2] and C.P. Novotny[2], Departments of Botany[1], and Microbiology and Molecular Genetics[2], University of Vermont, Burlington, VT 05405. Four master regulatory loci control development and differentiation in the life cycle of the basidiomycete, Schizophyllum commune. Each locus occurs in nature with many alternative forms (previously termed "alleles"). When two haploids mate by forming a fusion cell, any combination of different (i.e., nonself) alternatives for either of the regulatory loci promote development, whereas identical (i.e., self) alternatives do not. Our sequence analysis of three alternative forms (Aα1, Aα3 and Aα4) of one of the four regulatory loci, the Aα locus, shows that Aα is not a single gene with alternative alleles, but a small locus with up to three genes each with Aα-specific alleles. Each locus (6-9 kb) encodes up to three Aα-specific transcripts specifying two nonhomologous homeodomain proteins (HD1 and HD2) and a third product whose sequence has not yet been characterized. HD1 contains a proline-rich domain reminiscent of the CTF/ NF-1 family of transcription factors and two highly acidic domains. HD2 contains a lengthy basic domain similar to many DNA binding proteins. The third transcript is elevated in developmentally activated cells. Genetic data from transformation experiments suggest hybrid heteromultimers of HD1 and HD2 activate transcription within the fusion cells of nonself combinations, and the absence of hybrid heteromultimers and the activation of transcription in selfed combinations.

B 239 **LINK BETWEEN UP-REGULATION OF LYMPHOKINE mRNA AND DOWN-REGULATION OF TcR, CD4, CD8 AND LCK mRNA INDICATED BY THE EFFECT OF CYCLOSPORIN A ON ACTIVATED T LYMPHOCYTES**

*Catherine Vaquero, Florence Paillard and *Ghyslaine Sterkers. Institut Cochin de Génétique Moléculaire, Inserm U152, Paris and *CJF 9015, Hôpital Robert Debré, Paris, France.*

TcR, CD4, CD8 and p56^{lck} mRNA encode the molecules composing the Multi-Receptor Complex (MRC) which, in association with the CD3 molecules, is responsible for antigenic activation. These mRNA are constitutively expressed in resting T lymphocytes. Optimal activation of human T lymphocytes via the TcR/CD3 complex leads to a transient expression of lymphokine and IL-2Rα mRNA involved in functional differentiation and proliferation. Optimal activation leads also to a transient and specific down-modulation of MRC mRNA. This transient down-modulation was due to a decrease of both transcription and stability of these mRNA (1-3).

Down-modulation of the MRC genes appeared to be temporally correlated to transient accumulation of lymphokine mRNA. Furthermore, this inverse mRNA modulation appeared to be also quantitatively correlated. Indeed, the magnitude of lymphokine mRNA induction was proportional to the magnitude of MRC mRNA down-modulation.

This observation suggests that lymphokine and MRC genes might be inversely regulated through similar pathways. To investigate this putative relationship we took advantage of the immunosuppressive effect of cyclosporin A (CsA) that blocks lymphokine transcription. We showed that CsA inhibited MRC mRNA down-modulation also at transcriptional level, and had no effect on mRNA stability.

The fact that CsA inhibits lymphokine expression and MRC mRNA down-modulation both at transcriptional level favours the assumption that these two sets of genes might be alternatively regulated during activation by common pathways. A model of inverse regulation of MRC and lymphokine transcription will be presented.

[1]Paillard et al. 1988. Eur.J.Immunol. 18:1643; [2]Paillard et al. 1990. EMBO J. 9:1867; [3]Paillard et al. 1991. Nucleic Acids Res. in press.

B 240 EH-2, A HUMAN ZINC FINGER PROTEIN RELATED TO A MURINE REGULATOR OF RETINOIC ACID-INDUCED DIFFERENTIATION Robert H. Whitson, Ting Huang, Jinjun Dong and Keichi Itakura, Molecular Genetics Dept., City of Hope, Duarte, CA 91010. In human tera II teratocarcinoma cells retinoic acid (RA)-induced differentiation permits expression of human cytomegalovirus (HCMV). This expression is known to be triggered by transcriptional activation of the viral immediate early genes which are under the control of viral *cis*-acting factors and cellular *trans*-acting factors. Using labeled concatenated oligonucleotides which contain the putative *cis*-acting elements, we probed lambda gt11 expression libraries for proteins which bind these DNA sequences. We obtained a clone, EH-2, which when transcribed and expressed *in vitro* specifically recognized the probe sequence in gel retardation assays. Northern analysis revealed that this clone is part of a 2.3 kb transcript expressed in both RA-treated and untreated tera II cells and human fibroblasts. Sequence analysis revealed an open reading frame which coded for a protein with four zinc fingers of the $(cis)_2(his)_2$ type. In the 90 bases comprising the zinc fingers, the amino acid sequence had 77 exact matches with REX-1, a protein from mouse F9 teratocarcinoma cells whose expression is rapidly diminished by RA treatment (Hosler, et al. Mol. Cell. Biol. 9:5623-29; 1989). Outside the zinc finger region, the two proteins showed no significant homology. The 5' sequence of EH-2 is extremely G/C rich and codes for several clusters of his and pro residues. EH-2 may play a role in the regulation of tera II differentiation by RA.

Viral Gene Regulation in Pathogenesis; Transcriptional Control of Early Development

B 300 E2A/DAUGHTERLESS HOMOLOGOUS HELIX-LOOP-HELIX PROTEINS IN EARLY ZEBRAFISH DEVELOPMENT, Joel Bard and Ernest G.Peralta, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138
We have isolated and sequenced a fragment of a member of the helix-loop-helix family of transcription factors from a Zebrafish adult head cDNA library (gift of Greg Conway and Walter Gilbert). The 1.7 kilobase fragment contains a region which is highly homologous to the helix-loop-helix region of the E2A/Daughterless subfamily. A PCR expression assay was used to show that this transcript is expressed early in Zebrafish development and remains present at least through hatching. We have also used a PCR approach to clone fragments of two additional HLH genes which are expressed by the time epibole is completed. We have begun functional studies of all of these genes by injecting sense and anti-sense RNA's into embryos. Our preliminary work showing that injection of mammalian E2A/Daughterless homologues can cause specific defects in axial pattern formation in Zebrafish leads us to believe that these experiments may be fruitful.

B 301 HUCRBP, a human C2H2 type zinc finger protein that binds to a conserved regulatory sequence found in mammalian viruses and cellular genes.
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We have isolated a full length human cDNA , HUCRBP, which encodes a transcriptional regulatory protein. The protein is predicted to have four C2-H2 type zinc fingers at the C-terminal end, and a highly charged N-terminal region. The zinc finger region is most homologous to the murine zinc finger protein REX-1, while the N-terminal charged region shares a highly homologous stretch of histidines with the murine homeobox containing protein ERA-1. The molecular size of the endogenous protein, as determined by UV crosslinking is 44kd, which corresponds to the predicted protein size of our clone. The recombinant protein binds to an eight base site, GCCATTTT, found in greater than 30 mammalian retroviral LTRs, in many DNA viruses including Human Cytomegalovirus, as well many human cellular genes, including aldolase-A and superoxide dismutase. This clone is shown to act as a transcriptional activator in co-transfection experiments using HCMV-Cat as a reporter expression system.

B 302 REGULATION OF THE MURINE *HOX-3.3* GENE, P. Louise Coletta, Sebastian M. Shimeld, Mark W. Barnett and Paul T. Sharpe, Molecular Embryology Laboratory, Department of Cell and Structural Biology, Stopford Building, University of Manchester, Manchester M13 9PT, UK.

The murine *Hox-3.3* homeobox-containing gene and its *Xenopus* and human homologues produce two embryonic transcripts from two different promoters (PRI and PRII) located approximately 9kb apart in the genome. In order to understand how the *Hox-3.3* gene is regulated, we have sequenced 3kb of DNA immediately upstream of the PRII transcription start site and analysed this sequence for known transcription factor binding sites. Within this region are located a number of sequence motifs that match known *cis*-elements including those of the *Drosophila hunchback (hb)* and *Antennapedia/fushi tarazu (Antp)* binding sites. Using gel retention assays we have shown that proteins extracted from mouse embryos and some, but not all adult tissues, bind to these motifs in a sequence specific manner. However, protein binding cannot be related to the known spatial domains of *Hox-3.3* expression. A two base pair change in the *hb*-like binding site completely abolishes protein binding. Analysis of transgenic mice containing reporter gene constructs shows that these sequences are capable of directing position specific gene expression in the embryo suggesting that regulation of the *Hox-3.3* gene may, in part at least, involve binding of *hb*-like *i.e.* zinc finger proteins and *Antp*-like homeobox containing proteins.

B 303 CLONING OF A HUMAN HOMEODOMAIN GENE BY FUNCTIONAL COMPLEMENTATION IN YEAST: EVIDENCE FOR INTERACTIONS WITH MCM1 AND SRF

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We are studying the intracellular pathways by which signals travel from cellular receptors to the serum response element (SRE) of the *c-fos* protooncogene. Multiple cellular proteins bind to the SRE to regulate the expression of the *c-fos* gene, including the serum response factor (SRF). SRF binding is required for all activities of the SRE, and all or some of these activities are performed in conjunction with accessory proteins. A yeast homologue of SRF, called MCM1, has been identified. MCM1 also acts in conjunction with accessory proteins, including the DNA binding proteins, MATα1, MATα2, and STE12. The complex formed by MCM1 and MATα1 is analogous in organization and function to the complex formed by SRF and one of its accessory proteins, TCF, each accessory providing a signal-responsive activation function. To clone mammalian factors that interact with SRF, we have constructed a yeast strain in which the expression of a selectable marker (HIS3) is dependent on the MCM1/MATα1 protein complex. We have deleted MATα1 from this strain and selected for human cDNAs that restore HIS3 expression. Sequence analysis revealed that the majority of the His^+ clones contained the same cDNA, derived from a human gene, we have called *phox1*. The *phox1* gene contains a homeodomain that shares 70% identity with the the Drosophila *paired* gene. Mobility-shift asssays performed using extracts prepared from yeast cells expressing *phox1* suggest that MCM1 activity is modified in these cells relative to control cells and runs at a slower mobility. Assays performed using *E. coli*-expressed proteins show that Phox1 interacts with both MCM1 and SRF in vitro, enhancing the binding of these proteins to the SRE. SRF binding is also enhanced by the closely related Paired protein but not by the unrelated homeodomain protein Oct-2. We suggest that there may be general interactions between members of the SRF family and certain homeodomain proteins.

B 304 **PEA3, A NOVEL ETS TRANSCRIPTIONAL REGULATOR THAT IS DIFFERENTIALLY EXPRESSED DURING MOUSE DEVELOPMENT**, John A. Hassell, Ji-Hou Xin, Alison Cowie, and Paul Lachance, Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

The PEA3 motif, first recognized in the polyomavirus enhancer, is an oncogene, serum growth factor, and phorbol ester responsive element. Subsequently, an activity capable of binding to this sequence, termed PEA3 (polyomavirus enhancer activator 3), was identified in mouse 3T6 cell nuclear extracts. We have cloned cDNAs that encode PEA3 from a mouse FM3A cDNA library. A continuous open reading frame in the longest cDNA predicts a 555 amino acid protein with a calculated molecular mass of 61 kD. Cloned PEA3 binds to DNA with the same sequence specificity as that endogenous to mouse cells and activates transcription through the PEA3 motif in HeLa cells. Deletion mapping of the protein revealed that the DNA binding domain is located within a stretch of 102 amino acids near the carboxy terminus. This region shares extensive sequence similarity with the ETS domain, a region common to all *ets* gene family members. PEA3 is encoded by a 2.4 kb mRNA that is expressed to differing extents in murine and primate cell lines. Expression of the PEA3 gene is also restricted to certain tissues in the mouse; epididymus, brain and mammary glands were the principal organs that contained detectable amounts of its mRNA. Interestingly, the amount of PEA3 mRNA is down-regulated during retinoic-acid induced differentiation of mouse embryonic cell lines. These findings suggest that PEA3 may play a role as a regulator of transcription during mouse development.

B 305 CpG METHYLATION OF AN ENDOGENOUS RETROVIRAL ENHANCER INHIBITS TRANSCRIPTION FACTOR BINDING AND ACTIVITY, Chin C. Howe, Bruce T. Lamb, Davor Solter, Kapaettu Satyamoorthy, and Liye Li, The Wistar Institute of Anatomy and Biology, 3601 Spruce Street, Philadelphia, PA 19104

The endogenous retrovirus, intracisternal A-particle (IAP), is expressed at unique stages during murine embryogenesis and is also activated during the in vitro differentiation of F9 cells. We have examined the DNA elements and protein factors that control IAP expression during F9 differentiation. In the present study an IAP upstream enhancer (IUE) is identified by transient transfection assays and found to be active in both undifferentiated and differentiated cells. Further analyses reveal that a ubiquitous 65-kDa protein factor, the IUE binding protein (IUEB), binds with the IUE. Site-specific methylation within the IUEB binding site strongly inhibits both IUEB binding and IUE transcriptional activity, suggesting that methylation may regulate IUE function and IAP expression.

B 306 A GENE ENCODING TWO DNA-BINDING DOMAINS IS INVOLVED IN ORGANOGENESIS

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We describe the characterization of *BK27*, a gene in *Drosophila*, which contains two DNA-binding motifs, a homeodomain and a paired domain. Both motifs in *BK27* are homologous to corresponding regions of the products of *paired* and *gooseberry* segmentation genes. *In situ* hybridization reveals an expression pattern that is temporally and spatially restricted and is unrelated to that of *paired* or *gooseberry*. *BK27* is expressed predominantly in the ventral part of the labial segment in the placode that has been identified as the progenitor of the salivary gland. This expression is transient and preliminary evidence suggests that *BK27* is the first gene expressed specifically in the placode and is involved in the transcriptional regulation of *forkhead (fkd)*. *fkd* has been implicated in organogenesis of the salivary gland. *BK27* is also expressed in the tracheal placodes which are regions related to the salivary gland placode in other segments. A persistent patch is also present in the subantennal region. We describe the role of the homeotic gene *Scr* on specifying *BK27* expression in the labial segment as well as the role of other genes determining the dorsal-ventral position of *BK27* expression in the progenitor of the salivary gland. Although *paired* and *BK27* are highly similar they are involved in different developmental processes, segmentation and organogenesis, respectively.

B 307 THE *FTZ* HOMEODOMAIN IS REQUIRED FOR TRANSCRIPTIONAL ACTIVITY BUT NOT FOR THE GENERATION OF AN ANTI-FTZ PHENOTYPE, Henry M. Krause, Anthony Percival-Smith, V. Danial Fitzpatrick and C. James Ingles, Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5G 1L6 CANADA

The *Drosophila* pair-rule gene *fushi-tarazu* (*ftz*) functions as a DNA binding transcription factor in cultured *Drosophila* cells, in yeast and *in vitro*. We have found that, in cultured *Drosophila* Schneider 2 cells, *ftz* transcriptional activity was dependent upon one of two transcriptional activation domains that are located in the two terminal regions of the protein, as well as upon the DNA binding homeodomain. Each of the two activation domains conferred UAS_G-dependent transcriptional activity to the DNA binding domain of the yeast protein Gal-4 (amino acids 1-147). Interestingly, neither of these two domains functioned as transcriptional activation domains in yeast. In order to test whether the *ftz* N- and C-terminal activation domains were functional in *Drosophila* embryos, we expressed the *ftz* deletion constructs in embryos under the control of a heat shock promoter. It has been shown that ectopic *ftz* expression just prior to gastrulation causes a widening of the endogenous *ftz* stripes followed by widening of the even-numbered *engrailed* stripes and loss of the odd-numbered *wingless* stripes. This results in the apparent deletion of the odd-numbered parasegments, a phenotype that is nearly reciprocal to the *ftz* loss of function phenotype. This "anti-*ftz*" phenotype has been largely attributed to autocatalytic activation of the endogenous *ftz* gene by the ectopically induced protein. Surprisingly, we found that, although both transcriptional activation domains were required to generate the anti-*ftz* phenotype, the DNA binding homeodomain was not. This indicates that the anti-*ftz* phenotype is mediated via protein-protein interactions. This result emphasizes the modularity of transcription factor activities and raises several caveats to the interpretation of experiments involving ectopic gene expression.

B 308 GENE EXPRESSION DURING POST-NATAL DEVELOPMENT OF THE RAT PAROTID SALIVARY GLAND. K.W. Lazowski, P.M. Mertz, R.S. Redman and E. Kousvelari. CIPCB, NIDR, NIH, Bethesda, MD and VAMC, Washington, D.C.

Rat parotid salivary gland (RPSG) growth and differentiation occurs mainly after birth with serous acinar cell and duct maturation (e.g. RER, secretory granules). Slowing of proliferation takes place after weaning (Redman and Sreebny Dev. Biol. 25: 248-279 1971). The purpose of this study was to investigate the pattern of expression of the c-jun proto-oncogene and two parotid gland secretory proteins, proline-rich protein (PRP) and amylase during parotid gland post-natal development. Expression of c-jun, PRP and amylase was studied by northern blot analysis, in situ hybridization using ^{32}P labeled cDNA or cRNA probes and immunocytochemistry with PRP or amylase antibodies. Parotid glands from day 1, 7, 14, 21, 28, 42 and 90 were excised and used for RNA isolation and tissue sections. The levels of c-jun mRNA were high at days 1, 7 and 14 and barely detectable at later days. c-jun mRNA was localized in both the acinar cells and ducts. Amylase and PRP transcripts were present as early as days 7 and 14 respectively. An abrupt transition to much higher levels of expression of PRP and amylase was detected at day 21 with a small decrease at day 28 and constant levels thereafter. This pattern of PRP and amylase gene expression was followed by the presence of these proteins in the serous acinar cells. The data indicate that: a) the expression of c-jun may associate with parotid acinar cell and duct proliferation during development and b) the expression of PRP and amylase coincides with increased amount of RER and the appearance of secretory granules in the developing RPSG.

B 309 TRANSCRIPTION FACTOR PIT 1/GHF-1 SUPPRESSES SV 40 ENHANCER FUNCTION, Finian Martin, Sarah Larkin, Salem Giafri, Sarah Tait, Roisin Deane and Fergus Ryan, Department of Pharmacology, University College Dublin, Dublin 4, Ireland

The SV 40 enhancer/promoter system is silent in pituitary somatotroph/lactotroph tumour cells, GC cells. In addition we have previously shown that SV 40 enhancer/promoter sequences compete for Pit 1/GHF-1 binding, in pituitary extracts, with its natural binding elements (in the rat prolactin or rat growth hormone gene promoters). These observations, together with the fact that Pit 1/GHF-1 can bind to octamer elements, two of which are present in the SV 40 enhancer 72 bp repeat, suggested that the SV 40 enhancer may be actively suppressed in GC cells by the binding of Pit 1/GHF-1 to one or more such elements: We confirm that the SV 40 enhancer/promoter system is silent in GC cells but show that in these cells the SV 40 promoter can be activated by heterologous enhancers. This suggests strongly that it is the SV 40 enhancer, not the promoter, that is suppressed in these cells. We show that in non-pituitary cells, cotransfection of a Pit 1/GHF-1 expression vector with pSV2 CAT inhibits CAT gene expression. Furthermore, it was possible to demonstrate that Pit 1/GHF-1 binds to the 72 bp unit repeat of the SV 40 enhancer and to an octamer containing motif from the enhancer (the Sph II/Sph I motif) but not to a non-octamer containing motif (Core A/Core A) which binds transcription factor TEF 1, as does Sph II/Sph I. In cotransfection experiments Pit 1/GHF-1 was shown to selectively block the activity of a synthetic enhancer generated from multimerised Sph II/Sph 1 motifs; and, in addition, this latter enhancer was silent in GC cells. A similar construct containing multimerised Core A/Core A motifs was active. Our results strongly suggest that an interaction of Pit 1/GHF-1 with octamer motifs within the SV 40 enhancer contributes to its silence in somatotroph/lactotrophs. A comparative analysis of Pit 1/GHF-1 binding to *pit* and *octamer* binding elements showed it to form, predominantly, a large (probably dimeric) complex on the *pit* element but a smaller (monomeric) complex on the *octamer* element. This difference in ability to form the large complex on DNA may contribute to this inhibitory action of Pit 1/GHF-1.

B 310 TRANSCRIPTIONAL ACTIVATION OF THE SV40 PROMOTER REGION DURING MOUSE DEVELPMENT, Peter M. Mathisen, Leroy Hood, and Carol Readhead, California Institute of Technology, Division of Biology, 147-75, Pasadena, CA 91125.

SV40 has been a widely used model system for the study of transcription. Since it uses the host cell transcription machinery, it has been particularly helpful in dissecting the transcriptional regulatory mechanism of mammalian cells. Indeed, it was in the SV40 promoter sequences that the first enhancers were identified. These enhancing sequences are composed of 2 72-bp direct repeats, which are known to be functional in a broad range of cell types. Upon further dissection of the repeats, is has been found to be composed of a modular arrangement of enhancer segments and some of these segments possess different cell type specificity. Presumably each cell type would have a factor or set of factors that would bind to a specific enhancer segment depending on the availability of the factors present in that cell type.

In order to better study this cell-type specific expression of the SV40 promoter and better understand the factors responsible for its expression, we have begun a series of experiments that examine the activation of the SV40 promoter elements during mouse development. A series of transgenic mice were produced that contained the SV40 promoter elements fused to the bacterial reporter gene, *lacZ*. The activity of this promoter is then assayed by staining mouse embryos at various developmental stages with X-gal. To date, we have found restricted expression of the SV40 promoter in the embryo. This activity appears to be present later in development in specific regions of the embryo, such as the developing limbs and certain regions in the head. These data suggest that while SV40 promoter sequences may drive expression of genes in a number of different cell types, once this promoter is exposed to a developmental history, its expression is much less "constitutive". Other developmental stages are being examined and sectioning of the embryos are being performed to produce a more refined picture of the expression of the SV40 promoter region during mouse development.

B 311 **REGULATION OF mIL-2 GENE EXPRESSION BY HTLV TAX IS SERUM-DEPENDENT AND MEDIATED THROUGH COMPONENTS OF A COMPLEX THAT INTERACTS WITH TCE_d, A NF-κB-LIKE ELEMENT**, Leslie B. Overman and William Wachsman, Division of Hematology/Oncology, San Diego VAMC and UCSD School of Medicine, La Jolla, CA 92093.

Tax, an accessory gene product of the human T-lymphotropic virus (HTLV), is required for efficient viral replication. In addition to inducing proviral expression, *tax* also transactivates several cellular genes, including IL-2 and IL-2Rα. It has been proposed that Tax induces aberrant T-cell growth and leukemia via an IL-2 autocrine pathway. While the mechanism of Tax transactivation is unknown, the effect of Tax on the IL-2 and IL-2Rα promoters appears to be mediated through their respective NF-κB-like elements.

To define the mechanism of Tax action on IL-2 gene expression, we investigated its effect on the murine IL-2 (mIL-2) promoter in the EI-4 murine T-cell line. Transient expression assays unexpectedly revealed that Tax *decreases* expression of a mIL-2 promoter/CAT construct, with respect to basal, 6-fold. In addition, this negative regulatory effect of Tax is serum-dependent, being seen in cells grown at 10% fetal calf serum (FCS). In contrast, Tax *induces* mIL-2/CAT expression, when EI-4 media contains 2% FCS. Both the human and murine IL-2 promoters harbor a consensus NF-κB binding site, termed TCE_d, which is TPA-responsive in T cells, as well as, a potential target for the actions of Tax. Using a construct containing a 5X repeat of TCE_d placed upstream of a tk promoter, we observed that the serum-dependent effects of Tax on mIL-2/CAT expression were reiterated. This effect is independent of an 8-fold increase in mIL-2/CAT expression following TPA/ConA stimulation. UV photocrosslinking identified two major proteins, p50 and p85, in EI-4 nuclear extracts that interact with TCE_d. We found that p50 increases in response to TPA/ConA. On the other hand, in response to increasing FCS concentration, there is a relative increase in p85 component. Therefore, we propose that in murine EI-4 T cells (i) multiple proteins interact with the TCE_d to regulate expression of the mIL-2 promoter, (ii) p50 mediates an increased expression by TPA/ConA stimulation and (iii) p85 mediates decreased expression in the presence of Tax. How HTLV Tax operates on this regulatory protein complex to modulate its function, and, whether it does so directly or indirectly is, as yet, not known.

B 312 **HSV-1 ACTIVATES HIV-1 PROVIRUS BY NF-κB-DEPENDENT AND -INDEPENDENT MECHANISM**, **Paula M. Pitha, Waldemar Popik and Jaromir Vlach, Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD 21231**

Herpes simples virus type 1 (HSV-1) infection induces expression of the human immunodeficiency virus type 1 (HIV-1) provirus in the chronically infected T-cell line, ACH-2. The HSV-1-mediated induction correlates with the appearance of regulatory proteins binding to two HIV-1 LTR, κB enhancer region and to the untranslated leader sequence consisting of three LBP-1 binding sites. HSV-1 infection induced presence of two NF-κB-binding proteins of m.w. 55- and 85-kilodalton (kDa) in the nucleus; these proteins presumably represent the alternatively processed form of NF-κB p50 and the c-rel protooncogene, respectively. The leader sequence is a target for binding of a novel protein, designated HLP-1 that is present exclusively in HSV-1-infected, but not in 12-O-Tetradecanoylphorbol 13-acetate- (TPA) or tumor necrosis factor- (TNF) treated ACH-2 cells. Both the NF-κB and LPB-1 target sequences, when inserted either alone or together 5' of a heterologous (TK) minimal promoter, can confer inducibility by HSV-1 infection. Thus, it appears that the HSV-1-mediated activation of HIV-1 provirus proceeds through the binding of both NF-κB and HLP-1 proteins. The HSV-1 infection can also activate the expression of Tat-defected HIV-1 provirus, while interferon-α seems to inhibit the NF-κB-directed transcripts. The significance of these results will be discussed.

B 313 THE PATERNALLY DERIVED X-LINKED PGK GENE IS TRANSCRIPTIONALLY ACTIVE PRIOR TO EMBRYONIC X CHROMOSOME INACTIVATION, Judith Singer-Sam,[1] Verne Chapman,[2] Jeanne M. LeBon,[1] Aihua Dai[1] and Arthur D. Riggs,[1] Department of Biology, Beckman Research Institute, City of Hope, Duarte, CA 91010,[1] and Molecular and Cellular Biology Department, Roswell Park Memorial Institute, Buffalo, New York 14263 [2]

Fertilized female mouse eggs contain an active and inactive X chromosome, derived from the mother and father, respectively. Enzyme studies have shown the paternal alleles of the X-linked HPRT and alpha-galactosidase genes to be reactivated very early in embryogenesis. However similar studies on X-linked PGK have suggested that the paternal allele is not activated prior to 6 days of development, about the time when random X-inactivation occurs. We have found, using embryos heterozygous at the Pgk-1 locus, and a PCR-based quantitative allele-specific assay, that PGK RNA is transcribed from the paternal X chromosome in 8-cell mouse embryos. As predicted from isozyme studies, the paternally derived HPRT gene is also active at this time.

B 314 THE *XENOPUS LAEVIS* TRANSCRIPTION FACTOR FRGY2 BINDS RNA AND IS IDENTICAL TO THE P56 COMPONENT OF THE OOCYTE RNP FRACTION. Sherrie R. Tafuri and Alan P. Wolffe, Laboratory of Molecular Embryology, NIH, Bethesda, MD 20892

In *Xenopus laevis,* large stores of maternally derived components are accumulated for use during embryogenesis. These molecules are stored in the form of both mRNA and protein and include components of the transcription and translation apparatus. Most mRNA synthesis occurs during the early stages of oogenesis, however, only 5% of this message can be isolated from the polysomal fraction of immature oocytes. In later stages of the oocyte maturation, mRNA is recruited to the polysomes for translation. The mechanism for this translational regulation is unknown, however studies have indicated that packaging of mRNA within RNP storage particles inhibits translation *in vitro.* We have isolated a polymerase II transcription factor from *Xenopus* oocytes, FRGY2, and have shown that it is required for transcription of genes containing the Y-box DNA sequence element *in vitro.* In this study, we have shown that this factor is identical to the p56 component of the oocyte RNP fraction. The *E.coli* produced FRGY2 protein has the same mobility on SDS-polyacrylamide gels, antigenicity, and RNA and DNA binding properties as the p56 component of the oocyte RNP fraction. In addition, FRGY2 has a developmental profile identical to that of p56. This situation is analogous to that of the polymerase III transcription factor TFIIIA which regulates 5s RNA synthesis and is the protein component of the 5S RNP particle. Continuing studies are examining how FRGY2 interacts with both the protein and RNA components of the RNP particle and whether FRGY2 is involved in the coupling of transcription and mRNA translation within the oocyte.

B 315 REGULATION OF HIV-1 GENE EXPRESSION BY CELLULAR PROLIFERATION EVENTS. Sandra E. Tong, Dale Yuzuki, and B. Matija Peterlin. HHMI and SF VAMC, Dept. of Medicine, UCSF, San Francisco, CA 94121

Transcriptional mechanisms that control cellular genes newly expressed in activated T cells also activate the HIV-1 regulatory region, or long terminal repeat (LTR). T cell activation leads to release of interleukin (IL)-2 and surface expression of its high affinity receptor (IL-2R), which then allows proliferation of T cells through autocrine stimulation. Although the signals generated by the IL-2R are not clearly defined, similar physiologic events are mediated by another well-characterized receptor, the platelet-derived growth factor (PDGF) receptor. We have examined the response of the HIV-1 LTR to cellular proliferation induced by serum treatment of quiescent cells that express the PDGF receptor. A plasmid construction containing the HIV-1 LTR upstream from a reporter gene, the chloramphenicol acetyl transferase (CAT) gene, was used in transient transfections in a Chinese hamster ovary (CHO) cell line that was stably transfected with the wildtype PDGF receptor. The HIV-1 LTR responded 6-fold to stimulation with serum, PDGF, or the mitogen, phorbol myristate acetate (PMA). The HIV-1 enhancer, which consists of two nuclear factor κB (NFκB) binding sites that are required for the response to T cell activation signals, was also required for the serum response. This response was observed in the absence or presence of the HIV-1 regulatory protein, tat-1, which amplifies HIV-1 LTR directed gene expression. As an indicator of stimulation by each agent, a plasmid construction with the *fos* enhancer and promoter also responded 5-fold. Stimulation of the HIV-1 LTR in CHO cell lines that express mutated PDGF receptors are being examined. Thus, the HIV-1 LTR responds to cellular proliferation in a cell line regulated by a specific growth factor, PDGF; this system may serve as a model for regulation of a retrovirus by cellular proliferation events in activated T cells.

B 316 CLONING AND EXPRESSION ANALYSIS OF TWO *ZFY*-RELATED ZINC FINGER GENES FROM THE AMERICAN ALLIGATOR, A SPECIES WITH TEMPERATURE-DEPENDENT SEX DETERMINATION. Elizabeth M.A. Valleley, Ulrike Müller, Mark W.J. Ferguson and Paul T. Sharpe, Molecular Embryology Laboratory, Department of Cell and Structural Biology, Stopford Building, University of Manchester, Manchester, M13 9PT, UK.

In many reptiles, sex is not determined directly by the inheritance of sex chromosomes, but instead by the temperature of egg incubation. In American alligators (*Alligator mississippiensis*) there are no heteromorphic sex chromosomes, and incubation of eggs at 30°C produces 100% females, and 33°C produces 100% males. During recent years several candidate genes for the mammalian testis-determining factor have been isolated. One of these genes, the zinc finger gene *ZFY*, is a potential DNA-binding protein and transcription factor located on the human Y chromosome. *ZFY* has now been ruled out as the primary testis determining factor in mammals, but may function in germ cell development. To investigate whether related genes are involved in temperature-dependent sex determination, a fragment from the *ZFY* gene was used to isolate homologous genes from an alligator genomic library. Two alligator genes were isolated, *Azf-1* and *Azf-2*, both of which code for zinc finger proteins, and exhibit amino acid homologies to *ZFY* of 92% and 73% respectively. *Azf-1* codes for a protein with thirteen zinc fingers similar to *ZFY*. *Azf-2* has mutations in the third and seventh finger regions which may prevent the formation of these fingers in the putative protein. The expression of these genes throughout gestation was analysed using Northern blots of RNA from embryos incubated at 30°C and 33°C. Two transcripts were detected with *Azf-1*, and one with *Azf-2*. For both genes, transcripts were present at all stages of development, from day 3 (post-laying) to hatching. No sex-specific differences in expression were observed. This would indicate that these genes do not play a primary role in temperature-dependent sex determination, but they may function further down the sex differentiation cascade, or as general transcription factors.

Maintenance of Determined Cell States; Structural Features of Transciption Factors

B 400 CLONING OF 5'- UNTRANSLATED REGION AND ISOLATION OF PROMOTER OF A POU DOMAIN-CONTAINING XENOPUS GENE, XLPOU 1. Veena R. Agarwal and Sheryl M. Sato, Clinical Endocrinology Branch, NIDDK, NIH, Bethesda, MD 20892

A large family of transcription regulators have a conserved POU domain, consisting of a 76- to 78-amino acid POU-specific region and a 60-amino acid unique homeobox. Several of the POU domain-containing genes have been shown to be involved in tissue-specific gene regulation during development,as well as in the adult. Recently we have reported (Dev. Biol. Vol.147, 1991, in press) a brain specific POU domain-containing gene, XLPOU 1. Its expression starts at the neural plate stage during *Xenopus* development and is likely to regulate the early specification of neural tissues in *Xenopus laevis* embryos. This cDNA clone comprised of only one long open reading frame (ORF) containing POU domain and 205 5' flanking amino acids. To find the missing 5'-end of the gene, We screened *Xenopus* genomic library using outside POU domain region as a probe. One genomic clone has full open reading frame (ORF) and 5'-untranslated region as determined by sequence analysis. The ORF was checked by *in vitro* translation and the size of the single translation product was ~43.7 kD. Furthermore the transcription start site which was specified by primer extension, led us to analyse promoter. Experiments are in progress to characterize functional elements essential for XLPOU 1 gene transcription.

B 401 INVOLVEMENT OF LYSINE RESIDUES 289 AND 291 OF ΔCREB IN CRE RECOGNITION, Ourania M. Andrisani, Department of Veterinary Physiology & Pharmacology, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana, 47907.

The molecular interactions resulting in specific binding of transacting factors to distinct cis-acting elements is not well understood. Here the involvement of distinct amino acid residues of the basic domain of ΔCREB (cAMP-responsive-element binding protein) in the determination of binding towards the cAMP responsive element (CRE) is examined. Using *in vitro* mutagenesis, we constructed site directed mutants of distinct amino acid residues within the DNA contact region of ΔCREB. The activities of the mutant proteins were analyzed by gel retardation, methylation interference and CRE competion analyses. It is demonstrated that a single lysine to glutamine substitution at positions 289 and 291 of ΔCREB alters the methylation interference pattern of the mutant protein for the CRE site. Additional mutants constructed at these positions demonstrate that only identical basic residues at both positions 289 and 291 of ΔCREB can restore the wild type methylation interference pattern of the mutant ΔCREB protein for the CRE site. These observations point to the importance of the lysine residues at positions 289 and 291 in the process of CRE binding. In addition, this observation suggests that the symmetrical nature of the CRE site is reflected in the DNA contact region of the protein.

B 402 THE κ-CHAIN ENHANCER CONFERS DEMETHYLATION UPON THE Jκ REGION IN MATURE B-CELLS IN A *POSITION DEPENDENT* MANNER ONLY, Yehudit Bergman[1], Howard Cedar[2], and Michal Lichtenstein[1], Department of Experimental Medicine and Cancer Research[1], Department of Cellular Biochemistry[2], The Hebrew University Hadassah Medical School, Jerusalem 91010, ISRAEL

We are studying the mechanism and the role of DNA methylation in the regulation of Ig κ-chain gene expression during B-cell development. We methylated the κ-chain gene in vitro using bacterial methylases, and introduced the DNA into non-lymphoid and B-lymphoid cell lines. The methylation status of the Ig genes was not changed by the cellular non-lymphoid environment into which they were introduced. In contrast, the methylated κ-chain gene introduced into the B and plasmacytoma cell lines underwent demethylation. The ability to actively demethylate an exogenous κ-chain construct appears, however, to be not only lineage specific, but also cell-stage specific, since demethylation did not occur in a pre-B-cell line, where the endogenous gene is inactive and methylated. The demethylation was sequence specific; it was restricted to the gene region itself and did not spread into flanking sequences. Moreover, a methylated κ-chain gene deleted of its promoter undergoes specific demethylation in B-cells. Thus, transcription per-se is not sufficient for the demethylation process. In contrast, a methylated enhancer deleted κ-chain gene stays methylated in B-cells. Therefore, the κ-chain enhancer plays a vital role in the demethylation event. But most interesting, a fragment containing the κ enhancer can confer B-cell specific demethylation upon the Jκ region in an orientation independent but *position dependent* manner. More explicitly, the κ-chain enhancer could confer demethylation upon the Jκ region when it was cloned 3' to J5 only. Thus, the specificity of the demethylation resides within a fragment containing the transcriptional enhancer; similar to a transcriptional enhancer, it can demethylate from a distance and in an orientation independent manner, but unlike a transcriptional enhancer it can exert its effect in one position only.

B 403 CONE CELL DIFFERENTIATION IN TRANSGENIC MICE, Emil Bogenmann, David Hinton and Chandra Tucker, Department of Pediatrics and Pathology, Childrens Hospital Los Angeles, Los Angeles, CA 90027

The mature mammalian retina is a complex neuronal organ composed of various highly specialized cells which develop from primitive neuroblastic cells. Two types of photoreceptor cells are present in the retina, namely rod cells (vision in dim light) and cone cells (color vision). Although these photoreceptors have structural and functional similarities, they do differentiate at different times during development, and it is likely that they develop from different progenitor cells. Here we describe an experimental approach to study cone cell development using transgenic mice. Several strains were generated which express the β-galactosidase gene under the control of the human blue opsin promotor consisting either of a 3.5kb or a 1.1kb long DNA fragment. Expression is found in the retina at embryonic stage E13 which coincides with the onset of cone cell development in mice. In the developing post-natal animal, staining of β-gal is found in individual cone cells, few cells of the inner nuclear layer and some ganglion cells. Detailed characterization of the phenotype of expressing retina cells is currently being done using a panel of antibodies. These transgenic mice are, therefore, very useful to study cone cell development and our result may delineate the differentiation of a cone cell related visual system.

B 404 TRANSCRIPTION OF THE ALPHA-FETOPROTEIN GENE IN LIVER DURING RAT DEVELOPMENT

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Some information has been gained concerning the cis- and trans- acting elements involved in the expression of the alpha-fetoprotein gene(AFP) in liver and in its modulation in the course of the development.

Transfection experiments performed with CAT constructs indicated that the AFP promoter and a 170 bp enhancer located at -2.5 kb participate in the liver specificity of the AFP gene expression. The activities of these two regulatory elements are down regulated by glucocorticoids, which may contribute to the shut-off of the AFP gene transcription in the adult liver.

Fractionation of nuclear proteins from fetal and adult rat liver by Heparin Ultrogel chromatography followed by analysis of their binding to the rat AFP promoter revealed that :
-an as yet unidentified factor, specific to the fetal stage, elutes in the O.6M NaCl fraction of the column;
-several proteins can bind to regions which partially overlap Hepatocyte Nuclear Factor 1 (HNF1) binding sites on the AFP promoter.
In particular, the region -123 -97, which is target for Nuclear Factor 1 (NF1) can also be recognized by Liver Activating Protein (LAP) and C/EBP as demonstrated by using purified recombinant proteins (kindly provided by U.Schibler and S. Mc Knight).
One may hypothesize that variations in the degree of interference beetween HNF1, which plays a major role in the functioning of the AFP promoter, and NF1 or proteins from the C/EBP family susceptible to form homo-hetero-dimers can modulate the level of the AFP gene transcription in the course of the development.

This work was supported by grants from the C.N.R.S., the Association pour la Recherche contre le Cancer and the Fondation pour la Recherche Médicale.

B 405 SYNERGISTIC PHOSPHORYLATION OF cAMP RESPONSE ELEMENT-BINDING PROTEIN BY cAMP DEPENDENT PROTEIN KINASE AND GLYCOGEN SYNTHASE KINASE-3.

C.J.Fiol, Q.M.Andrisani, J.E.Dixon, and P.J.Roach, Department of Biochemistry and Molecular Biology Indiana University School of Medicine, Indianapolis and Department of Biochemistry, Purdue University, West Lafayette, Indiana

The cAMP response element-binding protein (ΔCREB) is known to be phosphorylated by cAMP-dependent protein kinase (PKA) at SER-119. Using a purified recombinant ΔCREB protein we observed synergistic phosphorylation of CREB by PKA and glycogen synthase kinase-3 (GSK-3). Based on the recognition determinants for GSK-3 phosphorylation, -SXXXS(P)-, Ser-115 of ΔCREB would be the predicted target for GSK-3. A peptide was synthesized with the sequence KRREILS(115)RRPS(119)YR containing these phosphorylation sites. As predicted, Ser-115 in the model peptide was phosphorylated by GSK-3 but only after previous phosphorylation of Ser-119 by PKA. GSK-3 had a K_m of 140 μM for the phosphopeptide with a V_{max} of 2.4 μmol/min/mg. A mutant ΔCREB having Ser119 substituted by an Ala was not phosphorylated by either PKA, GSK-3 or their combination thus confirming that phosphorylation by PKA at Ser-119 was required for a secondary phosphorylation of ΔCREB by (GSK-3). A second ΔCREB mutant with Ser-115 substituted by an Ala, was phosphorylated by PKA but not by GSK-3 confirming that Ser-115 of ΔCREB is the target for synergistic phosphorylation by GSK-3. This is the first example, to our knowledge, of an ordered or hierarchal multisite phosphorylation of a transcriptional factor. Phosphorylation of Ser-119 by PKA has been shown to be involved in controlling expression of genes containing CRE elements. Since this phosphorylation creates a site for GSK-3 in CREB, the possibility exists that GSK-3 may also have a role in regulating gene expression. (Supported by NIH grant DK27221, a Juveile Diabetes Foundation reserach grant to CJF and NIAAA grant AA07611 to OMA)

B 406 MyoD activates cardiac Myosin Light Chain 2 promoter through a mechanism independant of E-box requirement and involves expression of a new transcription factor.

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The role of MyoD and other members of the MyoD family in skekeletal myogenesis have been well investigated. It is believed that MyoD acts both as a myogenic specification and transcription factor through binding cooperatively to consensus sequence element (E-box) present in muscle genes. The lack of expression of MyoD in cardiac muscle, however, appears to preclude its involvement in regulation of cardiac muscle genes. We have recently reported that cardiac tissue specific expression of MLC2 promoter is determined by several positive and negative elements and their DNA binding proteins. Among them, a cardiac specific sequence (CSS), a CArG-like (element A) and a MEF-2 like sequence (element B) play a role in tissue specific transcriptional regulation of MLC2 gene. Here, we report that cotransfection of MyoD expression vector with MLC2 promoter/CAT recombinant carrying only the proximal promoter region containing the elements above resulted in MLC2 CAT expression in the fibroblasts cell line, C3H10T1/2, which, otherwise, does not support cardiac MLC2 transcription in the absence of MyoD. The MyoD-mediated activation is dependent upon the presence of intact element B (-42) sequence. Gel mobility shift assay using an oligonuclotide encompassing element B demonstrated that an element B specific DNA-binding protein is present in MyoD-expressing fibroblasts, but not in the parental 10T1/2 cells. It, therefore, appears that expression of cardiac MLC2 gene in 10T1/2 is induced by MyoD even in absence of E-box, through a cross activation network leading to the production of a regulatory trans-acting protein specific for element B.

B 407 CHARACTERIZATION OF THE *Drosophila* TFIID COMPLEX,

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The regulation of transcription involves interactions between promoter-selective factors and the general initiation factors. We have examined the requirements for transcriptional activation in *in vitro* reactions reconstituted with fractionated general factors derived from *Drosophila* embryos. Activation requires coactivators present in the endogenous TFIID fraction that are distinct from the purified TATA-binding protein (TBP). Immunopurification of endogenous TFIID indicates that it is a multi-protein complex containing TBP and six associated factors (TAFs). The immunopurified TFIID complex is able to mediate both basal and activated transcription. Preparations of TBP lacking TAFs after fractionation with denaturants retain basal transcription activity but no longer support regulated transcription. Addition of purified TAFs to free TBP restores activation controlled by regulatory factors. These results indicate that one or more of the TAF proteins confer coactivator function.

B 408 THE Lin-26 PUTATIVE ZINC FINGER PROTEIN MIGHT BE A HYPODERMAL DIFFERENTIATION FACTOR IN *C. elegans*. Michel Labouesse and H. Robert Horvitz, HHMI, Dept. Biology, MIT, Cambridge, MA 02139, USA.

We are interested in understanding how neurons and non-neural ectodermal cells are made different during *C. elegans* development. To address this question, we are studying the mutation *lin-26(n156)*, which transforms the normal hypodermal cell fate of 12 cells named P1.p-P12.p to a neuronal fate similar to that of their lineal sisters. We have cloned the gene *lin-26* and shown that a 9 kb DNA fragment can rescue the *lin-26* mutant phenotype upon germ-line transformation. This region encodes at least four transcripts: one set of three RNAs (1.4 kb, 1.45 kb and 1.5 kb) are very similar and arise via alternative splicing near their 3' ends; a fourth RNA (2.2 kb) does not overlap with the other three in its open reading frame. These two sets of transcripts encode proteins that are similar in structure. These proteins are likely to be transcription factors. Both sets of proteins contain: a Ser/Thr-rich N-terminal region, a Gln-rich central region, two copies of a Cys-His motif related to but distinct from the zinc fingers present in the TFIIIA transcription factor, and an acid-rich C-terminal region (which in the first set of transcripts varies as a consequence of alternative splicing). Transformation experiments show that only the 2.2 kb transcript is responsible for the *lin-26(n156)*-rescuing activity. The mutation *n156* changes a conserved Leu residue in the first zinc finger encoded by the 2.2 kb RNA to a Phe. We have constructed two *lin-26/lacZ* gene fusions. Staining of transgenic animals with X-gal shows for both fusions that ß-galactosidase activity is detected in most hypodermal cells and in most postembryonic-blast cells that divide to generate hypodermis and neurons. The staining is first observed during the period of embryogenesis when these cells are born. We have characterized the terminal phenotype of animals carrying the *n156* mutation in *trans* to a chromosomal deficiency that deletes the *lin-26* locus. Such animals die as L1 larvae with severe defects in organs composed of cells that express the *lin-26/lacZ* fusions (hypodermis, anus/rectum, excretory system). The *lin-26/lacZ* staining pattern, the presence of transcription factor motifs in Lin-26 proteins, and the terminal phenotype of *n156* hemizygous animals together suggest that *lin-26* could be a regulator of hypodermal differentiation.

B 409 CLONING OF A CANDIDATE GENE ENCODING A TRANSCRIPTION FACTOR DIRECTING LENS SPECIFIC GENE EXPRESSION, Quirong Liu, Dawn Bryce, Lap-Chee Tsui*, and Martin Breitman, Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Ave., Toronto, Canada M5G 1X5, and *The Hospital for Sick Children, Toronto, Canada M5G 1X8

During lens organogenesis, the mouse γF-crystallin gene is expressed in a population of terminally differentiating fiber cells that form the central nucleus of the lens. To study the elements regulating γF-crystallin gene expression, we carried out gene transfer studies in transgenic mice and demonstrated that sequences -67 to +45, which comprise the core promoter, contain sufficient information to direct gene expression specifically to fiber cells in the nucleus of the lens. Moreover, by conducting band shift and mutational analyses, we defined a 22 bp motif within this core promoter that binds a lens-specific transcription factor γF-1 and, which when multimerized, is capable of conferring lens-specificity to a heterologous promoter *in vitro*. To gain insight into the molecular mechanisms governing lens-specific expression, we cloned a candidate gene for γF-1 by screening a lens cDNA expression library using the 22 bp motif as probe. DNA sequence analysis revealed that this cDNA codes for a zinc finger protein which shows strong homology in its finger motif to *kr ppel*-related genes and extensive homology to human transcription factor ETR103. The characterization of this cDNA and its developmental profile of expression will be presented.

B 410 REGULATED DIMERIZATION OF A MAMMALIAN HOMEODOMAIN PROTEIN DETERMINES ITS TRANSCRIPTIONAL ACTIVITY, Dirk B. Mendel, Paul A. Khavari, and Arie Admon*, The Howard Hughes Medical Institute, Beckman Center for Molecular and Genetic Medicine, Stanford University, Stanford, CA 94305 and *The Howard Hughes Medical Institute, Department of Molecular and Cellular Biology, University of California at Berkeley, Berkeley, CA 94720.

Dimerization among transcription factors has emerged as a recurrent theme in the regulation of eukaryotic gene expression. Despite the potential advantages of regulating the association of transcription factors that share complementary dimerization motifs, to date no accessory proteins that aid in this process have been identified. Dimerization Cofactor of HNF-1α (DCoH) is a tissue-restricted, non-DNA-binding, 11-kDa protein that selectively stabilizes dimers of the homeodomain-containing protein HNF-1α. The formation of a stable tetrameric DCoH:HNF-1α complex, which requires the 32-amino acid NH_2-terminal dimerization domain of HNF-1α, does not change the DNA-binding characteristics of HNF-1α, but does enhance its transcriptional activity as much as 250 fold. DCoH does not, however, contain any discernable activation domain or confer transcriptional activity to the GAL4 DNA-binding domain. Thus, DCoH represents a novel type of accessory protein that enhances the activity of a transcription factor by stabilizing its dimerization.

B 411 FUNCTIONAL ANALYSIS AND REGULATION OF THE LIVER TRANSCRIPTION FACTOR HNF-3β, Luca Pani, David Overdier, Xiaobing Qian, Derek Clevidence and Robert H. Costa, Department of Biochemistry, University of Illinois College of Medicine, Chicago, IL 60612.

The transcription factor hepatocyte nuclear factor 3 (HNF-3) is involved in the coordinate expression of several liver genes. HNF-3 DNA binding activity is comprised of three different liver proteins (α,β and γ) which recognize the same DNA site. The HNF-3 genes possess strong homology in the DNA binding domain and contain conserved regions in the carboxyl and amino terminus. We have developed a cotransfection assay in HeLa cells, a cell line which lacks HNF-3 binding activity, to evaluate the functional domains involved in transactivation. We have demonstrated that conserved regions in the amino and carboxyl terminus are responsible for transcriptional activation through the HNF-3 binding site. We have also found that HNF-3 will interact with the transcription factor SP-1 to potentiate expression in a cotransfection assay. This activity uses a different region of the HNF-3 protein and does not require DNA binding activity. To understand the cell-type specific expression of HNF-3β we have defined the HNF-3β promoter sequences which requires the binding of four nuclear proteins including two ubiquitous factors. One of these promoter sites interacts with a novel cell-specific factor, LF-H3β, whose binding activity correlates with the HNF-3β tissue expression pattern. Furthermore, there is a binding site for the HNF-3 protein within its own promoter, suggesting that an auto-activation mechanism is involved in the establishment of HNF-3β expression. We propose that both the LF-H3β and HNF-3 sites play an important role in the initiation of cell-type specific expression of the HNF-3β gene.

B 412 THE CHROMO BOX GENE FAMILY, A GROUP OF GENES INVOLVED IN DEVELOPMENTAL MEMORY AND HETEROCHROMATIN FORMATION.

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The *Polycomb* (*Pc*) gene of *Drosophila melanogaster* has been shown to be a trans-regulatory repressor of the homeotic genes. The gene has recently been sequenced and the protein product shown to be bound to the polytene chromosomes of the salivary gland. Searches of the databases revealed only one significant homology, this is to a stretch of 37aa in Heterochromatin protein 1 (HP1). The region of protein homology has been termed the chromo domain and its coding sequence the chromo box.

Probes for the chromo boxes of both HP1 and *Pc* have been generated by PCR, using primers specific to the flanks of the homologous region, and with cDNA clones of the genes as the DNA template. Zooblot analysis using these probes has revealed that the chromo box is present in both the animal and plant kingdoms, it is therefore ancient in age and has been strongly conserved during evolutionary time. This finding led us to screen mouse embryonic cDNA libraries with the probes. We have cloned and sequenced genes belonging to both the HP1 and *Pc* subgroups of the chromo box family.

The finding of a region of homology between a gene involved in homeotic repression and a gene thought to be a component of heterochromatin has led to the development of new models of cellular determination. It is proposed that after an initial learning phase in which the patterns of expression of the homeotic genes are set up, the patterns are committed to memory by; encapsulating the inactive genes within heterochromatin like complexes, thus rendering them inexpressible; but leaving the active genes open and hence capable of expression. These different chromatin states are clonally inherited, thereby transmitting the state of expression of the homeobox genes down through time.

B 413 STRUCTURE OF THE HELIX-LOOP-HELIX PROTEIN DIMER: THE ROLE OF ELECTROSTATIC INTERACTIONS IN THE SPECIFICITY OF DIMERIZATION,

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Helix-Loop-Helix(HLH) proteins selectively form either homodimers or heterodimers to bind DNA and regulate transcription. The myogenic factor MyoD dimerizes to the E2A gene products, E12/E47, with a higher affinity than to itself. Analysis of mutated MyoDs in addition to chimeric MyoDs have shown that two amino acid replacements in Helix II change the specificity of MyoD/E12 dimerization. The mutated amino acids are located outside of a putative hydrophobic face of the helix, and each mutation reverses the charge of side chains (Lys → Glu, Gln → Glu). The results indicate that interactions between charged side chains stabilize the HLH dimer and also determine the specificity.

Based on our results and available information, we built a computer model for the MyoD/E12 dimer with QUANTA/ CHARM programs. In the model, the four helixes are aligned in parallel and the hydrophobic sides of the helixes face to the center of the helixes bundle. We also identify seven potential intermolecular interactions of the charged side chains that could stabilize MyoD/E12 dimer, two of which are desrupted by the above mutations. These findings lead us to a model defining the specificity of any two HLH proteins.

B 414 ROLE OF ETS- AND NOTCH-RELATED STRUCTURAL MOTIFS IN A HETEROMERIC DNA BINDING COMPLEX, Catherine C. Thompson, Thomas A. Brown, and Steven L. McKnight, Carnegie Institution of Washington, Department of Embryology, Baltimore, MD

GABP (GA binding protein) was identified as a factor that bound to a cis-regulatory element which facilitated VP16-mediated activation of herpes virus genes. cDNA clones encoding the two subunits of GABP were isolated. The predicted amino acid sequence of GABPα contains an 85 amino acid segment that is related to part of the c-*ets* proto-oncogene. The sequence of the second subunit, termed GABPβ, contains four repeats of an amino acid sequence present in the products of several developmentally important genes, including *Notch* of D. melanogaster and *lin-12*, *glp-1* and *fem-1* of C. elegans. Analysis of the DNA binding properties of GABP showed GABPα was capable of binding to DNA. GABPβ could not bind to DNA, but formed a complex with GABPα that bound to DNA more stably than GABPα alone. When complexed with GABPα, GABPβ could be crosslinked to DNA, suggesting that GABPβ directly contacts DNA. To investigate the role of ETS and Notch-related structural motifs, deleted variants of the two proteins were assayed for complex formation and DNA binding. The ETS domain of GABPα was sufficient for DNA binding, while an adjacent segment of 37 amino acids was required for interaction with GABPβ. The repeated motif of GABPβ mediated stable interaction with GABPα and contacted DNA. These observations reveal the interaction of distinctive sequence motifs normally associated with proteins located in different cellular compartments, suggesting the convergence of nuclear transforming proteins and proteins implicated in developmentally regulated signal transduction processes.

B 415 CHARACTERIZATION OF THE DNA BINDING AND TRANSCRIPTIONAL ACTIVATION PROPERTIES OF THE HMG TRANSCRIPTION FACTOR, TCF-1α. Marian Waterman, Peter Carlsson, Russell T. Boggs and Katherine A. Jones. Molecular Biology and Virology Laboratory, The Salk Institute, La Jolla California, 92037.

T cells develop from pluripotent stem cells into subsets of immuno-competent lymphocytes in stages defined by the absence or presence of various T cell-surface antigens. We recently cloned the gene for TCF-1α, a T cell-specific transcription factor that binds to many T cell antigen genes including the α subunit of the T cell receptor (TCRα). TCF-1α belongs to a subset of high-mobility group (HMG) DNA binding proteins that bind DNA specifically and influence transcription, which includes hUBF (an RNA pol I factor), and *SRY* (Y chromosome-specific, sex determination protein). We have mapped the TCF-1α DNA binding domain and regions important for its transcription activity. We find that the 68 amino acid HMG box is necessary but not sufficient for high affinity DNA binding. Thus, although amino acid sequences in the HMG box are important for binding specificity, a small region of basic residues distal to the HMG box is critical for high DNA binding affinity. Two alpha helices within the HMG box are conserved among those HMG proteins that bind DNA specifically, such as *SRY*. We have constructed sub-domain alpha helix swaps between TCF-1α and *SRY* to identify the residues important for DNA site recognition. In addition, we are analyzing sequences of the HMG box that confer different multimerization abilities to TCF-1α and *SRY*. Domains important *in vivo* for transcriptional activation by TCF-1α lie outside the HMG box. These regions are currently being mapped in T cells using a TCF-1α/Gal4 hybrid gene and a reporter construct in which the TCF-1α binding site within the TCRα enhancer has been replaced by the Gal4 DNA recognition sequence.

B 416 SERUM-DEPENDENT ACTIVATION OF CHICKEN CARDIAC MYOSIN LIGHT CHAIN-2 PROMOTER IS MEDIATED BY A CARDIAC SPECIFIC REGULATORY CIS-ELEMENT AND ITS BINDING PROTEIN. **Mingdong Zhou, S. Shafiq, S. Carlton and M.A.Q. Siddiqui, State University of New York Health Science Center, Brooklyn, N.Y. 11203**

We have previously reported that the tissue specific expression of chicken cardiac myosin light chain-2 (MLC-2) gene involves an upstream sequence element (CSS) which inhibits the gene promoter activity in skeletal muscle cells through interaction with negative regulatory protein factors (Shen et al., Mol. Cell. Biol., 11, 1676-1685, 1991). In this communication, we provide evidence that the MLC-2 gene expression in primary chicken cardiac cells is activated by serum in culture medium and this activation is dependent upon an upstream sequence element and its binding protein. In vitro footprinting, gel retardation and mutagenesis of the MLC-2 proximal promoter revealed several sequence elements with potential involvement in regulation of transcriptional activity. Among them, one sequence (element B) located at -42, is responsible for up-regulation of the promoter in cardiac, but not in skeletal muscle cells. A second positive element (element A) confers transcriptional activation in both cardiac and skeletal muscle cells. These two elements can function independent of each other through their interaction with respective DNA-binding nuclear proteins. Element B recognizes a protein factor (BBF01) present in cardiac, but not in skeletal cells. Lowering of serum concentration in post-transfection culture medium causes a significant reduction of the MLC-2 promoter activity in cardiac cells. Site specific mutagenesis revealed that the serum-dependent activation of MLC-2 gene is dependent upon an intact element B sequence. Nuclear extracts prepared from the primary cardiac cells cultured in low serum medium lack the element B-specific protein factor (BBF-1). These results, taken together, demonstrate that element B is a cardiac muscle s pecific cis-acting regulatory sequence which binds to a serum responsive trans-acting protein factor to mediate the tissue specific up-regulation of the cardiac MLC-2 gene promoter.

Environmental Induction and Cell-Cell Signalling

B 500 NF-IL6, A PLEIOTROPIC TRANSCRIPTION FACTOR INVOLVED IN INFLAMMATORY AND IMMUNE RESPONSES

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NF-IL6 was originally identified as a DNA binding protein responsible for IL-1 stimulated IL-6 induction. Direct cloning of the human NF-IL6 revealed its homology with C/EBP, a liver enriched transcription factor. C/EBP and NF-IL6 recognize the same nucleotide sequence, but exhibit distinct pattern of expression. C/EBP is found in liver and adipose tissues. In contrast, NF-IL6 is expressed at a minor level in normal tissues, but rapidly and drastically induced by LPS or inflammatory cytokines such as IL-1, TNF and IL-6. NF-IL6 is shown to be identical to IL-6DBP, a DNA binding protein which is responsible for IL-6 mediated induction in several acute phase proteins. Furthermore, the NF-IL6 binding motif is identified in several genes including IL-8, G-CSF, IL-1 and immunoglobulin genes. Evidence that NF-IL6 binding activities are increased after IL-6 stimulation without increased NF-IL6 protein synthesis demonstrates the importance of post-tranlational modification of NF-IL6 protein. Some data indicate that phosphorylation is involved in transcriptional and binding activities of NF-IL6. Recently NF-IL6β, another cytokine-inducible member of C/EBP family was isolated. NF-IL6 and NF-IL6β form a heterodimer via a leucine zipper structure. In conclusion NF-IL6 and NF-IL6β may be important transcription factors in immune and inflammatory responses.

B 501 COMPLEMENTARY DNA CLONING AND CHARACTERIZATION OF XENOPUS HOMOLOGUE OF NON MUSCLE MYOSIN HEAVY CHAIN GENE, Naina Bhatia-Dey and Igor B. Dawid, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda MD 20892. Nonmuscle myosins are distinct class of myosins which share common subunit composition and native structure with sarcomeric and nonsarcomeric myosins. They appear to contribute to force production in cellularization, various cell shape changes and movements during gastrulation that are necessary for embryogenesis in Drosophila. We describe cloning, cDNA sequence and expression of a nonmuscle myosin heavy chain gene from Xenopus. RNA blot analysis demonstrates a single transcript which is expressed maternally and in all embryonic stages including tailbud stage embryos. RNA is also expressed in various adult tissues including ovary, testis, stomach, liver, pancreas, lung, heart, spleen, brain, and eyes. The expression seems to be relatively abundant in lung, heart, brain and spleen. The localization of nonmuscle myosin heavy chain protein should elucidate its role in Xenopus embryogenesis.

B 502 HELIX-LOOP-HELIX TRANSCRIPTIONAL ACTIVATORS BIND TO A SEQUENCE IN GLUCOCORTICOID RESPONSE ELEMENTS OF RETROVIRUS ENHANCERS, B. Corneliussen, A. Thornell, B. Hallberg, C. Grundström and T. Grundström, Unit for applied Cell and Molecular Biology, University of Umeå, S-90187 Umeå, Sweden.

An important determinant of the T lymphomagenicity of the murine retrovirus SL3-3 is its enhancer which is preferentially active in T lymphocytes. The enhancer contains, among other domains, a Glucocorticoid Response Element (GRE). However, no or little stimulation of the enhancer activity was seen upon glucocorticoid induction in T lymphocytes, where the basal enhancer activity is high. In contrast, a strong glucocorticoid effect on the enhancer activity was evident in HeLa cells, where the basal activity of the enhancer is much lower. We have investigated whether nuclear factors other than the glucocorticoid receptor interact with the GRE in lymphoid cells. A family of proteins, designated SL3-3 Enhancer Factor 2 (SEF2), were found to interact with a sequence within the GRE. The important nucleotides for binding of SEF2 are conserved within most type C retroviruses. Various cell types displayed differences both in the sets of SEF2-DNA complexes formed, and in their amounts. Mutation of the SEF2 site decreased the basal enhancer activity in different cell types. A cDNA which encoded a protein that interacted specifically with the SEF2 binding sequence was isolated from thymocytes. The protein sequence predicted reveals that this SEF2-1 cDNA belongs to the helix-loop-helix class of DNA binding proteins. Several SEF2-1 mRNA transcripts of different sizes were identified. Molecular analysis of the SEF2-1 gene and many cDNA clones of SEF2-1 revealed many distinct mRNA species containing alternative coding regions as a result of differential splicing.

B 503 CYTOKINE INDUCTION OF ELAM-1 TRANSCRIPTION REQUIRES TWO FACTORS, ONE OF WHICH IS POTENTIALLY NOVEL, John F. DeLamarter, Rob Hooft van Huijsduijnen, James Whelan, Paola Ghersa, Wiweka Kaszubska, Michael Becker-André, Department of Molecular Biology, GLAXO Institute for Molecular Biology, 14 ch. des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland

Expression of several adhesion molecules present on the surface of inflamed endothelial cells is induced by cytokine activity. The tight regulation of their genes' transcription suggests a potent mechanism responsible for the dramatic changes in gene expression. We have shown that induction of Endothelial cell Leukocyte Adhesion Molecule (ELAM-1) expression by cytokines is transcription-initiation dependent, that the mRNA is relatively short lived (half life of approx. 2 hr), and that within the promoter sequence cytokine responsive elements are found in the first 200 bp upstream of the transcription start site, including a binding site for a member of the NFκB family of transcription factors. Mutation of this site showed that the NFκB factor is required for cytokine-induced transcription. Deletional truncations of the promoter showed that the NFκB site was insufficient to allow cytokine induction of ELAM-1 and that a second region is required. By DNase-I footprinting a second site (-150) within the required promoter fragment was identified as binding nuclear proteins. Using a panel of mutated oligonucleotides and binding of nuclear extracts showed 7 continguous bp in the region were involved. Blocked mutations of the sequence inhibited full activity of the promoter following cytokine treatment. This sequence has not been described previously as a recognition sequence for transcription factor binding. Efforts to isolate the protein binding this sequence are in progress.

B 504 NOVEL SEQUENCE ELEMENTS REQUIRED FOR TRANSCRIPTIONAL REGULATION OF CD45.

Jorge F. DiMartino, Department of Immunology, Memorial Sloan-Kettering Cancer Center, New York, NY.

The CD45 (Ly5, B220, T200, LCA) family of cell surface glycoproteins is among the earliest tissue specific antigens expressed by cells of the hematopoeitic lineage and plays a key role in lymphocyte activation. Surface expression begins at the pluripotent stem cell stage and increases or decreases with differentiation to more mature cell types. To understand the basis for this regulation at the level of transcription, we have begun to study the cis and trans-acting requirements for transcription of the murine and human CD45 genes. Genomic fragments of CD45 were subcloned upstream of a promoterless and enhancerless CAT reporter gene and their ability to drive CAT expression was measured after transient transfection into hematopoeitic and non-hematopoeitic cell lines. We found that all of the sequences required for appropriately initiated, tissue specific transcription of both human and murine CD45 are contained within a 200 bp region including the major start site, first exon and first intron. In contrast to the lack of requirement for sequences more than 40 bp upstream of the start site, sequences in the first intron appear to be crucial to expression. In addition, a minor start site, present in the first intron of the murine gene, can function independently as a tissue specific minimal promoter or initiator. No consensus binding sites for known transcription factors were found, suggesting that novel trans-acting factors are involved. The identification of these sequences and factors will be an important first step towards understanding the regulation of this gene, which is key to the development and function of the immune system.

B 505 A TRANSCRIPTIONAL RESPONSE ACTIVATED BY GENOTOXIC STRESS AND CELL GROWTH-ARREST.

Albert J. Fornace, Jr., M. Christine Hollander, Isaac Alamo, Jr., Joany Jackman, Barbara Hoffman-Liebermann* and Dan Liebermann*, Lab. of Molecular Pharmacology, N.I.H., Bethesda, MD, and *Dept. of Biochemistry, Univ. of Pennsylvania, Phila., PA.

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. We have isolated cDNA clones for 5 hamster genes that are coordinately induced either by DNA-damaging agents or by other cell treatments that induce growth arrest such as serum reduction or medium depletion; these genes have been designated *gadd* (growth arrest- and DNA damage-inducible)[1]. This "gadd" response has been found in every mammalian cell line/strain examined including human, hamster, mouse, and rat. Much of the induction is due to increased transcription, but we have recently found evidence for a post-transcriptional component. In most cases the *gadd* genes are not phorbol-ester responsive, but evidence for a role for a cellular kinase(s) other than protein kinase C has been found. Two of the gadd genes, *gadd45* and *gadd153*, have been sequenced from human and hamster cells. In the case of the *gadd45* gene, the promoter region has been highly conserved between human and hamster, and a 22 bp region of homology was found with *gadd153*. When linked to reporter genes, the promoter region of these genes conferred DNA-damage responsiveness in both human and rodent cells. Recently, the *gadd* genes were found to be related to another group of genes involved in negative growth control, the *MyD* genes. *MyD* cDNA clones were isolated on the basis of induction in murine myeloblastic leukemia cells following induction for terminal differentiation and growth arrest in the absence of protein synthesis[2]. *MyD116* was found to be the mouse homologue of hamster *gadd34*. In addition, *MyD118* was found to be related to *gadd45* with 60% homology to the hamster and human *gadd45* cDNA. Thus, the *gadd/MyD* genes represent a new class of genes involved in cell growth cessation.

[1] Fornace, A.J. Jr., Nebert, D., Hollander, M.C., Papathanasiou, M., Fargnoli, J., and Holbrook, N.: Molec. Cell. Biol. 9:4196, 1989.
[2] Lord, K.A., Hoffman-Liebermann, B., and Liebermann, D.: Oncogene 5:387, 1990.

B 506 REGULATION OF GENE EXPRESSION BY ETHANOL IN NEURAL CELL CULTURES.

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Chronic exposure to ethanol produces adaptive changes in the function of the central nervous system (CNS) in animal models and human alcoholism. We have explored the hypothesis that changes in neuronal gene expression underlie this adaptive response of the CNS. Recently we have identified several genes which are regulated by ethanol at the level of mRNA abundance in cultured neuroblastoma cells. Levels of ethanol observed in actively drinking alcoholics (25-100 mM) cause 50-300% increases in mRNA for tyrosine hydroxylase (TH) and the stress protein genes, Hsc70 and GRP94. Transient transfection and deletion analysis of the promoter regions for TH and Hsc70 identify that cis-acting sequences in the promoters of both genes may be involved in the regulation of these genes by ethanol. Interestingly, with the TH gene, this ethanol-responsive region can interact positively with the cyclic AMP regulatory element (CRE) such that ethanol together with 8-bromo-cyclic AMP produces a larger than additive increase in TH expression. Point mutation analysis suggests that the heat shock element (HSE) consensus sequence may be involved in the regulation of Hsc70 by ethanol. Gel retardation analysis, however, does not show an increase in heat shock factor (HSF) binding with ethanol treatment as compared to a typical heat shock induction. Thus, ethanol may regulate HSF in a novel manner. We are currently studying whether similar mechanisms may be involved in the regulation of TH and GRP94 by ethanol.

B 507 Transcriptional Regulation of the Mouse Aldehyde Dehydrogenase and Menadione Oxidoreductase Genes by Aromatic Hydrocarbon Receptor Agonists. Dan D. Petersen, Department of Environmental Health, Cincinnati, Ohio 45267-0056.

Metabolic detoxification of environmental contaminants such as benzo[a]pyrene, as well as toxic plant products like aflatoxin, is generally accomplished by a two-step pathway involving an activation step (Phase I) and a conjugation step (Phase II) ultimately resulting in the formation of an excretable derivative of the parent compound. Paradoxically, the end products of Phase I metabolism are often much more toxic than their precursors, and tight coupling of Phase I and Phase II activities is necessary to avoid toxicity or mutagenesis induced by Phase I products. We have examined the induction of two Phase II gene products, aldehyde dehydrogenase-3 (Aldh-3) and the NAPD-dependent menadione oxidoreductase-1 (Nmo-1) by the aromatic hydrocarbons dioxin (TCDD) and Benzo[a]pyrene (BAP).

Induction of Aldh-3 activity reached a maximum 10.5-fold above control 48 h after the addition of BAP. The induction curve occurred without an apparent time lag, and reached a level 8.5-fold greater than control within 24 h. Increases in the abundance of Aldh-3 mRNA measured by northern blot hybridization, were significantly more rapid, rising 6-fold by 6 h, and peaked at 11 times the normal level by 24 h. Transcription of the Aldh-3 gene, measured by nuclear run-on assays, increased 10-fold after the addition of BAP, and the maximal transcription rate occurred at the 6 h time point. The induction curves for TCDD were very similar except that less toxicity was noted at later time points (48 h).

Expression of Nmo-1 activity was also increased dramatically after the addition of BAP. Nmo-1 activity reached a plateau, 5- fold above control that was sustained for 48 h. Nmo-1 mRNA was induced 6.5-fold by 12 h, and was half-maximal at 3 h. Transcription of the Nmo-1 gene was also increased 6-fold, and in this case reached its maximal level by 4 h. Again, similar results were obtained using TCDD as the inducing agent. The maximal induction using TCDD was 10 % higher, but the shape of the induction curve was identical.

These results are consistent with the conclusion that induction of these Phase II genes by aromatic hydrocarbons is mediated entirely at the transcriptional level.

B 508 ANALYSIS OF THE CD20 PROMOTER: IMPORTANCE OF AN UNUSUAL OCT-2 BINDING SITE IN THE B CELL SPECIFIC EXPRESSION OF CD20. Claire Thévenin, Gaye L. Wilson, and John H. Kehrl, LIR, NIH, Bethesda, MD 20892.

Our laboratory has isolated cDNAs and genomic clones for a series of B cell specific genes including CD19, CD20, CD21, CD22. The promoter region of the CD20 gene was isolated, sequenced, and found to lack a TATA box and known regulatory DNA sequences. Deletion analysis of the promoter with fragments subcloned into a CAT vector localized the major positive CIS-acting element between bp -280/-186, a second positive element between -454/-280, and several negative regulatory elements between bp -454/-828. The sequence -280/-186 conferred B cell specific expression on a heterologous, TATA box containing promoter. Mobility shift assays (MSA) with probes spanning -280/-186 mapped a DNA binding site for a B cell specific factor. Mutational analysis of the binding site identified a TA rich DNA sequence, TTCTAATTAA, in which the core TAAT was essential for binding. Cross competition experiments with the octamer, the TA rich sequence from the CD20 promoter, and a TA rich sequence from the CD21 promoter revealed that all three sequences bound the same protein. Southwestern blot analysis of B cell nuclear extracts confirmed that both the octamer and the CD20 TA rich sequence bound the same nuclear proteins. The CD20 TA rich sequence also mediated phorbol ester induced CD20 expression in the pre-B cell line, PB-697. Transfection studies with the CD20 CAT plasmids demonstrated a PMA inducible element between bases -280/-186, the same region responsible for constitutive CD20 gene expression in mature B cells. MSA with the TA rich sequence revealed a PMA inducible protein. Further analysis by MSA of the region -454/-280 demonstrated 3 DNA binding elements, two of which bind B cell specific factors. Investigation of the nature of these binding factors is under investigation. Thus, Oct-2 and at least 2 other B cell specific DNA binding proteins are likely important in the B cell specific expression of CD20.

B 509 A "NONCLASSICAL' MODE OF INTERFERON-γ-ACTIVATED GENE EXPRESSION; REQUIREMENT FOR STEREOSPECIFIC ALIGNMENT OF INTERFERON-RESPONSIVE ELEMENTS, AND NEGATIVE REGULATION BY A CCAAT-BOX BINDING PROTEIN.

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Class II Major Histocompatibility Complex (MHC) genes are highly inducible by IFN-γ. We have shown that three conserved elements (designated as S, X and Y) separated by spacings that are conserved in lengths but not in sequences are required for this response. These elements are distinct from the "classical" interferon responsive consensus element (IRE) exemplified by class I MHC genes both in sequence and in the requirement for multiple elements of a specific arrangement. We present evidence that the specific arrangement of these elements is important for IFN-γ induction. For example, alteration of the spacing between X and Y elements by half a helical turn disrupted IFN-γ inducibility while alteration by a helical turn had no effect. This suggests that interactions between proteins specific for the X and Y elements may be required for this mode of IFN-γ induction. As additional support that this is a general mode of IFN-γ induction, we have also identified a similar set of S, X and Y elements in another interferon-γ-inducible gene that is unrelated in structure to class II MHC genes.

The Y element is eight bp in length, and contains an inverted CCAAT sequence. It interacts with a recombinant protein, YB-1, previously identified by screening a λgt11 library with a Y element probe. Upon IFN-γ treatment, the expression of YB-1 transcript decreases. To determine if YB-1 is a negative regulator of the IFN-γ induced class II MHC gene expression, YB-1 gene was subcloned into an expression vector, and co-transfected with a plasmid containing a human class II MHC promoter linked to a reporter gene. YB-1 protein suppressed IFN-γ-induced reporter gene expression by 80-90% compared to controls. In contrast YB-1 has no effect on the IFN-γ inducibility of the "classical" IRE from class I MHC promoter.

Pathogenic and Therapeutic Mechanisms Involving Transcription; The Nuclear Oncogenes

B 600 REGULATION OF PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1 EXPRESSION IN HT1080 AND HELA CELLS. INDUCTION OF PROMOTER/REPORTER GENE EXPRESSION WITH PHORBOLESTER, Koen A. Descheemaeker, Sabine Wyns, Luc Nelles, Johan Auwerx*, Tor Ny** and Désiré Collen, Center for Thrombosis and Vascular Research, University of Leuven, *Legendo, University of Leuven, B-3000 Leuven, Belgium and ** Unit for Applied Cell and Molecular Biology, University of Umeå, S-901 87 Umeå, Sweden.

Plasminogen activator inhibitor (PAI-1) is the primary physiologic inhibitor of tissue plasminogen activator (t-PA) and elevated levels of PAI-1 have been correlated with a thrombotic tendency. In the present study we have evaluated the role of promoter-elements in the responsiveness of the PAI-1 promoter to PMA.

Addition of 160 nM PMA to cells transfected with a 817 or 320 bp PAI-1 promoter fragment linked to a chloramphenicol acetyl transferase (CAT)-marker gene induced the CAT-activity in HeLa and HT1080 cells 10- and 4-fold, respectively, indicating that the first 320 bp of the PAI-1 promoter contain PMA responsive sequences. Site-specific mutagenesis of potential AP1 and AP2 sites in this 320 bp fragment abolished the PMA-induction in HT1080 cells and slightly affected the induction in HeLa cells. Gelshift experiments with oligonucleotides representing these potential AP1 and AP2 sites and nuclear extracts prepared from HeLa cells demonstrated the binding of AP1 and Sp1 like proteins. South-Western blotting experiments using the ^{32}P labeled AP1 oligonucleotide of PAI-1 hybridized to molecules with the same molecular weight as those recognized by labeled consensus AP1 oligonucleotide. However, the AP2 oligonucleotide of PAI-1 hybridized to proteins with molecular weight of ± 67 kD and 52 kD, whereas consensus Sp1 oligonucleotide hybridized to proteins with molecular weights of 105 kD and 52 kD. Labeled consensus AP-2 only recognized the 52 kD protein. In conclusion a new 67 kD DNA binding protein may be involved in the response of the PAI-1 gene to stimulation with PMA.

B 601 REGULATION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I GENE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM: THE ROLE OF ENHANCER BINDING FACTORS. Paul D. Drew, Kevin G. Becker, Margi E. Goldstein, Harold Gainer, Lois A. Lampson, Keiko Ozato, and Dale E. McFarlin, Neuroimmunology Branch, NINDS, NIH, Bethesda, MD 20892. Genes of the Class I Major Histocompatibility Complex are expressed in most somatic cells. However, MHC genes are not expressed in the central nervous system. In these studies, cells derived from human neuroblastomas have been used as a model for investigating the lack of MHC Class I expression in the brain. The neuroblastomas studied contain neurofilament proteins common to neurons and exhibit very low levels of MHC Class I RNA and protein cell surface expression. In addition, factors which bind the MHC Class I enhancer sequence which resembles an NFKB binding site are absent in the neuroblastomas. Interferon-gamma and tumor necrosis factor-alpha increase the levels of MHC Class I RNA and protein surface expression in the cells. Tumor necrosis factor-alpha also stimulates factor binding to the MHC Class I enhancer sequence. The cytokines act transcriptionally, at least in part, as determined by transfection studies utilizing MHC Class I- CAT reporter constructs. Factors which bind NFKB-like sequences in the neuroblastomas are being characterized by antibody and UV-crosslinking analyses.

B 602 DISPLACEMENT OF RB FROM THE TRANSCRIPTION FACTOR, E2F, BY HPV-16 E7, RBP-1 AND RBP-2 PROTEIN FRAGMENTS, P.S. Huang, K. Haskell, D. Heimbrook, R. Jones, A. Oliff, D. Defeo-Jones. Merck Sharp and Dohme, West Point, PA 19486.

The gene product of retinoblastoma, a tumor suppressor gene (pRb), has recently been shown to complex with the cellular transcription factor, E2f (1). The appearance of pRb-E2f complexes in cells can be correlated with a reduction in E2f stimulated transcription, suggesting a role for pRb in the control of growth specific transcription.

pRb can be displaced from E2f by the viral oncogene, adenovirus Ela protein (2). The ability of Ela to bind to pRb is at least partly dependent on a conserved amino acid sequence, LXCXE (3). This sequence also appears to be involved in the displacement of pRb from E2f. These results suggest that other proteins which bind to pRb and contain this sequence might be involved in the pRb-E2f interaction. Proteins which fall into this category include the HPV-E7 protein and the cellular pRb binding proteins, RBP-1 and RBP-2(4).

Using the gel shift assay for E2f binding to DNA, we have investigated the ability of the full-length HPV-E7 protein and smaller E7 protein fragments to disrupt pRb-E2f interactions. Additionally, protein fragments for RBP-1 and RBP-2 which bind to Rb have been assayed for the ability to interact with cellular E2f complexes. The results from these experiments will be presented.

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2. Bagchi, S. et.al. Cell 62, 659-669, 1990
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B 603 **A NOVEL GAMMA INTERFERON SPECIFIC RESPONSE ELEMENT REGULATES INDUCTION OF THE HUMAN CD64 GENE,** Ravi R. Iyer, Phillipe D. Benech, Kedarnath Sastry and Alan Ezekowitz, Div.of Hematology /Oncology, Childrens Hospital and Dana Farber Cancer Institute, 300 Longwood Ave., Boston, MA 02115.

The human CD64 gene for the high affinity FcγI receptor is expressed in a cell and stage specific manner in only γIFN activated macrophages (Mø) and neutrophils and presents an interesting system for the study of γIFN mediated effects on Mø gene transcription. We have identified a novel cis-regulatory γIFN specific response element (γIRE) in the 5' flanking region of the CD64 gene that functions as an enhancer and confers γIFN specific induction of CAT expression in both RAW 264.7 and H₂la cells. The sequence of the γIRE motif is distinct from all other known γIFN response elements such as the X-box in the MHC class II promoter or the GAF binding motif in the cytoplasmic guanylate binding protein gene promoter. The γIRE motif is highly conserved between the human CD64 promoter and its murine counterpart and interestingly the motif is also present in intron D of the MHC class II HLA DR1 gene, in intron B of the rat cellular retinol binding protein gene as well as the 5' flanking regions of the human leukocyte IFN alpha-a gene, the mouse Hox 3.1 gene and the human C4b-binding protein gene. Electrophoretic mobility shift assays indicate that the motif binds a protein factor (TFγ) that appears within minutes of γIFN stimulation in a specific and competible manner. Data on the preliminary characterisation of TFγ and the possible mechanisms of its activation by γIFN will be presented.

B 604 Regulation of Angiotensinogen gene expression in cardiac cells. Ashok Kumar, Yan-Yan Zhao, and Wei Gu. Depatment of Cell Biology and Anatomy, SUNY Health Science Center, Brooklyn, N.Y. 11203.

Angiotensinogen is the precursor molecule of the biologically active hormone angiotensin-II. Although angiotensinogen is normally synthesized in the liver, we have shown that it is also synthesized in the cardiac cells. In order to understand the regulation of angiotensinogen synthesis in cardiac cells, we have constructed expression vectors where different regions of the 688 b.p. 5'-flanking region of the rat angiotensinogen gene were attached to the coding sequence of the chloramphenicol acetyl transferase (CAT) gene. The promoter activity of the 5'-flanking regions was analyzed by measuring the CAT activity on transient transfection of these mutants in primary cardiac cells obtained from 13 day old chick embryos. These experiments have identified three cis-acting DNA sequences in the promoter region that are involved in the positive regulation of the CAT gene expression and three cis-acting sequences that are involved in the negative regulation of the CAT gene expression in cardiac cells. We have also used gel mobility shift assay to characterize trans-acting protein factors in cardia cells that are involved in binding with cis-acting nucleotide sequences present in the 5'-flanking region of the angioteninogen gene.

B 605 INDEPENDENT BINDING IN VIVO OF MHC CLASS II DRα GENE TRANSCRIPTION FACTORS. Catherine J. Kara and Laurie H. Glimcher. Department of Cancer Bio., Harvard School of Public Health, Boston, MA 02115

Transcription of the human major histocompatibility complex (MHC) class II gene DRα in B cells is mediated by factors interacting with multiple proximal promoter elements. By in vivo footprinting, we have previously identified four DNA binding sites: X1, X2, Y and octamer. Certain cases of the Bare Lymphocyte Syndrome (BLS) are characterized by lack of binding in vivo to all of these sites. In order to determine whether the factors recognizing these sites bind in a hierarchical order or must interact with each other to bind within the chromatin environment, we assessed the effect of mutation of each site individually on the binding to the other sites in vivo. This was accomplished by stably transfecting a B cell line with constructs containing either the wild type DRα promoter or single binding site mutations linked to a reporter gene and footprinting the integrated DNA by ligation mediated PCR. The transfected DNA was distinguished from the endogenous DRα gene by the use of a primer specific for the reporter gene. The wild type construct, containing 176 base pairs of DRα upstream DNA, displayed an in vivo footprint identical to the endogenous DRα promoter. Mutation of either the X1, X2, Y or octamer site eliminated binding in vivo to that particular site. However, for each construct, binding to the other, non-mutated sites was normal, indicating that the mutation did not influence binding to the adjacent sites. Mutation of the region upstream of X1, which is functionally important in transient transfection assays but to which no footprint is observed, also did not influence binding to the other elements. These results suggest that the DRα transcription factors can bind independent of each other in vivo, and furthermore suggest that the global lack of binding observed in BLS does not result from the loss of binding of only one of these transcription factors.

B 606 THE MOLECULAR BASIS OF HLA CLASS II NEGATIVE CONGENITAL IMMUNODEFICIENCY
Janet S. Lee, Sloan Kettering Institute, New York, N.Y.
Absence of HLA class II antigens on B lymphocytes is the basis of some congenital immunodeficiencies (CID). We have shown that mutations responsible for lack of expression fall into four complementation groups. In addition, the genetic defects for each group in HLA class II expression map outside the HLA class II region. Transient expression experiments using a 267bp fragment of the HLA DRA promoter demonstrate that the mutant cell lines are all defective at the level of transcription of HLA class II genes. We have characterized a large complex (C1) that binds to at least two independent sites in the DRA promoter. These sites have not been identified previously as cis-acting elements. Mutation of the sequences in the DRA promoter that bind the C1 complex reduced the ability of the DRA fragment to drive transcription of a reporter gene. A double stranded oligonucleotide that competes efficiently for formation of the C1 complex, but not for any other complexes that bind the DRA promoter, also specifically reduces DRA transcription when added to an in vitro system. These results support the putative function of the C1 complex as a positive trans-acting factor. Complexes with specificity similar to C1 also bind the DPA and DQA promoters suggesting that the C1 complex is involved in coordinate regulation of HLA class II A genes. Binding of the C1 complex and an X consensus element binding protein are quantitatively decreased in mutants within different complementation groups. Nuclear extracts from two groups fail to transcribe a DRA promoter construct in vitro accurately, reflecting their DRA negative phenotypes. Finally, though cell lines from different groups complement each other in vivo, no complementation was observed by mixing extracts for transcription in vitro.

B 607 AP-1 and PEA3 binding elements in the urokinase enhancer mediate regulation by TPA, cAMP and glucocorticoids in HepG2 cells.

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Urokinase is a serine protease controlling a proteolytic cascade important for regulation of peri- and extracellular proteolysis both in the normal organism and during tumor invasion. The urokinase gene is regulated by enhancer sequences located about 2kb upstream of the transcription initiation start site. This enhancer contains AP-1 and PEA3 binding sites necessary for both basal level and induced transcription mediated by the enhancer (1,2). We have found that a region of the enhancer situated between two AP-1 sites is required for the cooperation of AP-1 and PEA3 during TPA-induction of uPA transcription in HepG2 human hepatoma cells. This region contains several evolutionally conserved binding sites for nuclear proteins. Mutation of individual binding sites reduces the TPA-inducibility of the enhancer, and mutation of all of them abolishes it completely.
Investigation of the regulation of uPA expression by cAMP and glucocorticoids showed that the same enhancer sequences were required for regulation by these effectors.
These observations clearly indicate that the PEA3 and AP-1 binding elements in the uPA enhancer are of crucial importance to the transcriptional regulation of the uPA gene, and that additional sequence elements are involved in mediating their activity.

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B 608 ACTIVATION OF C-*FOS* GENE TRANSCRIPTION BY PKC-INDEPENDENT PATHWAYS: SEVERAL RECEPTOR TYROSINE KINASES RAPIDLY INDUCE SIF ELEMENT BINDING ACTIVITY. Henry B. Sadowski, Regina Graham and Michael Z. Gilman. Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, NY 11724.

Transcription of the c-*fos* gene is transiently induced by a variety of extracellular stimuli through the activation of multiple signal transduction pathways. Dissection of the c-*fos* promoter has revealed several discrete sequence elements that confer responsiveness to one or more of these pathways. In Balb/c 3T3 cells at least two distinct pathways act through proteins binding to the SRE. PKC-dependent signals act through a ternary complex composed of SRF and p62TCF. PKC-independent signals act through SRF either alone or in conjunction with other accessory proteins. We are investigating whether the previously characterized v/c-*sis* inducible factor (SIF; Hayes et al., PNAS, 84, 1272, 1987) is such an accessory protein. The SIF binding element is approximately 20 bp upstream of the SRE and coincides with an EGF-inducible in vivo footprint of the c-fos promoter in A431 cells (Herrera et al., Nature, 340, 68, 1989). In Balb/c 3T3 cells, mutations in the c-*fos* promoter that selectively disrupt ternary complex formation but still allow SRF binding lose the PKC-dependent component of the PDGF signal. A construct with mutations abolishing both SIF binding and ternary complex formation is almost completely un-inducible by PDGF; its activity is comparable to a construct lacking an SRF binding site. These and other data suggest that the PKC-independent component of the PDGF induction involves cooperation between the SIF-element and the SRE. Our gel shift experiments show that although the induction of SIF-element binding activity is limited to PDGF in Balb/c 3T3 cells, this activity is rapidly induced (maximal by 5 min) by EGF in A431 cells and by insulin in NIH 3T3 cells expressing the human insulin receptor. The direct activator of PKC, PMA does not induce binding in any of the cell types tested. However, expression of the *neu* oncogene, which encodes a constitutively activated receptor tyrosine kinase, in NIH 3T3 cells leads to constitutively high levels of SIF-element binding activity. Pretreatment of cells with the phosphotyrosine phosphatase inhibitor sodium orthovanadate further enhances both basal and activated levels of DNA binding. We are currently determining whether other tyrosine kinases are capable of inducing this DNA binding activity, but our data suggest that the SIF element and SIF activity may be a general pathway through which membrane receptor tyrosine kinases activate c-*fos* in a PKC-independent manner.

B 609 EGF RECEPTOR GENE REGULATION IN NRK CELLS X. Y. HOU+, A.C. Johnson#, I. Pastan# and M. R. Rosner+ *+The University of Chicago, The Ben May Institute, Chicago, IL 60637; #Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892*

The epidermal growth factor (EGF) receptor is a tyrosine specific kinase that mediates cell proliferation in fibroblasts and epithelial cells. To study regulation of EGF receptor gene expression in such a system, a human 5' proximal 1.1-kilobase fragment (-1109 to -16 relative to the ATG translation start site) and subfragments of this were subcloned into the CAT expression vector pSVoCAT and transfected into normal rat kidney (NRK) cells. The human EGF receptor gene promoter is fully functional in NRK cells. Deletion analysis indicated that the EGF receptor promoter activity is identical in rat NRK and human cell lines. CAT activity was stimulated by treatment of transfected NRK cells with EGF, TGF-β_1, dibutyryl CAMP, PDBU, retinoic acid, dexamethasone and serum. Additivity was observed in response to EGF plus retinoic acid, retinoic acid plus PDBU, and PDBU plus dibutyryl CAMP; the maximal response was observed with a combination of three inducers (EGF+TGF-β_1+Retinoic acid). Soft agar assays indicated that stimulated EGF receptor gene expression correlated with NRK cell anchorage independent growth. Deletion analysis localized the TGF-β_1 responsive element to a 61 bps promoter region (-911 to -850). This region contains a TIE (TGF-β_1 inhibitory element), which mediates TGF-β_1 inhibitory function in other gene promoters and binds the c-fos oncogene product. Cotransfection experiments indicated that E1A represses EGF receptor gene expression. These results suggest a model for the regulation of the EGF receptor gene by TGF-β and other stimulatory agents.

Late Abstracts

PURIFICATION AND CLONING OF A TRANSCRIPTION FACTOR WHICH ENHANCES THE cAMP RESPONSE IN THE PORCINE UROKINASE GENE PROMOTER, Pierre A. Menoud and Yoshikuni Nagamine, Friedrich Miescher Institute, P.O. Box 2543, CH-4002 Basel

Functional analysis of the promoter of the porcine urokinase type plasminogen activator (uPA) gene showed that a region located 3.4 kb upstream of the cap site contained a cAMP inducible enhancer complex. This complex is comprised of two CRE half-sites, regions A+B and a region C unrelated to the CRE consensus. The C region has specific DNA binding activity as shown by DNase I footprinting assays. The C binding protein enhances the binding of proteins to the A+B region indicating cooperative protein binding may be the mechanism increasing the cAMP response of the uPA gene. Using a luciferase gene reporter system, the C region has been shown to be essential to confer a good cAMP mediated induction in LLC-PK1 cells.

The C domain binding protein has been purified to homogeneity from LLC-PK1 nuclear extract by ion exchange and affinity chromatography. This protein appears to be about 10 to 100 times more abundant in the nuclei than other transcription factors. It shows an apparent molecular weight of 66 kd as determined by SDS-PAGE and South-Western analysis. It binds specifically a region of the porcine uPA promoter located at -3375 to -3342 bp as shown by DNase I footprinting experiment. The N-terminal of the protein was blocked, therefore tryptic peptides are currently being sequenced in order to obtain protein sequences to clone the cDNA encoding this protein. So its characteristics and mechanism of action can be further investigated.

TRANSCRIPTION ENHANCER FACTOR (TEF)-1 ACTIVATES THE HUMAN PAPILLOMAVIRUS-16 E6-E7 ONCOGENE PROMOTER, P97, AND CODETERMINES ITS KERATINOCYTE PREFERENCE, L.P. Turek[1], T. Ishiji[1], M.J. Lace[1], S. Parkkinen[1], R.D. Anderson[1], T.H. Haugen[1], T.P. Cripe[1,2], I. Davidson[3] and P. Chambon[3], Departments of [1]Pathology and [2]Pediatrics, [1]VAMC and [1,2]The University of Iowa College of Medicine, Iowa City, IA 52242, USA, and [3]Inst. de Chimie Biologique, Faculte de Medecine, 67085 Strasbourg Cedex, France

Transcription of the human papillomavirus (HPV)-16 oncogenes, E6 and E7, is limited to keratinocytes and cervical carcinoma cells due to a 5' enhancer. Within the keratinocyte-dependent enhancer fragment, we detected a 37 nt-long element active as a tetramer. An abundant cognate protein we enriched from keratinocytes by sequence-specific DNA chromatography was identified as transcriptional enhancer factor (TEF)-1 by complex size, affinity for *wt* and mutant SV40 and HPV-16 enhancer sequences, and antigenic reactivity with two anti-TEF-1 antibodies. Two TEF-1 binding sites within the HPV-16 37 nt element were necessary for its activation, and several additional TEF-1 sites were identified in the HPV-16 regulatory region by DNase I footprinting. TEF-1 itself is cell-specific, but also requires a cell-specific TEF-1 "adaptor" that can be titrated by excess TEF-1 in intracellular competition ("squelching"). We found that the prototype TEF-1-activated enhancer, $(GT\text{-}IIC)_8$, was active in HaCaT keratinocytes. Using TEF-1 and chimeric GAL4/TEF-1 expression vectors in intracellular competitions, both the $(GT\text{-}IIC)_8$ and HPV-16 P97 promoter and enhancer activity in keratinocytes as well as in cervical carcinoma cells were found to depend on the TEF-1 adaptor. In contrast, lymphoid B-cells which did not contain TEF-1 as a DNA binding protein did not support either GT-IIC or HPV-16-directed transcription. However, TEF-1 was present and activated the prototype GT-IIC enhancer not only in keratinocytes and cervical carcinoma cells, but also in hepatoma cells in which the HPV-16 enhancer functions poorly. TEF-1 and TEF-1 adaptor activities thus codetermine the keratinocyte dependence of HPV-16 transcription.