TRANSCRIPTIONAL CONTROL MECHANISMS

Darly K. Granner, Geoff Rosenfeld and Shing Chang, Organizers

April 6 — April 13, 1986

Plenary Sessions

Poster Sessions

DNA-Protein Interaction

01 STRUCTURE AND FUNCTION OF TRP REPRESSOR. P. B. Sigler, R. W. Schevitz, Z. Otwinowski, A. Joachimiak, C. L. Lawson, R.-G. Zhang and R. Marmorstein, Department of Biochemistry and Molecular Biology, University of Chicago, 920 East 58th Street, Chicago, Illinois, 60637.

The crystal structure of the E. coli trp repressor has been solved and refined to 2.2 A. The two subunits (107 residues each) are related by an exact crystallographic dyad. Each subunit is composed of six helices, five of which intertwine about each other in a way that may make it impossible to disengage the subunits without altering their tertiary structure. The two symmetrically related L-tryptophan binding sites are formed by this interface.

Tryptophan must bind to the protein for repressor function. Tryptophan acts as an allosteric effector as follows: (i) L-tryptophan is wedged between the amino end of the C helix of one subunit and the side of the E helix of the dimer-related subunit. This fixes the orientation of the E helix, the most important element in recognizing the operator. (ii) The polar substituents of the bound tryptophan mold the protein's polar residues near the region of the represor surface where the DNA backbone most closely approaches the protein. (iii) The charged amino group of L-tryptophan binds to the carboxyl terminus of the B helix and presumably mitigates a repulsive negative potential arising from the α-helical dipole.

To understand how repressor activity is induced by tryptophan, we are analyzing crystals of (i) aporepressor, the inactive dimeric protein that has no bound tryptophan; and (ii) pseudorepressor, a nearly isomorphous inactive adduct formed when tryptophan is displaced by analogues. Comparison of repressor and these inactive variants should show why bound tryptophan is essential for sequence-specific binding.

Modeling studies of the trp repressor/operator system show that hydrophobic interactions may play a more important role in sequence-specific DNA binding than has been reported for other regulatory proteins.

02 GENETIC ANALYSIS OF DNA-BINDING PROTEINS, Philip A. Youderian, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-1481

We have developed a general genetic strategy for the selection of bacterial clones that express DNA-binding activities corresponding to particular DNA recognition sites. This selection uses a "challenge phage" vector, P22 Kn9 arc-amH1605, into which is substituted a synthetic DNA-binding site for a site that controls transcription of the P22 antirepressor (ant) gene. Constitutive synthesis of antirepressor channels a challenge phage into lytic development and efficiently kills an infected host, unless the substituted site is bound by a specific protein. In this case, the challenge phage prefers lysogenic development, and the host survives and acquires an antibiotic-resistant phenotype.

Infections with challenge phages carrying the E. coli Lac operator, phage lambda O_{L1} operator, or synthetic, symmetric E. coli Trp, transposon Tn10 Tet, and E. coli Gal operators select clones that express each of the corresponding binding activities. The use of challenge phage vectors may be extended to select clones that express specific eukaryotic DNA-binding activities.

Biochemical Analysis of Transcription

03 INITIATION-SPECIFIC EVENTS IN MITOCHONDRIAL TRANSCRIPTION, David A. Clayton, David D. Chang and Robert P. Fisher, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

Mammalian mitochondrial DNA (mtDNA) maintains a novel displacement-loop (D-loop) structure at the origin of heavy-strand (H-strand) replication. This region is also the site of transcription initiation on both strands of mtDNA (1). The H strand contains a major promoter at the start site of H-strand transcription, very near the gene for phenylalanine tRNA. This promoter (the HSP) is likely used for transcriptional purposes only.

In contrast, the light-strand (L-strand) promoter (LSP) appears to serve as the priming site for H-strand DNA replication as well as the production of RNA transcripts that represent L-strand genes (2,3). An interplay between D-loop transcription and replication may represent a significant control point in organelle biogenesis.

This general mode appears to be true for different mammalian species and the relative positions of critical control regions are similar. Both the HSP and LSP can function bidirectionally (4). The nature of primary sequence determinants is quite variable. A possible mechanism by which this could occur would be the maintenance of transcriptional specificity factors involved in initiating transcription (5). The discrimination between efficiency of transcription and a role in accuracy requires a high level of enzyme purity. It seems clear that these organelle systems have multiple transcription proteins and closely-spaced DNA regulatory elements.

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04 GENE ENCODING THE σ^{37} SPECIES OF RNA POLYMERASE SIGMA FACTOR FROM *BACILLUS SUBTILIS*, A MEMBER OF A MULTIGENE FAMILY OF PARTIALLY HOMOLOGOUS GENES LOCATED AT DIVERSE CHROMOSOMAL POSITIONS, Craig Binnie, Mary Lampe and Richard Losick, Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

Sigma-37 (σ^{37}) is a minor species of RNA polymerase sigma factor from the gram-positive bacterium *Bacillus subtilis* that governs the transcription of a complex family of genes, including genes that are turned at an early stage in spore formation as well as other non-sporulation genes that are switched on at the end of the exponential phase of growth. To be able to study the role of σ^{37} in *B. subtilis* gene expression genetically, we have cloned the gene for this minor species of sigma factor in *Escherichia coli* by using as a hybridization probe a synthetic oligonucleotide, which was designed on the basis of a partial N-terminal amino acid sequence of σ^{37} protein. The cloned DNA was then used to determine the nucleotide sequence of the σ^{37} coding sequence, from which we infer that σ^{37} conforms significantly in its predicted amino acid sequence to other previously sequenced sigma genes in a region that is proposed to be the site of binding of sigma factors to core RNA polymerase. Genetic mapping experiments place the gene for σ^{37}, designated *sigB*, at 40° on the genetic map of Piggot and Hoch, a position well separated from other known sigma factor genes or genes for other components of the *B. subtilis* transcriptional machinery. Multiple species of RNA polymerase sigma factor in *B. subtilis* appear to be encoded by a multigene family of partially homologous genes, which are located at diverse positions on the *B. subtilis* chromosome.

05 DNA BINDING PROTEINS THAT MEDIATE SELECTIVE TRANSCRIPTION OF EUKARYOTIC PROMOTERS. R. Tjian, J. Kadonaga, M. Briggs, K. Jones, U. Heberlein and B. England, Department of Biochemistry, University of California, Berkeley, CA 94720

We have recently purified two promoter-specific transcription factors from HeLa cells on the basis of in vitro transcriptional activation and DNA binding. Using SV40 as a model template, we identified a factor, Sp1, which recognizes and binds to the 21 bp repeat elements of the viral promoter. The binding region recognized by Sp1 consists of six tandem GC-box domains. However, steric constraints and differences in binding affinities of the six potential sites results in only five occupied sites at any one time, with site IV largely unoccupied. In vitro transcription of binding site mutations indicates that sites I, II and III are responsible for activating early SV40 RNA synthesis, whereas transcription in the late direction is mediated by interaction of Sp1 at sites III, V and VI. Sp1 was also found to mediate transcription from a number of other viral and cellular promoters, including the HSV-tk promoter. A detailed genetic and biochemical analysis of the HSV-tk promoter revealed the presence of a second promoter selectivity factor, CTF (CAAT transcription factor), that is required to mediate transcription in vitro. CTF was subsequently shown to recognize and interact with the CAAT-box element, GCCAAT, which is also found in a number of other promoters, including α- and β-globin, ras, and human hsp70. Transcription of HSV-tk appears to require both Sp1 and CTF. Thus, unlike SV40, the TK promoter requires the coordinate action of at least two sequence-specific DNA binding proteins to modulate transcription.

We have also isolated and partially characterized a transcription factor from Drosophila tissue culture cells and embryos that is required for selective RNA synthesis of the distal alcohol dehydrogenase (Adh) promoter. This factor, termed Adf-1, binds selectively to a region of the distal promoter between -46 and -86 relative to the initiation site. Deletion and clustered base substitutions of the binding sequences inhibit transcription both in vitro and in transient transfection assays in vivo. More importantly, Adf-1 activity in extracts derived from embryos collected at different stages correlates with the pattern of Adh expression during embryonic development. We anticipate that further biochemical and genetic analysis of the Adf-1 promoter interactions may provide useful information regarding mechanisms of temporal and perhaps also spatial regulation of transcription.

Enhancers and Enhancer Binding Factors

06 Abstract Withdrawn

07 MULTIPLE ELEMENTS WITHIN THE SV40 ENHANCER CAN FUNCTION INDEPENDENTLY AND COMPENSATE FOR ONE ANOTHER. Winship Herr, Jennifer Clarke, Howard Fox, Brian Ondek, and Alyssa Shepard, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

We are studying the structure and function of elements within the SV40 enhancer that are responsible for activation of transcription. Our strategy has been to make point mutations within specific regions of the enhancer that weaken the enhancer and growth of SV40. We then select for and analyze the structure of SV40 growth revertants in which enhancer function has been restored. We have previously described the structure of 18 revertants of the enhancer mutant dpm12 (1). This mutant contains one copy of the 72 bp element and two point mutations within each of two different 8 bp stretches of alternating purines and pyrimidines ('PU/PY'); each revertant contains a tandem duplication ranging in size from 45 to 135 bp within the mutated enhancer region. A 15 bp region spanning the 'core' element was present in all the duplications, suggesting that this region plays a critical role in restoring activity to the dpm12 mutant.

To identify sequences that can compensate for point mutations within the 'core' element we selected growth revertants of the mutant dpm6, which contains a mutated core element (GTGGAAAG to GTCCAAAG) that partially inactivates the enhancer. SV40 growth revertants of this mutant likewise contain tandem duplications. The duplications span either one or both of two separate regions that we refer to as boxes A and B. Each of these boxes contains one of the 'PU/PY' sequences that were mutated in the dpm12 mutant. These and other results to be described suggest the SV40 enhancer contains multiple elements capable of functioning independently and compensating for one another.

We have examined the relative efficiency of the various mutants and revertants to activate β-globin gene expression in the simian cell line CV-1 and in HeLa cells. We find that both the dpm6 and dpm12 mutants yield similar levels of β-globin RNA after transient expression in either cell line. Revertants of these two mutants exhibit 2-6 fold higher activity in CV-1 cells. In HeLa cells the dpm12 revertants are also 2-6 fold more active but revertants of the dpm6 mutant are less than two fold more active than the dpm6 mutant. These results indicate that the revertant enhancers are differentially selective as to the cell lines in which they will function efficiently.

To determine whether or not the 15 bp 'core' region, that is commonly duplicated in the dpm12 revertants, can activate transcription in the complete absence of the A and B domains, we synthesized a 17 bp long SV40 fragment (GGGTGTGGAAAGTCCCC) containing 14 bp (underlined) of the 15 bp 'core' region. Four and seven tandem copies of this element efficiently stimulate transcription of the β-globin gene in both CV-1 and HeLa cell transient expression assays. We are presently determining whether the B element can also function independently.

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08 REGULATION OF THE HUMAN C-FOS GENE, Richard Treisman, MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, U.K.

Transcription of the c-fos gene is transiently activated when quiescent fibroblasts are stimulated by polypeptide mitogens or whole serum. Large amounts of c-fos mRNA are produced and subsequently rapidly degraded, reaching prestimulation levels within 1-2 hours (1-3). A cloned human c-fos gene transfected into mouse NIH3T3 cells is regulated in a similar manner (4). A short sequence in the 5' flanking region of the c-fos gene is essential for its correct regulation in response to serum factors. This 5' activating element is located 300 bp 5' to the mRNA cap site in a region absolutely conserved between the mouse and human c-fos genes. This region contains homologies to several transcription enhancers, and the 5' activating element has enhancer-like properties. However, correct regulation is not observed when the 5' activating element is replaced by viral enhancers from SV40 or MOMLV. The basis of these different properties is under investigation.

Experiments with gene fusions in which c-fos 5' flanking sequences are joined to heterologous transcription units such as CAT/SV40 or ß globin showed that elements in addition to the 5' activating element are required for transient c-fos RNA accumulation following serum stimulation. The study of hybrid c-fos/ß globin transcription units demonstrated that sequences at the 3' end of the c-fos gene are required for transient accumulation of c-fos RNA following serum stimulation. Deletion analysis of the c-fos gene showed that these sequences appear similar to those previously shown to inhibit transformation of fibroblasts by the c-fos gene (5,6). The c-fos 3' sequences act at least in part to destabilise c-fos mRNA. The properties and possible mechanisms of action of these sequences will be discussed.

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Transacting Factors

09 GCN4, AN ACTIVATOR PROTEIN THAT REGULATES AMINO ACID BIOSYNTHETIC GENES IN YEAST, Kevin Struhl, Department of Biological Chemistry, Harvard Medical School, Boston, Mass., USA 02115.

When yeast cells are subjected to conditons of amino acid starvation, transcription levels of *his*3 and many other amino acid biosynthetic genes are induced coordinatley. Detailed deletion analysis of the *his*3 promoter region has distinguished between elements that are necessary for the basal level of expression and those that are required for the regulation in response to starvation. Constitutive *his*3 transcription which is initiated at equal frequency from 2 site (+1 and +12), depends on TATA elements located between -35 and -55 and poly (dA-dT) stretch around -120. In contrast, the induction of *his*3 transcription depends on a separate upstream region between -86 and -102 and on a particular TATA element centered at -40; in addition, all of the induced transcription is intitiated at the +12 site. These and other lines of evidence suggest that *his*3 expression is mediated by two interlaced promoters that operate by different molecular mechanisms.

The *his*3 regulatory site contains the sequence TGACTC, which is found at similar positions in the promoters of other coregulated genes. Analysis of numerous single substitution mutations has defined the nucleotide sequence requirements of this site. The results are that the TGACT nucleotides are absolutely specified, the final C is strongly preferred, and the 3 nucleotides on either side of the core are also of some importance for *his*3 induction *in vivo*.

This general control mechanism requires the product of the *gcn*4 gene because *gcn*4 mutants are unable to induce any of the co-regulated genes. Radioactivity pure *gcn*4 protein, synthesized by transcription and translation *in vitro*, binds specifically to the *his*3 regulatory site and also the promoter regions of other amino acid biosynthetic genes. Analysis of the *his*3 point mutations indicates that the requirements for *gcn*4 binding *in vitro* are identical to those for *his*3 induction *in vivo*. A synthetic *gcn*4 mutant protein lacking the 40C-terminal amino acids fails to bind DNA; this correlates with a *gcn*4 mutant gene that is nonfunctional *in vivo*. By analyzing a series of deletions, the DNA binding activity of the *gcn*4 protein has been localized to the 92 amino C-terminal amino acids. However, this protein is unable to activate transcription *in vivo*. Thus, it appears that DNA binding and transcriptional activation are seperable properties of the *gcn*4 protein.

Regulation of Transcription I

010 REGULATION OF PHOSPHOENOLPYRUVATE CARBOXYKINASE GENE TRANSCRIPTION BY INSULIN, Daryl K. Granner, Kazuyuki Sasaki and David Chu, Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232

Considerable progress has been made toward understanding how insulin regulates the rate at which a specific protein is synthesized within a target tissue. There are now a number of examples of effects of insulin on mRNA metabolism and in several of these instances this hormone influences the rate of transcription of specific genes. Many of these involve multihormonal regulation, so it is now possible to study how several hormones, each with a unique mechamism of action, coverage to regulate a single gene.

The first, and most completely studied example of this action of insulin concerns the regulation of phosphoenolpytuvate carboxykinase (PEPCK), the rate-limiting enzyme in gluconeogenesis. The rate of synthesis of this enzyme in H4IIE hepatoma cells is subject to positive regulation by cAMP and glucocorticoid hormones, and to negative regulation by insulin and phorbol esters. These effects are directly due to alterations of PEPCK mRNA, which in turn are related to differences in the rate of transcription of the PEPCK gene. Transcription of this gene is increased 10-15 fold by cAMP and 5-6 fold by glucocorticoid hormones and is inhibited by insulin. This effect of insulin is exerted at physiologic concentrations of the hormone (1 pM-1 nM), occurs within 5 min., is rapidly reversible, does not require on-going protein synthesis, is highly specific, and is dominant over the actions of the positive regulators. Using the nuclear "run-off" assay, and probes directed against different regions of the PEPCK gene, it is apparent that insulin does not attenuate transcription. Insulin does result in a 3 fold slower rate of elongation of nascent PEPCK transcripts (as compared to that measured in cAMP treated cells) but this does not account for the total effect of the hormone. The effect of cAMP and insulin on transcript initiation was evaluated in an experiment in which PEPCK RNA transcripts were labeled in a nuclear "run-off" assay then allowed to hybridize to a genomic DNA fragment representing nucleotides -460 to +69. The extending single strands of DNA and RNA were digested with S1 nuclease and RNase plus T1 RNase, thus only those nascent transcripts associated with the first 69 bases of the PEPCK gene remained. This complex was sized by gel electrophoresis and analyzed by autoradiography. A significant increase of transcript labeling was noted in cAMP-treated cells and virtually no labeling was detected in insulin-treated cells. Our interpretation of these studies is that insulin inhibits transcript initiation.

011 REGULATION OF GENE EXPRESSION BY CYCLIC AMP-DEPENDENT PROTEIN KINASE SUBUNITS, Richard A. Jungmann, Andreas I. Constantinou, Stephen P. Squinto and Deborah M. Milkowski, Department of Molecular Biology, Northwestern University Medical School, Chicago, IL 60611

The mechanism of control of eukaryotic gene expression by cyclic AMP and cyclic AMP-dependent protein kinase is unknown. It has been proposed that the effects of cAMP on gene transcription involves phosphorylation of chromosomal proteins by the catalytic subunit, since these phosphorylative modifications can be correlated with altered transcriptional activity. However, no functional role for the regulatory subunits at the genomic level has been identified so far. We have recently found that the regulatory subunit RII (phospho-RII) from rat liver possesses intrinsic topoisomerase activity towards several DNA substrates. Like eukaryotic topoisomerases, phospho-RII can relax both positive and negative superhelical turns of DNA templates. Relaxation of superhelical DNA involves transient formation of a DNA-phospho-RII complex via DNA strand breakage and covalent attachment of the DNA to a tyrosine residue of phospho-RII. The topoisomerase activity of phospho-RII requires cAMP and is altered by changes in the degree of phosphorylation of RII.

Since these data suggest a putative role for phospho-RII in the transcriptional regulation of cyclic AMP-inducible eukaryotic genes through an alteration of DNA topology, we have examined the ability of phospho-RII to bind to and relax pBR322 and several recombinant plasmids containing eukaryotic gene sequences. Our results indicte that phospho-RII binds selectively to pRIII and p87-1, recombinant plasmids containing gene sequences for the cyclic AMP-inducible rat phosphoenolpyruvate carboxykinase and the rat lactate dehydrogenase A subunit genes. In contrast, phospho-RII fails to bind selectively to pBR322 RFI and gene sequences not regulated by cyclic AMP. Examination of the relaxation kinetics indicates that phospho-RII relaxes pRIII and p87-1 more efficiently than gene sequences which are not regulated by cyclic AMP.

We have furthermore investigated the association of the regulatory subunits RI and phospho-RII with DNAase I-sensitive regions of rat C6 glioma cell chromatin. We have found that the regulatory subunits are preferentially release from chromatin as the result of nuclease treatment, indicating the preferential association of the regulatory subunits with transcriptionally active regions of glioma cell chromatin.

These data considered in context provide first evidence for a functional interaction, especially of the regulatory subunit RII with DNA and strengthen the hypothesis that the subunits of cyclic AMP-dependent protein kinase are instrumental in the regulation of cyclic AMP-inducible genes.

Supported by grants from the NIH and the American Heart Association.

012 STEROID HORMONE CONTROL OF GENE TRANSCRIPTION AND mRNA STABILITY, David J. Shapiro, Michelle Barton, Denise Lew, Dennis McKearin and Martha Keller, Department of Biochemistry, University of Illinois, Urbana, IL 61801

We have shown that estrogen induction of the mRNAs coding for the egg yolk precursor protein vitellogenin in primary liver cultures of Xenopus laevis is achieved through both an increase of several thousand fold in the absolute rate of vitellogenin gene transcription, and by a selective 30 fold increase in the cytoplasmic stability of vitellogenin mRNA. Measurement of the absolute rate of nuclear vitellogenin gene transcription, and of the rate of cytoplasmic vitellogenin mRNA accumulation indicate that the efficiency with which each vitellogenin intron is excised in vivo cannot be less than 99%. Comparison of the promoter structure of vitellogenin and a second estrogen inducibe Xenopus gene we designate EISP should help to distinguish promoter elements required for hormone regulation of transcription from those required for efficient transcription.

In order to begin an analysis of vitellogenin transcription in vitro a high efficiency HeLa cell nuclear extract has been developed. Transription of the adenovirus major late promoter is approximately 20 times more efficient in this extract than in standard nuclear extracts. Under appropriate conditions using the strong adenovirus major late promoter more than half the label incorporated into nucleic acid is in the adenovirus major late transript. This should speed purification of transcription factors by eliminating the requirement for gel electrophoretic assays for many purposes. Application of this methodology to transcription of vitellogenin in homologous Xenopus extracts is under way.

Vitellogenin mRNA is degraded with a half life of 480 hours (approximately 3 weeks) in the presence of estrogen and exhibits a halflife of 16 hours when estrogen is removed from the culture medium. The mRNA stabilization is specific for vitellogenin mRNA and is a reversible cytoplasmic effect of estrogen which is not mediated by nuclear modification of the mRNA. Destabilization of vitellogenin mRNA does not appear to be accompanied by either progressive or complete loss of its 3' poly(A) tail.Comparative DNA sequencing of several cloned vitellogenin mRNAs and other data reveal a conserved secondary structure element in the 3' untranslated region of vitellogenin mRNA whose properties are consistent with a role in the regulation of mRNA stability.

Regulation of Transcription II

013 Hormonal and Environmental Control of Gene Expression in Mammalian Cells, Michael Karin, Alois Haslinger, Adriana Heguy, Masayoshi Imagawa, Rick Imbra, Therese Dietlin and Tracy Cooke, USC School of Medicine, Department of Microbiology, Los Angeles, CA 90033

The expression of metallothionein (MT) genes in animal cells is regulated at the transcriptional level by a large number of hormonal and environmental cues. Among these are steroid hormones (glucocorticoids and progesterones), interferon, interleukin 1, serum factors, heavy metal ions, phorbol esters and ionizing radiation. In addition to the complex regulation by a large number of inducers, the expression of the MT gene family is further complicated by the presence of a large number of genes which not only respond differently to induction, but also differ in their basal levels. To fully understand the mechanisms which control both the basal and induced transcription of the MT gene family, it is essential to characterize in detail the various cis and trans acting genetic elements involved in these regulatory circuits. To this end, we have used an integrated approach based on in vitro mutagenesis, gene-transfer and in vitro protein-DNA binding probed by DNase I.

The results indicate that diversity in expression of the hMT gene family is therefore caused by the presence of distinct promoter elements on each of its members. While some of the control signals are common to all MT promoters (MRE's) others are more specific (GRE's). Similar elements can be organized in a different fashion on each promoter. These results indicate the emergence of a regulatory code, composed of short regulatory signals which serve as binding sites for distinct trans acting factors, whose activity is modulated by different hormonal and environmental factors.

014 DEVELOPMENTAL AND REGULATED EXPRESSION OF PROLACTIN AND GH GENE TRANSCRIPTION, Michael G. Rosenfeld, Harry Elsholtz, Harry Mangalam, Christian Nelson, Bryan Crenshaw, Rodrigo Franco, and Ronald M. Evans, UCSD School of Medicine and Salk Institute, La Jolla, CA 92093

The rat prolactin and GH genes exhibit a very restricted pattern of expression, expressed only in a subset of cells in the pituitary gland, referred to as lactotrophs and somatotrophs, respectively. We have identified sequences in the 5'-flanking regions of both genes which act in a position- and orientation-independent fashion to transfer cell-specific expression of heterologous genes in vitro and in transgenic animals. The putative transactive factors required for the growth hormone gene enhancer function are apparently discrete because two lactotroph cell lines selectively fail to express fusion genes containing the GH gene enhancer sequence. The prolactin gene enhancer appears to contain two discrete regions, both of which are required for function and which appear to bind different transacting factors. A short 5'-flanking sequence is responsible for prolactin gene transcriptional regulation by growth factors, hypothalamic releasing hormones, and cAMP.

Regulation of Patterns of Gene Expression

015 MOLECULAR ANALYSIS OF AN ENHANCER OF RECOMBINATION, Reid C. Johnson, Michael F. Bruist, and Melvin I. Simon, Division of Biology, California Institute of Technology, Pasadena, CA 91125

The expression of certain genes is controlled by the rearrangement of specific DNA sequences. One such system involves the alternate expression of different flagellin genes in Salmonella typhimurium. This alternate expression is the result of inversion of a 995 bp segment of DNA. The site-specific recombination reaction generating the inversion has been shown to require the function of the hin gene which is located within the invertible segment.

The site-specific recombination reaction can be performed in vitro using extracts of E. coli in which the Hin protein has been overproduced. The in vitro system has been fractionated to determine the components necessary for the reaction. Maximum rates of recombination in the purified reconstituted system require 1) a supercoiled DNA substrate, 2) the Hin protein, 3) the E. coli histone-like protein HU, and 4) a 12,000 MW unidentified protein from E. coli.

The DNA substrate for the inversion reaction must contain two recombination sites in the proper configuration (inverted) plus an additional cis-acting sequence (a recombinational enhancer).

The recombinational enhancer increases the rate of recombination over 25 fold. It can be located on either side of the recombination site, in either orientation, and at variable distances (less than 100 bp to over 4000 bp) from the recombination site. The important sequences have been mapped by deletion analysis to be within two approximately 20 bp sequence domains within a 60 bp region. The 12,000 MW host protein specifically binds to these 20 bp domains as assayed by DNA footprinting. In addition to the 12,000 MW protein, the HU protein is probably required for the enhancer-mediated stimulation of recombination.

The recombination site consists of a 26 bp sequence which contains two 12 bp imperfect inverted repeats. The Hin protein binds to the recombination site and protects these 26 base pairs. The binding is mediated by the C-terminal end of the Hin molecule since a synthetic peptide consisting of the C-terminal 52 amino acids of Hin protects the DNA within the inverted repeats. It is presumed that the recombinational enhancer must be involved in bringing the recombination sites together in the proper synaptic complex to allow for strand exchange.

016 THE TISSUE SPECIFICITY OF DROSOPHILA P ELEMENT TRANSPOSITION IS REGULATED AT THE LEVEL OF mRNA SPLICING, Frank A. Laski, Donald C. Rio and Gerald M. Rubin, Dept. of Biochemistry, U. of Calif. at Berkeley, Berkeley, CA 94720

P elements are transposable elements found in Drosophila melanogaster that are interesting for a number of reasons. These include that they transpose at a very high rate, that they are under a very tight developmental regulation (they only transpose in germ-line cells and not in somatic tissues), that they are regulated genetically (the P-M hybrid dysgenesis system) and that they are the vector used in the Rubin-Spradling Drosophila transformation system.

An intact P element is 2.9kb long, has four major open reading frames (ORF), and encodes its own transposase. Previously, Roger Karess (in G. Rubin's lab) had shown that a 2.5kb transcript is the major transcript from the 2.9kb P element. He also showed, using site specific mutagenesis, that all four open reading frames (ORFs) in P elements are required to code for an active transposase. Primarily using S1 nuclease mapping techniques we have determined the structure of the 2.5kb mRNA. The interesting result from this study was that the first three ORF's in the genomic P element (ORF 0, 1 and 2) are spliced together in frame in the 2.5kb transcript but that no splice could be detected into ORF3. This presented the problem of how the information in ORF3 (information that is required for transposase activity) is expressed. For a variety of reasons we thought the best possibility was that there really was a splice connecting the first three ORFs to ORF3 but that it could not be detected because it was occuring only in germ line cells. The mRNA we were studying came from 0-12 hour embryos which are >95% somatic tissue, thus if germ-line specific splicing was occuring it is not surprising that we could not detect it. This germ-line splicing theory would also explain the germ-line specificity of P element transposition. If an ORF2 to ORF3 splice is required to make an active transposase and if this splice only occurs in germ-line cells then P element transposition would also be restricted to germ-line cells.

Using site specific mutagenesis and P element transformation techniques we have shown that the germ-line splicing theory is correct. The most compelling evidence uses a P element deletion mutant (designed and constructed *in vitro*) that exactly deletes out the intron between ORF2 and ORF3 of P element. This deletion mutant not only codes for transposase activity in germ-line tissues but also codes for transposase activity in somatic tissue.

017 REGULATION OF MEIOSIS IN YEAST BY THE MATING TYPE LOCUS AND THE PRODUCT OF THE RME1 GENE, Aaron Mitchell and Ira Herskowitz, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

The yeast S. cerevisiae may exist as either a haploid or a diploid. The transitions between these states, mating and meiosis, are controlled by a single genetic locus, the mating type locus (MAT). There are two haploid cell types, **a** and α, which are specialized for mating with one another; these differ genetically by carrying MAT**a** or MATα alleles, respectively. Fusion of an **a** cell with an α cell produces the third cell type, an **a**/α diploid, which is unable to mate but is uniquely capable of meiosis and sporulation. The MAT gene products dictate cell type through regulating expression of unlinked genes that encode cell specialization functions. We have focused on the target of MAT control that governs entry into meiosis, the RME1 gene (Regulator of Meiosis).

Expression of both MAT**a** and MATα alleles (in **a**/α cells) gives rise to **a**1-α2 activity, a repressor of several genes (such as HO and STE5). Previous genetic studies suggested that **a**1-α2 acts via the RME1 product to promote meiosis. We have cloned the RME1 gene and shown that its product is an inhibitor of meiosis: cells that lack RME1 product due to an insertion mutation constructed in vitro are able to initiate meiosis. Conversely, meiosis is blocked even in **a**/α cells if they express the functional RME1 gene.

How does **a**1-α2 control RME1 activity? Northern blots show that the RME1 transcript is repressed by **a**1-α2. **a**/α cells thus enter meiosis because **a**1-α2 turns off synthesis of the meiosis inhibitor, RME1 product. The other cell types (**a** and α) do not produce **a**1-α2 and therefore express RME1, which prevents initiation of meiosis.

Our current goal is to understand how RME1 product blocks meiosis. The RME1 product may be a repressor of meiosis-specific genes. Another possibility is that it is a protease or protein kinase that inactivates a meiosis-specific gene product. We are presently sequencing the RME1 gene to uncover clues about the structure, localization, and activity of its product.

Chromatin Structure and Transcription

018 ROLE OF NUCLEOPLASMIN MATURATION IN THE ORGANIZATION OF CHROMATIN STRUCTURE. R. Chalkley, M. Cotten, S. Felts, G. Kepa and L. Sealy, Department of Biochemistry, University of Iowa, Iowa City, IA 52242

In order to study the effects of various factors and enhancer binding proteins on the transcriptional activity of DNA, it seems unavoidable that eventually systems must be devised which employ reassembled chromatin, rather than simple naked DNA, as a template. Accordingly we have devoted much effort to developing approaches for obtaining correctly reassembled chromatin in vitro. Previous work on the role of nucleoplasmin in chromatin assembly required hyperphysiological amounts of histone. Conditions hence have been defined which permit assembly in the presence of equimass amounts of histone and DNA. We find that nucleoplasmin isolated from stage 6 oocytes is inactive in chromatin assembly, but that nucleoplasmin is activated during the final oocyte maturation steps at the time of GVBD. The activation process involves the modification of the amino terminal portion of pentameric nucleoplasmin by approximately 100 phosphate groups. This process can also be achieved in vitro if extracts containing maturation promoting factor are employed. Using this system the role of TFIII A, B and C in the organization of active yeast 5S chromatin have been studied. We find that there is an obligatory requirement for factor binding before histone addition and hence analyzed the required order of factor binding. The assays for appropriate binding and transcription have involved either addition of polymerase III and transcription in vitro, or injection of the reassembled chromatin template into oocytes.

019 SUBPOPULATIONS OF SV40 MINICHROMOSOMES AND TRANSCRIPTIONAL COMPLEXES, P. Oudet, E. Weiss, P. Schultz, C. Ruhlmann and E. Regnier, Institut de Chimie Biologique, Faculté de Médecine, U. 184 de l'INSERM, Laboratoire de Génétique Moléculaire des Eucaryotes du C.N.R.S., 11 rue Humann, 67085 STRASBOURG Cedex, France.

SV40 minichromosomes were extracted and purified from CV1 infected cells in a variety of conditions : either physiological or low ionic strengths. This material was characterised for its mass by STEM analysis, percentage of gap molecules, sensitivity to restriction enzymes and transcriptional activity.
The minichromosome and nucleosome masses of ungapped and gapped molecules were compared in the same conditions and not found significantly different. We directly show that transcriptional complexes prepared in 130 mM NaCl are preferentially sensitive to restriction enzymes, can be obtained in enriched fractions and contain an open free origin (gap).
The role of the late protein VP_1 on the transcriptional activity was previously described. The possible influence of VP_1 on the structure and the transcriptional activity will be discussed.

RNA Processing

020 THE INTERVENING SEQUENCE RNA OF TETRAHYMENA IS AN ENZYME,
Arthur J. Zaug and Thomas R. Cech, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215
A shortened form of the self-splicing ribosomal RNA intervening sequence of Tetrahymena thermophila acts as a true, regenerated enzyme in vitro. The enzyme (the L - 19 IVS RNA) catalyzes the cleavage-ligation of oligonucleotide substrates in a sequence-dependent manner with K_m = 42 μM and k_{cat} = 2 min^{-1}. The reaction mechanism resembles that of pre-rRNA self-splicing. With pentacytidylic acid as the substrate, successive cleavage and rejoing reactions lead to the synthesis of polyC. Thus, the RNA molecule can act as an RNA polymerase, differing from the protein enzyme in that it uses an internal rather than an external template. At pH 9, the same RNA enzyme has activity as a sequence-specific ribonuclease.

021 **Abstract Withdrawn**

Genetic Analysis of Gene Expression

022 CIS AND TRANS REGULATION OF A TYPE I COLLAGEN GENE, Benoit de Crombrugghe, Azriel Schmidt, Chiaki Setoyama and Pellegrino Rossi, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Transformation of fibroblasts by a number of oncogenes such as v-src, v-ras or v-mos results in changes in the expression of several genes. Among these is a strong transcriptional inhibition of type I collagen gene expression. To study the expression of these genes, we have constructed a recombinant gene consisting of the promoter of the mouse α_2(I) collagen gene (-2000 to +54) fused to either the chloramphenicol transacetylase gene (CAT) or the aminoglycoside phosphotransferase gene (neo). In experiments performed in collaboration with J. Khillan, P. Overbeek and H. Westphal (NIH), we observed tissue specific expression of the chimeric CAT gene in transgenic mice. Cell type specific expression of this gene was also found in tissue culture cells. Two segments of this promoter between -900 and -502 and between -346 and -104 are needed for optimal expression of the chimeric gene.

After stable transfection of an α_2(I) collagen promoter-neo chimeric gene in NIH 3T3 cells, these cells become resistant to G418. Transformation of these cells by v-mos results in a decrease in neo RNA which parallels the decrease in the expression of the endogenous α_2(I) collagen gene, presumably because a common mechanism inhibits both the endogenous and transfected promoter. In some of these cell lines, v-mos transformation causes a loss of G418 resistance. After mutagenesis of these v-mos transformed, G418 sensitive cells, G418 resistant colonies were selected. Two G418 resistant mutants showed an increased expression of the chimeric neo gene and of the endogenous type I collagen and fibronectin genes. No changes occurred in the levels of v-mos RNA and DNA in these cells or in the ability of these cells to induce tumors. We postulate that the mutations might alter cellular pleiotropic trans-acting factors that either directly or indirectly control the expression of the type I collagen and fibronectin genes.

023 REGULATION OF GENE EXPRESSION BY cAMP, Michael M. Gottesman, Irene Abraham* and Robert Fleischmann, National Cancer Institute, NIH, Bethesda, MD 20892 and *The Upjohn Company, Cell Biology Department, Kalamazoo, MI 49001

We have taken a genetic approach to analyze the involvement of cAMP dependent protein kinase (cADepPK) in regulation of gene expression by cAMP in cultured cells. A large number of independent cAMP-resistant mutants of Chinese hamster ovary (CHO) cells have been isolated (1). Most, if not all, of these CHO mutants contain alterations in activity of cADepPK. Mutants with affected catalytic (C) subunits (2) and Type I regulatory (RI) subunits (3) have been isolated. These mutations are dominant in somatic cell hybrids (4). The cAMP-resistance phenotype of these mutants can be transferred by DNA-mediated gene transfer into cAMP-sensitive CHO cells (5). We have cloned genomic sequences for RI and C from dominant RI and C subunit mutants in the cosmid vector pSV13:cos (M. McCormick and B. Howard, National Cancer Institute). The goal of these studies is to use the cloned mutant sequences as movable genetic elements for the introduction of genes encoding cAMP-resistance into cultured cell lines to study the role of cADepPK in affecting gene expression in differentiated cells.

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024 MOLECULAR GENETICS OF EUKARYOTIC RNA POLYMERASES, C.J., Ingles, M. Moyle, L.A. Allison, J.K.-C. Wong, J.D. Friesen, J. Archambault and E.M. Elliott. Banting and Best Department of Medical Research and Department of Medical Genetics, University of Toronto, Toronto, Ontario M5G 1L6, Canada.

Genetic and biochemical studies of eukaryotic RNA polymerases can contribute to an understanding of the molecular mechanisms of eukaryotic gene expression. The mutations in RNA polymerase II that conferred α-amanitin-resistant and temperature-sensitive phenotypes provided an entry for studies leading to the molecular cloning of several RNA polymerase genes. Using as probe DNA encoding the largest subunit of Drosophila polymerase II we have isolated related DNA sequences encoding both hamster and yeast RNA polymerases. Our analysis indicated that there is a family of genes in all eukaryotes encoding the largest subunits of RNA polymerase I, II and III. Nucleotide sequencing indicated that there are conserved regions in the amino acid sequences of the eukaryotic RNA polymerase subunit polypeptides that are remarkably similar in their primary structure to analogous portions of E. coli RNA polymerase. The largest subunit of both yeast and hamster RNA polymerase II has an additional C-terminal domain composed of an unusual tandemly repeated heptapeptide sequence. The importance of this novel domain in the eukaryotic enzyme is implied by studies demonstrating its requirement for transcription in vitro from class II promoters.

Mutations have been introduced into the yeast gene encoding the largest subunit of polymerase II (RPO21) by several techniques including the use of 12 bp oligonucleotide insertions at a variety of restriction enzyme sites. These mutations are being tested for lethality, ability to complement an RPO21 null allele, temperature-sensitivity and ability to sporulate. This genetic approach may provide information concerning the role of this subunit and its novel C-terminal domain in transcription.

025 THE HORMONAL CONTROL OF DEVELOPMENTALLY REGULATED ADIPOSE GENES, Gordon M. Ringold, Alger B. Chapman, David M. Knight, Marc E. Navre and Frank M. Torti, Department of Pharmacology, Stanford University School of Medicine, Stanford, California 94305

We have analyzed the basis for the control of adipocyte differentiation by glucocorticoids and cachectin (tumor necrosis factor). The cell line, TA1, derived from 5-azacytidine treated 10T1/2 mouse embryo fibroblasts, undergoes differentiation in culture after reaching confluence (1). Using cDNA clones corresponding to mRNAs that are induced during adipogenesis, we have found that dexamethasone elicits the precocious transcriptional activation of differentiation-specific genes. Since the hormone does not need to be present continuously, we propose that a regulatory factor(s) involved in triggering the conversion of pre-adipocytes to adipocytes is under glucocorticoid control.

In addition to their effects on differentiation, glucocorticoids also stimulate the specific transcription of a gene which is expressed only in mature TA1 adipocytes (2). The hormonal induction of this gene, that by sequence analysis we have identified as a member of the cytochrome P450 gene family, appears to be completely dependent on prior developmental activation.

Lastly, we have used TA1 adipocytes to study the control of adipogenesis by the macrophage hormone, cachectin (3). Production of cachectin (which is identical to tumor necrosis factor, TNF-α) is associated with a chronic catabolic state known as cachexia. Recent experiments indicate that cachectin not only blocks the differentiation of TA1 cells but rapidly and reversibly shuts off the transcription of adipose-specific genes. Moreover, the data suggest that this hormone may reverse the "terminal" differentiation step(s) associated with activation of adipogenic genes.

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Transgenic Animals

026 DEVELOPMENTAL REGULATION OF GLOBIN GENES IN TRANSGENIC MICE, Frank Costantini, Jeanne Magram, Marie Trudel and Kiran Chada, Columbia University, New York, N.Y.

The ability to introduce cloned genes into the mouse germline has been exploited to study the regulatory DNA sequences that control the tissue-specific and stage-specific expression of the beta-globin gene family. We first found that a cloned adult beta-globin gene in the mouse germline could be expressed specifically in erythroid cells[1], and also showed appropriate stage-specific expression[2]. These studies demonstrated that the cis-acting DNA sequences responsible for the tissue-specific and temporal expression pattern of the adult beta-globin gene are closely linked to the gene, and that the transgenic mouse can provide an assay system in which to identify these sequences.

We next studied the behavior of another member of the human beta-globin gene family, the G-gamma globin gene. In humans, the gamma-globin gene is expressed primarily during mid-to-late fetal development, but not in the earliest "embryonic" blood cells. We found, however, that the human gamma-globin gene behaved as an "embryonic" rather than a "fetal" gene in the developing transgenic mouse. This result can be explained by considering the evolutionary origins of the human and mouse gamma-like globin genes, and it allows us to suggest a model to explain the origin of fetal pattern of globin synthesis during evolution[3]. Since the cloned beta- and gamma-globin genes are differentially regulated in transgenic mice, we may now be able to define the DNA sequences which confer upon each of these genes a distinct pattern of stage-specific expression.

We have also shown that a hereditary defect in murine beta-globin synthesis, similar to human beta thalassemia, can be corrected by transfer of the human beta-globin gene into the mouse germline.

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027 **BIDIRECTIONAL TRANSCRIPTION AND DEVELOPMENTAL REGULATION OF THE RAT INSULIN PROMOTER/ENHANCER REGION IN TRANSGENIC MICE.** **Douglas Hanahan, Susan Alpert, Shimon Efrat. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724**

Transgenic mice harboring hybrid oncogenes composed of the 5' flanking region of the rat insulin II gene linked to protein coding information for the SV40 tumor antigens express large T antigen exclusively in the insulin producing β cells of the endocrine pancreas. These mice heritably develop well vascularized tumors, which are composed of proliferating β cells that co-express insulin and large T. Sequences extending to -660 from the insulin gene cap site are sufficient to direct correct cell type and tissue-specific expression. A second hybrid gene (RIR-Tag), in which the insulin promoter is inverted with respect to the large T coding information, also mediates tissue-specific expression in a comparable manner to versions with the normal promoter orientation (RIP1-Tag).

Analysis of two independent lines of transgenic mice harboring the reverse promoted large T gene has shown that RNA isolated from tumors contain two stable mRNAs for the spliced SV40 early region, and each is about 300 bp longer than those found in the RNAs derived from tumors in RIP1-Tag mice. A major site for initiation of transcription on the minus strand has been mapped to a location about 255 bp 5' to the cap site for normal insulin gene transcription. This site is near the 5' boundary of the transcriptional enhancer element. The insulin promoter itself remains active, thus resulting in bidirectional transcription from this region of the rat insulin gene.

The bidirectional activity of the 5' flanking region of the rat insulin II gene in transgenic mice raises the possibility that the "reversed promoter" element serves some role in its normal context of association with the insulin gene in its standard chromosomal location. Experiments in progress are examining endogenous mouse and rat insulin genes for bidirectional initiation of transcription and for the presence of stable transcripts extending on the opposite strand from this region.

In mouse development, insulin protein is first observed in the pancreatic rudiment at day 14 of embryogenesis. Immunohistochemical analysis of the RIP1-Tag gene in embryos of one line of transgenic mice has revealed that this hybrid gene begins to express at approximately the correct time, indicating that 660 bp of 5' flanking DNA from a rat insulin gene contains sufficient information for correct developmental regulation in a transgenic mouse. This analysis is currently being extended to additional transgenic lineages harboring either insulin promoted or reverse promoted T antigen genes, to assess whether bidirectional activity is developmentally regulated, and examine the possibility that position effects can influence developmental regulation.

028 TARGETED EXPRESSION OF CLONED GENES TO THE PANCREAS OF TRANSGENIC MICE.

Raymond J. MacDonald*, Robert E. Hammer†, Galvin H. Swift*, David M. Ornitz#, Brian Davis*, Ralph L. Brinster†, and Richard D. Palmiter#.
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To probe the molecular mechanisms that determine tissue-specific gene regulation in animals, we have introduced the rat pancreatic elastase I gene and fusion gene constructs into mice by microinjection into fertilized eggs. In adult transgenic mice the elastase I gene was expressed in a pancreas-specific manner that recapitulated expression in the normal rat. Rat elastase I mRNA levels in the pancreas of transgenic mice were generally as high or higher than the normal rat level. Non-pancreatic mRNA levels were at least 100-fold lower; differential tissue expression as high as half-a-million fold demonstrated rigorous control of multiple gene copies. The introduced genes were transcribed at high levels in pancreatic nuclei but not at detectable levels in liver nuclei. Progeny of original transgenic mice that inherited the introduced genes continued to express them in a pancreas-specific manner. A pancreas-specific regulatory region has been defined by fusing various regions of the elastase I 5' flanking region to the human growth hormone (hGH) gene. A fusion gene with only 130 bp of elastase I 5' flanking DNA, placed either within or outside the hGH gene, directs expression of hGH mRNA selectively to the pancreas. This elastase I regulatory region contains a sequence conserved among several pancreas-specific genes.

029 EXPRESSION OF IMMUNOGLOBULIN GENES IN TRANSGENIC MICE, Ursula Storb, *Carl Pinkert, Kindred Ritchie, Rebecca O'Brien, Benjamin Arp, Joanna Manz, Peter Engler, Katherine Gollahon, and *Ralph Brinster, Department of Microbiology and Immunology, Univ. of Washington, Seattle, Washington 98195, and *Laboratory of Reproductive Physiology, School of Veterinary Medicine, Univ. of Pennsylvania, Philadelphia, Pennsylvania 19104

Transgenic mice have been produced which carry microinjected functionally rearranged immunoglobulin κ light (L) and heavy (H) chain genes (1,2). The κ transgenes are expressed in completely normal tissue specific fashion, only in B lymphocytes (2,3). The H transgenes are also expressed in B lymphocytes, but in addition in T cells, presumably because transcriptional factors for T-cell receptor genes can transcribe H genes if they are correctly rearranged; correct rearrangement of H genes does not normally occur in T cells. The κ transgenes are under normal developmental control if present in a small number of copies, however large copy number transgenes are expressed prematurely in pre-B cells. The presence of transgenic κ protein together with endogenous H chains prevents rearrangement of endogenous κ genes; however in a B cell without endogenous H genes, transgenic κ chains alone do not cause allelic exclusion (5). We are now investigating the molecular mechanism of κ allelic exclusion by κH protein. Furthermore, the influence of a κ transgene on the expression of another L gene, λ, and the mechanism of κλ isotypic exclusion is being analyzed.

The transgenic mice are also a useful tool to study the mechanism of somatic hypermutation of immunoglobulin genes. Normally, expressed antibody genes are found to be mutated within the variable (V) region at a very high rate (up to 1% of nucleotides). Such mutations are not found in unrearranged V genes or in the constant (C) region of rearranged genes. Two alternative mechanisms have been proposed for this hypermutation. An "accumulation" model postulates that V regions are the target for a specific mutation process which operates after immunization and leads to accumulation of mutations during the immune response. A "rearrangement" model proposes that the mutations occur because of error-prone repair of free DNA ends during the process of VJ (or VDJ) joining. Analysis of mutations of κ transgenes which were microinjected in the rearranged form should allow to distinguish between these models.

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DNA-Protein Structure and Function

034 PURIFICATION AND CHARACTERIZATION OF SOME RNA POLYMERASE B TRANSCRIPTION FACTORS, J.M. Egly, N. Miyamoto, V. Moncollin, T. Tamura, X.M. Zheng and P. Chambon, U184 de l'INSERM - LGME du CNRS, Institut de Chimie Biologique, Faculté de Médecine - 11 rue Humann 67085 Strasbourg-Cédex (France).

We are working on the purification and characterization of factors involved in specific transcription catalyzed by RNA polymerase B (II). We have previously reported that the formation of a stable preinitiation complexes involves the TATA box element (Nature 1983, 301: 680-688) : one of these factors, the stimulatory transcription factor (43 kd STF) has been extensively purified and exhibits some actin properties (EMBO J. 1984, 3:2363-3271), the other the TATA box factor is partially purified; we will show its footprint and its interaction with other factors (T. Tamura et al., in preparation). In addition the purification and some properties of a tarnscription factor "GTF_2" absolutely required for in vitro transcription will be presented (X. M. Zheng et al., in preparation).

Stimulation of in vitro transcription by the upstream element of the Adenovirus-2 major late promoter, involves in addition to at least four general transcription factor, a specific trans-acting factor present in a HeLa whole-cell extract (Nucleic Acids Res. 1984, 12:8779-8799; N. Miyamoto et al. 1985, EMBO J. in press). The purification of the factor (M.W.: 57 kd) has been followed by the transcription stimulatory activity and a DNaseI footprint onto the upstream element of the Ad2MLP (V. Moncollin et al., EMBO submitted).

035 STRUCTURE AND FUNCTION OF NUCLEOLIN / ROLE IN rDNA TRANSCRIPTION. François Amalric, Henri Bourbon, Gérard Bouche, Michèle Caizergues-Ferrer and Bruno Lapeyre, C.R.B.G.C., 118 route de Narbonne, 31062 Toulouse cedex, France.

Nucleolin (100 kDa) is the major nucleolar protein in exponentially growing eukaryotic cells and is in amount directly related to the level of rRNA synthesis. Nucleolin is found associated with chromatin, preribosomes, and is involved in the structural organization of nucleolar subcomponents. cDNA encoding nucleolin has been isolated by probing a cDNA library of CHO cells constructed in the expression vector λgt11 with a polyclonal serum raised against nucleolin. The primary structure of nucleolin that was established by sequencing the cDNA (2650 nt) suggested the presence of at least five structural domains (A to E). Three of them possess unusual structural features that can be correlated to specific functions. B, contains 80 amino acids and presents a repetitive pattern : Ala-Val-Thr-Pro-Ala-Lys-Lys. This domain is involved in the interaction between nucleolin and pre rRNA and rDNA. C, is an acid-rich domain (132 aa). It contains 80 Asp and Glu residues, that are in three continuous stretches 23, 24 and 33 respectively. This domain interacts with histones and plays a role in the structuration of chromatin around rDNA genes. E, is the C terminal domain. Out of 67 aa, it contains 38 Gly residues interspaced with dimethyl Arg and Phe in a repetitive pattern. This domain could be involved in interactions of nucleolin with itself to produce the nucleolar network on which are bound the active ribosomal genes. The presence of separate domains is consistent with the multiple functions that have been described for nucleolin in rDNA transcription.

036 ISOLATION AND CHARACTERIZATION OF DNA METHYLTRANSFERASE FROM TRANSFORMED LYMPHOCYTES. Carol Anderson and Wendy Clough, Molecular Biology, University of Southern California, Los Angeles, CA 90089-1481

DNA methylation has been inversely correlated with gene activity in many eukaryotic systems. The exact mechanism by which DNA methylation may inhibit transcription is unknown but it is thought that the action of DNA methyltransferase may alter DNA-protein interactions, resulting in the inhibition of transcription.

We have partially purified and characterized DNA methyltransferase from HR-1 cells, which are Epstein-Barr virus (EBV) transformed human B lymphocytes, and compared the enzyme to partially purified DNA methyltransferase from Ramos cells, which are EBV-negative transformed B lymphocytes. DNA polymerase activity has been found to be associated with DNA methyltransferase in both purifications. Cell extract is treated with polyethyleneimine, then applied to hydroxylapatite, Cibicron blue agarose, and then DEAE Sepharose columns, using appropriate salt gradients in the purification procedure.

Further work is in progress to isolate DNA methyltransferase from EBV superinfected Raji cells, which contain large numbers of actively replicating viral genomes, to determine if there is an EBV-associated DNA methyltransferase. Other enzyme activities, such as nuclease and topoisomerase, are being tested for association with DNA methyltransferase.

037 INTERACTION OF THE ESHERICHIA COLI TRP APOREPRESSOR WITH COREPRESSOR, COREPRESSOR ANALOGS, AND OPERATOR DNA. Dennis N. Arvidson and Robert P. Gunsalus, Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

Transcription of the trp, trpR and aroH operons of E. coli is regulated in part by the Trp repressor in response to the intracellular L-tryptophan concentration. At high tryptophan levels, the Trp aporepressor binds the corepressor, L-tryptophan to form the active repressor which binds the trp, trpR and aroH operator DNAs. At low L-tryptophan levels these ternary complexes dissociate, allowing increased transcription by RNA-polymerase. We have measured the affinity of the purified aporepressor dimer for a variety of L-tryptophan analogs. The ability of each analog to displace L-[C^{14}]-tryptophan from the aporepressor was quantitated by equilibrium dialysis. The amount of bound L-[C^{14}]-tryptophan was measured in the presence of a fixed amount of free analog. Experiments were performed at various concentrations of free analog and Ki values were obtained. For example, the aporepressor bound indole propionic acid aproximately two times more strongly than L-tryptophan. The two binding sites were identical and independant based on Dixon plot analysis. Indoleamine was bound aproximately two times weaker. Indole was bound with the same affinity as L-tryptophan. D-tryptophan, L-phenylalanine and L-tyrosine were bound very weakly. The effect of these and other analogs on operator DNA binding has been tested by the ability of the aporepressor-analog complex to protect the trp operon operator from cleavage by the restriction enzyme RsaI.

038 PHOTOAFFINITY LABELING OF RNA POLYMERASE II TRANSCRIPTION COMPLEXES, Blaine Bartholomew, Claude F. Meares, and Michael E. Dahmus, University of California, Davis 95616.

Photoaffinity labeling of HeLa Cell RNA polymerase II transcription complexes has been done using thiouridine as a photoactivated crosslinker between RNA and protein. The nucleotide 4-thiouridine triphosphate is used as substrate by RNA polymerase II in the formation of promoter specific RNA transcripts. The length of transcript is controlled by addition of base specific RNA chain terminators, such as 3'-O-methyl guanosine triphosphate. The extract and DNA used are HeLa cell S-100 purified through heparin Sepharose and a DNA restriction fragment containing the adenovirus-2 major late promoter. After crosslinking for 5 minutes with >300 nm light, radiolabeled components are separated by polyacrylamide gel electrophoresis, blotted onto nitrocellulose, and visualized by autoradiography. Using this method of labeling the major proteins contacted by nascent RNA during its transcription, we have been able to detect specific labeling of proteins corresponding in mobility to subunits IIo and IIa. The photoaffinity labeling requires both the addition of exogeneous DNA and 4-thiouridine triphosphate, and is abolished by 1 μg/ml α-amanitin. Supported by NIH grant GM25909 (CFM) and NSF grant DMB85-44022 (MED).

039 MOLECULAR CLONING AND FURTHER CHARACTERIZATION OF SOME I^{-D} AND I^{TB} MUTANTS OF LAC REPRESSOR, Joan L. Betz, University of Colorado School of Medicine, Denver CO 80262

The lac repressor protein remains a prototypic example of a site specific DNA regulatory protein. Mutants of both the protein and of its recognition site (the operator) have been invaluable in defining functional domains. This laboratory had previously characterized and genetically mapped numerous repressor mutants of both tight-binding (i^{tb}) and transdominant i^{-} (i^{-d}) phenotypes. Using general recombination and molecular cloning, 13 i^{-d} and i^{tb} missense mutations of the lacI gene have been transferred from F'lac episomes to colE1 derivative plasmids. Deletion derivatives of the wild-type and of two i^{tb} lacI genes were also constructed. The mutant repressors were examined for polypeptide size, stability and aggregation state, as well as for binding to the inducer IPTG. Several of the i^{-d} repressors were shown to be partially degraded in vivo. The sizes of polypeptides produced by lacI deletion derivatives were consistent with expectations based on the extents of the deletions and the location of termination sites within the plasmid sequence. The first 400 nucleotides of several lacI mutant genes were sequenced, with identification of base substitutions and resultant amino acid changes. In keeping with genetic mapping data, several of these new changes were in the aminoterminal domain (the first sixty amino acid residues) of repressor, whereas other changes were located farther towards the carboxyterminus. Purified i^{tb} repressors were examined in vitro for their nonspecific affinity for DNA and for their specific affinity for the natural operator andseveral sequence-altered operators.

040 RNA POLYMERASE GENES OF C. ELEGANS, David Bird, Teresa M. Rogalski and Donald L. Riddle, Divison of Biological Sciences, University of Missouri, Columbia, MO 65211.

We are using a combination of genetic and molecular methods to study nematode RNA polymerase II. EMS-induced mutants resistant to alpha-amanitin have been obtained at a frequency of about 4 x 10^{-6}. Strains carrying the *ama-1(m118)* mutation produce an RNA polymerase II that is 150 times less sensitive to amanitin *in vitro* than the wild-type enzyme (Sanford *et al.*, JBC **258**: 12804, 1983). EMS-induced lethal and sterile mutants of *ama-1* have been obtained at a frequency of 2 x 10^{-3}. Of nine mutants examined thus far, two are arrested late in embryogenesis, three are early larval lethals, and four are adult steriles, one of which is temperature-sensitive. We also have isolated three gamma-ray-induced deficiencies that include *ama-1*. We have cloned two regions in the *C. elegans* genome that hybridize under non-stringent conditions to probes encoding the amanitin-binding subunit of *Drosophila* RNA polymerase II (Ingles *et al.*, PNAS **80**: 3396, 1983). One clone appears to encode *ama-1*, as quantitative blot analysis of genomic DNA from a strain heterozygous for the small deficiency, *mDf4* (which includes *ama-1*) reveals a dosage reduction in hybridization signal with the *C. elegans* probe. The second region is not within *mDf4*, but nevertheless hybridizes to the *Drosophila* probe encoding the carboxyl-terminus of the pol II large subunit. A similar pattern was observed in yeast (Allison *et al.*, Cell **42**: 599, 1985) where the *Drosophila* probe was found to detect both pol II and pol III subunit genes. Consequently, a yeast pol III probe was hybridized to both regions under non-stringent conditions. Several contiguous *Eco RI* fragments were strongly detected in one; no hybridization to *ama-1* sequences was observed. Thus, this gene probably encodes a *C. elegans* pol III subunit. Prior to sequencing these genes, we are attempting to define the extent of their transcription units by Northern blotting.

041 **INFLUENCES OF DNA STRUCTURE ON THE POSITIVE REGULATION OF THE YEAST ß-GALATOSIDASE GENE.** Karin D. Breunig, Maurizio Ruzzi, Annie Walker-Jonah, Sunil Das and C.P. Hollenberg, University of Düsseldorf, F.R.G.

The yeast ß-Galactosidase gene is comparable to the E.coli LacZ gene in its transcriptional regulation by lactose. In contrast to the prokaryotic system induction of the gene in the presence of lactose is mediated by a positive regulatory factor. Three non-complementing mutants affect regulation in trans resulting in a non-inducible (two loci) or in a constitutive phenotype (one locus). We have identified sequences in cis at around -350 bp upstream from the structural gene responsible for induction. They have characteristics of upstream activation sites (UAS) as found in several other yeast regulatory regions: they are functional in both orientations and confer lactose regulation to other yeast promoters when fused to the TATA-box functional element.

In the cloned gene the level of expression and the selection of transcription initiation sites is drastically affected by long distance plasmid effects. In a fusion of the LAC4 regulatory region with the bacterial bla gene regulation by lactose is no longer observed. Our data indicate that due to changes in chromatin and/or DNA structure the formation of functional complexes between UAS and regulatory proteins(s) can be impaired.

042 CHROMOSOMAL PROTEIN HMG 1 AND ITS INTERACTIONS WITH DNA, Andrew P. Butler, The University of Texas System Cancer Center, Science Park-Research Division, Box 389, Smithville, Texas, 78957.

The high mobility group (HMG) proteins are among the most abundant non-histone chromosomal proteins. The large HMG proteins, HMG 1, 2 and E, are believed to be associated with nucleosomal linker DNA. Although reported to be involved in chromatin assembly or as structural elements in transcriptionally active chromatin, their function remains obscure. Recent results from this laboratory will be presented in three areas: (1) Methods for the separation of HMG proteins by reverse phase HPLC have been developed to facilitate studies of functional differences in the individual proteins. (2) The structure and function of discrete proteolytic domains of HMG 1 are being studied, and kinetic studies of digestion with V8 protease will be presented. (3) Fluorimetric determination of the affinity of chicken erythrocyte HMG 1 for DNA suggest a ten-fold preference for single-stranded versus double-stranded DNA at physiological ionic strength. Studies of binding to a cloned chicken erythrocyte globin gene will also be presented. These results will be discussed in terms of possible involvement of HMG proteins in transcriptional complexes.

043 STUDY OF TRANSCRIPTION INITIATION BY RNA POLYMERASE II. H.Cai and D. Luse, Dept. of Biochem. & Molec. Biol., Univ. of Cincinnati, Cincinnati, OH 45267-0522

We have been studying transcription initiation by RNA polymerase II in vitro. We have isolated, using a BioGel A 1.5 m column, 3 different transcription complexes formed between template DNA, RNA polymerase II and protein factors required in transcription initiation. Complex 0, in which no nucleotide has been incorporated, is DNA-protein association paused immediately before the actual initiation of transcription. The next step that we have defined is a DNA-RNA Pol II complex paused very soon after initiation. It has already synthesized 2 phosphodiester bonds so we call it Complex 2. The step after Complex 2 that we have chosen to study is a complex between the template and the polymerase with a nascent transcript of approximately 10 nucleotides long (Complex 10), which is probably an elongation committed complex. We have studied the stability and probed the structural configuration of these complexes by subjecting them to high concentrations of salt, sarkosyl, DNAse I and protease K. We found that although Complex 0 remains active after prolonged incubation at 25°C, it is sensitive to high salt, sarkosyl and enzymatic digestions. Complex 2, in contrast, is resistant to high salt, sarkosyl and DNA I digestion but is heat (25°C) sensitive. Complex 10 shows a surprising resistance to extended proteolysis in addition to the resistances shown by Complex 2; Complex 10 is stable at 25°C. Current results on purification and footprinting studies with these complexes will be discussed.

044 THE PRIMARY STRUCTURE OF HGH GENE FAMILY, Ellson Chen, Yu-Cheng Liao, Dennis Eaton, Douglas Smith, R. Gelinas* and Peter Seeburg, Genentech, Inc., South San Francisco, CA 94080; *Fred Hutchinson Cancer Research Center, Seattle, WA 98104

The human growth hormone gene cluster is located within a 65 kilobase region (band q22-q24) of chromosome 17. This region contains two growth hormone genes (GH-N, GH-V) interspersed with three chorionic somatomammotropin genes (CS-A, CS-B and CS-L), all in the same transcriptional orientation. One of the GH genes (i.e., N gene) is expressed in the pituitary and two of the CS genes (i.e., A and B genes) are actively expressed in the placenta.

We have determined the complete nucleotide sequence of 66,491 bp encompassing this five-gene locus and observed the following structural features:

(a) All five genes exhibit the identical intron-extron structure, with each being divided into five exons. The sequences contained in both coding and non-coding areas are highly homologous, suggesting that they are derived from a common ancestral gene.

(b) Duplications of large regions of the locus are evident; i.e., a 20 kilobase segment encompassing the N and L genes is duplicated in the V and B genes region with occasional interruptions by the ALU repeating elements. A similar 17 kilobase region is also shared by the L, A and B genes.

(c) The whole locus has a high content of Alu repeating elements in the intergenic regions. All the above suggest that the cluster may have evolved quite recently and that the mechanism of gene duplication involved homologous but unequal exchange between the repetitive elements of the Alu family.

045 FUNCTIONAL CHARACTERIZATION OF MOUSE RNA POLYMERASE II: LOCALIZATION OF α-AMANITIN RESISTANCE TO THE 5' TERMINAL PORTION OF THE LARGE SUBUNIT GENE, Ken W.Y. Cho, Kamel Khalili, Steve Per, Alan Saltzman, and Roberto Weinmann, The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, PA 19104

With a human RNA polymerase II large subunit recombinant plasmid probe we have isolated two phages from a genomic library from an α-amanitin-resistant mouse mutant. These recombinants contain at least 18 kb of DNA, parts of which hybridize to an RNA of 7.3 kb, enough to code for the largest subunit (220 kDa) of the mouse RNA polymerase II. Most importantly, one of them is able to transfer the genetic information coding for α-amanitin resistance to sensitive Hela cells. The transfectant RNA polymerase II functions in the presence of α-amanitin and expresses a resistance level similar to the parental cell line. The enzyme is functional not only in vivo insuring cell survival but also in vitro in a specific transcription assay with a whole cell extract, retaining the transcriptional initiation specificity of the human parent in the presence of the fungal toxin. Analysis of the transfected cell DNA reveals that a homologous recombination event may be responsible for the α-amanitin resistance. DNA sequence analysis and comparison with the yeast and Drosophila large subunit genes allowed us to establish that this mutation responsible for encoding α-amanitin resistance is located towards the 5' end terminal portion of the mammalian large subunit gene.

046 PHORBOL ESTERS INHIBITION OF PEPCK GENE TRANSCRIPTION IN H4IIE HEPATOMA CELLS David Chu and Daryl Granner, Vanderbilt University, Nashville, TN 37232

Phorbol myristate acetate (PMA) provokes an insulin-like action on P-enolpyruvate carboxykinase (PEPCK) in cultured rat hepatoma H4IIE cells. PMA induces a rapid, reversible concentration dependent reduction cytoplasmic mRNA PEPCK with a half-maximally effective concentration of 50 nM. The inhibitory effect of PMA is specific, as biologically inactive phorbol esters (4α-phorbol 12, 13-didecanoate and 4β-phorbol acetate) do not suppress mRNA PEPCK. Like insulin, PMA overrides the stimulatory effects of cAMP and glucocorticoid analogs in regulating PEPCK gene expression. In vitro nuclear RNA transcription assays indicate that PMA selectively inhibits PEPCK gene transcription. A time course revealed a 2-fold reduction of cAMP stimulted PEPCK gene expression within 10 min. after PMA treatment and inhibition is complete within 30 min. PMA suppresses PEPCK gene transcription over a range of 1 nM-1 uM; a half-maximal effect was obtained with 50 nM PMA. The effect of PMA on PEPCK gene transcription is not influenced by the pretreatment of H4IIE cells with cycloheximide, a protein synthesis inhibitor, thus this action of PMA appears to be a direct effect on the PEPCK gene.

In conclusion, the tumor promoter PMA, presumably acting via protein kinase C, causes a specific repression of PEPCK gene expression in rat hepatoma H4IIE cells. This PMA effect resembles the action insulin has on PEPCK gene transcription.

047 **Cloning and Expression of Wildtype and Mutant Regulatory Subunit Genes of cAMP Dependent Protein Kinase.** **Christopher H. Clegg, Leslay A. Correll, and G. Stanley McKnight. Department of Pharmacology, University of Washington, Seattle Wa., 98195.** Variant S49 lymphoma cells have previously been isolated which possess altered forms of the regulatory subunit (R1) of cAMP-dependent protein kinase (Steinberg et al., Cell 10: 381-391.). These mutations decrease the affinity of R1 for cAMP. As a result holoenzyme dissociation does not occur at physiological concentrations of cAMP and kinase activation is inhibited. To enhance our understanding of the structure and regulation of cAMP-dependent protein kinase we have cloned and sequenced two classes of mutant R1 cDNAs. A comparison of the wild-type R1 sequence with the DNA sequence of the mutant R1 cDNAs shows a single base pair substitution that alters an amino acid in either the first or second cAMP binding site of the R1 protein. Since these mutations confer a dominant phenotype in S49 cells, we have constructed expression vectors in order to inactivate protein kinase in other cell types. These expression vectors which contain full length copies of the putative mutant or wild-type R1 genes were co-transfected with a G-418 resistance plasmid into Y1 adrenocarcinoma cells. Following the isolation of G418-resistant colonies, we observed that 10-20 % of the clones were resistant to the normally toxic effects of 100 uM 8-CPT-cAMP, thus suggesting a mutant R1 effect. No cAMP resistant colonies were obtained following transfection with wild-type R1 genes. Initial characterization of cAMP resistant cells shows specific transcription of mutant R1 genes, elevated levels of total R1 protein, and significantly reduced activity of cAMP-dependent protein kinase. These findings suggest that the identified alterations in the R1 gene affect cAMP binding and more importantly that we can modulate kinase activity in transfected cells. Expression of these mutant genes in cells may provide a specific means to explore the role of cAMP action and protein phosphorylation implicated in controlling various biological processes.

048 CHROMOSOMAL LOOP ANCHORAGE OF IMMUNOGLOBULIN GENES OCCURS ADJACENT TO ENHANCERS VIA DNA ELEMENTS POSSESSING TOPOISOMERASE II SITES. P.N. Cockerill and W.T. Garrard, Department of Biochemistry, The University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235.

Evidence indicates that DNA in the nucleus is organized into looped domains that are anchored to the nuclear matrix. It is believed that domains containing transcribed genes are under torsional stress. We have localized matrix association regions (MARs) within mouse immunoglobulin genes, before and after gene rearrangement and transcriptional activation, employing an assay for in vivo MARs and also an in vitro DNA binding assay in which MAR anchorage to the matrix is reconstituted. A single AluI fragment (254 bp) just upstream of the kappa IgL enhancer and two HinfI fragments flanking the IgH enhancer are specifically associated with the nuclear matrix in both B and non-B-cells, both in vivo and in in vitro assays. There appears to be evolutionary conservation of both the MAR binding sites (which number greater than 10,000 per nucleus), and the MARs as a class of elements which are A/T-rich and bear the recognition site for topoisomerase II and the sequence AATATTTTT. We suggest that MARs are of fundamental importance in facilitating the control of gene expression by co-operating with enhancers and auxilliary factors to regulate torsional stress introduced by topoiosmerase II. (Supported by NIH and The Robert A. Welch Foundation).

049 NOVOBIOCIN PRECIPITATION OF HISTONES, Matt Cotten, Linda Sealy and Roger Chalkley, University of Iowa, Iowa City, IA 52242.

Novobiocin has been shown to inhibit both bacterial and eukaryotic type II topoisomerases. Recently, the compound has been shown to inhibit chromatin assembly in vitro, in complex systems derived from Xenopus oocytes, suggesting that a type II topoisomerase is required for chromatin assembly. In our studies of this assembly system, we have found that in addition to any effects that novobiocin may have on topoisomerases present in the system, the compound interacts with histones. In the concentration range used to inhibit assembly (0.1-0.5 mg/ml), core histones bind approximately 20 novobiocin molecules per histone molecule and the subsequent charge neutralization causes the histones to leave the solution. Novobiocin can be used to precipitate histones from dilute solutions (0.001 mg/ml); the precipitation is enhanced by the presence of high salt concentrations (up to 2 M NaCl) and the novobiocin-histone interaction is disrupted by 1% SDS or 2 M urea. Thus, the precipitation of histones by novobiocin can be used as a convenient preparative technique. Furthermore, caution should be exercised in the interpretation of chromatin structure experiments involving novobiocin.

050 A STUDY IN THE REGULATION OF THE PYRUVATE KINASE GENE OF NEUROSPORA CRASSA, M. Devchand and M. Kapoor, University of Calgary, Calgary, Canada.

Pyruvate kinase (PK) of N. crassa, an allosteric enzyme is being used as a model system for studying the genetic regulation of constitutive enzymes. A partial genomic library was prepared by cloning size-selected Neurospora DNA in the pUC 13 plasmid and screened using a fragment from the yeast PK gene as a probe. Positive clones were analyzed by restriction mapping and sequence analysis is currently in progress.

Northern blot analysis of total cellular RNA showed the presence of two PK-specific mRNA species. Analysis by northern and dot blots of RNA prepared from mycelium grown for various times showed age-dependent variation in the level of PK-specific mRNA. The level of PK in mycelium at these stages was assessed by immunoprecipitation from [^{35}S]-methionine-labelled mycelium with anti-PK antibody. A correlation of PK protein and mRNA levels under different conditions suggests that control occurs mainly at the transcriptional level.

Western blots of total N. crassa proteins, electrophoresed on SDS-polyacrylamide gels and electroblotted onto nitrocellulose were hybridized with restriction endonuclease digested, [^{32}P]-labelled N. crassa DNA, as well as to labelled pUC13 DNA. When these blots were hybridized to an end-labelled PK gene fragment, a 30kdal polypeptide was found to bind strongly. It was found to bind weakly to pUC13 DNA and appeared as a faint band when probed with total N. crassa DNA. Polypeptide blot hybridizations and filter binding experiments with increasing levels of competing DNA and salt concentrations indicate that the binding of this protein to the PK gene is specific. Probing of the Western blots with different restriction fragments of the PK gene indicated that the protein binding occurs primarily in the 5'-noncoding region. DNase I footprinting and exonuclease III protection studies are currently in progress to delineate the precise site of binding.

051 A NOVEL E. COLI RECOMBINASE ENCODED BY fip STIMULATES SPECIFIC DNA INVERSION IN A DOSE RESPONSE MANNER IN VIVO. Cynthia S. Freitag,* John M. Abraham, and Barry I. Eisenstein. University of Texas Health Science Center, San Antonio, TX 78284.

A 314-bp invertible region of DNA controls the on-and-off transcription of fimA that is responsible for phase variation of type 1 fimbriae in E. coli. We recently cloned the gene fip (fimbriae inversion permissive factor) that is required in trans for the DNA inversion. We now find that the kinetics of the inversion event in vivo are dependent on the gene dosage of fip. When fip is present on a high-copy plasmid, the phase variation rate of $Lac^{+} \rightarrow Lac^{-}$ switching is unmeasurably high as measured with either a chromosomal fimA-lacZ operon fusion or a lytic infection with λ (fimA-lacZ). (In contrast fip in single copy gives switching rates of 10^{-3}-10^{-2} per cell-generation.) These results indicate that the fimbriae phase variation system is not rate limited by additional intracellular factors, as may be the case with the Salmonella flagellar system mediated by the product of hin, and provides further evidence that Fip is in a different class of DNA "invertases" from Hin, Gin, Pin, and Cin.

052 IN VIVO POINTS OF DNA-PROTEIN CONTACT WITHIN THE PROMOTER REGION OF THE YEAST HSP82 GENE. David S. Gross and William T. Garrard, Dept. Biochemistry, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Saccharomyces cerevisiae is an attractive biological system to dissect the cis-acting DNA sequence determinants of gene expression and to study the DNA contact points of trans-acting regulatory proteins. Genetic manipulation of this organism readily permits positive selection of site-directed integration, making it possible to change any base sequence within the normal chromosomal environment of a given gene. We have chosen to study the locus encoding an 82 kd heat shock protein, HSP82, and report here identification of regulatory protein contacts within the promoter of this gene. We have exposed living haploid yeast cells to dimethyl sulfate (DMS) and have assessed the extent to which the N-7 moiety of guanine, which resides in the major groove of DNA, is accessible to methylation. Mapping of piperidine-induced cleavage sites along an ~500 bp segment of the upstream region was by indirect end-labeling, using a modification of the genomic sequencing technique of Church and Gilbert (1984). Strikingly, this method reveals substantial reactivity of an A residue at -79 on the template strand, a position opposite the first T of the TATA box, as well as unusual reactivities of adenine clusters of 3-4 residues which flank the TATA box on the upper strand. These adenines are presumably methylated by DMS at the N-7 position, since they are unusually labile to treatment that normally results in chain cleavage exclusively at 7-methylguanine. We are currently constructing M13 clones that contain single base mutations at these sites.

053 SEQUENCES AND FACTORS INVOLVED IN POLYMERASE I TRANSCRIPTION TERMINATION. Ingrid Grummt and Ursula Maier, Institut für Biochemie der Universität Würzburg, Röntgenring 11, 8700 Würzburg, FRG

The 3' spacer of the mouse rDNA transcription unit contains a set of repetitive structural elements consisting of 18 bp conserved nucleotides (including a SalI restriction site) surrounded by stretches of pyrimidines. This repetitious region starts some 600 bp downstream from the end of the 28S RNA coding region. RNA polymerase I terminates transcription at position +565 relative to the 28S RNA terminus, i.e. 25 bp upstream of the first repetitive element (the "SalI-box"). This specific termination event can be duplicated in a cell-free transcription system. Analysis of several deletion and point mutants indicates that the "SalI-box" functions as polymerase I transcription termination signal. The integrity of the "SalI-box" is required for the binding of termination factor(s) and efficient termination. Transcription experiments in heterologous systems using human - mouse fusion genes show that the termination factor(s) are also present and active in human cell extracts.

054 SEQUENCE-DIRECTED CURVATURE OF DNA, Paul J. Hagerman, University of Colorado Health Sciences Center, Denver, CO. 80262

DNA sequences displaying apparent axial curvature have been found within the VP1 gene of SV40, at the site of integration and origin of replication of bacteriophage lambda, and in regions of conserved sequence in trypanosome kinetoplast DNA, to name a few. Moreover, disruption of a region of curvature upstream from the histidine tRNA gene in Salmonella appears to be associated with down-regulation of tRNA-His transcription. However, the functional significance of curvature per se remains obscure, as does its molecular origin. The presence of short runs of A residues in regions of curvature has led to various models for bending of the helix axis; these models include: cross-chain steric clash between purine residues, ApA dinucleotide wedge deformations, hyperflexibility of the oligo(dA) tracts, and junctional distortions between non-B and B DNA. We have begun to examine these models in a critical fashion using a wide variety of synthetic DNA polymers, some of which are substantially curved. Our results thus far indicate that neither hyperflexibility nor purine clash are responsible for the observed curvature of DNA. Moreover, while ApA wedges may exist in DNA, such local deformations are not sufficient to account for curvature. The possibility that thymidine methyl groups are responsible for curvature has been discounted by incorporating U residues in place of T residues in synthetic polymers displaying curvature. The U analogues display a greater degree of apparent curvature than do the T-containing polymers. As an offshoot of our studies of DNA curvature, we have been able to show that, contrary to recent reports based on model-building, psoralens do not kink DNA.

055 IDENTIFICATION OF TWO FACTORS WHICH BIND TO THE PROMOTER REGION OF THE α_2(I) COLLAGEN GENE, A. Hatamochi and B. de Crombrugghe, NIH, NCI, Bethesda, MD 20892

We have used an exonuclease III assay (C. Wu, 1985, Nature, 317, 84-87) to identify two factors which bind to the promoter of the mouse α_2(I) collagen gene. Soluble nuclear extracts were prepared from nuclei of NIH3T3 fibroblasts. One factor binds between -96 and -72 bp upstream of the start of transcription. This segment contains the sequence CCAAT on the anti-sense strand between -84 and -80. Binding to the CCAAT region in the α_2(I) collagen promoter is competitively inhibited by a segment containing the β-actin promoter but not by a segment containing the early promoter of SV40 or by a segment of the mouse α_1(III) collagen promoter. The β-actin promoter segment contains a sequence on the coding strand around -80 that corresponds closely to the consensus CCAAT box sequence. It is also a more effective competitor than the α_2(I) collagen promoter fragment itself in the DNA binding reaction. The sequence in the α_1(III) collagen promoter around -80 differs considerably from the consensus CCAAT-box sequence. The differential binding of a CCAAT-box binding protein to the α_2(I) and α_1(III) collagen promoters could account for the 5 to 10-fold difference in expression of these two genes in NIH3T3 cells.
The second factor binds to the α_2(I) collagen promoter between -421 and -410. Binding to this segment is not inhibited by an α_2(I) collagen promoter segment between -349 and +206. Hence, the factor which binds to this second site must be different from the factor which binds to the CCAAT box. Work is in progress to purify these factors and to determine their function in control of transcription.

056 SEQUENCE ELEMENTS REGULATING TRANSCRIPTION IN MAMMALIAN (ARTIODACTYL) MITOCHONDRIA, William W. Hauswirth, Sally L.D. MacKay, Steven C. Ghivizzani, Douglas M. McCarty, Philip J. Laipis, and Susan M. Tanhauser, University of Florida, Gainseville, FL, 32610

Accurate transcript initiation in mammalian mitochondria is regulated by a relatively simple set of sequences for each strand located near the origin of heavy-strand DNA replication. In detail, however, there is no conserved promoter sequence among the species thus far studied. It has therefore not been possible to identify additional, upstream (or downstream) conserved sequences which may regulate transcription efficiency. To circumvent this limitation we have analyzed the in vitro activity of mtRNA polymerase on homologous and heterologous mtDNA templates from a closely related group of mammals within the family Artiodactylia. We have determined the nucleotide sequence for putative transcription regulatory regions in species related to the cow, including yak (1 my divergent), American Bison (2 my), water buffalo (6 my), giraffe (17 my), and pig (42 my). In vitro initiation sites have been determined for both light- and heavy-strand transcription using mtRNA polymerases from cow and pig programmed by their respective DNAs using S1-nuclease and runoff analyses. These sites agree with the 5' termini of in vivo RNA. Despite poor overall homology between cow and pig mtDNA in this region (<30%), initiation sites for both polarity transcripts are within and surrounded by blocks of conserved sequences. In vitro analysis of each polymerase on heterologous artiodactyl templates, confirms that some or all of these conserved sequences control the accuracy and efficiency of transcription. We are currently analyzing each putative regulatory sequence by deletion and mutagenesis analysis to more precisely identify their function in mitochondrial transcription.

057 MOLECULAR STRUCTURE AND EXPRESSION OF THE HU-1 GENE OF ESCHERICHIA COLI, Fumio Imamoto, Yasunobu Kano, Morimasa Wada, and Setsuo Yoshino , Department of Molecular Genetics, Riken (The Institute of Physical and Chemical Research), 2-1, Hirosawa, Wako-city, Saitama, 351-01, Japan

The HU proteins constitute a major fraction of a set of DNA-binding proteins localized in the E. coli nucleoids. HU consists of two molecular species, HU-1 ans HU-2, and their amino acid sequences have been reported (Mende, L. et al., 1978; Laine, B. et al., 1980). Using mixed synthetic oligonunleotides (17-mer) predicted from a portion of peptide sequence of the HU protein, we cloned the HU-1 gene. Judging from the DNA sequence there seems to be an uninterrupted coding region of 90 codons starting at the GUG codon, specifying a protein with a molecular weight of 9,225 daltons. This is in good agreement with the chemical structure of the HU-1 protein reported previously. On the basis of the frequency of use of optimal codons for the HU-1 protein (Fop;0.797), it is estimated that there are 1 X 10^3 molecules of HU-1 protein per cell (Ikemura and Ozeki, 1983). This value is considerably lower than the previous estimate of 3 X 10^4 dimers of HU-1 and HU-2 proteins per cell (Rouvière-Yaniv, 1977). The sequence TAAXGAGG located 9 nucleotides upstream from the site of translational initiation is typical Shine-Dalgarno ribosomal binding site and could result in the production of a high level of the HU protein in the cell. Transcription is initiated at a site of 120 nucleotides upstream from the open reading frame, and the promoter sequence is constituted with TAAGTT for -10 bp region and TTGCCA for -35 bp region. The map position of the HU-1 gene and mode of expression are presented.

058 SCREENING FOR NOVEL TYPE II RESTRICTION ENDONUCLEASES, Christoph Kessler, Bryan J. Bolton, Michael J. Comer, Boehringer Mannheim GmbH, Biochemical Research Center Tutzing, Bahnhofstr. 9, D-8132 Tutzing, FRG.

Besides various species of lactic acid bacteria (Lactobacillus, Pediococcus and Leuconostoc) we have screened 252 different non-pathogenic species of the genera Achromobacter, Acinetobacter, Alcaligenes, Brevibacterium, Enterobacter, Flavobacterium and Herpetosiphon for the presence of potentially new type II restriction endonucleases. Among the above lactic acid bacteria screened, we could not detect any specific activities, whereas in all the other genera we found a high number of species producing different type II restriction endonucleases:

Lactic acid bacteria	Brevibacteria	Enterobacteria	Achromobacteria	Acinetobacteria	Flavobacteria	Alcaligenes	Herpetosiphon
0/40(0%)	1/14(7%)	5/46(11%)	45/120(38%)	11/24(46%)	7/13(53%)	13/23(56%)	12/12(100%)

For a more detailed determination of the different specificities of the new enzymes, we focussed our attention on the analysis of those genera which showed a high frequency of type II restriction endonucleases. These new specificities include isoschizomers of AvaII, ClaI, EcoRII, HaeIII, HindIII, HpaII, KpnI, NciI, PstI, ScaI, StuI, XhoI and XmnI.

One novel enzyme, Asp718, isolated from Achromobacter species 718, is an isoschizomer of KpnI. Asp718 recognises and cleaves specifically within the following nucleotide sequence:

5'-G/GTAC-C-3'
3'-C-CATG/G-5'

In contrast to KpnI, Asp718 generates fragments with 5'-protruding single-stranded ends. These 5'-terminal extentions may be efficiently labeled with T4 polynucleotide kinase, whereas the recessed 3'-ends are suitable substrates for the terminal labeling reaction applying Klenow enzyme.

Reference: Bolton, B.J., Nesch G., Comer, M.J., Wolf, W. and Kessler, C., FEBS Letters 182 (1985) 130-134.

059 **E. COLI Z-DNA BINDING PROTEINS: PURIFICATION, MONOCLONAL ANTIBODY GENERATION AND GENE ISOLATION. Eileen M. Lafer, Rui Sousa and Alexander Rich. Massachusetts Institute of Technology, Cambridge, Mass. 02139, USA.**

We have been studying proteins which specifically recognize and bind to Z-DNA in vitro. We are interested in finding out whether these proteins also bind specifically to Z-DNA in vivo, and in elucidating their biological functions. Because these questions can be best approached in a system which is amenable to both genetic and biochemical analysis, we applied the methodology that we developed for the purification of the wheat germ Z-DNA binding proteins (Lafer et al., Biochemistry 24, 5070-5076, 1985) to the bacterium E. coli. We found that Z-DNA affinity chromatography of a cleared E. coli lysate in the presence of a large excess of B-DNA competitor resulted in a dramatic enrichment for three large polypeptides (100K, 90K, 50K). This fraction displayed a higher affinity for a plasmid containing a Z-forming insert than the same plasmid lacking the Z-forming insert when compared at the same superhelical density, indicating the presence of Z-DNA specific binding activity in the mixture. We prepared monoclonal antibodies against each of these three proteins. Using these antibodies we measured the level of enrichment of these proteins on a column constructed of Z-DNA (Br-poly(dG-dC)·poly(dG-dC)), versus columns constructed of equal amounts of B-DNA or Br-B-DNA. All three proteins enriched substantially on the Z-DNA column when compared to the other columns: approximately 100 fold for the 50K, approximately 1000 fold for the 90K and a minimum of 1000 fold for the 100K. Immunoblots also revealed that they are all relatively rare proteins; an E. coli cell contains approximately 10-100 copies of the 100K protein, and 100-1000 copies each of the 90K and 50K proteins. Using these antibodies we have cloned the genes coding for all three proteins from an E. coli lambda GT11 expression library that we constructed. Characterization of these genes is now under way.

060 VARIATIONS IN DUPLEX DNA CONFORMATION ANALYZED BY THE BINDING OF MONOCLONAL AUTOIMMUNE ANTIBODIES, Jeremy S. Lee and Ralph P. Braun. Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0.

Four monoclonal antibodies which bound to duplex DNA, were prepared from two autoimmune female NZB/NZW mice. Their binding to various nucleic acids was investigated by competitive solid phase radioimmune assay which allows the estimation of relative binding constants. In all cases the binding constant to rRNA, poly(ADP-ribose), 'Z' DNA and single-stranded DNA was at least 100 fold less than to duplex calf thymus DNA. The binding constants to bacterial duplex DNAs showed little variation with base composition but one of the antibodies (Jel 241) bound only weakly to phage T4 and ØW14 DNAs which have obstructed major grooves. Further evidence for the interaction of Jel 241 in the major groove was obtained by comparing the binding constant to pairs of polymers such as poly d(AU)·poly d(AU) and poly d(AT)·poly d(AT) or poly d(GC)·poly d(GC) and poly d(Gm^{5}C)·poly d(Gm^{5}C) . In each case the binding constant to the methylated analogue was at least 30 fold higher than to the unmethylated DNA. However Jel 241 also recognizes conformational features of the DNA duplex since binding to poly(pyrimidine)·poly(purine) DNAs was barely detectable. This series of polymers also showed considerable variation in binding constants for the other three antibodies. In particular Jel 229 had binding constants which varied by 240 fold for the series poly(dG)·poly(dC) > poly d(TTC)·poly d(GAA) > poly d(TCC)·poly d(GGA) = poly d(TC)·poly d(GA) > poly(dA)·poly(dT). Thus (a) antibodies can be used to detect structural variations in both the major and minor grooves of duplex DNA and (b) poly(pyrimidine)·poly(purine) DNAs form a conformationally-diverse family. Supported by MRC Canada.

061 GENES, CODIND FOR ß SUBUNIT OF BACTERIAL RNA POLYMERASES, Nicholay A. Lisitsyn, Sergey O.Guryev, Galina S.Monastyrskaya, Eugene D.Sverdlov, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, 117871 GSP

Nine mutations, leading to E.coli RNA polymerase rifampicin resistance and one mutation, leading to streptolydigin resistance were localised by sequencing in the rpoB gene, coding for enzyme's ß subunit. It was discovered that amino acid residues Val(146), Asp(516), Ser(531) and Pro(564) are essential for RNA plymerase-rifampicin interaction and residues Gly(544) and Phe(545) - for interaction with streptolydigin. Several E.coli recombinant clones were obtained, carrying pNL 1 plasmid with rpoB gene containing fragment of Salmonella typhimurium. The primary structure of Salmonella rpoB gene was determined (4.2 kilobases in all) ; when compared with corresponding E.coli structure, some highly conservative regions were identified. Data obtained allowed to make some preliminary conclusions concerning the location of functional domains in the ß subunit of bacterial RNA polymerases and to bring up a hypothesis about space proximity of central part of the ß subunit with it's N- and C-terminal domaines.

062 THE PRESENCE OF NUCLEOSOMES ON A DNA TEMPLATE PREVENTS INITIATION BY RNA POLYMERASE II IN VITRO. Donal S. Luse and Joseph A. Knezetic, Dept. of Biochem. and Mol.Biol., Univ. of Cincinnati, 231 Bethesda Ave., Cincinnati, OH 45267-0522

RNA was synthesized in vitro using HeLa cell nuclear extracts and circular DNA templates on to which varying numbers of nucleosomes had been reconstituted with Xenopus oocyte extracts. Transcription reactions were done with one or more NTPs limiting; we have previously demonstrated (Coppola, et al., PNAS 80, 1251 (1983)) that this approach allows accurate initiation to be moritored with very little (<15 nucleotides) elongation of the nascent RNA chains. When fully reconstituted DNAs were used as templates in the NTP-limiting transcription protocol, no specific initiation by RNA polymerase II was detected. However, DNA exposed to the reconstitution extracts under conditions which did not allow nucleosome deposition was transcribed normally. A set of successively less reconstituted templates was also transcribed. No initiation occurred on reconstitutes with more than two-thirds of the physiological nucleosome density; reconstitutes with less than one-third of the physiological nucleosome density were transcribed as efficiently as naked DNA. Experiments are currently under way in our laboratory to determine whether the pre-binding of transcription factors or the pre-formation of complete preinitiation complexes will allow reconstitution of nucleosomes on DNA without loss of template activity.

063 ANALYSIS OF THE PROMOTER REGIONS OF TWO HUMAN PROTO-ONCOGENES: THE EPIDERMAL GROWTH FACTOR RECEPTOR GENE AND THE HARVEY-ras-1 GENE, G.T. Merlino*, S. Ishii*, A. Johnson*, J. Kadonaga†, R. Tjian†, and I. Pastan*. *Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD, 20892; †Dept. of Biochemistry, Univ. of California, Berkeley, CA 94720.

We have identified and isolated the promoter region of the human epidermal growth factor (EGF) receptor proto-oncogene. The 5'-flanking region, while lacking characteristic TATA and CAAT boxes, contains multiple RNA transcription start sites, and multiple GC box sequences: GGGCGG or CCGCCC. Several of these GC boxes are situated within a 10 bp sequence similar to the consensus sequence for binding Sp1, the transcription factor necessary for SV40 early transcription. Two EGF receptor GC boxes were capable of binding Sp1 by DNase I footprinting; however, little competition by SV40 promoter DNA for common transcription factors was observed using a transient transfection assay. The human Harvey ras (c-Ha-ras-1) proto-oncogene promoter was also identified and compared to that of the EGF receptor. The c-Ha-ras-1 promoter lacks a TATA box, and contains multiple RNA start sites and many GC boxes. Six GC boxes could bind Sp1 and, in contrast to the EGF receptor, this binding was very effectively competed by SV40 DNA containing the early promoter. Further analysis of the binding of Sp1 and other factors to various proto-oncogene promoters may provide clues to their normal and aberrant regulation.

064 STRUCTURE, FUNCTION AND APPLICATION OF THE PROMOTERS OF THE TOL PLASMID META-CLEAVAGE PATHWAY OPERON OF PSEUDOMONAS PUTIDA, N. Mermod and K.N. Timmis, Dept. of Medical Biochemistry, Univ. of Geneva, Switzerland. TOL plasmid of Pseudomonas putida contains a catabolic operon, the meta-cleavage pathway operon, that encodes enzymes for the cleavage and degradation of benzoate and toluates. Expression of this operon is positively regulated by the product of the xylS gene. We have characterized regulated and constitutive mutant promoters responsible for the expression of this operon and have derived a consensus sequence for P.putida promoters that is significantly different from that of E.coli (Mermod et al., EMBO J. 3, 2461-2466). We have constructed a broad host range regulated expression vector based upon the twin meta pathway operon promoters and the broad host range pKT231 vector plasmid. The regulated expression of a test gene cloned in this vector occurs in nearly all representative Gram-negative bacteria tested, but to an extent related to their phylogenetic distance from P.putida. We are currently developing a system for the in vitro regulation of TOL plasmid promoters transcription, and have isolated P.putida RNA polymerase and used it for in vitro run-off transcription. The xylS regulator gene product has been identified as a 32.5 kD protein; properties of this protein will be discussed.

065 EUKARYOTIC DNA TOPOISOMERASE I IS CATALYTICALLY ACTIVE AT SITES OF TRANSCRIPTION, M. Muller and D. Trask, Ohio State University, Columbus, Ohio 43210

Addition of a protein denaturant to nuclei arrests the catalytic reaction of topoisomerase I resulting in formation of a covalent complex. The distribution of the enzyme with respect to DNA sequence has been analyzed to reveal sites of catalytic activity of the topoisomerase in vivo. Covalent complexes were isolated by immunoselection using anti-topo I IgG and the associated DNA found to be heavily enriched in ribosomal genes. Topoisomerase enrichment at genes transcribed by RNA polymerase I was verified at the cytological level by immunofluorescence and at higher resolution by the protein A/colloidal gold method and EM. The distribution within the nucleolus appears non-random with substantially less enzyme in 'fibrillar centers'. Outside the nucleolus, pinpoint sites of intense fluorescence were also observed, thus, topo I distribution in the nucleoplasm is not uniform and the enzyme may be active on non-ribosomal genes. Dissociation and release of transcriptional complexes by actinomycin D treatment did not diminish the catalytic activity of endogenous topoisomerase indicating that the activity of the enzyme at specific sites is independent of actual transcriptional events and might be involved in maintaining chromatin structure at or near sites of transcription. Endogenous topo I is also catalytically associated with genes transcribed by RNA polymerase II since the globin gene is detected in complexes from erythrocytes but not from brain cells. These data are direct evidence that topoisomerase I is catalytically active at or near sites of RNA polymerase I and II transcription in a chromosomal setting.

066 **PROMOTER STRUCTURE AND FORMATION OF TRANSCRIPTION INITIATION COMPLEX OF RIBOSOMAL RNA GENE**, Masami Muramatsu, Toshimitsu Kishimoto, Masaru Nagamine, Hiroyuki Kato, Yoshiaki Ishikawa and Ryo Kominami, Dept. of Biochemistry, The University of Tokyo Faculty of Medicine, Hongo, Bunkyo-ku, Tokyo 113, JAPAN.

The "core promoter" region of mouse ribosomal RNA gene(rDNA) which is required for an accurate and efficient initiation of transcription in vitro lies between -40 and +40. Point mutations were introduced in this region and tested for the promoter activity with an in vitro system. At present, three mutants having a single mutation(G to A) at -7, -16 and -25 are found to have promoter activities 0,10 and 50% of the wild type. It was found, however, that mutations to different bases have different effects on the promoter activity; e.g. mutations to C and to T at -16 caused only 60 and 70% suppression, respectively. An extensive analysis of single point mutants in this region will be presented. Transcription factors have been purified partially and the steps of formation of initiation complex analyzed with preincubation and short-pulse experiments utilizing different promoter fragments. At least 2 factors besides RNA polymerase I(PolI) were found to be required for initiation reaction. A species-dependent factor(TFID) first binds with the promoter region of rDNA in a temperature-dependent manner. Then, a second factor (TFIA) binds with the rDNA·TFID complex, which is followed by the entry of Pol I. TFID remains bound at the promoter site together with TFIA while Pol I starts transcription repeatedly from the complex. Competition experiments indicate that different point mutants exhibit lesions at different steps of transcription, providing a new tool to dissect the initiation reaction.

067 INTERACTION OF THYROID SPECIFIC NUCLEAR EXTRACTS WITH THE PROMOTER REGION OF THE RAT THYROGLOBULIN GENE. Anna M. Musti & Roberto Di Lauro, C.E.O.S., Napoli, Italy & Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Maryland 20892.

The entire rat thyroglobulin gene has been previously isolated from the rat genome (1) and a 5' flanking region which allows transcription exclusively in a cloned rat thyroid cell line (FRTL-5) has been delimited (M. V. Ursini & R. Di Lauro, unpublished observations). In order to detect sequence specific DNA binding proteins the DNA fragment containing the above defined thyroglobulin promoter has been incubated with unfractionated FRTL-5 nuclear extracts and then subjected to either exonuclease III (2) or DNAase I footprint assay (3). Both methods detect three protected regions spanning approximatively 15 base pairs each and whose 5' border map at -73 (CAAT box), -119 and -139 respectively from the start of transcription. Furthermore, the protein components conferring the two protections at -73 and at -119 are present exclusively in the FRTL-5 nuclei, i. e. only in the cells actively expressing the endogenous gene.

References:
1) Musti, A. M. et al., Proc. Natl. Acad. Sci. U.S.A., in press.
2) Wu, C. (1985), Nature, 317, 84-87.
3) Galas, D. J. & Schmitz, A. (1978), Nucleic Acids Res., 5, 3157-3170.

068 POSITIVE AND NEGATIVE CONTROL OF PROKARYOTIC GENE EXPRESSION BY A METALLOPROTEIN. PURIFICATION AND CHARACTERIZATION OF THE MerR REGULATORY PROTEIN, T.V. O'Halloran and C.T. Walsh; Massachusetts Institute of Technology, Cambridge MA 02139

The merR gene product exerts dual control over expression of genes responsible for microbial resistance to mercurials. It represses the expression of the mer operon in the absence of mercuric ions and enhances its expression in the presence of mercuric ions. We report here the overproduction, purification and characterization of the small, cysteine containing, DNA-binding protein MerR. DNA fragments containing the merR gene were digested with Bal31 and inserted into the overproduction vector pKK223-3. Several milligrams of the 144 amino acid MerR protein were purified in a two step procedure. Gel filtration experiments reveal MerR forms dimers under non-denaturing conditions. Gel electrophoresis DNA binding experiments demonstrate that the MerR protein binds specifically to small, labelled DNA restriction fragments containing the 5' flanking control region of the mer operon. In the absence of mercury, the protein binds to a fragment containing the mer promoter but in the presence of mercury, it binds preferentially to a portion of the 5' flanking mer DNA further upstream. We will present footprinting results delineating the sites of MerR interactions with DNA in the presence and absence of mercuric ion and discuss the role of putative helix-turn-helix segments in the MerR protein. This system may serve as a paradigm for intracellular homeostasis of both toxic and essential inorganic complexes in bacteria and will be useful in understanding the regulation of the metal-induced metallothionein system in eukaryotes.

069 THE DNA-BINDING DOMAIN OF THE ESCHERICHIA COLI BIOTIN OPERON REPRESSOR, A. Otsuka, M. Buoncristiani, K. Uchida, P. Howard, J. Shaw, J. Ruppert, C. Johnson and R. Yamamoto, University of California, Berkeley, CA 94720.

Biotin operon transcription is negatively controlled by the bifunctional biotin operon repressor-biotin activating protein (BirA). Nucleotide sequencing of 19 birA mutations has shown that several repressor mutations change an amino acid in residues 32 to 34 lying within a predicted helix-turn-helix domain. The hyphenated 7-amino acid homology between BirA and the bacteriophage lambda Cro DNA-binding domains permitted computer modelling of the BirA and bio operator interaction. Ser32, Arg33 and Ala34 may interact with nucleotide pairs 14 and 15 of the bio operator to permit sequence-specific binding. Possible hydrogen bonding interactions of Lys38 with nucleotide pairs 12 and 13, and Arg44 with nucleotide pair 10, could provide additional specificity. van der Waals interactions are predicted between DNA and Met31, Ala34, and Ala35, and contacts may occur between Gln24 and Asn37 and the DNA phosphates. Mutations that affect enzyme function (positions 83 to 119 and 189 to 235) include changes in a sequence that is homologous to the conserved ATP-binding site sequence, Gly-X-Gly-X-X-Gly.

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                                R L E (representative BirA mutations)
BirA E Q L G E T [L G] M S R A [A I N K] H [I] Q
Cro  Y K T A K D [L G] V Y Q S [A I N K] A [I] H 41
     XXXXXXXXXXXXXX--------XXXXXXXXXXXXXX
  Backbone Binding Helix     Sequence Specificity Helix
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070 Control of transcription initiation of the ovalbumin gene in vitro : a transcription factor binds to the CAAT box. Martine Pastorcic, Heng Wang, Sophia Y. Tsai, Ming-Jer Tsai, Bert W. O'Malley. Baylor College of Medicine. Houston, Texas 77030.

We have used an in vitro system including HeLa cell extracts to characterize the transcription factors and promoter elements involved in initiation of transcription of the ovalbumin gene. By 5'-deletion mapping we demonstrated that a promoter element including the CAAT box (position -65 to -92) is required for quantitative transcription. The enhancement of transcription confered by the CAAT box region appears to be specifically competed in trans by DNA fragments containing the CAAT box. Furthermore, DNase I footprinting analysis identifies a protein binding site including the CAAT box in both crude nuclear extracts and a more purified fraction containing a transcription factor. We propose that a transcription factor activates transcription and binds to the CAAT box.

071 PURIFICATION OF A SINGLE YEAST GENE AS CHROMATIN, David S. Pederson, Fritz Thoma, Malabi Venkatesan and Robert T. Simpson, Laboratory of Cellular and Developmental Biology, NIADDK, National Institutes of Health, Bethesda, MD 20892 U.S.A.

The 1453 base pair TRP1ARS1 yeast plasmid is packaged as chromatin in high copy number in Saccharomyces cerevisiae. We have previously characterized the plasmid structure in terms of nuclease sensitive sites and nucleosome positioning [Thoma et al. (1984) J. Mol. Biol. 177, 715-733; Thoma and Simpson (1985) Nature 315, 250-252]. We now use this plasmid as a model to develop a general protocol to purify the chromatin of a unique gene in amounts sufficient for biochemical studies. TRP1ARS1 plasmid chromatin is selectively eluted from isolated nuclei and further purified by size and buoyant density separation techniques. Relative to total nucleic acid, a purification of about 10^4 is achieved, roughly the enrichment required for complete purity. Indirect end label mapping indicates that the positions of nuclease sensitive regions and nucleosomes are unchanged by the purification. Electron microscopic examination of the purified plasmid chromatin shows the presence of seven nucleosomes, consistent with the indirect end label mapping data. Electrophoresis of plasmid proteins reveals a relatively simple pattern of proteins. The most prominent proteins are the four yeast core histones. We will now use the procedures described here to study the chromatin structure of regulated genes cloned into similar plasmids.

072 Isolation of an Intact Transcription Complex from Nucleoli
Nancy Lynn Rosenberg, Baylor College of Medicine, Dept. of Pharmacology, Houston, Tx. 77030.

A deoxyribonucleoprotein complex was isolated from structurally intact nucleoli by restriction digestion with MspI followed by extraction with $MgCl_2$ (10mM). This particle, called a native transcription complex, NTC, was resolved by low ionic strength electrophoresis. Second dimension DNA and protein electrophoresis showed the DNA to be approximately 36 kbp long with 12-15 tightly associated proteins. Hybridization studies showed the DNA to be enriched in the repetitive sequences upstream to the rDNA genes. Sequences homologous to 18s and 5.8s rDNA were also detected. Electron microscopic analyses indicated the NTC to be approximately 340 angstroms in diameter. In vitro transcription studies using the NTC alone as well as the NTC DNA with a standard HeLa extract demonstrated specific transcription.

073 BIOCHEMICAL PROPERTIES AND DNA BINDING OF c-fos PROTEIN COMPLEX.
Lidia Sambucetti and Tom Curran, Department of Molecular Oncology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The oncogenic properties of the FBJ and FBR murine retroviruses are encoded by the v-fos oncogene. The products of the v-fos gene and its cellular homologue, c-fos, are acidic nuclear phosphoproteins that migrate on SDS-polyacrylamide gels with an apparent molecular weight of 55 kd. Both proteins form a high molecular weight complex with a 39 kd cellular protein (p39) in the nucleus. The c-fos protein, which differs from the v-fos protein, primarily in the carboxy-terminal region, undergoes more extensive post-translational modification. Treatment of a variety of cell types with polypeptide growth factors and other mitogens results in a rapid, but transient, induction of the c-fos gene. In particular, the c-fos protein complex can be readily detected in the pheochromocytoma cell line (PC12) following treatment with NGF in the presence of a peripherally active benzodiazepine. Using these cells as a source, we have extracted the fos protein complex by treatment with 200 mM sodium chloride in the presence of non-ionic detergents. This demonstrates that the complex is not tightly bound to the nuclear matrix. It migrates with an approximate molecular weight of 130 to 200 kd on high pressure liquid chromatography. The complex binds to double-stranded DNA cellulose and the majority is eluted with 500 mM salt. During all of these procedures p39 remains tightly bound to the fos protein and is only disassociated under highly-denaturing conditions. The data suggest that the function of the protein complex may involve interaction with DNA. The roles of the individual proteins in this interaction are currently under investigation.

074 IN VIVO FOOTPRINTING OF THE YEAST GAL 1-10 PROMOTER WITH LIGHT
S.B. Selleck and J.E. Majors, Washington University School of Medicine, Department of Biochemistry, St. Louis, MO 63110

We are using the "photofootprinting" procedure of Becker and Wang together with the "genomic sequencing" protocol of Church and Gilbert to identify transcriptional dependent changes that occur in vivo within the 600 bp GAL 1-10 promoter region of S. cerevisiae. For these experiments genomic DNA is isolated from UV irradiated cells and cut with an appropriate restriction enzyme prior to the chemical cleavage reactions that selectively recognize photomodified residues. These samples are run on a sequencing gel and electrophoretically transferred to a nylon membrane. Hybridization with a strand specific probe complementary to the restriction fragment that spans the target sequence permits detection and mapping of photomodification sites. To date we have examined those sequences between the UAS and the GAL 1 and 10 initiation sites. The active and silent promoters display several differences in the sensitivity to UV light-induced covalent modification at specific nucleotides. The most prominent of these occurs at the adjacent T residues in the sequence 3' TATATT 5'. This putative "TATA" box occurs twice in the GAL 1-10 promoter, 80 bp and 110 bp 5' to the GAL 1 and GAL 10 initiation sites, respectively. Photomodification of the underlined T residue within this sequence for both the GAL 1 and GAL 10 promoters is enhanced approximately 3 fold when these genes are activated either by growth in galactose or by mutation of the GAL 80 regulatory gene. This feature is not observed in gal 4 strains. Catabolite repression of the GAL 1-10 promoter by growth of a GAL 4/gal 80 strain in glucose does not abolish the enhanced sensitivity of this T residue.

075 SEQUENCE DETERMINATION AND FUNCTIONAL CHARACTERIZATION OF A HISTONE H1-DNA INTERACTION AT THE 5'-END OF THE ALBUMIN GENE, J. Sanders Sevall, Helicon Foundation, San Diego, California 92109.

Four rat liver histone H1 binding sites have been determined by DNase I protection assays at the 5'-end of the rat serum albumin gene. The strongest binding site is at the Z exon-intron junction 110 base pairs 3' from the initiation site of the albumin messenger RNA. The sequence of H1-binding has been determined on both strands of the DNA as $^{5'}$-GAGAAGCAC-gtaag-$^{3'}$, the capitals being exon sequences. A second binding site is 35 base pairs downstream from the initial binding site and has a sequence $^{5'}$-gtagtaacggaag-$^{3'}$. The third binding site lies 33 base pairs 3' from the second binding site (80 base pairs from the first binding site). The third binding site has a sequence $^{5'}$-ggtgatgatgattaa-$^{3'}$. The fourth binding site lies 150 base pairs 3' to the first site with a sequence of $^{5'}$-ctgagaatcc-$^{3'}$. A consensus binding sequence is $^{5'}$-gagaag/cg/cNc/gata/ta-$^{3'}$. The functional implication of the H1-DNA interaction is determined by indirect end-label analysis of the micrococcal nucleased rat liver nuclei. A strong hypersensitive site is located in the first intron of the albumin gene overlapping the first and second H1 binding sites. This site is being confirmed by end label analysis from the opposite side of the hypersensitive site. The relation between the H1 binding sites and the hypersensitive site location can be determined by in vitro reconstitution studies on the first intron of the rat albumin gene. (This work supported by NIH grant A32986-02A1.)

076 **TRANSCRIPTION OF THE rRNA GENE INVOLVES A LARGE AND COMPLEX INITIATION REGION**
Barbara Sollner-Webb, Jolene Windle, John Tower, and Sheryl Henderson
The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Studying the mouse and frog rRNA genes, we have delineated the sequences required for efficient initiation. In vitro analysis revealed a large promoter of the mouse rRNA gene that is composed of a proximal domain (residues ~-35 to +5) and at least two upstream domains contained between residues ~-145 and -40. Transient transfection studies have demonstrated an additional upstream promoter domain extending to residue -165. Within the proximal domain, the region -35 to -15 binds stably to the rDNA transcription factor 'D', which has been purified ~10,000 fold. The other identified mouse rDNA transcription factor, 'C', which is evidently a specifically activated subform of polymerase I, binds to this D/rDNA complex; the binding of these factors is augmented by the remaining region of the proximal promoter. Studies of the Xenopus laevis rRNA gene using Xenopus oocyte microinjection at various template concentrations again demonstrate a multidomain nature of the promoter. As is the case with the mouse gene, the proximal promoter domain directs efficient transcription under optimized conditions but the upstream domains become essential under more stringent conditions. Furthermore, analysis of Xenopus rRNA genes bearing linker scanner mutations across the entire promoter region confirm its multidomain nature. Data will also be presented that shows that the Xenopus rDNA enhancer is promoter-specific and acts in conjunction with an upstream domain of the promoter.

077 SCREENING OF COMPREHENSIVE EXPRESSION LIBRARIES FOR DNA BINDING PROTEINS. Joanne Tornow and George Santangelo, University of California, Santa Cruz, CA. 95064

The regulation of gene expression at the level of transcription is mediated by DNA-protein interactions. Methods have been described to identify specific DNA-binding proteins that have been separated on gels and transferred to nitrocellulose. We have developed a method which uses a bacterial expression library, containing 400-600 bp inserts of yeast DNA, as the source of protein. Colonies containing the recombinant plasmids are grown on nitrocellulose filters. The cells are lysed and the proteins are bound to the filters. The filters are then probed with the DNA fragment of interest (which has been labelled radioactively) in binding buffer. The plasmids from positive colonies can then be used to probe genomic libraries to clone the gene in its entirety. This method allows for the rapid and efficient cloning of genes which encode DNA-binding proteins. Furthermore, the DNA-binding domain is identified as part of the initial screen. This method was tested with the Gal 1 - Gal 10 promoter fragment from the yeast Saccharomyces cerevisiae, and in principle can be used with random fragments that contain yeast promoters to find DNA-binding proteins specific to these promotors.

078 Cell Cycle Regulation of Human Thymidine Kinase

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Thymidine kinase (TK) enzyme activity is regulated during the cell cycle and is tightly coupled with DNA synthesis during the S phase. Cultured cells synchronized by serum starvation and an aphidicolin block show an initial S phase peak in both tk-specific mRNA and enzyme activity with a decrease during G_2, M, and G_1 and a subsequent rise as the cells reenter the S phase. A human tk recombinant containing all tk coding sequences as well as the SV40 promoter and SV40 polyadenylation signals was stably transfected into tk mouse L cells. This cell line fails to show any quantitative changes in the tk-specific mRNA but demonstrates constitutive tk mRNA production throughout the entire cell cycle. This suggests that the construct is missing essential tk-specific sequences necessary for its regulation. A series of tk recombinant molecules have been constructed and are presently being analyzed to localize the critical regulatory sequences in the human tk gene.

079 CLONING THE GENES FOR THE SUBUNITS OF HUMAN RNA POLYMERASE II

Scott A. Watkins, John J. Cunniff, Kurt Disser, G. Michael Stillabower, Mark Wasserman, Susan Kupferman, Young I. Lee, and Stefan J. Surzycki, Department of Biology, Indiana University, Bloomington, IN. 47405

Previously, it has been demonstrated that mammalian RNA polymerase II is reasonably antigenic in chickens and that large quantities of IgG can be readily isolated from the egg yolks of immunized hens (Carroll and Stollar, J. Biol. Chem. 258: 24-26, 1983). A polyclonal antibody raised in chickens against human RNA polymerase II and shown to react with most of the subunits characteristic of mammalian RNA polymerase II has been used to screen a lambda gt11 human cDNA library (a gift from Drs. John O'Brien and Jeffrey de Wet). Initial screening of approximately 1.2 million clones produced 126 first round positives, of which 97 survived after 3 to 4 replatings. Seven of these clones hybridized at low stringency to a probe for the largest subunit of yeast RNA polymerase II (a gift from Dr. Richard Young). Furthermore at very low stringency, a probe for the second largest subunit of yeast RNA polymerase II (also a gift from Dr. Young) hybridizes to 2 of the 97 original clones. Currently, attempts are being made to confirm their identities by competitive Western analysis as well as characterizing them by standard Northern and Southern analysis.

080 ANALYSIS OF RANDOM YEAST PROMOTERS CLONED BY A NOVEL METHOD. Mark Wechser, George Santangelo, and Joanne Tornow, University of California, Santa Cruz, CA. 95064

A novel method, carrier-facilitated insertion, was used to generate a comprehensive library containing random small fragments from the Saccharomyces cerevisiae genome. Two size ranges of fragments (150-250 base pairs and 400-600 base pairs) were used to construct the library. A promoter-cloning vector was used to permit the detection of random promoters among these fragments. The vector contains promoterless indicator genes (coding for alcohol dehydrogenase and β-galactosidase) that permit the detection of promoters on either strand of the inserted fragments. Restoration of expression can be detected by genetic (ADH) or enzymatic (ADH and β-gal) criteria. ADH- and β-gal-positive colonies can be easily identified by using chromogenic substrates. We isolated plasmids from ADH-positive cells and showed by retransformation that they were responsible for the ADH-positive phenotype. We are analyzing several fragments that appear to contain random yeast promoters. The insert was always found to fall within the predicted size range. One of these fragments restores ADH activity when inserted in one of two orientations, and therefore activates transcription on one strand only. DNA sequencing and mapping of transcriptional start sites and chromosomal location is in progress.

081 SITE SPECIFIC BINDING OF A HERPES REGULATORY PROTEIN TO DNA, Kent W. Wilcox and Steve Faber, Medical College of Wisconsin, Milwaukee, WI 53226.

The viral polypeptide designated ICP4 (or Vmw175) is synthesized during the immediate early phase of productive infection by herpes simplex virus (HSV). ICP4 is a positive regulator of delayed early gene transcription and apparently is a negative regulator of immediate early gene transcription. The mechanisms by which ICP4 regulates transcription are not understood. We have found that native ICP4 forms a stable complex with specific nucleotide sequences in double-stranded DNA. This specific association is observed with ICP4 in crude extracts obtained from HSV-1 infected cells as well as with a partially purified preparation of ICP4. The binding of ICP4 to specific regions of HSV-1 DNA has been demonstrated with a DNA binding immunoassay. The specific nucleotide sequences from two different regions of the viral genome that are bound to ICP4 have been revealed by DNase footprinting. ICP4 protects a region spanning approximately 30 nucleotides from digestion with DNase. For the viral genes that have been investigated thus far, the binding sites for ICP4 are located within 150 nucleotides of the transcription initiation site. These results provide a functional assay for active ICP4 and suggest that ICP4 modulates viral transcription by interaction with specific cis-acting signals in viral DNA.

082 ON THE POSSIBLE PRESENCE OF HIGHLY UNDERWOUND DNA STRUCTURES IN REGULATORY GENE REGIONS. G. Yagil, Department of Cell Biology and J.L. Sussman, Department of Structural Chemistry, The Weizmann Institute of Science, Rehovot, Israel

Evidence has been accumulating that certain regions of DNA, both within and adjacent to active genes, may exist in conformational states different from bulk DNA. The possibility exists that DNA in these regions is in a highly underwound state. We have employed the techniques of structural modelling in order to explore whether reasonable structures for DNA in the underwound state can be proposed. Energy minimization by the EREF procedure of Levitt leads to two detailed structures - one with a single base pair and the other with a two base pair repeating unit. Energy differences of less than 4.6 and 3.3 Kcal/mole nucleotide between the two structures and standard B form DNA are indicated by the minimization procedure. Base pairing in the resulting structures is of the usual Watson-Crick type, with a high degree of base stacking. The backbone torsion angles have all except one (β) values found either in B DNA or in the CpG step of the ZI form of DNA. Detailed structural parameters will be described. The relative ease of strand separation makes the structures possible candidates for nuclease S1 sensitive sequences of active chromatin.

083 STUDIES ON FACTORS INVOLVED IN ACCURATE INITIATION OF HUMAN β-GLOBIN GENE TRANSCRIPTION, Yukio Yasukochi*, Shigetaka Kitajima* and Sherman M. Weissman°, *Tokyo Medical and Dental University, Tokyo, Japan and °Yale University, New Haven, CT 06510

To isolate factors involved in accurate initiation of transcription of the human β-globin gene, HeLa cell nuclear extract was subject to two successive chromatographic steps. On an anion exchange high performance liquid chromatography (HPLC) column, four fractions (Fractions I, II, III and IV in order of elution) were separated when linear gradient elution method was employed, and could reconstitute the activity toward the faithful transcription when assayed with partially purified calf thymus RNA polymerase II and stimulatory factor. After purified on an SP HPLC column, each fraction was further subject to the HPLC gel filtration. Fractions I, II and III had the activity in 41,000, 500,000 and 255,000 molecular weight regions, respectively, indicating that they were distinct. But the activity of Fraction IV could not be recovered from the gel filtration possibly due to its instability. The results suggest that four fractions were required for the accurate transcription of human β-globin gene in the addition to RNA polymerase II and stimulatory factor. The possible roles of these fractions will be discussed.

Enhancer Sequences and Transacting Factors I

084 **IMMUNOGLOBULIN ENHANCER DELETIONS IN MURINE PLASMACYTOMAS,**
Renato J. Aguilera, Tom J. Hope and Hitoshi Sakano, U.C. Berkeley, Berkeley, CA 94720.

We have analyzed immunoglobulin enhancer deletions and transpositions in murine plasmacytomas (1). This analysis revealed that the deletions occurred between the J_H region and the switch region, removing the Ig heavy-chain enhancer. As reported also by other investigators, the loss of the enhancer did not significantly affect the level of heavy-chain expression. Nucleotide sequence analysis of the area involved in one of the deletions revealed that an inverted heptamer (GTGACAC) was adjacent to the 5' recombination site. This along with the presence of nucleotide insertion at the deletion recombination site suggests that the deletion was mediated at least in part by the same recombination machinery used for V-D-J joining. Both the J_H and switch germline sequences involved in the deletion were further analyzed by an in vitro DNA cleavage system(2) with an endonucleolytic activity purified from mouse fetal liver nuclear extracts. It was found that the germline J_H sequence was strongly cleaved at the deletion recombination site. Strong cleavage was also observed at ATGGG repeats in the switch region and at germline c-myc DNA at translocation sites. We are currently attempting to identify and characterize DNA binding proteins that might be involved in these recombinations.

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085 Construction and expression of chimeric T-cell receptor genes.
Shizuo Akira, Michio Hagiya and Hitoshi Sakano
Department of Microbiology and Immunology, University of California, Berkeley, CA 94720

It is now well established that T lymphocytes express an antigen receptor molecule similar to immunoglobulin (Ig). Although the antigen-receptor interaction is well characterized for B-cells, little is known for T cells. In order to study the antigen-mediated T cell response at the molecular level, we have constructed chimeric receptor genes, containing the T cell receptor (TCR) constant region and the V region sequence from an Ig gene with a known antigen specificity. To obtain functional expression of the chimeric gene in T cells, we have studied those DNA regions of TCR genes which are essential for T cell-specific expression. A series of deletions were generated in the 5'- or 3'-side of the V gene, and analyzed for their effect on the expression in T cell lines. A possible transcriptional control element was identified about 200bp upstream of the TCR V gene. The function of this sequence was examined by connecting it to a promoter-deleted chloramphenicol acetyl transferase (CAT) gene.

086 U1 AND U2 snRNA GENES CONTAIN ENHANCERS, Manuel Ares, Jr., Marguerite Mangin, and Alan M. Weiner, Yale School of Medicine, New Haven, CT 06510

Genes encoding U1 and U2 RNAs, the major snRNA components of the pre-mRNA splicing apparatus, represent powerful RNA polymerase II transcription units. Based on the abundance of these stable RNAs, and the number of genes encoding them, initiation of transcription must occur at least once per gene every two to four seconds in growing cells. Transcription is initiated at a unique site, although a TATA-homology is not apparent in sequences upstream of the cap site. We have investigated promoter function by making human U1 and U2 promoters mutants, and testing them for the ability to direct transcription after transfection into Hela cells. We previously defined an element upstream of the U2 promoter which is required for efficient expression of U2 templates in microinjected frog oocytes. We now find that this element is an enhancer, because it stimulates transcription from the U2 promoter independent of orientation and position. Both a similar element from the human U1 gene and the SV40 enhancer region are able to replace the U2 enhancer and stimulate transcription from the U2 promoter. Comparison of snRNA genes from humans, rats, chickens, and frogs reveals a homology usually found between -210 and -250, with a consensus GGGCGRGNNYATGYAAAT. When 4 bp (YATG) are deleted from this sequence in the human U2 gene, the enhancer is destroyed. The consensus sequence appears to represent a composite of the GC-box known to bind transcription factor Sp1, and the "enhancer core" homology. We are attempting to identify the cellular factors which interact with the snRNA gene enhancer.

087 TANDEM KAPPA IMMUNOGLOBULIN PROMOTERS ARE EQUALLY ACTIVE IN THE PRESENCE OF THE KAPPA ENHANCER: IMPLICATIONS FOR MECHANISTIC MODELS OF ENHANCER FUNCTION.
Michael L. Atchison and Robert P. Perry, Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111

Immunoglobulin κ genes contain an enhancer sequence located in the large intron separating the J_κ region from C_κ. This enhancer and the immunoglobulin V_κ promoters function only in cells of lymphoid origin and thus operate in a tissue-specific fashion. We have studied the interaction of the κ enhancer and promoter sequences by introducing genes containing tandem V_κ promoters adjacent to a single κ enhancer into plasmacytoma cells and assaying for transient or stable transcription. We find that the promoters located proximal and distal to the enhancer function identically. Moreover, we find no difference in expression whether the promoters are separated by 440 bp or by 2.7 kb or whether they are located 1.7 or 7.7 kb away from the enhancer. Our results indicate that the immunoglobulin κ enhancer does not operate as a bidirectional entry site for RNA polymerase or other factors associated with the transcription complex. Rather, they suggest that the enhancer exerts its influence uniformly over large distances, and independently of the presence of intervening promoters.

088 A 278 BP DNA FRAGMENT CONTAINING THE UPSTREAM ACTIVATOR SEQUENCE DETERMINES PRECISE NUCLEOSOME POSITIONING OF THE YEAST PHO5 GENE. Lawrence W. Bergman, Dept. of Biological Sciences, University of Maryland Baltimore County, Catonsville, MD 21228.

The functional relationship of nucleosome positioning and gene expression is not known. Using high copy plasmids, containing the yeast phosphate-repressible acid phosphatase gene (PHO5) and the TRP1/ARS1 vector system, I have determined the nucleosomal structure of the 5'-region of the PHO5 gene and demonstrated that the nucleosomal positioning of this region is independent of orientation or position in the various plasmid constructions utilized. However, deletion of a 278 bp BamHI-ClaI fragment from the 5'-flanking sequences of the PHO5 gene cause the nucleosome positioning to become dependent on orientation or position in the plasmids tested. Use of PHO5-CYC1-LacZ fusions have demonstrated that this fragment contains the sequences responsible for the transcriptional regulation of the PHO5 gene in response to the level of phosphate in the growth media. Possible mechanisms involved in the precise nucleosome positioning will be discussed.

089 INFLUENCE OF 5'-END PROLACTIN GENES SEQUENCE OF GH CELLS ON THE AMPLIFICATION AND EXPRESSION OF NEIGHBORING GENES. D.K. Biswas; S.G. Pasion, and D.T.W. Wong. Pharmacology, Harvard Dental and Medical School, Boston, Mass 02115.

A 10.3kb segment containing DNA which flanks the 5'end of rat prolactin (PRL) gene of 5'-bromodeoxyuridine (BrUrd)-responsive GH cells can mediate amplification of linked sequences such as Herpes simiplex virus Thymidine Kinase (TK) and Human Growth Hormone genes. Cotransfection of this 10.3kb DNA segment and "Neo" gene (gene that confers G418-resistant phenotype) led to the amplication of "Neo" gene, in transfectants carrying the 10.3kb DNA segment and "Neo" gene stably intergrated in tandem in the chromosome of recipient mouse cells. The expression of "Neo" gene in mouse cells seems to be influenced by the 10.3kb 5'-end rat PRL gene in the similar fashion as observed in the parent GH cell strains. The basal level expression of "Neo" gene in transfectants carrying the 10.3kb DNA segment of PRL-non producing GH cells is less than that observed in transfectants with the homologous sequence of PRL producing cells. PRL synthesis in PRL producing GH cells is inhibited by hydrocortisone. Similar effect on the expression of "Neo" gene is observed following treatment of transfectants with dexamethasone.

090 REVERSAL OF ENHANCER ACTIVITY BY A HERPES SIMPLEX VIRUS "LATE"(VP5) PROMOTER. E.D. Blair and E.K. Wagner, University of California Irvine, Irvine, CA 92717.

Herpes simplex virus (HSV) promoters share many features with normal cellular promoters. To investigate whether promoter-regulator sequences down-regulate activity of the strong HSV-1 VP5 promoter, which is inactive in uninfected cells, we examined the response of various segments of this promoter to strong *cis*-acting transcriptional activation by SV40 enhancer sequences in transient expression assays. In uninfected cells, a plasmid containing 75 base pairs (bp) of sequences immediately upstream of the VP5 mRNA capsite linked to the chloramphenicol acetyl transferase (CAT) gene was activated by the SV40 enhancers to express high levels of CAT activity, but the inclusion of 50-168 bp of upstream sequence inhibited the response. The 240 bp promoter for the "early" mRNA encoding the viral alkaline exonuclease was strongly activated by SV40 enhancers to drive CAT gene expression. Sequences from the VP5 promoter/regulatory region, which are implicated in blocking the enhancer response, were added to the alkaline exonuclease mRNA promoter to determine mobility of the inhibition. Preliminary studies to identify *trans*-acting polypeptides that recognize the VP5 promoter will also be presented.

091 ANALYSIS OF THE HYPERSENSITIVE SITE IN A HUMAN hsp70 PROMOTER IN TRANSFECTED COS CELLS. Michael E. Brown, Paul C. Schiller, Richard W. Voellmy and Walter A. Scott, University of Miami School of Medicine, Dept. of Biochemistry, Miami, Fl 33101.

An intense hypersensitive site was observed in chromatin over the human hsp70 promoter contained in plasmids transfected into COS 7 cells (Voellmy et al., Proc. Natl. Acad. Sci. 82: 4949-4953, 1985). Comparable plasmids containing the *Drosophila* hsp70 promoter showed only a weak hypersensitive site in similar transfection experiments (relative to the intense hypersensitive chromatin structure formed over the SV40 sequences present in the same plasmids). We are currently carrying out a genetic analysis of the sequences responsible for the hypersensitive site over the human hsp70 promoter. Chromatin structural analysis using restriction enzymes and exonuclease III will also be presented.

(supported by NSF grant number DMB-8408619)

092 PROTEINS THAT BIND TO MAMMALIAN RNA POLYMERASE II ARE REQUIRED FOR ACCURATE TRANSCRIPTION FROM A CLASS II PROMOTER. Z.F. Burton, L.G. Ortolan and J. Greenblatt. Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada.

RNA polymerase II-associating proteins (RAPs) from a number of mammalian cell lines have been identified by affinity chromatography on immobilized calf thymus RNA polymerase II (Sopta et al. (1985) J. Biol. Chem. 260,10353-10361). The RAPs that we have identified elute from an RNA polymerase II column at moderate salt concentrations. HeLa cell nuclear extracts that support transcription from the Adenovirus major late promoter in vitro lose this accurate transcriptional activity by repeated passage over a column that contains covalently bound calf thymus RNA polymerase II. Chromatography of extracts on control columns with no bound RNA polymerase II has no effect on accurate transcription. A 0.5 M KCl eluate of an RNA polymerase II column completely restores activity to an extract depleted of RAPs by affinity chromatography. Accurate transcription from the Adenovirus major late promoter seems to require three RAPs:RAP30 (the number indicates molecular weight in kilodaltons as determined by SDS-polyacrylamide gel electrophoresis), RAP38 and a RAP of uncertain size that has DNA-dependent ATPase (and dATPase) activity. These RNA polymerase II-binding factors are required in addition to the DNA-binding factors identified by other laboratories as being necessary for accurate class II transcription in vitro.

093 NUCLEOTIDE SEQUENCE AND FUNCTIONAL ANALYSIS OF A TYPE D RETROVIRAL LONG TERMINAL REPEAT. Ing-Ming Chiu*, Susan Skuntz*, and Ram Narayan+, *Molecular Biology Division, Meloy Laboratories, Inc., Springfield, Virginia 22151; +Department of Pharmacology, NS285, Yale University School of Medicine, New Haven, Connecticut 06510. Nucleotide sequences of a DNA fragment containing the long terminal repeat (LTR) of squirrel monkey retrovirus (SMRV) were determined. Sequence analysis shows that the SMRV-LTR comprises a total of 413 base pairs (bp) in three sequence domains, 5'-U3-R-U5-3', and is bounded by 3 bp inverted repeats. Within the U3 region, there are two 43 bp repeats and two 42 bp repeats which are homologous to each other. These repeats are likely to provide enhancer activities commonly observed in other enhancer sequences. Following the repeats are transcriptional regulatory sequences including a CAT box, a Goldberg-Hogness box and a polyadenylation signal, all positioned within the U3 region of SMRV LTR. A 22 nucleotide sequence immediately downstream from the LTR was found to be complementary to lysine $tRNA_{1,2}$ suggesting that lysine $tRNA_{1,2}$ may serve as the primer for the reverse transcription of SMRV viral RNA. In order to test the functional properties and host cell specificity of SMRV-LTR enhancer-like sequences, a recombinant plasmid (pSMRVcat) was constructed using the SMRV-LTR and the coding sequence of chloramphenicol acetyl transferase (CAT). This plasmid DNA was transfected into a variety of cells and assayed for the enzymatic activity of CAT. The cis-acting element of SMRV-LTR and its host cell specificity will also be presented.

094 A TRANS-ACTING FACTOR NEGATIVELY REGULATES TRANSCRIPTION AT THE MOUSE MAMMARY TUMOR VIRUS LTR, Michael G. Cordingley, Helene Richard-Foy, Alex Lichtler, and Gordon L. Hager. Hormone Action and Oncogenesis Section, LEC, NCI, NIH, Bethesda, MD 20892.

We have previously reported that the LTR of mouse mammary tumor virus functions to negatively modulate the activity of a constitutive transcriptional enhancer (EMBO J. 3:1891, 1984) in the absence of glucocorticoids. Oligonucleotide-directed mutations in the hormone regulatory region (HRE) extensively impair the hormone response and destroy the repressive activity of the MMTV LTR. This indicated that the HRE mediates negative regulation of transcription. Experiments utilising protein synthesis inhibitors revealed that a labile protein was required for repression of transcription initiation at the LTR. Superinduction of transcription initiation occurs when protein synthesis is inhibited during hormone stimulation of the promoter. This effect is paticularly dramatic when the enhancer of Harvey murine sarcoma virus is present at the upstream boundary of the MMTV LTR but is also observed in the absence of an upstream enhancer element. Deletion of sequences which contain the HRE prevents superinduction of transcription initiation. We conclude that negative regulation at the MMTV LTR is due to a labile trans-acting factor which mediates its effect directly or indirectly through sequences at or closely associated with the HRE. These results suggest that both positive and negative regulatory factors interact with closely associated sequences in the MMTV HRE.

095 REGULATION OF IMMUNOGLOBULIN LIGHT CHAIN GENE TRANSCRIPTION IN VITRO R. Alexander Currie and W. Michael Kuehl, National Cancer Institute, National Institutes of Health, Bethesda, MD 20814

An in vitro transcription system has been established with a whole-cell extract from the human Daudi Burkitt's lymphoma cell line. This B cell extract has been compared with a HeLa S_3 whole-cell extract in an effort to identify lymphocyte specific regulatory factors of kappa light chain gene transcription. The B cell extract was capable of transcribing a viral gene template (adenovirus Ad2 late major promoter region), a human structural gene template (histone H4A), and 2 mouse immunoglobulin kappa light chain gene templates (V_k173 and V_k41), but not a viral SV40 early region gene template at a [DNA] of 50 µg/ml. The HeLa S_3 extract showed no discrimination as all 5 templates were transcribed. Alpha-amanitin at [0.1 µg/ml] completely inhibited the accumulation of transcripts in both systems. Two template constructions were used to examine the role of the C_k enhancer sequence in regulating transcription: a V_k173 template which contained 120 bp of 5' flanking sequence, the leader and variable region gene segments, and a V_k41 template which contained a 473 bp C_k enhancer sequence positioned upstream of 1.1 kb of 5' flanking seqence followed by the leader and variable region segments. At a [DNA] of 6.2 µg/ml the V_k41 enhancer-containing template was transcribed at a 3 fold higher level than the V_k173 template; at this [DNA] no transcriptional activity was detected with either of these templates in the HeLa S_3 extract. This 3 fold higher level of V_k41 transcription could be abolished by liberating the 473 bp enhancer fragment with Eco R1 digestion or by internal cleavage with Stu 1. Thus, a tissue specific trans-acting factor may be involved in the stimulation of kappa light chain transcription in vitro.

096 Glen A. Evans, Holly A. Ingraham, Kathy A. Lewis, and George M. Lawless. Enhancer-dependent activation of the Thy-1 glycoprotein gene in teratocarcinoma cells.

The Thy-1 glycoprotein is a small cell surface antigen expressed on fibroblasts, T lymphocytes and neurons of the mouse. Thy-1 is of interest primarily as a differentiation antigen in the immune and nervous systems and because it is a member of the immunoglobulin superfamily of genes and has a structure resembling a single immunoglobulin domain. The F9 teratocarcinoma cell line presents an *in vitro* model for Thy-1 gene activation during differentiation. Thy-1 is absent on F9 teratocarcinoma cells but is present on embryoid bodies after induced differentiation with retinoic acid. We investigated the expression of the Thy-1 gene in F9 cells by transfection using the intact 6.2 kb gene and constructions containing regulatory elements of the Thy-1 gene activating the chloramphenicol acetyl transferase (CAT) reporter gene. Constructions with bacterial CAT indicate that a 400 base pair region within a 2.2 kb intron has T lymphocyte-specific enhancer activity. A second enhancer element with a different specificity is also present within the Thy-1 gene. The T cell-specific enhancer is active in differentiated F9 cells but inactive in non-differentiated cells, suggesting that enhancer activation by a trans-acting factor is responsible for Thy-1 gene activity induced by retinoic acid.

097 ACTIVATION OF TRANSCRIPTION OF HTLV-I, HTLV-II AND LAV/HTLV-III BY VIRAL PROTEINS. Barbara K. Felber, Haralobos Paskalis, Connie M. Wright and George N. Pavlakis. LBI-Basic Research Program, National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701.

We have demonstrated that the Human T cell leukemia viruses HTLV-I, HTLV-II and LAV/HTLV-III encode proteins that activate transcription from the viral Long Terminal Repeats (LTRs) and provide an opportunity to study the interaction of regulated promoters with specific activators. We have expressed these activators in animal cells and have studied their interaction with the homologous and several heterologous promoters. The activators of HTLV-I and HTLV-II (TA I and TA II) are interchangeable. TA I is localized in the nucleus of transfected cells by indirect immunofluorescence.

We have also constructed mutants in the LTR and in the proteins in order to characterize the interaction between them. Deletion mutants in the LTR demonstrate that a region upstream of the promoter is necessary for the activation by TA I. This region has some characteristics of an inducible enhancer because it acts in both orientations 3' and 5' of heterologous promoters, as well as its own promoter, but only in the presence of the TA I activator. The interaction of the LAV/HTLV-III LTR with the homologous activator appears to be different in that only sequences in the R region 3' to the transcriptional start are required for the activation.

Overproduction of these activators in animal cells was achieved by Bovine Papilloma Virus (BPV) vectors. Overproducing cell lines are used for the biochemical isolation of these activators.

098 Point Mutants and Revertants of the Polyoma Enhancer Sequences. W. R. Folk, Shelley Berger, Wei Jen Tang, Steve Triezenberg and Maggie Sullivan. University of Texas at Austin, Austin, Texas 78712.

We have isolated and partially characterized a large number of bisulfite-induced point mutants in the polyomavirus A3 strain enhancer. Activation of transcription and of DNA replication are greatly reduced by simultaneous G→A transitions at nucleotides 5140, 5192 and 5218. Analysis of revertants of these mutants indicates that restoration of transcription and DNA replication occurs by any of the following three mechanisms: a) Reversion of the mutation at nt 5140; b) Acquisition of a new A→G mutation at nt 5258; c) Duplication of mutated sequences between nt 5146-5268.

These results indicate that the effect of the polyomavirus enhancer upon transcription and DNA replication are mediated by the same nucleotide sequences, and that nucleotides 5140, 5192, 5218 and 5258 are of key importance for these functions.

099 Fradkin, Lee G., Leong, Kahan, Morrow, Casey D., Berk, Arnold J., Dasgupta, A.
Depts. of Microbiology and Immunology & Microbiology.
Molecular Biology Institute, UCLA. L.A., CA 90024

A novel and sensitive assay for detection of eukaryotic RNA polymerase II has been developed. This assay depends on the ability of pol II to elongate a small RNA primer, oligo (U), hybridized to a single-stranded homopolymeric DNA template, poly (dA). The poly (dA)·oligo (U)-dependent RNA polymerase II from calf thymus has been purified approximately 10,000-fold using this assay. The purified enzyme contains four polypeptides of apparent molecular weights 180,000, 140,000, 24,000 and 16,000 and is fully active in accurate initiation of transcription from the adenovirus major late promoter in the presence of transcription initiation factors from HeLa cells. The poly (dA)·oligo (U)-dependent RNA polymerase activity can be detected in crude cell extracts from a variety of tissue culture cells and appears to be largely due to pol II since 90-95% of this activity is inhibited by a α-amanitin at a concentration of 1 ug/ml.

E. Coli RNAP will also use template-primer combinations with high efficiency in a manner sensitive to inhibition by rifampicin. Further characterization of primer-dependent transcription by both prokaryotic and eukaryotic polymerases will be presented.

0100 MOLECULAR ANALYSIS OF A TISSUE SPECIFIC TRANSCRIPTIONAL ENHANCER FROM THE RAT GROWTH HORMONE GENE,Rodrigo Franco,Chris Nelson,Sergio Lira,Ronald Evans and M.G. Rosenfeld,University of California at San Diego and the Salk Institute,La Jolla,CA

The rat growth hormone gene (RGH) in vivo is predominantly expressed in the somatotroph cell type of the anterior pituitary. Pituitary cell lines which express the growth hormone gene have been used to characterize a DNA element capable of determining cell type specific expression. Using a transient expression assay to measure the expression in various growth hormone producing cell lines of transfected fusions of RGH genomic sequences and the bacterial Chloramphenicol acetyltransferase gene, an element which stimulated enzyme expression was localized between nucleotides 235 and 48 5' of the transcriptional CAP site. The RGH element is cell type specific on the basis of its failure to function in various cell lines of non-pituitary origin. Furthermore, this cell type specific element exhibits position and orientation independance characteristic of transcriptional enhancers as shown by its activity upon transfer from 48 base pairs 5' of the RGH CAP to 422 base pairs 5' of the rat prolactin gene CAP in either orientation.
Current studies on the RGH enhancer focus on deletion and linker scanning mutagenesis in conjunction with characterization of protein binding regions through nuclease protection experiments.

0101 MOLECULAR DISSECTION OF THE LY-4 LOCUS FROM B2M BY USE OF IMMUNOSELECTED MUTANTS
Wayne Frankel, Terry A. Potter, and T.V. Rajan, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Mutants in the b allele of β_2-microglobulin ($B2m^b$) in a $B2m^a/B2m^b$ heterozygous murine cell line were analyzed for expression of the tightly linked alloantigen, Ly-4.2. Two types of mutants were analyzed for Ly-4.2 expression, retroviral insertions and spontaneous deletions. The wild type clone, 439.4.2, expresses Ly-4.2, as determined by microcytotoxicity. Clone C1, which contains a deletion extending at least 10 kb from the first intron, thus deleting the first exon of $B2m^b$, does not express Ly-4.2. However, three retroviral insertion mutants, which each contain either a Moloney or Abelson leukemia provirus near the first exon of $B2m^b$, thus inactivating the β_2-m^b phenotype, continue to express Ly-4.2. In addition, two of these mutants appear to express more Ly-4.2 than the wild type clone 439.4.2. These two also synthesize novel, high molecular weight, poly A^+ transcripts as detected by a sense-strand B2m leader sequence probe, while appearing to make no $B2m^b$ message. These transcripts do not contain proviral LTR sequences, and it is possible that the LTR is acting as an enhancer to stimulate transcription of a previously untranscribed segment of DNA, initiating antisense to B2m in the leader portion of the normal B2m message. We hypothesize that the novel RNA's splice into the endogenous Ly-4.2 transcript, which may actually reside not more than 10 kb from, but in the opposite transcriptional orientation of $B2m^b$. Regardless of the quantitative difference in Ly-4.2 expression between the insertion mutants and the wild type clone, these results imply that the gene encoding the Ly-4.2 alloantigen resides upstream of B2m, within the region spanned by the C1 deletion. These results argue, contrary to previous suggestions, that Ly-4 and B2m gene products are distinct molecules, and not different determinants on the same molecule.

0102 DELETION ANALYSIS OF THE STRONG PROMOTER FROM THE CHICKEN CYTOSKELETAL BETA-ACTIN GENE, Nevis Fregien and Norman Davidson, California Institute of Technology, Pasadena, CA 91125

The cytoskeletal beta-actin gene is expressed at high levels in most cells. We have found that the promoter of the beta-actin gene is very strong. We have tested the beta-actin gene for enhancer sequences. Transient and stable transformation experiments indicate that sequences in the region of the beta-actin promoter can stimulate transcription from other promoters after stable chromosomal integration but not during the transient phase of expression. To further understand the mechanisms for the strong promoter activity of the beta-actin promoter we have done deletion analysis. The promoter fragment tested consists of 1.6 kb of 5' flanking DNA including the CAAT and TATA boxes, the first (untranslated) exon, the first intron, and 6bp of the second exon up to the (now deleted) ATG for the initiation of translation of the beta-actin protein. Deletions in the 5' flanking DNA indicate that much of the DNA 5' of the CAAT box can be deleted with decreasing the level of expression. Deletions in the first intron show that removal of the 5'splice site (and/or nearby sequences) resulted in the decrease of the level of CAT expression.

0103 POLYOMA VIRUS ENHANCER-BINDING FACTOR, Frank K. Fujimura, La Jolla Cancer Research Foundation, La Jolla, CA 92037

Using retention on nitrocellulose filters as an assay, at least one factor that binds specifically to the enhancer region of polyoma virus DNA has been detected. This factor is present in nuclear extracts of mouse F9 embryonal carcinoma cells. A similar binding activity is present in mouse 3T6 cells. The binding of the factor is specific for the Pvu II-4 fragment of polyoma DNA which contains the B element of the polyoma enhancer. No binding activity to other putative elements of the polyoma enhancer was detected by the filter-binding assay. Footprint analyses using DNase I and Exo III indicated that the factor binds to a region of the polyoma genome containing a nine-base-pair inverted repeat located between sequences having homology to core sequences of the immunoglobulin heavy chain and the SV40 enhancers. No difference in binding of factor has been detected between wild-type polyoma enhancer and the mutated enhancers of the PyEC mutants, F101, F111 and F441, which were selected for growth in undifferentiated F9 cells. However, both DNase I and Exo III analyses show a difference between wild-type and mutant footprint patterns around the site of the point mutation common to these three PyEC DNAs, suggesting that these mutant genomes have structural features located within the enhancer region that can be distinguished by these nucleases from structures in the corresponding region of the wild-type polyoma enhancer.

0104 **THE UNIT SEQUENCE WHICH FUNCTIONS AS VIRUS-INDUCIBLE ENHANCER FOR THE HUMAN β-INTERFERON GENE EXPRESSION** Takashi Fujita, Hiroshi Shibuya, Haku Hotta, and Tadatsugu Taniguchi, Inst. Mol. Cell. Biol. Osaka Univ. Japan Human β-interferon (IFN-β) gene is transcriptionally activated by induction such as virus infection, otherwise the gene is non-functional. We previously identified a DNA sequence which mediates viral induction (between -117 and -39 from transcription initiation site, **Cell**, **41**, 484-496, 1985). The sequence essentially consists of seven blocks of hexanucleotides whose sequences are homologous to each other (consensus: $AA\begin{matrix}AT\\GG\end{matrix}GA$). This time we chemically synthesized the consensus sequence and examined its function [$(AAGTGA)_8$]. First we constructed a chimeric gene in which the bacterial chrolamphenicol acetyl transferase (CAT) structural gene is connected at downstream of IFN-β 5' upstream sequence (-55 to +22). Since the gene lacks the DNA sequence required for viral induction, CAT gene is not expressed by viral induction. However, when synthetic repeats are present in its upstream, CAT activity as well as correctly initiated transcript is induced by virus with efficiency similar to the natural gene. The repeat is found to be functional in either orientation and in certain circumstances, from downstream of the gene. Thus, the synthetic repetitious oligomers function as an inducible enhancer. Furthermore, the repeats are also operative when joined to human IL-2 gene upstream sequence (-80 to +51);i.e. the IL-2 gene became virus inducible.

0105 CLONING A BACTERIOPHAGE T4 GENE ENCODING A 15 kd PROTEIN TIGHTLY BOUND TO E. COLI RNA POLYMERASE, Christopher G. Goff, Haverford College, Haverford, PA 19041

Bacteriophage T4 causes a number of changes in E. coli RNA polymerase after infection; for example, several T4-coded polypeptides are found noncovalently attached to the transcription enzyme. Two of these, gp33 and gp55, have well documented roles in regulating transcription of T4 late genes. Another T4 protein, MW ~15 kd, is bound very tightly in stoichiometric amounts, but has no proven function (see Horvitz, Nature New Biol., 244, 137); no gene for it has been identified. However three T4 mutants which produce an altered form of this protein have been discovered by examining (on SDS gels) T4 proteins bound to RNA polymerase after infection (Horvitz, Ratner, and Goff, unpublished). These mutants are rII deletions with one endpoint in common, adjacent to gene 60. All three mutants produce a "doublet" of 15 kd protein bands which have slightly different mobilities on SDS gels from the wildtype protein (one larger, one smaller, by ~500 daltons). The "doublet" phenotype cannot be separated from the rII deletions by recombination in vivo. The mutation responsible is cis-acting so it is not a defect in a protein-processing enzyme. These phage are rII but otherwise grow normally.

My lab cloned and restriction-mapped about 10 kb of the T4 gene 60 region (from gene 39 to rIIb) in lambda phage vectors. We have now subcloned DNA from the gene 60-rIIA junction region into M13 vectors and have begun DNA sequencing to investigate the nature of this doublet protein (are there overlapping genes here?). We hope to determine the function of this protein by expressing it in an uninfected cell.

0106 TRANS-ACTING REGULATORY FACTOR FOR THE MOUSE IMMUNOGLOBULIN KAPPA GENE PROMOTER, T.V. Gopal, National Institutes of Health, Bethesda, MD 20892

The J-C intron sequences of both heavy and light chain immunoglobulin genes contribute to their tissue-specific expression. Recently we have shown that the mouse immunoglobulin kappa gene promoter also contributes to its tissue-specific expression (T.V. Gopal et al, Science, 239, 1102-1104, 1985). Tissue-specificity of cis-acting kappa gene regulatory sequences is thought to be due to their interaction with lymphoid cell specific trans-acting regulatory factors. We devised a strategy to establish unequivocally the presence of a lymphoid cell specific trans-acting regulatory factor that interacts with the mouse immunoglobulin kappa gene promoter (which we designate as Promoter Competence Factor, PCF) and then clone the gene for such a factor. A hybrid gene consisting of mouse kappa gene promoter with a neutral enhancer derived from Harvey murine sarcoma virus and a dominant selectable bacterial neomycin (Neo) resistance gene was constructed. This was introduced into thymidine kinase (TK) deficient 3T3 cells. Stable transformant 3T3 cell clones containing the hybrid gene, but not expressing it were selected using another selectable marker, herpes simplex virus TK gene, linked to the same plasmid. The ability of the lymphoid cell specific PCF to activate the kappa gene promoter of the non-expressing hybrid gene was tested by fusion of these 3T3 clones with both mouse and human lymphoid cells as well as non-lymphoid cells followed by selection with G418. Hybrids expressing the neomycin resistance gene were obtained upon fusion only with mouse or human lymphoid cells, but not with human non-lymphoid cells such as HeLa, HT1080, HL60 and mouse 3T3 cells. These results suggest the presence of a lymphoid cell specific trans-acting regulatory factor(s) that can activate the mouse immunoglobulin kappa gene promoter. Also, the presence of immunoglobulin kappa gene specific PCF in these hybrids was confirmed by an independent transient expression system using bacterial CAT gene linked to kappa gene promoter with the same neutral viral enhancer. These human lymphoid cell activated hybrids are currently being analysed for chromosomal mapping and cloning of kappa PCF gene.

0107 CHARACTERIZATION OF A MOUSE MITOCHONDRIAL TRANSCRIPTION SYSTEM, Michael W. Gray* and David A. Clayton, Dept. of Pathology, Stanford University, Stanford, CA 94305

Although different mammalian mitochondrial (mt) genomes are organized and expressed in a very similar fashion, a pronounced species specificity in mammalian mt transcription is indicated by the fact that (1) mouse mtDNA is not transcribed in a human mt run-off transcription assay that is highly active when programmed with human mtDNA, and (2) sequences identified as the human light (L-) and heavy (H-) strand promoters do not appear in the comparable region of mouse mtDNA, or elsewhere in the mouse mt genome. To investigate the molecular basis of this specificity, we have isolated a fraction of mouse mt enzymes that is capable of accurate initiation of transcription when programmed with cloned mouse mtDNA. Fractionation on phosphocellulose separates a nonspecific RNA polymerase activity from a "transcription factor" that is essentially devoid of RNA polymerase activity. The combination of nonspecific polymerase + transcription factor reconstitutes promoter selectivity and accurate initiation from the mouse mt L-strand promoter. These results reproduce similar observations made recently with a human mt transcription system [Fisher, R.P. & Clayton, D.A. (1985) J. Biol. Chem. 260, 11330-11338]. Continued purification of the separate polymerase and transcription factor activities will permit a critical assessment of the degree to which each fraction contributes to the accuracy and efficiency of initiation. These studies also open the way to defining the role of the separate polymerase and transcription factor activities in determining the species specificity of mammalian mt transcription. Supported by NIH and MRC. *Permanent address: Dept. of Biochemistry, Dalhousie University, Halifax, N.S. B3H 4H7 (Canada).

0108 SPECIFIC STIMULATION OF SV40 LATE TRANSCRIPTION BY A FACTOR BINDING THE SV40 21 BP REPEAT REGION. Ulla Hansen, Chung H. Kim, Catherine Heath and Alison Bertuch, Dana-Farber Cancer Institute, and Department of Pathology, Harvard Medical School, Boston, MA 02115

The three direct, adjacent, 21 bp repeats of the SV40 early and late promoters have been shown to be required for optimal transcription from these promoters, both *in vitro* and *in vivo*. We have identified a novel cellular transcription factor, the late SV40 transcription factor (LSF), which specifically binds to the SV40 21 bp repeats and activates transcription from sites utilized late during an SV40 lytic infection (subsequent to SV40 DNA replication). The late SV40 transcription factor LSF is distinguished in three ways from the early SV40 transcription factor SP1:

1) LSF and SP1, when added to a reconstituted transcription system, activate transcription very differently from the SV40 early and late promoters. LSF stimulates transcription from sites used post-DNA replication (mainly the major late start sites L325 and L264, but also the late-early start site). The early SV40 factor, SP1, stimulates transcription from the early-early start site, used prior to DNA replication, and from a minor upstream late start site (L167). Both factors stimulate transcription bidirectionally.
2) LSF and SP1 form complexes with the 21 bp repeats of different apparent molecular weights, as analyzed by the DNA-binding gel retardation assay.
3) LSF and SP1 elute at different salt concentrations from a heparin affinity column.

In addition, LSF and SP1 seem to interact with different cellular promoters.

0109 BIOCHEMICAL ANALYSIS OF THREE cAMP-INDEPENDENT cAMP RECEPTOR PROTEINS (CRP): CYCLIC NUCLEOTIDE-INDEPENDENT *lac* PROMOTER ACTIVATION *IN VITRO*. James G. Harman, Keith McKenney and Alan Peterkofsky. NIH, Bethesda, MD 20892

Lactose operon promoter (*lacP*) activation requires the cAMP-modified form of wild-type CRP. Some *crp* mutants eliminate the cAMP requirement for *lacP* activation *in vivo*. To gain a better understanding of the mechanism of cAMP-independent promoter activation we have: 1) determined, through cloning and DNA sequence analysis, the primary structure of three cAMP-independent forms of CRP; 2) purified the mutant proteins and assessed the effect of mutations on CRP tertiary structure; and 3) studied their requirements for *lacP* activation *in vitro*. Protease sensitivity has been used to distinguish the inactive form of wild-type CRP from the active form of the protein. Wild-type CRP is protease sensitive in the presence of cAMP. The mutant proteins were found to be sensitive to proteases in the absence of cAMP. We infer from this that the conformations of the mutant proteins resemble, in the absence of cAMP, the active conformation of wild-type CRP. Consistent with this interpretation, we have found that the mutant forms of CRP stimulate *lac* mRNA synthesis *in vitro* in the absence of cAMP. Wild-type CRP activated *lacP* only in the presence of cAMP. This was observed in transcription reactions with *lacP* contained on either a 203 bp DNA fragment or on a supercoiled plasmid. In addition, the altered cyclic nucleotide specificity for *lacP* activation observed *in vivo* was reproduced *in vitro* in reactions that contained mutant CRP. The results of this study show that mutational modification of CRP structure is sufficient for *lacP* activation *in vitro* and can account for cAMP-independent *lacP* activity *in vivo*.

0110 DNA SEQUENCES OF TISSUE SPECIFIC DNaseI HYPERSENSITIVE SITES IN THE T-CELL RECEPTOR. YASUHIRO HASHIMOTO#, Ethan Bier*, Mark I. Greene# and Allan M. Maxam*. #University of Pennsylvania, Philadelphia, PA 19104. *Dana-Farber Cancer Institute, Boston, MA 02115.

The T-cell receptor ß-chain gene has nuclease hypersensitive sites in T cells that do not appear in B cells. Conversely, the k immunoglobulin gene shows a known nuclease hypersensitive site at its enhancer element in B cells, but this site is absent in T-cells. The T cell hypersensitive site, like the B cell nuclease hypersensitive site is located in the intron separating the joining and constant region segments of each respective gene. Overall, the DNA sequences of ß -chain J-C intron do not show significant homology to the immunoglobulin J-C intron. However, the DNA sequences of a small region that includes the major T-cell nuclease hypersensitive site are found homologous to sequences of the immunoglobulin heavy and kappa chain nuclease hypersensitive sites at their enhancer elements. In particular, a seven nucleotide sequence (C-A-G-G-T-G-G) in the T-cell receptor nuclease hypersensitive site may be the potential protein binding site which is observed in the enhancer element of the immunoglobulin heavy chain J-C intron. This was demonstrated by *in vivo* DMS treatment and *in vivo* genomic sequence methods.

0111 THE POLYOMAVIRUS ENHANCER COMPRISES MULTIPLE FUNCTIONAL ELEMENTS, John A. Hassell, William J. Muller and Christopher R. Mueller, McGill University, Montreal, Quebec, Canada, H3A 2B4.

The polyomavirus (PyV) enhancer comprises at least three genetic elements. Individual elements function poorly or not at all to enhance transcription of marker genes, but pair-wise combinations of any two elements augment transcription nearly as well as all three elements together do. Each element contains either the SV40 or adenovirus enhancer core sequence within its borders as well as a common sequence that appears as an inverted repeat in two of the elements. Two of the enhancer elements have previously been defined as the auxiliary replication elements alpha (α) and beta (β). Either the α or β element must be juxtaposed next to the PyV core to form a functional origin for DNA replication. Like the enhancer elements, the auxiliary elements activate replication independent of orientation relative to the PyV core. By contrast to their effect on transcription, neither the individual auxiliary elements nor a combination of both elements can activate replication from a distance 200 base pairs removed from the late border of the core, or when placed at the early border of the core. We suggest a model for enhancer function which takes these observations into account.

0112 CIS-REGULATORY ELEMENTS INVOLVED IN MURINE ADENOSINE DEAMINASE (ADA) EXPRESSION. Diane E. Ingolia, Cho-Yau Yeung, Muayyad R. Al-Ubaidi, Hélène A. Bigo*, David A. Wright* and Rodney E. Kellems, Baylor College of Medicine and M.D. Anderson Hospital*, Houston, TX 77030.

Congenital abnormal ADA gene expression regulation in man is always associated with genetic hematopoietic dysfunctions. Mammalian ADA expression patterns show complex tissue-specific and developmentally stage-specific regulation. We have isolated 46 overlapping cosmid clones containing the entire 29 kb murine ADA gene and ~15 kb each of 5' and 3' flanking sequences from an ADA gene amplification mutant genomic library. Murine ADA mRNAs were found by S1 mapping and primer-extension techniques to initiate at 5 major start sites located within 94 to 162 nucleotides upstream of the initiating ATG codon. The ADA promoter region contains no CAT or TATA boxes, is extremely G-C rich and displays two sequence motifs reputed to be binding sites for the transcription initiation factor SP1. Either 0.25 kb or 2.5 kb of upstream sequence flanking the ADA start codon was ligated to the chloramphenicol acetyltransferase (CAT) gene and introduced into cultured human cells or Xenopus oocytes for promoter enhancing function studies. In both bioassays, the 2.5 kb promoter fragment enhanced CAT expression by more than 40 fold over the construct containing only 0.25 kb of 5' flanking sequences. Deletion mapping to define a possible tissue-specific enhancer is currently underway.

0113 ENHANCER SEQUENCES AND SPECIFIC BINDING PROTEINS IN THE PROMOTER OF THE MOUSE H-2K^b GENE - A. Israël, A. Kimura, J. Kanellopoulos, O. Yano and P. Kourilsky - Institut Pasteur - 75724 Paris Cédex 15 - France.

Several potentially important regions have been defined in the promoter of the H-2K^b gene. Two of them, sequence A (-159 to -193) and sequence B (-61 to -120) display enhancer activity in mouse fibroblasts but not in undifferentiated teratocarcinoma cells, in which H-2 genes are not expressed.
A consensus interferon response sequence (IRS : -137 to -165) overlaps with enhancer A and is necessary for transcriptional stimulation by type I interferon : this IRS functions only when associated with a functional enhancer A sequence and potentiates its action in the presence of interferon.
In a nuclear extract derived from mouse fibroblasts, we have shown the presence of factors that specifically bind to enhancer A and IRS sequences, even in the absence of interferon in the medium. The binding of these two factors is cooperative, i.e. the IRS site can be occupied only if the enhancer factor is already bound to its site.

0114

TRANSCRIPTIONAL REGULATION OF HUMAN FACTOR IX GENE
Larry Gelbert, Mary Ann Shallcross, Rajinder Kaul and Pudur Jagadeeswaran
Center for Genetics, University Of Ilinois at Chicago, College of Medicine, 808 South Wood Street, Chicago, Il 60612

We have been interested in molecular genetics of blood clotting factors. As a first step we have isolated the entire human factor IX gene. In order to localize the promoter elements we have cloned the 5' flanking region into CAT vectors and analyzed the sequences responsible for promoter activity. The characterization of the promoter will be presented.

We have also identified ectopic expression of factor IX transcripts in a lymphoblastoid cell line GM 1416B. We are currently studying the factors responsible for activation of the factor IX gene transcription. The identification of such factors by the band competition assay will be discussed.

0115 AD12 E1A PROMOTER: A CONTROL ELEMENT WITHIN THE ITR IS FUNCTIONALLY REPLACED BY HAMSTER DNA, Inge Kruczek and Sabine Domrath, Institut für Biochemie der Universität, D-8000 München 2, FRG.

A hamster-Ad12 DNA junction molecularly cloned from an Ad12 induced tumor line lacks the leftmost 174 bp of the Ad12 genome, yet Ad12 E1A is transcribed in these cells. We thus tested the ability of the flanking hamster DNA to functionally replace the deleted part of the E1A promoter. The promoter activity of hamster-E1A was compared to that of the wt Ad12 E1A promoter and of a deletion promoter lacking the leftmost 152 bp using the CAT transient expression assay after transfection into different cell lines. The deletion of the leftmost 152 bp reduced E1A promoter activity between 1%-70%, depending on the cell line. The presence of Ad12 E1A gene products did not restore the reduced activity. The hamster-E1A promoter showed 40%-150% of wt activity. A detailed quantitative analysis will be presented.

Upstream of the hamster transcriptional control sequences (HTC) a class III gene coding for the 4.5S RNA was found. Southern analysis of hamster genomic DNA showed that the HTC homologous DNA is arranged in clustered repeats and are probably all flanked by the class III gene.Experiments to test the influence of the adjacent class III gene on the E1A promoter are in progress.

Finally, using an appropriate subclone of a hamster gene library as probe in Northern analysis, we found, in addition to the 4.5S RNA, a 3.5 kb mRNA transcribed closely to the HTC homologous DNA in 2 hamster cell lines.

0116 TRANSCRIPTIONAL CONTROL ELEMENTS OF A MAMMALIAN GENE REGULATABLE BY CALCIUM IONOPHORE, GLUCOSE AND A TEMPERATURE-SENSITIVE MUTATION. Amy S. Lee, Augustine Y. Lin, Cindy S. Chang, Janet W. Attenello, Elpidio Resendez, Jr. Department of Biochemistry, University of Southern California, School of Medicine, Los Angeles, CA 90033. Our laboratory has been interested in the transcriptional regulation of two cellular genes, p3C5 and p4A3, that are highly inducible in a variety of mammalian cell types by the calcium ionophore A23187, and by glucose starvation. We have isolated from the rat genome the p3C5 gene and examined its regulatory/promoter sequences. After induction by A23187, this promoter was 10 to 100 fold more active than the SV40 early promoter. Within this regulatory/promoter region, a DNA fragment immediately 5' to the TATA sequence with enhancer-like properties was identified. This 291 nucleotide fragment acted in cis to enhance expression of a heterologous fusion gene in an orientation independent manner. In addition, this fragment could confer A23187 inducibility to the heterologous gene and effectively compete for positive regulatory factors involved in A23187. When transfected into the ts mutant cell line K12, this fragment could also compete for the factor involved in the temperature induction of this gene. Sequence analysis of this promoter revealed homology with viral core enhancer sequences and the apparent organization of direct repeat domains similar to those observed in viral enhancers. Deletion analysis of the entire promoter region suggested the existence of both positive and negative regulatory elements which influenced the basal level transcription and specific induction of this gene.

0117 CHARACTERIZATION OF THE BINDING SITES OF TRANS-ACTING FACTORS WITHIN THE IMMUNOGLOBULIN HEAVY CHAIN AND KAPPA LIGHT CHAIN GENES. Michael J. Lenardo, Ranjan Sen and David Baltimore, Whitehead Institute for Biomedical Research, Cambridge, MA 02142

During the differentiation of B lymphocytes, the genes for the heavy and light immunoglobulin chains are transcriptionally activated at the pre-B and B cell stages, respectively. Important elements for tissue-specific expression are regions of 300-400 bp within the J-C introns of the heavy chain and kappa light chain genes. These regions contain several conserved sequence motifs, in particular, multiple copies of a conserved octonucleotide 5' CAGGTGGC 3' which has been shown to interact with a factor in B cells. Further studies have revealed at least two different nuclear factors which bind to these and other sites within the enhancer regions of the heavy chain and kappa light chain genes (R. Sen, unpublished results). We have carried out a systematic mutagenesis of the conserved binding sites to establish a correlation between enhancer activity in a transient cell transfection assay and the binding of nuclear factors to various mutant enhancer restriction fragments. These studies reveal an internal structure to the enhancer region and suggest mechanisms by which it responds to trans-acting factors.

0118 IDENTIFICATION OF A LENS-SPECIFIC ENHANCER ELEMENT IN THE MOUSE GAMMA-2 CRYSTALLIN GENE. Si Lok[1,2], Wendy Stevens[1], Martin Breitman[2,3], Reynold Gold[2] and Lap-Chee Tsui[1,2]. [1]Dept. of Genetics, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada, M5G 1X8. [2]Dept. of Medical Genetics, University of Toronto. [3]Dept. of Research, Mount Sinai Hospital, Toronto.

Crystallins, the major structural proteins of the vertebrate eye lens are encoded by several multigene families. Their expression is highly lens-specific and is differentially regulated during lens development. We previously demonstrated,byDNA transfection experiments, that the mouse γ 2-crystallin promoter is active in chicken lens epithelial explants, but is inactive in a variety of nonlens cell cultures, including mouse L cells, monkey COS-1 cells and human 293 cells. Here we report that the crystallin promoter contains an enhancer-like element between positions -31 and -226 with respect to the transcription start site. This DNA sequence when inserted in either orientation upstream of the enhancer-less SV40 early promoter activates transcription from this promoter in lens explant cultures In addition, deletion analysis of the crystallin promoter region revealed that sequences required for optimal expression can be separated into two domains, one extending from nucleotide positions +45 to -105 and the other from -125 to -190. Both domains appear to be required for the lens-specific activity of the γ2-crystallin promoter. Further analyses are underway to identify the critical sequences involved.

0119 Analysis of the Human Autosomal Phosphoglycerate Kinase Gene Promoter, John R. McCarrey, Beckman Research Institute of the City of Hope, Duarte, CA 91010
The mammalian autosomal phosphoglycerate kinase gene (PGK-2) is a tissue-specific gene expressed only in the late stages of spermatogenesis. This is in contrast to the X-linked PGK-1 gene which is expressed as a housekeeping gene in all somatic cells and premeiotic germ cells. The promoter sequence of the autosomal PGK-2 gene contains no TATA-box, but does have a single GC-box at -55 to -45. In addition, a single CAAT-box is located 65 bases upstream from the GC-box, and a single transcriptional start site has been identified. The promoter of the X-linked PGK-1 gene is also reported to contain no TATA-box (Singer-Sam et al., Gene 32, 409-417), but does have two GC-boxes and two CAAT-boxes arranged as overlapping pairs similar to the single pair found in the PGK-2 promoter. Multiple transcriptional start sites have been reported for the PGK-1 gene. The correlation between a single CAAT/GC-box pair and a single start site for the PGK-2 gene versus multiple CAAT/GC-box pairs and multiple start sites for the PGK-1 gene suggests that CAAT/GC-box pairs may act in lieu of a TATA-box to initiate transcription in these genes. Neither GC-box in the promoter of the PGK-1 housekeeping gene is a member of the consensus decamer enhancer sequence reported by Kadonaga et al. (TIBS, in press) to be required for binding of the Sp1 transcription factor. The fact that the GC-box in the promoter of the tissue-specific PGK-2 gene does conform to the Sp1 binding sequence may represent an important difference leading to the significantly higher (>50x) level of expression of this gene over that from the PGK-1 gene, and may also contribute to the tissue-specific nature of the PGK-2 promoter as compared to the housekeeping nature of the PGK-1 promoter.

0120 **FACTORS REQUIRED FOR REGULATION OF TRANSCRIPTION BY GLUCOCORTICOIDS**, David D. Moore and P. Reed Larsen, Department of Molecular Biology, Massachusetts General Hospital, and Brigham and Women's Hospital, Harvard Medical School, Boston, Ma. 02114

Competition experiments have shown that several constitutive viral enhancers increase transcription rates via a common factor (or factors). We have used this approach to show that the glucocorticoid dependent expression directed by the mouse mammary tumor virus long terminal repeat requires a factor that is strongly bound by an enhancer containing fragment from the Rous Sarcoma virus long terminal repeat. The RSV promoter is not responsive to glucocorticoids, and the enhancer containing fragment does not bind to the glucocorticoid receptor in vitro. The factor identified by these experiments is, therefore, required to mediate the induction of transcription directed by the binding of receptor, and could be a basic element generally required to mediate enhancer action.

0121 REGULATION OF RAT INSULIN 1 GENE EXPRESSION: EVIDENCE FOR NEGATIVE REGULATION IN NON-PANCREATIC CELLS. U. Nir, M.D. Walker & W.J. Rutter. Hormone Research Institute University of California, San Francisco, CA 94143-0534

Two cis-acting elements, the enhancer and the promoter, independently contribute to the tissue-specific expression of rat insulin 1 (rINS1) gene (Edlund et al., Science Nov 1985). Intracellular competition experiments carried out with fibroblasts (COS-7 cells) suggest the presence of negative factor(s) that down regulate the enhancer activity in these cells. In these experiments the test plasmid contained the chloramphenicol acetyltransferase (CAT) gene under the control of the thymidine kinase promoter and the insulin enhancer; the competitor plasmid contained the SV40 virus origin of replication to permit replication in COS cells. The presence of insulin enhancer sequences in the competitor replicating plasmid led to 5-6 fold increases in CAT activity as compared with activity detected when insulin enhancer was absent from either the competitor plasmid or from the test plasmid. Similar effects were seen when the rat amylase enhancer was present on the competitor plasmid but no effect was seen with the SV40 enhancer. Thus the specificity of binding of the putative repressor appears to be less stringent than the factor binding to the enhancer. Efficient derepression requires 5' rINS1 DNA sequences from both the rINS1 enhancer and the promoter regions. However sequences upstream of nucleotide -219 do not seem to play a key role, implying that the binding site does not appear to be congruent with the enhancer sequences. We propose that the activity of the rINS1 enhancer is modulated by negative factor(s) which are present in all cells and by dominant positive trans-acting factor(s) present in insulin-producing cells.

0122 MULTIPLE COPIES OF AN SV40 ENHANCER "CORE-17MER" ELEMENT CONFER TRANSCRIPTIONAL ACTIVATION. Brian Ondek and Winship Herr, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

We have previously reported the structure of 18 revertants of the SV40 enhancer mutant dpm12 (Herr and Gluzman, Nature 313, 711). This mutant contains one copy of the 72 bp element and has double point mutations (dpm) within each of two different 8 bp alternating purine/pyrimidine (PU/PY) sequences. These mutations decrease enhancer function and SV40 virus viability. Each revertant contains a simple tandem duplication within the mutated enhancer region ranging in size from 45 to 135 bp. A 15 bp sequence spanning the consensus "core" element (GTGGAAAG) is duplicated in each of the 18 revertants.

We have tested whether this conserved region can confer enhancer activity in the absence of other enhancer sequences; specifically the 'PU/PY' elements. We synthesized a 17 bp oligonucleotide (GGGTGTGGAAAGTCCCC) containing 14 bp (underlined) of the 15 bp sequence that is duplicated in each of the dpm12 revertants. Four and seven tandem copies of this 'core-17mer' were assayed for transcriptional activition of the human β-globin gene in HeLa cells. Four tandem copies of the 'core-17mer' yielded similar β-globin RNA levels as those produced by the SV40 enhancer with a single 72 bp element. Seven 'core-17mers' contains 75-85% the enhancer activity of the wild-type SV40 enhancer. Similar results were observed in CV-1 cells. We are presently testing another SV40 enhancer domain defined by revertant analysis of a core mutant.

0123 Studies on a cholesterol regulated promoter in animal cells.
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We are interested in the role of negative feedback regulation in the maintenance of cholesterol homeostasis. To this end we are studying the process whereby excess intracellular cholesterol inhibits transcription of the gene encoding 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the enzyme that catalyzes the rate limiting step in the biosynthesis of cholesterol. We have already demonstrated that transcriptional inhibition in vivo is a property of the 5' flanking region of this gene. Using in vitro transcription and foot printing assays, we have now defined specific and unique DNA-protein interactions in the 5' flanking-promoter region that are essential to basal promoter activity. The relationships between the different DNA-protein interactions and the mechanism of cholesterol mediated suppression of transcription will be discussed.

0124 TRANSCRIPTIONAL REGULATION OF THE ENDO B CYTOKERATIN IN DIFFERENTIATING MURINE EMBRYONAL CARCINOMA CELLS, Robert G. Oshima*, Stuart Leff, and Michael G. Rosenfeld, La Jolla Cancer Research Foundation, La Jolla, CA 92037, and University of California, San Diego, CA 92037

Endo B is a type I cytokeratin that is first expressed at approximately the 4-8 cell stage of preimplantation mouse embryos and in extraembryonic endodermal derivatives of differentiating murine embryonal carcinoma (EC) cells. Nuclear run on assays of nuclei derived from F9 EC cells and F9 cells which had been induced to differentiate by exposure to retinoic acid indicate that the Endo B gene is transcribed at a low level in F9 cells and at a higher level in induced cells. A combination of both transcriptional and post-transcriptional regulation may account for the very large difference in the steady state levels of the Endo B mRNA in the two cells types. In contrast the murine CGRP (calcitonin gene related peptide) gene is transcribed approximately equally in both the stem cell and its retinoic acid induced derivatives. However, the CGRP mRNA was undetectable by Northern analysis in the differentiated cell population indicating that post-transcriptional regulatory mechanisms also contribute to differential gene expression in this system.

0125 IDENTIFICATION OF A CELLULAR TRANSCRIPTION FACTOR INVOLVED IN E1A INDUCTION
R. Reichel, I. Kovesdi, and J.R. Nevins, The Rockefeller University, New York, N.Y. 10021

The mechanism for transcriptional induction by the adenovirus E1A gene has been investigated with an in vivo exoIII mapping technique to assay for proteins that interact with an E1A-inducible promoter. A protein bound to the early E2 promoter was detected in wild-type infected cells. In the absence of E1A induction, specific interactions at the promoter could not be detected. However, if conditions were established that allowed transcription of the E2 gene in the absence of E1A, the same exoIII protection was observed as was found in the presence of E1A. Furthermore, we have employed a gel-retardation assay for the detection of the transcription factor in nuclear extracts of adenovirus infected cells that interacts with the E2 promoter. After titration of non-specific binding proteins, the assay allowed the detection of a protein that retarded the migration of a DNA fragment containing the E2 promoter. E2 promoter sequences deleted to -70 could compete for binding, whereas a -28 deletion could not. Footprint analysis revealed a protection from DNase cleavage between -33 and -74. Within this region there was cleavage at -55 suggesting two seperate binding domains. The factor could be detected in extracts of uninfected cells, although at greatly reduced levels, as assayed by a sensitive exoIII mapping technique. These results suggest that the E2 binding activity is a cellular transcription factor whose concentration or binding activity increases as a result of adenovirus infection.

0126 A TRANSCRIPTIONAL ROLE FOR THE NONTRANSCRIBED SPACER OF RAT RIBOSOMAL DNA. Lawrence I. Rothblum, Brandt G. Cassidy, and Hsin-Feng Yang-Yen. Dept. of Pharmacology, Baylor College of Medicine, Houston, Texas 77030.

The nontranscribed spacer (NTS) of rat rDNA contains at least one region that enhanced the rate of transcription in vitro. A 3.4 kb segment (contained in clone p3.4) of the 5' NTS (-287 to -3.7) was attached to a 5.1 kb fragment (p5.1) extending from -287 to +4.9, yielding a new clone designated p8.5. p8.5 was transcribed up to 15 times more efficiently in vitro than templates without the NTS segment attached. When the orientation of the NTS and the promoter was reversed, the enhanced rate of transcription was maintained. Transcription of a series of double-digested templates mapped the region responsible for this affect to between -1000 and -300. To test for the formation of stable complex between the enhancing factor and the DNA, an order-of-addition experiment was carried out. Preincubating cell extracts with the NTS (p3.4) reduced transcription of p8.5 60%. Preincubation with pBR322 or adding p3.4 after preincubation with p8.5 had no affect on transcription. We attempted to visualize the DNA-protein complex using the gel electrophoretic method. A radiolabelled Hinf 1 digest of p3.4 was preincubated with nuclear extracts of rat Novikoff hepatoma ascites cells, in the presence of a 300-1000 fold excess of pBR322, and electrophoresed on a 5% acrylamide gel. The mobility of one Hinf 1 fragment was retarded. This mobility shift was sensitive to protease K or cold p3.4 as competitor. The fragment that was retarded included that region identified as the enhancer. These data suggest that a trans-acting factor forms a complex on sequences found in NTS, and this complex can modulate the efficiency of ribosomal RNA synthesis. These studies were supported by Cancer Research Center Grant, CA10893,P4.

0127 REGULATION FACTORS IN THE IMMEDIATE EARLY TRANSCRIPTION OF HUMAN CYTOMEGALOVIRUS. R. Rüger, J. Stoerker, A. Gmeiner and B. Fleckenstein. Institut für Klinische Virologie, Universität Erlangen-Nürnberg, West-Germany.

A very strong enhancer element was identified in the upstream region of the dominant immediate early (IE) gene of human cytomegalovirus (HCMV) (Boshart et al., 1985). A series of deletion mutants from the enhancer sequence was cloned upstream of CAT to test relative strength. IE genes of HCMV stimulate transcription of the native and some foreign promoters. Cotransfection experiments with cloned IE genes of HCMV and enhancer/CAT constructs showed a trans-activating effect of an IE gene on a specific region defined by the deletion mutants from the enhancer region. DNA binding assays with extracts from HCMV infected cells have indicated specific interactions with IE proteins not seen under early conditions or with uninfected cells. Footprinting experiments with Exo III, DMS and DNaseI protocols have identified the parts of DNA-protein binding. A correlation is made between the sites of binding and trans-activation seen in the CAT assays.

Reference: Boshart, M., Weber, F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B. and Schaffner, W. (1985). A Very Strong Enhancer is Located Upstream of an Immediate Early Gene of Human Cytomegalovirus, Cell, 41, 521-530.

0128

TISSUE SPECIFICITY OF TRANSCRIPTION FROM THE HUMAN $^{G}\gamma$- AND β-GLOBIN GENE PROMOTERS

Tim Rutherford and A.W.Nienhuis Clinical Hematology Branch, NHLBI, NIH, Bethesda MD 20205

We have assayed human globin gene promoter function by using the promoters to drive the neo gene, and measuring their ability to generate G418 resistant colonies after transfection into different cell lines. Promoter sequences between -380 and +20 of the cap site were sufficient for tissue specific expression. Thus in human K562 erythroleukemia cells, which express the endogenous γ but not the β genes, the $^{G}\gamma$ promoter but not the β promoter will allow colony formation. In murine erythroleukemia (MEL) cells the β promoter allows more efficient expression than the γ promoter. Neither promoter functioned efficiently in a variety of non-erythroid cell lines. We are using this system to further study trans- regulation of human globin promoter function.

0129 EXPRESSION OF STEROID-RESPONSIVE GENES IN PRIMARY OVIDUCT CELL CULTURES, Michel M. Sanders and G. Stanley McKnight, Dept. of Pharmacology, University of Washington, Seattle, WA, 98195

Four classes of steroid hormones, estrogens, androgens, glucocorticoids and progestins, increase the transcriptional activity of the chicken egg white genes when given *in vivo* . To examine the mechanisms whereby steroids regulate the expression of these genes, we have established a primary oviduct cell culture system in which the genes for ovalbumin and transferrin are induced by these steroids. Surprisingly, the induction of the ovalbumin gene requires a second steroid, which is probably corticosterone *in vivo,* and insulin in addition to estrogen to obtain a physiologically relevant increase in ovalbumin mRNA ($mRNA_{ov}$). When estradiol, corticosterone and insulin are included in the culture medium, a 200-fold increase in $mRNA_{ov}$ occurs during the first two days, representing about a 20-fold increase in the transcription rate and a 10-fold increase in the stability of the mRNA. The steroid response elements in the ovalbumin and transferrin genes are being mapped by transfecting mutated genes into the cultured cells. The promoter and 5'-flanking regions from the ovalbumin (-1342 to +7), lysozyme (-208 to + 15), and mouse mammary tumor virus (MMTV, -630 to +120) genes have been fused to the structural gene for chloramphenicol acetyl transferase (CAT). Expression of the transfected DNA is assessed by an assay based on the enzymatic activity of the CAT protein. With estrogen and corticosterone in the medium, about a 20-fold increase in CAT activity is observed with the ovalbumin construct, a 12-fold increase with the lysozyme construct and a 40-fold increase with the MMTV construct. Thus, steroid-responsive genes can be transfected into primary oviduct cells and regulated by steroids. In addition, the increase in CAT activity of the ovalbumin construct is comparable to the increase in transcriptional activity observed with the endogenous ovalbumin gene. Deletions in the ovalbumin and transferrin 5'-flanking regions are currently being tested to determine which sequences are responsible for regulation by each class of steroids.

0130 GENE SPECIFICITY OF THE HUMAN TRANSCRIPTION FACTOR USF. Michele Sawadogo and Robert G. Roeder, The Rockefeller University, New York, NY 10021

Maximum expression of the major late promoter of adenovirus in vitro requires a cellular gene-specific transcription factor (USF) in addition to the general transcription factors TFIIB, TFIID, TFIIE and RNA polymerase II (1). We showed that USF interacts specifically with an upstrem element in the ML promoter and that this interaction is stabilized by - and in turn stabilizes - the interaction of TFIID on the TATA box (1). Using deletion and point mutations in the ML promoter, we have defined a minimum sequence requirement for USF binding (2). We are now analyzing the possible involvement of USF in the transcription of other cellular and viral genes as well as pursuing the purification and characterization of the USF protein.

(1) Sawadogo, M. and Roeder, R.G. (1985) Cell, in press.
(2) Sawadogo, M. and Roeder, R.G., in Cancer Cells, vol 4, in press.

0131 ASSAYS FOR THE STUDY OF REGULATORY FACTORS INTERACTING WITH THE MOUSE METALLOTHIONEIN I GENE. Carl Seguin* and Dean H. Hamer. Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 and *Laboratory of Molecular Endocrinology, Laval University Medical Center, Quebec, Que, Canada GIV 4G2

Metallothionein (MT's) are small cysteine-rich proteins that bind to and are induced by heavy metals such as zinc, cadmuim and copper. Mutational analysis shows that the upstream flanking DNA contain multiple heavy metal control elements with a conserved DNA sequence that may act as a core recognition site for regulatory factors. We recently reported the detection of cellular factors involved in the cadmium induction of the mouse MT-1 gene by an in vivo competition assay and we showed that at least one class of the cellular factors acts by a positive regulatory mechanism depending on the same region of the 5^1 flanking DNA required for maximal transcription. In an attempt to locate the boundaries of regulatory protein-binding sites on the mouse MT-1 gene, we used an exonuclease III (EXO III) protection assay. We show that a crude mouse L cell nuclear extract can protect against EXO III digestion regions of the 5' flanking DNA required for proper expression of the gene.

0132 INTERACTION OF TISSUE-SPECIFIC AND NON-SPECIFIC FACTORS WITH THE IMMUNOGLOBULIN ENHANCERS, Ranjan Sen, Harinder Singh, Philip Sharp and David Baltimore, Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge, MA 02142

One of the three lymphocyte-specific regulatory elements present in an immunoglobulin gene is the enhancer. We had observed a tissue specific suppression of in vitro transcription from templates carrying the immunoglobulin enhancers. To explain this phenomenon, we have used a binding assay to detect proteins that interact with these sequences. Two tissue non-specific proteins have been identified in nuclear extracts that bind to sequence motifs in the μ enhancer. One of these proteins interacts with the κ enhancer as well; thus there is at least one common protein interacting with the μ and κ enhancers. The binding sites of these factors have been mapped using the technique of methylation interference, and coincide closely with those identified in vivo. A third factor has been identified that binds only to a sequence in the κ enhancer. Examination of extracts made from a variety of cell lines indicates that this factor is not only tissue specific (B cells), but also stage specific within that lineage (it is not found in pre-B cells where κ is not expressed, but found in B cells and myelomas where κ is expressed).

0133 TRANSCRIPTIONAL REGULATION OF THE TISSUE-SPECIFIC CHICKEN HISTONE H5 GENE, M.F. Shannon, P. Wigley, J. Scott and J.R.E. Wells, University of Adelaide, Adelaide, S.A. 5001.

The linker histone H5 protein is found only in erythroid cells of birds, fish, amphibians and reptiles. Increasing levels of H5 during erythroid cell maturation is correlated with chromatin condensation and a shutdown of replication and transcription. The chicken H5 gene has been isolated and sequenced in this laboratory.

The transcriptional regulation of the gene will depend on at least two factors: specific sequences within the H5 gene and trans-acting molecules which would interact with these sequences and both of these are being investigated with a number of approaches: (a) Construction of appropriate mutations in the H5 gene, followed by transfection into erythroid and non-erythroid cells in culture is helping to define gene sequences which control tissue-specific expression. (b) Trans-acting factors involved in H5 gene expression are being investigated using the Xenopus oocyte system. Chromatin wash fractions from a transformed erythroblast cell line (actively expressing H5) have been shown to substantially stimulate H5 transcription when introduced into oocytes with the H5 gene.

Current work involves the use of 'footprinting' to locate the binding sites of the trans-acting molecules. Purification of these molecules is also being undertaken.

Transcriptional Regulation and RNA Processing I

0134 A MUTANT RNA POLII AFFECTS SPECIFICALLY THE TRANSCRIPTION OF MYOGENIC GENES, E. Arpaia, T. Smith, M. Pearson, E. I. Du Pont de Nemours & Co., Inc, Experimental Station, E328/138, Wilmington, DE 19898

In rat L6 myogenic cells, α-amanitin selects for at least three classes of resistant mutants carrying a lesion in their RNA polII. These mutants are designated as Myo^+, Myo^- or conditional Myo with reference to the maintenance or loss of the myogenic differentiation phenotype in the presence of α-amanitin. We are in the process of characterizing the molecular nature of the myogenic defect in Ama27, a representative of the conditional Myo class of mutants. We have been able to show that in Ama27 cells the mutant RNA polII affects selectively the transcription of myogenic marker genes encoding α- and β-actin, creatine kinase, and myosin heavy chain, without altering the transcription of constitutive "housekeeping" genes, such as glyceraldehyde dehydrogenase, α-tubulin and vimentin. We have also constructed a complete genomic library of Ama27 DNA in λEMBL3. This library will be screened for the mutant RNA polII rat gene with three different hybridization probes, two Drosophila probes covering the coding region of the large subunit of RNA polII and a CHO probe cross-hybridizing to one of the Drosophila probes. In this manner, we hope to isolate genomic sequences coding for Ama27 RNA polII carrying the lesion responsible for the α-amanitin resistant phenotype. These sequences will then be used in transfection experiments into WT L6 myoblast cells in order to demonstrate conclusively that the mutated purified RNA polII gene is responsible for the conditional Myo phenotype. This mutant gene should be useful in analyzing the molecular interactions responsible for the selective expression of muscle-specific genes during development.

0135 Stable repression of a Gal-K gene in Hamster cells by expression of antisense RNA

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*Univ. of Pennsylvania ; and +Dept. of Molecular Genetics, Smith Kline and French Labs, Philadelphia PA

We describe here the complete and stable repression of a specific gene in cultured hamster cells by placing an antisense transcription unit into a BPV expression vector. Gal-K $^{-}$ R1610 hamster cells were stably transformed to the gal-K $^{+}$ phenotype by transfection with an *E. coli* gal-K gene (under the control of the SV40 promoter). A BPV vector carrying an antisense gal-K transcription unit and a neomycin resistence gene was introduced into these cells under G418 selection. Transfectants were cloned and the gal-K protein was quantified enzymatically and immunologically. In 5 clones galactokinase was undetectable (< 5% of gal-K activity in parental cell lines); however, in one clone ,activity was reduced by 60%. By northern analysis this latter clone contains a relatively low level of antisense RNA. Quantitation of sense and antisense RNAs,their localization and characterization of gal-K DNA sequences are in progress.

0136 EXPRESSION OF THE MUSCLE CREATINE KINASE GENE IN MUTANT AND WILD TYPE MYOGENIC CELLS

Pamela Benfield, David Graf, Peter Korolkoff and Mark L. Pearson

Muscle creatine kinase gene expression is induced as differentiating muscle cells withdraw from the cell cycle and fuse to form multinucleate myotubes. Transcription from this locus is selectively impaired in rat L6 myoblasts containing mutations in RNA Polymerase II that confer alpha amanitin resistance on these cells. We have shown that both the timing and levels of ckm mRNA production can be modified by the presence of such RNA Polymerase II mutations. Transfer of alpha amanitin resistant DNA from mutant to wild type L6 cells results in the transfer of this transcriptional defect. Furthermore, certain transformants produce abnormal creatine kinase message suggestive of mRNA processing defects. In order to examine the regulation of muscle creatine kinase gene transcription in mechanistic detail we have isolated and characterized genomic clones for rat muscle creatine kinase. This single copy gene is structurally homologous to the gene encoding the brain isoform of creatine kinase that has also been isolated in our laboratory. We have begun to examine the regulation of this gene using gene transfer and in vitro transcription technology.

0137 EXPRESSION OF MULTIPLE PROTEIN PRODUCTS CODED BY POLYCISTRONIC MESSAGES IN TRANSIENTLY AND STABLY TRANSFECTED MAMMALIAN CELLS, K.L. Berkner+, S. Yarnold+, M. Insley+, and E. Boel*, +ZymoGenetics, Seattle, WA 98199, *Novo Industries, Copenhagen, Denmark

The ability of mammalian cells to translate multiple products from polycistronic mRNA has been tested by juxtaposing different cDNAs behind a single promoter sequence - the adenovirus 2 major late promoter (Ad2 MLP). Constructions that generate dicistronic mRNA encoding chloramphenical acetyltransferase (CAT) followed by dihydrofolate reductase (DHFR) efficiently express both proteins. Expression has been detected transiently in COS and BHK cells, and in stably transfected BHK cells. Efficient expression of the downstream cDNA (DHFR) occurs even with an in vitro mutagenized CAT cDNA that contains an optimized translational signal immediately upstream of the initiation methionine. Southern analysis has been performed on a number of the stably transfected BHK cell lines. Several copies are integrated per genome, with no evidence for rearrangement of the transcriptional unit. Northern and S1 analysis of RNAs present in transiently or permanently-expressing cells detects only a polycistronic message, initiating from the Ad2 MLP. Thus, translation of the internal cistrons appears to be a consequence of initiation at internal methionines, rather than to the rearrangement of transcriptional sequences or internal splicing events.

A construction that generates a tricistronic message encoding factor IX, CAT and DHFR, in that order, has also been positioned behind the Ad2 MLP. The effect of location within the polycistronic unit on the relative efficiency of DHFR expression, as well as the effect of sequence context around the initiation methionines in upstream cistrons, will be discussed.

0138 THE STRUCTURE AND REGULATION OF THE ACTIN 5C GENE OF DROSOPHILA, Beverley J. Bond and Norman Davidson, California Institute of Technology, Pasadena, CA 91125

At least six developmentally regulated mRNAs are made from the Drosophila actin 5C gene. We have examined the structure of these RNAs in detail and have determined that they are heterogeneous at both their 5' and 3' ends. At the 5' end there are two nonhomologous leader exons which are alternately spliced to the remainder of the gene. At the 3' end of the gene there are three different sites of polyadenylation. This 3' variation is the principal cause of the length diversity observed in the transcripts. Our data indicate that in whole animal RNA the two leader exons are used with approximately equal probability in all stages of development and with all three polyadenylation sites. There is some developmental variability in the use of the three polyadenylation sites. We have begun to study the sequences necessary for the initiation of transcription from each start site. We have made fusions between putative actin 5C promoter sequences and the bacterial CAT gene and tested these by transient transformation experiments in the Kc line of Drosophila cultured cells. We have found that there is a promoter upstream of the first leader exon and have begun to narrow down the sequences necessary for efficient expression from this promoter. Our current experiments are aimed at determining whether the two start sites each have independent promoters or whether they share common regulatory sequences.

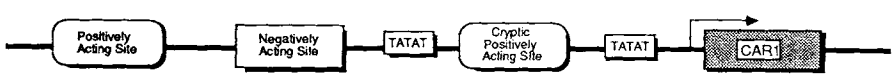
0139 TRANSCRIPTION AND REGULATION OF THE δ-CRYSTALLIN GENES. T. Borras, E. F. Wawrousek, D. Parker, J. Piatigorsky, National Institutes of Health, National Eye Institute, Bethesda, MD 20892.

There are two δ-crystallin genes in the chicken (5'-δ1-δ2-3') which are expressed in the developing lens. The two genes are separated by 4 Kb of DNA and each contains 17 exons. We have made gene-specific oligonucleotide probes for δ1 and δ2. Only δ1 mRNA was found in the cytoplasm of the 15-day-old embryonic chicken lens. Current experiments suggest that the δ2 transcript is made in the lens but not processed to mature mRNA. We have analyzed the δ-crystallin promoters in primary cultures of cells derived from partially trypsinized lenses. Co-transfection with an RSV-β-Gal expression vector allowed standardization of the results. We found that 344 bp of 5' flanking sequence of the δ1 gene introduced into the pSVO-CAT expression vector promoted twice as much CAT activity as 345 bp of flanking sequence of the δ2 gene. These δ1 flanking sequences were 2.7 times as effective in the lens cells than in chicken fibroblasts. Interestingly, 601 bp of 5' flanking sequence of the δ1 gene was 3.1 times less efficient in the lens cells than the 344 bp of sequence. In fibroblasts, however, the larger δ1 promoter was only 1.5 times less effective than the smaller promoter. Our results indicate that the 5' flanking sequences of the δ1 gene contain positive and negative regulatory elements which both display lens-specific preference.

0140 TRIPARTITE CONTROL OF ARGINASE GENE TRANSCRIPTION IN S. cerevisiae, T.G. Cooper & R.A. Sumrada, University of Tennessee, Memphis, Tennessee 38163.

Expression of the S. cerevisiae CAR1 & CAR2 genes is subject to induction & repression. We have shown that expression is both positively & negatively controlled. Sites mediating this regulation are situated as shown in the figure below, which is based, in part, on the following observations. A C-G transversion at position -153 or a 13 bp deletion including this position resulted in constitutive expression of a CAR1-LACZ fusion, thereby defining a negatively acting site. Deletion of sequences 5' from position -219 had little effect on normal regulated expression of the fusion, whereas deletion of sequences 5' from position -190 resulted in total loss of expression, suggesting the presence of a positively acting UAS between positions -219 & -190. However, when deletions continued past position -153, the location of the putative negatively acting site, constitutive expression of the fusion was restored. This result suggested the presence of a second, cryptic UAS that functioned only when the negatively acting site was mutationally inactivated. Such a UAS was identified by a deletion covering positions -118 to -50. Fragments predicted to contain a UAS were cloned into an expression vector to directly demonstrate their ability to support gene activation.

MODEL ORGANIZATION OF THE CAR1 GENE



0141 CHARACTERIZATION OF THE MOUSE PREALBUMIN GENE
R. Costa and J.E. Darnell, Jr. The Rockefeller University, New York, N.Y. 10021

Prealbumin is a liver specific protein containing 127 amino acids and it is involved in the plasma transport of both vitamine A and thyroxine. This protein is encoded by a 700 base pair mRNA whose abundance in the liver during development is proportional to its transcription rate. The expression of the prealbumin gene was investigated in different tissues and it was found predominately in the liver with minor amounts of mRNA detectable in the kidney. A prealbumin cDNA was used to select a mouse genomic clone and the mRNA cap site was localized. To investigate liver specific gene expression, plasmids containing various amounts of DNA sequences upstream of the prealbumin 5' end were constructed to promote expression of a test gene. These constructions were transfected into either a mouse (BW1J) or human (HEPG2) hepatoma cell line to determine sequences involved in tissue specific gene expression. A DNA sequence comparison was made between several liver specific promoters to determine concerved sequences amoung these promoters.

0142 **NUCLEOTIDE SEQUENCE DETERMINING THE FIRST CLEAVAGE SITE IN THE PROCESSING OF MOUSE PRECURSOR RIBOSOMAL RNA.** **Nessly Craig, Susan Kass*, and Barbara Sollner-Webb*. University of Maryland Baltimore County, Catonsville, Maryland, 21228, *The Johns Hopkins University School of Medicine, Baltimore, Maryland, 21205.**

Previous work has shown that the first step in the processing of 45S precursor ribosomal RNA in mouse cells involves a cleavage at a site corresponding to residue ~+650 and that this processing is reproduced in an S-100 in vitro transcription reaction (Cell 27:165). Comparison of the mouse and human rDNA sequences showed that the region between residues +650 and +850 (mouse sequence; +414 to +614, human sequence) is highly conserved, while surrounding regions are not, suggesting that this region may be important. To test this hypothesis, we constructed systematic series of deletion mutants of this region from both the 5' and 3' directions and analyzed the processing of their transcripts in vitro. The 5' boundary of the region required for processing is sharp; it precisely corresponds to the in vivo/in vitro rRNA cleavage site which demarks the 5' end of the conserved DNA sequence. The 3' boundary is more gradual and is within the conserved DNA sequence region. 3' deletions extending up to residue +850 process about at control levels, progressive deletions extending to residues +723 cause a progressive decrease in the amount of processed RNA, and deletions to or beyond residues +691 eliminate processing. Our working hypothesis is that this region may represent a binding site for proteins involved in processing. Finally, we have found that the primary rRNA processing, unlike rDNA transcriptional initiation, is not highly species specific since the putative human rRNA processing site functions in the mouse cell extract. (Supported by a grant from the March of Dimes)

0143 CHARACTERIZATION AND TRANSGENIC EXPRESSION OF A HEAT INDUCIBLE STRESS GENE FROM SOYBEAN, Eva Czarnecka, Luis A. Mosquera, and William B. Gurley, Microbiology and Cell Science, University of Florida, Gainesville, FL 32611, Ronald T. Nagao and Joe L. Key, Botany, University of Georgia, Athens, GA 30602.

Soybean stress gene Gmhsp27a is a member of a multigene family comprised of 4 to 5 members encoding proteins of 27 kD. This gene is constitutively expressed in soybean, and is further induced (5- to 50-fold) by a variety of stresses including arsenite, heavy metals (Cd, Cu), auxin, polyamines, and others. Gmhsp27a has been sequenced and transcript mapped (S1 nuclease). A comparison of cDNA and genomic sequences indicates the presence of at least one intron. The 5' flanking region contains multiple TATA motifs each corresponding with a distinct 5' RNA terminus. This multipromoter structure seems complex and contains regions of homology with both the metal responsive element (MRE, 8 out of 12) of the mammalian metallothionein gene (Stuart et al., PNAS 81:7318) and the heat shock consensus element (HSE; 5 out of 10; Pelham, Cell 30:517). The relationship of these consensus homologies to transcriptional control in this soybean gene is unclear.

Transcriptional activity of the soybean Gmhsp27a has also been characterized in a heterologous expression system. The gene was transferred to sunflower cells via an Agrobacterium T-DNA-based vector system. Transgenic expression of Gmhsp27a is similar to that seen in soybean with the exception of a 3°C lowering of the temperature optimum and an elevation in basal transcription. Supported by Agrigenetics Research Associates, Ltd.

0144 Synthesis of recombinant human placental alkaline phosphase-specific antibodies

De Waele, P., Molemans, F., Van de Voorde, A. and Fiers, W.
Laboratory of Molecular Biology, State University of Ghent, Belgium

We previously constructed a murine hybridoma cell line (E6) secreting highly specific IgG2b, K class antibodies against hPLAP, an oncodevelopmental protein present on the cell surface of certain tumors and in sera of cancer patients. The mRNAs coding for the H and L chains of the E6 antibodies were isolated and cloned as cDNA copies, and full-length cDNA clones of both chains were obtained. Transient expression studies in COS cells with SV40-based vectors, more specifically by using the SV40 early promoter and small t splice signals and polyadenylation signals, could only demonstrate H-chain synthesis. An SP6-based transcription-coupled translation system confirmed H-chain synthesis but indicated a deletion of the L-chain, as a protein of only 14 Kd was immunoprecipitated instead of an expected 23 Kd protein; DNA-sequencing confirmed a 4-bp deletion. An SP6-based system for screening L-chain insertion mutants produced by oligonucleotide-directed mutagenesis was developed. Permanent expression studies by cotransfection with DHFR plasmids in CHO-DHFR-minus cells and with the 'neo' dominant selection marker for myelomas for the production of recombinant antibodies are under way.

0145 Myelin Proteolipid Expression in Normal and Jimpy Mouse Brain Development.

P.L. Deininger, M.V. Gardinier and W.B. Macklin
Dept. of Biochemistry and Molecular Biology, L.S.U. Medical Center, 1901 Perdido St., New Orleans, LA 70112

Myelin proteolipid mRNA is not expressed in the mouse brain until about 5 days after birth. By about 3 weeks in age it peaks in expression as one of the major brain mRNAs. Fairly strong expression of the gene does continue into the adult animal. Northern blot analysis shows two major RNA species of 3600 and 2500, with a minor species at 1500 nucleotides in length.

In the jimpy mouse stain, which is deficient in myelination, only very low levels of PLP mRNA are ever expressed. These levels drop off further prior to the death of the animal. Since low levels of apparently normal PLP protein can also be detected, the genetic defect only affects PLP expression and not structure. Our studies show the jimpy PLP mRNA to be 100 bases smaller than that from the normal mRNA which suggest the defect may be found directly with the jimpy PLP gene. Genetic characterizations are underway. This work was supported by NMMS grant RG1747 and PHS grant CA37673.

0146 THE ORIGIN OF REPLICATION AND EARLY PROMOTER OF BK VIRUS

Karen Deyerle and Suresh Subramani, U.C.S.D., La Jolla, Ca 92037

Papovaviruses (SV40, polyoma, BKV, JCV) have served as model systems for the study of viral transformation of mammalian cells and the cellular machinery involved in DNA replication and gene expression. While SV40 and polyoma have been studied extensively, little is known about the biology of the human papovaviruses BKV and JCV. Approximately 75% of the human population acquires antibodies against BKV at an early age. Following this initial inapparent infection, the virus persists in a latent form, and can be reactivated in immunosuppressed or immunodeficient individuals. Although the DNA sequences of SV40 and BKV are 70% homologous, the two viruses have different host ranges and oncogenic potentials. Interestingly, they share only 34-43% DNA sequence homology in their regulatory regions.

We are interested in the factors that determine the different host ranges of SV40 and BKV. Our initial studies have focused on the origin of replication and early promoter of BKV. A fragment of BKV spanning this region was cloned into the expression vector pSV2Acat in place of the SV40 ori-promoter. The BKV early promoter was defined by deletion analysis and measurement of CAT expression following transient transfections of CV1 cells. The early mRNA start sites were defined by S1 analysis and primer extension. The origin of replication was localized in both COS and L603 (human fibroblast) cells. The results indicate that the BKV early promoter and origin of replication show similarities but are not identical to analogous elements in polyoma and SV40.

0147 TRANSCRIPTIONAL ACTIVATION OF A POTENTIALLY NEW PROTO-ONCOGENE BY MMTV, Clive Dickson, Robert Moore, Mark Dixon, Sharon Brookes, Graham Casey and Gordon Peters, Imperial Cancer Research Fund, London, U.K.

Sixty percent of breast tumors induced by mouse mammary tumor virus (MMTV) show proviral insertions at a specific locus, int-2. Integrations at this locus occur over a range of 30kb and result in the transcriptional activation of the int-2 gene. Taken in concert, the proviral insertions fall into two clusters either side of the int-2 gene with viral transcription directed away from the cellular gene, suggesting that activation is the result of an enhancement-type mechanism. There are a few examples of tumors where the provirus appears to have integrated in a promoter insertion mode. DNA sequence analysis of the genomic locus and cDNA clones have revealed part of the exon structure for this potentially novel oncogene. Using DNA probes from the 3' exon, two major RNA species of 3.2 and 2.9kb were detected in the tumors. However, a 5' exon probe only detects the larger RNA species, indicating a potential for differential exon usage. Northern blots often detect additional, but less abundant, int-2 related RNAs of both larger and smaller size. To date, int-2 specific transcripts have been detected only in mouse mammary tumors. To elucidate the complex transcriptional control and processing of this gene we are continuing to isolate and sequence more cDNA clones and developing in vitro culture systems to facilitate a direct examination of the sequences necessary for expression.

0148 SPECIFIC α-TUBULIN GENE EXPRESSION IN TESTIS AND SKELETAL MUSCLE, Paul R. Dobner, Edward Kislauskis, and Lydia Villa-Komaroff[+], University of Massachusetts Medical Center, Worcester, Ma. 01605, [+]Children's Hospital Medical Center, Boston, Ma. 02115. We have isolated cDNA clones from a macaque testis cDNA library which encode a novel α-tubulin. Using a subcloned probe (MT5'), derived from an unique sequence at the 5' end of one of the cDNA clones, we have isolated the corresponding human gene, designated Hα44. The MT5' probe detects a 2.0 kb poly(A)$^+$ RNA which is testis-specific. In two previously characterized α-tubulin genes, the only coding capacity of the first exon is the initiator methionine codon, AUG. Hα44 contains a long (470 bp) 5' exon which contains a considerable extension of the α-tubulin open reading frame but does not contain an in phase AUG codon. Translation of synthetic, full-length RNAs, corresponding to the Hα44 2.0 kb RNA, in rabbit reticulocyte lysates, indicates that the 5' exon encodes a 14,000 dalton amino-terminal extension of the α-tubulin protein and that these RNAs are translated efficiently. Since there is no in phase AUG codon within this exon, it appears that the initiation of translation occurs at a triplet codon other than AUG. The 5' exon is present only once per haploid genome; surprisingly a probe derived from the 3' untranslated region of Hα44 detects 3 sequences in the human genome. This same probe detects the testis 2.0 kb transcript but in addition reveals two additional transcripts of 2.2 and 1.8 kb which are specifically expressed in skeletal muscle. Whether or not these transcripts arise from the Hα44 or a different gene(s) with related 3' untranslated regions is currently under investigation.

0149 **THE PRO-OPIOMELANOCORTIN GENE: SEQUENCE ELEMENTS RESPONSIBLE FOR TISSUE-SPECIFICITY AND GLUCOCORTICOID INHIBITION OF TRANSCRIPTION,** Jacques Drouin, Jean Charron, Lucie Jeannotte and Richard K. Plante, Institut de recherches cliniques de Montréal, Canada and Orjan Wrange, Karolinska Institute, Stockholm.

Pro-opiomelanocortin (POMC), the precursor to ACTH, β -endorphin and the melanotropins, is expressed in the anterior (AP) and intermediate lobes (IL) of the pituitary, in certain brain areas, in the testis, ovary and placenta. Glucocorticoids decrease POMC gene transcription rate in AP, but not in IL. POMC transcription is stimulated in these tissues by CRF and cyclic AMP. Tissue specificity and regulation of rat POMC (rPOMC) gene transcription was studied by gene transfer in cells in culture using rPOMC-neo fusion genes. Whereas the rPOMC promoter is as inefficient in L cells as a promoter-less control, it is 20 and 5 times more efficient in AtT-20 and GH_3 cells, respectively. Sequences up to -478 bp are required for this activity which can be confered to an heterologous promoter by a 350 bp POMC fragment that behaves as a tissue-specific enhancer element. Transcription from the rPOMC promoter starts at the correct site as demonstrated by RNAase mapping. Glucocorticoid (DEX) treatment of pools of AtT-20 cells electroporated with pPOMCneo plasmids similarly inhibits mRNA levels of endogenous mouse POMC and exogenous POMCneo. Glucocorticoid-sensitive elements (GSE) were defined by deletion analysis as above and by glucocorticoid receptor (GR) binding to POMC DNA. Five GR binding sites were defined by DNAaseI and exonuclease III footprinting and by DMS protection; at least one of these sites can confer full glucocorticoid sensitivity. The data suggest models for the mechanism of glucocorticoid inhibition of POMC transcription.

0150 REGULATION OF HUMAN ALCOHOL DEHYDROGENASE GENE EXPRESSION BY GLUCOCORTICOIDS. Gregg Duester, Department of Micro. & Mol. Gen., Univ. of California, Irvine 92717

Human alcohol dehydrogenase (ADH) is encoded by five genes which display developmental and tissue-specific regulation. To analyze the regulation of ADH gene expression genomic clones encoding the α, β, and γ subunits of human ADH were identified. Human genomic DNA hybridization results indicated that the α, β, and γ ADH genes form a closely related gene family and suggested that the other known human ADH genes (those encoding the π and χ subunits) share a more distant evolutionary relationship. Nucleotide sequence analysis of the β ADH gene revealed that the coding region was interrupted by eight introns and spans 15 kilobases. A transcription initiation site for the β ADH gene was located by S1-nuclease mapping at a position 70 base pairs upstream of the start codon. The 5' flanking region possesses a TATA box promoter element as well as two tandem DNA sequences which display homology to previously examined glucocorticoid responsive elements (GRE) such as those found in the mouse mammary tumor virus. In the β ADH gene the GRE sequences are located at positions -226 and -187 relative to the site of transcription initiation. Nucleotide sequencing of the α and γ ADH genes indicated a high degree of homology between the 5' flanking regions of α, β, and γ ADH up to position -226. Each of the three genes thus contain two tandemly situated GRE sequences, suggesting that all may be inducible by glucocorticoid hormones. The DNA sequences upstream of position -226 appear to be unique for each gene and may thus harbor the signals responsible for their differential expression during development and their tissue-specificity.

0151 MOLECULAR BASIS OF TRANSCRIPTIONAL SPECIFICITY IN MAMMALIAN MITOCHONDRIA, Robert P. Fisher, David D. Chang and David A. Clayton, Stanford University School of Medicine, Stanford, CA 94305

The process of mitochondrial gene expression, as reflected in the organization of genes and of regulatory elements for transcription and replication, has been conserved throughout mammalian evolution. In contrast, the control sequences themselves are evolutionarily remarkably labile. To understand the molecular basis of this flexibility, we have dissected both the protein machinery and the DNA sequences required for selective transcription, using soluble extracts of human and mouse mitochondria. We have isolated and characterized a specificity factor (mtTF) required for selective initiation at both major promoters (HSP and LSP) of human mtDNA. Three properties of this factor suggest a DNA-binding mode of action: factor alone, but not mtRNA polymerase alone, can sequester template DNA in a preinitiation complex; transcriptional stimulation by factor requires sequences 16-60 bp upstream of the start sites; and isolated mtTF protects approximately 25 bp of these control regions from DNAase digestion. Factor-binding is necessary, but not sufficient for selective initiation; mutations which prevent binding impair transcriptional selectivity, but several mutants show severe losses of transcriptional capacity while retaining essentially normal factor-binding function. The two regions (LSP-proximal and HSP-proximal) protected by mtTF are positioned identically in relation to their respective start sites, but show no apparent sequence homology, suggesting a flexible "recognition code", or perhaps the copurification of two separate specificities.

0152 **CHARACTERIZATION OF CHROMATIN FRAGMENTS AS TEMPLATES FOR IN VITRO TRANSCRIPTION.** Margaret A. Flanagan, Merrell Dow Research Institute, Cincinnati, OH 45215, Helena Mishoe and Alan N. Schechter, LCB/NIADDK, National Institutes of Health, Bethesda, MD 20205.

The primary sequence of DNA is generally not sufficient to direct tissue specific or developmentally specific expression. The assembly of histone and nonhistone proteins onto DNA in the cell nucleus appears to contribute significantly to differential gene expression. In vitro transcription of micrococcal nuclease (MNase)-generated chromatin templates shows differential expression of the 5S RNA genes, polymerase III genes present in high copy number [Schlissel, M.S. and Brown, D.D. (1984) Cell 37:903]. However, MNase-generated templates have several disadvantages which significantly limit the sensitivity of any assay for specific initiation of transcription. They are therefore not well-suited for the study of single-copy genes. In this study, nuclei isolated from human K562 cells have been digested with EcoR1 to yield chromatin fragments which can be well characterized and do not have the inherent insensitivity associated with MNase-generated fragments. These fragments can be fractionated into three physical types: small soluble material that is mostly RNA (S1), soluble material that is mostly DNA (S2) and an insoluble matrix of DNA and nuclear structural proteins (P). S2 contains the full complement of histone proteins, arranged on the DNA as nucleosomes. Active genes (ε- and γ-globin) and an inactive gene (β-globin) are present in S2 in approximately the same proportion, although they are depleted relative to the total genomic DNA. Soluble chromatin generated by EcoR1 digestion should prove to be a superior template for in vitro transcription studies of differential gene expression.

0153 STRUCTURE AND EXPRESSION OF HUMAN METALLOTHIONEIN GENES. Foster, R., Jahroudi, N., Sadhu, C., Varshney, U. and Gedamu, L. The Department of Biology, The University of Calgary, Calgary, Alberta, Canada.

Two human metallothionein genes (MT-IG and MT-IF) have been isolated from a human genomic library. These two genes are separated by approximately 7.0 Kb of sequence and are orientated in a head-to-head manner. Both genes contain three exons separated by two introns. The introns interrupt the coding sequences at the same position as all other mammalian MT genes and are flanked by the consensus splice sequence GT--AG. The 5'-flanking region of these genes is very G+C rich. In this region MT-IG has a TATAAA consensus sequence while MT-IF possesses a variant, TATCAA. These genes also exhibit metal regulatory elements and putative SP1 binding sites, GGGCGG. In their 3'-untranslated region these genes have a variant of the polyadenylation signal, AATTAA. Furthermore, MT-IG has a mRNA processing signal, YGTGTTYY, while MT-IF has a variant, YGTGCTYY. The variation in these sequences may be important in cell-specific regulation. These genes were shown to be functional and inducible by heavy metals, but not by dexamethasone in mouse LMTK-cells after their transfer on a plasmid containing the herpes simplex virus thymidine kinase gene. S1 nuclease studies using MT-specific probes identified transcripts of these genes in a Cd-induced human hepatoma cell line, Hep G2. Further expression studies using MT-specific probes and different cell lines suggest that the expression of these genes is differentially regulated in response to the various inducers and appears to be cell-type specific.

0154 ANALYSIS OF MECHANISMS BY WHICH INTERFERON-γ INCREASES MHC mRNA IN MURINE MACROPHAGES. Yvonne R. Freund & Patricia P. Jones, Dept. of Biological Sciences, Stanford University, Stanford, CA. 94305.

Expression of class II MHC antigens on the surface of macrophages is essential for the process of antigen presentation to T cells, and for the initiation of antigen-specific immune responses. The class II antigens are not constitutively expressed on macrophages (mφ); rather, they can be induced by treatment of the cells with gamma interferon (IFN-γ). We have demonstrated in the mouse mØ cell line, WEHI-3, that induction results in elevated levels of mRNA for the 4 class II (Ia) genes. Levels of mRNA increase abruptly at 8 hours post-IFN-γ-treatment in a tightly coordinated manner for all 4 Ia chains and the Ia-associated invariant chain. Nuclear run-off experiments indicate that regulation is at the level of increasing rates of transcription; however, a lag period of roughly 8 hours occurs between the time that IFN-γ binds to the cell surface receptor and the time that increases in transcription rates are detected. This raises the possibility that synthesis or activation of a second factor is required to initiate the increase in Ia transcription. We have also observed that IFN-γ induces increases in levels of class I (H-2) mRNA. Kinetics of this induction differ from those observed for the Ia genes in that increases are observed soon after addition of IFN-γ and mRNA accumulates with more gradual kinetics. This indicates the possibility that IFN-γ may regulate expression of Ia and H-2 genes by different mechanisms. Experiments are underway to test this hypothesis.

0155 EXPRESSION OF MYOSIN AND ACTIN GENES DURING MYOGENESIS IN THE MOUSE, Ian Garner, Serge Alonso, Philippe Daubas, Adrian Minty and Margaret Buckingham, Institut Pasteur, 75724 Paris Cedex 15, France.

We are interested in the expression of actin and myosin genes during myogenesis in the mouse. Promoter regions of a number of genes have been isolated. Conserved sequences in these regions are evident between species for a given gene but not between different genes expressed in the same phenotype, with the exception of a polyoma type enhancer core-sequence (Daubas et al. NAR 13 p.4623, 1985). For one such promoter, that of the cardiac (α_c) actin gene, we demonstrate that a short fragment (-331 to +113 bp) is sufficient to confer differential expression on -CAT gene fusions in differentiating myogenic murine cell lines. In some strains of mice (DBA2 and BALB/c) this promoter is duplicated in a 5 Kb direct repeat upstream of the bona fide gene. Also duplicated are the coding sequences up to amino acid 151. We have sequenced these regions in the duplication and the real gene. They are identical. Furthermore, the duplication is transcriptionally active in cardiac tissue and results in splicing artifacts. In the hearts of these mice, there is an abnormally low level of mature α_c mRNA (20% that of control mice). Finally, we observe an accumulation of skeletal actin gene transcripts in these hearts. This situation mimics that observed in foetal hearts and suggests that the developmental control of the expression of these actin genes is a reversible process and can respond to abnormalities in mRNA levels.

0156 CLONING AND EXPRESSION OF A HUMAN MONOCLONAL ANTIBODY IN MURINE MYELOMA CELLS, Stephen D. Gillies and John Wesolowski, Damon Biotech Needham Heights, MA 02194

A human-human hybridoma line was isolated following fusion of a non Ig-producing human lymphoblastoid line, UC 729-HF2, with peripheral blood lymphocytes. The lymphocytes had been previously stimulated by an in vitro exposure to antigen. Human hybridoma lines were obtained that produced monoclonal antibody reactive with the J5 endotoxin antigen. One such line, which produces an IgMκ antibody, was used as a source of mRNA for the preparation of a cDNA library using the phage vector gt10. Two different μ cDNA and two different κ cDNA clones were identified by oligonucleotide hybridization and subsequent DNA sequence analysis. One of the μ cDNA clones contained a stop codon in what appeared to be the leader sequence coding region while the second μ cDNA had an open reading frame and could be expressed in transfected murine myeloma cells. Vectors have been constructed for the expression of both the heavy and light chains in the same myeloma transfectant and are currently being tested.

0157 DELINEATION OF THE STRUCTURAL REQUIREMENTS FOR THE TISSUE RESTRICTED AND STAGE SPECIFIC TRANSCRIPTION OF THE ALPHA-SKELETAL ACTIN GENE PROMOTER, James M. Grichnik, Derk J. Bergsma, Robert J. Schwartz, Baylor College of Medicine, Houston, Tx 77030

Alpha-skeletal actin mRNA levels have been shown to be tightly regulated in chicken primary myoblast cultures. To test for gene elements required for muscle specific expression, DNA sequences containing the 5′ flanking region of the chicken alpha-skeletal actin, beta-cytoplasmic actin, and the histone H2b genes were linked to the coding sequences of the chloroamphenicol acetyl-transferase gene and transfected into myogenic and non-myogenic cells. In contrast to the beta-cyt-, H2b-, and SV2-CAT hybrids, alpha-skeletal actin-CAT constructs displayed stage specific activation during myoblast differentiation, and restricted expression in non-myogenic cells. Through a series of 5′ deletions of the alpha-skeletal promoter, tissue restricted and stage specific activation is shown to be maintained within a 190 base pair fragment. This fragment displays an imperfect dyad symmetry about a central axis and may function bidirectionally. Two pairs of sequence elements can be identified which are symmetrical and conserved in both chicken and rat sequences. In the chicken promoter, the upstream element containing an inverted CCAAT-like sequence and the region between the CCAAAT and ATAAA sequences appear to be important in the expression of the chicken alpha-skeletal actin gene.

0158 IDENTIFICATION OF PROMOTER ELEMENTS NECESSARY FOR TRANSCRIPTIONAL REGULATION OF A HUMAN HISTONE GENE IN VIVO, Sarah Hanly and Nathaniel Heintz, Rockefeller University, New York, NY 10021.

The replication dependent hisone genes have been shown to be cell cycle regulated at both transcriptional and post-transcriptional levels. Transcriptional induction of histone gene expression in S phase has been reproduced in vitro using S phase extracts (Heintz and Roeder). Furthermore our analysis of deletion mutants in the promoter of an H4 gene has identified elements upstream from the TATA box which are necessary for S phase specific transcription (Hanly et al). Recently, we have identified at least two and possibly three proteins which bind to different upstream elements of this gene (Dailey, Hanly and Heintz). We are currently addressing the question of how these promoter elements function in vivo, in particular, to discern which distal element confers cell cycle regulation on this gene. Stable cell lines containing H4 promoter mutant genes have been constructed and initial results indicate that the steady state levels of histone message in cell lines containing H4 genes which lack the upstream promoter elements fluctuate normally during S phase. This result is not surprising considering the powerful post-transcriptional regulatory mechanisms which operate to control histone mRNA concentration. Therefore we have begun an analysis of the rate of transcription of H4 mRNA from the mutant genes and have demonstrated that the wild type gene is normally regulated. Our present efforts are directed toward analysis of the mutant genes and identification of the upstream sequence elements responsible for transcription regulation.

0159 TRANSCRIPTIONAL CONTROL REGIONS OF MOUSE HISTIDINE tRNA GENES. ACTIVITY OF ISOLATED A AND B BLOCKS AND FLANKING REGIONS. John Harding, Mary Morry, Barbara Ross and Robert Rooney. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

We have recently shown that transcription of a mouse $tRNA^{His}$ gene in a HeLa cell extract is greatly reduced when 5' flanking residues between positions -4 and -9 are deleted (M. Morry and J. Harding, *Mol. Cell. Biol.* Jan. '86). To further analyze the transcriptional control regions of this gene we prepared the following deletion mutants and analyzed their transcription and ability to compete with an intact $tRNA^{His}$ gene for transcription factors in vitro. The following results were obtained. 1). The 5' flanking region alone is not transcribed and does not compete. 2). The A block with or without 5' flank is not transcribed and does not compete. 3). A clone containing the A + B blocks + 3' flank in the L orientation in M13 has c. 5% of wild type transcriptional activity and c. 12% of competitive ability. The R orientation clone is transcribed much more efficiently. This probably occurs because the proximal 5' flank normally required for efficient transcription is partially reconstructed by vector sequences in the R clone. 4). Clones containing the B block + 3' flank in either orientation in M13 are transcribed at c. 5% wild type levels. The R orientation clone competes very inefficiently while the L orientation clone shows anomalously high competitive ability.

Taken together these experiments suggest that at least 3 loci -- the 5' flank from -9 to -4, the A block and the B block -- must be present together for efficient transcription and competition. However, vector sequences can sometimes contribute to anomalously high activity in some clones. Experiments to test the binding of factors by means other than competition are currently in progress.

0160 SINGLE BASE PAIR CHANGES WITHIN A CONSERVED REGULATORY ELEMENT ALTER HIS3 REGULATION, David E. Hill[1,2], Ian Hope[1], & Kevin Struhl[1]; [1]Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass. 02115; [2]Genetics Institute, Cambridge, Mass. 02140.

The Yeast His3 gene is transcriptionally regulated in response to amino acid starvation. The sequence TGACTC, or minor variations found in all coordinately regulated amino acid biosynthetic genes, has been implicated as important for transcriptional regulation of His3. In addition, regulation of His3 requires the action of the GCN4 gene product. In vitro, His3 DNA containing the sequence TGACTC is capable of binding to in vitro-synthesized GCN4 protein. Using a novel method of preparing a library of single and multiple base changes in the TGACTC sequence and surrounding DNA, cloned in homoduplex form as dsDNA, we have investigated the sequence requirements for His3 regulation. In vivo, point mutations in the TGACTC sequence abolish regulation of His3, whereas single base changes outside the element have little or no effect. The sequence 3' to TGACTC is oligo(dT_9). Removal of six or nine T residues reduces or abolishes regulation suggesting that the sequences surrounding the TGACTC may be important for regulation. Mutations which do not function in vivo do not bind GCN4 protein in vitro. Quantitative changes in regulation observed in the in vivo assay are preserved in the in vitro binding analysis. Our results suggest that transcriptional regulation of His3 is dependent upon effective binding of the GCN4 protein to the TGACTC element in His3, and that the TGACTC sequence is critical for proper regulation of His3 mediated by GCN4 binding.

0161 TISSUE SPECIFIC EXPRESSION OF MOUSE RETROTRANSPOSONS LTR-IS/MuRRS. Ivan Horak, Thomas Baumruker, Thomas Wirth and Claudia Gehe, Institut für Virologie und Immunbiologie der Universität Würzburg, Versbacher Str. 7, 8700 Würzburg, FRG

Murine retrovirus-related sequences (MuRRS) is a newly described family of mouse retrotransposons (1). The long terminal repeats of MuRRS, called LTR-IS, exist as solitary elements in high copy number dispersed in the genome. Evidence for mobility of the LTR-IS as well as for presence of functional RNA polymerase II promoters within the LTR-IS has been documented. Northern-blot analysis revealed that the expression of the LTR-IS/MuRRS is limited to B-lymphoid cells only. Screening of a cDNA library, made from polyA-containing RNA of an expressor B-cell line lead to the identification of several LTR-IS/MuRRS-hybridizing clones. The characterization of these cDNA clones will be presented and the role of the LTR-IS/MuRRS elements in insertional mutagenesis will be discussed.

1) Schmidt, M., Wirth, T., Kröger, B. and Horak, I., Nucleic Acids Res. 13 (1985) 3461.

0162 EFFECT OF CELL PROLIFERATION ON METALLOTHIONEIN GENE EXPRESSION. Richard J. Imbra and Michael Karin. Metallothionein (Mt) synthesis begins during early embryonic development and continues in most cells throughout life. Expression of the human Mt genes is induced by several agents, including heavy metal ions, glucocorticoid hormones, interferon and interleukin-1, and the DNA sequences required for induction by some of these agents are characterized. Since metallothionein binds essential trace elements, such as zinc, which is required for both DNA and RNA polymerases, we began to investigate Mt gene expression during stimulation or inhibition of cell proliferation.

We report that serum starvation results in a decreased level of Mt mRNA as compared to the level in cells grown under standard conditions. This effect is not due to inhibition of DNA synthesis, since Mt mRNA is inducible in the presence of mitomycin-C and in temperature sensitive mutants defective in DNA synthesis. Furthermore, preliminary data suggests that Mt gene expression is induced by the phorbol ester, TPA, and that Mt mRNA is over-expressed in some virally transformed cells. These results suggest a role for protein kinase C in Mt gene expression.

0163 IDENTIFICATION OF THE DNA SEQUENCE ELEMENTS RESPONSIBLE FOR ANAEROBIC INDUCTION OF MAIZE *ADH1*, John C. Ingersoll (1), Robert J. Ferl (2), and William B. Gurley (1), (1) Department of Microbiology and Cell Science (2), and Department of Botany, University of Florida, Gainesville, FL 32611.

Transcription of maize alcohol dehydrogenase 1 (*Adh1*) is a highly regulated event. In maize roots transcript corresponding to this gene is present at low constitutive levels. When the plant is exposed to anaerobic conditions, such as flooding, transcription is increased up to 50-fold.

Agrobacterium tumefaciens was used as a vector to incorporate the monocot (maize) *Adh1* gene into the genome of transformed cells of sunflower, a dicot. The 5' flanking region of *Adh1* was systematically deleted from 5' to 3' in order to identify the upstream border of the promoter element responsible for anaerobic inducibility. The altered genes were assessed for promoter activity in sunflower tumors. Induction was obtained by flooding for various lengths of time. Poly $(A)^+$ RNA was extracted and analyzed by RNA blot hybridizations and S1 nuclease hybrid protection.

Transcription of the introduced *Adh1* gene containing approximately 1100 bp of upstream sequences is anaerobically inducible. Although the start of transcription corresponds to the site utilized in maize, processing of the RNA in sunflower is aberrant. RNA blot analysis revealed the presence of two high molecular transcripts not seen in control tumors or in maize roots. Readthrough transcription originating from far upstream of the normal start was also present in induced tumors. USDA 81-CRCR-0656.

0164 HETEROLOGOUS TRANSCRIPTION AMONG FUNGI, Michael A. Innis, Peter C. McCabe and Michael J. Holland* Department of Microbial Genetics, Cetus Corporation, 1400 53rd St. Emeryville, CA 94608. *Department of Biological Chemistry, School of Medicine, University of California, Davis CA 95616.

The 5'-flanking region of the *Aspergillus awamori* glucoamylase gene shares considerable homology with conserved elements in the 5'-flanking region of several highly expressed yeast genes. In spite of these homologies, we could not detect any transcription from the natural *Aspergillus* glucoamylase gene in *Saccharomyces cerevisiae*. Substitution of the 5'-flanking region of a yeast enolase gene (*ENO1*) for the glucoamylase 5'-flanking region allowed high level expression of a cDNA copy of the gene in yeast. Based on these observations, we speculated that yeast were unable to recognize the glucoamylase upstream activation sequences. To test this hypothesis, a 210bp fragment containing a yeast enolase (*ENO2*) upstream activation sequence was inserted in both orientations at a position 170bp upstream from the natural glucoamylase TATA element. Yeast strains bearing either hybrid promoter fused to glucoamylase cDNA secreted low levels of glucoamylase to the medium. Transcription starts from the hybrid promoters were mapped and compared to those we found prevously in mRNA isolated from *A. awamori*. Four major transcription starts were seen: one is the same as a major start in *Aspergillus*, the other three are at new positions.

0165 STUDIES ON HUMAN METALLOTHIONEIN GENE EXPRESSION. N. Jahroudi, U. Varshney, R. Foster, C. Sadhu and L. Gedamu. The University Biochemistry Group and The Department of Biology, University of Calgary.

We have studied the expression of two human metallothionein (MT) genes of the MT-I family (MT-IF and MT-IG) in two human hepatoma and a lymphoblastoid cell lines in response to heavy metals (Cd, Cu and Zn) and Dexamethasone (Dex). We have used the coding region of a human MT-II processed gene to detect total MT-mRNA while the 3'-non-coding regions of MT-IF and MT-IG genes were employed as specific probes for the analysis of MT-IF and MT-IG mRNAs.

In the lymphoblastoid cell line (WI-L2), the time course of induction of total MT-mRNA in response to heavy metals (Zn, Cd and Cu) indicates that the maximum time of induction is about 12 hours. Dex, however, does not induce total MT-mRNA above basal level. In the hepatoblastoma (Hep G2) and hepatocarcinoma (Hep 3B2) cell-lines the maximum time of induction of total MT-mRNA in response to heavy metals Zn, Cd and Cu is between 5 to 9 hours. Dex also causes a substantial level of induction of total MT-mRNA in Hep G2 but not in Hep 3B2 and the maximum time of induction is between 9 and 12 hours. Furthermore, there is a high level of basal total MT-mRNA synthesis in Hep G2 compared to that of WI-L2 and Hep 3B2. Using specific probes for MT-IF and MT-IG mRNAs, we have clearly demonstrated that both genes are expressed in hepatoma cells in reponse to the various inducers, and total MT-mRNA induction in response to Dex is regulated in a cell-type specific manner. In addition, the MT-IF and MT-IG genes are not expressed in the lymphoblastoid cell-line upon induction with either metal or Dex. These results suggest that both genes show cell-type specific expression. We are currently investigating the role of methylation in the expression of these MT genes in various human cell lines.

0166 STRUCTURE AND EXPRESSION OF THE MOUSE THYMIDYLATE SYNTHASE GENE, L. F. Johnson, C.-H. Jenh, D. Li, and T. Deng, The Ohio State University, Columbus, Ohio 43210.

We have studied thymidylate synthase (TS) gene expression in a FUdR-resistant 3T6 cell line, LU3-7, that overproduces TS and its mRNA 50-fold due to TS gene amplification. When serum-arrested LU3-7 cells were stimulated to proliferate, the rate of synthesis of TS increased at least 9-fold when the cells traversed S phase. TS mRNA content increased 20-40-fold as cells progressed from G0 to late S phase. This was the result of an 8-fold increase in the rate of production of poly(A)+ TS mRNA, although the rate of labeling of poly(A)- TS mRNA changed very little. The rate of transcription of the TS gene, as determined in isolated nuclei, increased only by a factor of 3-4 during S phase. Since the content of the message increased to a much greater extent than the rate of transcription of the gene, posttranscriptional controls must also play a role in regulating the content of TS mRNA under these conditions. Our results suggest that the cell may regulate the distribution of TS mRNA between a stable poly(A)+ RNA species and a labile poly(A)- RNA species. Sequence analysis of TS cDNA revealed several unusual features. The predicted amino acid sequence of mouse TS showed a remarkable degree of homology (50-55%) with TS from E. coli or L. casei, indicating that TS is among the most highly conserved proteins studied to date. Furthermore, the UAA stop codon was followed immediately by a poly(A) sequence, suggesting that the mouse TS mRNA may lack a 3' untranslated region. The TS gene has been cloned from mouse cells. The intron-exon boundaries as well as the 3' and 5' flanking regions of the gene have been sequenced. The ability of the cloned TS gene and various constructs to complement a TS(-) phenotype in hamster cells is being studied.

0167 ANALYSIS OF THE GLUCOCORTICOID RESPONSIVE PROMOTER OF THE RAT α_1-ACID GLYCOPROTEIN GENE, Elliott S. Klein and Gordon M. Ringold, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305

The increase in the abundance of mRNA encoding the acute phase reactant α_1-acid glycoprotein (AGP) upon treatment of rat hepatoma derived cell lines with dexamethasone (DEX) has been previously shown to require ongoing protein synthesis. In addition, analysis of the transcriptional rate at the AGP locus has led us to propose that a post-transcriptional mechanism is involved in the regulation of AGP expression. In more recent studies we have analyzed the expression from a fusion gene (pAGPCAT) which includes the 5'-upstream portion (-763 to +20) of the AGP gene attached to the CAT structural gene. Stable transformants of pAGPCAT in the cell line JZ.1 yield cat enzyme activity which is at least 20-fold inducible upon DEX treatment. CAT activity is also DEX inducible from an AGP-CAT construction which includes the region -380 to +1 of the AGP gene. Measurement of poly(A)+ RNA from pAGPCAT transformants shows both CAT and AGP RNAs to be detectable only upon addition of DEX. Unlike the endogenous AGP gene however, two (2) RNA species (approximately 1.1 and 1.5 Kb) are synthesized from the pAGPCAT fusion gene. In addition, this induction of the two CAT RNAs does not require ongoing protein synthesis, but appears to be enhanced with cycloheximide treatment. In contrast to the nuclear transcription assays these data suggest that promoter activation plays a role in the induction of AGP mRNA by glucocorticoids. Moreover, the difference seen in the induction of AGP and CAT RNAs from the same promoter, in regards to their respective sensitivities to inhibition of protein synthesis, presents an additional paradox that needs to be resolved.

0168 THE FIM GENES FROM ESCHERICHIA COLI: REGULATION OF FASE VARIATION AND MODULATION OF EXPRESSION.

Per Klemm, Department of Microbiology, Technical University of Denmark, DK-2800 Lyngby-Copenhagen, Denmark.

Type 1 fimbriae, produced by the majority of Escherichia coli strains, are subjected to phase variation. This phase variation, which is an all- or none way of regulating the transcription of the fimbrial subunit gene, is due to the specific inversion of a 300 bases DNA-fragment harboring the promotor of the subunit gene. Two genes, located adjacent to the subunit gene play a role in the modulation of the phase switch and the expressional control of type 1 fimbriae. The potential function of these genes and their products will be presented.

0169 THE STABILITY OF BACTERIOPHAGE T4 GENE 32 mRNA; A 5' LEADER SEQUENCE THAT CAN STABILIZE mRNA TRANSCRIPTS.

K. Gorski, E. Mudd, D. Belin, P. Prentki, and H.M. Krisch, Department of Molecular Biology, University of Geneva, Geneva, Switzerland.

The gene 32 monocistronic mRNA has the unusual property of being extremely stable. To study the molecular basis for this stability, we have constructed chimeric plasmids containing the monocistronic promoter and the gene 32 translation initiation sequence fused to either prokaryotic (lac operon) or eukaryotic (TK of Herpes Simplex Virus) coding segments. The coding sequence is followed by the transcription terminator of gene 32. The hybrid mRNAs from these gene fusions are not stable in uninfected cells. In phage-infected cells, however, the hybrid mRNAs are at least as stable as the gene 32 mRNA itself. An unidentified early phage function is implicated in this stabilization. Analysis of other plasmid constructs indicates that the sequences on the gene 32 mRNA from its 5' end to slightly beyond the initiation codon suffice to stabilize hybrid mRNA molecules. This was confirmed by studies with a series of deletions of the gene 32 leader sequence which indicate that an RNA sequence in the vicinity of the gene 32 initiation codon is involved. One may envisage stabilization occurring in a variety of different ways; these various mechanisms are discussed.

0170 POST-TRANSLATIONAL AND PRE-TRANSLATIONAL REGULATION OF THE SYNTHESIS OF THE MAJOR PHOSPHORYLATED GLYCOPROTEIN EXCRETED BY RAT HEPATOCYTES IN ACUTE INFLAMMATION, Alphonse Le Cam, Ginette Le Cam, Richard Benarous, Pierre Leopold, Nicolas Glaichenhaus and Cedric Galup, INSERM Institute, Nice, France

Hepatocytes isolated from normal rats secrete a major phosphorylated, fucose-rich glycoprotein (PP63) also present in plasma. Polyclonal antibodies were raised and used to study PP63 biosynthesis: a) after translation of the mRNA in a rabbit reticulocyte translation system; b) in intact cells labeled with (35S)-methionine, (3H)-fucose and (32P)-orthophosphate. A cDNA clone containing a 630 bp insert was isolated by immunological screening of a rat liver library constructed in the λgt11 expression vector. This cDNA insert was labeled by nick-translation and used as a probe to measure PP63 mRNA levels in hepatocytes by northern blot analysis. Production of the mature phosphorylated protein was considerably reduced in hepatocytes prepared from inflamed rats as compared to controls. This defect was not due to a blockade in excretion of the protein but to inhibition of its biosynthesis. Two sequential mechanisms appeared to account for this inhibition. During the first hours following turpentine injection, glycosylation of the precursor polypeptide was impaired at different levels, indicating that PP63 biosynthesis was regulated at a post-translational level. In a second step, a large decrease in amounts of translatable and hybridizable PP63-mRNA present in hepatocytes was detected. This indicated that biosynthesis of PP63 was also regulated at a pre-translational level, in inflammation.

0171 EXPRESSION OF THE ompR-envZ OPERON OF SALMONELLA TYPHIMURIUM, Peter Liljeström, Department of Genetics, University of Helsinki, Finland

The regulatory operon ompB (ompR-envZ) of S.typhimurium and E.coli is required for the proper expression of the ompC and ompF genes, which code for the corresponding outer membrane porin proteins OmpC and OmpF. The ompB operon directs the synthesis of two proteins, OmpR and EnvZ. OmpR is a positive regulatory protein acting at the ompC and ompF promoters while EnvZ is situated in the cytoplasmic membrane. Analysis of in vitro constructed ompR-lacZ and envZ-lacZ gene fusions showed that the OmpR protein is produced 10 times more than the EnvZ protein. The ompR gene was modified in vitro to change the position of the OmpR translation termination site. The effects of these changes on the envZ expression was monitored by employing an envZ-lacZ gene fusion. The envZ expression was abolished in all cases where the OmpR translation was terminated prematurely. The basis for this translational coupling will be presented.

0172 Expression Cloning of Plastid Genes Specifying Chloroplast RNA Polymerase Subunits Michael Little and Richard B. Hallick, Dept. Biochemistry, University of Arizona, Tucson, AZ 85721. During the course of sequencing chloroplast DNA fragments, two genes having strong homology to E. coli RNA polymerase subunits have been identified. A spinach DNA clone with homology to alpha subunit (rpoA) and a Euglena gracilis clone with homology to beta' (rpoC) were used in constructions designed to express these genes as fusion products using a pATH 3 expression vector. The constructions were designed to allow the expression of fusion proteins containing a trpE leader peptide of approximately 37,000 Da with the respective translational products of the chloroplast DNA sequences. Five independent clones containing the correctly oriented DNA sequence for alpha subunit and one containing the correct orientation for beta' subunit were examined for the expression of fusion proteins upon induction with 3-indole acrylic acid. All clones of alpha subunit expressed a major protein (8-10 mg/liter cells) of the expected mass (58,000 Da) and that or beta' expressed a protein (2-3 mg/liter cells) of mass (61,000 Da) nearly equal to that calculated upon the basis of DNA sequence (68,000 Da). Presently, rabbit antibodies to these fusion proteins are being elicited for use in experiments to probe the roles of these subunits in chloroplast RNA polymerase from Euglena and spinach.

0173 ISOLATION OF cDNA CLONES SPECIFIC TO CYTOTOXIC T LYMPHOCYTES, C.G. Lobe, V.H. Paetkau and R.C. Bleackley, Dept. of Biochemistry, University of Alberta, Edmonton, Canada

Different subsets of T cells are responsible for cytotoxic, helper and suppressor activities. Despite extensive serological studies, little is known of how these functionally distinct cells differ at the molecular level. We have screened a cDNA library by differential hybridization to identify clones representing mRNAs specific to cytotoxic T cells (CTL). Two of these were chosen for more extensive analysis. On a Northern blot, these clones detect bands in mRNA from CTL lines but not in mRNA from two different helper T cell lines or thymocytes. Further experiments have shown that these mRNAs are not expressed at a detectable level in non-lymphoid or B cells. Most important, we have found that the level of expression correlates with cytotoxic activity in a mixed lymphocyte culture. Therefore we postulate that this tissue-specific expression is crucial for activation of CTL to the lytic state. We have now identified genomic clones corresponding to each of the cDNA clones in order to study how these genes are regulated as CTL differentiate and respond to antigen.

0174 CELL CYCLE REGULATION OF HISTONE TRANSCRIPTION IN YEAST, D. Lycan, M.A. Osley, and L. Hereford. Dana-Farber Cancer Institute and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.

In eukaryotic cells, histone RNA levels fluctuate with the cell cycle, with maximum accumulation occurring in S phase. In most cells, this pattern is achieved by a combination of transcriptional and post-transcriptional mechanisms. In yeast, the sequences required for transcription of both H2A and H2B have been defined by deletion analysis to a single 300 bp region between the two divergently transcribed genes. A cell cycle specific region required for periodic transcription has been confined to a 70 bp core. Deletion of this core results in elevated and constitutive expression of both genes. In addition, there are three positive elements which are required for expression and which may also have a cell cycle specific role. We are currently generating point mutations in the promoter to define more clearly the relative roles of these elements in regulating histone gene transcription. At the post-transcriptional level, we had previously proposed that histone RNA stability was linked to the rate of DNA replication, accounting for the very rapid drop in RNA levels observed when DNA synthesis is interrupted in mid-S. However, we have now been able to show that this dramatic drop in levels is due largely to the inhibition of transcription. H2B-lacZ fusion genes containing the promoter, 5' leader and first 20 amino acids are periodically expressed in a pattern identical to the intact H2B gene. Furthermore, the fusion RNA levels are sensitive to the inhibition of DNA synthesis. However, if the histone promoter is replaced with the Gal10 promoter leaving the RNA structure intact, the RNA levels are no longer coupled to DNA replication. In addition, promoter deletions which eliminate sequences required for periodic transcription also uncouple RNA levels from DNA replication. Thus, we believe that the major control of histone gene expression occurs at the transcriptional level and that the same sequences involved in cell cycle control are used for the coupling of RNA levels to DNA replication.

0175 **DUAL ROLE OF SIGMA FACTOR IN TRANSCRIPTION INITIATION, S. Malik & A. Goldfarb, Dept. of Microbiology, Columbia University, NY, NY 10032**

To assess the role of initiation factors in the steps of promoter utilization we studied the interaction with the same promoter by two forms of RNA polymerase containing either host σ (Eσ) or bacteriophage T4-induced factor, the product of T4 gene 55 (Egp55). The promoter studied combined features of gp55-dependent late T4 promoters and of weak sigma-dependent E. coli promoters. The rate of open promoter complex formation was much higher with Egp55 than with Eσ. However, open complexes formed with Eσ had a much longer half life in the presence of heparin than those formed with Egp55. Hence we argue that, in addition to its ability to recognize the promoter via weak sequence-specific interactions, host σ participates in strong sequence-independent interactions which stabilize the open complex. The unstable open complex formed by Egp55 can be stabilized by the addition of σ in the presence of heparin indicating that σ can bind to Egp55. The resulting complex containing both factors (Egp55σ) continues "cycling" but cannot enter into the elongation mode and clear the promoter. We suggest that σ contains two functional domains, one responsible for promoter recognition and the other for anchoring RNA polymerase at the promoter, thereby preventing both reversal (closing) of the complex and transition into the elongation mode. In contrast, gp55 which is less than half the size of σ, is equivalent to the specificity domain of σ but lacks the stabilization domain.

0176 INTERACTIONS OF E. COLI TRANSCRIPTION TERMINATION PROTEIN, RHO WITH RNA: PHYSICAL AND ENZYMATIC STUDIES. James A. McSwiggen and Peter H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The mechanism of rho-dependent termination can be conceptually divided into two steps: (i) selection of a potential termination site through pausing of RNA polymerase at certain template sites, and (ii) release of RNA from a subset of these sites through the action of rho factor. To effect release, rho must first bind to RNA and then activate an RNA-dependent ATPase. Thus, the inability of rho to terminate at every pause site may be due to an inability to interact with the RNA near some sites.

Using well-defined, synthetic RNA as cofactors, the RNA binding and ATPase activity of rho has been examined as a function of RNA size, concentration, and composition to discover: (i) what constitutes the rho binding site on natural RNA, (ii) how RNA binding causes activation of the ATPase, and (iii) how ATPase activation brings about RNA release. The effect of salt concentration has also been examined to investigate the magnitude of the ionic contributions to binding free energy.

These studies reveal (among other things) that ATPase activation does not require cooperative activation among all six subunits of the hexamer, but that full saturation of the hexamer RNA binding site is still favored over partial saturation due to a more stable binding interaction. The implications of these results are discussed with respect to models for rho-dependent transcript release.

(Supported by USPHS grant GM15792 (to PHvH) and an NSF Predoctoral Fellowship (to JAM).)

0177 STRUCTURAL CHANGES IN THE 5' CHROMATIN OF THE J CHAIN GENE DURING B CELL DIFFERENTIATION: ASSOCIATION WITH REGULATORY SEQUENCES, Mark E. Minie and Marian E. Koshland, University of California, Berkeley, CA 94720

The murine immunoglobulin J chain is a 15 kd protein that mediates polymerization of IgM to its pentameric secreted form. The gene for J chain is transcriptionally silent during the antigen independent phases of B cell differentiation, but becomes active when the mature B cell is triggered to secretion. The mechanism of activation was investigated by analyzing J chain gene chromatin structure before and after the activation of expression. The active gene was found to contain a region of open structure that extended 230 bp upstream of the leader exon. This region was defined by both its high sensitivity to digestion by DNase I and accessibility to cleavage by several restriction enzymes. By use of these nuclease accessibility assays, it was found that the hypersensitive site correlated with the pattern of J chain gene expression in cell lines representing various stages of B cell differentiation. Furthermore, the site was found to be co-inducible with the activation of J chain gene transcription both in normal mouse splenocytes stimulated to secretion with LPS, and in BCL1 cells triggered to secretion by rIL-2. Analysis of the DNA sequence of the hypersensitive zone revealed the presence of potential regulatory elements that are homologous to known immunoglobulin transcriptional control sequences. Transient transfection experiments, in which the chromatin structure of the vector was analyzed, indicate that the hypersensitive site is generated by transacting factors.

0178 EXPRESSION OF HEPATITIS B SURFACE ANTIGEN WITH A RECOMBINANT ADENOVIRUS: Alan R. Davis, Berverley Kostek, Paul P. Hung; Wyeth Laboratories, Inc., Microbiology Division, P.O. Box 8299, Philadelphia, PA 19101.

Early region 1 of adenovirus type 5 was substituted with a cassette containing the gene coding for the hepatitis B surface antigen HB_sAg flanked by the adenovirus 2 major late promoter and processing and polyadenylation signals from SV40 virus. In one type of hybrid virus only the adenovirus 2 major late promoter, including just 33 base pairs of the adenovirus type 2 tripartite leader, preceded the coding region of the HB_sAg gene. In another, this region was preceded by both the adenovirus major late promoter and almost the entire tripartite leader. The structure of the cassette in each of the recombinant viral DNAs was identical to that in the plasmids used to construct the viruses. Approximately equivalent amounts of HB_sAg-specific mRNA were produced late in infection with each recombinant virus. Although HB_sAg production was detected late in infection of the hybrid virus not containing the full tripartite leader sequence, its level was 70-fold lower than that obtained with the hybrid virus containing this sequence. This suggests that the presence of the tripartite leader at the 5'-end of this mRNA is critical for the synthesis of HB_sAg polypeptide in the late stage of infection. HB_sAg produced upon infection with the hybrid adenoviruses was glycosylated and secreted into the culture medium as particles that were essentially indistinguishable from the 22 nm particles found in human serum.

0179

Transcriptional Regulation of Fibronectin Gene Expresssion
Noelynn Oliver, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111

Glucocorticoids may play essential roles in tissue repair and embryonic development by stimulating fibronectin gene transcription. HT1080 human fibrosarcoma cells attach more rapidly to tissue culture plastic, show changes in cell morphology and develop an extensive fibronectin-containing matrix upon exposure to physiological levels of a synthetic glucocorticoid, dexamethasone. These changes are due, at least in part, to a glucocorticoid-induced increase in the rate of fibronectin mRNA synthesis.

Studies of fibronectin biosynthesis in mouse-HT1080 hybrids indicate that this stimulation requires trans acting factors in addition to the glucocorticoid-receptor complex. Mouse RAG cells, which do not produce any detectable fibronectin, were fused with HT1080 cells, and hybrids selected. Hybrids containing the human chromosome encoding fibronectin, #2, had acquired the ability to produce fibronectin. These cells have high affinity glucocorticoid receptors which are likely to be functional since they translocate to the nucleus upon activiation. Even though these hybrid cells contain the gene encoding fibronectin as well as apparently functional hormone receptors, fibronectin biosynthesis in these hybrids is not enhanced by glucocorticoid treatment. This finding implies that glucocorticoid regulation of fibronectin gene expression is indirect, possibly requiring trans acting factors besides the glucocorticoid-receptor complex. In addition, glucocorticoid regulation of fibronectin gene transcription may be tissue specific since biosynthesis of fibronectin is also not stimulated by glucocorticoids in human cell lines of neural origin.

0180 THE ROLE OF THE RNA POLYMERASE IN CHROMOSOME REPLICATION OF E.COLI.

Svend Petersen, Teknologirådets Gensplejsningsgruppe/Technical University of Denmark, 2800 Lyngby/Copenhagen, Denmark.

To examine the nature of the regulatory role of transcription in replication from the chromosomal origin, oriC, the conditional transcription initiation mutant rpoC 907 was studied. The mutation was precisely mapped by plasmid-chromosomal recombination and DNA sequencing, and its influence on different transcriptions assessed by lacZ-fusion expression and C-14 uracil incorporation in stable RNA. The doubled DNA/mass ratio of the mutant at semipermissive temperature correlates with increased expression of the replication initiation gene dnaA and transiently decreased expression of genes subject to stringent (rel dependent) control in shift experiments. The significance of the latter with respect to transcription of the origin-contained mioC (16 k) gene is discussed.

0181 CHARACTERIZATION OF MOUSE DIHYDROFOLATE REDUCTASE RNA Shelley Sazer and Robert T. Schimke, Stanford University, Stanford, California 94305

Using primer extension and nuclease S1 mapping techniques we have reexamined the 5' termini of RNA transcribed from the mouse dihydrofolate reductase (DHFR) gene. We characterize a previously undescribed transcription initiation site at position -55 relative to the AUG codon, in addition to the previously identified start site at position -115. Differences in the 5' noncoding regions of these two transcripts with respect to their length and relative G + C content result in their differential ability to form stable hybrids with the DNA probe used in previous analyses of these transcripts and thus precluded the detection of transcripts initiated at -55. We show that changes in the temperature of the hybridization reaction result in the ability to detect the RNA having a shorter noncoding region and a lower G + C content. That position -55 represents an authentic transcription start site is confirmed by use of a DNA probe with which the two transcripts can form S1 resistant hybrids of equal stability and by primer extension analysis using an oligonucleotide primer that hybridizes near the AUG codon. These analyses also demonstrate that the transcript with a 5' end mapping near position -55 accounts for the majority of cellular DHFR RNA.

The hexanucleotide GGGCGG which has previously been shown to bind the transcription factor SP1 lies 40 - 50 basepairs 5' of the transcription initiation sites at -55, -115 and a minor site at -165. We are currently investigating the relationship between the interaction of regulatory factors with the DHFR promoter and the cell cycle regulation of the DHFR gene.

0182 REGULATION OF INTACT AND HYBRID β AND α ACTIN GENES INSERTED INTO MYOGENIC CELLS. S. B. Sharp, Caltech, Pasadena, CA 91125; T. A. Kost, Norden Labs, Lincoln, NB 68501; S. H. Hughes, Frederick Cancer Research Facility, Frederick, MD 21701; C. P. Ordahl, UCSF, San Francisco, CA 94143; N. Davidson, Caltech, Pasadena, CA 91125.

In order to determine which regions of cytoplasmic and skeletal muscle chicken actin genes contain cis-acting sequences important in regulating their developmentally timed expression, we have transferred intact and hybrid genes into myogenic BC3H-1 cells, made pools of stable transformants, and measured changes in the steady state levels of transcripts from each of the genes over eight day time courses of differentiation. The intact β and α genes were differentially regulated. Chick β mRNA was present in undifferentiated cells, and like endogenous β mRNA, showed substantial reduction during myogenesis. In contrast, chick α message, while not always appropriately upregulated, did not show the overall pattern of down-regulation typical of β. The amount of transcript from an α5'β3' hybrid decreased in a manner similar to intact β transcript. In pools with the converse β5'α3' gene, the amount of transcript first decreased and then reaccumulated to near initial levels. However, the majority of clones thus far isolated from this pool showed only a decrease, with no reaccumulation. One interpretation consistent with these results is that the 3' half of the β gene contains sequences which confer down-regulation on transcripts from the α promoter, but which are not required for down-regulation of transcripts from the β promoter. Experiments are underway to determine whether the observed regulation is transcriptional, and to further describe the regulation of expression of the β5'α3' gene during the later stages of differentiation.

0183 AN X-LINKED GENE FAMILY DIFFERENTIALLY REGULATED IN LYMPHOCYTE DEVELOPMENT, Jeffrey N. Siegel, Alfred D. Steinberg, William E. Paul, Mark M. Davis and David I. Cohen, Natl. Institutes of Health, Bethesda MD 20892

A cDNA isolate has been identified by screening a T cell-B cell subtracted cDNA library with a T_H hybridoma-T_S hybridoma subtracted cDNA probe. This cDNA represents one member of an X-linked gene family of 10-20 members. Transcripts from this XLR (X-linked lymphocyte regulated) gene family can be identified in certain B and T lymphocytes but not in normal kidney, liver or in non-lymphoid tumors. Studies of tumors of B-lymphocyte lineage demonstrate transcription in most late stage B cell tumors (e.g. secretory and pre-secretory plasmacytomas) but not in early stage B cell tumors (e.g. pre-B cells), suggesting that transcription of XLR genes is regulated in development.

We have screened cDNA libraries from a secretory and a pre-secretory plasmacytoma, from normal thymus cells and from spleen cells with the XLR gene probe. By DNA sequencing, we have determined that in all these cell types there is one common XLR transcript present. In addition, unique cDNA clones were identified which potentially represent distinct, cell type specific XLR transcripts. Sequence data comparing these cDNA isolates will be presented and the possible role of these distinct transcripts in differentiation will be discussed.

Hormones and Transcription, Expression Systems and Transcriptional Regulation and RNA Processing II

0184 CYCLIC AMP INDUCES CELL-TYPE SPECIFIC MESSENGER RNA ACCUMULATION BY INTERACTING AT A CELL SURFACE RECEPTOR IN THE CELLULAR SLIME MOLD, DICTYOSTELIUM DISCOIDEUM, Daphne D. Blumberg and Masakazu Oyama, National Cancer Institute, Frederick, MD 21701-1013.

The accumulation of many post-aggregative mRNA species in Dictyostelium discoideum is dependent upon the continuous presence of elevated levels of cAMP. Analysis of the cyclic nucleotide specificity of this requirement shows that it is similar to that of cell surface receptor and distinct from the specificity displayed by the cAMP-dependent protein kinase. The same specificity is displayed for the accumulation of two classes of prespore mRNAs, a prestalk mRNA and for the shut-off of a growth phase mRNA. Half-maximal accumulation of prestalk mRNA is obtained at cAMP concentrations of 320 to 520 nM, while 1 to 2 μM is required for half-maximal accumulation of the prespore mRNAs and shut-off of the growth phase mRNA. The cell surface receptor can, in the presence of cAMP and a GTP binding protein, interconvert from the high affinity form (Kd=60 nM) responsible for chemotaxis to a lower affinity form (Kd=450 nM) (Van Haasert and DeWit, J. Biol. Chem. 259:13321, 1984), precisely in the range at which cAMP induces expression of prestalk mRNAs. While accumulation of prestalk mRNAs occurs in response to cAMP as soon as cells reach aggregation stage, accumulation of prespore mRNAs requires some form of cell-cell interaction. We suggest that cell adhesion is responsible for a further change in the receptor or its environment which allows it to recognize the higher concentration of cAMP necessary for prespore mRNA induction. These effects of cAMP on gene expression have been obtained under conditions where activation of the receptor associated adenylate cyclase is completely inhibited.

0185 EPSTEIN-BARR VIRUS INFECTION INDUCES fgr PROTO-ONCOGENE mRNA IN B LYMPHOCYTES Marc S. C. Cheah, Steven R. Tronick and Keith C. Robbins. National Cancer Institute, Bethesda, Maryland 20892

Several acute transforming retroviruses encode tyrosine specific protein kinases which possess structural and functional relationships to cell surface receptors for certain growth factors. One such tyrosine kinase is encoded by the onc gene, v-fgr of Gardner-Rasheed feline sarcoma virus (GR-FeSV). Recently we described the isolation and characterization of the human gene, c-fgr corresponding to the viral onc sequence and showed that human c-fgr is a unique gene located on chromosome 1p36.1-36.2 (PNAS 82:6595,1985). Utilizing DNA probes which represent the GR-FeSV tyrosine kinase gene, v-fgr, we have surveyed human tumor cells for expression of the fgr proto-oncogene. A single transcript, 3kb in length, was detected in approximately 50% of the Burkitt's lymphomas but in none of the sarcomas and carcinomas examined. Further analysis showed that American Burkitt's lymphomas were uniformly negative, whereas the fgr proto-oncogene was transcriptionally active in all African Burkitt's lymphomas tested. It was of interest that expression correlated with the presence of the Epstein-Barr virus (EBV) genome in all the Burkitt's lymphomas analyzed. Normal umbilical cord or peripheral blood lymphocyte lines established in vitro by EBV infection were also found to contain detectable c-fgr mRNA. Moreover, a 50-fold induction of steady state c-fgr mRNA concentration was observed when uninfected Burkitt's lymphoma cell lines were deliberately infected with EBV. These findings demonstrate for the first time the induction of a proto-oncogene in response to infection by a DNA tumor virus.

0186 **Abstract Withdrawn**

0187 RETINOIC ACID INDUCED EXPRESSION OF TISSUE TRANSGLUTAMINASE IN PERITONEAL MACROPHAGES, Joseph P. Stein and Peter J. A. Davies, University of Texas Medical School, Houston, Texas 77030

Retinoids are a class of lipid molecules that have dramatic effects on the growth and differentiation of both normal and neoplastic cells. We have recently demonstrated that retinoic acid acts as a direct and acute regulator of the expression of a specific enzyme, tissue transglutaminase (TGase), in mouse and human myeloid cells. Specifically, exposure of resident mouse peritoneal macrophages to nanomolar levels of trans-retinoic acid causes the enzyme to accumulate to 1% of total cellular protein within 24 hours. Two arguments suggest that this induction of TGase activity occurs at the level of transcription: actinomycin D completely blocks the induction, and exposure of the cells to retinoic acid causes a significant increase in the amount of translatable TGase mRNA within 30 minutes. Further evidence that retinoids have a direct effect on TGase expression is the fact that cycloheximide does not block the stimulation of TGase mRNAs. Retinoic acid and intracellular cyclic AMP act synergistically to stimulate TGase expressions in both normal and leukemic myeloid cells. We believe the induction of tissue TGase in myeloid cells is the first example of how retinoids might act in a direct fashion to regulate specific gene expression.

0188 Don J. Diamond and Susumu Tonegawa, Center for Cancer Research, M.I.T., Cambridge, MA 02138

Recently, the analysis of the sequence organization of the T-cell receptor beta- and gamma-chain gene families has been reported. We now present an initial characterization of the murine T-cell receptor alpha-chain gene family, and conclude that although it is related to both immunoglobulin and other T-cell receptor gene families, it shows unique characteristics. There is only a single constant (C) region gene segment which in mnay cases is an exceptionally large distance (ca. 20-40 kilobases) from joining (J) gene segmanets. The J-cluster is extremely large with as many as 25 distinct J-gene segments already identified.

The expression of some lymphocyte-specific genes has been shown to be controlled by an element called an enhancer located within the intron between J and C gene glusters of both heavy and light chain immunoglobulin genes. We are investigating whether an analagous situation occurs within the intron of both T-cell receptor alpha and gamma genes. In the case of the gamma genes a typical sized intron (ca. 6 kilobases) is present between the J and C clusters, and we have constructed vectors using the CAT assay system to test for T-cell specific enhancing elements. The situation of the alpha-gene is more enigmatic since the intron between a given J segment and the single C segment is as large as 40 kilobases. We have adapted a similar approach of cloning successive fragments from the intron using our alpha gene cosmid into CAT expression vectors, and then testing for T-cell specific enhancer elements in transfected T-cell lines.

0189 GLUCOSE-DEPENDENT INDUCTION AND REPRESSION OF THE YEAST ENOLASE I STRUCTURAL GENE. Michael Holland, Regina Cohen, Teresa Yokoi, Alan Penner, Paul Brindle and Janice Holland, Department of Biological Chemistry, University of California, Davis, CA 95616.

There are two yeast enolase genes, designated ENO1 and ENO2. Both genes are expressed at similar levels in cells grown on gluconeogenic carbon sources. ENO2, but not ENO1, is induced more than 20-fold in cells grown on glucose. The cis-acting regulatory sequences within the 5' flanking regions of both genes have been identified by deletion mapping analysis. Both genes contain functionally similar bipartite upstream activation regions located 500bp upstream from the respective translational initiation codons. These activation regions are required for expression of each gene in cells grown on glycolytic or gluconeogenic carbon sources and they mediate glucose-dependent induction of gene expression. The ENO1 gene also contains an upstream repression region located 210bp upstream from the initiation codon which has been mapped to a 25-30bp sequence. ENO1 genes carrying a deletion of all or a portion of this repression region are induced in cells grown on glucose to levels comparable to the wild type ENO2 gene. The wild type ENO1 gene, but not the ENO2 gene, is derepressed when glucose is exhausted from the growth medium. ENO1 genes carrying a deletion of the repression region are not further derepressed when glucose is exhausted from the growth medium. Glucose-dependent repression of ENO1 is not mediated by the same mechanism involved in glucose-dependent repression of the yeast alcohol dehydrogenase II gene. From this analysis we conclude that the ENO1 structural gene is simultaneously induced and repressed in cells grown on glucose.

0190 TRANSIENT AND STABLE EXPRESSION OF HUMAN CLASS II HISTOCOMPATIBILITY ANTIGENS IN CELLS TRANSFECTED WITH cDNA CLONES, Eric O. Long and Rafick Sekaly, Laboratory of Immunogenetics, NIAID, Bethesda, MD 20892

Class II antigens of the major histocompatibility complex (MHC) are required for the recognition of foreign antigens by certain T lymphocytes. Three class II MHC antigens consisting of distinct alpha and beta chains exist in man, called HLA-DP, -DQ and -DR. To define the requirements for their proper assembly and cell surface expression and for their function in antigen presentation we have used expression systems in transfected cells. Full-length cDNA clones for the alpha and beta chains of DP, DQ and DR antigens have been cloned in expression vectors carrying either the SV40 early promoter or the promoter from the LTR of Rous Sarcoma Virus (RSV). No splice site exists on either vector. Using transient expression in COS cells we have shown that assembly and cell surface expression of alpha-beta dimers do not require the presence of the invariant chain. Transfected human and murine cell lines were selected for cell surface expression of class II antigens. Stable clones were used to show that a single class II antigen was sufficient to present viral antigens to specific T lymphocyte clones.

0191 Expression of the E. Coli D-xylose isomerase gene in S. cerevisiae

V. V. Mackedonski,
Solar Energy Research Institute, Golden, Co 80401

The D-xylose isomerase (EC 5.3.1.5) gene fron Escherichia coli was cloned on a 1.6 KB Bgl II DNA fragment. The bacterial control regions of the gene were enzymatically removed and the resulting 1.4 KB DNA fragment was ligated downstream of the GAL1 or GAL10 promoter.

The GAL promoter - xylose isomerase gene constructions was cloned in two diffrent yeast cloning vectors, pBM258 (M. Johnston and R. Davis, Molec. Cell. Biol., 4:1440,1984) and pSEY101 lacZ fusion vector (M. G. Douglas et al, PNAS 81:3983, 1984).

The expression of the bacterial xylose isomerase gene in yeast S. cerevisiae was followed by the indicator blue color on X-Gal plates, measuring the xylose isomerase activity in cell extracts and antibodies made against purified xylose isomerase protein.

0192 Promoter Elements located within the coding region of yeast genes.
J. Mellor, M.J. Dobson, N.A. Roberts, S.M. Kingsman & A.J. Kingsman.
Dept. of Biochemistry, University of Oxford, South Parks Road, Oxford, U.K.

We have created a number of hybrid transcriptional units consisting of the 5' and 3' non-coding regions of the yeast phosphoglycerate kinase (PGK) gene flanking the coding regions of other genes such as DEX, Ty, TRP1 and human interferonα-2 (IFNα-2). These hybrid genes have been placed in a 2μ based high copy number plasmid and the steady-state RNA levels compared to the intact PGK gene on a similar plasmid. Hybrid genes containing Ty or DEX coding regions produce high RNA levels, similar to those from the intact PGK gene. In other hybrid genes we have examined, the steady-state RNA levels are 5 to 10 fold lower than those from the intact PGK gene. Using a PGK:IFNα-2 hybrid gene we have shown that this difference in RNA levels is not due to differences in plasmid copy number, nor to an increased rate of turnover of the hybrid transcripts, but is due to a 5 to 10 fold reduction in the rate of initiation of transcription. This reduction suggests that the PGK gene has promoter functions located within the coding region.

Using the IFNα-2 structural gene fused to deletion end points throughout the PGK coding region we have located this promoter function, the downstream activator sequence DAS within the first 235bp. We have compared the sequences within this region of the PGK gene with those present in the coding region of the two yeast genes Ty and DEX which do not show low RNA levels and synthesised an oligonucleotide containing the core of the DAS. This 18bp oligonucleotide is able to partially restore full promoter activity to a PGK - IFNα-2 hybrid gene.

0193 MULTIHORMONAL REGULATION OF MILK PROTEIN GENE EXPRESSION, Jeffrey M. Rosen, Craig H. Couch, Yvonne David-Inouye, Sheldon M. Campbell, Kuo-Fen Lee, Chester A. Bisbee and Li-Yuan Yu-Lee, Baylor College of Medicine, Houston, TX 77030
Milk protein gene expression is regulated by the complex interplay of several peptide and steroid hormones, as well as cell-cell and cell-substratum interactions. Both casein and whey acidic protein (WAP) gene expression are induced in explant cultures by a combination of insulin (I), hydrocortisone (F) and prolactin (M). However, in both primary mammary epithelial cells and non-transformed cell lines, maximal levels of casein gene expression are observed only when cells are cultured on a floating type I collagen gel matrix, but not when cells are grown on a plastic substratum. In contrast, WAP gene expression is repressed under both of these conditions. In order to examine these regulatory events, we have transfected the entire rat β-casein and WAP genes, an α-casein minigene and 5' flanking CAT fusion gene constructions into several mammary epithelial cell lines and primary cultures, as well as introduced the rat β-casein gene into transgenic mice. Only a 2-fold induction of fusion gene constructs has been observed following the addition of F and M to cultures containing I. Both the entire β- and α-casein minigenes were expressed, but the RNA transcripts observed appear to be incorrectly processed. This may reflect the failure of prolactin to elicit the expected post-transcriptional regulation in these transfectants. In contrast, WAP gene expression was not observed in the majority of transfectants analyzed. (Supported by NIH CA16303)

0194 ALTERATIONS OF cAMP AND DNA METHYLATION CONCOMITANT WITH INDUCTION OF DIFFERENTATION OF MURINE NEUROBLASTOMA, NB15, Herman S. Shapiro, New Jersey Medical School, Newark, N.J. 07103

Morphological and biochemical differentiation of murine neuroblastoma cells may be induced by growth in serum-free medium or by a variety of chemical agents among which is RO 20-1724; an inhibitor of phosphodiesterase. NB15 cells maintained for 48 hours in serum-free medium or in full medium supplemented with RO 20-1724 show elevated levels of both acetylcholinesterase activity and intracellular concentration of cAMP compared to control cultures.

An inverse correlation between gene expression and the level of methylated cytosine near promoter regions of eukaryotic DNA is recognized. Our analyses of methylcytosine in DNA isolated from differentiated NB15 cell (formation of neurite extensions) showed a statistically significant hypomethylation compared to DNA from undifferentiated cells. We had previously shown that hypomethylation contributes to profound changes in the conformation of chromatin in murine erythroleukemic cells. The present study indicates that factors known to contribute to modification of DNA and protein conformation; methylation or cAMP; simultaneously accompany cellular differentiation of a neuroblastoma cell line and may synergistically influence diffential transcription of the total genomic material.

0195 Abstract Withdrawn

0196 ANALYSIS OF HUMAN GLUCOCORTICOID RECEPTOR MUTANTS BY USE OF MONOCLONAL ANTIBODIES AND cDNA's, E. Brad Thompson, Javed Ashraf, Bahiru Gametchu, Dept. of Human Biol. Chem. & Genetics, Univ. of Texas Medical Branch, Galveston, TX 77550; R. Evans, The Salk Institute, La Jolla, CA; and Jeffrey M. Harmon, Dept. of Pharmacol., USUHS, Bethesda, MD. After in vitro mutagenesis with a frame-shift mutagen, clones of glucocorticoid-resistant cells were isolated from the glucocorticoid-sensitive parental clone, CEM C7. The sensitive cells are lysed by glucocorticoids; the resistant cells grow unabated in 10^{-6}M dexamethasone. The predominant receptor phenotype of the mutagenized, resistant clones was paucity of steroid binding sites (r^-), usually < 10% wild type. This binding phenotype could be due to a down regulation of receptor transcription, to non-receptor factors that cause a decrease in binding, or to loss of receptor mRNA and/or protein. A typical r^- clone, ICR 27, was chosen for detailed analysis. Somatic cell hybrid and cytosol mixing experiments between ICR 27 (r^-) and CEM C7 (r^+) cells have shown no evidence for dominance of the r^- phenotype. Labelling with the affinity ligand dexamethasone mesylate showed a small amount of receptor of normal size in ICR 27 cells. But immunoblotting with polyclonal and monoclonal anti-receptor antibodies showed a large amount of an immunologically reactive protein the size of holoreceptor. Analysis of the cellular RNA's from CEM C7 and ICR 27 cells showed that they had comparable amounts of glucocorticoid receptor-specific RNA. Thus the r^- phenotype in ICR 27 cells is not due to down-regulation of receptor transcription nor to a net loss of receptor protein. Their resistance to glucocorticoids appears to correlate with a loss of ability to bind the steroid, and they are candidates for mutants in the steroid binding site of the receptor.

0197 ANALYSIS OF THE TRANSCRIPTION TERMINATION REGION OF THE HISTONE H5 GENE
Markus Affolter and Adolfo Ruiz-Carrillo, Cancer Research Center and Department of Biochemistry, School of Medecine, Laval University, Quebec, Canada.

We have previously defined the transcription unit of the chicken H5 gene and found that approximately 90% of the engaged RNA polymerase B molecules terminate transcription within a region of 500 base pairs immediately downstream of the polyadenylation site. S1 nuclease protection experiments indicate that the downstream sequences are also transcribed in vivo, since they are present in steady-state RNA, and that their relative amounts reflect the distribution of RNA polymerases observed in vitro. No discrete site(s) of transcription termination could be identified. No obvious region of homology was observed when we compared our sequence with other RNA polymerase B termination regions or with yeast terminator-related sequence elements. In the region where termination occurs, we identified 1) an S1 hypersensitive site in supercoiled plasmids, 2) two closely (~50 nucleotides apart) DNase I hypersensitive sites which occur only in H5 expressing chromatin, and 3) a sequence that could form a very stable hairpin in pre-mRNA molecules. To examine whether these structures are involved in the process of transcription termination, RSV-H5 wildtype and mutant genes lacking one or several of these features, have been transfected into an established chicken embryo fibroblast cell line. Analysis of the transcription pattern of the mutants as well as the mapping of DNase I hypersensitive sites in the chromatine of the transfected genes will be presented.

0198 Studies on the Expression of the Somatostatin Gene. Ourania M. Andrisani and Jack E. Dixon. Purdue University, West Lafayette, IN 47907.

The genes of the endocrine pancreas are responsible for the synthesis of several peptide hormones which have pronounced effects on the overall homeostasis of the animal. One of these genes encodes peptide somatostatin which regulates insulin and glucagon release. The rat somatostatin gene has been isolated from a λ phage library. In order to study the regulation of the somatostatin gene, a fragment corresponding to 750 bp 5' to the start of transcription was cloned in front of the bacterial chloramphenicol acetyl transferase gene. Promoter deletions were constructed using conveniently located restriction sites or by Bal 31 nuclease digestion. The activity of the somatostatin promoter and its deletions were tested in two cell lines; Hela cells which do not express any endogenous somatostatin, and CA-77 cells (derived from a rat medullary thyroid carcinoma) which produces endogenous somatostatin. Plasmid DNA was introduced into the Hela and CA-77 cell lines by calcium phosphate precipitation and electroporation, respectively. Briefly, the following results were obtained: 1) the 750 bp fragment harboring the somatostatin promoter (+1 to -750) is transcriptionally inactive in Hela cells, but is active in CA-77 cells; 2) the plasmid containing the promoter sequences between +1 and -250 is active in CA cells but not in Hela cells; 3) removal of the sequences between -200 and -750 renders the somatostatin promoter active in Hela cells; and all the deletions show promoter activity in CA-77 cells, except the deletion which contains sequences between +1 and -43. These results will be discussed with respect to somatostatin promoter expression and regulation.

0199 CELL-TYPE SPECIFIC EXPRESSION AND HORMONAL REGULATION OF THE RAT PROLACTIN (PRL) GENE, Carter Bancroft and Thomas C. Lufkin, Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029

To investigate cell-type specific expression, a PRL-CAT chimeric gene consisting of 1.95 kb of PRL upstream flank fused to the bacterial CAT gene (pPRL-CAT) was employed. Either during transient expression or in stably transfected cells, PRL promotor activity was strong in either GH_3 rat pituitary cells or their PRL-negative GC cell variants, but was low or undetectable in glial, kidney, or fibroblast cell lines. In contrast, neither pRSV-CAT, pSV_2-CAT, nor a mouse metallothionein-CAT plasmid (pmMT-CAT) were expressed especially strongly in GH_3 cells. RNase protection experiments showed that in the GH_3 cells, transcription of PRL-CAT is initiated correctly at the PRL cap site. Deletion analysis of the PRL promotor region in pPRL-CAT yielded a complex structure, with alternating positive and negative regulatory regions lying within the first 600 bp upstream of the cap site. Preliminary results suggest that DNA sequences required for regulation by a number of hormones lie within the first 300 bp upstream of the PRL cap site.

0200 CIS AND TRANS ACTIVATION OF OPIOID PEPTIDE GENES. Neal C. Birnberg, Michael J. Comb, Howard M. Goodman. Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

Our interests are in characterizing pathways in which ligand-bound cell surface receptors couple with membrane-localized enzymes to activate intracellular second messenger systems and regulate transcription of specific genes. The second messengers are thought to induce a cascade of covalent modifications of cell components which activates effector molecules to interact with specific DNA sequences to regulate transcription. We have identified a *cis* acting DNA sequence in the human proenkephalin A gene between positions -80 and -160 which confers significant inducibility to a linked gene in the presence of cAMP. This genetic element is active in both orientations, shows distance independence and can be transferred to heterologous promotors. We have also characterized a property of a putative mutant coupling protein encoded by the activated c-Ha-Ras proto-oncogene. This gene acts in *trans* to increase expression of co-transfected opioid peptide genes in a highly cell-type specific fashion. This activity is specific to the activated form of the proto-oncogene. The DNA sequence in the promotor of the proenkephalin gene required for *trans* activation has been mapped to a region within 60 bp of the site of transcription initiation.

0201 FUNCTIONAL ANALYSIS OF THE GLUCOCORTICOID REGULATORY REGION OF MOUSE MAMMARY TUMOR VIRUS (MMTV) DNA. E.Buetti, B.Kühnel and H.Diggelmann, Swiss Institute for Experimental Cancer Research,CH-1066 Epalinges, Switzerland

Starting from two series of 5' and 3' resected molecules, internal deletions and substitutions using molecular linkers were introduced in the glucocorticoid regulatory region of the MMTV LTR. Their effect on the MMTV promoter, fused to the coding sequence of the HSV thymidine kinase gene, was studied in Ltk$^-$ cells either stably or transiently transfected with the altered DNA using a quantitative S1 nuclease mapping of the transcripts.Deletion of both in vitro binding sites for hormone-receptor complexes (-193 to -70bp) abolished the hormone response. Deletions of the proximal(-129 to -78) or the distal (-193 to -162bp) binding sites had only$\sim$10% of the wild-type level. Insertion of a synthetic distal element at the site of the proximal one restored the hormone response, either partially or completely, depending on the extent of the initial deletion. In the distal region,no decrease in the response was seen with linker-scanning mutations 5' of-186 or 3' of-172.A reduction to $\sim$10% of wt was found in mutants between -177 and -173bp, affecting a conserved hexanucleotide of in vitro binding sites. A significant reduction (to$\sim$20% of wt) was also seen in mutants between -185 and -177, which retain the hexanucleotide. In the proximal region, a reduction to$\sim$20% was found in mutant LS-125/-116 and in deletion -90/-70, while others did not affect the hormone response. No decrease was seen in mutants between -161 and -132 bp.

0202 TRANSFER OF THE FUNCTION OF THE TCDD RECEPTOR BY TRANSFECTING HUMAN DNA INTO MOUSE CELLS. Fong-Fong Chu, Fred Sander and Oliver Hankinson, Lab of Biomed. & Environ. Sciences and Dept. of Pathology, UCLA, Los Angeles, CA 90024

High m.w. DNA prepared from a human hepatoma cell line (HepG2) was used to transfect a mutant line of mouse hepatoma cells (Hepa-1). This mutant has been characterized as aryl hydrocarbon hydroxylase (AHH) non-inducible, and its receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is deficient in nuclear translocation. TCDD receptor is required for the induction of AHH activity. Usually DNA from pSV2gpt DNA was cotransfected with human DNA. On average, the frequency of 4×10^{-4} was obtained for pSV2gpt DNA as assessed by selection with HAT medium, while for the TCDD receptor gene the frequency of stable transfection was 6×10^{-8}. The selection for human TCDD receptor gene is based on our previously described procedure for isolating AHH$^+$ cells.

Seven AHH$^+$ clones were obtained. Among them, three are HAT^r, while four remain HAT^s. Although one of the HAT^s clones is derived from a transfection without the addition of pSV2-gpt DNA, the three other HAT^s were derived from cotransfections that were only selected for AHH$^+$ at the initial screening. All three of the HAT^r clones contain considerable amounts of human DNA. Only one of the HAT^s clones contains human DNA, and its amount is only 1/30th of that in the HAT^r clones. It is clear that when cells only take up small amounts of DNA, they may not take both high m.w. and plasmid DNAs simultaneously. The human transfectants should provide a means for us to clone the human TCDD receptor translocation gene. *(This investigation was supported by PHS Grant # CA-09030 awarded by the National Cancer Institute.)*

0203 DNA SEQUENCE REQUIREMENTS FOR THE EFFICIENT FORMATION OF POLYADENYLATED mRNA 3' TERMINI J. Barklie Clements & John McLauchlan, MRC Virology Unit, Glasgow G11 5JR, Scotland

Polyadenylation of eukaryotic mRNA requires the poly A signal (AATAAA) and other further downstream signals. Using a modular transcriptional assay system we have identified a consensus DNA sequence (YGTGTTYY) located approximately 30 base pairs downstream from the AATAAA signal (McLauchlan et al, Nucl. Acids Res. 13, 1347-1360, 1985), beyond the poly A site, which is required for efficient formation of mRNA 3' termini. To a lesser extent, the removal of further downstream DNA also reduced the level of 3' end formation.

To examine the properties of processing signals at mRNA 3' termini: (i) we have altered the distance between the AATAAA and the YGTGTTYY signals and have separated the signals to test their individual funcitons (ii) we have inserted DNA fragments containing these signals at different locations on DNA constructions containing transcriptional activators and promoters.

0204 ANALYSIS OF GLUCOCORTICOID RECEPTORS FROM WILD TYPE AND VARIANT MOUSE CELL LINES USING A MOUSE GLUCOCORTICOID RECEPTOR cDNA CLONE, Mark Danielsen, Jeffrey P. Northrop and Gordon M. Ringold, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305

We have previously characterized WEHI-7 and S49 mouse lymphoma cell lines, which have altered glucocorticoid responsiveness, using affinity labelling and Western blots (J. Biol. Chem. 260, 6398-6403, 1985). To further these studies we have isolated glucocorticoid receptor (GR) cDNA clones from wild type and variant cell lines. These clones represent 6 Kb of the approximately 7 Kb receptor mRNA. Northern blot analysis of poly A+ mRNA reveals two forms of GR mRNA in wild type cells, a 7 Kb major form and a 5 Kb minor form. The 5 Kb form lacks 2 Kb of 3' non-coding sequences. We have characterized the GR transcripts in both S49 (nt^i, nt^-, r^-) variants and in W7 variants (r^-). All the variants except S49 nt^i have qualitatively similar GR transcripts. The nt^i cells, however, have an extra transcript of approximately 5.5 Kb. Detailed analysis of this transcript reveals that it lacks 1.5 Kb of 5' receptor sequence. This is consistent with the presence in nt^i cells, of a truncated 40 Kd receptor protein in addition to a 90 Kd receptor. The 40 Kd protein retains DNA and hormone binding activity suggesting that the C-terminal half of the receptor protein contains both the DNA-binding and hormone-binding domains. In addition, the so-called modulatory domain of the GR, which is missing in the 40 Kd nt^i receptor protein, is most likely contained within the N-terminal half of the GR.

0205 **ASSOCIATION-DISSOCIATION PROPERTIES OF THE E. COLI TRANSCRIPTION TERMINATION PROTEIN RHO,** **J. Geiselmann, T. Yager, S. Gill, J. McSwiggen, W. Baase and P.H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229**

Essential steps in the mechanism of action of the transcription termination protein rho of E. coli include binding to the nascent RNA transcript and a concomitant hydrolysis of ATP. It is not yet understood how these events (and possibly others) combine to cause transcript release. The active form of rho appears to be a hexamer of 46.1 kD protomers. We are using various physical techniques to define and analyze the association equilibria involved in generating the active form of rho.

Our results (at [rho] = 1 mg/ml) are as follows: (i) At [KCl] > 0.20 M, rho exists predominantly as a 9.5 S species. (ii) Upon lowering [KCl] to 50 mM, the 9.5 S species is converted to an 11-12 S species. This process is reversible. (iii) Addition of Mg^{++} or ATP at 0.5 M KCl has no effect on the association state. (iv) At 0.2 M KCl, addition of oligo(rC) (n = 23±1) causes the conversion of the 9.5 S form to the 11-12 S form. This can be reversed by raising the KCl concentration. (v) Association to the 11-12 S form does not stimulate the ATPase activity, in the absence of RNA (2.5% glycerol present).

Currently we are working to relate these observations to additional studies of the RNA binding site size of rho (13±1 nucleotides/protomer) and of the dependence of ATPase activity on RNA length. We ultimately seek a detailed molecular picture of how rho functions in transcript release. [Supported by USPHS Research Grant GM-29158 (to PHvH) and USPHS Postdoctoral Fellowship GM-10227-02 (to TY).]

0206 **STUDIES OF IgM TRANSCRIPTION, ASSEMBLY AND SECRETION FROM HUMAN HYBRIDOMA CELL LINES,** Michael Glembourtt, Mark Jahnsen, Edward Max* and James Larrick, Cetus Immune Research Laboratories, 3400 West Bayshore Road, Palo Alto, CA 94303; *National Institutes of Health, Bethesda, MD 20205

We have generated a number of anti-lipopolysaccharide human monoclonal antibody secreting cell lines which provided an opportunity to study human immunoglobulin gene expression. Peripheral blood B cells were transformed by Epstein-Barr virus followed by fusion to a ouabain- and 6-thioguanine-resistant cell line (F3B6) of mouse (NS1)/Human (B cell) origin. These chimaeric cell lines produce functional pentameric IgMs. These predominantly murine cell lines contain both human and murine heavy- and light-chain genes. Genomic blots utilizing human and mouse J-chain probes showed the presence of only the murine J-chain gene. Northern blots showed that murine J-chain, a murine K light chain and human heavy and light chains were transcribed. The secreted IgM was assembled with mouse J-chain and contained only human heavy and light chains. No secretion of IgM was observed from hybridomas made with another mouse/human cell line (MTP) which has very low transcription of mouse J-chain RNA. Studies using antisense expression of J-chain cDNA are in progress to further delineate the role of J-chain gene expression in IgM assembly and secretion.

0207 ALTERNATIVE RNA SPLICING AND POLYADENYLATION ARE RESPONSIBLE FOR THE GENERATION OF NONMUSCLE AND MUSCLE TROPOMYOSIN ISOFORMS FROM A SINGLE GENE, David M. Helfman, Steve Cheley, Esa Kuismanen, Linda A. Finn, and Yuriko Yamawaki-Kataoka, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Full-length cDNA clones encoding tropomyosins from rat embryonic fibroblasts (REF) and skeletal muscle were used to study the relationship of tropomyosins expressed in these cell types. Sequence comparison of cDNA clones encoding REF tropomyosin 1 (TM-1) with skeletal muscle beta-tropomyosin revealed virtual identity between these isoforms except for two carboxy-proximal regions (amino acid residues 189-213 and 258-284), and completely different 3'-untranslated regions. These isoform-specific regions delineate the troponin-T binding domains of skeletal muscle tropomyosin. Northern transfer and S1 analyses indicate that smooth muscle (stomach, uterus and vas deferens) express the same isoform as fibroblasts. Analysis of genomic clones revealed that a single gene containing 11 exons and spanning ~10 Kb encodes TM-1 and skeletal muscle beta-tropomyosin. Sequences common to all mRNAs were found in exons 1-5 (amino acids 1-188) and exons 8-9 (amino acids 214-257). Exons 6 and 11 encode amino acids 189-213 and 258-284, respectively, in fibroblast and smooth muscle mRNAs. Exons 7 and 10 encode amino acids 189-213 and 258-284, respectively, in skeletal muscle mRNA. In addition, exons 10 and 11 contain the entire 3'-untranslated sequences of the respective mRNAs. S1 and primer extension analyses indicate all mRNAs expressed from this gene are transcribed from a single promoter. Supported by NIH grants CA40599 and CA29569, MDA and EMBO.

0208 TRANSCRIPTIONAL CONTROL OF THE P-ENOLPYRUVATE CARBOXYKINASE GENE. Y. Hod, J.M. Short, A. Wynshaw-Boris and R.W. Hanson, Case Western Reserve Univ. Cleve, OH 44106

The gene encoding P-enolpyruvate carboxykinase (EC 1.1.1.32) (PEPCK) is a model to study the mechanism of hormone control of gene expression. The transcription rate of the PEPCK gene can be induced by glucagon and epinephrine (acting via cAMP) or by Bt_2cAMP. Enhancement of PEPCK gene transcription by Bt_2cAMP is dependent on a nucleotide domain located in the 5'-flanking region of the gene. Analysis of several graded deletions in this region, maps a core element (CTTACGTCAGAG) essential for induction of PEPCK gene transcription by cAMP between positions -91 to -80. Further analysis of the deletion mutants suggests the presence of an inhibitory sequence which may function to maintain low basal levels of gene transcription. To study the molecular basis of cAMP regulation of PEPCK gene transcription, we have developed a cell-free transcription assay using extracts from hormone-responsive hepatoma cells. Initial fractionation of cellular extracts show that both a nuclear and the post-polysomal fraction are required to confer accurate transcriptional initiation at the CAP site. This activity is sensitive to 1 μg/ml of α-amanitin, suggesting the involvement of RNA polymerase II. Modest induction of PEPCK gene transcription was observed when cAMP was included in the incubation medium. Attempts to optimize the *in vitro* effect of cAMP will be discussed. Results with *in vitro* transcription of the neomycin-resistance gene driven by a deleted PEPCK promoter, correlate with our *in vivo* data and suggest the presence of an inhibitory sequence in the PEPCK promoter. Development of a reconstituted transcription complex will allow identification of regulatory elements required for control of gene transcription.

0209 OPTIMIZATION OF EXPRESSION OF GENES TRANSFERRED USING RETROVIRAL VECTORS. J-K.Yee,[+] J.C.Moores,[°] A.D.Miller,[+] T.Friedmann,[+] D.J.Jolly,[~] [+]Univ. of California San Diego,Ca 92093,[°]Hutchinson Cancer Ctr. Wa 98104, [~]INSERM U33 Bicetre 94270, France. We have used the human HPRT gene as a model to explore factors affecting the introduction and expression of genes in retroviral vectors. We have examined:(1) the use of different cell lines as helper lines to increase HPRT viral titers and avoid accidental and unwanted production of competent helper virus;(2) alterations in the configuration of the viral genome. Expression has been tested on the Lesch-nyhan lymphoblast system already described (Willis et al. J.B.C.(1984) 259, 7842). We have transfected the defective helper genome pPAM (Miller et al. MCB (1985) 5, 431) into human LNSV and mouse SCTG1 cells. We have also further disabled the helper genome and put this into several mouse and human lines. Titers between 10^5-10^7 per ml. of HPRT virus were obtained. With the LNSV derived lines no competent retrovirus was produced from many independent transrecants suggesting that the frequency of patch repair of the defecive helper genome is low. Several arrangements of LTR, enhancers and promotors with rhz HPRT gene were assayed using such a packaging line, infecting Lymphoblasts and assaying bulk infectants for HPRT activity. The activity assay is fast, economical and quantitative, allows accurate estimation of absolute and relative enzyme activities and hence, presumably, gene expression. Immunoglobulin enhancers did not stimulate expression further. The metallothionine promotor (human MT IIA) did not confer inducibility to dexamethasone or Cd in the presence of murine LTR's and the largest increase in expression was achieved by deletions of oligo dG-dC stretches in the viral constructs.

0210 A NOVEL MECHANISM OF POST TRANSCRIPTIONAL, SEQUENCE-SPECIFIC REGULATION OF mRNA STABILITY, Robert Kamen*, Ian Kerr+, Gray Shaw*, *Genetics Institute, Cambridge, MA +Imperial Cancer Research Fund, London, UK.

AU-rich sequences within the 3' untranslated regions of murine and human GM-CSF (granulocyte-monocyte colony stimulating factor) mRNAs are more extensively conserved than protein encoding sequences (1,2). Similar AU-rich tracts are present in the 3' untranslated regions of c-fos (3), IL1, IL2, IL3, c-sis and α, β type interferons. To study the functional significance of these sequences, they were synthesized and inserted into the 3' non coding region of a rabbit β globin gene. As a control, we used an identical synthetic DNA except with G's and C's interspersed among the A and T's. Quantitative S1 analysis of mRNA produced after transient expression in a variety of cell types or in stabley transformed mouse cell lines revealed that insertion of the 50 nucleotide AU tract specifically reduced the accumulation of β globin mRNA's by more than 10 fold in all cases. Pulse-chase experiments suggest that the effect is caused by accelerated degradation of RNA containing the AU sequences. To test the hypothesis that the AU sequence is the target for a processing nuclease, SP6 generated GM-CSF mRNAs were incubated in mouse L cell extracts. Preliminary data indicate cleavage occurs at the AU sequences. The nuclease responsible is not stimulated by 2'-5' oligoadenylate nor inhibited by analogues of 2'-5'A, hence is distinct from the 2'-5'A dependent ribonuclease involved in the interferon response (4,5).

(1) Wong, G.G. et al. Science 17, 810-815 (1984), (2) Gough, N.M. et al. Nature 309, 763-767 (1984), (3) Meijlink, F. et al. P.N.A.S. 82, 4987-4991 (1985), (4) Wreschner, D.H. et al. Nature 289, 414-417 (1981), (5) Watling, D. et al. EMBO 4, 431-436 (1985).

0211 HORMONAL AND TISSUE SPECIFIC CONTROL OF RAT α_{2u} GLOBULIN: Kurtz, D., Addison, W., Danna, D. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

My laboratory is studying the molecular mechanisms underlying the hormonal and tissue specific control of the transcription of a rat gene family called α_{2u} globulin. Using in vitro mutagenesis of the promotor region of cloned α_{2u} globulin genes, we have succeeded in identifying a small upstream region ($\sim$ -150 to -120) which is absolutely required for glucocorticoid induction. Attempts are currently underway to isolate the protein factor(s) which interact with this region. In addition we are attempting to identify specific regions in or around cloned α_{2u} genes which are responsible for the tissue-specific expression of specific α_{2u} globulin gene sets.

0212 IDENTIFICATION OF PROMOTER AND REGULATORY REGIONS OF THE MURINE THYMIDINE KINASE GENE, Howard B. Lieberman, Pin-Fang Lin, Dong-Bor Yeh and Frank H. Ruddle, Department of Biology, Yale University, New Haven, CT 06511

Cytosolic thymidine kinase (TK) activity is increased in quiescent cells induced to enter S phase by the addition of serum. We have isolated functional genomic and cDNA clones encoding murine TK, and have characterized the structure of the gene. When our genomic clone is transfected into mouse L cells deficient in TK activity (LTK^-), the gene is expressed at much higher levels in rapidly proliferating cells compared to those in quiescence, indicating that the DNA sequences responsible for regulation are retained. In order to define promoter and regulatory regions of the gene, we have constructed a series of genomic/cDNA hybrid minigenes, introduced them into LTK^- cells, and tested for TK activity in stable transformants either rapidly proliferating or in quiescence. Using this strategy, we have identified a DNA fragment at the 5' end of the gene that has promoter activity, and have analyzed it by DNA sequencing and S1 mapping. In addition, we have found that an internal region of the gene enhances the level of TK expression in rapidly proliferating cells. Detailed analyses of these regions are in progress.

0216 LIVE, RECOMBINANT VACCINIA VIRUS ENCODING EXPRESSION OF THE FELINE LEUKEMIA VIRUS ENVELOPE PROTEIN, J.H. Nunberg and J.H. Gilbert, Cetus Corporation, Emeryville, California 94608

As part of our effort to develop a vaccine to protect domestic cats from feline leukemia disease, we have constructed an infectious, recombinant vaccinia virus expressing the envelope gene of Feline Leukemia virus (FeLV).

The FeLVs comprise a group of horizontally transmitted retroviruses associated with a variety of naturally occurring malignant and degenerative diseases in domestic cats. The major envelope glycoprotein of this virus, gp70, is believed to be involved in host-receptor binding and has been implicated as a major target for antibody-mediated virus-neutralization. Using methods developed by B. Moss and colleagues, we have inserted, into the vaccinia virus genome, the envelope (env) gene of the Gardner-Arnstein strain FeLV (subgroup B) under the transcriptional control of the vaccinia virus early '7.5K gene' promoter. Studies of the expression of the FeLV env gene have revealed no qualitative differences in the processing and intracellular transport of the vFeLVenv-encoded env gene product compared to that of FeLV. Proteolytic processing of the env gene product gp85 precursor protein, to yield mature gp70 and p15E, occurs early during processing within intracellular membranes; only the proteins accumulate on the cell surface.

Immunogenicity studies were performed using the vFeLVenv virus in mice and cats; preliminary results will be presented.

0217 TISSUE-SPECIFIC EXPRESSION OF THE CAT GENE DIRECTED BY THE LTR OF ROUS SARCOMA VIRUS IN TRANSGENIC MICE, Paul A. Overbeek[1] and Heiner Westphal[2], 1) Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030, and 2) Laboratory of Molecular Genetics, NIH, Bethesda, MD 20892

Transgenic mice were generated using pRSV-CAT, a plasmid that contains the 3' long terminal repeat (LTR) of Rous sarcoma virus (RSV) fused to the bacterial gene encoding chloramphenicol acetyltransferase (CAT). Five of the families of transgenic mice were found to express the CAT gene in a tissue-specific fashion. CAT enzymatic activity was detected primarily in tissues containing tendon, bone and muscle cells. This pattern of expression parallels the disease specificity of the intact Rous sarcoma virus. Although there are a wide range of factors that may influence viral disease specificity, these results suggest that the tissue-tropism of retroviruses may be determined primarily by the existence of tissue-specific transcriptional control factors that regulate expression of genetic information linked to the retroviral LTR.

The different families of RSV-CAT transgenic mice were bred to homozygosity. One family was found to have a recessive mutant phenotype. Homozygous mice of this family have fused toes in all four feet. Breeding experiments have indicated that the mutation is allelic to a previously described mutation named fused phalanges (sy^{fp}). We are currently cloning out and characterizing the genomic sequences flanking the RSV-CAT insertion in order to study the possibility that the insertion has inactivated a gene essential for normal toe development.

0218 CONSTRUCTION AND ANALYSIS OF BPV ..CTORS FOR THE EXPRESSION OF t-PA IN MOUSE CELLS. V.B. Reddy, A.J. Garramone, N. Hsiung and C.M. Wei, Integrated Genetics, 31 New York Avenue, Framingham, MA. 01701.

We have made t-PA expression vectors containing the entire BPV genome which is essential for maintaining the DNA in high copy number and episomal form. The t-PA transcriptional unit in these vectors consists of a mouse metallothionien (MT-1) promoter at the 5' end of the entire coding sequence of t-PA cDNA flanked at the 3' end by different transcriptional signals. In one vector, the 3' end contains MT-1 DNA with two introns and polyadenylation signal which were deleted and replaced, in other vectors, by SV40 early introns and poly A signal or by SV40 early poly A signal alone. DNA transfections into C127 cells and selection and analysis of transformed foci revealed that t-PA is expressed 100-200 fold more with the expression vector containing SV40 early polyadenylation signal alone. mRNA analysis indicated that the cells secreting more t-PA contained more t-PA mRNA. DNA analysis showed that the cells transformed by different vectors contained approximately the same number of DNA copies. Nuclear run-off experiments of different cell lines also revealed that there is not significant difference in the rates of transcription from the MT-1 promoter of three vectors containing different introns and poly A signals. We conclude in this report that (1) introns are not an essential part of gene expression and (2) the rate of transcription from the same promoter is not affected by the presence or absence of introns.

0219 ENHANCERS IN E. COLI: NR_I AND ACTIVATION OF glnAp2, Lawrence J. Reitzer and Boris Magasanik, Massachusetts Institute of Technology, Cambridge, MA 02139

E. coli genes that are expressed during nitrogen-limited growth require the glnG and glnF gene products. The glnALG operon is special among nitrogen-regulated systems because low levels of NR_I, the glnG product, can activte the major promoter of the glnALG operon, glnAp2, but not other nitrogen-regulated systems. There are three high affinity NR_I binding sites in the glnALG operon, two preceding the start of transcription by 100 to 150 bases, and one downstream from the glnA structural gene in glnA-glnL intercistronic region. Sequential deletion of the three NR_I binding sites weakens glnAp2 and higher levels of NR_I are required for activation. Full activation of glnAp2 is also achieved when the two NR_I sites preceding glnAp2 are instead placed 1400 bases upstream or downstream from the start of transcription. The NR_I binding sites activate transcription independent of distance or orientation and therefore regulate transcription like eukaryotic enhancers.

0220 EXPRESSION AND BIOLOGICAL ACTIVITY OF A MOUSE/HUMAN CHIMERIC ANTIBODY, B.Sahagan, H.Dorai, J.Saltzgaber, F.Toneguzzo, C.Guindon, S.Lilly, K.McDonald, D.Morrissey, B.Stone, G.Davis, G.Moore, Section for Molecular Biology, E.I. duPont de Nemours & Co., 331 Treble Cove Rd., N.Billerica, MA 01862

Chimeric immunoglobulin genes containing murine variable region exons fused to human constant region exons were constructed. Our goal was both to study the expression of novel immunoglobulin genes in lymphoid cells and to produce an antibody capable of escaping surveillance by the human immune system while retaining the antigen specificity of a murine monoclonal. The murine variable regions were therefore isolated from the functionally expressed kappa and gamma 1 immunoglobulin genes of the murine hybridoma cell line B6.2. The monoclonal antibody secreted by B6.2 reacts with a surface antigen from human breast, lung and colon carcinomas. The kappa and gamma 1 chain fusion genes were cloned into pSV_2gpt and pSV_2neo, respectively, and co-introduced into the non-antibody producing murine myeloma cells J558L, P3X63-Ag8.653 or SP2/0-Ag14 by electroporation. Analysis by ELISA of transfectants expressing the gpt gene demonstrated that more than 50% were secreting antibody reactive not only with anti-human IgG but also with the human tumor antigen recognized by murine B6.2 antibody. Furthermore, the chimeric and murine B6.2 antibodies have identical cell type specificities and affinity for antigen as demonstrated by immunofluorescence and competition ELISAs. Western analysis showed that the level of expression of chimeric kappa and gamma chains varies extensively. Experiments to understand this range of expression are in progress.

0221 COORDINATELY REGULATED EXPRESSION OF A SET OF SINGLE-COPY GENES ASSOCIATED WITH A COMMON REPETITIVE ELEMENT. Stephen A. Saxe and Alan R. Kimmel, LCDB, NIADDK, NIH, Bethesda, MD 20892

The organization of the M4 repetitive element in Dictyostelium has been previously described (Kimmel, A.R. and Firtel, R.A. (1985) Mol. Cell. Biol. 5: 2123). The entire M4 repeat sequence is (AAC/GTT)n which is present in ~100 copies in the genome, interspersed with single-copy DNA. 50-100 different mRNAs are transcribed from single-copy sequences with an associated M4 repeat. The M4 repeat is asymmetrically transcribed with only (AAC)n sequences represented on these compound mRNAs. We have isolated cDNAs corresponding to 10-20% of the M4 mRNA family. Each cDNA is specific to a unique member and contains sequences derived from both single-copy and M4 DNA. Each mRNA is expressed at a very low level during normal cell growth. During a specific developmental period all of the mRNAs demonstrate an apparent coordinate increase in expression levels. These data suggest a role for the M4 repeat as a common element mediating the developmental regulation of these genes. Preliminary in vivo experiments would support this conclusion. Data will also be presented describing the specific organization of these genes and possible relationships with similar gene families in other organisms.

0222 FUNCTIONAL ANALYSIS OF TRANSCRIPTIONAL AND TRANSLATIONAL REGULATORY SIGNALS AT THE 5' END OF THE MOUSE α_2(I) COLLAGEN GENE, Azriel Schmidt, Pellegrino Rossi and Benoit de Crombrugghe, Laboratory of Molecular Biology, NIH, Bethesda, MD 20892

A chimeric gene was constructed in which sequences between -2000bp upstream of the start start site of transcription of the mouse α_2(I) collagen gene to +54 downstream of this site are fused to the chloramphenicol acetyl transferase gene. This segment is sufficient for cell specific expression of the chimeric gene in tissue culture cells, as also suggested by the analysis of its expression in transgenic mice. A deletion analysis led to the identification of several cis-acting elements important for transcriptional control of the promoter. Two DNA segments between -979 and -502, and between -346 and -104 are needed for optimal expression of the chimeric gene. The results obtained with other deletions are consistent with an additional negative control of the promoter. Using constructions with a similar α_2(I) collagen promoter segment extending to +156, a second transcriptional start site was identified at position +100. This second start site is also present in the corresponding endogenous gene. Furthermore, a sequence of about 50 bp, which contains an inverted repeat element around the translation initiation site, is strongly conserved in three different interstitial collagen genes from bird to man. A partial or total deletion of this inverted repeat causes a 30-fold increase in translational efficiency of collagen mRNA in NIH 3T3 cells. This increase in translational efficiency is much less pronounced in v-mos transformed fibroblasts, suggesting that the conserved inverted repeat might be the target of a trans-acting mechanism that would regulate collagen mRNA translation.

0223 TRANSCRIPTIONAL REGULATION OF THE HUMAN PROENKEPHALIN GENE. Audrey Seasholtz, Michael Comb*, Howard Goodman*, and Edward Herbert, Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR., and *Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA.

The expression and regulation of the human proenkephalin gene, one of the opioid peptide genes, are being examined in two different transient expression systems: 1)chloramphenicol acetyltransferase (CAT) fusion genes and 2) Xenopus oocytes. The CAT fusion gene contains the first 403 bp of the human proenkephalin gene (200 bp of 5' untranscribed sequences and the first two untranslated exons) linked to the coding sequence of the CAT gene. This fusion gene has been used to examine the expression of the human proenkephalin gene in several different cell lines and in the presence of a variety of regulators. These studies revealed two control elements in the 5' untranscribed region of the gene. One is a positive transcriptional control element and the second is an element which confers cAMP regulation on the proenkephalin gene. A series of 5' deletion mutants have been constructed and are being used to further define these regulatory sequences.

Xenopus oocytes provide an alternative and complementary system to examine the regulation and expression of the human proenkephalin gene. The proenkephalin gene is efficiently and accurately transcribed in oocytes after injection of the wild-type gene into the nucleus. The 5' deletion mutants are also being injected into oocytes to examine the role of the two control elements in these cells.

0224 Murine and human cells transduced with amphotropic retrovirus containing hepatitis B virus (HBV) RNA transcribe the 2 major HBV mRNA's from chromosomally integrated HBV DNA. M.A.Sells, P.M.Price & G.Acs. Mount Sinai School of Medicine, NY, NY 10029.

Although several genes of HBV are expressed efficiently in cell culture systems, no viral replication has been detected. One of the prominent distinctions between these in vitro systems and liver infected in vivo is the relative amount of the two major HBV transcripts produced. In vivo, a greater than genome length RNA (3.5 kb), which is considered an intermediate in viral replication, is produced in ∿ equal amounts to that of the 2.3 kb RNA, which codes for the envelope protein, HBsAg, whereas cells transfected in vitro with cloned HBV produce ∿ 1000X more of the 2.3 kb RNA than the 3.5 kb. We report here the establishment of a culture system in which HBV RNA's of these lengths are detected in ∿ equal amounts.

The system was established by: 1) inserting tandem copies of HBV DNA into a plasmid (pDolmpl0) between the LTR's of Moloney murine leukemia virus (MoMLV); 2) transfecting this vector into ψAM22b cells [Cone and Mulligan (1984) Proc Natl Acad Sci, USA 81, 6349]; 3) selecting clones of cells that grow in G418 and produce HBsAg and HBeAg, a proteolytic product of an HBV nucleocapsid protein; and 4) infecting fresh ψAM22b cells and 293 cells (a human embryonal kidney cell line) with replication defective MoMLV-HBV recombinant viruses produced by the transfected ψAM22b cells. G418 resistant clones of these infected cells that synthesize HBV proteins were studied in detail. Analysis of cellular DNA and poly A(+) RNA revealed the presence of several complete copies of HBV DNA per cell and the production of two major HBV transcripts in ∿ equivalent amounts. The significance of these findings will be discussed in relation to HBV replication. Supported by grant number CA34818, NCI.

0225 **Control of Drosophila 5S RNA Gene Transcription.** Stephen Sharp, Alonzo Garcia, and Anita O'Connell. UC Irvine, California College of Medicine, Irvine, CA 92717.

Transcription of isolated repeat units of D. melanogaster 5S DNA in a Drosophila KcO cell extract revealed three types of template activities: 5SI DNA encodes the known 5S rRNA of D. melanogaster and has a relatively high transcription efficiency. 5SII DNA is identical to 5SI DNA except for a two-nucleotide deletion at 5S rRNA positions 28 and 29. The efficiency of transcription is approximately 40% that of 5SI DNA and because of the deletion, the primary transcript is two nucleotides shorter. 5SIII DNA, which does not support in vitro transcription (<2% 5SI DNA), has the same sequence as 5SI DNA except for a single G to A transition at position 86. Greater than 50% of the 5S DNA repeats in D. melanogaster appear to be the transcriptionally inactive 5SIII DNA type. Given the abundance of 5SIII DNA in the genome and that this DNA is not transcribed and probably is a poor substrate for TFIIIA binding, we wondered as to the function of 5SIII DNA in binding the other transcription factors, TFIIIB and TFIIIC. With the aim of identifying transcription control sequences and transcription factor recognition and binding sequences in Drosophila 5S DNA, we have constructed linker-scanner mutations across the 5SI DNA repeat unit. These templates have been analyzed in vitro using transcription and stable complex formation assays.

0226 TRANSCRIPTION AND EXPRESSION OF MYOSIN LIGHT CHAIN GENES, Ana-Maria Zarraga, Kenneth Danishefsky, Armurt Deshpande, C. Chandra Kumar, Diarmuid Nicholson, Chandrika Saidapet, Charmaine Mendola and M.A.Q. Siddiqui, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

Myosin polypeptides, the major constituents of muscle proteins, are encoded by multigene families and are expressed differentially in different muscle and nonmuscle cells. The genes coding for these proteins undergo a complex program of regulation and gene switching. In order to study the mechanisms underlying the differential expression of genes for myosin light chain (MLC) isoforms in development and to delineate the cis-acting regulatory sequences involved in tissue-specific expression, the genes for MLC_2 of the chicken and rat cardiac muscle tissues were isolated and characterized. Two phage recombinants, λLC13 and λLC5, were isolated which together contain the entire chicken MLC_{2A} gene sequence. A segment of λLC5 DNA corresponding to the 5'-terminus region of the gene containing the putative promoter region was introduced into an expression vector, and the transcription initiation activity was analyzed by the S1 nuclease protection assay following transient expression in appropriate host cell lines. The transcription initiation site was also identified by the primer-extension assay utilizing a chemically synthesized DNA primer and the endogenous RNA. A recombinant phage (λRLC4B) encompassing the entire rat cardiac MLC_2 gene was isolated from the rat genomic library. The regulation of expression of these genes is being analyzed by constructing mutant DNAs and assaying for the transcriptional activity.

0227 ANTIBODIES TO HN-RNP PROTEINS INHIBIT SPLICING OF PRE-MRNA IN VITRO, Halina Sierakowska[1], Paul Furdon[2], Wlodzimierz Szer[1] and Ryszard Kole[2], Department of Biochemistry, New York University School of Medicine, New York, NY 10016[1], Department of Pharmacology and Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC 27514[2]

Antibodies raised against purified proteins isolated from heterogeneous nuclear ribonucleoprotein particles (hnRNP) were used to inhibit splicing of a truncated human beta-globin pre-mRNA in vitro. Pretreatment of the extract with anti-hnRNP antibodies before addition of the substrate RNA led to complete inhibition of the splicing reaction. Generation of the spliced product was inhibited about 60% if the same antibodies were added to the reaction mixture after the formation of the splicing complex. Cleavage at the 5' splice site was inhibited by the anti-hnRNP antibodies in a similar fashion. These results suggest that hn-RNP proteins are necessary for in vitro splicing, may play a role in the formation of the splicing complex and that they are associated with the spliceosome during the splicing reaction.

0228 TRANSIENT AND STABLE TRANSFECTION OF RAT PITUITARY GH3 CELLS, M. Silberklang, J. Kopchick, R. Malavarca, S. Munshi, A. Lenny, F. Pasleau, T. Livelli and F. Leung, Merck Sharp and Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065

We have found the rat pituitary cell line, GH3, to be an efficient host for expression of foreign genes, and especially useful for secreted proteins, such as Bovine Growth Hormone (BGH). An adherent cell subpopulation of the parental GH3 suspension line was first derived, which can be grown as a stably, albeit loosely, attached monolayer culture. Under the appropriate growth conditions, this subpopulation is amenable to both transient DNA transfection with DEAE-dextran, and stable DNA transfection with calcium phosphate.

When the major parameters of the DEAE-dextran transient expression assay are optimized for these cells, using BGH vectors, levels of secreted BGH of 200-800 ng/ml/24 hr are routinely reached by days 4-6 post-transfection. We have used this procedure to compare transcriptional promoters as to efficiency in these cells, and have found the Cytomegalovirus immediate early promoter to be particularly strong (Pasleau, F.; Tocci, M.J.; Leung, F.; and Kopchick, J.J., 1985, Gene, in press).

We have also used the calcium phosphate method to develop stable BGH-secreting GH3 clones. Levels of secretion of 10-25 $\mu g/10^6$ cells/24 hr are typical and are generally reached at a gene dosage of only 1-3 copies. We conclude that GH3 cells are particularly well-suited to BGH expression and secretion. In other experiments, we have extended these observations to truncated, "anchorless" viral glycoprotein antigen genes. Though we have also developed GH3 clones expressing non-secreted proteins, levels of expression, in these cases, are not as striking.

0229 *In Vitro* Transcription of the *Drosophila engrailed* Gene. Walter C. Soeller, Stephen Poole, and Thomas Kornberg, University of California, San Francisco, CA 94143-0554.

Over the past several years this laboratory has demonstrated that the *Drosophila engrailed* gene, one of a number of genes controlling pattern formation, is itself temporally and spatially regulated at the transcriptional level throughout embryonic development. We are examining the basis for this regulation through establishment of an *in vitro* transcription system for *engrailed* using nuclear extracts derived from *Drosophila* embryos. These extracts support accurate transcription from *engrailed* genomic DNA templates at levels up to 50% of that seen for actin 5C and alcohol dehydrogenase (ADH) templates. Initiation of transcription *in vitro* is identical to that seen *in vivo* and these extracts require at most 236 bp of DNA upstream from the initiation site and they can be chromatographically resolved into at least 2 components which retain promoter fidelity when recombined. DNAse protection experiments reveal the presence of at least one *engrailed* promoter-specific DNA binding activity in one of these components. Futher purification of these components should allow us to identify and characterize novel transcription factors which mediate the temporal and spatial regulation observed *in vivo*.

0230 IN VITRO TRANSCRIPTION PROMOTED BY THE αA-CRYSTALLIN GENE 5' FLANKING SEQUENCES. Bernd Sommer, Ana B. Chepelinsky, and Joram Piatigorsky, Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20892.

The αA-crystallin gene is expressed in the vertebrate eye lens. We have demonstrated earlier (Chepelinsky et al., Proc. Natl. Acad. Sci. U.S.A. 82, 2334-2338, 1985) that sequences between positions -364 and +45 of the murine αA-crystallin gene can promote bacterial chloramphenicol acetyl transferase (CAT) activity when inserted into the pSVO-CAT expression vector and assayed in transfected, explanted chicken lens epithelia. By contrast, sequences between positions -87 and +45 did not promote CAT activity in the transfected epithelia. Here we show that the plasmids containing the -364 to +45 promoter fragment and the -87 to +45 fragment can initiate transcription in a Manley Hela cell extract. Assays were performed by primer extension. Supercoiled plasmids were preferentially transcribed. A deletion mutant containing 64 bp of 5' flanking sequence was still transcribed *in vitro*. Further deletions are being analyzed. The initiation site of *in vitro* transcription with 364, 87 and 64 bp of flanking sequence was the same as observed *in vivo* in the mouse lens. Footprinting experiments are being conducted in order to identify precisely which nucleotides are binding transcription factors in the Hela cell extract. We hope that by comparing lens extracts with heterologous extracts we will gain more information about factors which regulate the expression of this lens gene.

0231 THYROID HORMONE RESPONSIVE REGION OF THE RAT GROWTH HORMONE GENE, Stephen Spindler, Mark Crew and Jennifer Nyborg, Univ. of California, Riverside, CA 92521.

The region of the rat growth hormone gene responsible for thyroid hormone transcriptional induction has been identified using two approaches. First, chimeric genes were constructed consisting of various regions of rat growth hormone (rGH) gene and 5' flanking region linked to the coding sequence of the dominant, selectable bacterial gene neo. These constructs were stabily transfected into growth hormone-producing pituitary tumor cells. Northern blot, dot blot, S_1 nuclease mapping and transcription run-on studies have led to identification of a 5' region of the gene responsible for thyroid hormone-inducible transcription. Second, an analysis of chromatin structure in and near the rGH gene has localized a unique thyroid hormone-induced, DNase I-hypersensitive site at or near the gene sequence identified as hormone-responsive by the deletion-transfection studies.

0232 ALTERNATIVE SPLICING OF SV40 EARLY PRE-mRNA IN VITRO: T and t ANTIGEN mRNAs ARE PRODUCED BY DIFFERENT BIOSYNTHETIC MECHANISMS. Richard A. Spritz and Vicky L. van Santen, Laboratory of Genetics, University of Wisconsin, Madison, WI 53706.

Simian virus 40 (SV40) early pre-mRNA is spliced using either of two alternative 5' splice sites and a common 3' splice site to produce two different mRNAs that encode the T and t antigens. By studying the splicing of synthetic SV40 early pre-mRNA in vitro using a HeLa cell nuclear extract system, we have found that the mechanisms of production of T and t antigen mRNAs are substantially different. The optimal conditions for T antigen mRNA splicing are similar to those for other pre-mRNA splice events; under these conditions the ratio of T to t antigen mRNA biosynthesis in vitro is approximately 6:1, similar to that observed in vivo. Transcript cleavage at the T antigen 5' splice site apparently involves production of a typical branched "lariat" splicing intermediate, and is inhibited by increased concentration of mono- or divalent cations, elevated temperature, pre-incubation of the HeLa cell nuclear extract at 45°C, low levels of vanadyl ribonucleoside complexes, and requires ATP. In contrast, cleavage of SV40 early pre-mRNA at the t antigen 5' splice site is insensitive to all of these manipulations, does not require ATP, and apparently does not involve a lariat splicing intermediate. However, cleavage at the t antigen 5' splice site does require at least one heat-insensitive component of the HeLa cell nuclear extract. The molecular structure of the excised t antigen intervening sequence is currently under investigation. The differential requirements for production of T and t antigen mRNAs in vitro may relate to differences in sizes and potential secondary structures of the T and t antigen intervening sequences.

0233 RETINOIC ACID INDUCED EXPRESSION OF TISSUE TRANSGLUTAMINASE IN PERITONEAL MACROPHAGES, Joseph P. Stein and Peter J.A. Davies, University of Texas Medical School, Houston, Texas 77030

Retinoids are a class of lipid molecules that have dramatic effects on the growth and differentiation of both normal and neoplastic cells. We have recently demonstrated that retinoic acid acts as a direct and acute regulator of the expression of a specific enzyme, tissue transglutaminase (TGase), in mouse and human myeloid cells. Specifically, exposure of resident mouse peritoneal macrophages to nanomolar levels of trans-retinoic acid causes the enzyme to accumulate to 1% of total cellular protein within 24 hours. Two arguments suggest that this induction of TGase activity occurs at the level of transcription: actinomycin D completely blocks the induction, and exposure of the cells to retinoic acid causes a significant increase in the amount of translatable TGase mRNA within 30 minutes. Further evidence that retinoids have a direct effect on TGase expression is the fact that cycloheximide does not block the stimulation of TGase mRNA. Cycloheximide appears to increase the levels of other retinoid inducible mRNAs. Retinoic acid and intracellular cyclic AMP act synergistically to stimulate TGase expressions in both normal and leukemic myeloid cells. We believe the induction of tissue TGase in myeloid cells is the first example of how retinoids might act in a direct fashion to regulate specific gene expression.

0234 CHICKEN U4 RNA GENES HAVE AN ALTERED PROMOTER STRUCTURE RELATIVE TO U1 AND U2 RNA GENES, William E. Stumph and Michael L. Hoffman, San Diego State University, San Diego, CA 92182

We have cloned and sequenced a region of the chicken genome that contains two homologies to U4 small nuclear RNA. These two U4 homologies are closely linked within 465 base pairs (bp) of each other and have the same transcriptional orientation. The downstream U4 homology is a true U4 RNA gene since it is expressed when injected into Xenopus oocytes and it agrees perfectly in sequence with chicken U4B RNA (Reddy, Nucl. Acids Res. 13, r155, 1985). The upstream coding sequence, however, contains 7 base changes relative to the U4B sequence. This gene may thus code for a variant U4 RNA, or, alternatively, it may be a non-expressed pseudogene. The promoter regions of the two U4 RNA genes possess very limited sequence homology with U1 and U2 RNA gene promoters. U1 and U2 RNA genes of vertebrates contain two conserved regions of homology that have been shown to be required for proper gene expression. These sequences are located approximately 55 and 200 bp upstream from the transcriptional initiation sites. A search for similar sequences upstream of the two chicken U4 RNA genes (or gene + pseudogene) revealed that both U4 RNA genes contain the -55 region homology. However, they both lack the conserved -200 region homology that is shared by the U1 and U2 RNA genes of chickens and other vertebrates. This latter finding suggests that U4 RNA gene expression may be controlled by mechanisms partially different from those involved in U1 and U2 RNA gene expression.

0235 CELL SPECIFIC EXPRESSION OF THE CLONED HUMAN GASTRIN GENE . Lars Eyde Theill,Ove Wiborg,Jens Vuust,Molecular Piology Institute, Aarhus University, DK-8ooo Aarhus,Denmark.

5'-flanking sequences of the cloned human gastrin gene(Wiborg,Ove,et al.PNAS 81 1o67 1984)was combined with the coding sequence of the CAT gene(Gorman,C.M Mol.Cell.Bio.,2,1044,1982).In the murine neuroblastoma cell line N18TG2(Nelson,P.,PNAS 73,123,1976)the CAT expression of this construct,was 10 to 100 fold higher than measured in other murine or human cell lines.Inclusion,on the vector,of SV40 enhancer segments,did not further augment the CAT expression in N18TG2. 5'end deletions in the gastrin gene promoter,resulted in a gradual lowering of the cell specific expressin.Almost 50% of maximum promoter activity was contributed however by a -17 to +65 bp(as measured from the cap site) gastrin fragment.This fragment could not without complete loss of activity,be sustituted by other DNA fragments.Primerextension analysis revealed,that the hybrid RNA did start at the autentic gastrin gene cap site.These findings suggests,that transciptional control sequences in the gastrin gene, are situated,not only in the 5'-flanking regions,but also in the cap/exon 1 region of the gene.

0236 TRANSCRIPTIONAL REGULATION OF THE S. CEREVISIAE COPPER - METALLOTHIONEIN GENE, Dennis J. Thiele and Dean H. Hamer, Laboratory of Biochemistry, NCI, NIH, Bethesda, Maryland 20892.

The S. cerevisiae CUP1 gene encodes a cysteine-rich, low molecular weight copper - binding protein denoted copper - metallothionein (MT). The biosynthesis of copper - MT is induced at the level of transcription by the addition of exogenous copper to the growth medium. A 430 base - pair DNA fragment derived from the 5'- noncoding region of CUP1 contains all of the information required to direct copper - inducibility in cis. By analyzing deletion and linker-fusion mutant derivatives of a CUP1/galK promoter fusion, we have identified repeated cis-dominant upstream control sequences necessary for copper- induced transcription. A synthetic version of one of these elements confered efficient copper-inducibility on a heterologous yeast promoter when present in two tandem copies. This transcriptional control region, identified by in vitro mutagenesis, may serve as an interaction site for copper-dependent transcriptional factors.

0237 TRANSCRIPTIONAL REGULATION IN YEAST MITOCHONDRIA Baruch Ticho, D. Mueller, T. Biswas, J. Wettstein, J. Backer and Godfrey Getz The University of Chicago, Chicago, IL 60637

There are three well established mechanisms for the control of transcription: promoter strength, attenuation, and trans acting factors; all three operate in yeast mitochondria.

We have identified a nine nucleotide consensus promoter, 5'ATATAAGTA3', that includes the initiating nucleotide (+1), upstream of all known mt transcripts. The single G, at position -2, and the A at -4, are essential for transcription since substitution by any other nucleotide leads to loss of activity. The invariability of other nucleotides within the promoter is being tested. Various promoters with identical upstream sequences have differing strengths both in vitro and in vivo. In vitro run-off assays using cloned mitochondrial promoters can distinguish strong and weak promoters. A novel technique, in which hybridized M13 probes protect in vivo labeled RNA from RNase degradation, showed that the rates of synthesis were in agreement with the in vitro data. Sequence comparison and point mutation showed that the nucleotides at positions +2 and +3 modulate promoter strength with the strong promoters having a purine at +2 and a pyrimidine at +3. In vivo analysis of individual genes of polygenic transcription units revealed asymmetric synthesis rates, indicating that attenuation occurs between these genes. We observed that mitochondrial transcription is uniformly induced upon derepression, due to increased synthesis. The RNA polymerase, which we have purified over 4500 fold from isolated mitochondria, has a selective form and a non-selective form which can be separated by chromatography. The selective enzyme forms a heparin resistant complex with mt promoter DNA. Polymerase activity sediments at a native molecular weight of 100,000 - 150,000 in glycerol gradients. The non-selective enzyme is thought to be a monomer of 150,000d molecular weight. An inactive fraction containing only a 70,000d peptide can be added back to the non-selective activity to regenerate activity selective for the mt promoter.

0238 THE EXPRESSION OF THE IMMUNOGLOBULIN J CHAIN GENE IN A B LYMPHOMA LINE IS INDUCED BY INTERLEUKIN 2, Michael A. Tigges, Marcia A. Blackman and Marian E. Koshland, University of California, Berkeley, CA 94720

Exposure of a mature resting B lymphocyte to antigen and T cell hormones converts it to a plasma cell secreting pentamer IgM. Three molecular events in this conversion have been identified; a shift in μ mRNA from primarily the membrane to the secreted form that occurs early in the response and induction of J chain gene transcription followed by dramatic increase in immunoglobulin and J chain mRNA levels that occurs later. These late events can be elicited in a dose-dependent manner simply by exposure of the B lymphoma BCL_1 to recombinant Il-2(rIl-2). In uninduced BCL_1 cells, transcription of the J chain gene is undetectable in a run-on assay. Treatment with rIl-2 causes a transient increase in J chain transcription initiation. This increase is reflected in a 64-fold increase in cytoplasmic J chain mRNA levels and a 20-fold higher concentration of secreted IgM in culture supernatants after four days of rIl-2 treatment. That Il-2 delivers the differentiative signal is demonstrated by the ability of anti-Il-2 receptor antibody to block the response. These results show that Il-2 induction of BCL_1 cells provide an excellent system for analyzing the molecular mechanisms by which induction of gene transcription is regulated during differentiation.

0239 THE PROTEIN FACTORS REQUIRED FOR ACCURATE IN VITRO TRANSCRIPTION OF THE MOUSE rRNA GENE John Tower and Barbara Sollner-Webb
The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Previous studies had identified three chromatographic fractions derived from S100 extracts of cultured mouse cells which appeared to be required for in vitro rRNA synthesis (Mishima, et al, Nuc. Acids. Res. 10:6659). We find that two of these ("C" and "D") are necessary and sufficient for accurate initiation, and we have purified these factors several thousand fold. D is responsible for template commitment/stable complex formation. Specific binding of D to the mouse rDNA promoter requires the region from residues ~-39 to ~-15 and is augmented by additional sequences extending to residue ~+9. C will stably associate with the D/rDNA complex but not with the naked rDNA. C appears to be a form of polymerase I, for C activity and polymerase I copurify to virtual homogeneity of the polymerase I polypeptides. This identification is also supported by experiments utilizing α-polymerase I antibodies. However, C is evidently a subset of polymerase I, for C may be separated from the bulk of the polymerase I by co-precipitation with the stable transcription complex. C is capable of restoring activity to transcriptionally inactive extracts derived from both quiescent cells and cells in which protein synthesis has been inhibited by a one hour cyclohexamide treatment. These transcriptionally inactive extracts contain normal amounts of D and bulk polymerase I, but the polymerase I isolated from these extracts lacks C activity. Taken together, this suggests that rRNA synthesis may be regulated in vivo by the availability of factor C -- a functionally distinct subpopulation of RNA polymerase I.

0240 Using band competition assays to monitor the purificatin of CAAT box transcription factors from HeLa cells. Sophia Y. Tsai, Ikuko Sagami, Milan Bagchi, Ming-Jer Tsai and Bert W. O'Malley. Baylor College of Medicine, Houston, Texas 77030.

Recently, we have identified a protein factor in the nuclear extracts of HeLa cells which binds specifically to the CAAT box region of the ovalbumin gene. This CAAT box binding transcription factor is essential for efficient transcription of the ovalbumin gene. To assay for the functional activity of this factor, an in vitro reconstituted transcription assay was used. However, these assays are cumbersome and require a substantial amount of material. To facilitate the purification of this transcription factor, we have used the band competition assay developed by Garner and Revzin (NAR 1, 3047 1981) to monitor for the presence of the CAAT box transcription factor. These assays identified specific protein-DNA complex by retardation of the mobility of the labeled DNA fragment in a polyacrylamide gel. The interference of nonspecific DNA binding protein in these assays can be minimized by including a large excess of nonspecific DNA fragment in the reaction mixture. Using this simple assay we have been able to monitor the purification of the CAAT box transcription factor through DEAE-Sephadex, phosphocellulose, Sephacryl-300 and Heparin Sepharose column chromatography. The results are similar to those obtained by in vitro reconstituted system. In addition, this assay is not affected by the presence of RNase which will facilitate the isolation of the CAAT box transcription factor from the homologous oviduct tissues.

0241 TRANSCRIPTION OF CLONED NEUROSPORA GENES IN VITRO.
Brett M. Tyler. Dept.Genetics, Research School of Biological Sciences, Australian National University, Canberra, ACT 2601, Australia.

Neurospora crassa in vitro transcription systems have been developed for RNA polymerase I (1), RNA polymerase II (2), and RNA polymerase III (3). The RNA polymerase III system has been used to assay the effects of 'linker-scanning' mutations spanning two Neurospora 5S rRNA genes. The results indicate that Neurospora 5S genes contain three internal promoter elements as well as an upstream 'TATA-box' at -29 which fixes the start-point of transcription.

The RNA polymerase I system has been used to localize the promoter region of Neurospora 45S rRNA genes. This region has been shown to contain sequences homologous to three out of the four promoter elements of Neurospora 5S genes, raising the possibility that in Neurospora, the 45S rRNA and 5S rRNA genes may be co-regulated via common promoter elements.

The RNA polymerase II system has been used to examine in vitro the regulation of the inducible qa (quinic acid) genes of Neurospora.

(1) Tyler,B.M. and Giles,N.H. (1985) Nucl. Acids Res. 13, 4311-4332
(2) Tyler,B.M. and Giles,N.H. (1985) Proc. Natl. Acad. Sci. USA 82, 5450-5454
(3) Tyler,B.M. and Giles,N.H. (1984) Nucl. Acids Res. 12, 5737-5755

0242 YEAST TRANSPOSABLE ELEMENT SIGMA FUNCTIONS AS A HORMONE-INDUCIBLE PROMOTER, Scott W. Van Arsdell and Jeremy Thorner, University of California, Berkeley, CA 94720.
We have characterized a genomic clone from Saccharomyces cerevisiae (λScG7) that encodes a 650-base polyA+ RNA which is induced about 50-fold in MATa cells that have been treated with the mating pheromone, α-factor. The 650-base RNA is transcribed from a cluster of repetitive sequences: a sigma element and three delta elements adjacent to a tRNA-trp gene. Northern analysis using strand-specific probes indicates that this RNA is transcribed in the direction away from the tRNA gene and contains sigma sequences at its 5'-end and delta sequences at its 3'-end. In addition to the 650-base transcript, MATa cells contain two other abundant α-factor-inducible polyA+ RNA species (500 and 5300 bases) that are homologous to the same strand of sigma, but are transcribed from other locations in the genome. Pulse-labeling experiments indicate that the dramatic induction of the sigma-related transcripts can be accounted for by an increase in their rate of synthesis. Induction of the sigma RNAs is rapid, requires a functional STE2 gene product (putative α-factor receptor), and does not require new protein synthesis, suggesting that sigma transcription is a primary response to the pheromone. In MATa cells transformed with a plasmid in which the λScG7 sigma element has been inserted just upstream of the coding sequence for the internal form of invertase (SUC2 gene) that lacks its own promoter, a new prominent polyA+ RNA appears in response to α-factor that hybridizes to both sigma and SUC2 probes and intracellular invertase activity is induced 10-20-fold. Primer extension indicates that synthesis of the hybrid transcript initiates uniquely within the sigma sequence. When the sigma element is inserted in the opposite orientation, pheromone-induced transcription of vector (pBR322) sequences occurs.

0243 TERMINATION OF TRANSCRIPTION BY POLYMERASE I IN YEAST, Harm van Heerikhuizen, Annemarie E. Kempers-Veenstra, J. Klootwijk and Rudi J. Planta, Biochemisch Laboratorium, Vrije Universiteit, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

We have been studying 3' end formation of the 37S precursor rRNA of *Saccharomyces* in yeast transformed with artificial *minigenes*.
It appeared that a 140 bp fragment from -36 to +101 (relative to the 3' end of 26S rRNA) is sufficient to generate the correct 3' ends. Deletion analysis was used to narrow down the sequences involved in 3' end formation: the 5' border of these sequences is located between -35 and -5, whereas the 3' border is located between +43 and +73.
These date are in good agreement with the strong sequence conservations we observe between three different Saccharomycetoideae in the region between +10 and +55. The conserved sequence elements are part of an extensive dyad symmetry. We believe, however, that the sequences between -35/-5 and +43/+73 are not involved in termination of transcription but rather in processing of a longer precursor for two reasons: 1) Studies in collaboration with dr. P.N. Piper (London) with minigenes transformed in an rna 82 strain that is impaired in 3' processing of 5S rRNA shows that transcription proceeds at least 50 nucleotides beyond the 3' end of 26S rRNA. 2) 5' deletions to -5 or further, specify a new 3' end, located at +210; the rna 82 mutation does not affect the formation of this 3' end, which therefore may be formed by genuine termination rather than by processing. This putative termination site appears to be very closely associated with sequences involved in enhancement of the RNA polymerase I initiation (Elion and Warner (1984), Cell **39**, 663-673). A model for the regulation of the transcription of yeast rRNA genes will be discussed.

0244

DEVELOPMENT OF IN VITRO METHODS TO STUDY KAPPA IMMUNOGLOBULIN GENE TRANSCRIPTION AND REGULATION, Brian Van Ness, Laurie Berdahl, and Leslie Hattig, University of Iowa, Iowa City, IA. 52242.

The study of immunoglobulin gene expression in lymphoid cells which serve as models of transcriptional regulation could be facilitated by experimental approaches in cell-free systems. Transcription in isolated nuclei offers a potential advantage in that the nuclear environment can be altered. Typically, however, transcriptional initiation by endogenous RNA polymerase II is very inefficient. We are establishing conditions which initiate kappa immunoglobulin gene transcription by the addition of purified RNA polymerase II to isolated nuclei. Using the MPC-11 lymphoid cell line, we see a consistent increase (2-3 fold) in total RNA synthesis when RNA pol II is added, as well as a significant increase in kappa immunoglobulin transcription (up to 20 fold), suggesting exogenous pol II is contributing to label incorporation by initiating new RNA synthesis. We are currently using this method of approach to study the effect on transcription of adding cell extracts from cell cultures in which immunoglobulin gene transcription can be regulated. This could provide a useful means of fractionating extracts to identify regulatory factors. Whole cell and nuclear extracts are also being examined for their ability to transcribe a cloned kappa gene. Our preliminary results suggest that, in cell extracts, transcription only occurs on DNA templates which lack the intronic enhancer sequence.

0245 TRANSCRIPTIONAL CONTROL OF MOUSE MAMMARY TUMOUR VIRUS, Paul Webb, Maurice Needham, Roger White, Philippa Darbre and Malcolm Parker, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

Transcription from the mouse mammary tumour virus (MMTV) promoter is stimulated by the steroids dexamethasone, progesterone and dihydrytestosterone (DHT). We have transfected a MMTV-LTR chloramphenicol acetyltransferase (CAT) fusion gene into a series of breast tumour cell lines which possess receptors for each of the classes of steroid hormone. CAT enzyme activity was assayed in transient expression experiments in the presence or absence of various hormones.

Three types of hormone responsiveness have been demonstrated. In mouse S115 cells CAT activity is increased five- to ten-fold over background levels by both DHT and dexamethasone. In human T47D cells, progesterone markedly stimulates CAT levels by up to fifty-fold. There is no effect of either dexamethasone or DHT in these cells. Similarly in ZR-75 cells we have shown that CAT levels are not stimulated by any of these three classes of steroid despite the presence of the appropriate receptors.

We conclude that (a) MMTV transcription can be regulated by steroids other than glucocorticoids, (b) the presence of a functional steroid receptor is necessary, but insufficient, to allow the corresponding hormone to stimulate expression of MMTV.

We have preliminary data to suggest that other transcription factors can regulate MMTV expression.

0246 EXPRESSION OF A MILK PROTEIN MINIGENE, Li-Yuan Yu-Lee, Sara Rupp, Craig H. Couch and Jeffrey M. Rosen, Baylor College of Medicine, Houston, TX 77030

In the mammary gland, the peptide hormone prolactin modulates tissue-specific function, i.e., casein mRNA production, by increasing nuclear casein mRNA stability 17- to 25-fold. To investigate these effects, a milk protein minigene has been constructed into a BPV-neomycin expression vector (BMNα), and transfected into a mouse mammary cell line, COMMA ID, cultured on floating type I collagen gels. The rat α-casein minigene (@ 4 kb) contains 680 base pairs of 5' flanking DNA, the first two exons and introns fused in phase through an internal restriction site to the rest of the α-casein cDNA. Since the in vivo splice signals have not been altered, a functional α-casein mRNA is expected. Analysis of the transfected DNA from individual foci as well as total populations showed that BMNα (21.5 kb) remained unrearranged and possibly episomal. RNA analysis indicated that the minigene is expressed; however, the transcripts appear to be largely unprocessed. Experiments are underway to map these transcripts and to determine the basis for the apparent lack of correct processing. (Supported by ACS BC-425A)

Prokaryotic Transcription, Oncogenes, Development and Transcription and Gene Expression

0247 REGULATION OF mRNA PRODUCTION BY ESTRADIOL, TAMOXIFEN AND ANTIMETABOLITES. R.D. Armstrong, Y.Y. Tan, and Y.S. Choi, Univ. of California, San Francisco, CA 94143.

We have initiated a project in MCF-7 human breast carcinoma cells to investigate the regulation of mRNA levels by Estradiol (E_2) and its antagonist Tamoxifen (TAM) at post-transcriptional sites (eg. processing, nucleocytoplasmic transport), and to determine how the antimetabolites FUra and 6-TG may modulate these actions. Our preliminary studies have focused on the estrogen-inducible pS2 mRNA, although analogous experiments with cMyc oncogene, DHFR and actin mRNA are currently being completed. To determine if E_2 stimulates ATP-dependent mRNA transport, isolated nuclei were used in a standard in vitro mRNA transport assay. Non-specific mRNA transport was measured by release of ^{3}H-mRNA (20 min treatment of cells with ^{3}H-Urd). E_2 did not alter the efflux of this "rapidly-labelled" mRNA at concentrations up to 10 nM. However, E_2 markedly stimulated the selective efflux of pS2 mRNA (identified by ^{32}P-cDNA hybridization) by 61% and 93% for 1 nM and 10 nM E_2, respectively. This effect was antagonized completely by 1 uM TAM. When exposed to whole cells for 24 hr, 1 uM TAM produced a 51% suppression in pS2 mRNA, while 10 uM FUra produced a 36% reduction. When both were administered together, an 86% reduction in pS2 mRNA levels was observed. 6-TG was found to stimulate the level pS2 mRNA, although the distribution among the various pS2 mRNA sized species was different than for E_2 alone. Our studies suggest that post-transcription modulation sites are an important target for regulation of pS2 mRNA, and perhaps other mRNA in estrogen sensitive cells, and may act to trigger transcriptional changes, which are being measured in nuclear run-on procedures.

0248 Regulation of Albumin Gene Expression in Disaggregated Hepatocytes. Friedman, J.M., Babiss, L. E., Clayton, D. F., and Darnell, J. E. The Rockefeller University, New York, N.Y. 10021.

Previous studies have suggested that the transcription of liver specific genes is dependent on the presence of cell-cell and cell-extracellular matrix contacts. Thus the transcription rate of liver specific genes decrease rapidly in primary cultures of disaggregated hepatocytes. We have constructed a recombinant adenovirus in which the viral E1A gene and the E1B promoter have been replaced by the rat albumin promoter. We have used this virus to infect primary hepatocytes that have been in culture for one hour (when the transcription rate of the endogenous albumin gene is high) and twenty four hours (when the transcription rate of the albumin gene is low). We have found that the activity of the viral albumin promoter, as scored by the synthesis of E1B containing RNAs, is fifty times greater in freshly plated hepatocytes as compared to hepatocytes in culture for one day. These data show that the activity of the trans acting factor that controls albumin transcription diminishes rapidly after primary culture of hepatocytes and that the activity of this factor depends on the maintenance of cell-cell and or cell-matrix contacts. The mechanism by which this extracellular signal controls the factors involved in albumin transcription are unknown.

0249 DNASE I FOOTPRINTING OF THE ACANTHAMOEBA RIBOSOMAL RNA GENE PROMOTER, Erik Bateman and Marvin R. Paule, Colorado State University, Fort Collins, CO 80523

Accurate transcription of Acanthamoeba ribosomal RNA genes in vitro is dependent on binding of a transcription initiation factor (TIF) to the promoter region of rDNA. DNase I footprinting was used to examine the interactions between the species specific TIF, RNA polymerase I and the rRNA gene promoter. TIF protects a region extending from -12 to -69 on the non-coding strand, and from -14 to -67 on the coding strand. The TIF footprint can be competed out by wild type promoters but not by promoters containing 5' deletions which do not stably bind TIF. Pol I extends the footprint obtained with TIF to +20 on the non-coding strand and to +18 on the coding strand. No gap between the two protected regions is discernible. Pol I alone does not protect any DNA region from digestion. Parameters which affect the DNA binding by TIF and Pol I were examined.
Transcription of rRNA in vivo is shut down during encystment of Acanthamoeba in response to growth conditions. Pol I modification is responsible for this regulation. While vegetative polymerase is completely functional, pol I from cysts is unable to accurately initiate transcription from the rDNA promoter, but is unimpaired in its ability to non-specifically transcribe DNA. Using the footprinting assay, it was found that cyst pol I does not bind to the transcription start site, and therefore cannot initiate transcription. Since TIF from the same source is unaltered by encystment, this result suggests a mechanism by which commitment to rRNA transcription can be maintained, while allowing the cell to respond to environmental conditions. Supported by NIH Grant GM22580.

0250 THE ROLE OF THE 21 BASE PAIR REPEATS OF SV40 ON TRANSCRIPTION IN VIVO. S. Batson[1], K. Detmer[2], R. Kingston[3], and U. Hansen[1], [1]Harvard Medical School, and Dana-Farber Cancer Institute, Boston, Massachusetts; [2]Department of Biology, MIT, Cambridge, Massachusetts: [3]Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts.

The 21 base pair repeats within the promoters of SV40 stimulate transcription in vivo and in vitro in an orientation independent manner at nearby initiation sites. To further understand the stimulatory properties of this region, we have cloned the 21 base pair repeats in both orientations at several positions both 3' and 5' of the Ad EII with and without its own upstream promoter sequences present.

In the two constructs used, the Ad EII promoter is fused either to the chloramphenicol transacetylase gene or to the dihydrofolate reductase gene. In the CAT constructs, the 21 base pair repeats stimulate transcription 2-3 fold 5' of the promoter when the Ad EII's immediate upstream region is present, and 7-8 fold when it is absent. The 21 base pair repeats have no effect on transcription when 1000 bp 3' of the promoter. These results are observed when the repeats are in both orientations.

These results will be extended by comparison with the levels of gene expression seen in the stable transfection assay. The quantity of RNA produced will be measured by S1 mapping and reverse transcriptase extensions.

0251 EXPRESSION OF TWO MEMBERS OF THE RAT KALLIKREIN GENE FAMILY, JAMES M. BRADY AND RAYMOND J. MACDONALD, UNIV. OF TX. HEALTH SCIENCE CENTER-DALLAS, DALLAS, TEXAS 75235-9038

RAT KALLIKREIN GENE FAMILY MEMBERS ARE DIFFERENTIALLY EXPRESSED IN A NUMBER OF EXOCRINE TISSUES. THE RAT KIDNEY EXPRESSES TWO MEMBERS OF THIS GENE FAMILY, TRUE TISSUE KALLIKREIN (PS TYPE), AND A CLOSELY RELATED MEMBER (K1 TYPE). THE KIDNEY K1 MRNA NUCLEOTIDE SEQUENCE IS 86% HOMOLOGOUS TO PS MRNA. THE ENCODED K1 ENZYME IS 80% HOMOLOGOUS TO TRUE TISSUE KALLIKREIN AND RETAINS KEY AMINO ACID RESIDUES CHARACTERISTIC OF KALLIKREIN GENE FAMILY MEMBERS. THE SITES OF EXPRESSION OF THE PS AND K1 MRNAS HAVE BEEN DETERMINED BY NORTHERN ANALYSIS. THE LEVEL OF EXPRESSION OF THE PS AND K1 MRNAS IN VARIOUS TISSUES HAVE BEEN DETERMINED BY SOLUTION HYBRIDIZATION STUDIES. RAT GENOMIC CLONES CONTAINING THE PS AND K1 GENES HAVE BEEN TENTATIVELY IDENTIFIED. EXPERIMENTS ARE IN PROGRESS TO DETERMINE TRANSCRIPTIONAL REGULATORY SEQUENCES NECESSARY FOR TISSUE-SPECIFIC EXPRESSION OF THE RAT KALLIKREIN GENE FAMILY.

0252 RARE NON-POLYADENYLATED TRANSCRIPTS UNIQUE TO RAT BRAIN, Murray H. Brilliant[1], Christina A. Harrington[2], and Dona M. Chikaraishi[2], [1]The Jackson Laboratory, Bar Harbor, ME 04609; [2]Department of Neurology and Neurosurgery, Tufts University School of Medicine, Boston, MA 02111

Although the existence of a large class of non-polyadenylated transcripts unique to brain was indicated some time ago, until very recently no individual representatives of this class has been cloned. We have previously described techniques aimed at cloning DNA sequences corresponding to this class (M. H. Brilliant, N. Sueoka and D. M. Chikaraishi, Mol. Cell. Biol. 4:2187-2197, 1985). In this study we present two clones in detail. Clones rg100 (rat genomic 100) and rg13 correspond to rare non-polyadenylated polysomal transcripts unique to rat brain. Transcripts of rg100 are found in whole brain, cerebellum, cerebral cortex, and areas underlying the cerebral cortex, but not in neural tumor cell lines. Previously, transcripts of rg13 were shown to exhibit the same pattern. In this study, finer dissection has revealed rg13 transcripts to be enriched in the tectum of the midbrain, although transcripts are found in the tegmentum of the midbrain, hippocampus, hypothalamus and striatum. No transcripts corresponding to rg13 were detected in the pons medulla. Both rg100 and rg13 transcripts appear to be enriched in membrane bound polysomes. Southern hybridization revealed both transcripts were derived from unique-sequence DNA and that the short restriction fragments cloned had no close homologies with mouse DNA sequences. The sequence of rg13 showed an open reading frame, however, the rg100 sequence had stop codons in all three reading frames, even though both transcripts were associated with polysomes.

0253 NUCLEOTIDE SEQUENCE OF VACCINIA VIRUS RNA POLYMERASE LARGE SUBUNIT PREDICTS HOMOLOGY TO EUKARYOTIC AND PROKARYOTIC RNA POLYMERASES, Steven S. Broyles and Bernard Moss, NIAID, Bethesda, MD 20892. Vaccinia virus encodes a multisubunit RNA polymerase which bears some similarity to cellular RNA polymerases. We have determined the nucleotide sequence of a 6,067 base pair region of the viral genome known to encode two RNA polymerase subunits. Among the four open reading frames identified, two predicted polypeptides of 22,000 and 147,000 daltons as expected from previous studies. All open reading frames are closely spaced and ends of the polymerase mRNAs, and presumably the transcription promoter and termination signals, mapped within adjacent open reading frames. The predicted amino acid sequence of the 147K subunit shows significant homology to the largest subunit (β') of *Escherichia coli* RNA polymerase, yeast RNA polymerase II and III and Drosophila RNA polymerase II. The regions of homology between the four RNA polymerases can be subdivided into six separate domains which span most of the length of each. In all six domains, the vaccinia large subunit has greater homology with the eukaryotic RNA polymerase than with that of bacteria. The large subunits of vaccinia virus and eukaryotic RNA polymerases may therefore have evolved from a common ancestor after eukaryotes diverged from procaryotes.

0254 T-DNA 780 GENE: A MODEL FOR CONSTITUTIVE NUCLEAR GENES OF HIGHER PLANTS, Wesley Bruce and William B. Gurley, Microbiology and Cell Science, University of Florida, Gainesville, Fla. 32611.

Transgenic expression of T-DNA genes from *Agrobacterium* represents a model system for examining *in vivo* DNA sequence elements involved in transcriptional control of constitutive nuclear genes in higher plants. The 780 gene of T-right was used in a systematic deletion and linker scan study to delineate subelements within the promoter. A homologous reference gene/test gene vector system was utilized to precisely quantify the level of transcription for each mutation. The 5' flanking region of the 780 gene resembles a typical constitutive eukaryotic promoter with 5'-TATAAA-3' 31 bp upstream and 5'-CCGAATT-3' 74 bp upstream from one of the two transcriptional start sites. No apparent GC box or enhancer sequence motifs are present in the sequence. Deletion studies suggest that the 5' boundary of the functional promoter is located at -320 (+/-30bp). Transcriptional activity was drastically reduced to 0.1% of the wild-type level with removal of 5' sequences to position -171 bp, and was completely abolished by deleting to -74 bp. Removal of the CAAT box by an internal deletion resulted in a increase in transcription originating at the upstream (60 bp) minor start site relative to the wild-type level. Supported by USDA grant #81-CRCR-1-0656.

0255 TRANSCRIPTIONAL REGULATION OF ACETYLCHOLINE RECEPTOR GENES DURING MUSCLE DEVELOPMENT, Andres Buonanno* and John P Merlie, Department of Pharmacology, Washington University Medical School, St. Louis, MO 63110

The quantities of acetylcholine receptors (AchR) measured by α bungaratoxin binding on the surface of mouse skeletal muscle C2 cells increase by approximately 10 to 100 fold during their terminal differentiation in culture. By Northern blot analysis, we have determined that the steady-state levels of the AchR α and δ subunits mRNAs increase by approximately 15 fold during differentiation of C2 cells. To determine if the differences in message levels during myogenisis are due to changes in transcriptional rates, nuclear run-on experiments were done using nuclei isolated from 3 day old undifferentiated cells and 7 day old differentiated cells. The rates of transcription for both the α and δ subunit genes changed from levels that could not be detected over background in nuclei from undifferentiated cells, to levels that were 5 to 7 fold over background in differentiated nuclei. As internal controls we measured the rates of transcription of mouse β actin and histone genes. The signals obtained from both undifferentiated and differentiated nuclei were approximately 100 fold over background for both genes, indicating that the absence of detectable transcription of receptor subunit genes in undifferentiated C2 is specific. Interestingly, transcription from the opposite strand of both α and δ subunit genes was also observed in nuclei from differentiated, but not from undifferentiated cells. Further work using nuclear run-on may help to define important mechanisms which regulate the expression of AchR genes. *Supported by the Consejo Nacional de Investigaciones Cientificas y Technologicas, Republic of Venezuela.

0256 ALDOSTERONE CAUSES HYPOMETHYLATION OF DNA AND GENE EXPRESSION IN A-6 KIDNEY CELLS, Peter K. Chiang, Richard K. Gordon, and John P. Johnson, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

A-6 cells, derived from Xenopus kidney, express a differentiated response to steroid hormones in culture. When incubated with 10^{-7} M aldosterone, net transepithelial Na^+ transport in A-6 cells was increased within 2 h, and reached a maximum at 6 h and was maintained over 24 h. The activity of Na^+-K^+ ATPase was not changed until 18 h, at which time it was increased by 50%, hence suggesting pleiotropic effects of aldosterone in A-6 cells. Aldosterone also induced a decrease in DNA methylation in A-6 cells at 18 h, but not in the early time course. 5-Azacytidine or 3-deazaadenosine (inhibitor of methylation via the inhibition of S-adenosylhomocysteine hydrolase) alone had no effect on basal DNA methylation, but inhibited both the hypomethylation of DNA induced by aldosterone and the increase in Na^+ transport seen at 18 h. There was thus a correlation between DNA hypomethylation and increased Na^+ transport in A-6 cells. Hypomethylation of DNA might be necessary for the prolonged expression of aldosterone action in A-6 cells. While basal incorporation of [^{14}C]thymidine in confluent A-6 cells was low, aldosterone stimulated [^{14}C]thymidine incorporation at 18 h but not before. 5-Azacytidine inhibited the incorporation of [^{14}C]thymidine, suggesting that 5-azacytidine inhibited de novo hypomethylation of DNA by this mechanism. Amiloride, which blocked the increased Na^+ transport induced by aldosterone, did not inhibit [^{14}C]thymidine incorporation.

0257 THE MINUTE VIRUS OF MICE (MVM) P39 TRANSCRIPTION UNIT CAN ENCODE BOTH CAPSID PROTEINS AND THE THREE MAJOR VIRAL TRANSCRIPTS POLYADENYLATE AT A SINGLE SITE, Karen E. Clemens, Laura Labieniec-Pintel and David Pintel, University of Missouri-Columbia, Columbia, MO 65212

We have undertaken an examination of the organization and expression of the P39 transcription unit of the single stranded autonomous parvovirus Minute Virus of Mice (MVM). A double stranded segment of the MVM genome expressing only the P39 transcription unit was cloned into the Bovine Papillomavirus (BPV) shuttle vector and used to transform murine C127 cells. Analysis of cell lines transformed by these chimera has indicated that the MVM R3 message (which normally is the most abundant message seen in viral infection) contains sufficient information to authentically encode both virion proteins VP1 and VP2, in the same regulated ratio seen in a normal viral infection. Electron microscopy has indicated that these proteins can assemble into empty capsids at high efficiency (Labieniec-Pintel, L., and Pintel, D., J. Virol, in press). We are currently examining whether both proteins are produced from a singly spliced message or from a previously unidentified set of overlapping, differentially spliced transcripts.

We have also examined the site of polyadenylation of the three major messages encoded by MVM. The right hand end of the MVM genome contains four cannonical AATAAA signals, however, S1 analysis using MVM single stranded probes metabolically labelled in M13 vectors, has indicated that all the messages polyadenylate at a single site at approximately nucleotide 4905, 20 nucleotides beyond the final AAUAAA.

0258 **MECHANISMS INVOLVED IN REGULATING THE ADENOVIRUS EARLY REGION IB GENE,** **Barbara L. Dalie and M.L. Harter, New Jersey Medical School, Newark, New Jersey 07103**

We have generated a series of SV40 expression vectors which direct the synthesis of adenoviral early region IB gene products in animal cells. The E1B transcription unit encodes 2 major mRNAs, a 22S and 13S species which differ by alternate splice sites with the respective proteins terminating upstream of the splice junctions; the 22S mRNA directs the synthesis of both E1B proteins. To examine the role of introns in the generation of mature, stable E1B mRNAs, two type of vectors were constructed. The pSVDNA vectors contain a genomic DNA fragment encompassing the entire E1B transcription unit, including sequences downstream from the poly A addition site, fused to the SV40 early promotor. In the second type (pSVcDNA) the E1B genomic fragment was replaced with cDNA corresponding to the E1B-22S mRNA. This strategy also allowed us to ascertain whether specific sequences downstream of the polyadenylation signal (AAUAAA), absent in the cDNAs, were required for the matuation of these mRNAs.

The recombinants were transfected into COS-1 cells and both the RNA species and proteins produced were analyzed in order to provide direct comparisons between expression of genomic DNA and that of the homologous cDNA. Quantitative analysis of RNA indicated that steady state levels of cytoplasmic E1B-mRNA remained constant while accumulation of nuclear precursors varied between cDNA and genomic recombinants. Moreover, immuno assays showed that the 22S-mRNA was preferentially translated into the larger polypeptide suggesting that the choice of splice site may have an indirect effect on initiation codon usage. These and other results of experiments, which include S1 nuclease analysis for determining the structure of E1B mRNAs in the various transfectants, will be presented.

0259 AN UNUSUAL GENETIC CODE IN RIBOSOMAL PROTEIN GENES OF TETRAHYMENA. Jan Engberg, Karsten Kristiansen, Per H. Andreasen, Hanne Dreisig and Henrik Nielsen. Universities of Odense and Copenhagen, Denmark.

The coordinated regulation of the ribosomal proteins in Tetrahymena is a result of a regulational control at the level of transcription, processing and translation (cf. Eur.J.Biochem. 140, 485-492 (1934)). To get an understanding of these phenomena at the molecular level we have cloned and characterized structurally some of the r-protein genes. A c-DNA library was constructed using m-RNA preparations from Tetrahymena thermophila enriched for r-protein mRNA's. Individual cDNA clones for r-proteins were identified by in vitro translation of hybrid selected mRNA followed by 2-D gel electrophoretic characterization of the translation products. Several hybrid selected mRNAs gave rise to 2 or 3 proteins upon in vitro translation using a reticulocyte system. When the system was supplemented with a pH 5 cytoplasmic fraction from Tetrahymena, single translation products were obtained which co-migrated in 2-D gels with authentic Tetrahymena r-proteins. These observations are believed to reflect an unusual codon usage of some of the r-protein mRNAs. A c-DNA clone for r-protein S-20 has been sequenced as well as used to pull out the (single) chromosomal gene copy from a genomic gtWES library of Tetrahymena nuclear DNA. Comparison of the cDNA and the genomic gene copies revealed an intron in this gene close to its 5'end. We are in the process of sequencing one of the r-protein clones which show evidence of an unusual codon usage and expect by the time of the meeting to present sequence data related to transcription initiation, splice sites, polyadenylation sites as well as to the nature of the unusual codons.

0260 IN VITRO REGULATION OF THE DHFR GENE. Peggy J. Farnham and Robert T. Schimke, Stanford University, Stanford Ca 94305.

We have developed an in vitro transcription system for the murine dihydrofolate reductase gene. The dhfr gene does not have a typical TATA box but instead has 4 GGGCGG hexanucleotides within 300 base pairs 5' of the AUG codon. Although transcription in vitro from a linearized template initiates at the same two major start sites as in vivo, the correct ratios are more closely approximated when a supercoiled template is used in the in vitro reaction. In addition, whereas the dhfr promoter functions bidirectionally in vivo, all of the GC boxes direct unidirectional transcription in vitro. Deletion analysis indicates that each of the GC boxes specifies an initiation ~40-50 nucleotides downstream. A time course of the in vitro transcription reaction demonstrates that the dhfr promoter differs mechanistically from typical TATA box promoters. The reaction products are maximal by ~ 2 min with no preincubation, whereas the major late promoter of adenovirus 2 accumulates transcription products for 30 min. We are beginning to study the cell cycle regulation of the dhfr gene in vitro. We have demonstrated that nuclear extracts prepared from cells blocked in S phase by aphidicolin have enhanced transcriptional activity with respect to the dhfr gene. This increase of in vitro transcription products mimics the increase in steady state levels of dhfr mRNA seen in S phase cells. These results suggest the presence of a cell cycle-specific factor which stimulates transcription from the dhfr gene.

0261 ARCHITECTURE OF THE MAIZE Adh 1 GENE PROMOTER AND ITS CHROMATIN: REGULATORY ASPECTS
Robert J. Ferl, Beth J. Laughner, Anna-Lisa Paul and Harry Nick, Department of Botany, University of Florida, Gainesville, FL 32611

The maize alcohol dehydrogenase-1 gene offers a convenient system to study the chromatin and DNA structural parameters of gene expression in plants. Adh1 is environmentally induced in roots by anaerobiosis and is developmentally inactivated in leaves. We can therefore compare and contrast the mechanisms of environmental and developmental gene regulation.

To that end we have described the chromatin structure of the promoter by probing nuclei with DNAase-1 and various restriction enzymes. We have found two clusters of DNAase-1 hypersensitive sites, but have noted little if any change in these sites upon activation of the gene. However, using restiction enzymes that have recognition sites within the hypersensitive regions, we have detected quantitative modulation of the chromatin structure. We have also mapped two separate and distinct S1 hypersensitive sites within supercoiled clones of the promoter. The positions of these sites correlate with certain of the hypersensitive sites in chromatin. One of the S1 sites is a classic homopurine/homopyrimidine run at -65bp from the start of transcription. The other site is located at -330 and has the sequence structure and S1 signature of Z-DNA. The relationships among the S1 sites, DNAase-1 sites, chromatin modulation, methylation and gene regulation will be discussed.

0262 TRANSCRIPTIONAL CONTROL OF CMP-N-ACETYLNEURAMINIC ACID SYNTHETASE GENE EXPRESSION,
Charles W. Finn, *Keith McKenney, Wendy Aaronson, and Richard P. Silver, OBRR, FDA, Bethesda, MD 20892 and *NINCDS, NIH, Bethesda, MD 20892

The Escherichia coli K1 capsular polysaccharide is an α 2-8-linked homopolymer of N-acetylneuraminic acid (NeuAc). CMP-NeuAc Synthetase catalyzes the activation of NeuAc to CMP-NeuAc, a precursor of the K1 polysaccharide. The gene encoding this enzyme has been localized on a 3.3 Kb Hind III fragment. Strains containing multiple copies of the gene show no gene dosage effect, suggesting that full expression probably requires an additional factor. To study the mechanism of regulation we have begun to characterize a 621 bp region of DNA preceding the structural gene. Cloning this region onto the promoter detection vector pKO1 resulted in low, but consistently measurable promoter activity. Further studies utilizing subclones of the 621 bp fragment fused to the galactokinase structural gene indicate that each half of the fragment contains 5-10 times more promoter activity than observed with the intact fragment, suggesting the presence of two promoters upstream from the CMP-NeuAc synthetase gene. In addition, we have identified a transcription terminator located between the two promoters. The potential role these signals may play in the regulation of expression of CMP-NeuAc Synthetase will be discussed.

0263 Sequence and Analysis of the Human Thymidine Kinase Promoter.
Erik Flemington, Harvey Bradshaw+, Vicki Traina-Dorge, Valerie Slagel and Prescott Deininger
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The entire human thymidine kinase (tk) gene has been sequenced along with 500 base pairs (bp) of 5' flanking sequence which contains the tk promoter. The promoter contains a "TATAA" and "CCAAT" box 24 and 69 bp, respectively, upstream from the CAP site. At ca. 250 bp upstream from the CAP site there is a large inverted repeat (24 of 27 bp homology). Within each repeat there are two G-C clusters, one of which is a G-C box. The promoter is generally very G+C rich and has a high number of CpG dinucleotides. The first 150 bp upstream from the CAP site is 73 G+C and has 18 CpG dinucleotide pairs. Finally, the promoter contains a 12 bp repeat which is found in the promoter region of the chicken tk gene as well as several other cell-cycle regulated genes.

0264 Transcription from the rat albumin promoter on a type 5 adenovirus vector in human hepatoma cells is increased due to the viral E1A enhancer and proteins. Lee E. Babiss, Jeffrey M. Friedman, and James E. Darnell, Jr. The Rockefeller University, New York, N. Y. 10021.

We have recently shown that a type 5 adenovirus vector containing the rat albumin promoter sequences positioned at the left-end of the viral genome can be activated by trans-acting factors that dictate specificity in both hepatoma cells and disaggregated cultures of liver hepatocytes. While tissue-(cell)-specificity was demonstrated, the rate of expression from the viral-albumin promoter was greatly reduced relative to the endogenous albumin gene. We therefore introduced the Ad5 E1A enhancer element 5' to the albumin promoter to investigate the effect on the rate of albumin transcription. We have found that the Ad5 E1A enhancer element increases the rate of expression from the viral-albumin promoter 4-fold in hepatoma cells, and that the addition of viral E1A proteins in trans increases the level of expression another 25-fold. This E1A protein effect depends on the presence of the E1A enhancer since the E1A proteins have no effect when the enhancer is absent. While the E1A enhancer and proteins boost expression in hepatoma cells, they do not increase albumin expression in myeloma cells. Thus, it appears that the rate of expression from a tissue-specific gene can be enhanced, if a tissue-specific factor(s) first initiate a low level of expression.

0265 5' NUCLEOTIDE SEQUENCES INFLUENCE EXPRESSION OF A HUMAN DIHYDROFOLATE REDUCTASE MINIGENE, Merrill E. Goldsmith and Kenneth H. Cowan, Clinical Pharmacology Branch National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

A human dihydrofolate reductase minigene was constructed from DHFR genomic and cDNA sequences and was stably transfected into DHFR gene-deleted CHO cells. These minigene transfected cells have been used to study the modulation of DHFR levels in response to release from serum and amino acid deprivation. The minigene transfected cells respond like normal cells with a synchronous round of DNA replication and an increase of DHFR enzyme levels. When enzyme levels were assayed at the time of maximum DNA synthesis, DHFR levels rose 2.4-3.7 fold for serum and 3.2-4.9 fold for amino acids. In contrast, DHFR levels in cells transfected with a DHFR expression construct made from mouse DHFR cDNA and viral promoter, intron, and termination sequences were not inducible under the identical conditions. Minigene deletions in the promoter, intron 1, 3' nontranslated, and 3' flanking regions were made and used to determine which regions of the DHFR gene were responsible for the regulation. Deletion of nucleotide sequences upstream from 322 bp 5' to the start of transcription do not affect DHFR expression by either modulator. However, deletion of sequences between -322 and -113 bp alters DHFR modulation by both serum and amino acids. This deletion leaves intact the repeated DNA sequences immediately 5' to the start of transcription. In addition to 5' regulatory sequences, nucleotide sequences within the 3' nontranslated region of the DHFR gene also appear to affect amino acid modulated expression.

0266 CHARACTERIZATION OF THE MINIMAL BOVINE GROWTH HORMONE POLYADENYLATION SIGNAL, Edward C. Goodwin and Fritz M. Rottman, Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland OH 44106

The Bovine Growth Hormone (bGH) gene yields mRNA which is polyadenylated at a discrete site both in the anterior pituitary and in transiently expressed bGH constructs. Previous work in this laboratory established that 3' flanking sequences are required in addition to the common hexanucleotide AATAAA for accurate polyadenylation. To define a minimal sequence required for accurate polyadenylation, deletion mutants were constructed which contained variable amounts of bGH sequence extending from both the 3' and 5' sides of the wild-type polyadenylation site. Analysis of a series of 3' deletion mutants has defined an essential element located between 14 and 18 nucleotides 3' of the wild-type site of polyadenylation. Utilizing the bGH expression vector containing only 18 nucleotides of 3' flanking sequence, a series of deletions containing progressively shorter 5' extensions of the presumed polyadenylation signal were constructed. S1 mapping of the transiently expressed mRNA demonstrated that a deletion extending to 11 nucleotides 5' of the AATAAA sequence yields wild-type polyadenylation. Thus, a minimal sequence of 52 nucleotides containing bGH sequences from 11 nucleotides upstream of the hexanucleotide to 18 nucleotides downstream of the polyadenylation site is sufficient to accurately direct polyadenylation. This cassette of 52 nucleotides has been chemically synthesized and utilized as a polyadenylation signal in chimeric constructs. The small size of this cassette has made it ideal for probing the essential components of the polyadenylation signal via site-directed mutagenesis.

0267 STRUCTURE AND EXPRESSION OF THE RAT BRAIN CREATINE KINASE GENE
David Graf, Peter Korolkoff, Pamela Benfield and Mark L. Pearson

The brain creatine kinase gene is differentially regulated in developing muscle cells. Although the gene is typically expressed in myogenic precursor cells (myoblasts), it is down regulated as these cells withdraw from the cell cycle and fuse to form multinucleate myotubes. Instead myotubes express the gene for the muscle specific isoform of creatine kinase. We have isolated and characterized the gene for rat brain creatine kinase. The brain isoform of creatine kinase appears to be coded for by a single copy gene. The structure and sequence of this gene have been determined. One interesting feature of this gene appears to be the presence of two potential promoter regions. In addition, we have identified a genomic sequence corresponding to a processed pseudogene for the rat brain enzyme. In order to examine the mechanisms responsible for the differential regulation of this gene we have examined its expression upon reintroduction into myogenic and non-myogenic cell lines. A preliminary report of these studies will be presented.

0268 Expression of Murine Interleukin 3 in E. coli using a Temperature Inducible Runaway Plasmid: The Effect of Various E. coli Host Strains. ROBERT GREENBERG, KEITH GEWAIN, KAREN JOY SHAW, JAMES ANAGNOST, SATWANT NARULA AND PAUL LEIBOWITZ, Department of Molecular BiologY, Schering Corporation, Bloomfield, NJ 07003

Murine Interleukin 3 (IL-3) or mast cell growth factor (MCGF) was originally cloned and expressed in E. coli using a TAC promoter at the DNAX Research Institute. Significant increases in expression levels have been achieved through the use of the TAC promoter in a vector possessing a cloacin plasmid derived temperature inducible runaway replicon. The effect of various host backgrounds on expression levels and protein stability was investigated. Bioassay data and Western analysis show that specific E. coli host strains, e.g., a minicell producer and a lon$^-$ strain show significantly higher expression levels than E. coli 294. The IL-3 producing strains were grown and treated with chloramphenicol to examine the stability of this protein in vivo. Western analysis of chloramphenicol treated and control samples indicate significant differences in the stability of IL-3 in various strains of E. coli.

0269 ANALYSIS OF 5' DELETIONS OF THE PROMOTER OF A HEAT SHOCK GENE FROM SOYBEAN, Eva Czarnecka and William B. Gurley, Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611.

The heat shock gene Gmhsp17.5-E encodes a low molecular weight (17.5 kD) heat shock protein in soybean. The 5' flanking region of the gene contains both TATA-proximal and -distal heat shock consensus elements (HSEs) with 70 to 90% homology to the Drosophila consensus CT-GAA--TTC-AG (Pelham). Sequential 5' deletions were analyzed in sunflower tumors using an Agrobacterium T-DNA based vector system. Efficient thermal induction (40°C, 2 h) of transcription was dependent on sequences located upstream from the TATA-proximal HSE. A deletion at position -95 bp reduced activity by over 95%. Basal activity (28°C, 2 h) was dramatically increased by removal of sequences from -1175 to -3250 bp. A reference gene containing an altered 5' leader was used as an internal control to precisely quantify transcriptional activity of the altered promoter. Transcriptional activity was assessed by S1 nuclease hybrid protection mapping using a single end-labeled probe able to discriminate between transcripts originating from the test gene (altered promoter) and the reference gene.

Primary crown gall tumors are well suited as a system to study transgenic expression of heat shock promoters since the T-DNA is integrated in low copy number (1-5 per genome). RNA from 50 to 100 tumors were pooled in order to average out variations in transcriptional activity due to the "position effect" of random integration.

Research supported by Agrigenetics Research Associates, Ltd.

0270 DNA SEQUENCES REQUIRED FOR REGULATED EXPRESSION OF A HERPES SIMPLEX VIRUS TYPE 1 γ_2 GENE LIE WITHIN BASES -35 TO +124 RELATIVE TO THE RNA CAPSITE, P.L. Homa, J. Glorioso M. Levine. University of Michigan, Ann Arbor, MI 48109-0618.

The herpes simplex virus genome consists of 3 groups of genes; α, β and γ, whose expression in infected cells is regulated in a cascade fashion. α genes are expressed first and are required for the expression of β and γ genes. γ genes of which there are two subclasses, γ_1 and γ_2, require viral DNA synthesis for expression. The expression of γ_2 genes is stringently dependent on viral DNA synthesis while γ_1 gene expression is not. The DNA sequences required for transcriptional activation of a γ_2 gene during viral infection were studied using viruses containing specific deletions in the 5' nontranslated and/or upstream regions of the HSV-1 glycoprotein C gene (gC), a model γ_2 gene. Eight mutant viruses which have variable 5' and 3' deletions within bases -569 to +124 relative to the start of gC transcription have been isolated. Analysis of RNA extracted from cells infected with these mutants indicate that the DNA sequences required for regulated expression of this γ_2 gene lie within bases -35 to +124. This 259 base pair fragment is sufficient to confer accurate and quantitative expression of gC mRNA and to maintain the stringent requirement on viral DNA replication. The only recognizable concensus regulatory sequence within the residues upstream of the RNA capsite is the TATA sequences at -30. To determine if this element can function as a γ promoter when moved to another region of the HSV-1 genome the 259 base pair fragment was substituted for the normal tk regulatory sequences in the tk locus. Transcription of this chimeric gene was regulated as a γ_2 gene in that its expression in infected cells was dependent on viral DNA synthesis.

0271 TRANSCRIPTION TERMINATION BY CALF THYMUS RNA POLYMERASE II. Caroline M. Kane, Russell L. Dedrick, Daniel Reines, and Michael J. Chamberlin, Department of Biochemistry, University of California, Berkeley, CA 94720.

We have begun a survey of DNA sequences that direct transcription termination by RNA polymerase II *in vitro*. These sequences are found in plasmid and bacterial DNA's as well as in eukaryotic genes. The terminators for the ribosomal operon rrnB and the tryptophan attenuator of *E. coli* are recognized by RNA polymerase II; however, the specificity and efficiency of termination differ dramatically from that of the bacterial RNA polymerase. Studies of *trp* attenuator mutations suggest that the termination sequences but not the self-complementary RNA hairpin region are involved in RNA polymerase II termination. In addition, there are termination sites in both bacterial and eukaryotic DNA which are recognized efficiently by the eukaryotic but not the prokaryotic enzyme. These can occur within eukaryotic gene coding sequences and 3'-untranslated regions as well as downstream from polyadenylylation sites; the sites are often in T-rich regions. Analysis of these sequences indicates that RNA polymerase II can be halted by some but not all T-rich regions in the template. Also, the context of the sequences surrounding the termination sites is more important than secondary structures in the transcript.

0272 CHARACTERIZATION AND FRACTIONATION OF A U2 snRNA 3' PROCESSING ACTIVITY FROM HeLa CELL CYTOPLASMIC EXTRACTS. Ann M. Kleinschmidt and Thoru Pederson, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We have recently reported the initial characterization of an activity from HeLa cell cytoplasmic extracts that accurately processes the 3' end of U2 snRNA precursor molecules (Wieben *et al.*, *J. Mol. Biol.* 183:69-78, 1985). To produce sufficient amounts of substrate with which to characterize this processing activity in more detail, we have constructed an SP6-human U2 gene plasmid (pSPU2^{pre}) from which we can transcribe RNA molecules (SPU2^{pre}) ending either at +5 or +11 nucleotides with respect to the mature 3' end of U2. When incubated in the S100 fraction of a cytoplasmic extract under appropriate conditions, both of these precursors yield RNA products with an electrophoretic mobility indicative of an accurately processed 3' end. Using electrophoretic analysis of processed SPU2^{pre}+11 as an assay, we have determined optimal concentrations of monovalent and divalent cations for the activity. Using this assay we have also shown that the activity is inactivated by moderate heat or micrococcal nuclease treatment. The processing activity has been partially purified using ammonium sulfate precipitation and chromatography on DE52-cellulose, heparin-agarose and Cibacron blue-Sepharose. At all stages of purification the activity fractionated as a single peak, indicating that it is a single molecular species or complex. We are now using this system to further define the processing activity and the reaction mechanism.

0273 HUMAN GLOBIN GENE EXPRESSION IN TRANSGENIC MICE, George Kollias, Nick Wrighton, Jacky Hurst, Ernie de Boer and Frank Grosveld, MRC, Mill Hill, UK

Although many aspects of globin gene expression have been studied, relatively little is known about the cis-acting regulatory DNA sequences involved in the tissue specific and developmental regulation of gene expression. We have chosen the approach of introducing cloned globin genes into the germ line of mice and to examine their expression in different tissues during development. The results of experiments with the human embryonic ε-, the foetal γ-, the adult β- globin and hybrid globin genes will be presented.

0274 A HUMAN U6 SMALL NUCLEAR RNA GENE IS TRANSCRIBED BY RNA POLYMERASE III. Gary R. Kunkel[1], Robin L. Maser[2], James P. Calvet[2] and Thoru Pederson[1], [1]Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, [2]Department of Biochemistry, University of Kansas Medical Center, Kansas City, KS 66103.

We have investigated the species of RNA polymerase responsible for transcription of human U6 small nuclear RNA. A human genomic DNA clone homologous to U6 RNA was sequenced and found to correspond exactly to the known sequence of mouse U6 RNA (human U6 RNA has not been sequenced, but has a RNase T1 oligonucleotide fingerprint identical to mouse). The immediate 5'-flanking region of the human U6 DNA clone has significant homology with a potential mouse U6 gene (Ohshima, Y. et al. (1981) Nucl. Acids Res. 9, 5145-5158), including a TATA box at position -29. Although this sequence element is characteristic of a RNA polymerase II promoter, we have found that human U6 is in fact transcribed by RNA polymerase III. First, the human genomic U6 DNA clone is accurately transcribed in a HeLa cell S100 extract under conditions in which other known pol III transcription units run in parallel are also transcribed, but in which templates for pol II are inactive. Second, U6 RNA transcription in the S100 is resistant to α-amanitin at 1 μg/ml but is completely inhibited at 200 μg/ml. Third, U6 RNA synthesis in isolated HeLa cell nuclei shows an identical sensitivity to α-amanitin, in contrast to U1 and U2 RNA transcription which is inhibited >90% at 1 μg/ml. Finally, U6 RNA synthesized in isolated nuclei is quantitatively immunoprecipitated by La antibody, a property common to almost all other RNA polymerase III transcripts. We are investigating the role of 5' flanking sequences in U6 gene transcription in addition to U6 processing and ribonucleoprotein assembly.

0275 IN VITRO STUDIES OF TRANSCRIPTION IN TRYPANOSOMA BRUCEI, Judith R. Levin, Kenneth P. Watkins, and Nina Agabian, Naval Biosciences Laboratory, School of Public Health, 140 Warren Hall, U.C.Berkeley,CA 94720

The mRNAs of Trypanosoma brucei are unusual in that most, if not all, contain a 35 nucleotide 5' leader sequence that appears to be transcribed as a separate 135 base RNA. The gene encoding the small RNA is found as a 1.4kb tandem repeat in the genome and is unlinked to the majority of protein coding genes. It therefore seems likely that mRNA maturation in trypanosomes involves novel mechanisms at the level of transcription, splicing, or both. As a first step towards addressing this problem, we are characterizing the transcription machinery of trypanosomes by studying RNA synthesis in isolated nuclei as well as in soluble extracts. The -amanitin sensitivity and divalent cation requirements of transcription in nuclei suggest that the small leader-containing RNA is transcribed by a different polymerase than that transcribing mRNA coding regions. While RNA polymerase activity can be detected in vitro, the characterization of this activity (or activities) is hampered by the unavailability of in vivo data on transcriptional initiation sites. We are taking a number of approaches toward identifying in vivo start sites in order to develop appropriate promoter-containing templates for in vitro transcription studies.

0276 TRANSCRIPTION OF DINOFLAGELLATES rRNA GENE, Luc Maroteaux, Laboratoire Arago 66650 Banyuls/Mer France

Dinoflagellates are a group of protists characterised by an unusual organization of their nuclei: Their permanently condensed chromosomes are associated with a low amount of basic proteins (Nuclear basic proteins/DNA< 0.1), which are different from typical eukaryotic histones. Their chromatine is not organized in nucleosomal structure (No repetitive pattern of digestion by endonucleases, smooth DNA filament in EM). In contrast Dinoflagellates genome present several eukaryotic characteristics: repetitives DNA sequences, U1 to U6 snRNA, α-amanitine sensitive RNA polymerase, poly ADPribose polymerase activity. So, one of the main problem of those protists is their transcription regulation in absence of histones. We recently cloned rRNA gene from a Dinoflagellate, Prorocentrum micans. This gene is organized in a tandem repeat of 6 kbp separeted by a non transcribed spacer(NTS) of variable length. Sequence data of this gene is compatible with an early divergence of this group among eukaryotic lineage. NTS sequence around the promoter do not shows any relationship with other rRNA promoter. I start construction of an eukaryotic vector (derived from pSV-neo) where the neomycine resistance gene is under control of the dinoflagellate rRNA promoter, allowing selection of transformants G418 resistant. In parallel, I prepared Manley extracts of dinoflagellates cells to performed in vitro transcrption experiments and I am now characterizing their transcription conditions.(supported by the CNRS)

0277 EVOLUTION AND REGULATION OF THE SGS-3 GLUE GENE OF DROSOPHILA MELANOGASTER, Christopher H. Martin and Elliot M. Meyerowitz, California Institute of Technology, Pasadena, CA 91125

The polytene chromosomes of a third instar Drosophila melanogaster larvae contain a prominent puff at chromosomal location 68C. This puff contains genes which code for components of the proteinacious glus which is synthesized in the larval salivary gland and is used in affixing the puparium to a solid surface. These genes are regulated in tissue, time and by the steroid hormone ecdysone. In one approach to determine the signals used to regulate this gene cluster, the evolution of one of these three glue genes, sgs-3, has been studied in three other species of Drosophila: D. simulans, D. erecta and D. yakuba. Comparison of the upstream sequences has revealed regions which are relatively highly conserved and are candidates for interaction with the trans-acting factors which are presumed to confer tissue and time specificity and regulation by hormone. These regions will be subjected to mutagenesis and re-introduced to flies by P-factor mediated transformation in order to correlate conserved regions with actual regulatory sites.

0278 SPECIFIC NUCLEAR PROTEINS BIND TIGHTLY TO PRE-mRNA DURING SPLICING IN VITRO. Sandra Mayrand, Nina Pedersen and Thoru Pederson, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Pre-messenger RNA transcribed in vivo is packaged into a specific ribonucleoprotein complex termed hnRNP. We have investigated RNP formation during the splicing of human β-globin pre-mRNA in vitro. Capped, ^{32}P-labeled transcripts were synthesized with SP6 RNA polymerase from a truncated human β-globin gene. During incubation in the splicing system, approximately 50% of the β-globin pre-mRNA formed a ribonucleoprotein complex that banded at 1.3 g/cm^3. No such complex formed when RNA was incubated under splicing non-permissive conditions (0°C). Analysis of RNA from this RNP complex by primer extension mapping revealed unspliced pre-mRNA and splicing intermediates, including the characteristic lariat structure generated after cleavage of the 5' donor splice site. RNA-protein cross-linking of the ∿1.3 g/cm^3 RNP complex, followed by nuclease digestion and SDS gel electrophoresis, revealed major, ^{32}P-nucleotide tagged proteins of 38,000 and 41,000 mol. wt., which are in the size range of hnRNP core proteins. To further characterize the protein components, RNP complexes assembled during splicing were crosslinked and reacted with antibodies against either hnRNP core proteins or snRNP proteins. ^{32}P-tagged proteins in the antibody-selected RNP complexes were visualized as above. The major labeled protein components had, with both antibodies, molecular weights characteristic of hnRNP core and snRNP proteins. These results show that hnRNP proteins bind to pre-mRNA during in vitro splicing and, in addition, indicate that the snRNP proteins make direct contact with pre-mRNA in the splicing complex.

0279 Cyclic AMP Induction of Gonadotropin Alpha Subunit mRNA.

Niles Fox, Craig McKnight*, Lih-Hwa Hwang and Davor Solter

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Expression and cyclic AMP (cAMP) mediated induction of gonadotropin alpha subunit mRNA was compared in homologous, human choriocarcinoma cells versus heterologous, alpha gene transfected mouse fibroblast cells (L-cells). In choriocarcinoma cells 5mM dbcAMP or 20uM forskolin (activates the adenylate cyclase-cAMP effector pathway) induced a 10-12 fold increase in alpha subunit mRNA production over a 24 hour period which was also accompanied by an 8-10 fold increase in gonadotropin beta subunit mRNA. Surprisingly, efficient expression of alpha subunit was observed in mouse fibroblast cells stably transfected with the alpha subunit gene. Moreover, induction of alpha subunit expression by dbcAMP or forskolin in these heterologous host cells closely paralleled that observed in choriocarcinoma cells. Together these findings support a pretranslational level for cAMP mediated induction of gonadotropin subunit expression and indicate that cAMP induction of alpha subunit gene expression is mediated by a pathway lacking strict species or tissue specificity.

0280 TRANSCRIPTIONAL CONTROL OF HERPES SIMPLEX VIRUS GENES IN AN IN VITRO SYSTEM, Robert Millette, Jaya Lahiri, Kathy Sampson, and Diane Tomar, Portland State University, Portland, OR 97207

To identify and study viral and cellular proteins involved in the "cascade" regulation of herpes simplex virus type I (HSV-1) gene expression, we have used partially purified RNA polymerase II prepared from HEp-2 cells either mock-infected or infected with HSV-1 under different conditions (harvested at different times post infection, ± cycloheximide or phosphonoacetate), or infected with an immediate-early (ICP4) ts mutant of HSV-1 at permissive or non-permissive temperature. Western blot analyses were used to identify major viral polypeptides present in each enzyme preparation. To further assess the role of IE gene product ICP4, we have used whole cell extracts from mouse L cells that either lack or produce ICP4 (kindly provided by R. Persson & J. Smiley). With these enzyme systems we have transcribed in vitro cloned fragments of HSV DNA each containing a specific class of viral promoter--immediate-early (IE), delayed-early (DE), or late (L). The results of these experiments demonstrate that a) host cell RNA polymerase, in the absence of viral polyproteins, preferentially transcribes IE genes in vitro, b) ICP4 alone is insufficient for transcription of DE & L genes, c) IE proteins, including ICP4, are required for expression of DE and L genes, and d) one or more DE proteins is required for the turn-off of IE gene transcription. By fractionation of HSV-infected HEp-2 cells, we have begun to identify and characterize the viral and cellular factors that allow the uninfected cell RNA polymerase to recognize DE and L viral gene promoters in vitro.

0281 PARADOXICAL EFFECTS OF INSULIN ON TYROSINE AMINOTRANSFERASE, Patrick Moore and John Koontz, Dept. of Biochemistry, University of Tennessee, Knoxville, TN 37996

The hepatic enzyme tyrosine aminotransferase (TAT) is inducible by glucocorticoids, cAMP, and insulin. Glucocorticoids and cAMP appear to increase TAT activity by increasing transcription of the TAT gene. While it has been reported that functional TAT mRNA increases during insulin stimulation (Kenney, 1981), it was not clear whether the increase was due to increased transcription, stabilization of the pre-existing messenger, or even some other mechanism. We have obtained evidence that shows insulin decreases the amount of TAT mRNA in H-35 hepatoma cells. This evidence comes from Northern analysis on total cellular RNA using a cDNA probe for TAT mRNA. The decrease in TAT messenger is most obvious when insulin is added to cultures pre-induced with dexamethasone. The reported half-life of the TAT messenger is about 2 hours. Within 2 hours of insulin addition to dex induced cells, TAT mRNA levels decrease by about 50%, while in the control cultures (dex alone), TAT mRNA levels are relatively unchanged. This is most surprising in that enzyme activity increases under the same conditions. The same phenomena also occurs in cultures that have been pre-induced with cAMP. Insulin alone appears to have little or no effect on TAT mRNA levels.

These results suggest that the increase in TAT enzyme activity under insulin stimulation is due to a post-transcriptional effect. The decrease in hybridizable TAT mRNA is contradictory to the increase in enzyme activity.

This work is supported by NIH Grant #AM30512.

0282 **TRANSCRIPTIONAL CONTROL OF THE RAT FIBRINOGEN LOCUS** John G. Morgan, Elaine Evans, and Gerald R. Crabtree. Stanford University, Stanford, CA 94305

The three non-identical polypeptide chains which constitute the major clotting protein fibrinogen are encoded by three genes, α, β, and γ, which are linked on a 50 kb region of human chromosome 4. In vitro transcription studies, in isolated rat liver nuclei, indicate that the genes exist as individual transcriptional units. Furthermore, following induction of the acute phase response, the three mRNA's accumulate with almost identical kinetics suggesting a mechanism for coordinating their expression in the rat liver. Analysis of the 5' flanking region of each gene has demonstrated the presence of three sequence homology blocks common to all three genes despite the fact that the three chains originated by triplication nearly one billion years ago. To study the role of these 5' homologies in coordinate gene expression, a series of 5' deletion and linker-scanner mutants was constructed and fused to the coding sequence of the chloramphenicol acetyl transferase gene. Following transfection, into a rat hepatoma cell line, these deletion mutants were tested in a transient assay for promoter function. A sequence essential to in vivo transcription was localized to between 50 and 118 bp upstream of the γ chain gene transcription initiation site. A similar analysis of the 5' end of the β chain gene indicates that a region essential to in vivo transcription lies between 51 and 117 nucleotides of the cap site. Preliminary analysis of the 5' end of the α chain gene indicates that its promoter may be considerably weaker than either the β or γ chain promoters. The 5' flanking regions of the 3 genes (-1550 to +32 bp for α, -502 to +7 bp for β, and -860 to +30 bp for γ) do not possess a function capable of conferring inducibility upon the chloramphenicol acetyl transferase gene.

0283

mRNAS FOR UREA CYCLE ENZYMES ARE REGULATED BY DEXAMETHASONE AND cAMP IN RAT LIVER AND IN CULTURED HEPATOCYTES. S.M.Morris, Jr.[1], C.L. Moncman,[1], V.L. Nebes,[1] and W.O'Brien[2]; [1]University of Pittsburgh, Pittsburgh, PA 15261, and [2]Baylor College of Medicine, Houston, TX 77030.

Levels of the urea cycle enzymes are known to be modulated by dietary factors and by various hormones. In order to investigate the basis for this regulation, we have used cloned cDNA probes for 4 urea cycle enzymes to examine effects of dibutyryl cAMP and dexamethasone on mRNA levels for these enzymes in rat liver. Increases of 4- to 6-fold in relative mRNA levels were seen within 5 hr after injection of dibutyryl cAMP, with approximately 2-fold increases within 1 hr. A single injection of dexamethasone elicited a 2- to 3-fold increase within 5 hr. Using primary rat hepatocytes cultured in serum-free medium, we find that either dibutyryl cAMP or dexamethasone alone are necessary and sufficient to produce more than 50-fold changes for some-but not all-of the urea cycle enzyme mRNAs. These results demonstrate significant and relatively rapid regulation at the pre-translational level for these mRNAs.

0284 VIRUS INDUCED STABILIZATION OF A INTRINSICALLY UNSTABLE mRNA, Joseph D. Mosca, The Johns Hopkins University Oncology Center, Baltimore, MD 21205

The human β interferon (IFN) gene has been shown to be post-transcriptionally regulated on the mRNA level. Significance accumulation of IFN mRNA, after poly rI.rC induction occurs only in the presence of inhibitors of protein synthesis. Removal of the protein synthesis inhibitor results in rapid degradation of IFN mRNA within 1 hour. To examine the molecular mechanisms involved in the regulation of gene expression by mRNA stability, we constructed hybrid genes where either the human β-IFN coding sequence or the chloramphenicol acetyl transferase (CAT) coding sequence was inserted behind the simian CMV IE-94 promoter. This promoter has been shown to direct strong constitutive expression in transfected cells. Both hybrid constructs were transfected together with SV_2neo into mouse L-cells and permanent cell lines were established after G418 selection. Constitutive expression of CAT, but never IFN, was observed on the RNA level of several pooled (>100 clones) cell lines. Superinfection of these cell lines containing both the CMV-IFN and CMV-CAT hybrid genes, with HSV, resulted in substantial accumulation of IFN, but not CAT specific mRNA over the untreated controls. The accumulation of IFN-RNA by HSV was dependent on the m.o.i. used. A requirement for a viral gene function for IFN-RNA accumulation was demonstrated using HSVts mutants. Run-on transcription in isolated nuclei argues against induction on the transcriptional level and supports HSV dependent stabilization of human β-IFN transcripts. The use of reconstructed model systems of this type should lend insight into the interactions which occur between viruses and the host cell during virus infection.

0285 Coordinate Regulation of Gonadotropin mRNA Expression by cAMP, J.H. Nilson, A. Milsted, and R.P. Cox, Case Western Reserve University, Cleveland, OH 44106

Chorionic gonadotropin (CG) is a heterodimeric glycoprotein hormone composed of a non-covalently associated common α subunit and a unique β subunit. As α subunit and CGβ genes are located on different chromosomes, we have begun to address whether their expression is differentially or coordinately regulated. To initiate these studies we have used a human choriocarcinoma cell-line (BeWo) which synthesizes and secretes high levels of α and CGβ subunits. Treatment with 8-bromo cAMP causes at least a 10-fold increase in both α and CGβ mRNA levels; the magnitude of the responses are dose-dependent. Furthermore, both mRNAs accumulate with the same kinetics and are maximally induced by the same concentration of 8-bromo cAMP, suggesting their regulation is coordinated. In addition, treatment with either forskolin or cholera toxin stimulates accumulation of both mRNAs, confirming that intracellular generation of cAMP can efficiently regulate gonadotropin mRNA levels in BeWo cells. Preliminary results from experiments in progress indicate that at least part of the effect of cAMP is due to an increase in transcription of both the α subunit and CGβ genes. Since the α subunit and CGβ genes do not share any obvious regions of nucleotide sequence homology, it will be of interest to define the nature and location of nucleotide sequence elements which mediate the action of cAMP.

0286 BOVINE IGF I: GENOMIC STRUCTURE AND EXPRESSION IN LIVER, Sharon D. Ogden and Gwen G. Krivi, Monsanto Co., Chesterfield, MO 63198

The insulin-like growth factors (somatomedins) are primary mediators of growth hormone action throughout mammalian development and are also known to be potent mitogens for many types of cells in culture. We have focused our research on trying to elucidate the molecular mechanisms by which IGF1 gene expression is regulated in the bovine. As a prelude to our gene expression studies, we have investigated the structure of the bovine IGF1 gene. We have found the structure to be remarkably similar to the human gene in terms of the number of exons, sizes and positions of introns, and nucleotide sequence of the coding exons (>90% homology).

Our initial experiments on IGF1 gene expression in bovine liver have shown that 3 major transcripts of approximately 1.5, 4.5 and 6.5 kb are present in both the whole organ and in primary hepatocyte cultures. The mRNA's are very rare, comprising only .001% of the total mRNA's in untreated liver samples.

0287 TRANSCRIPTION OF HUMAN TYPE I COLLAGEN GENES, Anne S. Olsen and Darwin J. Prockop, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854

Type I procollagen is composed of two proα1 chains and one proα2 chain. Although the proα1 and proα2 genes are located on different chromosomes, they are apparently coordinately regulated, since the two polypeptides are synthesized in a ratio of 2:1, and the steady state levels of the two mRNAs are also 2:1. In order to study the regulation of transcription of these genes, we have used a runoff transcription assay in nuclei isolated from cultured human fibroblasts. Control experiments indicate that for a given preparation of nuclei the transcription ratios of the proα1/proα2 genes do not vary significantly with varying reaction conditions. However, a significant variation has been detected between preparations of nuclei isolated from cells grown under different conditions. Cells at lower densities generally transcribe the two genes in a ratio of about 2:1, while cells at very high densities transcribe the two genes in a ratio of about 4:1. Analysis of the amount of proα1 and proα2 RNA transcribed suggests that it is the transcription of the proα1 gene that is sensitive to cell density. Examination of fibroblasts from several patients with osteogenesis imperfecta has revealed one line in which the proα1/proα2 transcription ratio is only about 1:1 and does not increase with cell density. The data suggest a possible transcriptional defect in one or both of the proα1 genes of this patient.

0288 5'-REGULATORY ELEMENTS OF THE HUMAN HPRT GENE. Pragna I. Patel, Twee Tsao, C. Thomas Caskey and A. Craig Chinault, Baylor College of Medicine, Houston, TX 77030

The 5' flanking sequences influencing expression of the human hypoxanthine phosphoribosyltransferase (HPRT) gene have been examined by introducing HPRT minigenes into HPRT-deficient hamster fibroblasts by either $Ca(PO_4)_2$-mediated gene transfer or by microinjection. Positive regulatory (presumably the promoter) as well as negative regulatory sequences have been identified. The minigene, pHPT36 used in these studies comprises 1600 bp of 5'-flanking sequences of the human HPRT gene ligated to human HPRT cDNA with a polyadenylation signal from hamster HPRT cDNA. $Ca(PO_4)_2$-mediated introduction of pHPT36 into $HPRT^-$ hamster cells produced $HPRT^+$ transformants at a frequency of 1.7×10^{-6} per μg of DNA, indicating the presence of a functional promoter. To allow delineation of the promoter region, deleted derivatives of pHPT36 with variable amounts of 5'-flanking sequence were examined for their ability to produce $HPRT^+$ transformants. These studies suggest that (i) the promoter is located within a region 234 bp upstream from the AUG codon that includes four copies of the sequence, GGGCGG and (ii) a negative regulatory element is located between 356 and 234 bp upstream from the AUG codon; deletion of the latter sequence causes a ten-fold increase in the frequency of $HPRT^+$ transformants. Similar results were obtained with a transient expression assay in which these minigenes were microinjected into $HPRT^-$ cells and examined for incorporation of (^{3}H)hypoxanthine into nucleic acid by autoradiography. Further dissection of the negative regulatory sequence and the promoter is in progress and should further our understanding of housekeeping gene expression.

0289 Glucocorticoids increase the rate of degradation of type I procollagen messenger RNAs. Rajendra Raghow and Andrew H. Kang, Departments of Pharmacology and Biochemistry, UTCHS and VA Medical Center, 1030 Jefferson Avenue, Memphis TN 38104

The effect of dexamethasone on the synthesis of cellular and extracellular proteins and specifically on the synthesis of type I procollagen chains and fibronectin was examined in cultured rat fibroblasts. A slight but consistent inhibition of total protein synthesis by dexamethasone was dose and time dependent. Treatment of cells with 1 μM dexamethasone for 24 hr abolished procollagen synthesis nearly completely (<95%) without significantly affecting synthesis of fibronectin. The steady-state levels of translatable mRNAs coding for procollagens and fibronectin corresponded well with the protein data. Quantitative northern blot analysis of pro α1(I), fibronectin and β-actin mRNAs also corroborated the data obtained from cell-free translation experiments. As judged by run-off transcription assays, glucocorticoid treatment did not affect the rate of transcription of type I collagen genes; similarly, the rate of transcription of fibronectin and cytoplasmic β-actin genes also remained unaltered. We tested the sensitivity of our transcription assay and could detect 30-fold stimulation of mouse mammary tumor virus (MTV) gene transcription in dexamethasone-treated cells. Finally, we analyzed the kinetics of decay of radiolabeled mRNA coding for pro α1(I), pro α2(1) and fibronectin in hormone-treated cells and found that procollagen mRNAs were turned over more rapidly in dexamethasone-treated cells. These data suggest that dexamethasone regulates type I collagen gene expression by preferentially destabilizing pro α1(I) and pro α2(I) mRNAs.

0290 TRANSCRIPTS, CODING REGION AND FUNCTIONAL DOMAINS OF THE RAT GLUCOCORTICOID RECEPTOR. Sandro Rusconi and Roger Miesfeld, Department of Biochemistry, UCSF, San Francisco, CA 94143-0448.

The glucocorticoid receptor activates transcription by sequence-specific binding to a class of hormone-dependent enhancers (1). We have extended our initial cDNA clones (2) to include 6.4 kb corresponding to a major rat glucocorticoid receptor mRNA. Complete DNA sequence analysis revealed the putative initiator codon and an open reading frame of 794 aa. The coding sequence is followed by 2 kb or 4 kb untranslated segments terminating at alternative poly-A sites. In vitro translation yielded an immunoreactive protein that co-electrophoreses with purified intact receptor. In addition, expression of fusion proteins containing discrete regions of the receptor coding sequence allowed initial localization of epitopes recognized by two distinct monoclonal antibodies, and of functional domains for DNA and hormone binding. The DNA and hormone binding domains appear to reside, respectively, near the middle and toward the C-terminus of the protein. In contrast, the N-terminal half of receptor, which includes a major epitope, may specify transcription regulatory functions, since it is selectively lacking in one class of mutant receptors that fails to mediate glucocorticoid effects.

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2) Miesfeld R, Okret S, Wikstrom AC, Wrange O, Gustafsson J-A, Yamamoto KR (1984). Nature 312, 779

0291 EXPRESSION OF A MODIFIED MOUSE α-GLOBIN GENE REINTRODUCED INTO THE MOUSE GERM LINE IS AFFECTED BY THE SIZE OF THE CO-TRANSFERRED FLANKING SEQUENCES. Sandro Rusconi*, Institut fuer Molekularbiologie II der Universitaet Zuerich , CH-8093 Hoenggerberg, Switzerland. To specifically detect the expression of a newly introduced homologous gene in transgenic animals, the 3' untranslated trailer of a cloned mouse α1-globin gene was mutated by insertion of synthetic linker or a corresponding segment of rabbit ß-globin. The modified gene and variable portions of its flanking sequences were reintroduced into the germ line of ICR/Z mice; gene transfer and expression could be monitored by Southern blots and nuclease protection, respectively. Analyses of independent transgenic lines indicated that little or no expression was detected in 35 lines in which the gene was introduced together with 4 kb of flanking DNA upstream and downstream, either with or without linked vector or SV40 enhancer sequences. In contrast, expression at a level comparable to that of endogenous α-globin genes was observed in three transgenic lines transformed with a 40 kb DNA segment of the α-globin locus containing an embryonic α-globin gene upstream of the modified α1 gene and the α2-globin gene downstream. The results suggest that cis-acting elements relatively distant from the mouse α1-globin gene might be required for its efficient expression when inserted at aberrant chromosomal sites in transgenic animals.

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0292 A RAPID AND EFFICIENT METHOD TO CLONE RANDOM YEAST PROMOTERS, George Santangelo and Joanne Tornow, University of California, Santa Cruz, CA 95064

We have begun a comprehensive analysis of the genome of Saccharomyces cerevisiae, involving the identification and isolation of RNA polymerase II-specific promoters and coding regions. We first developed a method to generate libraries of small random fragments of genomic DNA. This method, carrier-facilitated insertion, employs a carrier fragment of vector DNA to circumvent the difficulties in ligating two fragments together to generate a recombinant circle efficiently. We have made libraries in several vectors by using this method. The expression vector pMR200X was used to detect open reading frames (ORFs), which allowed the calculation of a minimum estimate for gene number in Saccharomyces cerevisiae (3970). Similar estimates for single chromosomes or other genomes can be obtained by screening the appropriate library for ORFs. This comprehensive genomic expression library has also been used to clone DNA that codes for a specific yeast antigenic determinant. When used in combination with a method to detect constitutive, repressible, and inducible yeast promoters, and methods developed by others to map cloned regions unambiguously to specific regions of yeast chromosomes, carrier-facilitated insertion should permit a comprehensive analysis of the yeast genome and its expression. Several libraries were also constructed with various promoter-cloning vectors that contain silent indicator genes. Activation of these indicator genes can be detected after insertion of either known promoters or a subset of yeast random fragments. One of these random fragments appears to contain a bidirectional yeast promoter. In principle, this methodology can be used to isolate families of promoters (and genes) by virtue of their identical response to states of induction or repression.

0293 MODULATION OF RECOMBINANT H-γ-IFN EXPRESSION BY 3' UNTRANSLATED SEQUENCES. Nava Sarver, Molecular Biology Division, Meloy Laboratories, Springfield, VA 22151.

Previously, we developed a bovine papillomavirus (BPV) based shuttle vector which supports a sustained, high-level expression of human gamma-interferon (H-γ-IFN) cDNA in compatible host cells [Sarver, et al., Papilloma Viruses: Molecular and Clinical Aspects (1985), in press]. In addition to the BPV genome and the prokaryotic origin of replication, the vector contains several obligatory and optional regulatory elements to direct γ-IFN expression. Using this vector, we assessed the effect of 3' untranslated sequences and of mRNA processing elements (splice sites and polyadenylation signal) in modulating cDNA expression. Sequences downstream of the insert were systematically deleted in a 5' to 3' direction, removing a discrete element 3' to the cDNA with each deletion step. We then analyzed each deletion for γ-IFN expression. Our data indicate that untranslated sequences placed downstream of the cDNA insert interfere with gene expression. The extent of interference is directly correlated with the length of the untranslated sequences. Moreover, the presence of donor acceptor sites downstream of the cDNA has an augmenting effect on γ-IFN expression when compared to a vector containing only the poly(A) signal. Taking advantage of these findings, a modified vector was designed which is capable of directing ten-fold higher levels of γ-IFN than the original vector reported.

0294 UNFAITHFUL HERITABILITY OF GENE METHYLATION PATTERNS AND LEAKY GENE EXPRESSION IN DIPLOID HUMAN FIBROBLASTS, R.J. Shmookler Reis, J.W. Hardin, and S. Goldstein, University of Arkansas for Medical Sciences and McClellan VA-GRECC, Little Rock, AR 72205

We have observed unfaithful somatic inheritance of DNA methylation patterns in clonal lineages of normal human diploid fibroblasts (PNAS 79:3949-3953, 1982; Nucl. Acids Res. 10:4293-4304, 1982, and 13:7055-7065, 1985). We have focused on the genes for the α- and β-subunits of human chorionic gonadotropin (hCG), which are frequently derepressed in tumor cells. Fibroblast clones differed markedly in their maintenance of DNA methylation in the α-hCG gene region. Several clones underwent progressive and apparently random hypomethylation, with distinctive patterns appearing in each subclone, while other clones maintained essentially complete methylation of these sites. Methylase assays of clones are currently in progress, although methylase levels alone cannot account for our data, because clones which maintained stable methylation for one gene region could nevertheless become hypomethylated at other loci.

We have also investigated the possibility of leaky or ectopic expression of the α- and β-hCG genes in normal human fibroblasts. Several strains secreted significant levels of immuno-assayable β-hCG, but none produced detectable α-hCG. Data thus far have shown little or no mature mRNA corresponding to the α- or β-hCG genes, by either Northern blot or S_1-nuclease analysis.

We conclude that hypomethylation per se is insufficient to allow derepression of these genes, at least in normal cells. Low levels of β-hCG detected by us and others, in several fibroblast strains, may thus represent immunologic cross-reactivity of related proteins.

0295 Methylation of the α2(I) Collagen Gene in Chemically Transformed Rat Liver Epithelial Cells, Barabara D. Smith and Erika Marsilio, Veterans Administration outpatient Clinic and Boston University School of Medicine, Boston, Mass 02118

The W8 cell line, a chemically transformed variant of K16 rat liver epithelial-like cells, does not produce a normal type I collagen but instead secretes an α1(I) trimer and no α2(I) chains. There are no steady state levels for proα2(I) RNA in nuclear or cytoplasmic extracts from W8 cells as demonstrated by northern hybridization analysis using probes for the 5' and 3' regions of the proα2(I) gene. The gene for proα2(I) collagen is present in the W8 cells. There are no large deletions, insertions or rearrangements in the promotor-5' or 3' region of the gene by Southern hybridization analysis. However, the promoter-5' region of the W8 DNA is methylated at several Hpa II sites whereas the same region is not methylated in the K16 DNA. This methylation may inhibit the transcription of proα2(I) collagen gene in the chemically transformed cells.

0296 EXPRESSION OF YEAST RANDOM PROMOTERS IN MAMMALIAN CELLS. Mary Zavanelli and George Santangelo, University of California, Santa Cruz, CA 95064.

Attempts to study the control of expression of mammalian genes has been hampered by the lack of a method to rapidly and easily clone mammalian random promoters. We have developed such a system for use in the yeast Saccharomyces cerevisieae. These promoter-cloning vectors contain silent indicator genes which can be activated upon insertion of known promoters. One indicator gene is the yeast alcohol dehydrogenase I gene (ADH-I). Monoclonal antibodies against this gene product have been produced in Balb/c mice. These are used in an enzyme-linked immunosorbent assay to detect ADH-I expression in transformed cultures. We used an African green monkey kidney cell-derived line, Cos-1, as the target for transformation. This cell line produces the SV-40 T antigen protein which allows the propagation of plasmids which contain the SV-40 origin of replication. The expression of these yeast vectors in mammalian cells will enable us to use this system to isolate mammalian random promoters. Any promoters which are cloned in this fashion can be propagated in bacteria and tested for activity in S. cerevisiae.

Enhancer Sequences and Transacting Factors II

0297 POSITIVE AND NEGATIVE REGULATORY ELEMENTS OF THE CHICKEN LYSOZYME GENE
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The chicken lysozyme gene is expressed constitutively in macrophages and under steroid hormone control in the oviduct. To study the mechanisms responsible for the specificity we have introduced recombinants of this gene into homologous and heterologous cells.

Fusions of several lysozyme 5'-deletions with the coding region of the bacterial CAT-gene were transfected into different cell types. Transient expression of the hybrids was measured as enzymatic CAT-activity in cell extracts. Cells transfected with a plasmid containing the HSV-TK-promoter in front of the CAT-gene were used as reference.

In every case expression was low compared to TK-CAT. Exceptions were lysozyme deletion mutants retaining approximately 200 bp immediately upstream of the start site: Their expression was high in oviduct cells only after steroid application and in MCF-7 cells (mammary gland; endogenous gene activity not yet determined), in which expression was not affected by hormone treatment.

Two different regulatory elements seem to be present in addition to the steroid control regions:

a) an inhibitory sequence upstream of -200 bp

b) an activator sequence downstream of -200 bp, for which a tissue specificity was shown.

0298 CIS-ACTING REGULATORY ELEMENTS WITHIN THE GAG GENES OF AVIAN SARCOMA VIRUSES,
Karen Beemon, Salvatore Arrigo, Kristen Carlberg, Thomas Lee, Alison Lin, and Mary Yun, Biology Department, Johns Hopkins University, Baltimore, Maryland 21218

Cis-acting regulatory sequences have been detected within the gag genes of several avian retroviruses, including Rous sarcoma virus, Fujinami sarcoma virus, and the endogenous virus RAV-0. Five to twenty-fold enhancement of CAT gene expression was observed in a transient assay when an internal gag coding region (nucleotides 532 to approximately 1150) was inserted into a plasmid containing the CAT gene under control of the SV40 promoter. This enhancement was independent of the position of the insert. However, there was a pronounced orientation dependence, with higher levels of CAT expression from constructs in which the 5' end of the gag fragment was nearest to the promoter. A slightly smaller gag fragment (nucleotides 532-1015) did not show this orientation dependence, but enhanced in either orientation effectively. To test the possibility that the gag insert in these plasmids was encoding a trans activator in an alternate reading frame, insertional mutations were made resulting in frameshifts in the gag gene. These mutations did not alter the CAT gene expression. Since the presence of the gag fragment did not affect the level of plasmid DNA in the nucleus of transfected cells, we propose that it may be acting at the level of transcription. We are currently performing site-directed mutagenesis of this region of the viral genomes to assess the significance of this sequence for viral replication.

0299 TRANSCRIPTIONAL REGULATION OF INTERLEUKIN 2 (IL 2) RECEPTOR EXPRESSION ON HEMATOPOIETIC CELLS BY INTERLEUKIN 3 (IL 3).
M. C. Birchenall-Sparks, W. L. Farrar, D. Rennick and F. W. Ruscetti. Laboratory of Molecular Immunoregulation, BRMP, NCI, Frederick, MD 21701-1013 USA

Numerous reports have suggested a possible role of IL 2 and receptors for IL 2 in the growth and differentiation of a number of different cell types. We have investigated the activation of genes for IL 2 receptors using three IL 3-dependent murine cell lines (granulocyt and macrophage progenitor cells, FDC-P1, and newly derived murine mast cell lines, DX-1 and DX-2).

Our studies indicate that FDC-P1, DX-1 and DX-2 cell lines when stimulated by IL 3, all develop high levels of the IL 2 receptor on their surface. ^{32}P-labeled run off RNA transcripts were isolated from nuclei between 0 and 2 hrs after growth factor addition to confluent cells. Plasmids containing cDNA for IL 2 receptors were bound to nitrocellulose and were hybridized with ^{32}P-labeled RNA. Increased levels of IL 2 receptor transcripts were observed 1 hr after IL 3 stimulation of quiescent FDC-P1 cells. An increase in the expression of IL 2 receptor mRNA was also seen by Northern blot analysis at 1 and 2 hrs after stimulation of cells with IL 3. Analysis of the binding characteristics of these receptors using ^{125}I-labeled recombinant IL 2 revealed that only receptors with low affinity for IL 2 were present on these cells. The role of this receptor in hematopoietic cells remain to be established.

0300 POSITIVE AND NEGATIVE TRANS-ACTING TRANSCRIPTION CONTROL FACTORS AND THEIR ROLE IN THE RAPID INDUCTION AND SUPER INDUCTION OF GENES BY GROWTH FACTORS. ISOLATION AND IN VITRO TRANSCRIPTION OF β-ACTIN GENES. Stanley P. Blatti, Paula K. Elder and Michael J. Getz, Department of Cell Biology, Mayo Clinic/Foundation, Rochester, MN 55905 Growth factors have been shown to rapidly induce (within 10 min) β-actin transcription in AKR-2B cells. Other genes such as VL30 have been shown to be induced at later times but are still part of the primary response since CH (cycloheximide) does not abolish the induction. Thus positive control factors are thought to act to increase transcription of these genes. β-actin and c-fos are thought to be controlled by labile negative factors as well since protein synthesis inhibitors such as CH result in a much higher level of mRNA synthesis in nuclei and c-fos and β-actin mRNA accumulation. The trans-acting repressor, SV40 T antigen, binds to a GGXGG -7 nt spacer- GGXGG sequence 25 bp downstream from the start site of SV40 early gene expression. Interestingly, the rat β-actin gene contains a GGXGG -8 nt spacer- GGXGG sequence 35 bp downstream from the start site. To elucidate further these possible control mechanisms, we have isolated a mouse β-actin genomic clone which retains several putative control sites. In vitro transcription of the β-actin clones with purified calf thymus RNA polymerase II and transcription factors from Hela resulted in specific run-off transcripts of 8.5 Kb. These results suggest that (1) the start site for β-actin should be 8.5 Kb from the end of the 11 Kb insert and (2) that the positive control sequences should be present. Subsequent restriction enzyme mapping demonstrates that the putative start site is 8.5 Kb from one end of the insert and that a putative control sequence is conserved between human, rat and mouse β-actin.

0301 ADR1 Encodes an ADH2-Specific Transcription Factor, Hal Blumberg, Toinette Hartshorne, Marie Tavianini, Wayne Taylor, Josie Yu and Ted Young, University of Washington, Seattle, WA 98195

The glucose-repressible ADH2 gene in the yeast Saccharomyces cerevisiae is transcriptionally regulated. An upstream activator sequence(UAS) is required for ADH2 transcription and is the site of action of the positive regulatory protein ADR1. The ADR1 gene could encode a 1323 amino acid protein but an amino-terminal 302 amino acid polypeptide is sufficient to activate ADH2 expression. ADR1 activity is likely to be regulated at the post-translational level in a carbon source-dependent manner. An ADR1-lacZ gene fusion encodes a bifunctional nuclear protein. ADR1 has striking homology with the Xenopus RNA Polymerase III transcription factor TFIIIA. The region of homology contains contiguous thirty amino acid repeats(nine in TFIIIA,two in ADR1) which are proposed to form a "finger structure" involved in DNA binding. Experiments are in progress to:

1) define an ADH2 sequence which is sufficient to confer ADR1-mediated activation on a heterologous promoter.
2) define the domain structure of the ADR1 protein
3) generate and characterize mutations in the "finger structure."
4) determine whether the ADR1 protein binds to the UAS of ADH2.
5) determine how ADR1 activity is regulated.

0302 ACTIVATOR SEQUENCES OF THE MURINE αA-CRYSTALLIN GENE, Ana B. Chepelinsky and Joram Piatigorsky. Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20892.

There are two α-crystallin genes, αA and αB, which are differentially regulated during lens cell differentiation. Transient assays using the pSVO-CAT expression vector in explanted chicken lens epithelia (Chepelinsky et al., PNAS 82, 2334, 1985) and transgenic mice (Overbeek et al., PNAS, in press) have demonstrated that sequences between position -364 and +45 of the murine αA-crystallin gene promote CAT (chloramphenicol acetyl transferase) activity in a lens tissue-specific manner. CAT expression is not promoted by sequences between positions -87 and +45 of the αA gene. Our present experiments indicate that sequences between -110 and +45 of the αA promoter are sufficient to promote CAT activity in the lens explants. CAT activity was still promoted when a 57 bp spacer was inserted at position -87 in a fragment spanning positions -286 to +45. The upstream sequences between position -286 and -87 functioned more effectively when their orientation was not reversed; these sequences did not promote CAT activity when the spacer was increased to 2871 bp. Furthermore, the -286 to -87 fragment did not activate the SV40 promoter in the pA10-CAT2 expression vector. This suggests that the activating influence of the -286 to -87 region of the αA-crystallin promoter may be affected positively by sequences between position -87 to +45. Taken together, our experiments suggest that the 23 bp between -87 and -110 are critical for αA-crystallin gene expression. In addition, our results suggest that there are at least 2 separate regions (one upstream and one downstream of position -87) which are needed for tissue-specific expression of the αA-crystallin promoter, and that the upstream sequence (-286 to -87) share some properties of a cellular enhancer.

0303 CHARACTERIZATION OF A NOVEL HOST RANGE MUTANT OF POLYOMA CONTAINING a 2500bp INSERT CAPABLE OF EXPRESSION ON EMBRYONAL CARCINOMA CELLS, Larry A. Couture, John M. Lehman, Albany Medical College, Albany NY 12208.

Murine embryonal carcinoma (EC) cells are refractory to infection by polyoma virus. Repeated selection and cloning of cells, from pcc4 azal or F9-22, expressing T antigen after innoculation with polyoma strains A2 or large plaque toronto (LPT) by this and other laboratories has yielded a number of mutant viruses which show host range specificity for EC lines. These mutant polyoma viruses show host specificity for the cell line from which they were isolated with little or no cross-selectivity. All reported mutants have shown alterations in the enhancer region and exhibit high mutation homology with other polyoma mutants with the same host range. A LPT mutant, pyF9340, recently isolated in this laboratory which shows host selectivity for F9-22 cells has a 2500bp insert on the early side of the origin between the HaeII restriction sites located at position 84 and 95. LPT shows a degree of selectivity for pcc4 azal cells which is retained in this multi-host selective mutant. Restriction mapping has characterized the insert as composed of 5 copies of a 500 bp region presumably spanning the origin 100bp to the late side and 400 bp to the early side of wild type polyoma LPT or A2. Further characterization of the insert by sequencing and cloning of various fragments of the insert to identify the necessary regions which confer the altered host range will be presented.

0304 FUNCTIONAL CHARACTERIZATION OF PURIFIED HTLV-III TAT-III PROTEIN EXPRESSED IN ESCHERICHIA COLI, C. Debouck, T. Chao[+], A. Aldovini[o], J. Culp, J. Lewis, G. Sathe, F. Wong-Staal[o] and M. Rosenberg, Departments of Molecular Genetics and [+]AP&S Chemistry, SmithKline & French Laboratories, Swedeland, PA 19479; [o]Laboratory of Tumor Cell Biology, NIH-NCI, Bethesda, MD 20205.

Human T-lymphotropic virus type III (HTLV-III) is the etiological agent of the acquired immune deficiency syndrome (AIDS) and related disorders. This retrovirus encodes a trans-acting factor (tat-III) that activates transcription initiated in the viral long terminal repeat. We have constructed a plasmid expression vector, pOTS-tat-III that permits the regulated and high level expression in Escherichia coli of the product of the tat-III gene. We have purified the E. coli-expressed tat-III protein and initiated detailed characterization of its function. Its interaction with nucleic acids and its cellular localization will be presented.

0305 AN RNA POLYMERASE I ENHANCER IN YEAST, Elaine A. Elion and Jonathan R. Warner, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461

The transcription of many eukaryotic genes is regulated by a variety of cis-acting positive control elements which can stimulate transcription 10 to 200-fold. Until recently, positive control elements had been characterized exclusively for RNA polymerase II genes. By the use of an artificial rDNA gene we have found that transcription of the major ribosomal precursor RNA in yeast is stimulated 15-fold by a positive control element located 2 kb upstream of the transcription initiation site. Analysis of in vitro run-off transcripts suggests that the promoter element stimulates the frequency of initiation by RNA polymerase I molecules. A 190 bp fragment encompassing the promoter element can stimulate transcription on a centromere plasmid in either orientation, upstream or downstream of the transcription initiation site, suggesting it is an enhancer element, although its function is strongly influenced by adjacent sequences. Finally, sequences lying on each edge of the 190 bp fragment are essential for activity of this RNA polymerase I enhancer. In particular, a 5 bp deletion at the extreme 3' boundary of the 190 bp fragment abolishes the activation of transcription. These results implicate both a 'Z-prone' region of DNA as well as a set of inverted repeats that bear strong homology to the SV40 core consensus sequence.

0306 YEAST CELL TYPE REGULATION OF TY1 ENHANCER FUNCTION, B. Errede, M. Company, C.A. Hutchison III, M.A. Teague, University of North Carolina, Chapel Hill, NC 27514.

Insertions of Ty1 frequently are found to activate expression of the adjacent gene. Activation occurs in haploid cell types but is repressed in the MATa/MATα diploid cell type. We have applied deletion and reconstruction methods to define the DNA sequences within Ty1 that are responsible for this effect. Our results indicate that the Ty1 activating region consists of multiple components. One component shows homology to mammalian enhancer sequences and to the diploid control sequence identified at MATα. This component, in either orientation, functions to activate gene expression, but only when present with other Ty1 sequences. However, this component by itself can mediate repression of adjacent gene expression in diploid cells. We chemically synthesized a 28 bp regulatory element corresponding to this component of the activator. The method of synthesis produces random mutations throughout the synthesized region. We have identified single base pair substitutions at 22 of the 28 possible positions and are in the process of characterizing the enhancer and diploid control phenotype of each.

Transacting factors are required for the activating function of Ty1 in haploid cells. We have sequenced the STE7 gene which encodes one such factor. The predicted amino acid sequence has significant homology to the catalytic subunit of cAMP dependent kinase and to the mos family of oncogenes. These findings suggest there may be a direct relationship between protein kinases and cell type control of enhancer function.

0307 SYNERGISM BETWEEN IMMUNOGLOBULIN PROMOTERS AND ENHANCERS, Victor Garcia, Jeannine Stafford and Cary Queen, National Cancer Institute, Bethesda, MD 20892

To investigate the relationship between particular transcriptional enhancer elements and particular promoters, we constructed a set of plasmids in which different promoters and enhancers were attached to the gene for chloramphenicol acetyltransferase (CAT). The SV40 early promoter, a kappa light chain promoter, and a metallothionein promoter were each combined with the polyoma, kappa, and immunoglobulin heavy chain enhancers, or with no enhancer. All the plasmids were transfected into a line of mouse myeloma cells, and transcription from the promoters was assayed by measuring the amount of CAT synthesized after 48 h. We found that the kappa and heavy chain immunoglobulin enhancers stimulated transcription from the kappa promoter 10-30 times more than transcription from the SV40 or metallothionein promoters. In contrast, the polyoma enhancer stimulated all 3 promoters the same amount, to within a factor of 2. Our results imply that the immunoglobulin enhancers work synergistically with the kappa promoter and suggest that one or more proteins have binding sites in both the immunoglobulin promoter and enhancers.

0308 STUDIES ON THE REGULATION OF EXPRESSION OF THE T3δ GENE OF THE T3/T CELL RECEPTOR COMPLEX. Katia Georgopoulos, Peter van den Elsen and Cox Terhorst. Lab of Molecular Immunology, Dana-Farber Cancer Institute, Boston, MA 02115.

Antigen recognition by thymus derived lymphocytes (T cells) and the subsequence T cell activation are mediated by a group of integral membrane proteins, the T cell receptor/T3 complex. The T cell receptor α and β chains comprise the clone specific structures involved in recognition and binding of the foreign antigen in the context of polymorphic MHC antigens and the γ,δ and ε chains of the T3 complex are thought to be involved in signal transduction and subsequent T cell activation. The cDNA clones coding for the human and mouse 20kD glycoprotein (T3-δ) of the T3/T cell receptor complex have been isolated (van den Elsen, et al) and the genetic organization of the human and mouse genes has been determined. The gene coding for the human T3δ chain is a single copy gene approximately 4kb long and consists of five exons. The 5' flanking region from the CAP site of the T3δ gene does not contain the TATA and CAAT boxes found in many eukaryotic promoters. The mouse T3δ gene is organized in an identical fashion. The immediate 5' flanking region from the major mRNA initiation sites (CAP sites) exhibits high levels of conservation of nucleotide sequences in both human and mouse T3-δ genes. Preliminary experiments have revealed the existence of a DNase I hypersensitive site within the T3δ gene in T lymphocytes. We are currently investigating the regulation of expression of the T3δ gene in the T lymphocytes by studying the activity and tissue specificity of the promoter region and by screening the gene for tissue specific enhancer elements. The results of these studies will be discussed.

0309 INTRACELLULAR MEDIATORS OF C-FOS INDUCTION BY GROWTH FACTORS.

Michael Z. Gilman and Robert A. Weinberg*, Whitehead Institute for Biomedical Research and *Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA.

We are investigating the mechanism of induction of the mouse c-fos gene by serum growth factors. We find that fos expression is induced by a variety of agents which activate protein kinase C, including the tumor promoter TPA and synthetic diacylglycerols. Expression of fos is also induced by the calcium ionophore A23187. However, our data suggest that this induction is not due simply to elevation of intracellular calcium. Concentrations of these agents sufficient to induce fos expression to physiological levels fail to induce appreciable expression of c-myc. As an alternate approach to this problem, we have begun to identify potential regulatory regions in the mouse c-fos gene using a variety of in vitro and in vivo assays. We have detected activities in nuclear extracts which bind specifically to fos 5'-flanking sequences. These binding sites have been defined by footprinting and competition experiments. We have constructed a variety of fos promoter derivatives carrying deleted or mutated binding sites and have begun to use these mutants in gene transfer studies to assess the functional significance of the binding interactions we detect in vitro.

0310 AN ACTIVATOR OF PYRUVATE DECARBOXYLASE EXPRESSION IS ITSELF TRANSCRIPTIONALLY REGULATED

Jeremy B.A. Green, Anthony P.H. Wright, Wing Y. Cheung & Brian S. Hartley.
Centre for Biotechnology, Imperial College, London SW7, England.

Pyruvate decarboxylase (PDC) is a key fermentative enzyme in S. cerevisiae whose structural gene, PDC1, is 20-fold inducible at the trancriptional level (Schmitt et al 1983, MGG 192,247-252). We have cloned a second gene, PDC3, which rescues some pdc1 mutant alleles when present at high copy number. We have shown that such strains over-produce a defective PDC enzyme and conclude that the PDC3 gene product functions as an activator of PDC1 expression. In addition to this positive control, we also have evidence for negative regulation of PDC1. Thus, in many respects, the PDC system is ideal for the study of trancriptional control of metabolic genes.

Interestingly, PDC3 expression is itself regulated at the transcriptional level. As with PDC1, PDC3 expression is about 20-fold greater during fermentation than under respiratory conditions. Studies using promoter fusions to the lacZ gene have delineated upstream DNA sequences sufficient for regulation and we are examining the effects of deletions and point mutations on them. We are using mutagenesis of fusion-containing strains to identify trans-acting genes involved in the regulation of PDC3 and by implication PDC1. Other studies involving gene disruption and in vitro DNA binding assays are in progress to determine the precise function of the PDC3 gene product.

0311 THE HOMOLOGOUS DNA OF THE BACULOVIRUS *AUTOGRAPHA CALIFORNICA* NUCLEAR POLYHEDROSIS VIRUS (ACNPV) *TRANS*-ACTIVATES VIRAL TRANSCRIPTION. Linda A. Guarino and Max D. Summers, Texas A&M University, College Station, TX 77843

Five regions of homologous (hr) DNA, rich in EcoRI sites, are interspersed in the AcNPV genome. Expression of chloramphenicol acetyl transferase (CAT) under the control of a delayed early (39K) promoter was stimulated by cotransfection of the 39CAT plasmid with plasmids containing hr DNA. Expression from several heterologous viral promoters was also activated by hr DNA. The stimulation of CAT activity was not increased by cloning the hr sequences into the 39CAT plasmid, indicating that the hr's are *trans*-acting. Quantitative S1 mapping indicated that the hr's act at the level of transcription. RNA transcripts homologous to hr DNA have not been detected and the nucleotide sequence of hr DNA indicates that hr's do not encode a protein product. The presence of a repeated 28 bp palindrome in hr DNA supports our theory that the hr's *trans*-activate indirectly by binding and inactivating a host factor which inhibits viral transcription.

0312 DNA-PROTEIN INTERACTIONS ASSOCIATED WITH A WEAK TISSUE-SPECIFIC PROMOTER, Deborah L. Gumucio and Miriam H. Meisler, Department of Human Genetics, University of Michigan, Ann Arbor, MI 48109-0618

Tissue-specific expression of the mouse Amy-1 gene is unusual in that transcription can be initiated from two distinct promoters: a strong parotid-specific promoter and a weaker promoter which is active in liver. To study the trans-acting factors which interact with the liver promoter, we have examined the surrounding chromatin for hypersensitive sites using the indirect end-labelling technique of Wu (Nature 286: 854, 1984). Two hypersensitive sites are consistently found in liver nuclei, but are not present in parotid nuclei. One of these sites maps between -65 and +100 and thus includes both the TATA box and cap site. The other site is located in an intron which interrupts the 5' nontranslated portion of the transcript. We have subcloned and sequenced the one kilobase of DNA surrounding these hypersensitive sites. Using the exonuclease III procedure described by Wu (Nature 317: 84, 1985), we have partially purified several proteins from liver nuclei which interact with this DNA.

0313 ISOLATION OF A NOVEL TYPE OF GENE-ACTIVATOR FROM HUMAN GENOME BY FUNCTIONAL SELECTION. Hiroshi Hamada, Memorial University of Newfoundland, St.John's, Canada A1B 3V6.

In an attempt to isolate enhancer-like elements from human genome, an enhancerless plasmid containing a selectable gene(gpt) was used as an "enhancer-trap". When the enhancer-trap was transfected to HeLa cells, gpt -transformants appeared with a very low frequency. From six of the transformed cell clones that have the single integrated gene and express high level of gpt activity and gpt mRNA, transcribed from the native initiation site, human DNA sequences flanking the integrated gpt-gene have been moleculary cloned. In three of the six clones the gpt-gene was closely associated with a common human DNA sequence (GA-1).GA-1 was located 5' to the gpt gene in one clone and 3'-to the gpt-gene in other two clones. GA-1 sequence seemed to exist 2-5 times in the human genome. GA-1 had a strong (as strong as the SV40 enhancer) enhancer-activity when assayed in a stable transformation system. However, unlike viral and other cellular enhancers, GA-1 showed no activity when assayed in transcient expression system. I suggest that GA-1 is a novel type of gene activator that is active in human chromosome but inactive in transfected non-integrated plasmid DNA.

0314 CIS REGULATORY FUNCTIONS OF A GIBBON APE LEUKEMIA VIRUS (GALV) LONG TERMINAL REPEAT (LTR) IN THE INTERLEUKIN 2 (IL-2) GENE OF MLA 144 CELLS, Nikki Holbrook,* Alberto Gulino,* Gerald Crabtree,† Su Chen++ and Yuan Lin,++ *National Institute of Aging, Bethesda, MD 20892, †Stanford University, Stanford, CA, and ++E.I. duPont, Glenolden, PA.

IL-2 is ordinarily produced by a subset of T-lymphocytes only upon stimulation with antigen or mitogen. The MLA 144 cell line is unique in that it constitutively produces IL-2. We have found that MLA 144 cells contain two GALV insertions in or near the IL-2 gene. One of these is located 5' to the gene in an antisense orientation. The second occurs in the 3' untranslated region of the gene and results in a composite mRNA made up of protein coding sequence for IL-2 and the viral LTR. To study the role these viral sequences play in influencing IL-2 expression we tested the GALV LTR for its ability to directly activate expression of the bacterial chloramphenicol acetyl transferase (CAT) gene, or to enhance expression of CAT under control of the SV40 early or IL-2 promoters. The LTR acts as a functional promoter in a variety of cell types, both lymphoid and nonlymphoid, with its greatest activity seen in MLA 144 cells. The LTR also enhances expression of CAT under control of the SV40 early promoter in a manner consistent with enhancer elements. When placed near the CAT gene under control of IL-2 promoter/regulatory elements, expression of CAT is also enhanced, but only in specific cell types capable of producing IL-2 and only when the cells are appropriately stimulated with mitogen. These results suggest that while the presence of the LTR alone is not sufficient to activate IL-2 expression, enhanced expression due to viral sequences could significantly affect the level of IL-2 produced in these cells.

0315 BINDING OF SPECIFIC PROTEIN FACTORS TO THE HUMAN c-K-ras GENE PROMOTER.

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The promoter region of the human c-K-ras gene has been characterized by deletion mutagenesis and gene transfer experiments. DNA sequence analysis and chromatin studies have revealed a striking similarity betwen the c-K-ras gene and the SV40 promoter regions. Thus, the c-K-ras gene promoter contains several CCGCCC boxes, clustered in a region that resembles the 21 base repeats of SV40, and two nearly-perfect enhancer core sequences (GGTGTGGAAAG) are also present 5' and 3' of the 21 bp like sequences, respectively. At the same time, the c-K-ras promoter region exhibit several DNAse I hypersensitive sites which are coincident with the SV40 homologous sequences, and the size and overall organization of the hypersensitive region is similar to the equivalent region described for the SV40 promoter. The c-K-ras promoter hypersensitive region shows an inner structure with a protected DNA fragment of subnucleosomic size flanked by DNAse I hypersensitive sites. A 5' distal DNAse I hypersensitive site which is coincident with one of the two SV40 enhancer homologous sequences, shows a variable sensitivity in the different cell lines tested. These results suggest the presence of specific protein factors binding to the c-K-ras gene promoter region which has been confirmed by in vitro "band-competition" binding assays using low ionic strength nuclear protein extracts. Footprinting experiments are in progress to determine the specific DNA sequences involved in the binding to these factors.

0316 REGULATION OF EXPRESSION OF THE HUMAN HEAT SHOCK PROTEIN 70 PROMOTER

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Expression of the 70 kd mammalian heat shock protein (hsp70) is elevated after infection by certain DNA tumor viruses as well as in some transformed cell lines. We have identified two polyomavirus large T antigen binding sites in a human hsp70 promoter between positions -110 and -170. Expression from this promoter is elevated 10 fold by the polyomavirus early region. This stimulation is partially caused by the polyoma large T antigen. Promoters deleted for the large T antigen binding sites are still fully stimulated by large T antigen. The sequence CCAAT at -64 of this promoter must be intact for full basal activity. Replacement of this sequence with a 21 base sequence encompassing one of the upstream T antigen binding sites results in reactivation of the promoter in cell lines that either express or do not express large T antigen. These data suggest that polyomavirus large T antigen and a cellular transcription factor both recognize the same sequence.

0317 STUDIES ON ALTERED DNA STRUCTURES WITH BROMOACETALDEHYDE, Terumi Kohwi-Shigematsu, Jay Nelson, Mohammad Majlessi and Yoshinori Kowhi, La Jolla Cancer Research Foundation, La Jolla, CA 92037

We have previously shown on the chicken adult β^A globin gene system that the altered DNA conformation originally detected by DNasel, contains effectively unpaired DNA bases employing bromoacetaldehyde (BAA), a chemical probe to detect unpaired DNA bases. Such "unpaired" DNA bases were detected only in cells in which this gene was actively being transcribed. Using the BAA method, those DNA sequences which confer an altered conformation in chromatin were shown to possess an intrinsic tendency to form an altered DNA structure in supercoiled plasmid DNA. We have extended this study to other putative regulatory sequences and various enhancer sequences and found that they also adopt "single-stranded" structure in supercoiled plasmid DNA. The BAA reactive sites within these DNA sequences were determined to a single nucleotide resolution. During the course of this study, poly(dG)-poly(dC) sequences such as those found in the 5' flanking region of the chicken adult β^A globin gene was shown to not only adopt a "single-stranded" structure within itself, but it also induces an altered DNA structure upon its neighboring DNA sequences in a direction specific manner. The effect of sequence specific binding protein(s) on such conformation is studied.

0318 POINT MUTANTS OF THE ACANTHAMOEBA rRNA PROMOTER: FUNCTIONAL ASSAYS, P. Kownin, P.R. Nicholson, C.T. Iida and M.R. Paule, Colo. St. Univ., Fort Collins, CO 80523

Single point mutations were introduced into the promoter region of the Acanthamoeba rRNA gene by chemical mutagens: nitrous acid, formic acid and hydrazine. The promoter mutants were tested for transcription initiation factor (TIF) binding by a template commitment assay and for transcription by an in vitro runoff assay. Point mutants within the TIF interaction region (between -31 and -20, motif A) at -31(T→ C) or -30(A→ G) decreased the amount of the runoff transcript while changes at -27(C→ T), -24(G→ A) and -20(G→ A) increased the specific transcription product. Point mutations within the region required for the formation of a stable complex with TIF (between -32 and -47, motif B) at -45(T→ C), -39(C→ T), -34(C→ T), -33(C→ T) or -32(A→ G) lowered the quantity of the specific runoff transcript, whereas mutation at -44(C→ T) and -37(G→ A) increased the specific RNA product. Alterations of the base sequence around the transcription start site at -1(T→ C), +1(A→ G), +2 (A→ G), +3(A→ G) or +4(G→ A) also increased specific RNA synthesis in vitro, but changes at -8(G→ A), -5(T→C) and -4(A→ G) lowered synthesis. Point mutations outside motifs A, B and the start motif as well as some within these regions did not affect the ability of the promoter to direct specific transcription in vitro. Results from the template commitment assay confirmed footprint data (Bateman et al, PNAS, Dec. 1985) that the nucleotide sequences at -45, -41, -32, -31, -30, -24 and -20 were required for the formation of the stable preinitiation complex, since these mutants do not sequester TIF in as stable a complex as wild type DNA. (Supported by NIH Grant GM 26059 to MRP).

0319 TRANS-ACTIVATION OF A CLASS I MHC GENE IN SV40 TRANSFORMED CELLS, Matthew C. Lorence and Robin A. Robinson, University of Texas Health Science Center, Dallas, TX 75235

Several cellular genes appear to be activated during SV40 transformation, one of which appears to be a class I MHC gene. To further examine this observation, a cDNA library prepared from SVT2 (an SV40 transformed BALB-3T3 cell line) poly(A)-selected mRNA was initially screened with a class I MHC "public" probe to pre-select all cDNA clones containing sequences homologous to class I MHC genes. Candidate cDNA clones were screened further by differential hybridization analysis to identify those clones exhibiting increased steady-state transcript abundancies in SV40 transformed cells as compared to that in the non-transformed parental cells. DNA sequence analysis was performed on one of these clones, and an oligonucleotide probe specific for this cDNA clone hybridized to a single Tla gene, TL-10, in a class I MHC cosmid cluster library. This gene, which exhibited a 50 - 100-fold increase in transcript abundancy in SVT2 cells, also showed similar levels of activation in SV40 tsA mutants under permissive conditions. The mechanism of TL-10 transactivation in SV40 transformed cells is currently being pursued in vitro by transcriptional assays utilizing purified SV40 lg TAg and various cell-free nuclear extracts, and in vivo by transient CAT assays utilizing TL-10 promoter-CAT constructs in SV40 transformed cell lines.

0320 TRANSCRIPTIONAL REGULATION OF THE HCMV MAJOR IMMEDIATE-EARLY GENE IS ASSOCIATED WITH INDUCTION OF DNASEI HYPERSENSITIVE SITES AND TRANSCRIPTION OF UPSTREAM ENHANCER SEQUENCES. Jay A. Nelson.

Human teratocarcinoma cells were used to examine structural features associated with expression of the major IE gene of HCMV. By immunofluorescence, comparison of RNA levels and in vitro transcription of nuclei, we have shown that the major IE gene is inactive in undifferentiated but active in differentiated cells. Therefore the block in HCMV replication in teratocarcinoma cells appears to be at the transcriptional level, in one of the initial genes transcribed. In addition, the in vitro transcription experiments demonstrated that in permissive infections the gene was transcriptionally inactive late in infection. Comparing structural features of the promoter region with the active and inactive IE gene showed the presence of constitutive and inducible DNaseI hypersensitive sites. The majority of the constitutive sites existed in an area at -175, -275, -375, -425 and -525 relative to the cap site in an area which has been shown to be capable of SV40 enhancer function. In contrast, the inducible DNaseI sites were located outside this region at -650, -775, -875 and -975. The inducible sites at -650 and -775 map close to four NF-1 consensus sequences which bind the cellular factor in vitro. Finally, the in vitro transcription of permissive nuclei has identified transcription in the 5' noncoding region which contains enhancer function for the major IE gene. This transcription is α-amanitin sensitive and occurs in an area with no known steady-state transcript. Analysis of this transcription with single-stranded probes indicates that transcription is on opposite strands on either side of the enhancer.

0321 MULTIPLE TRANSCRIPTIONAL REGULATORY ELEMENTS AS MEDIATORS OF DIFFERENTIAL TRANSCRIPTION, David O. Peterson, Mark G. Toohey and Kimberlin L. Morley, Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843.

The rate of transcription from the promoter of mouse mammary tumor virus (MMTV), a retrovirus, is under the control of glucocorticoid hormones. DNA sequences associated with this control are located in the proviral long terminal repeats (LTRs) and are termed the glucocorticoid response element (GRE). In the proviral state, LTR sequences with their respective GREs are located at each end of the retroviral genome. Utilizing a transient expression assay system, the transcriptional properties of this and other gene structures containing multiple GREs were investigated. Results indicate that multiple GREs either 5' or 3' to the regulated transcription unit can differentially activate the MMTV promoter and that this increase is additive with respect to the number of GREs present. In addition, deletions of LTR sequences have served to identify a negative transcription control element within the LTR that decreases the activity of the MMTV promoter in the absence of glucocorticoids. This negative element is able to function at multiple positions in both orientations and is contained on a 344bp Sau3A restriction fragment that also contains a functional GRE; however, sequences required for negative regulation are not identical to those of the GRE. The role of this element may be to maintain the transcriptional inactivity of the MMTV promoter in the absence of glucocorticoids and ensure a high level of differential gene expression after hormone induction.

0322 TRANSCRIPTION OF THE TWO δ-CRYSTALLIN PROMOTERS IN A HELA CELL EXTRACT. Gokul C. Das and Joram Piatigorsky, Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20892

δ-Crystallin is the first crystallin synthesized in the embryonic chicken lens and is the major structural protein in the lens before hatching. δ-Crystallin synthesis ceases several months after hatching. There are 2 linked δ-crystallin genes in the chicken (5'-δ1-δ2-3). Only the δ1 gene has been shown definitively to be active in the lens. We show here that both the δ1 and δ1 promoters are active in a Manley Hela cell extract. The δ1 promoter appeared several-fold stronger than that of δ2 under the present conditions. Transcription of deletion mutants showed that the sequences necessary for the functioning of the δ1 promoter in a Hela cell extract are located upstream from the RNA initiation site between nucleotide positions -121 and -38. This region includes a number of G + C-rich motifs, including one hexanucleotide sequence CCGCCC which is repeated 6 times in the SV40 promoter. Competition experiments with purified fragments from the δ1 promoter showed that binding of transcription factor(s) from the Hela cell extract to this G + C-rich region is required for promoter activity in vitro. Furthermore, competition experiments using 3 different fragments from the SV40 promoter suggested that the δ1 transcription factor(s) is similar to that which stimulates transcription by binding to the G + C-rich 21 bp repeats of the SV40 promoter (Sp1) and differs from that which interacts with the SV40 enhancer region.

0323 ENHANCER ELEMENTS IN YEAST, John F. Pulitzer and Maria Ciaramella
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In yeast RNA polymerase II promoters, there is a flexible spatial relationship between TATA box and upstream activation sites (UAS), suggesting a loose analogy between UAS and the enhancers of higher eukaryotes. We address the soundness of this analogy directly by asking whether transcription in yeast can be driven by the polyoma enhancer. Using as tester a plasmid-borne HIS3 gene transcriptionally inactivated by deletion of all upstream promoter sequences, we find that transcription is restored beyond the control wild-type level by insertion of the polyoma enhancer, in either orientation, at the 5' and 3' ends of the gene. By further genome manipulation we show that: 1) enhancer activation in yeast is likely to be homologous to that in mammals because, as as has been observed in mouse fibroblasts, reduction of the composite polyoma enhancer to its B element abolishes activation while a mutant B element (ex PyFLC78) carrying a 36 bp transposition of the element A core restores transcription as well as the holoenhancer;2) we find permissive and non-permissive domains for enhancer function within the genic segment we study, but no evidence for directional barriers to activation ; 3) by its strength enhancer activation is unequivocally distinguishable from non-specific position effects shown to restore some function to promoterless his3 mutants. (Supported by a Grant from C.N.R. Progetto Finalizzato Ingegneria Genetica)

0324 Purification of Plasmodium falciparum cathepsin D. Identification of cathepsin D specific mRNA and genetic homology in different strains with a human cDNA probe.

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Malaria parasites during their erythrocytic development, digest host cell haemoglobin to meet their amino acid requirements. P.falciparum posess predominant proteolytic activity with acid pH optima which has been identified to be cathepsin D. Using anticathepsin D-Sepharose affinity matrix the parasite cathepsin D has been partially purified. The parasitic origin has been confirmed by metabolically labelling parasite proteins with [^{35}S] methionine. Poly A+RNA has been isolated from P.falciparum (Honduras I CDC) infected erythrocytes. The mRNA preparation was electrophoresed for Northern blots and probed with cathepsin D specific human cDNA probe. A 2.5 Kb mRNA coding for cathepsin D has been identified. P.falciparum DNA from three strains- Honduras I, 6 252 and FVO (SD) has been isolated. Determination of genetic homology by restriction enzyme analysis (RFLP) and Southern blotting is in progress.

UCLA Symposia on molecular and cellular biology,Park City, Utah, Jan. 26-31, 1986.

0325 An Approach For The Molecular Cloning Of Genes Encoding Sequence-Specific DNA Binding Proteins. Harinder Singh, Jonathan H. LeBowitz and Phillip A. Sharp, Massachusetts Institute of Technology, Cambridge, MA 02139

Many prokaryotic and eukaryotic transcriptional regulatory proteins function in part by binding with high affinity to cognate DNA elements in their target genes. We are attempting to exploit this property in an approach that may make possible direct cloning of mammalian transcriptional regulatory protein genes. The approach depends upon the conditional high level expression of eukaryotic cDNAs in an E. coli expression vector. Clones expressing functional sequence-specific DNA binding domains can then be identified in situ using radiolabeled cognate DNA probes by virtue of a selective and high affinity interaction between the DNA binding domain and its congnate DNA template. To demonstrate the feasibility of this methodology we have employed a test gene that encodes the bacteriophage λ O protein - a DNA replication initiator that binds with high affinity to the λ origin of replication (ori λ). We have established conditions for the specific detection of E. coli clones expressing high levels of the λ O protein using labeled ori λ DNA. We are attempting to extend this methodology to the detection of DNA binding domains of the SV40 large T antigen and the EBV nuclear antigen EBNA-I. Finally we plan to screen mammalian cDNA libraries with viral and cellular enhancer DNA probes for genes that encode proteins that interact with these transcriptional regulatory elements.

0326 **DIFFERENTIAL ACTIVITY OF ADENOVIRUS PROMOTERS IN CELL-FREE EXTRACTS CONTAINING E1A, Rudolph Spangler, Mark Bruner and M.L. Harter, New Jersey Medical School, Newark, New Jersey 07103**

In an effort to understand the mechanism(s) by which the adenovirus (Ad) E1A protein (encoded by the 13S-mRNA) is able to regulate RNA transcription, we devised two experimental approaches that would allow us to study its effect on several adenovirus promoters. Both approaches included using cell extracts for use in priming in vitro transcription from DNA templates containing either the Ad E2A or major late and VA RNA promoter; the only difference being that one of the extracts was derived from human 293 cells (expressing E1A) and the other from HeLa cells, which in some cases, was supplemented with E1A protein that had been produced in E. coli from a plasmid carrying the cloned E1A gene (1).

Under varied experimental conditions, we observed that the activity of the VA RNA promoter, in contrast to the major late, was markedly different in extracts of 293 cells when compared to HeLa; and in the company of the E2A promoter the activity of VA in 293s decreased approximately 3-4 fold while that of the major late remained essentially unaffected. When by itself, the activity of the E2A promoter was more pronounced in extracts of 293 cells than in HeLa. The results of these and other experiments using E1A specific antibodies for dissecting the contribution of the bacterially synthesized E1A in up-regulating transcription in HeLa will be presented.

(1) Ko, J.-L. and Harter, M.L. (1984) Mol. Cell. Biol. 4, 1427

0327 A TEMPERATURE SENSITIVE CHINESE HAMSTER CELL LINE WITH A DEFECT IN TRANSCRIPTION, Thelma C. Slezynger and Immo E. Scheffler, University of California at San Diego, La Jolla, CA 92093.

Our laboratory is characterizing a temperature-sensitive hamster mutant cell line with a defect in transcription. After 12 hours at 40.2 C there is a 40-50% decrease in RNA synthesis in whole cells, cell extracts and isolated nuclei. We hypothesize that the defect might be in transcription by RNA polymerase II.

We have tested in vitro heat inactivations of partially purified RNA polymerases I and II, and found that they are not temperature sensitive, suggesting that the defect might be in a transcription factor. We are using cloned probes specific for genes transcribed by RNA polymerases I, II, and III to look at steady state levels of RNAs and rates of transcription of these genes after a temperature shift. After 12-15 hours at 40.2 C there is a decrease in the mRNA levels for myc, actin and a ribosomal protein. The induction of thymidine kinase and histone mRNAs in synchronized cells can be shut off by a temperature shift just 1-3 hours prior to the onset of transcription of these genes. To determine how the rate of transcription of RNA polymerase I, II and III genes is affected, measurements of run-on transcription in isolated nuclei are in progress. We would like to know whether the postulated defect in a transcription factor discriminates between different RNA polymerase II promoters.

0328 FACTORS THAT BIND TO THE M-MULV AND MPSV ENHANCERS IN TRANSCRIPTIONALLY PERMISSIVE AND NONPERMISSIVE (EC) CELL EXTRACTS, Nancy A. Speck, Wolfram Ostertag and David Baltimore, Whitehead Institute for Biomedical Research, Cambridge, MA 02142 and Universitat Hamburg, FRG

The M-MuLV enhancer is transcriptionally inactive in undifferentiated embryonal carcinoma cell lines. Transcriptional inactivity could be caused by either 1) the absence of a positive regulatory factor in undifferentiated EC cells that is present in permissive cells, or 2) presense of a negative regulatory factor in EC cells that binds directly to the DNA, or alternatively interacts with transcription factors found in both permissive and nonpermissive cells. To distinguish between these alternatives, we have employed a binding assay to identify factors in both permissive and nonpermissive EC cell extracts that bind to the M-MuLV 72/73 bp repeat. We detect at least 3 distinct factors present in both permissive and nonpermissive cell extracts. The closely related myeloproliferative sarcoma virus (MPSV) enhancer is transcriptionally active in EC cells, and contains several point mutations compared to the M-MuLV enhancer. Binding studies on the MPSV enhancer indicate that one of the factors found to bind the M-MuLV enhancer does not bind the corresponding region on MPSV, suggesting that negative regulation of M-MuLV is mediated through this site. In vivo experiments to confirm the correlation between loss of binding with transcriptional activity in EC cells are in progress.

0329

LYMPHOID-SPECIFIC AND UBIQUITOUS OCTAMER BINDING PROTEINS AND THEIR RELATION TO IMMUNOGLOBULIN GENE TRANSCRIPTION. Louis M. Staudt, Harinder Singh, Phillip A. Sharp and David Baltimore. Whitehead Institute, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142.

The octamer sequence homology, 5'-ATGCAAAT-3', or its inversion has been found upstream of every immunoglobulin gene at approximately position -70 relative to the transcription start site. The same sequence homology is also found upstream of all U1 and U2 genes at position -200 to -260. The region surrounding the octamer has been implicated in transcriptional enhancement in immunoglobulin genes and U1 and U2 genes as well as in the lymphoid specificity of the immunoglobulin promoters. We have used a gel electrophoresis DNA binding assay to identify proteins which specifically bind to the octamer in nuclear extracts from a variety of lymphoid and non-lymphoid cell lines. In this assay, the migration of a radiolabelled kappa promoter fragment is retarded by binding to proteins from the extract. Two bands were found in every extract tested so far and a third band was found only in extracts from B and T lymphocytes. All three bands were competed by unlabelled homologous fragment and by a fragment of the heavy chain enhancer which only shares the octamer motif with the promoter fragment but not by fragments lacking the octamer. The lymphoid specific band was found in extracts from early and late pre-B, B and myeloma cells and in half of the T lymphoma cell lines tested. This band was not found in extracts of Hela, 3T3, Cos or Mel cell lines.

0330 CELLULAR ENHANCER ELEMENT ACTIVE IN MURINE EMBRYONAL CARCINOMA CELLS, Makoto Taketo and Masafumi Tanaka, The Jackson Laboratory, Bar Harbor, ME 04609

We isolated rare G418-resistant embryonal carcinoma (EC) cell clones that express the integrated neomycin resistance (neo) gene by infecting EC cells (F9tk⁻ or PCC4.azalR lines) with a recombinant retrovirus that carries the neo gene linked to the Moloney murine leukemia virus (Mo-MuLV) LTR. The expression of the neo gene appeared to be due to cis-acting mechanism(s) (Taketo et al., Proc. Natl. Acad. Sci 82, 2422-2426, 1985). The DNA segments containing the integrated proviral genomes and their cellular flanking sequences were molecularly cloned from 6 independent G418-resistant EC cell clones into λ phage vectors. Upon transfection on parental EC cells, some of these λ clones showed 100 times higher efficiency of transfection, suggesting existence of cellular enhancer elements flanking the proviral genomes. From one such λ clone exhibiting the highest frequency, the 5'-flanking cellular sequence (2.5 kb) was recloned into a test plasmid that carried the neo gene linked to the Mo-MuLV LTR. Transfection experiments using the recloned plasmids revealed that the 2.5 kb segment increases the number of transfectant colonies 100 times in a manner independent of the orientation and its position relative to the LTR promoter. Data obtained using deletion analyses suggested that the putative enhancer activity resides in a subregion of about 0.5 kb. The results of further analyses on the activity and structure of this enhancer-like element, including the DNA sequence, will be presented.

0331 CHANGES IN DNase I HYPERSENSITIVITY WITH INDUCTION OF THE RAT METALLOTHIONEIN I GENE, Susan J. Taplitz, Kathryn L. Calame and Harvey R. Herschman, University of California, Los Angeles, L.A., CA 90024

The rat metallothionein I (MT-I) gene is inducible by a variety of agents including glucocorticoid hormones and heavy metals. To begin looking at the three dimensional interactions governing the regulation of this gene, we have examined the pattern of DNase I hypersensitivity of the MT-I gene in cultured rat hepatoma cells treated with different inducers. In the absence of induction there is a low basal level of MT-I expression. When chromatin from uninduced cells is treated with DNase I and analyzed, a single hypersensitive is present. This site maps approximately 250 bp 5' of the transcription start site and is positioned midway between the consensus sequences for the glucocorticoid responsive element (GRE) and the basal level element (BLE) identified by Haslinger and Karin (in press). When cells are treated with cadmium prior to analysis, a second DNase I hypersensitive is observed. This second site is positioned very near the transcription start site and flanks, but does not include the metal responsive elements (MREs). This new hypersensitive site is also induced in cells treated with zinc, indicating that the formation of this site is associated with induction of the gene by heavy metals. In contrast, induction of cells with dexamethasone does not lead to the appearance of this hypersensitive site. Thus the formation of a new DNase I hypersensitive site is specific to transcriptional activation of the MT-I gene by heavy metals and is not involved in a general induction response.

0332 TRANS-REGULATION OF A CHIMERIC LIVER-SPECIFIC GENE IN HEPATOMA HYBRID CELLS, Mathew J. Thayer, Tracy Gross Lugo and R.E.K. Fournier, University of Southern California School of Medicine, Los Angeles, CA 90033

Hybrid cells formed by fusing dissimilar cell types generally fail to express tissue-specific products of either parent, a phenomenon known as extinction. Our laboratory has investigated the genetic basis of extinction in hepatoma cell hybrids, and has recently identified discreet genetic loci that regulate liver-specific gene expression (Killary, A.M., and Fournier, R.E.K., Cell 38, 523-534, 1984). In the present study, we attempt to define cis elements required for extinction of a liver-specific gene in trans.

A chimeric gene was constructed in which a 620 bp fragment of the 5'-end (-547 to +73) of the phosphoenolpyruvate carboxykinase (PEPCK) gene was ligated to the coding sequence of the Herpes simplex virus thymidine kinase (TK) gene. When transfected into rat hepatoma cells this chimeric gene has been shown to initiate mRNA synthesis at the PEPCK start site and to be inducible by Bt_2CAMP (Wynshaw-Boris, A., et al., J.B.C. 19, 12161-12169, 1984). Hybrid cells were formed by fusing such rat hepatoma transfectants with TK⁻ mouse L-cells. The TK phenotype of the hybrids was determined utilizing three criteria: 1) relative plating efficiency in HAT versus BrdU, 2) TK enzyme activity, and 3) Northern blot analysis of total cytoplasmic RNA. We report here that these hybrids were extinguished for TK expression by all three criteria. Therefore, the cis elements of this liver-specific structural gene required for its extinction in trans are localized to a specific region at the 5'-end of the gene.

0333 THE HOMEO BOX OF THE DROSOPHILA ENGRAILED GENE MAY ENCODE A SEQUENCE SPECIFIC DNA BINDING DOMAIN. James F. Theis, Claude Desplan, and Patrick H. O'Farrell, University of California, San Francisco.

Genetic analyses in Drosophila have identified two classes of zygotic genes involved in pattern formation: segmentation genes, which establish the segmented body plan, and homeotic genes, which specify the identity of the segments. Recent molecular studies, together with the genetics, indicate that these genes interact in a combinatorial fashion to specify the fates of cells. An intriguing feature of several of these genes is the presence of a highly conserved sequence, the homeo box. This common sequence may imply that the products of these genes carry out similar functions. Utilizing lacZ fusions expressed in E. coli, we have demonstrated that a fusion containing a 140 amino acid fragment from the engrailed protein (containing the homeo box) and a full-length fusion both show sequence specific DNA binding. These fusions recognize sites 5' to the engrailed coding region and within its first intron; sites 5' and 3' to the fushi tarazu coding region are also recognized. We are currently determining the sequence of these binding sites. Preliminary results indicate that protein dimerization is required for sequence specific binding. We are using similar fusions to determine which parts of the protein are involved in DNA binding, dimerization, and protein-protein interactions.

0334 MOLECULAR CHARACTERIZATION OF AN UPSTREAM ENHANCER ELEMENT INVOLVED IN rbcS GENE PHOTOREGULATION, Michael P. Timko and Anthony R. Cashmore, Laboratory of Cell Biology, Rockefeller University, New York, NY 10021

The nuclear genes encoding the small subunit polypeptide of ribulose-1,5-bisphosphate carboxylase (rbcS) are expressed in a light-regulated, tissue-specific manner in pea (Pisum sativum). In order to define the nucleotide sequence requirement for photoregulated expression a chimaeric gene consisting of a 5'-noncoding fragment (-4 to -973 bp upstream of the cap site) from the rbcS 3.6 gene was fused to the coding sequence of the bacterial cat gene and its expression studied in tobacco callus. These studies demonstrated that nucleotide sequences within 973 bp of the rbcS cap site contain sufficient information required for the photoregulated expression of the cat gene. Subsequent deletion analysis has shown that sequences at least 722 bp 5' to the cap site are required for high levels of photoregulated expression (80 % of wild-type 973 promoter level) and that sequences within 92 bp of the cap site are capable of directing low levels (5% of wild-type) of photoregulated expression. Furthermore, sequences directing high levels of photoregulated expression and contained within a fragment extending -90 to -973 function independently of orientation when fused to their homologous promoter. These same sequences also confer high levels of photoregulated expression when fused to a normally non-photoresponsive heterologous promoter in an orientation independent manner. These data suggest that rbcS photoregulated gene expression involve a genetic element with some of the properties attributed to animal viral and cellular enhancers. A more detailed analysis of the rbcS enhancer sequences is presently underway.

0335 IDENTIFICATION OF A GLUCOCORTICOID RESPONSIVE ELEMENT IN THE HEPATITIS B VIRUS GENOME, Ran Tur-Kaspa, Robert D. Burk, Yosef Shaul and David A. Shafritz, Liver Research Center, Albert Einstein College of Medicine, Bronx, N.Y. and the Department of Virology, Weizmann Institute of Science, Rehovot, Israel

It has previously been shown that Hepatitis B Virus (HBV) contains a transcriptional enhancer element. In order to determine whether this enhancer responds to glucocorticoids, a series of plasmid derivatives of $pA_{10}CAT_2$, containing the HBV enhancer and variable lengths of further upstream sequences, has been constructed. Transient expression of chloramphenicol acetyl transferase (CAT) was determined following introduction of these plasmids into PLC/PRF/5, Hep 3B, Hep G2, HeLa and L cells. Highest CAT activity was noted in the human hepatocellular carcinoma line, PLC/PRF/5, containing integrated HBV DNA. Dexamethasone augmented CAT expression in all cell lines tested with 40% of maximal induction at 10^{-8}M and maximum stimulation (3-8 fold) at 10^{-6}M Dexamethasone. Dexamethasone augmentation of CAT expression was observed only when constructs contained HBV DNA sequences residing upstream to map position 735 from the Eco RI site. This indicates that the putative glucocorticoid responsive region is distinct from the previously defined HBV enhancer sequence located at map position 1080-1234. Furthermore, a sequence nearly identical to the consensus sequence of glucocorticoid responsive genes is found in the HBV genome at map position 594-605. These studies indicate that HBV DNA probably contains a glucocorticoid responsive element which may mediate expression of HBV genes in infected mammalian cells.

0336 HEME REGULATES THE EXPRESSION IN SACCHAROMYCES CEREVISIAE OF CHIMAERIC GENES CONTAINING A 5' FLANKING SOYBEAN LEGHEMOGLOBIN SEQUENCES. Ingrid S. Villadsen, Department of Microbiology, The Technical University of Denmark, DK-2800 Lyngby, *Kjeld A. Marcker, and *Erik Østergaard Jensen, Department of Molecular Biology and Plant Physiology, University of Aarhus, C.F. Møllers Allé 130, DK-8000 Aarhus C.

The TM1 yeast mutant was transformed with a 2 μ derived plasmid (YEp24) which carries a chimaeric gene containing the E. coli chloramphenicol acetyl transferase (CAT) gene fused to the 5' and 3' flanking regions of the soybean leghemoglobin (Lb) C_3 gene. Expression of the chimaeric CAT gene is controlled specifically by heme at a posttranscriptional level, most likely by regulating the efficiencies of translation. Expression of another chimaeric gene consisting of the neomycin phosphotransferase (NPTII) gene fused to only the 5' flanking region of the Lbc_3 gene is regulated by heme in a similar way. Thus, in yeast heme modulates the translation of the chimaeric mRNAs through interactions with the 5' Lbc_3 noncoding region.

0337 **MUTAGENESIS OF THE HTLV *x* GENE MODIFIES ITS *TRANS*-REGULATORY PHENOTYPE.** W. Wachsman, A.J. Cann, D.J. Slamon and I.S.Y. Chen, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

The human T-cell leukemia viruses (HTLV) are postulated to induce T-cell transformation via the *x* gene, a unique gene at the 3' end of the proviral genome. Recent studies have shown that the HTLV-I and -II *x* gene products, $p40^{xI}$ and $p37^{xII}$, differentially *trans*-regulate various promoters. The $p37^{xII}$ induces transcription from both the HTLV-I and HTLV-II long terminal repeat (LTR), as well as from the adenovirus E3 promoter, while the $p40^{xI}$ *trans*-regulates the HTLV-I LTR but not the HTLV-II LTR or the E3 promoter. To investigate the mechanism of *trans*-regulation by $p40^{xI}$ and $p37^{xII}$, we generated mutants of both *x* genes by site-directed mutagenesis. Transient co-transfection CAT assays of *x* cDNA with LTR/CAT recombinants reveal that specific mutants in the amino-terminus of $p40^{xI}$ and $p37^{xII}$ are unable to induce transcription from either of the HTLV LTRs. Furthermore, mutants of the $p37^{xII}$ amino-terminus were observed to inhibit *trans*-activation of the HTLV-II LTR by wild-type $p37^{xII}$. These mutant $p37^{xII}$ were not observed to inhibit the *trans*-activation of the HTLV-I LTR by wild-type $p37^{xII}$ or $p40^{xI}$. Our results demonstrate that the amino-terminus of $p40^{xI}$ and $p37^{xII}$ is involved in the *trans*-regulatory properties of these molecules. It is unclear whether the altered *trans*-regulatory phenotype induced by mutant $p37^{xII}$ is due to competitive inhibition of wild-type $p37^{xII}$ or to an inherent property of the molecule previously obscured by its *trans*-activating function.

0338 X. laevis globin gene transcriptional activation and chromatin structure. M. Walmsley J. Allan, A.C. Brewer, T. Enver & R. Patient. King's College, London, England.

Although the globin genes of X. laevis are transcriptionally inactive when transfected in to Hela cells, rescue of the globin gene has been achieved by linkage to SV40 control sequences. Emerson and Felsenfeld have suggested that tissue specific trans-acting factors may be responsible for setting up the chromatin structure peculiar to actively transcribed genes. However, in our SV40- globin construct, transcriptional rescue was accompanied by the appearance of a 5' DNase 1 HS (absent from the unexpressed globin gene) at precisely the position found in Xenopus erythrocytes, in a background where trans-acting factors were unchanged. Using SV40 origin and enhancer cassettes plus inhibitors of DNA replication, we have found that full transcriptional activation requires both the enhancer and DNA replication. We are now investigating which SV40 sequences are responsible for the induction of DNase 1 hypersensitivity. Microinjection of the SV40- globin construct in to Xenopus oocytes results in no enhancement of transcriptional activity and the 5'DNase 1 HS is found at an (AT)46 run located 400bp upstream of the in vivo HS, both in the globin gene alone and in the SV40-globin construct. Using SV40-CAT constructs, we have shown that the SV40 enhancer is inactive in oocytes, possibly in a manner analogous to F9 cells. To investigate the role of trans-acting factors, we have coinjected the chicken globin gene and erythrocyte nuclear protein extracts in to oocytes. We find that a 5'DNase 1 HS is established and is dependent on the nuclear extract but, to date, no stimulation of correctly initiated transcription has been detected.

0339 Purification and characterization of a CAAT box binding protein from HeLa cells
Heng Wang, Ikuko Sagami, Sophia Y. Tsai, Ming-Jer Tsai and Bert W. O'Malley. Baylor College of Medicine, Houston, Texas 77030.

Using deletion mutants and a cell-free HeLa cell transcription system we have demonstrated that a distal promoter containing the CAAT box is required for efficient transcription of the chicken ovalbumin gene. Competition assays utilizing DNA fragments containing the CAAT box region and exonuclease footprinting indicate the presence of a trans-acting factor in the HeLa cell extracts. To further investigate the existence of such a transcription factor and facilitate its purification, we have carried out DNase I footprinting analysis. Our results identify a protein factor in HeLa cell extracts which binds specifically to the CAAT box region between positions -65 and -92 of the ovalbumin gene. We have partially purified this CAAT box binding transcription factor using DEAE-Sephadex, phosphocellulose, Sephacryl -300 and heparin Sepharose column chromatography. This CAAT box binding protein indeed confers upstream sequence dependent transcription of the ovalbumin gene in our in vitro system. In addition, this factor is also necessary for the efficient transcription of the β-globin gene which contains the CAAT box but not required for efficient transcription of the SV40 early gene which lacks the CAAT box. Taken together, our studies indicate that this CAAT box binder may be involved in regulating genes which possess such a conserved sequence.

0340 Identification Of An Immunoglobulin Heavy Chain Enhancer DNA-Binding Activity. Judah Weinberger, Harinder Singh, Ranjan Sen, David Baltimore and Phillip A. Sharp. Massachusetts Institute of Technology and the Whitehead Institute for Biomedical Research, Cambridge, MA 02139

Using a sensitive DNA-binding assay described by Fried and Crothers (J. Mol. Biol. 172:241), we have identified a DNA binding activity in crude nuclear extract from various B lymphocyte cell lines and HeLa cells which bind to specific sequences in the immunoglobulin heavy chain enhancer. Methylation interference has allowed identification of critical guanine residues necessary for binding:

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  *  **  *
GATGGCCGATC
CTACCGGCTAG
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Ephrussi et al. (Science 277:134) have identified four regions of the heavy chain enhancer which in B cells only interfere with methylation in vivo. The site identified by our experiments corresponds closely with the residues identified by Ephrussi et al. in the 5'-most region of the enhancer. Other experiments indicate that the activity identified herein binds with highest affinity to this binding site. Experiments are currently underway to test for binding of this activity by related sequences in the immunoglobulin kappa light chain enhancer.

0341 A Frog Virus 3 trans-acting Protein Induces Transcription from Methylated Adenovirus Promoters. DB Willis, JP Thompson, and A Granoff, St. Jude Children's Research Hospital, Memphis, TN 38101

The genomic DNA of the Iridovirus frog virus 3 (FV3) is highly methylated at CpG sequences. FV3 uses the host RNA polymerase II for transcription of early viral mRNA, suggesting that infection with FV3 allows transcription of methylated promoters by RNA polymerase II. To test this hypothesis, we used the bacterial methylases HpaII and HhaI to methylate plasmids containing the promoter sequences for the adenovirus E1A or E2A genes 5' to the coding sequence for chloramphenicol acetyl transferase (CAT). These plasmids were introduced into HeLa cells by CaPO4-mediated transfection, and the transfected cells were infected 24 hr later by FV3. Extracts were prepared for CAT assay 4 hr after infection. Cells transfected with the methylated plasmids did not promote the synthesis of CAT unless they were superinfected with FV3 at 30°C, the permissive temperature for viral replication. Southern blot analysis did not reveal any demethylation of the transfected DNA. Experiments carried out in CHO cells having an α-amanitin resistant or sensitive pol II showed that the host enzyme was required, directly or indirectly, for transcription from the methylated promoters. FV3 did not induce transcription of CAT specific RNA at 37°C, when UV-irradiated, or in the absence of protein synthesis, suggesting that the required FV3 product is an early viral protein. We conclude that an FV3 protein acts in trans with either the template or the host RNA pol II to induce transcription from methylated promoters.

0342 TERMINATION SEQUENCES IN THE CONTROL REGION OF THE AD2 SPECIFIC VARNA2 GENE, GUANG-JER WU and RONALD E. CANNON, Emory University School of Medicine, Atlanta, Ga.

Both VARNA genes in adenovirus genome are transcribed by host DNA-dependent RNA polymerase III. Nevertheless, the mass ratio of VARNA2 to VARNA1 in the late phase of the adenovirus infected human cells is about 1:40. This quantitative difference is most likely due to their difference in promoter strength which is most likely attributed to the sequence difference in the control regions of the two genes. To investigate this possibility, two genes are cloned into separate plasmid molecules and their transcription efficiencies were compared in vitro. VARNA2 gene was transcribed in vitro at least 50 times less efficiently than VARNA1 gene; this agrees well with the in vivo result above. By analyzing the hybrid RNAs transcribed from a hybrid gene and twenty-seven fused genes in which only the control region of VARNA1 gene was fused to various lengths of the 5'-flanking and the coding regions of VARNA2 gene, one major and several minor termination sites, which may cause premature termination of transcription, were revealed downstream of the A block sequence and in the 5'-flanking region of VARNA2 gene, respectively. The presence of termination sequence in the control region may attribute in part to a weaker promoter in VARNA2 gene. Other features in this control region which may attribute to this weak promoter also will be discussed.

0343 DIFFERENTIAL DNASE I SENSITIVITY OF THE MOUSE HPRT PROMOTER REGION ON THE ACTIVE AND INACTIVE X CHROMOSOMES. Thomas P. Yang, C. Thomas Caskey, Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030.

Dosage compensation of mammalian X-linked gene products is accomplished by inactivation of one X chromosome in females. We have investigated the DNase I sensitivity of the X-linked mouse hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene promoter region on the active and inactive X chromosomes in mouse nuclei. DNase I treatment of mouse liver nuclei yields a DNase I hypersensitive site within or in close proximity to the promoter in chromatin from the active male X chromosome. This hypersensitive site is near an S1-sensitive site mapped in supercoiled plasmids containing the mouse HPRT promoter region. In order to investigate the differential DNase I sensitivity of this region in chromatin from the active and inactive X chromosomes, we have utilized two female mouse hybrid cell lines generated from interspecies crosses between Mus musculus and Mus caroli (Chapman, Shows, 1976). One hybrid cell line contains an active M. musculus X chromosome and inactive M. caroli X; the other cell line carries the converse arrangement. The HPRT promoter region in these two species can be differentiated in Southern blots by a TaqI restriction fragment length polymorphism (RFLP). Using the RFLP in these two cell lines, we demonstrate moderately increased DNase I sensitivity of the active HPRT promoter region as compared to the inactive promoter. Furthermore, the DNase hypersensitive site is present only on the active X. These results suggest that certain characteristics of the chromatin structure of genes on the active and inactive X chromosomes appear to be similar to those in other differentially regulated genes, despite the unusual features of X inactivation.

0344 REGULATION OF RIBOSOMAL GENE EXPRESSION IN YEAST, Michele Yip, Rosie Mestel, Janice Holland, and Michael Holland, University of California, Davis, CA 95616

The yeast 18S, 5.8S and 25S ribosomal RNAs are derived from a precursor, designated 35S pre-rRNA. Sequences within the spacer region of yeast ribosomal cistrons enhance 35S pre-rRNA synthesis *in vivo*. We previously identified a 22-base pair sequence within the spacer region which is both required and sufficient for RNA polymerase I-dependent selective transcription *in vitro*. This sequence, designated the spacer promoter, is located 2.2kb upstream from the sequences which encode the 5' terminus of 35S pre-rRNA. Utilizing plasmids containing spacer sequences and the 35S pre-rRNA initiation region, we have shown that the 22-base pair spacer promoter sequences alone are not sufficient to enhance 35S pre-rRNA synthesis *in vivo*. The spacer promoter is, however, contained within sequences which are required for enhancer activity *in vivo* suggesting a role for the spacer promoter in enhancer function. A partially purified extract obtained after phosphocellulose chromatography was used to study transcription from plasmid templates containing the enhancer region and the 35S pre-rRNA initiation region *in vitro*. No transcripts were detected which initiated from the 35S pre-rRNA initiation region in the presence or absence of a functional enhancer region. Transcripts initiated from the spacer promoter read through the 35S pre-rRNA initiation region and terminate within the 35S pre-rRNA termination region.

0345 ADENOVIRUS E1A FUNCTIONS STIMULATE TRANSCRIPTION OF CLASS III GENES BY INCREASING THE ACTIVITY OF TRANSCRIPTION FACTOR IIIC: S.K. Yoshinaga, N. Dean, and Arnold Berk. Molecular Biology Institute, UCLA, Los Angeles, CA 90024

Adenovirus E1A proteins function as positive regulators of transcription. These transacting E1A proteins stimulate transcription of early viral genes as well as non-viral genes newly introduced into cells by infection or transfection. Transcription from early viral promoters is greatly delayed in cells infected with an adenovirus mutant deleted in the E1A region (dl312). Previous studies demonstrated E1A regulation of class II genes (genes transcribed by RNA polymerase II), here we report evidence that the E1A proteins also stimulate transcription of class III genes. Recently we found E1A functions to stimulate the transcription of transfected class III genes. In order to more precisely analyze the nature of these *in vivo* results, we utilized an *in vitro* transcription system using S100 protein extracts. We found that extracts made from wild-type adenovirus infected HeLa cells demonstrated 5-10 fold greater specific class III transcriptional activity than extracts made from the E1A deletion mutant, dl312 infected cells. Fractionation of the S100 extracts indicates that the increased *in vitro* activity was not due to E1A itself but rather due to the increased activity of the rate limiting transcription factor TFIIIC. Based on these results, we proposed that E1A proteins stimulate transcription *in vivo* by increasing the activities of rate limiting transcription factors. This model can explain many findings related to class II gene as well as class III gene activation by the E1A proteins.

0346 VACCINIA VIRUS REGULATORY SEQUENCES RESPONSIBLE FOR TERMINATION OF TRANSCRIPTION, Leonard Yuen and Bernard Moss, LVD, NIAID, Bethesda,MD

A soluble template-dependent system derived from vaccinia virus cores that accurately transcribes early vaccinia virus genes was used to identify sequences responsible for transcription termination. The precise 3' polyadenylated ends of the mRNA encoding the vaccinia virus growth factor (VGF) were identified by cDNA sequencing. A series of three tandem oligo(dT) stretches are present immediately downstream of the translation termination codon and 50 to 90 bp upstream of the polyadenylation sites. The entire VGF gene was cloned into pUC13 and deletion mutants were constructed using exonuclease III to systematically delete sequences at the 3' terminus of the gene. In vitro termination still occurred when the templates retained the first oligo(dT) sequence. To further identify the regulatory signals, deletions starting from the coding region and progressing toward the 3' terminus were made. In vitro assays demonstrated that the presence of the second oligo(dT) sequence also was sufficient for termination. The combined results indicate that the signals for regulation of termination occur upstream of the termination site and include oligo(dT) sequences. Two tandem copies of the termination signal are present at the end of the VGF gene.

Late Additions

0347 REGULATORY MUTATIONS IN THE S10 LEADER REGION OF THE *E. COLI* S10 R-PROTEIN OPERON THAT AFFECT L4-MEDIATED AUTOGENOUS CONTROL. Leonard P. Freedman, Janice M. Zengel and Lasse Lindahl, Department of Biology, University of Rochester, Rochester NY 14627.

The 11 gene S10 r-protein operon of *E. coli* is autogenously regulated by L4, the product of the third gene of the operon. L4 regulates *in vivo* by stimulating an attenuation of transcription at a site in the S10 leader approximately 30 bases upstream from the beginning of the first structural gene. L4 also inhibits translation of the operon *in vitro*. A 43 base stem-loop structure, followed by four U's, can be drawn from the sequence from this region of the leader. Moreover, within this putative secondary structure is a 9 base sequence which is identical to a sequence on 23S rRNA very close to where L4 binds. We have used oligonucleotide site-directed mutagenesis to test the biological relevance of the predicted stem-loop structure and 9 base homology sequence in L4-mediated regulation. Our results suggest that the region of the putative terminator is crucial for L4-mediated control of both attenuation and translation. Although the targets for both levels of L4 regulation map within this stem-loop structure, in two mutants we have been able to genetically separate attenuation and translation controls.

0348 BROAD HOST RANGE EXPRESSION VECTORS, Joachim Frey and Henry M. Krisch, University of Geneva, CH-1211 Geneve, Switzerland.
We have constructed an expression vector, pαΩ-001, for use in Gram-negative bacteria. This plasmid contains the replicon and conjugal mobilization functions of the plasmid RSF1010. The expression casset consists of the bacteriophage T4 gene 32 promotor and transcription terminator separated by a unique restriction site. To test the system we have inserted at this site the coding sequence for the xylE gene of the Pseudomonas putida TOL plasmid pWW0. This gene encodes the catechol 2,3-dioxygenase enzyme (C23O) of the meta-cleavage degradation pathway of touluene. The composite plasmid was subsequently introduced by conjugal mobilization into a variety of gram-negative bacteria. In each strain, the C23O enzyme activity was quantitated and the amount of C23O gene product was determined directly by polyacrylamide gel electrophoresis of cell extracts. Extremely high levels of expression of the catechol 2,3-dioxygenase gene was observed in all species tested. The bacteriophage T4 promoter is apparently active constitutively in a wide variety of bacteria (Pseudomonas, Erwinia, Agrobacter, Paracoccus, Xanthamonas, and Aeromonas) in addition to E. coli. We conclude that this expression vector can thus be used all in these species with the advantages of stable maintenance, easy and efficient transfer between bacterial species, and strong expression of the cloned genes.

0349 FUNCTIONAL SIMILARITIES OF TRANSCRIPTIONAL ACTIVATION BY THE HTLV II pX AND ADENOVIRUS EIA PROTEINS. R.B. Gaynor, ISY Chen, Foon Wu, UCLA Department of Medicine, Divisions of Hematology-Oncology, UCLA School of Medicine.
Both the adenovirus EIA and HTLV pX proteins are capable of activating transcription from their respective viral genomes. We have recently shown that the pX protein from HTLV II will activate transcription of early adenovirus genes, and that the EIA protein will weakly activate transcription of the HTLV LTR. A 12 base pair sequence repeated three times upstream of the early region II gene of adenovirus has strong homology with the 21 bp repeats in the HTLV I and HTLV II LTR. We will present data concerning the effect of upstream sequences on activation by the EIA and pX proteins. Using exonuclease III protection and gel retardation assays, DNA binding proteins in the upstream regulatory regions of the viral LTR and early adenovirus genes have been studied. By these methods, we hope to determine whether these DNA binding proteins are altered by the action of pX and EIA proteins.

0350 TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL EFFECTS OF DEXAMETHASONE ON AN IN VIVO MODEL OF FIBROGENESIS, Mark A. Zern, Mark J. Czaja, Shizuko Takahashi and Francis R. Weiner, Albert Einstein College of Medicine, Bronx, NY 10461

Recently we have shown that dexamethasone (DEX) increases albumin mRNA and decreases collagen mRNA steady state levels in rat hepatocyte cultures. These studies have now been extended by evaluating an *in vivo* model of hepatic fibrogenesis (murine schistosomiasis) and by determining more precisely the level of gene expression responsible for these changes. Control mice and their littermates infected with 50 cercaria of *S. mansoni* were evaluated at a time when the livers of the infected mice become progressively fibrotic and their serum albumin levels fall significantly. The addition of 4 ug/ml of DEX to the drinking water of half of the infected mice led to a 75% decrease in the liver collagen protein content as determined by HPLC. RNA was then extracted from the livers of mice under 4 conditions, control ± DEX or infected ± DEX and hybridized with cDNA probes to determine steady state levels of specific mRNAs. In the infected mice, addition of DEX suppressed the mRNA level of type I collagen by 50% but induced albumin mRNA content by more than three-fold. To analyze transription rates we isolated labeled nuclear transcripts from the mice livers and did nuclear run-off studies. DEX decreased transcription rates of type I collagen by half but did not induce a change in the transcription of albumin mRNA. These data suggest that DEX exerts its effect on different genes in the injured liver *in vivo* by separate mechanisms; i.e., decreasing collagen synthesis at a transcriptional level and increasing albumin mRNA content by a posttranscriptional mechanism. These corticosteroid-induced changes lead to a stabilization of liver function and inhibition of fibrogenesis and may explain why corticosteroids are beneficial in some forms of chronic liver disease.

0351 NUCLEAR FACTOR I SERVES AS A TRANSCRIPTIONAL FACTOR FOR THE MURINE MAMMARY TUMOR VIRUS LTR PROMOTOR. R. Miksicek[1] and J. Nowock[2], [1]German Cancer Research Center, D-6900 Heidelberg, F.R.G. and [2] Heinrich-Pette Institute, D-2000 Hamburg 20, F.R.G..

Nuclear Factor I (NF 1), also referred to as the TGGCA-binding protein, is a nuclear protein ubiquitous among higher eukaryotes which shows high binding affinity in vitro for homologues of the sequence 5'-PyTGGCANNNTGCCAPu-3'. Though its only described function is an ability to stimulate the initiation of adenovirus DNA replication, the presence of NF I binding sites in the MMTV LTR and in the BK virus enhancer suggests that NF I may play a role in the initiation of transcription. In order to test this possibility, mutations were introduced into the MMTV binding site and were analyzed by transient expression in mouse L and human MCF7 cells. Mutations which eliminated NF I binding in vitro strongly reduced the glucocorticoid-dependent expression of the MMTV promoter. Restoration of NF I binding by the insertion of oligonucleotides containing synthetic or natural binding sites led to a restoration of induced expression, dependent on the number and position of binding sites reintroduced. Taken together, these data support the conclusion that NF I is capable of serving as a general transcription factor and that its interaction with the MMTV LTR is required for the hormone-induced expression of this viral promoter.

0352 CHARACTERIZATION OF MUTATIONS IN THE YEAST mRNA SPLICING SYSTEM. A.J. Lustig, R.J. Lin, and John Abelson. Biology Division, California Institute of Technology, Pasadena, California 91125.

The temperature-sensitive yeast rna mutations have been implicated in the process of mRNA maturation. The nature of this involvement, however, has remained unclear. Utilizing the in vitro splicing system, recently developed in our laboratory, we have demonsrated that several of the rna mutants (rna 2,5,7, and 11) are temperature-sensitive for splicing in vitro. The defects in all cases appear to be in early stages of the reaction. We have further shown that pairwise addition of heat inactivated extracts from different mutants result in the reacquisition of activity. These data provide the first evidence for the direct involvement of rna gene products in the process of splicing. The role of the rna products in "spliceosome" formation, as well as the identification and purification of these proteins is also currently being pursued.