MECHANISMS OF CONTROL OF GENE EXPRESSION

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Poster Sessions

Gene Regulation in Prokaryotes - I

L 001 REGULATION OF RIBOSOME BIOSYNTHESIS IN ESCHERICHIA COLI, Masayasu Nomura, Department of Biological Chemistry, University of California-Irvine, Irvine, CA 92717

The synthesis of ribosomes in E. coli is regulated so that the cellular concentration of ribosomes is roughly proportional to the growth rate. In addition, the synthesis rates of all the ribosomal components are balanced and, like the synthesis of ribosomes, respond coordinately to changes in environmental conditions. This second aspect of the regulation can now be explained by the translational feedback regulation of ribosomal protein (r-protein) synthesis. When r-protein synthesis rates exceed those needed for ribosome assembly, certain "free" r-proteins act as translational repressors on their respective mRNAs to inhibit further translation. The first aspect of the regulation, the growth rate dependent regulation of ribosomes biosynthesis, can also be explained by another feedback mechanism, which involves products of rRNA operon to feedback inhibit unnecessary synthesis or rRNA (and tRNA). Some recent experiments relevant to the two aspects of the regulation of ribosome synthesis will be discussed.

L 002 REGULATION OF THE HEAT SHOCK RESPONSE IN E.COLI, David B. Straus, Deborah W. Cowing, James W. Erickson, Alan D. Grossman, Yan-ning Zhou and Carol A. Gross, Department of Bacteriology, University of Wisconsin, Madison, WI. 53706.

When *E. coli* cells are shifted from 30° to 42° the synthesis of about 17 proteins, the heat shock proteins, is transiently induced. Synthesis of these proteins increases between 5 and 20 fold by 5 minutes after temperature shift, and then drops to a new steady-state rate of synthesis, slightly higher than the 30° rate, by about 15 minutes after shift. The transient induction is the result of increased transcription initiation at heat shock gene promoters.

The *E. coli rpoH* (*htpR*) gene encodes a 32Kd sigma factor which directs RNA polymerase ($E\sigma^{32}$) to initiate transcription at heat shock gene promoters. Heat shock gene promoters have a consensus sequence distinct from the one for promoters recognized by RNA polymerase containing the predominant, 70Kd, sigma factor. Cells lacking σ^{32} are unable to initiate transcription at heat shock gene promoters.

The existence of a unique set of promoters for heat shock genes, recognized by a minor form of RNA polymerase, provides an explanation for the coordinate expression of heat shock genes. These results by themselves do not explain the transient induction of heat shock gene mRNA synthesis following a temperature shift. We have examined the role of σ^{32} in heat shock gene expression. The results lead us to believe that the heat shock response is regulated by changes in the level of σ^{32}. The level of σ^{32} increases 10-20 fold by about 5 minutes after upshift. This increase is comparable to the increase in heat shock RNA synthesis. Two independent effects lead to the transient accumulation of σ^{32} after temperature upshift: 1) σ^{32} is usually unstable($T_{1/2} \approx 1$ minute), however, σ^{32} is transiently stabilized immediately after upshift. 2) The synthesis of σ^{32} transiently increases after temperature upshift. The increased rate of σ^{32} synthesis does not involve increased transcription of *rpoH* and may result from a change in the rate of translation of *rpoH* mRNA after temperature upshift. Studies with mutations that affect the heat shock response support these conclusions.

L 003 REGULATION OF THE METHIONINE REGULON, Robert Shoeman, Mary Maxon, Nathalie Jacques, Tim Coleman, Betty Redfield, Nathan Brot, and Herbert Weissbach, Roche Institute of Molecular Biology, Nutley, New Jersey 07110.

In *Escherichia coli* the genes coding for the enzymes involved in methionine (met) biosynthesis are scattered throughout the *E. coli* chromosome, and only two of the nine *met* genes are organized as an operon. The expression of the genes in the met regulon is regulated primarily in two ways. High levels of methionine in the growth medium repress all of the genes in the biosynthetic pathway whereas growth in the presence of vitamin B_{12} represses the expression of two of the *met* genes, *metE* and *metF*, that code for non-B_{12} N^5-methyltetrahydrofolate:homocysteine S-methyltransferase and 5,10-methylenetetrahydrofolate reductase. Previous genetic studies indicated that there are two regulatory genes involved in the repression by methionine: the *metJ* gene coding for a repressor protein and the *metK* gene which codes for S-adenosylmethionine (AdoMet) synthetase, the enzyme that catalyzes the synthesis of AdoMet from methionine. These results suggested that AdoMet (corepressor) and the MetJ protein (repressor protein) are involved in the regulation of the met regulon.

We have used highly defined *in vitro* DNA directed protein synthesis systems capable of synthesizing the entire protein, or the first dipeptide of the gene product, to study the regulation of the met regulon. Our efforts have concentrated on the expression of the *metE* and *metF* genes because of their dual regulation by met and vitamin B_{12}. For the initial studies, plasmids containing the *metF* gene were used as templates in the *in vitro* systems. At low concentrations of MetJ protein, there was essentially no repression of *metF* gene expression in the absence of AdoMet. However, with increasing levels of the MetJ protein, significant repression was obtained even without AdoMet. There was no evidence of methylation of either the MetJ protein or the DNA template during the reaction with AdoMet or the formation of a stable MetJ-AdoMet complex. Thus, the data suggest that AdoMet functions as an allosteric effector of the MetJ protein. The *metF* gene also contains a common sequence (met box) present in other *met* genes that appears to be the recognition site for the MetJ protein.

The *metE* gene has recently been cloned and the 5' region of the gene sequenced. Although the gene can be expressed *in vitro* it has not been possible to obtain significant repression with the MetJ protein and AdoMet under the same conditions in which the *metF* gene is repressed. In addition, the expression of both the *metE* and *metF* genes are unaffected by vitamin B_{12} or the B_{12}-dependent transmethylase (*metH* gene product) which has been implicated in the repression by vitamin B_{12} from *in vivo* studies.

Gene Regulation in Prokaryotes - II

L 004 DNA LOOPS AND REGULATION OF THE L-ARABINOSE OPERON IN E. COLI, Robert Schleif, Biochemistry Department, Brandeis University, Waltham, Massachusetts 02254.

In the absence of arabinose, AraC protein binds to two sites in the ara regulatory region separated by 210 base pairs, the $araO_2$ site and the araI site. A DNA loop that forms between these two proteins holds AraC protein at the araI site from entering its inducing conformation.

1. The upper and lower limits to DNA loop size have been determined for this system.

2. Helical twist experiments suggest that the DNA constituting the loop may have a helical repeat of 11 bp/turn.

3. Upon the addition of arabinose, the repression loop opens, releasing AraC at araI to assume an inducing conformation from which it stimulates transcription. Meanwhile a second DNA loop forms, this loop still involving $araO_2$, as well as a third site in the ara regulatory region, $araO_1$. In vivo footprinting, helical twist experiments, and the physiological behavior of relevant mutants support the above picture.

4. Mutations in AraC have been isolated that repress much better than wild type, (can be induced only in the absence of $araO_2$) or much worse than wild type. Purified protein of these mutants shows the expected behavior. The super-repressor oligomerizes better than wild type, and the sub-repressor oligomerizes less well than wild type.

Mechanisms of Control of Gene Expression

L 005 TRANSCRIPTION ATTENUATION MECHANISMS IN BACTERIA, Charles Yanofsky, Department of Biological Sciences, Stanford University, Stanford, CA 94305.

We are studying transcription attenuation in three operons; the trp and tna operons of Escherichia coli, and the trp operon of Bacillus subtilis and Bacillus pumilus. Investigations with the trp operon of E. coli have focused on transcription pausing in the leader region and its role in the coupling of transcription and translation essential to attenuation control. We have demonstrated formation of leader pause RNA in vivo; thus transcription pausing is a physiological reality. We have isolated in vitro transcription pause complexes and have used ribonuclease T1 digestion to examine the structure of leader RNA. Our findings suggest that formation of an RNA hairpin at the 3' end of the growing RNA chain disrupts the RNA-DNA heteroduplex in the transcription elongation bubble, thereby preventing the 3' end of the transcript from participating in polymerization. Robert Landick performed most of these studies; Jannette Carey participated in the in vivo pause analyses. The tna operon of E. coli encodes two proteins; an enzyme that degrades L-tryptophan to indole and the utilizable carbon and nitrogen sources, pyruvate and ammonia, and a tryptophan permease. Transcription of the tna operon is regulated by catabolite repression and attenuation. In cells growing in the absence of endogenous tryptophan rho-mediated transcription termination occurs in the leader region of the operon. The presence of tryptophan prevents this termination. The leader region of the tna operon encodes a 24-residue leader peptide; synthesis of this peptide is essential for normal attenuation control. Mutational studies and in vitro S-30 experiments suggest that the nusA protein participates in tna regulation by facilitating rho-mediated transcription termination. These studies were performed by Valley Stewart and Paul Gollnick. The trp operon of B. subtilis and B. pumilus contains a 200 bp leader region. The transcript of this region can fold to form alternative secondary structures which function as antiterminator and terminator. Unlike the trp operon of enteric bacteria the leader region does not encode a leader peptide. In vivo experiments suggest that the mtr locus specifies a polypeptide which when activated by tryptophan can bind to the promoter-proximal segment of the RNA antiterminator. This binding prevents formation of the antiterminator and promotes formation of the terminator, resulting in transcription termination. These studies were performed by Mitzi Kuroda of my group in collaboration with Hidenori Shimotsu and Dennis Henner of Genentech.

Gene Regulation in Eukaryotes: Control Mechanisms - I

L 006 EVIDENCE FOR TRANS-ACTIVATION OF MUSCLE GENES, Helen M. Blau, Bruce Blakely, Edna Hardeman, Steve Miller, Grace Pavlath, Kim Poffenberger, Laura Silberstein, Marilyn Travis and Steve Webster, Department of Pharmacology, Stanford University Medical Center, Stanford, CA 94305.

There is considerable plasticity in the expression of the differentiated state. The expression of nine different muscle genes can be induced in human nonmuscle cells following fusion with C_2C_{12} mouse muscle cells in nondividing heterokaryons. The mechanisms of activation differ among cell types, suggesting differences in lineage decisions and the steps required to generate and maintain them. Changes in chromatin structure associated with DNA replication do not appear necessary for trans-activation of muscle genes in all cell types tested, except HeLa cells. However, there are marked differences in gene dosage effects and in the kinetics of novel muscle gene expression which may be due to differences in the requisite number or amount of trans-acting factor(s). It appears that the activated human fibroblasts incorporated into heterokaryons produce their own muscle regulatory factors. The pattern of human α-cardiac actin transcript accumulation in heterokaryons is quite distinct from that of the mouse muscle cells that induce it and strikingly similar in time course and relative amounts to that in human primary muscle cultures. In addition, the usual decline in the level of mouse α-cardiac actin transcripts is not observed; instead, after fusion with human fibroblasts the levels increase. Using cells isolated with the fluorescence-activated cell sorter, we have shown that developmentally regulated transitions in myosin expression are amenable to analysis in pure populations of human primary muscle cells that can be isolated in quantity from normal and diseased muscle tissues. The potential existence of stage-specific trans-acting factors is now being investigated and in vivo assays for identifying and isolating the requisite tissue-specific trans-acting factors developed.

L 007 CIS-ACTING REGULATORY ELEMENTS ASSOCIATED WITH A CLASS II MHC GENE, Virginia Folsom, Jacki Goldman and Susumu Tonegawa, Center for Cancer Res., M.I.T., Cambridge, MA 02139

We have been investigating the regulation of transcription of the mouse class II major histocompatibility complex gene, E_β. Using the CAT assay, we have evaluated the function of E_β-derived DNA sequences in the activation of either the homologous E_β promoter or the SV40 early region promoter, in the macrophage tumor cell line $P388D_1$ that expresses E_β in response to γ-IFN, or in the B lymphoma cell line A20-2J that expresses E_β constitutively. A 2.6 kb DNA fragment from the 5' flanking region of the E_β gene (-2679 to -66 relative to the start of transcription can confer a 15 to 20 fold induction of CAT activity (in response to γ-IFN) on either the E_β promoter or the SV40 early region promoter. The ability to respond to γ-IFN can thus be transferred to a promoter (SV40) that is not normally responsive to this agent. A 126 bp subfragment from the 5' flanking region (-192 to -66) confers 6-7 fold γ-IFN induction on the SV40 promoter-driven expression of CAT. Using a series of overlapping oligonucleotides, we have tentatively identified a 17 bp interval (-158 to -144) that is required for γ-IFN inducible expression. A macrophage-specific negative regulatory element that represses activity in either the absence or presence of γ-IFN has been mapped upstream (between -666 and -344) of the γ-IFN responsive region. Removal of sequence in this interval increases CAT activity in $P388D_1$ macrophage cells, but has no effect on the activity measured in A20-2J B cells. The putative negative element has been shown to function independently by virtue of its ability to down regulate (in crs) the signal from a highly active (E_β/SV40 promoter/CAT) construct that lacks the negative region. Agents which are known to down-regulate class II gene expression in macrophage may operate at the negative regulatory site.

L 008 REGULATORY PROTEINS THAT GOVERN CELL SPECIALIZATION AND MATING TYPE INTERCONVERSION IN YEAST. Ira Herskowitz, Paul Sternberg, Ira Clark, and Brenda Andrews. Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

Cell type in the yeast Saccharomyces cerevisiae is determined by alleles of the mating type locus, MAT**a** and MATα (reviewed in **1**). Cells with MAT**a** have the **a** mating type, and cells with MATα have the α mating type. **a** and α cells mate efficiently with each other to form the third cell type, the **a**/α diploid cell. MATα codes for two regulatory proteins: α1, a positive regulator of expression of α-specific genes, and α2, a negative regulator of **a**-specific genes. α2 is a sequence-specific DNA-binding protein (**2**). In addition, a third regulatory activity, **a**1-α2, is responsible for repression of another set of genes.

Yeast cells switch between **a** and α in a process that involves genetic rearrangement. Mating type interconversion is catalyzed by the HO gene product, which encodes a site-specific endonuclease. This process is regulated in several different ways, which come about by regulation of the HO gene (**3,4**). (1) **Cell type control**: HO is expressed in **a** and α cells but not in **a**/α cells. (2) **Cell cycle control**: HO is expressed only in late G1. (3) **Mother-daughter control**: HO is expressed in mother cells but not daughter cells.

The upstream regulatory region of HO is approximately 1.4 kb (**5**). Products of at least five SWI genes are necessary for expression of HO (**6**). We have identified at least six other genes, SIN1-SIN6, which may be repressors of HO transcription (**7**). We propose that the SWI5 product activates transcription of HO by antagonizing action of the SIN3 product and that the SWI4 product activates transcription by antagonizing action of the SIN6 product. Our studies (**7**) indicate that SWI5 and SIN3 are responsible for mother-daughter control, which is exerted at the most upstream region (URS1) and that SWI4 and SIN6 are responsible for cell cycle control, which is exerted at the adjacent region (URS2).

1. Herskowitz, I (1985). CSHSQB **50**:565. **2.** Johnson, A and I Herskowitz (1985). Cell **42**:237. **3.** Jensen, R, G Sprague Jr, and I Herskowitz (1983). PNAS **80**:3035. **4.** Nasmyth, K (1983). Nature **302**:670. **5.** Nasmyth, K (1985). Cell **42**:225. **6.** Stern, M, R Jensen, and I Herskowitz (1984). JMB **178**:853. **7.** Sternberg, PW, MJ Stern, I Clark, and I Herskowitz (1987). Cell, in press (Feb.)

REGULATION OF EMBRYONIC MUSCLE GENE EXPRESSION, M.A.Q. Siddiqui,
L 009 Ana Maria Zarraga, Kenneth Danishefsky, Mariadele Noe and
Charmaine Mendola, 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 non-muscle cells. The genes coding for these proteins undergo a complex program of regulation and gene switching. In order to study the mechanism underlying the differential expression of gene for the myosin light chain (MLC) isoforms in development and to delineate the cis-acting regulatory elements involved in tissue-specific expression, the genes for MLC_2 of the chicken and rat cardiac muscles were isolated. Two recombinant clones, λLC5 and λLC13, encompassing the entire regulatory myosin light chain 2 (MLC_{2A}) gene of chicken heart muscle were characterized by partial nucleotide sequence analysis. Based on primer extension reaction with a synthetic 20-mer corresponding to the 5'-leader sequence and total poly(A^+) RNA, the transcription initiation site in the gene was located. The gene promoter activity was demonstrated following transient expression of recombinant genomes containing the chicken upstream sequence fused to the bacterial chloramphenicol acetyltransferase (CAT) or to the rat preproinsulin II genes used as markers. The extracts from a Quail fibroblast cell line (QT35) transfected with the construct (pLCo5.2iCat) containing the putative chicken promoter and the CAT gene promoted the formation of 3'-acetate chloramphenicol. Another construct (pBC12LC5.2f) contains the rat preproinsulin II gene, placed under the control of the chicken promoter, and a simian virus 40 origin of replication. Transfection of COS cells with pBC12LC5.2f DNA resulted in an efficient expression of rat preproinsulin mRNA initiating from the chicken promoter. The transfection assay also allowed detection of chicken MLC_{2A} gene transcripts by S1-nuclease protection of end-labeled DNA probes. An examination of the 5'-flanking region revealed the presence of two distinct inverted repeat sequences which can form remarkably stable hairpin structures. Whether such structures are biologically significant is being examined with mutant DNA constructs introduced into appropriate host cells for transcription assays. A comparison of the MLC_{2A} upstream gene sequence with those available for skeletal MLCs revealed no common sequence elements, suggesting that the cardiac MLC_{2A} gene promoter region has diverged considerably from its counterparts in skeletal muscle.

Gene Regulation in Eukaryotes: Control Mechanisms - II

TRANS-ACTIVATION OF HSV-1 IMMEDIATE EARLY GENE EXPRESSION
L 010 Steven J. Triezenberg, Karen L. LaMarco, and Steven L. McKnight, Dept. of Embryology, Carnegie Institution of Washington, 115 W. University Parkway, Baltimore, MD 21210

The HSV-1 virion contains a virally-encoded protein, termed VP16, that acts in trans to stimulate expression from immediate early (IE) genes following virus entry into permissive host cells. Each IE gene of HSV-1 is endowed with an enhancer that mediates trans-activation by VP16. We have mapped the cis-acting regulatory elements associated with an IE gene (ICP4) that facilitate response to VP16. Two adjacent, yet qualitatively distinct, DNA sequence elements are required for the ICP4 enhancer to respond maximally to VP16 induction. Mutated enhancers were constructed in which either or both of the VP16 response elements were selectively eliminated. Analyses of these mutants suggest that either VP16 response element is sufficient for a minimal level of trans-activation, but that together they act in a synergistic manner.

The trans-activating protein, VP16, does not appear to bind to either of the cis-acting elements identified by mutational analysis. However, using a DNAase I footprinting assay on nuclear proteins prepared from uninfected cells, we have found and fractionated two activities that bind to the two VP16 response elements of the ICP4 gene. Thus, we provisionally conclude that VP16 is a highly specific trans-acting factor which induces transcription indirectly.

Control of Eukaryotic Gene Transcription: Trans-Acting Elements

L 011 MOLECULAR ASPECTS OF REPLICATION AND PATHOGENESIS OF THE HUMAN LEUKEMIA AND AIDS RETROVIRUSES, William A. Haseltine, Joseph Sodroski, Craig Rosen, Wei Chun Goh, Ernest Terwilliger, Andrew Dayton, Roberto Patarca, Laboratory of Biochemical Pharmacology, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115

Retroviruses have been shown to be the etiologic agents of human leukemias and lymphomas and the acquired immune deficiency syndrome and related disorders. These viruses are unusual amongst retroviruses both with respect to virus genomic structure and replication cycle. The human leukemia virus is poorly transmissable and poorly infectious. It has the capacity, upon co-cultivation with infected cells, to transform primary T4+ lymphocytes in vitro. The virus contains a 1,500 region located between the envelope gene and the 3' LTR. This region encodes several proteins, one of which is an activator of LTR transcription. This gene product also induces - directly or indirectly, genes associated with T cell proliferation. The role of X-region encoded genes in virus replication and transformation will be discussed. The AIDS virus encodes at least four genes (sor, 3' orf, art, and tat) in addition to gag, pol, and env genes common to all retroviruses. Mutation analysis reveals that the 3' orf gene is not required for virus growth. The viruses lacking the sor gene can replicate slowly. The tat and art genes are absolutely required for viral growth. Both the tat and art genes appear to be post transcriptional regulators of expression of virus structural proteins. The mechanism of action of these genes will be discussed. The envelope gene protein of the AIDS virus has been shown to be cytotoxic to T4+ cells. The mechanism of the AIDS virus T4+ cell killing will be discussed.

L 012 COMPARISON OF THE REQUIREMENTS FOR SPECIFIC DNA SEQUENCES OF TWO TRANS-ACTING REGULATORY PROTEINS OF HERPES SIMPLEX VIRUS 1, **Thomas M. Kristie and Bernard Roizman, The Marjorie B. Kovler Viral Oncology Laboratories, The University of Chicago, Chicago, IL 60637.**

The genes of herpes simplex virus-1 (HSV-1) form five major groups (α, β_1, β_2, γ_1, and γ_2) whose expression is coordinately regulated and sequentially ordered in a cascade fashion. The α genes are the first to be expressed in the infected cell and their expression is induced by a structural component of the HSV-1 virion. The induction of α gene expression by α-trans-induction factor, designated as α-TIF, requires a small DNA sequence element. The consensus sequence [(G C/T ATGNTAATGA G/A ATTC T/C TTGNGGG) (α-TIC; α-trans-induction cis site)], present in the regulatory domains of HSV-1 α genes, has been defined by deletion analysis and by studies of test promoters with or without α-TIC sites. The α-TIC binds at least one HeLa host cell protein, but does not appear to interact with the α-TIF protein as determined by the DNA-protein gel electrophoresis technique. Competition analysis of the α-TIC-host cell protein complex with the α-TIC sites present in the regulatory domains of the α4, 0, and 27 genes indicates that all tested α-TIC sites bind the same major host cell protein. In addition, an α-TIC homolog, present within the SV40 72bp enhancer elements, also competes efficiently for the same host cell protein(s). Thus, it appears that α-TIF may mediate the induction of α genes by an indirect mechanism, utilizing available host cell factors.

The expression of the later groups of HSV genes (β and γ) is dependent upon the prior expression of functional α gene products (α4, 0, 22, 27, and 47). Of these, the α4, 0, 27, and 22 proteins have been implicated in the positive regulation of β and γ gene expression. Evidence from temperature sensitive viral mutants and transient expression systems have shown that the α4 protein is a major regulatory protein of HSV and has both positive(β and γ) and negative (α) regulatory characteristics. In contrast to the apparent indirect regulation of the α genes by α-TIF, the α4 protein binds to specific sequences within the promoter-regulatory domains of HSV-1 α and γ_2 genes. Mapping of the binding sites within the α gene promoter and regulatory domains has indicated that there are multiple binding sites for the α4 protein. Thus, the positive or negative regulatory effect of this protein on the expression of HSV genes may be determined by its interaction with specific binding sites over the course of viral infection.

Post-Transcriptional Control of Eukaryotic Gene Expression

L 013 MECHANISM OF ACTION OF THE HIV TRANS-ACTING GENE PRODUCT TAT III.
Bryan R. Cullen, Department of Molecular Genetics, Hoffmann-La Roche, Nutley, NJ 07110

Human Immunodeficiency Virus (HIV) encodes a trans-acting gene product, tat III, that is able to enhance the expression of genes transcribed from the HIV long terminal repeat (LTR). This enhanced expression is mediated both by a specific increase in mRNA levels and by an increase in the efficiency of translation of mRNAs containing HIV specific leader sequences. This presentation will address various aspects of the bimodal action of the HIV tat III gene product including the stage at which target mRNA steady-state levels are enhanced. I will also describe the effects of site-directed mutagenesis of the HIV LTR target region on the trans-activation phenotype and present data on the sub-cellular location of tat III. These results will be discussed with reference to the possible mechanisms of action of tat III at the transcriptional and post-transcriptional levels.

L 014 ANALYSIS OF THE INTERACTION BETWEEN ADENOVIRUS VAI RNA AND THE INTERFERON-INDUCIBLE P1/eIF2α KINASE, Jan Kitajewski and Thomas Shenk, Department of Molecular Biology, Princeton University, Princeton, NJ 08544

Adenovirus VAI RNA is a small, RNA polymerase III-transcribed species required for efficient translation of mRNA late after infection (Thimmappaya et al., Cell 31, 543, 1982). Mutant dl331 fails to produce this RNA and, as a result, grows poorly. VAI RNA facilitates translation by preventing phosphorylation of eIF2α (Reichel et al., Nature 313, 196, 1985, Schneider et al., PNAS 82, 4321, 1985, Siekierka et al., PNAS 82, 1959, 1985). Phosphorylation of eIF2α results in a decrease in initiation events, presumably by sequestering the GTP recycling factor (eIF2B) in an inactive complex. The protein responsible for phosphorylating eIF2α during dl331 infection is the interferon-induced, double stranded RNA (ds RNA) dependent, P1/eIF2α kinase (O'Malley et al., Cell 44, 391, 1986, Kitajewski et al., Cell 45, 195, 1986).

The P1/eIF2α kinase is activated by low levels of ds RNA, high levels of ds RNA block activation. This protein binds tightly to ds RNA, presumably as part of the activation event. We had previously shown that VAI RNA prevents activation of latent kinase by ds RNA in crude extracts of interferon-treated cells. We have now purified this kinase in a latent form to near homogeneity and show that purified VAI RNA blocks activation of the purified kinase by ds RNA. Using a nitrocellulose filter binding assay, radiolabeled VAI RNA was found to bind to the purified kinase and this binding can be specifically competed by unlabeled VAI RNA. Further analysis of the interaction of VAI RNA with the P1/eIF2α kinase are in progress.

Control of Eukaryotic Gene Transcription: DNa-Protein Interactions

L 015 IDENTIFICATION AND FUNCTIONAL ANALYSES OF COMMON AND GENE SPECIFIC TRANSCRIPTION FACTORS FOR REGULATED EUKARYOTIC GENES. R. Roeder, M. Sawadogo, M. Horikoshi, S. Abmayr, H. Sive, C Fletcher, F. LaBella, N. Heintz, J. Mizushima-Sugano, C. Scheidereit, A. Heguy, and R. Prywes, The Rockefeller University, New York, NY 10021.
Our studies of eukaryotic transcriptional mechanisms have emphasized (i) the development of cell-free systems in which purified genes are both accurately transcribed (by common factors) and regulated (by gene- or cell type-specific factors) and (ii) the subsequent purification and characterization of the various factors. I. Adenovirus Major Late (Ad ML) Promoter. This promoter employs a set of common factors (employed by all class II genes) and a promoter specific binding factor (USF or MLTF) which interact cooperatively with the bound TATA factor TFIID. USF has been purified to homogeneity and shown to interact with several other promoters. TFIID has also been shown to interact with a number of promoters. The mechanism by which a viral immediate early protein stimulates transcription from the ML promoter (in vitro) has also been further analyzed. II. Histone H2B and Immunoglobulin K Promoters. The regulatory elements which mediate the cell cycle control (S phase activation) of the H2B gene and the developmental control (B cell activation) of the K promoter contain a common octamer (ATTTGCAT) sequence. Both ubiquitous (B cell and HeLa cells) and B cell-specific octamer binding factors have been identified, separated and partially purified. Surprisingly, these factors do not discriminate between the respective sequence elements in simple binding (gel shift) analyses, but they are selective in functional (transcription) assays. Thus, the purified HeLa factor stimulates transcription only from the H2B gene while the high level of octamer-dependent K transcription is observed only in the presence of the B cell factor(s). The structural relationships of the octamer binding factors and the basis for their differential function in the H2B and K genes are being investigated. These observations raise obvious and highly significant questions regarding the diversity and inter-relationships of factors which mediate control of distinct types of genes. III. C-Fos Gene. The induction of c-fos transcription by growth factors has been shown (by others) to involve a specific enhancer. Gel mobility shift/footprint analyses have demonstrated a concomitant 10-fold increase in transcription and in an enhancer binding activity in A431 cells by EGF, although the subsequent drop in transcription is not accompanied by a decline in the enhancer binding activity. These and other studies with protein synthesis inhibitors suggest that c-fos activation by EGF involves a two-step modification of the enhancer factor (effecting both binding and function) and/or the involvement of other factors. In addition, the enhancer binding activity in A431 cells is unaltered by other agents (TPA and the calcium ionophore) which markedly induce fos transcription--suggesting that these agents induce fos by non-identical mechanisms that presumably involve other factors and control sequences.

L 016 **TRANSCRIPTION OF DEVELOPMENTALLY REGULATED *DROSOPHILA* GENES *IN VITRO*,** Ulrike Heberlein, Bruce P. England, Mark D. Biggin, Karen K. Perkins and Robert Tjian. Department of Biochemistry, University of California Berkeley, CA 94720.

We are interested in studying the biochemical mechanisms involved in determining the spatial and temporal specificity of gene expression. As model systems we have chosen regulated *Drosophila* genes such as Alcohol dehydrogenase (Adh) , and the homeotic genes *Ultrabithorax (Ubx)* and *Antennapedia (Antp).*

The Adh gene is transcribed from two tandem promoters (distal and proximal) which are regulated in a complex manner throughout the development of the fly. We have developed cell-free transcription extracts from staged embryos in an attempt to reproduce the phenomenon *in vitro.* We find that the efficiency of initiation at the distal promoter in a series of such staged extracts follows the pattern observed *in vivo.* Analysis of promoter mutants together with DNase I footprinting experiments suggest that the regulation observed *in vitro* is at least partly due to the levels of the transcription factor Adf-1. This factor, which binds to and activates the distal promoter has been purified to near homogeneity from *Drosophila* Kc cells using specific DNA-affinity chromatography. Surprisingly, the purified Adf-1 also binds strongly to the *Antp* P1 promoter and its role in *Antp* transcription is being investigated.

We have also analysed the signals involved in regulating transcription from the *Ubx* promoter in embryo and Kc cell extracts. Sequences located both 5' and 3' to the *Ubx* start site affect the efficiency of initiation *in vitro.* DNase I footprinting revealed the presence of several factors, some of which are embryo specific, that selectively recognize these sequences. We are in the process of purifying and characterizing these factors in order to further study their role in regulating the temporal progress of transcription during Drosophila embryogenesis.

RNA Processing

L 017 ALTERNATIVE SPLICING: SPLICE JUNCTIONS ARE NOT FUNCTIONALLY HOMOLOGOUS, THEIR USE IS REGULATED BY CIS- AND TRANS-ACTING ELEMENTS. B. Nadal-Ginard; R.E. Breitbart; M. Gallego; A. Andreadis; T. Endo and C. Smith. Laboratory for Molecular and Cellular Cardiology, Howard Hughes Medical Institute, The Children's Hospital, Harvard Medical School, Boston, MA 02115.

Several contractile protein genes, including those coding for myosin light chain 1 and 3 (MLC 1/3), α -tropomyosin (α -TM) and troponin T (TnT) provide favorable systems to study the types and mechanisms of alternative splicing. In these systems, alternative splicing results in the generation of multiple tissue- and developmental-specific contractile protein isoforms from single genes. A multitude of in vitro prepared mini-gene constructs containing MLC 1/3, TnT and α -TM gene sequences, driven by strong and promiscuous promoters such as SV40 and the LTR of retroviruses, have been prepared and expressed in muscle and non-muscle cells. The picture emerging from these experiments is intriguing: The constructs analyzed are alternatively spliced both in non-muscle and undifferentiated myogenic cells, but the pattern of splicing differs according to the construct and, in most cases, is different from the pattern exhibited by the differentiated muscle cells.

Three patterns of splicing have been detected: **a) Cis-regulated splicing** with little or no influence of cell-specific trans-acting factors. This pattern is exhibited by the MLC 1/3 gene. The donors of exon 1 (MLC1-specific) and exon 2 (MLC3-specific) appear to determine whether exon 3 (MLC3-specific) or exon 4 (MLC1-specific) are utilized. The selectivity of the donor sites is so stringent that when the appropriate exon is removed, the alternative one is **not** utilized. **b) Trans-regulated splicing,** exhibited by constructs that include exons 4 to 8 of TnT, either "in toto" or in different combinations. In general, most combinatorially spliced exons are excluded in non-muscle cells and myoblasts, although different combinations are included at low levels of efficiency. During differentiation, there is a progressive increase in the number of combinations of TnT mRNAs generated by the constructs until the basic pattern of the endogenous gene is attained. In addition, in these constructs exons that are normally constitutively spliced become alternative in a developmentally regulated fashion. **c) Cis- and trans-regulated exons.** The combination of these two elements is more clearly demonstrated by the α - and β -exons at the 3' end of the TnT gene. These two exons are spliced in a mutually exclusive manner in all muscle cells. This is also the case for all the minigenes that have been prepared so far. Yet, in both muscle and non-muscle cells the mini constructs always include the β exon while excluding α . More interestingly, the α exon is not included in any mRNA even when the β exon has been deleted. These results strongly suggest that in the absence of specific trans-acting factors the α exon is not recognized by the splicing machinery of the cell and this exon is constitutitvely excluded. Trans-acting factors present only in adult muscle, however, make this exon recognizable and able to successfully compete with the β exon. Taken together these results demonstrate that splice junctions in a gene are not equivalent but express different affinities for each other even when they have a consensus sequence. This affinity can be modulated by cis- and trans-acting elements. The trans-acting factors are modulated in a developmental and tissue-specific manner.

L 018 ASSOCIATION BETWEEN THE MYC ONCOGENE PROTEIN AND RIBONUCLEOPROTEIN PARTICLES. Neil F. Sullivan,[1] David L. Spector[1] and Rosemary A. Watt[2]. Cold Spring Harbor Laboratory(1), Cold Spring Harbor,New York 11724 and Molecular Oncology Group(2), Smith Kline and French,709 Swedeland Road, Swedeland PA 19479.

The subnuclear immunolocalization of the myc oncogene protein has been examined in quail cells (Q8) retrovirally transformed with the avian myelocytomatosis virus MC29, using a polyclonal antibody directed against the recombinant human c-myc protein (1). The punctate appearance of v-myc in the nucleus is similar to that seen for certain autoimmune disorders; subsequent double label immunofluorescence using the anti myc antibody and a monoclonal directed against the Sm antigen of the snRNP (Y79, 2) indicated a precise colocalization of these two antigens in nuclear regions which excluded the nucleoli and the bulk of the nuclear DNA. Subsequently we examined the localization of c-myc in cells of the human colon adenocarcinoma COLO320 and show that indeed c-myc colocalizes with the Sm antigen. In addition, when the bacterially produced recombinant c-myc protein was microinjected into the cytoplasm of quiescent rat fibroblasts it not only migrated into the nucleus but precisely localized to the RNP containing regions. Serial sectioning followed by immunoelectron microscopy reveals that myc may be associated with a three dimensional reticulum which traverses the nucleoplasm. Futhermore, in situ nuclease digestion shows that RNAse A but not DNAse I digestion of Q8 cells results in a reduction of both the myc and Sm immunostaining. Continuous sucrose gradient centrifugation of nucleoplasmic extracts prepared from Q8 cells shows that v-myc and Sm sediment in similar, but not identical fractions across these gradients although the sedimentation of both antigens appears to be sensitive to predigestion with RNAse A. These data raise the possibility that the myc gene product may be associated either with the RNA processing machinery per se or a common reticulum upon which other nuclear processes are carried out.

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Role of Oncogene Proteins in the Pathways that Control Gene Expression

L 019 REGULATION OF PROTEIN-TYROSINE KINASES, Jonathan A. Cooper, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Increased protein-tyrosine kinase activity, due to growth factor stimulation or oncogene expression, commonly lead to alterations in gene expression. Although the steps linking kinase activation to transcriptional and translational stimulation are obscure, much has been learned regarding regulation of the kinases themselves.

Expression of the protein-tyrosine kinase pp60$^{v\text{-}src}$ (product of the v-src viral oncogene) in fibroblasts leads to malignant transformation. In contrast, the related normal cellular protein, pp60$^{c\text{-}src}$, may be expressed to high level in normal fibroblasts without causing transformation. When assayed in vitro, pp60$^{c\text{-}src}$ has lower specific kinase activity than pp60$^{v\text{-}src}$, perhaps explaining their different transforming abilities.

Observations from several laboratories suggest that pp60$^{c\text{-}src}$ is regulated by tyrosine phosphorylation of its carboxy terminus. (a) Mutated genes that code for pp60$^{c\text{-}src}$'s in which the carboxy terminus is removed and an unrelated sequence is substituted are transforming. (b) Mutation to phenylalanine of a tyrosine (Y527) that lies in this region is sufficient for transformation. (c) This tyrosine is phosphorylated to high stoichiometry in pp60$^{c\text{-}src}$ of normal fibroblasts. (d) Enzymatic dephosphorylation of Y527 in vitro stimulates pp60$^{c\text{-}src}$ kinase activity. (e) Some mutations of c-src do not affect the carboxy terminal sequence yet are transforming. In the resulting mutant proteins, Y527 is not phosphorylated. (f) In cells transformed by the DNA virus, polyoma virus, a viral transforming protein binds to pp60$^{c\text{-}src}$: the bound pp60$^{c\text{-}src}$ has increased kinase activity and decreased phosphorylation at Y527. Thus the extent of Y527 phosphorylation correlates inversely with kinase activity. One view, consistent with kinetic data, is that the phosphorylated carboxy terminus of pp60$^{c\text{-}src}$ acts as a product analog, covalently attached to the enzyme. In the cell, specific unknown stimuli might reduce Y527 phosphorylation stoichiometry (by increasing phosphatase or decreasing kinase activity), or induce protein binding to the carboxy terminus (mimicked in vitro by a specific antibody), and turn on the pp60$^{c\text{-}src}$ kinase activity.

At least 4 other protein-tyrosine kinases are closely related to pp60$^{c\text{-}src}$, and bear a similar carboxy terminal sequence. pp56^{lck} is one example. Very high expression of this protein in a lymphoid cell line (LSTRA) appears to be the cause of transformation in these cells. In LSTRA cells, preliminary indications are that some pp56^{lck} molecules lack phosphate at the carboxy terminal tyrosine. The kinase activity of these molecules may be enhanced. Perhaps in these cells, the enzymes that usually keep pp56^{lck} inactive are incapable of coping with the over-abundance of pp56^{lck}.

L 020 C-FOS INDUCTION IS MEDIATED BY DIVERSE BIOCHEMICAL PATHWAYS, T. Curran[1] and J. Morgan[2], Department of Molecular Oncology[1] and Department of Neurosciences[2], Roche Institute of Molecular Biology Nutley, NJ 07110

Transcriptional activation of c-fos has been detected in many different biological situations. It has been linked to mitogenesis, differentiation and cellular depolarization. These observations indicate that c-fos might play a rather general role in coupling short-term events to long-term alterations in gene expression. Using c-fos expression as an early nuclear marker, we have investigated stimulus-transcription coupling, particularly in the PC12 phaeochromocytoma cell line, in an attempt to identify intracellular signals involved in the activation of c-fos expression. Two major pathways have been distinguished that promote c-fos induction in PC12 cells. Depolarization, either by elevation of extracellular potassium, or using the drugs veratridine or Bay K 8644, leads to a calcium and calmodulin-dependent stimulation of c-fos expression. Nerve growth factor (NGF), on the other hand, induces c-fos independently of the presence of calcium in the extracellular medium and is not affected by calmodulin antagonists. In addition, NGF-stimulated c-fos expression is not affected by down regulation of protein kinase C or alterations in cAMP levels. Although activation of protein kinase C using phorbol esters and activation of cAMP-dependent kinase by addition of dbcAMP lead to only modest elevations of c-fos expression in PC12 cells, these agents are potent inducers in other cell types. Thus, depending on the particular cell line studied; calcium flux, calmodulin activation, stimulation of protein kinase C, stimulation of cAMP-dependent kinase, and other unknown signals, such as those triggered by NGF, can all lead to c-fos induction. It is not yet known whether these diverse messenger systems activate c-fos expression independently or whether they all act through a common intermediary.

L 021 **INITIAL EVENTS IN THE MECHANISM OF ACTION OF RAS PROTEINS,** James R. Feramisco and Dafna Bar-Sagi, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.
In order to identify the cellular activities that are controlled by ras proteins, we have studied the early morphological and biochemical events that occur in response to microinjection of ras proteins. Within 30 minutes after microinjection of the human H-ras proteins into rat fibroblasts, the injected cells show an increase in surface ruffles and fluid-phase pinocytosis. The rapid enhancement of ruffling and pinocytosis is induced by both the protooncogenic and the oncogenic forms of the H-ras protein. However, the effects of the oncogenic protein persist while the effects of the proto-oncogenic protein are short-lived. The stimulatory effect of ras proteins on ruffling and pinocytosis is dependent on the amount of injected protein, requires extracellular calcium, and is independent of protein synthesis. Injection of the ras protein results in the stimulation of phospholipase A_2 activity as indicated by an increase of 2-fold in the levels of lyso-PC and lyso-PE within 1 hour after injection. These changes in cell membrane activities are the earliest ras-mediated effects detected to date and may represent primary events in the mechanism of action of ras proteins.

L 022 THE EXPRESSION OF GENES INVOLVED IN THE MITOGENIC RESPONSE TO PLATELET-DERIVED GROWTH FACTOR, Lewis T. Williams, Shaun R. Coughlin, Jaime A. Escobedo, and Raymond L. Kacich, Howard Hughes Medical Institute, Box 0724, Department of Medicine, University of California, San Francisco, 94143.
The mechanism by which platelet-derived growth factor (PDGF) stimulates the proliferation of mesenchymal cells is unknown. The interaction of PDGF with its receptor elicits a diverse group of responses including enhanced expression of the c-myc and c-fos genes, increase in cyclic AMP levels, activation of tyrosine kinase, and stimulation of protein kinase C. We have shown that one pathway that mediates PDGF-stimulated c-myc gene expression involves receptor mediated phosphoinositide turnover and activation of protein kinase C. When protein kinase C is depleted by chronic exposure of the cells to phorbol esters and c-myc induction by subsequent phorbol ester exposure is abolished, PDGF-stimulated c-myc expression is attenuated by 60%. Thus the phosphoinositide turnover-protein kinase C pathway is one of several pathways from PDGF receptor to c-myc gene expression. We have now shown that there are kinase C - independent pathways that mediate the expression of the c-myc gene. Because cyclic AMP potentiates the action of a number of growth factors and is elevated in PDGF-stimulated Swiss 3T3 cells, we examined the ability of cyclic AMP to stimulate c-myc expression. Forskolin (50μM), a direct activator of adenylate cyclase, stimulates an increase in c-myc levels within 30 min.

Although the expression of the c-myc and c-fos genes may be necessary for the mitogenic response to PDGF, it is not sufficient because some agents that stimulate phosphoinositide turnover and c-myc expression to the same extent as PDGF are poor mitogens. Furthermore, when cells are depleted of protein kinase C, the stimulation of c-myc expression by PDGF is attenuated by 60% while the mitogenic response to PDGF is unchanged.

The PDGF receptor has a large cytoplasmic region that mediates the effects of PDGF on c-myc expression and activates the other pathways necessary for PDGF-stimulated proliferation. To determine the role of the tyrosine kinase domain and non-kinase domains in these responses, we have expressed the PDGF receptor cDNA in Chinese hamster ovary (CHO) cells that normally lack PDGF receptors. Stable transfectants of the cDNA expressed under the control of SV40 promotor express receptor mRNA that is translated and processed to a 195 kDa mature receptor protein. The transfected receptor mediates a number of PDGF-stimulated responses. This CHO cell expression system for the PDGF receptor provides an approach to study the pathways that mediate c-myc gene expression and other pathways involved in the mitogenic response to PDGF.

Mechanism and Regulation of Stable RNA Synthesis

L 023 MECHANISM OF REGULATION OF rRNA SYNTHESIS IN ACANTHAMOEBA, Marvin R. Paule, Calvin Iida, Erik Bateman, Preecha Kownin, Deborah Knoll and William Kubaska, Department of Biochemistry & The Cellular and Molecular Biology Program, Colorado State University, Fort Collins, CO 80523.

When starved, the free-living eukaryote Acanthamoeba ceases growth and undergoes differentiation into a dormant cyst. In the process, transcription of rRNA stops. This is accomplished by a direct modification of RNA polymerase I (pol I) which prevents its binding to the rRNA promoter. We have shown that the mechanism of rRNA transcription initiation involves the binding of a trans-acting protein factor (TIF) to the region between -67 and -14 of the template, with sequence-specific contacts in a region between about -45 and -17. These conclusions are based upon the effects on transcription and on template commitment of a series of BAL-31 deletions and point mutations within the promoter, and from DNase I and methidiumpropyl-EDTA-Fe(II) (MPE) footprinting. This preinitiation complex is next recognized by pol I which interacts with the promoter in a region bounded by TIF and +18. The strongest contacts are adjacent to the TIF binding site and around the start site (+1) as shown by MPE footprinting. The final steps in initiation involve DNA melting, nucleotide binding, de novo chain initiation and translocation. Translocation of the polymerase was demonstrated by movement of the pol I footprint a predicted distance during short RNA chain synthesis halted by the chain terminator 3'-O'methyl CTP. The TIF footprint remains unchanged following pol I translocation implying the preinitiation complex's stability in the cell. Pol I isolated from cysts is functional in elongation, but is unable to faithfully initiate at the promoter. Footprinting shows that this is a consequence of the inability of cyst pol I to bind to the promoter. The homogeneous cyst pol I is also less heat stable than pol I from vegetative cells suggesting covalent modification. The results of structural comparisons of the cyst and vegetative pol will be presented.

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L 024 TERMINATION OF RNA POLYMERASE I TRANSCRIPTS IN XENOPUS, Ronald H. Reeder, Paul Labhart, and Brian McStay, Hutchinson Cancer Research Center, Seattle, Washington 98104.

Transcription of the ribosomal genes of Xenopus laevis begins at the gene promoter and proceeds across both the 40S precursor coding sequence and the entire intergenic spacer to terminate at a site about 215 bp upstream of the transcription initiation site for the next repeating unit. We have identified a 12 bp sequence element which is necessary for this termination event. Evidence will be presented that the termination signal also acts as an upstream element of the adjacent promoter. Models for termination will be discussed including the possibility that polymerase is conservatively passed from the terminator to the promoter without entering the free pool.

L 025 **TRANSCRIPTION OF MOUSE AND FROG RIBOSOMAL RNA GENES** Barbara Sollner-Webb, Sheryl Henderson, Jolene Windle, Val Culotta, Louise Pape and John Tower. The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We are studying the DNA sequences and factors that direct transcription of rRNA genes. Analysis of 5' and 3' deletion mutants of both mouse and frog rDNA showed the promoter to consist of an essential domain surrounding the initiation site and additional stimulatory domains extending ~140 bp further upstream (Cell 35:199; MCB 5:554; MCB 6:1228). Moreover, the same large promoter region is utilized to direct initiation on Xenopus rDNA both in Xenopus and mouse cell extracts.

Transcription of Xenopus rDNA linker scanning mutants in microinjected oocytes demonstrates that a core promoter domain (residue ~-35 to +6) and a far upstream domain (~-140 to -128) are most crucial, while mutants bearing substantial sequence alterations in the 100 bp region between these domains initiate actively (MCB 6: Dec.). Surprisingly, however, any spacing change of >2 bp abolished initiation. In vitro analysis shows that the core domain dictates the precise initiation site, while the upstream domain specifies the approximate distance and the face of the helix on which initiation can occur.

Fractionation of mouse cell extracts reveals two activities that are necessary and sufficient to direct in vitro rDNA transcription. One is a subform of RNA polyerase I that is specifically activated to participate in accurate transcription. The other component binds to the upstream half of the core promoter domain and allows attachment of the active polymerase (MCB 6: Oct.). Regulation of rDNA transcription appears to be mediated by alterations in the levels of both of these transcription factors.

Sequences surrounding mouse rDNA residue -168 are essential for appreciable levels of transcription in transfected cells and are also stimulatory in vitro. This promoter domain evidently functions by the novel mechanism of providing a polymerase I terminator. It acts cotranscriptionally, inhibiting transcripts from reading into this region from upstream and instead causing these RNAs to end and be released (Cell, Dec. 1986). Most likely, polymerases on the tandem genomic rRNA genes do not detach after transcribing each 40-47S RNA, but traverse the spacer to the next promoter-terminator which makes them available for concerted reinitiation. Through such polymerase recycling plus additional binding of free polymerase, these tandem genes could achieve their characteristic high transcription levels.

In summary, these data show that rRNA transcription is a complex process, well conserved across different species, which involves a large multi-domain promoter with very precisely positioned elements, at least two trans-acting factors that are both essential and regulatory, and a novel and efficient recycling of polymerase.

Gene Regulation in Prokaryotes

L 100 MECHANISMS OF rRNA TRANSCRIPTION IN ACANTHAMOEBA, Erik Bateman and Marvin R. Paule, Colorado State University, Fort Collins, CO 80526.

Acanthamoeba rRNA transcription is dependent on a promoter, a transcription factor (TIF) and RNA polymerase I. Using MPE and DNase I footprinting under various conditions, as well as other assays, we obtained data suggesting that TIF directs polymerase I to the transcription start site through protein-protein interactions. TIF produces sequence-dependent structural alterations to the DNA surrounding the start site, in the apparent absence of direct interaction with this region. The presence or production of these structural changes correlate well with the efficiency of transcription. TIF may fulfill two roles: positioning of RNA polymerase I at the transcription start site with stabilization of the complex, and alteration of the DNA helix to facilitate transcription initiation.
Supported by USPHS Grants GM22580 and GM26059.

L 101 OXYGEN CONTROL OF BACTERIOCHLOROPHYLL BIOSYNTHESIS OCCURS BY REGULATING TRANSCRIPTION THROUGH *PUFQ*, A NEWLY IDENTIFIED *PUF* OPERON GENE PRODUCT: Carl E. Bauer and Barry L. Marrs; Central Research and Development, E.I. du Pont de Nemours & Co.

Photosynthetic growth by the purple nonsulfur photosynthetic bacterium *Rhodobacter capsulatus* is regulated by the level of molecular oxygen. Under anaerobic conditions the cells synthesize the necessary components for photosynthesis whereas under aerobic conditions they are repressed. Deletion analysis of the *puf* operon, which encodes the light harvesting I (LH-I) and reaction center genes, demonstrated the existence of an additional open reading frame (*pufQ*) that is located between the promoter and the LH-I beta gene. Translational fusions of *lacZ* with genes in the *puf* operon demonstrates that the level of transcription is regulated approximately 30-fold by molecular oxygen. Internal deletion mutagenesis of the *puf* operon demonstrates that *pufQ* encodes a regulatory protein that regulates the level of bacteriochlorophyll biosynthesis approximately 40-fold. Thus, transcription through the *puf* operon couples the amount of bacteriochlorophyll biosynthesis to the level the, bacteriochlorophyll binding, light harvesting and reaction center polypeptide biosynthesis. Analyses of *pufQ* demonstrates that it does not effect the biosynthesis of cytochromes even though they are though to share a common pathway of biosynthesis with bacteriochlorophyll. Additional studies demonstrate that *pufQ* does not appear to regulate the level of bacteriochlorophyll by regulating transcription through the biosynthetic genes. The mechanism whereby *pufQ* regulates bacteriochlorophyll biosynthesis most likely occurs through the formation of a structural component required for synthesis.

L 102 CHARACTERIZATION OF A TRANSCRIPTION UNIT FOR A VARIANT-SPECIFIC SURFACE GLYCOPROTEIN (VSG) OF TRYPANOSOMA BRUCEI, P. Borst, J.M. Kooter, P.J. Johnson, C. d' Oliveira, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Trypanosomes evade the immune defense of their mammalian host by varying VSGs. VSG genes are transcribed in one of several telomeric expression sites (ES). To assess how ES are controlled to avoid co-production of several VSGs, we have extended our analysis of the ES of the telomeric VSG gene 221 in T. brucei 427 (1,2). By chromosome walking we have obtained 60 kb of overlapping clones upstream of telomeric VSG genes. Analyses of nascent RNA (cf. 1) and UV-inactivation of transcription indicate that the promoter of the 221 ES is located 50 kb upstream of VSG gene 221. The transcription unit yields at least 5 poly(A)$^+$ RNAs, carrying a mini-exon sequence at their 5' end, besides the 221 mRNA. These must be produced from a long primary transcript by processing and this may involve transsplicing. Inactivation of the ES does not lead to alterations in the promoter area that are detectable in restriction digests of nuclear DNA. It remains possible, however, that minor alterations in this area are responsible for inactivation and are introduced by gene conversion, since the promoter area is moderately repetitive (more than 15 copies).

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L 103 Small RNAs sharing homology with the primary transcript of the trypanosome mRNA spliced leader. Gregory A. Buck and Cathleen McCarthy. Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, Virginia 23298.

Most or all trypanosome mRNAs share an identical 35 base spliced leader sequence (SL). This SL seems to be added to the mature transcripts by a novel process of inter-molecular (or trans-) splicing. We have examined this phenomenon in Trypanosoma cruzi, the causative agent of Chagas Disease in Central and south America, and in T. equiperdum, an African trypanosome that causes disease primarily in equine species. We have identified the primary transcripts of the SL in these species (∿110b in Tc.; ∿140b in Te) by Northern Hybridization with radiolabelled synthetic oligonucleotides homologous to their respective SLs. Using similar oligonucleotides as probes, we cloned and sequenced the gene encoding the T. cruzi SL. Synthetic oligonucleotides homologous to various regions of the SL primary transcript were hybridized to Northern Blots of total trypanosome RNA to identify potential RNA co-factors of the splicing reaction. With one of these probes, two small transcripts in T. cruzi (∿160b and ∿170b) and one in T equiperdum (∿170b) were identified. We have mapped the approximate locations of the portions of these RNAs that are homologous to the SL primary transcript by hybridization with the oligonucleotides and digestion with RNAseH and are currently directly sequencing these transcripts by primer extension and chain termination.

L 104 SELF-SPLICING RNA IN BACTERIOPHAGE, Frederick K. Chu, Gladys F. Maley and Frank Maley, New York State Department of Health, Albany, NY 12201

Structural and functional studies of the T4 phage thymidylate synthase gene (td) have revealed the following findings:

1) The presence of a 1017 bp intron,
2) td expression involves intron-excision and exon ligation at the RNA level,
3) td precursor RNA self-splices in vitro, and
4) td intron is a group I intron

Although td RNA self-splicing in vitro requires only guanosine and Mg^{++}, it is likely that other components, protein or otherwise, may aid the splicing process in the cell.

The property of guanosine addition to the 5'-end of the intron RNA segment during splicing has been exploited in screening for other procaryotic self-splicing RNAs. In this manner, multiple candidates for self-splicing introns were observed only in the T-even phages. Those introns whose genomic origins have been identified include the thymidylate synthase gene in T2, T4 and T6 phages and the nrdB gene (ribonucleotide reductase small subunit) of T4 phage. (Supported in part by grants GM26387 (NIH) and PCM8402005 (NSF)).

L 105 FEEDBACK REGULATION OF rRNA SYNTHESIS IN E.COLI REQUIRES IF2, James R.Cole[1], Chris Olsson[2], John Hershey[2] and Masayasu Nomura[1], [1]University of California, Irvine 92717 and [2]University of California, Davis 95619.

During exponential growth the amount of ribosomes in E.coli is proportional to the rate of protein synthesis. Stable RNA synthesis is normally regulated by a negative feedback loop that balances the rate of rRNA accumulation to the demand for increased protein synthetic capacity. This negative feedback control of rRNA synthesis by excess rRNA requires assembly of rRNA into complete ribosomes. We now report that rRNA feedback regulation breaks down in cells deficient in translation initiation factor 2. We have placed the chromosomal copy of the infB gene, coding for IF2, under the control of the inducible lac promoter. By varying the concentration of the lac operon inducer IPTG we were able to vary the in vivo level of IF2 and the cellular growth rate (μ). When the level of IF2 was insufficient to support a normal rate of growth the cells displayed an elevated RNA to protein ratio, indicating an increase in the level of ribosomes. Furthermore, polysome profiles revealed a massive accumulation of monosomes and free ribosomal subunits, confirming the accumulation of ribosomes beyond the level needed for translation. These accumulated ribosomes were evidently insufficient to cause feedback regulation of stable RNA synthesis in the absence of sufficient IF2. This breakdown in rRNA feedback regulation shows that IF2 is apparently necessary for proper stable RNA regulation and implies that excess rRNA must increase the capacity for translation initiation, not just the amount of assembled ribosomes, for feedback regulation of stable RNA synthesis to occur.

L 106 REGULATION OF EXPRESSION OF CONJUGATION GENES IN SEX FACTOR F AND PLASMID R100 BY FinO AND INTEGRATION HOST FACTOR. Walter B. Dempsey, VA Medical Center and Univ. of Texas Health Science Center, Dallas TX 75216.

Expression of the 33kb long traY-Z transfer operon of both the sex factor F and the antibiotic resistance plasmid R100 requires the plasmid-specific TraJ protein. traJ is immediately upstream of traY-Z in both plasmids. Its ORF is 0.68 kb and its leader is 0.10 kb long. traJ expression is regulated by the plasmid specific gene finP and the non-specific R100 gene finO. The finP genes encode 4S RNAs that are antisense to the ribosome binding site of their traJ mRNAs. (McIntire, Fee and I have sequenced finO but do not know what its product is.) Using a 0.103 kb ss RNA probe for early traJ mRNA, I examined Northern blots of extracts of E.coli mutants carrying R100 or F and chromosomal mutations known to affect transfer (dye, cpx, himA and hip.) I found the traJ gene makes a set of 8 transcripts in all but cpx strains. Sizes are 3.3, 1.6, 1.05, 0.83, 0.67, 0.41, 0.295, 0.160 kb. Only the amounts are strain dependent. The predominant transcript is always 3.3 kb long. Thus most of traJ mRNA ends beyond traY rather than between traJ and traY. The shortest four transcripts end inside the J ORF and, for F, have lengths that agree with the positions of inverted repeats. (The R100 traJ sequence is unknown.) Cells lacking Integration Host Factor (himA and hip) and cells containing finO have increased amounts of the 4 short transcripts and decreased amounts of the others. When finO is present in either a himA or hip mutant but not otherwise, a large amount of the transcripts are 295 bases long. This size is normally just detectable in these mutants without finO. Based upon these data, control of traJ appears to be at least partly translational.

L 107 CONTROL OF GENE EXPRESSION IN CHLAMYDIA Joanne Engel, Lisa Sardinia, and Don Ganem, UCSF, San Francisco, CA 94143.

Chlamydia are medically important pathogens which cause venereal disease, infertility, blindness, and pneumonia. Along with the rickettsiae, these gram negative bacteria are the only known obligate intracellular parasites which infect human cells. To understand the control of gene expression in this pathogen, we have begun a study of the structure and organization of constitutively and developmentally expressed genes. rDNA genes were chosen as examples of constitutively expressed genes. A partial Eco RI library of chlamydial DNA (Mouse Pneumonitis strain) was cloned in λgt WES and screened with a rDNA probe made by reverse transcribing random primed total chlamydial RNA. On a genomic Southern blot of chlamydial DNA cleaved with Eco RI, 4 different sized bands of hybridization are observed. Recombinant phage containing each of these 4 Eco RI fragments were isolated when the library was screened with this probe. Each of these Eco RI fragments was shown to hybridize to rRNA on a Northern blot. Further restriction enzyme analysis and Southern and Northern blot analysis of subfragments indicate that chlamydia contain two rDNA cistrons, not closely linked in the genome, with a gene order 16 S- 23 S. The 5' end of the 16 S rDNA of one of the cistrons has been mapped by S1 nuclease and primer extension studies. DNA sequence analysis demonstrates that while the 16 rDNA gene is homologous to those of *E. Coli*, the upstream regions bear no resemblance to those in *E. Coli*. We are currently studying the promoter sequences. To study developmetnally regulated genes, we have chosen to isolate the genes encoding the major outer membrane proteins, which are known to be expressed late in the chlamydial life cycle. A library of chlamydial DNA was screened by immunoblotting with a polyclonal serum made to EB's. This serum was shown to recognize primarily the outer membrane proteins. Several clones encoding putative outer membrane porteins have been identified and are being further studied.

L 108 A CONSTITUTIVELY EXPRESSED HEAT SHOCK PROTEIN MAY FORM THE PROTEIN CORE OF A MAJOR SURFACE ANTIGEN IN *TRYPANOSOMA CRUZI*

David M. Engman[1], Louis V. Kirchhoff[2], Kim H. Brown[1], and John E. Donelson[1], Departments of [1]Biochemistry and [2]Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52242

Gp72 is a 72,000 M_r surface glycoprotein in *Trypanosoma cruzi*, the etiologic agent of Chagas' disease. Because the in vitro transformation of non-infective to infective stage parasites can be inhibited by a monoclonal antibody specific for gp72, this molecule is thought to be involved in regulating parasite differentiation. Also, gp72 is one of the two immunodominant surface antigens of *T. cruzi*, and all infected patients have high titers of antibodies against gp72. A *T. cruzi* λgt11 expression library was screened with polyclonal anti-gp72 rabbit serum, and several recombinant phage were isolated. Further analysis of these cDNA's revealed that they encode a new member of the heat shock protein family. The gene for this protein is distinct from that encoding the major stress protein (hsp70), although they are similar in size and sequence, and are tandemly repeated on the same size chromosomes in *T. cruzi*. This gene is expressed under both heat shock and non-heat shock conditions in African trypanosomes and *Leishmania*, suggesting that gp72 may function in regulating differentiation of hemoflagellate parasites in general.

L 109 TRANSCRIPTIONAL CONTROL SEQUENCES OF THE CHELATIN GENE IN YEAST Tina Etcheverry and Wayne Forrester, Genentech, Inc. South San Francisco, CA. 94080

The promoter and transcriptional regulatory sequences from the chelatin gene have been used to express heterologous proteins in yeast (Biotechnology *4*, 726, 1986). The promoter is regulated by copper ions in the media. A region necessary for copper regulation has been identified by studying the effects of varying promoter lengths. BAL31 digestion from outside the promoter sequence generated truncated promoters exhibiting constitutive expression; no longer requiring copper induction for expression. Linker- scanning mutations were introduced throughout the 300 base pairs preceding the transcription start site. In this region there are six repeated elements containing the consensus sequence TCTTTTTGCT. The orientation and spacing of these elements is similar to other upstream activator sequences (UAS). Deletions of some, but not all of these repeated elements interfere with gene expression suggesting that the sequences are required for positive regulation of the gene. The close proximity of the sequences to the trancription start site make them possible candidates for binding sites of copper-mediated regulatory proteins.

L 110 **REGULATION OF THE Escherichia coli dnaZX LOCUS: A SINGLE OPEN READING FRAME ENCODES TWO PROTEIN PRODUCTS** Ann M. Flower and Charles S. McHenry, Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, CO 80262

The *E. coli dna*ZX gene provides a unique opportunity to study genetic regulation as this region encodes two protein products from one open reading frame. *dna*Z and *dna*X were originally identified as two separate genes based on complementation analysis (Henson *et al.* (1979) *Genetics* **92**: 1041). The region of DNA necessary to complement mutations in both genes was defined as a 2.2kb fragment that produces two protein products with molecular weights of approximately 77,000 and 52,000 in the "maxicell" system (Kodaira *et al.* (1983) *Mol.Gen.Gen.* **192**:80 and Mullin *et al.* (1983) *Mol.Gen.Gen.* **192**:73). The 52,000 dalton protein corresponds to γ and the 77,000 to τ. We have determined the DNA sequence of *dna*ZX and found it to be one long open reading frame of sufficient length to encode a 71,147 dalton protein. This raises the question of how both of these proteins are produced from this region. We have identified two potential transcriptional promoters preceding the *dna*ZX gene that are interspersed with sequences with homology to the *dna*A binding site. There is also an additional possible translational initiation site that could encode a protein of 68,451 daltons in the same reading frame. We have mapped the ends of the mRNA transcripts and have constructed both transcriptional and translational fusions between *dna*ZX and *lac*Z to monitor the expression of *dna*ZX regulatory sequences and to examine the possibility of posttranslational processing of the large gene product to produce the smaller.

L 111 **BROAD SPECTRUM GENETIC REGULATORY ELEMENTS IN GRAM-NEGATIVE BACTERIA,** **Joachim Frey, Rémy Fellay and H.M.Krisch** Departments of Biochemistry and Molecular Biology, University of Geneva, Geneva 4 Switzerland.

Our objective has been to develop techniques for the genetic analysis, manipulation, and expression of cloned genes in Gram-negative soil and water bacteria. As part of this effort we have constructed a series of derivatives of the Ω interposon (Prentki and Krisch, Gene *29*:303). Each of these DNA fragments carries a different antibiotic resistance gene (Ap^r, Cm^r, Tc^r, Km^r or Hg^r) which is flanked, in inverted orientation, by transcription and translation termination signals and by synthetic polylinkers. The DNA of these interposons can be easily purified and then inserted, by *in vitro* ligation, into a linearized plasmid. Plasmid molecules which contain an interposon insertion can be identified by their drug resistance. The position of the interposon can be precisely mapped by the restriction sites in the flanking polylinker. The properties of these Ω derivatives were verified by *in vitro* mutagenesis of a broad host range plasmid which contains the entire *meta*-cleavage pathway of the toluene degradation plasmid pWW0 of *Pseudomonas putida*. We show that when a plasmid containing a Ω interposon is transferred by conjugal mobilization from *E.coli* to other Gram negative species the appropriate interposon drug resistance is usually expressed and the interposon acts as an extreme polar mutation. Thus, the selection and/or characterization of Ω insertional mutations can be carried out in these bacterial species. In a related project, we have developed an expression vector called p-αΩ, for use in these same organisms. The expression casset of this vector comes from phage T_4D gene *32* and promotes highly efficient expression in all Gram-negative species tested.

L 112 THE PROCESSING OF SYNTHETIC $tRNA^{His}$ PRECURSORS BY THE CATALYTIC RNA COMPONENT OF RNase P, Christopher J. Green and Barbara S. Vold, SRI International, Menlo Park, CA 94025.

Several variants of Bacillus subtilis $tRNA^{His}$ genes were chemically synthesized and cloned into an SP6 RNA polymerase promoter plasmid. These plasmids were used as templates for the in vitro transcription of $tRNA^{His}$ precursors. The precursors were then used as substrates for processing by the catalytic RNA component of RNase P from B. subtilis and Escherichia coli. Our results show that the $tRNA^{His}$ precursor patterned after the natural B. subtilis gene is cleaved by both catalytic RNAs to leave an extra G residue at the 5'-end of the aminoacyl stem. This extra base is found in all $tRNA^{His}$ species so far sequenced and usually results in an eight base pair aminoacyl stem, compared with the typical seven base pair stem in most other tRNAs. Changing the 3'-proximal CCA sequence to increase the length of the aminoacyl stem did not alter the cleavage site. However, when the G residue at the expected 5'-mature end was replaced by an A residue in one of the synthetic precursors, the processing site was changed, resulting in the cleavage occurring after this base.

These experiments indicate that the extra G residue in prokaryotic $tRNA^{His}$ is left on by RNase P processing because of the precursor's structure at the aminoacyl stem and that the cleavage site can be altered by a single base change.

L 113 **TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL CONTROL OF CHLOROPLAST GENE EXPRESSION,** Wilhelm Gruissem, Xing-Wang Deng, Helen Jones, David Stern and John Tonkyn, Department of Botany, University of California, Berkeley, California 94720

Expression of chloroplast genes in higher plants is regulated at different levels. At the transcriptional level, we have studied the promoter regions for several tRNA and protein genes. The trnM2, rbcL, atpB and psbA genes share DNA sequences in their promoter regions that have homology to the prokaryotic consensus promoter. The mutational analysis, however, has revealed striking differences in promoter requirements of the chloroplast RNA polymerase. The basic transcription pattern of the four chloroplast genes is established by the different strengths of their promoter regions. Mobility shift experiments with the psbA promoter indicate, however that multiple components may bind to this regions during transcription initiation. In addition to the genes with a typical -10/-35 promoter arrangement, we have identified a class of chloroplast tRNA genes (trnR1, trnS1) that do not contain 5' upstream promoter elements. Their transcription pattern in vitro resembles the transcription of eukaryotic nuclear tRNA genes.

We have used a plastid run-on transcription system to determine the regulation of chloroplast gene expression at the transcriptional and post-transcriptional level. First, transcription activity changes during light-induced leaf development and maturation are independent of plastid DNA copy number. Second, the relative transcription of genes is maintained at all developmental stages. Third, significant changes in mRNA accumulation are regulated at the post-transcriptional level. We will present an overview of our current work.

Supported the National Institute of Health and the Department of Energy.

L 114 LOCALIZATION AND IDENTIFICATION OF LATE PROMOTERS OF BACTERIOPHAGE MU. Martha M. Howe, Steven F. Stoddard, and William Margolin, University of Tennessee, Memphis, TN 38163.

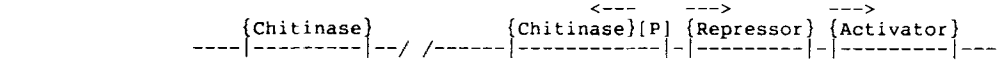
The approximate locations of transcription initiation sites on the Mu genome were determined by hybridization of "capped" 5' end-labeled mRNAs to Mu DNA restriction fragments. RNAs isolated at various times after heat induction of a Mu cts62 prophage were labeled using vaccinia virus guanylyl transferase, which adds (^{32}P)GMP onto the 5' di- or tri-phosphate ends of RNAs. Hybridization of shortened "capped" RNAs to Mu DNA restriction fragments on Southern blots revealed six major transcription initiation sites, two active at both early and late times and four active only at late times and only in the presence of Mu DNA replication and the C gene product. S1 nuclease mapping was used to localize more precisely the 5' ends of three of these late transcripts (the fourth, the mom transcript, has already been characterized). Examination of the DNA sequences upstream from the 5' ends revealed little or no homology to the Escherichia coli promoter consensus sequence (for RNA polymerase with sigma 70), but demonstrated sequence homologies among the Mu late promoter regions. Plasmids containing these promoter regions inserted upstream of lacZ were found to be transactivated for B-galactosidase synthesis in the presence of a plasmid expressing the Mu C gene product.

L 115 COORDINATED TRANSCRIPTIONAL REGULATION OF THE SYNTHESIS AND NOVEL SECRETION OF ENTEROBACTERIAL HAEMOLYSINS. Colin Hughes and Vassilis Koronakis, Department of Pathology, Cambridge University, England. The production of cytotoxic haemolysins by pathogenic Enterobacteria is encoded by 4 contiguous hly genes; hlyC and hlyA determine synthesis of active intracellular toxin, hlyB and hlyD its specific secretion across both bacterial membranes without the aid of a conventional N-terminal signal sequence. Cloning of the hly determinants from 4 different enterobacterial species has revealed conservation of the core transcriptional unit hlyC, hlyA, hlyB and although upstream sequences are quite divergent sequence and S1 analysis indicate retention of the main promotor and transcription start sites. Mutations upstream of this promotor increase the level of both haemolytic activity and specific transcription, and these map in a region of direct and inverted repeat sequences which we regard as a operator site. A second class of mutations suggest that this operator may be the target of more than one factor and we are analysing regulatory and specific DNA-binding activity of putative effectors. A further level of transcriptional control is indicated as complete transcription of the operon to express the primary secretion gene, hlyB (the sequence of which determines a highly conserved ATP-binding domain) appears to be inhibited by an intergenic rho-independent terminator. Specific modulation of this transcription termination prior to the hlyB gene may explain the observed responsiveness of specific secretory activity to the level of intracellular haemolysin accumulation.

L 116 EXPRESSION AND REGULATION OF CLONED CHITINASE GENES FROM SERRATIA LIQUEFACIENS, Sadhna JOSHI and Maya KOZLOWSKI, Allelix Inc., 6850 Goreway Drive, Mississauga Ont., L4V 1P1 Canada

S. liquefaciens chitinase gene(s) were cloned by expression in E. coli. Transposon mutagenesis was performed to tag and clone regulatory gene(s) controlling all chitinase expression in the cell.

Two different chitinase genes were cloned (pSJ12 and pSJ21) and expressed in E. coli. The expression and regulation of cloned gene(s) was studied by Tn5 mutagenesis, subcloning and/or complementation experiments. Chitinase expression from pSJ12 is regulated both positively and negatively. In S. liquefaciens in the absence of Activator, a Repressor is synthesized which turns off all chitinase genes. In the presence of Activator and Inducer(s) no Repressor is made or is inactivated and the chitinase(s) are synthesized by the cell. The chitinase expression from pSJ21 was constitutive in E. coli. In S. liquefaciens this gene is also regulated by the elements present on pSJ12.



L 117 SECRETION OF MURINE AND HUMAN INTERLEUKIN-2 BY E. COLI, Rob A. Kastelein and Anita Van Kimmenade, DNAX Research Institute of Molecular and Cellular Biology, 901 California Ave., Palo Alto, CA 94304
We have fused the mature coding sequences of mouse and human IL2 to the ompA bacterial signal peptide in an attempt to direct the expression of these lymphokines to the periplasm of E. coli. Although both proteins are highly homologous, only mIL-2 is efficiently translocated across the cytoplasmic membrane with concomitant cleavage of the signal peptide. The periplasm is highly enriched for mIL-2, from where it conveniently can be recovered by an osmotic shock. On the other hand, hIL-2 is not detected in the periplasm and is present mainly in the uncleaved form. In an attempt to understand this difference in localization and processing we have initially focused on the N-terminal regions of the proteins. The effect of substitutions in this region on translocation and processing will be discussed.

L 118 NOVEL PURIFICATION OF PROTEINS INVOLVED IN TRANSCRIPTION OF RIBOSOMAL DNA IN THE EUKARYOTE ACANTHAMOEBA CASTELLANII, William M. Kubaska, Laura Hoffman, Calvin Iida, Deborah Knoll and Marvin R. Paule, Colorado State University, Fort Collins, CO 80526.

In vitro studies of rRNA transcription initiation in eukaryotes have shown that expression of this gene is regulated by a polymerase modification [Nuc. Acid Res. 12, 8161-8180 (1984); Cell 47, 445-450 (1986); Tower et al Mol. Cell Biol. 6, 3451-3462 (1986)]. A modified extraction procedure has resulted in the purification of both RNA polymerase I (RNAP I) and a transcription initiation factor (TIF) from the same batch of either rapidly growing vegetative or starved cells. A 0.35 M KCl extraction of crude cell homogenate is followed by polyethyleneimine precipitation. Chromatography on phosphocellulose (PC) completely resolves RNAP I from TIF and RNA polymerase III. RNAP I is purified to homogeneity by DEAE-52, heparin agarose, DEAE-Sephadex chromatography followed by sedimentation on a 5-20% sucrose density gradient. Milligram quantities of RNAP I are obtained. Comparisons of subunit structure of RNAP I from vegetative and starved cells were performed in order to identify the site of the modification involved in regulating rRNA transcription. TIF fractions from PC chromatography are extensively purified by heparin agarose, malachite green agarose and Biorex 70 chromatography followed by sedimentation on a 15-45% glycerol gradient. Binding to promoter-containing DNA has also been utilized to identify the active protein component of the highly purified TIF preparation.

L 119 ANALYSIS OF THE MECHANISM OF mRNA TURNOVER IN ESCHERICHIA COLI K-12. Sidney R. Kushner, Cecilia M. Arraiano, and Stephanie D. Yancey. Department of Genetics, University of Georgia, Athens, Georgia 30602

mRNA turnover in E. coli is thought to occur through a sequence of endo- and exonucleolytic steps. Recent work has demonstrated that two of the enzymes involved in the degradation of mRNAs are polynucleotide phosphorylase (pnp) and ribonuclease II (rnb). In addition, in the absence of normal mRNA turnover cells become inviable when grown in rich medium. Through the construction of a series of multiple mutants containing pnp, rnb and ams (altered message stability) mutations, it has been possible to study general mRNA turnover as well as the degradation of specific mRNAs. Of most interest was the pnp-7 ams-1 rnb-500 triple mutant grown in rich medium in which the half-life ot total pulse-labelled RNA was 15.8 min at the nonpermissive temperature. In comparison, the half-lives in the control strains were: wild type, 3.4 min; ams-1, 7.9 min; and pnp-7 rnb-500, 5.3 min. Cell growth and net RNA synthesis stopped simultaneously at the nonpermissive temperature. RNA-DNA hybridization analysis of several specific mRNAs such as trxA (thioredoxin), ssb (single-stranded DNA binding protein) and uvrD (DNA helicase II) indicate a 2-4 fold increase in chemical half-life. Our results suggest that the ams, pnp and rnb gene products are required for normal mRNA turnover but are not involved in the initial steps of degrdation. (This work was supported by grant GM28760 to S.R.K. and PF-2749 to S.D.Y.).

L 120 DETERMINATION OF THE TRANSCRIPTIONAL TERMINATION SITE OF THE LAC OPERON OF ESCHERICHIA COLI,
Catherine Kwan, Matthias A. Hediger and Donald P. Nierlich, UCLA and Mt. St. Mary's College, Los Angeles, Ca.

We have identified the transcriptional termination sites destal to the lac A gene by S1 mapping and in vitro transcription. A strong rho dependent terminator was found 110 nucleotides distal to the translational stop codon and several weak terminator were found in its vicinity.

S1 mapping was done to map the 3' end of the lac messenger RNA. A plasmid carrying the distal end of thelac A gene was used as probe. The experiment indicates that there are several ends points with various degrees of strength. In vitro transcription experiments were carried out using a fragment of a plasmid with the lac promoter joined to the distal end of the lac A gene as template. Purified RNA polymerase, cAMP binding protein and rho protein were added to the transcription system. The primary RNA's synthesized without rho are RNA s of 185 and 240 nucleotides, with the addition of rho, a RNA of 170 nucleotides was found. The result agrees with the S 1 mapping experiments indicating a rho dependent terminator at a site 110 nuclsotide distal to the translation stop site.

L 121 **TRANSSPLICING IN TRYPANOSOMES**, Peter W. Laird, Joost C.B.M. Zomerdijk and Piet Borst, Netherlands Cancer Institute H-8, Amsterdam, The Netherlands.

Messenger RNA synthesis in trypanosomes differs from that of other eukaryotes in that protein-coding transcription units can be multi-cistronic, while the processing of individual mRNAs from the primary transcript entails the acquisition of a 5' RNA segment from a different transcription unit. This RNA segment, common to all mature mRNAs is 35 nt, carries a 5' cap and is encoded by separate mini-exons found in about 200 copies in the trypanosome genome. The mini-exon repeats yield a primary transcript of 140 nt called the mini-exon-derived RNA (medRNA). The mini-exon sequence at the 5' end of the medRNA is somehow transferred to pre-mRNAs. We have studied the nature of this last step by pulse-chase labelling of RNA in vivo. Analysis of specific RNA species by hybridization selection indicates a half-life for the 140 nt medRNA of less than 10 min. in pulse-chase and approach to equilibrium experiments. The average half-life of high MW intermediates containing the 3' 105 nucleotide segment of medRNA is estimated at less than 1 minute, which rules out a discontinuous mRNA synthesis mechanism involving priming of transcription of very long pre-mRNAs by full-length medRNA. The structure of high MW RNA molecules containing the 105 nt segment was analyzed by treating steady-state high MW RNA free of medRNA with debranching enzyme followed by Northern electroblot analysis. Upon debranching, the majority of the signal in high MW poly(A)+ RNA detected with a 3' medRNA probe is converted to a 105 nt and a 90 nt band. These results are consistent with a transsplicing mechanism involving very short-lived intermediates containing the 105 nt segment as a branch in a forked molecule analogous to lariats in cissplicing systems.

L 122 BACTERIO-OPSIN GENE EXPRESSION. Diane Leong, Mary Betlach, and Herbert Boyer, Department of Biochemistry, University of San Francisco, San Francisco, California, 94143-0554.

Bacteriorhodopsin (BR) in the purple membrane of the archaebacterium Halobacterium halobium functions as a light-driven proton pump. Cellular levels of BR increase 4 to 5-fold on limitation of the oxygen concentration. We are investigating regulation of bop, the gene encoding bacterio-opsin which is the protein moiety of BR. Another gene (brp) affecting the synthesis of bacterio-opsin is located 526 bp upstream of the bop gene and is transcribed in the opposite orientation. In addition to experiments involving immunological detection of the brp protein, we have developed a procedure for quantitating bop and brp mRNA levels in H. halobium. This procedure is being applied to the analysis of a series of spontaneous Bop mutants and to wild-type cells grown under anaerobic vs. aerobic conditions.

We have also characterized three spontaneous Bop mutants which have alterations in a region further downstream of brp and upstream of bop. Analysis of wild-type halobacterial mRNA indicates that transcription occurs in this region and in the same direction as for bop. These observations suggest that this region contains yet another gene affecting bop expression. Nucleotide sequencing of the region is in progress.

L 123 TRANSCRIPTIONAL CONTROL OF CELLULASE SYNTHESIS IN THERMOMONOSPORA FUSCA, Ershen Lin and David B. Wilson, Cornell University, Ithaca, NY 14853

Our study shows that T. fusca produces at least five different extracellular cellulases and their synthesis is coordinately regulated both by induction and by repression. The inducers of cellulase synthesis are cellulose and its hydrolysis products. Repression can be relieved by limitation of carbon, nitrogen or phosphorus. Two mutants which are constitutive for cellulase synthesis were isolated after chemical mutagenesis and both strains are sensitive to repression. We used a cloned T. fusca cellulase gene as a probe to measure its mRNA level in T. fusca cells grown on a number of different carbon sources. The level of mRNA and enzyme activity varied in parallel under various growth conditions, suggesting that both the induction system and the repression system regulate transcription. The size of this mRNA is 1.5 kb while the corresponding cellulase has a molecular weight of 45 K dalton, therefore the mRNA is monocistronic. The boundaries of the transcription unit were determined by S1 mapping and the 5'-end of the mRNA is just downstream from a 60 bp region that has an A-T content of 57%, which is significantly higher than the average A-T content (30%) of T. fusca DNA. There is some homology between this region and the nitrogen regulated promoters present in Klebsiella, Rhizobium and E. coli. We have also found a protein in extracts of wild-type cells by band-shifting experiments. This protein specifically binds to the putative cellulase promoter and can only be detected in extracts of induced cells but not in extracts of uninduced cells.

L 124 MECHANISM OF LEXA REPRESSOR CLEAVAGE. J.W. Little, S.N. Slilaty, L. Lin and S. Hill. Department of Biochemistry, University of Arizona, Tucson AZ 85721.

LexA and λ cI repressors are inactivated by a specific cleavage in the middle of each protein. In vitro, cleavage requires RecA protein at pH 7 and proceeds spontaneously at higher pH. We have proposed that RecA stimulates autodigestion, rather than itself being a conventional protease; by this model, RecA is a positive effector but the actual active site lies in the repressors. The hinge and C-terminal domains of the two proteins are involved in cleavage, and share about 20% homology.

We have isolated non-cleavable (Ind$^-$) mutants of LexA protein, using both a classical screen and site-directed mutagenesis. The screen for Ind$^-$ mutants isolated mutants normal in repressor function but defective in RecA-mediated in vivo cleavage. It identified ten different residues important in cleavage, lying in three different blocks in the primary sequence: around the Ala84-Gly85 cleavage site, and in the largest blocks of conserved residues, around Ser119 and Lys156. All these mutant proteins are defective in both autodigestion and RecA-mediated cleavage in vitro, supporting our idea that the two reactions have a close mechanistic relationship. The choice of residues for site-directed mutagenesis was guided by pH data and analogy with other proteases; Lys-156 and Ser-119 were changed to an unreactive residue, Ala, resulting again in proteins with normal repressor function but no cleavage activity. We postulate, first, that the regions around Ser119 and Lys156 make up a protease active site that interacts specifically with the cleavage site and cuts it; and second, that Ser-119 is a nucleophile attacking the peptide bond and an unprotonated Lys-156 activates Ser-119.

L 125 TRANSLATIONAL FEEDBACK REGULATION OF THE SPC OPERON IN E. COLI. L. Matthews, M. Nomura, Department of Biological Chemistry, University of California, Irvine, CA 92717.

The spc operon of E. coli encodes 10 ribosomal protein genes in the order: L14, L24, L5, S14, S8, L6, L18, S5, L30, L15, followed by secY, a secretion protein. Protein S8 is a translational repressor that interacts at a single mRNA target site near the translation start of L5, the third gene in the operon, to inhibit L5 translation. As a consequence, the translation of the distal genes, S14 through L15, are also inhibited.

We have been studying three aspects of this regulation. First, we have shown that the mechanism of regulation of the distal genes, S14 through L15, is by translational coupling. Using plasmids that carry the entire spc operon, we have shown that blocking L5 translation results in a 20-fold decrease in the protein synthesis rates of distal genes S14 through L15 but only a 40% decrease in distal mRNA synthesis rates.

Second, we have analyzed the mRNA secondary structure of the S8 target site at L5 using deletion and mutational analysis and have examined structural homologies to the known S8 binding site on 16S rRNA.

Third, we have also studied the regulation of the promoter-proximal genes L14 and L24. We have found that S8 also inhibits the expression of L14 and L24 in vivo, probably by acting at the downstream target site near L5. We are currently studying the mechanism of this apparent retroregulation.

L 126 MUTAGENESIS BY OLIGONUCLEOTIDE-DIRECTED DOUBLE-STRAND BREAK REPAIR IN PLASMIDS OF ESCHERICHIA COLI: A STUDY OF THE MECHANISM. J. O. McCall and W. Mandecki, Corporate Molecular Biology, Abbott Laboratories, Abbott Park, IL 60064

Synthetic oligonucleotides can be used to direct the repair of double-strand breaks introduced into plasmid DNA by restriction enzyme cleavage. This new technique, termed "bridge-mutagenesis", allows the efficient generation of predefined mutations within plasmid DNA (W. Mandecki, Proc. Natl. Acad. Sci. USA 83:7177 (1986)). The method does not require enzymatic manipulations in vitro, and up to 98% of the transformants obtained are found to contain the predefined mutation.

The present study addresses the biochemical mechanisms involved in bridge-mutagenesis. Strains known to be defective in various aspects of DNA replication, recombination, transcription, and repair were tested for their efficiency and fidelity in this repair process. In addition, a determination was made as to which plasmid strand (with polarity identical to or opposite that of the oligonucleotide) is necessary for mutagenesis. By performing the mutagenesis on phage M13 single-stranded DNA it was found that the oligonucleotide must be complementary to the DNA strand that participates in mutagenesis. Also, the plasmid sequence surrounding the double-strand break was found to have an effect on the efficiency of repair and mutagenesis, as does the condition of the ends of the linear plasmid molecule (blunt or staggered ends, phosphorylation). These results favor a gap repair model for bridge-mutagenesis.

L 127 SENSE AND ANTISENSE TRANSCRIPTION OF T4 GENE 32, Elisabeth A. Mudd, Dominique Belin, Pierre Prentki, Yu Yi-Yi and Henry M. Krisch, Department of Molecular Biology, University of Geneva, Geneva, Switzerland.

Bacteriophage T4 gene 32, whose gene product is essential for phage DNA replication, recombination and repair, is transcribed as several mRNAs. During a wild-type phage infection, gene 32 is initially transcribed from early polycistronic and monocistronic promoters, while later a monocistronic mRNA predominates. The early mRNA is post-transcriptionally processed. Both the processed product of the early mRNA and the late transcript can be stable; their eventual degradation is temporally regulated by the phage.

Transcription of gene 32 from plasmids in uninfected bacteria initiates primarily at a strong promoter which is not recognised after infection. The transcription termination region of gene 32 can also function in plasmids as an antisense promoter, both *in vitro* and *in vivo*. We are currently investigating the role of this antisense promoter in the regulation of gene 32 expression.

L 128 MAPPING NUCLEOTIDE CONTACTS BETWEEN THE *lacZ* MESSENGER RNA AND THE 70S RIBOSOME WITH RNA ETCHING, George J. Murakawa and Donald P. Nierlich. University of California, Los Angeles, CA 90024.

With the use of low molecular weight reagents and a footprinting procedure, RNA etching, we have characterized the binding site of the 70S ribosome to the *E. coli lacZ* mRNA ribosome binding site. Purified 70S ribosomes were bound to *lacZ* messenger and the complex was treated with limiting amounts of ribonuclease or a RNA-modifying reagent. Ribosomes were removed by phenol extraction and the reaction sites were mapped by primer extension with reverse transcriptase. By employing RNase A, T1, and Phy M, we have defined the general ribosome-protected region as about 34 nucleotides. Additionally, small chemical probes, both DMS and diethyl pyrocarbonate, were used to determine specific nucleotide contacts between the ribosome and messenger RNA. Using these reagents, protection was seen in the Shine and Dalgarno region, the fMet initiation codon, and in a region 10-13 nucleotides distal to the initiation codon. Not only are protected nucleotides seen, but also, specific enhancements within the ribosome binding site occur. The data suggest that the *lacZ* mRNA is held by the ribosome at specific nucleotide contacts within the aforementioned regions, and, that nucleotides not associated with these regions are highly accessible to chemical modification.

L 129 STABILIZATION OF mRNA BY PROKARYOTIC "REP" SEQUENCES AND THEIR EFFECT ON GENE EXPRESSION. Sarah F. Newbury and Christopher F. Higgins, Department of Biochemistry, University of Dundee, Dundee, Scotland. DD1 4HN.

REP (Repetitive Extragenic Palindromic) sequences are small, highly conserved inverted repeats found in the genomes of *Escherichia coli* and *Salmonella typhimurium*. At least 500 REP sequences are present on the *E. coli* chromosome; all in transcribed intercistronic regions or at the end of transcription units.

We have used Northern blotting to show that REP sequences cause upstream mRNA to accumulate. Experiments indicate that REP sequences protect mRNA from 3′-5′ exonucleases. A number of upstream RNA′s can be stabilised artifcially by cloning REP sequences within their transcription units. The protected upstream mRNA is translatable, as shown by pulse-labelling after rifampicin treatment. Therefore, the stabilization of mRNA by REP sequences can increase the amount of protein products from upstream transcripts. Experiments showing the mechanisms by which REP sequences affect mRNA stability and gene expression will be presented.

L 130 TRANSCRIPTION OF HEAT SHOCK GENES ON MULTICOPY PLASMIDS IN YEAST, David S. Pederson and Robert T. Simpson, Laboratory of Cellular and Developmental Biology, National Institutes of Health, Bethesda, MD 20892.

Saccharomyces cerevisiae carrying the HSP26 gene on a multicopy plasmid [TRP1ARS1/HSP26] and grown at 23°C contain about as much HSP26 mRNA as do cells carrying just one [chromosomal] copy of the gene. During heat shock, cells carrying the TRP1ARS1/HSP26 plasmid accumulate more HSP26 message and degrade it faster than do cells carrying only the single endogenous gene. Taking into account this reduced half-life, we calculate that, during heat shock, cells containing TRP1ARS1/HSP26 plasmid synthesize HSP26 message at least 45 fold faster than do cells containing a single heat shock gene. These observations suggest that most of the episomal HSP26 genes are regulated and transcribed normally and that neither repression nor activation of transcription of HSP26 in normal cells is limited by the availability of regulatory factors. Thus, we feel that multicopy plasmids carrying the HSP26 gene can be purified, as chromatin, in a transcriptionally active form, by methods we have recently described [D.S. Pederson, et al. 1986. Proc. Natl. Acad. Sci., U.S.A. 83, 7206-10.]. Such purified material should permit isolation of HSP26 gene transcription factors in biochemical quantities, and characterization of those factors in the context of native chromatin.

L 131 TRANSCRIPTIONAL COUPLING OF ENTEROBACTIN BIOSYNTHETIC AND TRANSPORT GENES IN ESCHERICHIA COLI, Gregg S. Pettis and Mark A. McIntosh, University of Missouri, Columbia, MO 65212.

The expression of the native E. coli siderophore enterobactin and its transport components, including its outer membrane receptor FepA and the Fes product, which is responsible for intracellular release of iron from ferric enterobactin, is regulated at the transcriptional level by an unknown mechanism. We have placed a small fragment containing the extreme 5' region of the biosynthetic locus entF ahead of a cloned structural lacZ gene. This construct (pITS301) expresses low constitutive amounts of a hybrid EntF-LacZ protein. A similar clone (pITS312) consists of the same entF-lacZ fusion but also contains a much larger upstream region, including the entire fes gene and part of the fepA gene, which is divergently transcribed relative to entF. This construct yields high, yet iron inducible, amounts of the hybrid protein. Transposon insertions both in and 3' to the fes gene in pITS312 reduce production of the hybrid protein to the low constitutive amount seen for pITS301. We suggest that an iron-responsive operator located between the fepA and fes genes bi-directionally controls the inducible expression of both fepA and a polycistronic transcript that includes the fes and entF genes, thus providing a single controlling element that coordinates the expression of siderophore biosynthesis and transport genes.

L 132 IDENTIFICATION OF THE *SALMONELLA TYPHIMURIUM metR* GENE PRODUCT AND BIOCHEMICAL CHARACTERIZATION OF THE *metR-metE* CONTROL REGION, Lynda S. Plamann, Mark L. Urbanowski, and George V. Stauffer, Department of Microbiology, University of Iowa, Iowa City, IA 52242

We have identified a new methionine regulatory gene, designated *metR*, whose product acts in trans to activate the *Salmonella typhimurium* and *Escherichia coli metE* and *metH* genes. A P1 transduction analysis indicated that the *metR* gene is closely linked to the *metE* gene. Plasmid pGS47, which carries the *S. typhimurium metE* gene and flanking regions, was found to complement a *metR* mutation. The *metR* gene was subcloned from pGS47 into the plasmid vector pBR322 on a 2 kb *Sal*I-*Hind*III fragment. This new plasmid was designated pGS191. *Xho*I linker insertion mutations that inactivate the *metR* gene in pGS191 were isolated and mapped by restriction enzyme analysis. Plasmid pGS191 and the mutagenized derivatives were used as templates to direct protein synthesis in a minicell system. SDS polyacrylamide gel electrophoresis of plasmid-encoded polypeptides showed that the *metR* gene encodes an approximately 34,000 *M*r polypeptide. The transcription start sites for the *metR* and *metE* genes were determined by Mung Bean nuclease mapping. The *metR* and *metE* genes are divergently transcribed, with only 25 bp separating the transcription start sites. The overlapping nature of the two promoters suggests that there may be common regulatory signals for the *metR* and *metE* genes.

L 133 A STAGE-SPECIFIC NUCLEAR FACTOR BINDS TO THE UNIVERSAL 5′ LEADER SEQUENCE DNA OF TRYPANOSOMA BRUCEI. Alistair M. Ross, Vassilis Koronakis, Richard W.F. Le Page, and Colin Hughes.

The mRNAs of the African pathogenic trypanosomes and other trypanosomatids include a short 5′ leader sequence which is discontinuously transcribed from the structural gene. The 140nt primary transcript is derived from a repetitive DNA sequence. To study regulation of the synthesis of leader sequence RNA we have cloned an entire 1.35kb repeat from T.brucei and have used restriction fragments in gel-retardation assays in order to identify sequences which may be involved in binding proteins capable of controlling transcription. Incubation of an end-labelled probe derived from the cloned repeat sequence with nuclear extracts from procyclic organisms resulted in the formation of a protein/DNA complex. When incubated with the same probe trypomastigote nuclear extracts formed two protein/DNA complexes, one of which co-migrated with the procyclic complex. A 10-fold excess of un-labelled fragment dissociated both complexes. DNAase footprinting and methylation studies have enabled us to locate the sites of protein binding. Since the unique trypomastigote factor enhances the binding of the common factor to the probe we are investigating the possibility that this protein is a differentially expressed regulator of spliced leader RNA synthesis.

L 134 MOLECULAR CLONING AND EXPRESSION OF GENETIC DETERMINANT(S) RESPONSIBLE FOR THE ENHANCED SIDEROPHORE ACTIVITY PRODUCED BY A STRAIN OF VIBRIO ANGUILLARUM, P.C. Salinas, M.E. Tolmasky and J.H. Crosa, The Oregon Health Sciences University, Portland, OR 97201.

The highly virulent fish pathogen Vibrio anguillarum 775 harbors the plasmid pJM1 which encodes a very efficient iron uptake system, consisting of the siderophore anguibactin and several transport components.
We have recently identified other virulent V. anguillarum strains with the ability to produce higher activity of the siderophore anguibactin as compared to the 775 prototype strain.
To characterize the nature of the siderophore enhancing activity (SEA) we cloned the iron-uptake region from pJHC-1, a pJM1-like plasmid isolated from one of these strains. A recombinant clone carrying the pJHC-1 iron-uptake region conferred the SEA phenotype to an iron-uptake deficient derivative of V. anguillarum 775. Transposition mutagenesis of the recombinant clone from pJHC-1 and complementation analysis allowed us to locate the *sea* locus, responsible for the SEA phenotype, within a 7.1 kb strech of the pJHC-1 plasmid DNA. Furthermore, a mutation in this pJHC-1 region resulted in a reversion of the SEA phenotype back to that of the prototype strain 775, suggesting that the *sea* region may play a role in the regulation of the production of siderophore activity.

L 135 MAPPING OF THE Bacillus subtilis spoIIA PROMOTERS Demetris Savva and Elizabeth Pontin, The University, Reading 2AJ, U.K.

Results obtained using "Northern" hybridisations to study the synthesis of the spoIIA mRNA in Bacillus subtilis indicated that the spoIIA operon may be subject to transcriptional control from two promoters which are separated by 1000 base pairs of DNA.

Nuclease S1 mapping is now being used to identify these two promoters. The promoter nearest the translational start site has been mapped at a position 51 base pairs upstream from the AUG initiation codon for the spoIIAA gene; transcription from this promoter starts at about 1 hour after the initiation of sporulation. The sequences at the "-10" and "-35" regions are 5' GGAATTCAT 3' and 5' AGTTTT 3' respectively; these are in good agreement with the sequences of those promoters which are recognised in vitro by the σ^{37}-containing RNA polymerase of B. subtilis.

L 136 TRANS-SPLICING OF THE 35-MER SEQUENCE TO THE 5′ END OF T. BRUCEI VSG GENE mRNA CAN OCCUR AT MULTIPLE SITES, J.K. Scholler, R. Aline Jr., and K. Stuart. Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, Wa. 98109.

In Trypanosoma brucei, the VSG genes are apparently transcribed as large precursors which include the 5′ co-transposed segment sequences. These precursors are processed to yield mature transcripts from the 5′ flank as well as the mature VSG mRNA. We have previously shown that for two IsTat VSG genes these transcripts contain the 35-mer sequence (also known as the spliced leader) and are polyadenylated. Thus, processing appears to include both the attachment of the 35-mer to the 5′ end and the addition of the poly A tail to the 3′ end of each transcript. We have sequenced cDNAs from the IsTat 1.1 VSG gene and its 5′ flank transcript. Comparison to the genomic DNA sequence indicates that there is an apparent 120 nucleotide 'gap' between the polyadenylation site of the 5′ flank transcript and the 35-mer attachment site of the VSG mRNA. An oligonucleotide corresponding to a sequence in this 'gap' region hybridizes with a series of transcripts between 1.7 and 2.0 kb. These are larger than the 1.7 and 1.8 kb mature flank transcript and VSG mRNA, respectively. In addition, this oligonucleotide also hybridizes with RNA which has been hybrid selected using sequences from the VSG coding region. These results suggest that the 1 VSG mRNA might have multiple 5′ ends, some of which contain the 'gap' sequences. To test this, we did primer extension sequencing of 1 VSG mRNA using the oligonucleotide as a primer. We discovered that there are two additional 35-mer attachment sites 79 and 98 nucleotides 5′ to the site used in the cDNA. All these sites are 3′ to the polyadenylation site of the 5′ flank transcript and are characterized by an AG 5′ to the splice site. This suggests that processing utilizes multiple 35-mer attachment sites at the 5′ end of the VSG mRNA.

L 137 CLONING AND EXPRESSION OF HUMAN ALPHA-1-ANTITRYPSIN IN E. COLI AND OPTIMIZATION OF ITS PRODUCTION IN FERMENTATION PROCESSES. Allan R. Shatzman, Edward J.Arcuri, Robert G. Gerber, W. Blair Okita, and Dane W. Zabriskie, Smith Kline & French Laboratories, 709 Swedeland Rd., Swedeland, PA 19479

The human alpha-1 antitrypsin gene, when expressed as a full length, non-fusion gene product in E. coli, accumulates to levels $\leq$ 0.1% of total cellular protein. Deletion of the 5' end of the gene or synthesis as a fusion protein increases expression at least 200 fold. Levels of message for the full length, deletion, and fusion genes were nearly identical and disruption of putative RNA secondary structure had only minor affects on expression levels. However, use of an E. coli galK translational fusion system (pASK) and mutagenesis of the 5' end of the A1-AT gene, has permitted selection of several point mutations which each increase AAT translation and expression individually up to 20 fold and in combination up to 200 fold. Cells induced in shake flasks or 15L fermentors at an OD_{650} of 0.4, showed product yield and volumetric productivity of 50 mg/g and 11 mg/L respectively. In shake flasks, both the product yield and volumetric productivity fell off dramatically with increasing OD, with no production detected at an OD of 2.2. In fermentation studies, if the dissolved oxygen concentration was zero, no A1-AT production was detected. If the dissolved oxygen concentration was controlled at 20% and the cells induced with 60 ug/mL of nalidixic acid (Nal)at an OD of 4.7, the product yield and the volumetric productivity were 5.5 mg/g and 18mg/L, respectively. Increasing major nutrient concentrations and Nal to 180 µg/ml boosted A1-AT product yield and volumetric productivity to 36 mg/g and 115 mg/L respectively.

L 138 STUDIES ON THE EXPRESSION OF HUMAN CONNECTIVE TISSUE-ACTIVATING PEPTIDE-III (CTAP-III) IN E. COLI. Indira Sohel, Christopher Green, Paul H. Johnson, Department of Molecular Biology, SRI International, Menlo Park, CA 94025

CTAP-III, an α-granule protein secreted by human platelets, is known to stimulate mitogenesis, extracellular matrix synthesis, and plasminogen activator synthesis in human fibroblast cultures. From its primary sequence, a synthetic gene was constructed, then cloned and expressed in E. coli using a new expression vector containing regulatory elements of the colicin E1 operon (Waleh and Johnson, 1985, PNAS 82, 8389).

CTAP-III was expressed at high levels in vivo and in vitro, as determined by SDS gel electrophoresis and immunodetection on western blots. Upon induction with low levels of mitomycin C or nalidixic acid to stimulate the SOS response, CTAP-III was produced as approximately 30% of the total cellular protein. The presence of L-[^{35}S] cysteine during induction results in preferential labeling of CTAP-III. This system is convenient for producing large quantities of labeled product and is a good alternative to the maxicell system.

Because radiolabeled CTAP-III of high specific activity is useful for cell receptor binding studies, efforts were made to increase the level of expression by modifying the regulatory region of the expression vector. Nucleotide sequence changes in the transcriptional promoter region dramatically increased the level of CTAP-III protein synthesis in vitro but not in vivo.

L 139 TRANSLATIONAL REPRESSION IN VITRO BY THE BATERIOPHAGE T4 REGA PROTEIN, E.K. SPICER AND H.A. ADARI, YALE UNIVERSITY, NEW HAVEN, CT 06510

The bacteriophage T4 regA protein has been shown by genetic analysis to control the expression of a number of T4 early genes at the level of translation. Examination of the sequence of six regA-regulated T4 genes fails to indicate a common conserved sequence which could serve as a target for regA protein recognition. In order to study the mechanism of regA protein recognition and binding to specific mRNA, we have purified regA protein from an overexpression plasmid. Previously, in simple in vitro protein synthesis reactions, regA protein was found to specifically inhibit the translation of T4 genes 44, 45 and ORF 45-1 in a concentration dependent manner. Synthesis of T4 gene 32 protein and ORF 45-1 protein in vitro, was unaffected by the presence of regA protein. In an effort to determine which nucleotides of the gene 44 mRNA serve as a recognition region for regA protein, we have constructed a plasmid in which the 5′ end of gene 44 (containing 37 upstream nucleotides) was fused in-phase with the 5′ end of the β-galactosidase gene present on phage M13 mp18. Expression of the 23 kd fusion protein of 44P- β-galactosidase was found to be regulatable in vitro by the regA protein. Removal of nucleotides from the leader region of the g44-gal mRNA (by Bal 31 digestion of the template plasmid) up to the ribosome binding site (at position -9) preceding the g44 AUG start codon did not eliminate regA protein repression in vitro. Thus the regA target must lie downstream of the -9 position. Experiments are in progress to further delineate the nucleotides required for regA regulation of gene 44 expression.

L 140 DEVELOPMENTALLY REGULATED ADDITION OF Us TO MITOCHONDRIAL TRANSCRIPTS OF TRYPANOSOMA BRUCEI, Kenneth Stuart, Jean E. Feagin, and Douglas P. Jasmer, Seattle Biomedical Research Institute, Seattle, WA 98109.

T. brucei regulates the production of the mitochondrial respiratory system and specific mitochondrial transcript abundance during its life (developmental) cycle. Also, two size classes of transcripts occur for all but one of the mitochondrial genes that encode proteins. For example, the smaller class of cytochrome b (CYb) transcripts is present in RNA from procyclic forms (PF) which have a complete respiratory system and from bloodstream forms (BF) which lack cytochromes and a Krebs cycle. The larger CYb transcript class is in very low abundance in BF RNA. Two CYb primer extension products were found with RNA from both life cycle stages using a primer from near the initiation codon. These appear to occur in the smaller size class of RNA. Two additional runoff products were found in PF RNA. Thus, multiple CYb transcripts vary at their 5' end. The most abundant CYb transcripts in PF RNA have a sequence that differs from the mitochondrial gene sequence by the presence of additional Us in the 5' non coding region. Sequences corresponding to that with additional Us were not found in genomic DNA. A less abundant RNA with a sequence like that in mitochondrial genome was discernable in both BF and PF RNA. The nucleotide sequence of CYb cDNA clones and Northern analysis using oligonucleotides complementary to the major and minor transcript sequences indicates the presence of three classes of CYb transcripts. One of these corresponds to the genomic sequence, a second has additional Us but the same apparent size as the former, while the third has additional Us and a larger apparent size. Thus, nucleotides not encoded in the gene can be added in a developmentally regulated fashion to mitochondrial RNA during or after transcription. This process may be involved in regulating the production of the mitochondrial respiratory system.

TRANSCRIPTIONAL ANALYSIS OF THE dnaE GENE OF Escherichia coli.

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L 141

ABSTRACT: DNA polymerase III holoenzyme is the multisubunit complex responsible for replicating most of the *E. coli* chromosome. Our lab is interested in the regulation of the synthesis of the replicative complex and the coordination of this process with the global control of cell growth and division. As a model, we have been studying the regulation of *dnaE*, the structural gene for α, the catalytic subunit of the holoenzyme. We have determined the nucleotide sequence of *dnaE* and have found it to encode a protein of 129,920 Daltons. A promoter for the *dnaE* gene was identified based upon sequence homology with known promoters. Deletion of the -35 region of this putative promoter from plasmids lacking all upstream sequences abolished expression of *dnaE*. There are two sequences proximal to *dnaE* that are homologous (8/9 nucleotides) to the *dnaA* box raising, the possibility that *dnaE* expression is regulated by cellular levels of the *dnaA* protein . Sequence analysis of this region indicates *dnaE* may be part of a complex operon with the following organization: 5'-*lpxB*-orf$_{23}$-*dnaE*-orf$_{37}$-3'. *Lpx*B encodes lipid A disaccharide synthase, an enzyme involved in the biosynthesis of the essential lipopolysaccharide component of the outer membrane (Crowell *et al.* J. Bact. (1986) **168**: 152-159). The first three genes appear to be translationally coupled. A bidirectional ρ-independent transcriptional termination signal is present at the 3'-terminus of orf$_{37}$. We have identified the termini of the *dnaE* transcripts.

L 142 THE COMMON 5' TERMINAL SEQUENCE OF TRYPANOSOME mRNAS: A TARGET FOR ANTI-MESSENGER OLIGONUCLEOTIDES,

J.J. Toulmé, P. Verspieren, A.W.C.A. Cornelissen#, N.T. Thuong*, P. Borst# and C. Hélène, INSERM U201, MNHN, F-75005 Paris, France, #Cancer Institute, 1066CX Amsterdam, The Netherlands, *CBM, CNRS, F-45071 Orléans cédex, France.

Trypanosomes are protozoan parasites which are entirely coated by a single variable surface glycoprotein that is periodically switched to evade the host's immune response. All trypanosome mRNAs have a common 5' terminal sequence of 35 nucleotides. We showed that synthetic oligonucleotides, complementary to part of this 35 nucleotide "mini-exon", inhibited *in vitro* translation of trypanosome mRNAs. This was very likely due to the formation of [oligonucleotide/mRNA] hybrids as the investigated "anti-messengers" had no effect on translation of non-target mRNAs. The inhibition increased with the length of the oligonucleotide *i.e.* with the hybrid stability.

We then synthesized a 9-mer, complementary to the mini-exon sequence, covalently linked to an acridine derivative (Acr-9-mer). This Acr-9mer specifically inhibited *in vitro* trypanosome protein synthesis to a larger extent than the homologous unmodified 9-mer. This was ascribed to the stabilization of the [anti-messenger/mRNA] complex by acridine intercalation in the duplex. Preliminary experiments showed that this Acr-9-mer entered cultured trypanosomes and reduced their viability. Neither the homologous 9-mer nor acridine-modified 9-mers not complementary to the mini-exon had any effect in the same conditions.

L 143 MECHANISM OF REGULATION OF E. COLI TOPA GENE EXPRESSION.

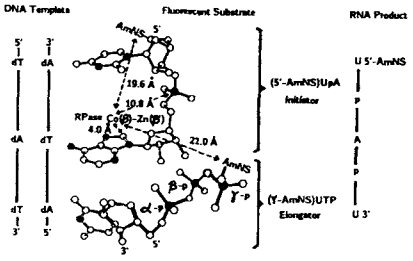
Y.-C. TSE-DINH, E. I. du Pont de Nemours & Co., Wilmington, Delaware 19898, Central Research & Development.

We have demonstrated previously, by fusing the transcriptional control region of the E. coli DNA topoisomerase I (topA) gene to the galactokinase (galK) gene coding region in a recombinant plasmid, that negative supercoiling of the DNA template is required for efficient transcription of the topA gene. Deletion studies on the plasmid show that more than one promoter present in the 5' upstream region can initiate transcription. Mapping of the in vivo m-RNA start sites by avian reverse transcriptase confirms the presence of multiple promoters. Examination of the DNA nucleotide sequence shows that the transcriptional start site closest to the translational starting methionine corresponds to a promoter of unusual sequence. It has a near-consensus -35 nucleotide sequence (TTGATA) but the -10 nucleotide sequence (CATATC) departs from the consensus E. coli promoter sequence at both the first and sixth positions, something not previously observed in other prokaryotic promoters that do not require positive regulators. Deletion of the other promoters further upstream reduces the level of transcription by two to three fold but negative supercoiling is still required for efficient transcription. However, upon deletion of the promoter closest to the starting methionine, transcription from the remaining promoters still remains dependent on negative supercoiling. The role of the multiple promoters is presently still under investigation.

L 144 PROXIMITY RELATIONSHIP BETWEEN THE INTRINSIC METAL ION OF *Escherichia coli* RNA POLYMERASE AND FLUORESCENT ANALOGS OF INITIATOR AND ELONGATOR OF RNA CHAIN.

Suresh Tyagi and Felicia Y.-H. Wu, Dept. Pharmacol. Sci., SUNY at Stony Brook, NY 11794

E. coli DNA-dependent RNA polymerase (RPase) contains 2 mol Zn/mol holoenzyme ($\alpha_2\beta\beta'\sigma$) with one Zn each in the β and β' subunits. A new method for selectively replacing Zn in the β subunit with Co^{2+},Cd^{2+} and Cu^{2+} was developed. Reconstituted Co(β)-Zn(β') RPase retained 91% enzyme activity and had absorption max. at 395 and 465 nm. Fluorescent analogs, (5'-AmNS)UpA and (γ-AmNS)UTP, with a fluorophore, 1-aminonaphthalene-5-sulfonic acid (AmNS), attached to 5' and γ-P, respectively, were synthesized. They can be incorporated into RNA with 30 and 73% efficiency as compared to UpA and UTP respectively. Their respective fluorescence spectra (max. 445 and 460 nm, λ_{ex} = 340 nm) overlap with the absorption spectrum of Co(β)-Zn(β') RPase, allowing us to study the spatial relationship between the metal (energy acceptor) and fluorescent substrate (energy donor) by fluorescence resonance energy transfer. The results obtained for the interaction of Co(β)-Zn(β') RPase with the (5'-AmNS)UpA (initiator)-UTP and UpA-(γ-AmNS)UTP (elongator) pairs in the absence and presence of poly(dA-dT), and those by our earlier NMR studies (JBC,259,284-289,1984) are shown in the Figure.



L 145 HORMONAL REGULATION OF GENE EXPRESSION IN YEAST, Scott W. Van Arsdell and Jeremy Thorner, University of California, Berkeley, CA 94720.

In the yeast Saccharomyces cerevisiae, haploid cells of opposite mating type (MATa and MATα cells) must exchange oligopeptide pheromones before the mating process can occur, to yield diploid (MATa/MATα) cells. These intracellular signal molecules (MATa cells secrete a-factor and MATα cells secrete α-factor) cause specific physiological changes in their target cells, including the modulation of gene expression. We have shown that α-factor induces the transcription of three polyA+ RNA species (500, 650, and 5300 bases) in MATa cells which are homologous to the same strand of the repetitive element sigma (Van Arsdell et al., 1987, Mol. Cell. Biol., in press). Analysis of a recombinant clone encoding the 650 base pheromone-induced RNA revealed that transcription initiates within a full-length sigma element (sigma-650) and terminates within or distal to a full-length delta element. In MATa cells containing a plasmid in which the sigma-650 element has been inserted just upstream of an invertase (SUC2) gene lacking its own promoter, invertase expression is α-factor-inducible. Deletion analysis of the 5'-flanking sequences of the sigma-SUC2 fusion gene indicate that a region located entirely within the sigma element (between 113 and 177 bp upstream of the transcription initiation site) is essential for pheromone induction. The region required for induction contains three copies of the 8 bp consensus sequence ATGAAACA, which is also found at multiple sites upstream of several other pheromone-inducible genes (STE2, STE3, MFa1, MFa2, BAR1, and BIK2). This conserved DNA sequence motif may be a cis-acting regulatory site that plays a role in hormonal induction of gene expression.

L 146 Human Apolipoprotein E: Bioengineering and bioactivities of recombinant analogs. T. Vogel, H. Giladi, R. Guy, K. Weisgraber, A. Levanon, B. Amit, R. Oren, D. Kanner, Z. Yavin, N. Zeevi, T.L. Ineratiry, A. Lalazar, S.C. Rall, R.W. Mahley & M. Gorecki. Bio-Technology General Ltd., Rehovot, Israel & Gladstone Foundation Labs., San Francisco, CA. We have genetically engineered and purified, the Met derivatives of the plasma Apo-E3 (Met Apo-E). This material is as active as authentic Apo-E3 in Apo-B,E (LDL) and Apo-E receptor binding (RB) assays (Vogel et al. PNAS, 82, (1985) 8696). We would like now to report on two series of genetically engineered Met Apo-E analogs. (1) Apo-E analogs in which the N-terminal of Met Apo-E was fused to various short peptides consisting of 5 to 45 amino acids were constructed. In contrast to Met Apo-E, which exhibits both a short half-life and a high toxicity in E. coli, the analogs exhibited higher stability and lower toxicity. A representative of this group of analogs the Met Leu3 Met Apo-E analog, was also assayed for RB activity and displayed essentially identical binding properties as Met Apo-E. As the Met Leu3 Met Apo-E analog accumulates in E. coli several fold more than Met Apo-E it is now being evaluated for its biological activity in animal models. (2) Site directed mutagenesis of the Met Apo-E gene in the vicinity of the receptor binding domain was performed. Purified Met Apo-E mutant proteins were tested in the Apo-B,E (LDL) RB assay, and the results obtained with several of these proteins together with the data from naturally occuring mutants, indicate that the suggested α helical structure and the positive charges of the RB domain (around amino acid residues 136 to 160) are crucial for the normal biological activity of Apo-E3.

L 147 TRANSLATION THROUGH A RHO-INDEPENDENT TERMINATOR ABOLISHES TRANSCRIPTIONAL TERMINATION, Joanna J. Wright and Richard S. Hayward, University of Edinburgh, Edinburgh, UK.

The "early" transcription of coliphage T7 DNA in vivo is carried out by the host RNA polymerase, initiating near the physical map position 1%, and stopping with about 75% efficiency at a rho-independent terminator (tec1) mapping near 19%. When we inserted tec1 between the galactose promoter and the assayable galactokinase gene in a plasmid of the pKO family, the termination efficiency was found to be very much as in T7 itself, despite the altered promoter and genetic environment. However, when we constructed derivatives of this plasmid in which the translation phase was altered such that the ribosomes were allowed to read through, instead of stopping prior to the terminator as in the wild type situation, termination was completely abolished. We suggest that this translational read-through interferes with an obligatory step in rho-independent termination: either the formation of the stem-loop structure in the RNA transcript, thought to be responsible for the pausing of RNA polymerase prior to dissociation of the nascent RNA from the DNA template; or access of auxiliary termination factors to the mRNA.

L 148 STRINGENT CONTROL OF RNA TRANSCRIPTION OF BACTERIOPHAGE PROMOTERS, M. Yamagishi*, J. R. Cole*, M. Nomura*, F. W. Studier† and J. J. Dunn†, *University of California, Irvine, CA 92717 and †Brookhaven National Laboratory, Upton, NY 11973.

The deprivation of amino acids causes a large inhibition of rRNA and tRNA synthesis in relA$^+$ but not in relA$^-$ E. coli cells, a phenomenon called stringent control. We have found in vivo transcription of hybrid rrnB operons, in which the normal promoters were replaced by either the λ phage P_L promoter or the T7 phage φ10 promoter (the latter was transcribed by T7 RNA polymerase) was under stringent control. Transcription of T7 genes directed by T7 φ9 and φ10 promoters was also stringently controlled. Furthermore, T7 phage infection experiments showed that transcription by T7 RNA polymerase in general is subject to stringent control. These results are consistent with the idea that stringent control acts in a relatively nonspecific manner to inhibit some step(s) in transcription that are often rate limiting for very active transcription.

L 149 CHARACTERIZATION OF PROMOTER AND REGULATORY ELEMENTS IN YEAST ATP2, Patrice O. Yarbough and Michael Douglas, Dept. of Biochemistry, UTHSC at Dallas, Dallas, Texas 75235.

ATP2, encoding the mitochondrial F_1 ATPase β subunit precursor, is a member of a gene family coordinately regulated by glucose. To study the promoter and regulatory elements of ATP2 in S. cerevisiae, the 5' upstream region of this gene was sequenced and its transcription start site was determined by primer extension. Transcription initiation occurs at position -191 from the translational start site. ATP2 promoter sequences appear to belong to the class of DNA sequences in which a pyrimidine flanked by two purines on either side serve as preferred sites for transcription initiation. Putative TATA sequences lie upstream at positions -249 and -289. The effect of deletions within the 5'region of ATP2 on expression was determined by gene fusions to E. coli lacZ. Deletions within the ATP2 regulatory regions, upstream of the TATA sequences, reduced the expression of ATP2 by 10-fold. This provides support for a positive control element in enhancing ATP2 activity. Further, an additional promoter fusion to lacZ at -99 in ATP2 suggests that an open reading frame within the 5' leader of ATP2 may be translated at low levels. This in conjunction with sequence data suggest that an open reading frame could be involved in modulating ATP2 expression. Deletion analysis of this region is currently in progress to test this possibility. (Supported by NIH Postdoctoral Fellowship GM11020-01 to POY).

L 150 UPSTREAM ACTIVATION SEQUENCES IN YEAST MAY BE DIVIDED INTO FUNCTIONALLY DISTINCT REGIONS. H. Yoo, F. Genbauffe, & T.G.Cooper, University of Tennessee, Memphis, Tennessee 38163.

Upstream activation sequences (UAS) of the inducible DAL7 and DUR1,2 genes consist of two functionally distinct regions. The first region, designated UES (upstream expression sequence), supports constitutive B-galactosidase production when placed in a CYC1-LACZ expression vector lacking the CYC1 UAS. The second region, designated UIS (upstream induction sequence), will not support B-galactosidase production when placed in the expression vector, but confers inducibility on the UES when placed adjacent to it. The 5' flanking regions of the highly inducible DAL7 and DUR1,2 genes contain both UES and UIS sequences. The partially inducible DAL1 and DAL2 genes also contain both sequences. However, the UISs possess relatively poor homology to the UISs of the inducible DAL7 and DUR1,2 genes. The constitutively expressed DAL3 and DAL5 genes, contain only UES sequences. These data argue that inducible genes differ from constitutively expressed genes by virtue of possessing target sites for additional regulatory proteins that bring about induction. According to this model, the UAS of non-inducible genes, such as HIS3 and other genes responding to general amino acid control, are predicted to possess only the UES component. In contrast, the inducible GAL, CAR, and CYC1 genes are predicted to possess composite promoter structures similar to those described above.

Gene Regulation in Eukaryotes - A

L 200 INSULIN REGULATES HUMAN GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE GENE TRANSCRIPTION THROUGH SPECIFIC DNA SEQUENCES, Maria Alexander-Bridges, Massachusetts General Hospital, Boston, MA 02114.

Insulin increases the content of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in hepatoma and mouse 3T3-F442a cell lines. The induction of GAPDH in 3T3 adipocytes is the result of a 10-fold increase in gene transcription. There are 200 GAPDH pseudogenes in the human genome. We have cloned a 10 Kb BAM fragment of a human GAPDH gene and transfected this gene in 3T3 preadipocytes. The transfected human GAPDH gene has been shown to encode a functional protein product. The human GAPDH mRNA encoded by the transfected gene is identical in length to endogenous human placental GAPDH mRNA. The start site of transcription of the transfected gene is identical to that of endogenous human placental GAPDH mRNA. The expression of human GAPDH mRNA is increased 10-fold by insulin in transfected 3T3 adipocytes. These studies indicate that sites required for specific regulation of the GAPDH gene by insulin are present in our clone. A 600 BP fragment of DNA containing the promoter and 5'-flanking sequences of the GAPDH gene was fused to the chloramphenicol acetyl transferase (CAT) gene. This construct is regulated by insulin when transfected into hormone-sensitive cells. This is the first demonstration of insulin regulation of gene transcription through specific DNA sequences.

L 201 STUDIES ON INDUCIBLE AND SUPPRESSIBLE DHFR GENES, John Abrams and Robert Schimke, Department of Biology, Stanford University, Stanford, Ca. 94305

As an approach to study the effects of transcriptional activity upon frequencies of gene amplification, we have constructed vectors which place the murine DHFR cDNA under the control of either an inducible or a suppressible promotor. Preliminary analyses of steady state RNA and protein levels in cells transfected with these constructs indicate that these minigenes regulate as expected. Positive regulation of pHSiD6, a minigene which places the murine DHFR cDNA downstream of the human metallothionein II_Apromotor, is observed when transformants harboring this construct are treated with cadmium. Alternatively, cells transformed with pHMGpD11+, a construct placing the DHFR cDNA under control of the hamster HMGCoA reductase promotor, respond to the presence of sterols by suppressing the expression of these minigenes. The manipulation of DHFR expression parameters in these cell lines will enable us to explore the consequences of induced or suppressed gene activity upon frequencies of spontaneous amplification events.

L 202 IDENTIFICATION AND CHARACTERIZATION OF THE MURINE ADENOSINE DEAMINASE TRANSCRIPTION UNIT, M. R. Al-Ubaidi, D. E. Ingolia and R. E. Kellems, Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030.

Adenosine deaminase (ADA) is an enzyme of purine metabolism that is found in virtually all mammalian tissues, with significant variation in a tissue-specific manner. To address a number of questions concerning ADA gene structure, expression and developmental regulation, we obtained overlapping lambda and cosmid clones which encompass the entire murine ADA structural gene. The transcription unit boundaries were determined using cell lines with highly amplified copies (20,000) of functional ADA structural genes. Nuclease protection, primer extension and DNA sequence analyses indicate that transcription initiates at multiple locations within a highly G:C rich promoter region devoid of any TATA or CAAT boxes. The transcription termination region was localized by hybridization of nascent labeled RNA to an ordered set of genomic subclones spanning over 40 kb of the ADA locus. The results of these experiments indicate that transcription occurs at a constant level throughout the structural gene and terminates within a discrete restriction fragment situated approximately 5 kb downstream of the major polyadenylation site. The fact that transcription terminates within the same restriction fragment for virtually all 20,000 ADA structural genes implies that a distinct genetic signal is associated with this process.

L 203 CHARACTERIZATION OF TWO ENHANCERS IN THE CHICKEN LYSOZYME GENE

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The chicken lysozyme gene is constitutively active in macrophages and under the control of steroid hormones in the oviduct.To investigate which DNA-elements are involved in the control of its expression we performed DNA-transfer experiments with the bacterial CAT(chloramphenicol- acetyl-transferase)-gene as an expression marker. Two different types of plasmids were used for transient transfections into cell lines and primary bone-marrow macrophages of chickens: 5'- deletion mutants of the upstream region of the chicken lysozyme gene placed in front of the CAT coding sequences and different fragments from this area in front of the HSV-TK-promoter. Two enhancers (E1 and E2) were characterized. They are active in macrophages, oviduct cells (only E1 tested) and, surprisingly, in mammalian mammary gland cells, but not in other cell types tested. E1 is located between -208 and -66; it contains one binding site for the progesterone receptor, but shows a high basic level enhancement even without hormonal stimulus (Miksicek, Altschmied, Renkawitz, Schütz unpublished). E2 is located around -2700 and shows higher activity in macrophages than in mammary cells. Its position is correlated with a DNaseI- hypersensitive (HS-)site that appears only in macrophages. Furthermore a negative element was identified at -2400, which is active in promyelocytes, but not in mature macrophages. This coincides with another HS-site found in all tissues but disappearing in mature macrophages (Fritton et al., Nature **311**, 163-165 (1984)).

L 204 CHARACTERIZATION, IN VIVO AND IN VITRO, OF TRANSCRIPTION FACTORS BINDING THE RAT METALLOTHIONEIN-1 (MT-1) GENE.

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An analysis of dimethyl sulfate (DMS) protection in rat hepatoma cells, using genomic sequence methodology, reveals a cadmium-inducible protection pattern *in vivo* in five metal responsive elements (MREs) in the rat MT-1 gene. A site of strong methylation enhancement also occurs only after metal-ion induction. These results indicate that metal induction involves the metal-dependent binding of a sequence-specific binding transcription factor to the MRE sequences. A labeled fragment containing MRE sequences shows two retarded bands in an acrylamide gel when mixed with a protein extract from the nuclei of rat hepatoma cells. Competition studies show that this binding *in vitro* is sequence-specific. It appears that this fragment contains a minimum of two distinct protein binding sites. One of these sites is also present in fragments containing TATA and glucocorticoid regulatory sequences. DMS protection analysis *in vivo* also reveals a binding site located 5' of the distal MRE at a consensus sequence for the Sp1 transcription factor.

L 205 RE-EXPRESSION OF DEOXYCYTIDINE KINASE (dCk) IN CEM/dCk(-) CELLS AFTER TREATMENT WITH 5,6-DIHYDRO-5-AZACYTIDINE (DHAC).

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Hypomethylation of DNA has been associated with gene expression. In this study the human leukemia cell lines CEM/0 and CEM/dCk(-) were treated with DHAC. The phosphorylation and incorporation of DHAC into DNA and the effect on DNA methylation and dCk expression were examined. In both cell lines DHAC was anabolized to its triphosphate and deoxy-triphosphate. After 4 hours incubation with 100 µM DHAC at 37° C, the cellular concentrations of DHACTP and DHAdCTP in the CEM/0 cells were 120 µM and 16 µM, respectively. The anabolite concentrations in the CEM/dCk(-) cells were between 30 and 50 % lower after incubation with 200 µM DHAC. Purified DNA from 10^7 CEM/0 and CEM/dCk(-) cells contained 16±9 and 17±12 pmol DHAdCMP, respectively. The methylation levels in untreated CEM/0 and CEM/dCk(-) cells were 3.72 % and 3.06 % 5-methyl-cytosine (5-mC), respectively. Treatments with 100 and 200 µM DHAC decreased the methylation level to 1.49 % and 0.72 % 5-mC in CEM/0 and 1.94 % and 1.40 % 5-mC in CEM/dCk(-) cells, respectively. The dCk activity increased in CEM/0 cells approximately 10 % and in CEM/dCk(-) cells between 10 and 20 % compared to untreated controls. Although the hypomethylation was transient, the increase in dCk activity was persistent and essentially constant at the level of re-expression for at least 8 days. Repetitive treatments with DHAC could not further increase DNA hypomethylation or dCk activity.

REGULATED EXPRESSION OF TRANSFECTED MUSCLE SPECIFIC GENES

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During the differentiation of muscle tissue a complex set of gene regulations takes place resulting in the muscle specific phenotype of the cell. Whereas, for instance, the genes coding for contractile proteins are switched on, others like the cytoplasmic ß-actin gene are down-regulated. In order to study the molecular mechanisms underlying the regulation of gene expression, we have constructed chimeric CAT genes with upstream DNA segments of the cardiac specific chicken myosin light chain 2 gene. Upon transfection into primary chicken skeletal or cardiac muscle cells with or without BUDR we observed regulated expression of the CAT gene in differentiated muscle cells, if the appropriate upstream DNA segment of the LC_2 gene was placed in front of CAT.

The down-regulation of the ß-actin gene has been studied in C2 mouse myoblasts stably transfected with the neomycin resistance gene plus either ß-actin promoter containing CAT constructs or SV40 promoter containing ß-actin gene body hybrids. The-down regulation of the ß-actin gene as measured by S1 analysis was always observed when the 3'end region of the gene was present in the hybrid. The promoter or upstream sequences of the ß-actin gene appear not to be required for proper down-regulation during muscle differentiation.

POLYBRENE-ASSISTED GENE TRANSFER: DEVELOPMENT OF A SIMPLE AND GENERAL PROCEDURE

L 207 ENSURING REPRODUCIBLY HIGH TRANSFECTION FREQUENCIES IN CULTURED FIBROBLASTS, Remy Aubin, Michael Weinfeld and Malcolm Paterson, Molecular Genetics and Carcinogenesis Laboratory, W.W. Cross Cancer Institute, 11560 University Ave., Edmonton, Alberta, CANADA, T6G 1Z2.

The recent advent of gene transfer methodology has greatly facilitated the analysis of eucaryotic gene structure, function and regulation. Yet, in comparison to established rodent lines, human fibroblast strains remain notoriously refractory to transfection unless sophisticated or costly strategies are employed. Inspired by the initial report of Kawai and Nishizawa [Mol. Cell. Biol. **4**:1172 (1984)] we have now developed a simple, inexpensive, and reproducibly efficient gene transfer procedure which promises to be readily adaptable to a diverse array of cultured mammalian cell types. The basic protocol entails adsorption of purified DNA to cell monolayers in the presence of the cationic polymer Polybrene followed by cellular permeabilisation with dimethyl sulfoxide. Cell density, culture conditions, concentrations and exposure times to Polybrene, plasmid DNA, and especially DMSO are critical determinants of success. Under optimal conditions the procedure routinely yields between 10^3 and 10^4 stable transfectants per µg pSV2neo DNA using immortalised cell lines as recipients for the bacterial **neo** gene, whereas non-established human dermal fibroblast strains show transfection frequencies of 10^2 per µg plasmid DNA. Transfection experiments with the bacterial chloramphenicol acetyl transferase gene also demonstrate that Polybrene-assisted gene transfer results in elevated levels of transient expression of foreign genes in human skin fibroblasts.

PURIFICATION OF CHICKEN OVALBUMIN GENE UPSTREAM PROMOTER (COUP)

L 208 BINDING PROTEIN FROM CHICK OVIDUCT, Milan K. Bagchi, Sophia Y. Tsai, Ming-Jer Tsai and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Previous studies on the in vitro transcription of chicken ovalbumin gene carried out in HeLa nuclear extracts established the requirements of a transcription factor binding to sequences between positions -70 to -90 of the gene. By employing gel retardation and DNase I footprinting assays we have now purified the protein(s) binding to this sequence from homologous chick oviduct tissue. The purification scheme starting from oviduct cell nuclear extract involved DEAE Sephadex, phosphocellulose and heparin sepharose column chromatography and finally two consecutive sequence-specific DNA affinity chromatography steps. The resulting purification was 80,000 - 100,000 fold starting from the nuclear extract. The purified protein preparation showed multiple polypeptide bands upon SDS-polyacrylamide gel electrophoresis. Renaturation of separated polypeptides after extraction from the gel matrix was carried out. The majority of the renatured polypeptides exhibited specific binding to the COUP element. These observations indicate the presence of multiple COUP binding polypeptides in our purified protein preparation.

L 209 **USE OF TRANSIENT CELL FUSION TO IDENTIFY PROLACTIN GENE PROMOTOR SEQUENCES WHICH INTERACT WITH POSITIVE TRANS-ACTING CELL TYPE-SPECIFIC TRANSCRIPTIONAL FACTORS,** **Carter Bancroft and Thomas C. Lufkin, Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029**

Previous experiments employing cell fusion (stable or transient) have been employed for studies of cell-type specific expression of endogenous genes. However, the inability to readily manipulate endogenous genes has prevented further characterization of the regulatory factors amd target DNA sequences involved. We have investigated whether transient cell fusion can be employed to activate a transfected gene construct which exhibits cell type-specific expression. Mouse fibroblast (C127) cells were stably transfected with pPRL-CAT, which contains two kbp of prolactin (PRL) 5'-flanking sequence. Transient fusion of a polyclonal culture of transfected fibroblasts to GH_3 rat pituitary cells yielded a strong (>1000-fold) induction of expression of CAT enzymatic activity by the formerly quiescent PRL-CAT construct. Assay by this technique of 5' deletion mutants of pPRL-CAT showed that activation of CAT expression requires a short DNA sequence upstream of the prolactin gene. RNase protection showed that fusion leads to induction of correctly initiated transcripts, while transcription in isolated nuclei showed that fusion yields transcriptional activation. Similar cell fusion experiments failed to activate a rat growth hormone (GH) promotor construct which is also active only in GH_3 cells, implying different mechanisms for cell-type specific regulation of the PRL and GH genes. Fusion to GH_3 cells also did not activate expression of the endogenous mouse fibroblast genes for PRL or GH, implying a different chromatin milieu for the endogenous and transferred PRL gene promotors.

L 210 EXPRESSION OF Mn-SUPEROXIDE DISMUTASE IN HUMAN CELLS Yaffa Beck, Rachel Oren, Boaz Amit, Avigdor Levanon, Marian Gorecki and Jacob R. Hartman, Biotechnology General Ltd, Kiryat Weizmann, Rehovot 76326 Israel.

cDNA clones containing the entire coding region for human MnSOD were isolated from a human T-lymphocyte cDNA library by hybridization to ^{32}P-labelled oligonucleotide probes synthesized according to known amino-acid sequence. Nucleotide sequence analysis suggests a mature protein of 198 amino acids preceeded by a 24 amino acid prepeptide, in accordance with processing required for transport into mitochondria. Southern blot hybridization to human DNA digested with several restriction endonucleases suggest a single copy gene. The gene for human MnSOD has been isolated from a human genomic library and its organization has been determined.

The human MnSOD cDNA was hybridized to poly A^+ RNA from human cell lines, human placenta, mouse WEHI-3 cells and bovine liver. A major transcript of human mRNA for MnSOD about 1100 nucleotides (nt) long was identified. The mouse mRNA for MnSOD is similar in size to the human transcript, whereas mRNA of bovine MnSOD is about 300 nt longer.

The proportion of both Cu/Zn and Mn SOD mRNAs in various cell lines was in the order of 10^{-3}%, as determined by hybridization of the SOD cDNA probes to dot blots of serially diluted poly A^+ RNA. The MnSOD message was most abundant in Hepatoma cells (2.5×10^{-3}%) and Cu/Zn SOD transcripts were most abundant in the T-lymphocyte line (4×10^{-3}%).

The above studies may facilitate analysis of oxygen-inducibility and transcriptional control of the SOD genes during development.

L 211 FUNCTION AND REGULATION OF CHICKEN ERYTHRO SPECIFIC HISTONE H5. Mathias G. Bergman, Mårten Winge, and Edgar Wawra. Med. Cell Genetics, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden; Inst. Mol. Biol. der Universität Wien, Wasagasse 9, A-1090 Wien, Austria.

During erythropoesis in chicken, linker histone H5 accumulates in the maturing erythrocyte (CE), in paralell with a compaction of the chromatin and inactivation of transcription and replication. H5 replaces H1 to ca 70% in this process. In contrast to most histone genes, H5 is a single copy gene coding for a polyadenylated mRNA. Transcription is not S-phase dependent. Since H5 protein has been suggested to cause the inactivation of the CE nucleus, purified H5 was microinjected into proliferating cells. H5 accumulated in the nuclei and blocked both transcription and replication substantially and persistently. Studies of transcriptional rate show that H5 mRNA is still produced at a low rate in "inactive" CE; also in erythroblasts the rate is low. The mechanism of the massive accumulation of H5 mRNA and protein seems to be the extreme stability of the mRNA (half life 16-18 h.). Although the rate of transcription is low in mature CE, the transcriptional potential is high; the gene is associated with a large number of transcription complexes that can be activated by Sarkosyl. To study the stability of the differentiated state CE were fused with rat myoblasts to form heterokaryons; in these cells the H5 gene is faithfully transcribed, despite the myoblast cytoplasma. Prel. res. indicate that the gene is specifically rearranged in these cells. In search for factors controlling H5 yranscription we have mapped 5 CE-specific DNaseI hypersensitive sites flanking the gene. We are presently studying binding of possible factors to these regions by DNase footprinting.

L212 REGULATION OF MURINE MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II GENES. Michael A. Blanar and Richard A. Flavell. Biogen Research Corporation, Fourteen Cambridge Center, Cambridge MA 02142.

The tissue-specific expression of MHC class II genes is restricted primarily to macrophages, B cells, dendritic cells, and cells of the thymic epithelium. Various treatments can augment the levels of class II mRNA in mature B cells and plasma cells whereas γ interferon (IFN-γ) enhances levels of class II mRNA in macrophages but not in B cells. It is likely that class II genes have separate B cell and macrophage responsive regulatory sequences. The molecular mechanism of induction has not been fully determined, but it has been shown that trans-acting factors are necessary for induction of class II genes following treatment with IFN-γ.

DNA fragments from the region 5′ of the E^d_β structural gene have been analyzed by 'gel retardation' assays utilizing nuclear protein extracts from IFN-γ induced and uninduced cells from the macrophage-like line, WEHI-3. At least one protein has been shown to bind specifically to a 70 bp fragment, located approximately 400 nucleotides 5′ of the ATG of exon 1. DNase footprinting experiments have led to the suggestion that a 14 bp A-T run of DNA may be important for binding of this nucleoprotein. Competition binding experiments utilizing poly(dI-dC)-poly(dI-dC) and poly(dA-dT)-poly(dA-dT) suggest that discrimination of the binding site probably is not due to minor groove characteristics of this A-T run. Further characterization of the binding site and its importance to expression is being pursued.

L213 REGULATION OF SURFACTANT APOPROTEIN GENE EXPRESSION IN FETAL LUNG, Vijay Boggaram and Carole R. Mendelson, Univ Tex Hlth Sci Ctr, Dallas TX 75235.

Pulmonary surfactant, a developmentally- and hormonally-regulated lipoprotein, reduces surface tension at the alveolar-air interface. The major apoprotein of surfactant, a sialoglycoprotein, M_r ≈ 35,000, is believed to serve an important role in surfactant function. Previously, we found that surfactant apoprotein gene expression is initiated in fetal rabbit lung tissue after day 26 of gestation. Cortisol and cyclic AMP analogues increase the levels of surfactant apoprotein and its mRNA in lung explants from 21-day fetal rabbits. To investigate further the regulation of surfactant apoprotein gene expression in fetal lung tissue, we have isolated a cloned cDNA specific for the major rabbit surfactant apoprotein. This cDNA hybridizes to two major species of mRNA (2.0 and 3.0 kb in length) that are coordinately induced in rabbit lung tissue during development and with hormonal treatment. The two mRNA species appear to be encoded by a single gene. Cortisol treatment of lung explants from 21-day fetal rabbits caused an induction of surfactant apoprotein mRNA levels that was first observed after 24-48 h of incubation. Bt_2cAMP caused a marked induction of surfactant apoprotein mRNA levels by 4-6 h of incubation; a stimulatory effect of cyclic AMP was often detectable as early as 2 h after its addition to the culture medium. Cycloheximide (CHX, 2μg/ml) markedly reduced the levels of surfactant apoprotein mRNA in both control and Bt_2cAMP-treated explants after 4 h of incubation. This inhibitory effect of CHX was reversed within 6 h of its removal from the medium. These findings are suggestive that a protein with a relatively short half-life mediates the expression of the surfactant apoprotein gene and its induction by cyclic AMP.

L214 REGULATORY ELEMENTS OF THE ANTENNAPEDIA GENE OF D. MELANOGASTER, Anne Boulet, David Mastronarde and Matthew Scott, U of Colorado, Boulder, CO 80309-0347.

The Antennapedia gene plays a fundamental role in the development of the fruit fly, Drosophila melanogaster. Embryos homozygous for recessive lethal Antp alleles show partial transformations of the second and third thoracic segments towards first thoracic segment. Somatic clones lacking Antp function generated in portions of the thorax are abnormal in the adult fly. Thus, the Antp gene product appears to be required for proper thoracic development in the larva and in the adult. The ability of some Antp mutants to completely complement each other suggests that the gene is composed of discrete functional units. The 100 kb Antp gene contains two promoters which can direct the expression of the same protein products. The genetic behavior of mutations which affect only promoter 1 or promoter 2 indicate that the two promoters serve very specific functions with respect to the development of different regions of the thorax. Examination of the localization of mRNAs initiated at each promoter by in situ hybridization shows that promoters 1 and 2 have distinct spatial and temporal patterns of expression (A. Martinez-Arias, personal communication; E. Jorgensen & R. Garber, personal communication). We have constructed fusions between the Antp promoters and the coding region for β-galactosidase in order to identify the regulatory elements required for proper Antp expression. The fusion genes, containing various amounts of DNA upstream and downstream of the transcription start site are introduced into flies by P-element mediated transformation. By examining the patterns of expression of β-galactosidase protein driven by different constructs, we have begun to identify sequences which are involved in proper timing and spatial regulation of Antp expression.

L 215 EXPRESSION OF THE EMBRYONIC MYOSIN HEAVY CHAIN GENE IS REGULATED BY TWO DISTINCT REGULATORY SEQUENCES. **Patrice Bouvagnet, Glenn E. White, Emanuel E. Strehler, Bernardo Nadal-Ginard and Vijak Mahdavi**, Howard Hughes Medical Institute, Children's Hospital, and Harvard Medical School, Boston. The embryonic skeletal myosin heavy chain (MHC) gene is expressed in a tissue and stage specific manner. In order to determine the sequences involved in the regulation of its expression, MHC minigenes constituted by 5' upstream sequences, exons 1 to 3 and 37 to 41 as well as CAT gene constructs fused to the first exon of the embryonic MHC gene were used for transient gene expression assays in cultured Hela cells, C2 myoblasts and myotubes. Deletion of sequences upstream of position -671 from the cap site decreased drastically the level of expression of the minigene in C2 myotubes. No construct of this deletion series was expressed in C2 myoblast or Hela cells. However, sequences from -2600 to -299 do not restore high level of expression in C2 myotubes when reintroduced at the 5' end in a reverse position or in either orientation into the first intron of the deleted minigene. These sequences do not function as enhancers when fused to other MHC gene constructs or to the enhancer-less pA10CAT2 gene. However, introduction of the SV40 enhancer to the deleted minigene constructs restore high level of expression without altering the tissue and developmental specificity. These data suggest that a cis-acting regulatory region located between -1141 and -671 behaves as a position-, orientation- and promoter-dependent enhancer. There is a secondary regulatory region located downstream of -303 which determines the tissue specific expression of the embryonic gene. The nucleotide sequence of the 5' upstream region has been determined. It was compared to homologous region of the cardiac alpha and beta MHC genes. The embryonic MHC upstream sequences (-730) contain a CCGCCC hexanucleotide known to be a core sequence of the binding site for the promoter-specific protein factor Sp1. In order to provide a more detailed analysis of these two cis-acting regulatory regions, constructs have been made by ligation of a series of successive deletions of the embryonic sequences to the CAT gene.

L 216 TOPOISOMERASE ACTIVITY AND RNA POLYMERASE II TRANSCRIPTION, Therese Brendler, John A. Thompson, Susan Garfinkel, Judy DiPietro, Kathryn Anderson, W. French Anderson and Brian Safer, Section on Protein Biosynthesis, LMH, NHLBI, NIH, Bethesda, MD 20892.

Transcriptional activity of the adenovirus major late promoter (Ad-2 MLP) was studied in nuclear extracts from partially hepatectomized rat livers. Supercoiled DNA templates of the Ad 2 MLP were specifically transcribed 3- to 5-fold more efficiently than linear templates. Using agarose gel electrophoresis, the transcriptionally active forms of Ad 2 MLP supercoiled templates were detected as DNA structures associated with radiolabeled nascent RNA transcripts. To characterize the relationship between transcriptional activity and DNA template topology, rat liver nuclear extracts were fractionated by Mono S cation-exchange chromatography and analyzed for topoisomerse I, topoisomerase II and transcriptional activities. We have identified two fractions that were required for high levels of specific transcription. Neither fraction contained topoisomerase II nor camptothecin sensitive topoisomerase I. By using extracts depleted of transcriptional activity using either α-amanitin or DNA affinity chromatography, one fraction was shown to contain RNA polymerase II and associated transcription factors. The other fraction required for functional transcription contained a camptothecin resistant topoisomerase I activity. The characterization of this topoisomerase I-like activity and its relationship to transcriptionally active DNA templates are currently being investigated.

L 217 HIGH MOBILITY GROUP (HMG) PROTEINS 14 AND 17 BIND SELECTIVELY TO MONONUCLEOSOMES OF ACTIVE DOMAINS AND MEDIATE DNASE I SENSITIVITY, Timothy Brotherton and Gordon Ginder, University of Iowa, College of Medicine, Iowa City, IA 52240.

HMG 14 and 17 proteins are ubiquitous non-histone nuclear proteins that have been proposed to be the mediators of selective DNase I digestion of chromatin containing transcriptionally active genes. We have previously shown that binding by these proteins in vitro is selective for mononucleosomes containing genes that are DNase I sensitive in vivo (Brotherton and Ginder, 1986, Biochem. 25, 3447). In addition, we have shown that HMG 14/17 binding is not determined by DNA methylation, linker DNA length, single-stranded DNA nicks, or bulk histone acetylation. In this study we have examined both the binding of HMG 14/17 proteins to chicken reticulocyte mononucleosomes under a variety of buffer conditions using formaldehyde fixation and the DNase I sensitivity of HMG-bound and unbound monosomes separated by gel electrophoresis. Results show that selective binding in vitro was at least partially salt dependent, but did not require changes in nucleosome conformation. Further, HMG 14/17-bound monosomes were digested by DNase I at a rate at least twice that of unbound. Digestion was dependent on HMG binding and not gene content. Taken with previous work, these results suggest that selective binding by HMG 14/17 proteins to monosomes of active domains is mediated by ionic interactions between these proteins and the core histones, and that binding determines intermediate-type DNase I sensitivity.

L 218 THE FEMALE-SPECIFIC EXPRESSION OF *SCHISTOSOMA JAPONICUM* EGGSHELL GENES MAY BE MEDIATED BY CONSERVED REGULATORY SEQUENCES.

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Adult *Schistosoma japonicum* male-female pairs live *encopula* in the hepatic portal system of their mammalian host, producing as many as 3,500 eggs per day. Among the genes involved in egg production are those encoding the eggshell proteins. We have isolated several members of the *S. japonicum* eggshell gene family, and have shown that transcription of these genes is developmentally regulated, female-specific, and coincident with the time of female maturation and formation of worm pairs. The genes are not sex-linked, suggesting that the coordinate transcriptional regulation may be mediated by an interaction between cis-acting genomic sequences and a female-specific transcription factor(s). The nucleotide sequences of two distinct, homologous genomic clones were determined; each contains a TATA box and a polyadenylation sequence. The two genes encode proteins that are extremely glycine rich, but that contain differences in amino acid sequence. There is a 240 bp sequence in these genes, beginning 140 bp upstream of the initiating methionine and extending through the putative signal peptide coding region, that is nearly identical. This region contains the transcription start point and sequences also found in *Drosophila* and silk moth chorion genes. The high conservation of this 5' region, particularly in contrast with the differences in the coding region and the complete lack of homology farther upstream suggests that it may contain control elements involved in the precise developmental regulation of these genes in the female.

L 219 REGULATORY ELEMENTS OF THE αA-CRYSTALLIN GENE PROMOTER. Ana B. Chepelinsky, Bernd Sommer 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 lens of all vertebrates and is the first crystallin to appear during mouse lens development. A hybrid gene containing a DNA fragment (-366 to +46) from the mouse αA-crystallin gene fused to the bacterial chloramphenicol acetyl transferase (CAT) gene is expressed specifically in lens cells in transient experiments using explanted embryonic chicken lens epithelia and in transgenic mice. Deletion experiments showed that the transcription of the αA-crystallin-CAT hybrid gene in a Hela whole cell extract required only the TATA box and downstream sequences; additional upstream sequences did not improve transcription. On the contrary, mapping experiments in transfected explanted lens epithelia indicated that two different regulatory elements must interact to activate the αA-crystallin promoter: one proximal (-88 to +46) and one distal (-88 to -111). Deletion tests showed that the sequence between position -88 and -60 is critical for the proximal element to function. The distal element activated the proximal element even when separated by a 57 bp spacer; and this spacer did not alter the transcription initiation site. The distal element contains a dyad of symmetry surrounding a 6 bp stretch. When the mouse αA-crystallin DNA fragment -111 to +46 was incubated with chicken lens nuclear extracts a mobility shift was observed in acrylamide gels. This mobility shift was prevented by competition with the -111 to -55 αA-crystallin DNA fragment but not by competition with the -111 to -84 fragment. Present mutagenesis experiments will allow us to characterize more precisely the bases involved in the activation of this promoter.

L 220 IN-VITRO TRANSCRIPTION AND DNA-PROTEIN BINDING STUDIES ON A HUMAN GENE REGULATED BY INTERFERON. Yuti Chernajovsky, Andrew C.G. Porter, Ian M. Kerr and George R. Stark, Imperial Cancer Research Fund, Lincoln's Inn Fields London WC2A 3PX, U.K.

We have characterised the promotor region of the gene 6-16, which is induced by Type I interferons. The sequences upstream of the TATA box contain a nearly perfect direct repeat 41 nucleotides long (from -89 to -172). The transcripts initiated IN-VITRO start at the same point as the natural mRNA. We could not detect differences between extracts of control or interferon treated cells. Removal of the tandem repeat up to position -94 has no effect in the transcription efficiency of this promotor, but 5' deletion to position -39 abolishes transcription. DNA-retardation assays, DNAse-footprinting and chromatographic separation of nuclear proteins allowed us to identify at least three proteins which bind to different parts of the promotor region but not to the first exon or following intron. The binding regions are around positions -470 to -447, the direct repeat and a CCAAT box located between -83 to -62 in the non coding strand. We are trying to purify these proteins by sequence specific DNA affinity chromatography.

L 221 Developmental and Tissue-Specific Expression of Aldolase A in Brain and Muscle of the Mouse. Elena Ciejek, Ann Maine, Jim Stauffer, Tony Mestek, University of Rochester, Rochester, NY 14642.

Aldolase is a glycolytic enzyme necessary for normal metabolism in all cells and absolutely indispensable in brain and muscle tissue. There are three isozyme forms of aldolase in vertebrate organisms. These forms, A, B and C are expressed in a tissue-specifi developmental-specific and carcinogenic-specific manner. Aldolase B is expressed in adult liver, C is expressed almost exclusively in the brain and A is expressed in muscle, brain and fetal tissues. Two different mRNA sizes have been detected for aldolase A and these mRNAs appear in a tissue specific manner. In the rat it has been proposed that these two mRNAs arise from transcription which initiates at a set of alternative promoters in front of a single gene. We have investigated the gene structure of the aldolase isozyme family in the mouse and have subsequently isolated and sequenced a cDNA for aldolase A from the brain. We have been able to identify that aldolase B exists as a single copy in the mouse genome whereas aldolase A is apparently a multigene family or one gene and several pseudogenes. The sequence for aldolase A expressed in the brain and its comparison to mouse muscle aldolase A mRNA supports the proposal that different promoters are being utilized in a tissue-specific manner in the brain and muscle. We are presently looking at the possible use of the muscle specific promoter during brain development as well as isolating genomic clones to further verify the presence of these two promoters in the mouse genome and to continue an investigation into their activation during development, differentiation and carcinogenesis.

L 222 IDENTIFICATION AND PURIFICATION OF TWO PROTEINS WHICH RECOGNIZE THE UPS OF THE AD 2 MLP. Roger B. Cohen, John A. Thompson, Therese Brendler, Susan Garfinkel, Linda Yang and Brian Safer, Section on Protein Biosynthesis, LMH, NHLBI, NIH, Bethesda, MD. 20892

A rapid procedure using Mono Q, Mono S and DNA-Mono Q affinity chromatography has been developed to identify and enrich two distinct DNA binding proteins from K562 nuclear extracts and HeLa whole cell extracts. Specific and competitive binding of two proteins to the upstream promoter sequence (UPS) of the Adenovirus 2 major late promoter (MLP) is demonstrated by DNase I footprinting, the formation of distinct DNA-protein complexes in gel electrophoresis mobility shift assays, and a new affinity radiolabel transfer technique. By the latter procedure, the two factors interacting with the UPS are identified as M_r = 40,000 and 116,000 polypeptides. The two factors can also be distinguished by different patterns of hypersensitivity within the UPS footprint and differential stability to Mg^{++}. Incubation of HeLa whole cell extracts or K562 nuclear extracts with DNA sequences containg the entire Ad 2 MLP causes specific depletion of both the 40 kDa and 116 kDa proteins. In HeLa whole cell extracts specific transcription of the MLP is also abolished by a synthetic oligonucleotide containing the UPS sequence. Both the 40 kDa and 116 kDA proteins are specifically depleted by this sequence. The potential role of the 40 and 116 kDa proteins in modulating transcription from the MLP is being investigated.

L 223 UBIQUITOUS EUCARYOTIC UPSTREAM REPRESSION SEQUENCES CONTROL ACTIVATION OF THE INDUCIBLE ARGINASE GENE. T.G. Cooper and R.A. Sumrada, University of Tennessee, Memphis, Tennessee 38163

The arginase gene (CAR1) responds to both induction and nitrogen catabolite repression. This dual regulation is mediated through sequences that both positively and negatively modulate CAR1 transcription. A 13 bp sequence possessing characteristics of a repressor binding site, plays a central role in the induction process. This upstream repression sequence (URS) repressed gene expression when placed either 5' or 3' to the upstream activation sequence of a heterologous gene (CYC1). Action of the URS and its cognate repressor were overcome by CAR1 induction when the URS was situated cis to the CAR1 flanking sequences. This was not observed, however, when it was situated downstream of a heterologous gene UAS indicating that URS function is specifically neutralized by cis-acting events associated with CAR1 induction. Analysis of various gene banks revealed that the 13 bp upstream repression sequence is found ubiquitously, occurring in the regulatory regions of bacteriophage, yeast, mammalian, and viral genes. In a significant number of cases, the sequence has been shown to be contained in a region associated with negative control of yeast gene expression. These data suggest that the URS identified in this work is probably a generic repressor target site that may have been conserved through the evolution of transcriptional regulation.

L 224 LINKER-SCANNING MUTAGENESIS OF THE RAT INSULIN II GENE'S 5'-FLANKING SEQUENCES, David T. Crowe and Ming-Jer Tsai, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

There are two nonallelic insulin genes (I & II) in rats, and at least three cis-acting elements which regulate tissue-specific expression have been identified in the 5'-flanking sequences of the rat insulin I gene. These elements include a tissue-specific enhancer (-333 to -103), an upstream promoter (-113 to +1), and perhaps two negative regulatory elements which serve to repress insulin expression in inappropriate tissues. In order to determine which sequences in the rat insulin II gene are necessary for such diverse activities, a series of linker-scanning mutants have been constructed (along with a battery of 5' and 3' deletions) which reside within the 5'-flanking sequences of a rat insulin II-CAT hybrid gene. These mutants have been transfected into both endocrine (HIT) and non-endocrine (CHO) cell lines, and CAT activity determined. Our results indicate that the 5' boarder of the insulin II gene's enhancer occurs between -218 to -110. Additional experiments will examine if those mutations which abolish the in vivo expression of insulin-CAT hybrids also perturb protein-DNA interactions occuring within the 5'-flanking region.

L 225 TRANSCRIPTIONAL REGULATION OF THE HUMAN PRO-α1(I) COLLAGEN GENE. W.N. de Vries, C.M.S. Rossouw, W.P. Vergeer and W.J. de Wet, Department of Biochemistry, Potchefstroom University, Potchefstroom 2520, South Africa.

Type I collagen is a major constituent of most connective tissues. The heterotrimeric protein is first assembled as type I procollagen, a precursor comprised of two pro-α1(I) and one pro-α2(I) chains. Cloning of the pro-α1(I) collagen gene revealed a large, highly elaborate multi-exon structure containing 51 exons. To identify potential regulatory sequences in the pro-α1(I) gene, transcription of various chimeric gene constructs was investigated in transfected cells and in microinjected *Xenopus laevis* oocytes. Our functional analysis showed that *cis*-acting sequences are present in three segments broadly located between -440 and -330, between -253 and -15, and between +720 and +1260. Exceptional stimulation of transcription was observed with the latter region; the enhancer effect is independent of orientation and functions over long distances, both from upstream or downstream positions relative to the initiation site of transcription. Sequencing and DNase protection studies revealed the presence of Sp1 binding sites, an enhancer core sequence and a nuclease hypersensitive site in the intronic enhancer. The other two regulatory regions contain binding sites for CAAT binding protein as well as for other *trans*-acting DNA binding proteins. Our analysis also revealed differential interaction between the regulatory sequences and DNA-binding proteins present in nuclear extracts from different cell types, indicating that some of these DNA binding proteins are involved in cell-specific expression of the pro-α1(I) gene. Identification of these proteins are in progress.

L 226 A COMPOSITE ENHANCER REGULATES THE GLYCOPROTEIN HORMONE α SUBUNIT GENE PROMOTER. Angelo M. Delegeane and Pamela L. Mellon, Salk Institute, La Jolla, CA. 92037

Human chorionic gonadotropin is a placental glycoprotein hormone comprised of an α and β subunit. Using a transient expression assay, we have examined tissue-specific expression and hormonal regulation of the cloned α subunit gene in cultured cells derived from a variety of tissues. Hybrid genes were constructed by placing the 5' flanking region of the α subunit gene on the marker gene, chloramphenicol acetyl transferase (CAT). We have demonstrated tissue-specificity of the 5' flanking sequence by showing strong expression in cell lines of placental origin and weak expression in non-placental cell lines. In addition, we have observed regulation of the gene by cyclic AMP (cAMP). Treatment of the transfected placental cells with forskolin, which activates adenylate cyclase, induced a 10-fold increase in the level of CAT activity. Deletion analysis of the 5' flanking sequence mapped the tissue-specificy and the cAMP response to a region from -168 to -100. Analysis of the sequence revealed an 18 bp direct tandem repeat in this region which contained the consensus sequence TGACGTCA previously shown to be involved in cAMP response (Montminy et al.(1986) PNAS U.S.A. 83,6682). Only one copy of this repeat is necessary for the full cAMP response and elimination of both copies destroyed the response. Tissue-specificity is also dependent on the 18 bp repeat but requires a larger region than the cAMP response. When placed in the reverse orientation upstream of a heterologous promoter, this region confers not only cAMP reponsiveness but tissue-specific enhancement of the promoter. cAMP response and tissue-specific enhancement are also seen when the region is placed at the 3' end of the gene but enhancement occurs at lower level.

SIMILAR MODULATION OF MOUSE GLYCEROPHOSPHATE DEHYDROGENASE mRNA DURING FAT AND
L 227 MUSCLE CELL DIFFERENTIATION, Deborah E. Dobson and Bruce M. Spiegelman, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115

Glycerophosphate dehydrogenase (GPD), a key enzyme in triglyceride biosynthesis, is transcriptionally activated during the differentiation of mouse 3T3-F442A cells into adipocytes (Cook et al. (1985) J. Cell. Biol. 100, 514). We find the adult GPD gene is also activated when mouse C2 muscle cells differentiate in culture. GPD mRNA is undetectable in C2 myoblasts and appears as the cells differentiate into myotubes. Thus, GPD gene activation may be generally linked to the process of cell differentiation. Modulators of GPD expression in adipocytes regulate GPD mRNA levels in muscle in a similar manner. Treatment of adipocytes and myotubes with either dibutyrylcAMP or 8-bromocAMP and theophylline (0.5 mM, 18 hr) results in an 80-90% decrease in GPD mRNA in both cell types. Sodium butyrate (5 mM, 18 hr) also causes a 75-95% decrease in GPD mRNA in treated adipocytes and myotubes. Tumor necrosis factor (TNF, 2500 U/ml) effects a rapid reduction in adipocyte GPD mRNA (75-95% decrease by 6.5 hr) and a reduction in myotube GPD mRNA, though with somewhat different kinetics (12% decrease in 6.5 hr, 55-60% decrease in 18 hr). These results suggest that 2 distinct differentiated cell types, fat and muscle, may use similar mechanisms to control the expression of a single gene (GPD) during development and in response to several modulators. Experiments are in progress to determine whether genomic sequences necessary for regulation of GPD expression in adipocytes are the same required for regulating GPD expression in muscle.

TISSUE-SPECIFIC CONTROL OF MOUSE ALCOHOL DEHYDROGENASE EXPRESSION
L 228 Howard J. Edenberg, Lisa S. Patterson, Ke Zhang, and William F. Bosron, Indiana University School of Medicine, Indianapolis, IN 46223.

There are three isozymes of alcohol dehydrogenase in mice, which differ in both catalytic properties and tissue distribution. ADH-AA, encoded at the *Adh-1* locus, is the main ethanol-active alcohol dehydrogenase. It is expressed at high levels in mouse liver, and is responsible for most of the ethanol oxidation in the mouse. We have isolated and sequenced a cDNA encoding the mouse liver ADH-A subunit, and are using it to examine the control of ADH-AA expression in different tissues.
The amount of the ADH-AA isozyme in mouse liver is under genetic control: there are strains such as C57BL/6J that have twice as much liver ADH-AA as other strains such as DBA/2J. We find a corresponding twofold difference in the steady-state level of ADH-A mRNA in their livers. The mRNA extracted from all strains tested was the same length, approximately 1.4 kb. The excellent correspondence between mRNA level and enzyme level suggests that control is exercised through regulation of the steady-state level of mRNA. We have found RFLPs in the 5' portion of the *Adh-1* gene that correlate with this genetic control of quantitative expression, and are analyzing genomic clones to determine the differences between high and low-activity strains.
We are comparing the ADH-AA enzyme activity, immunoreactive protein, and the steady-state level of its mRNA in other tissues. There is little or no ADH-AA activity and no detectable ADH-A mRNA in heart, brain, or testis. There is both ADH-AA activity and mRNA in kidney. ADH-A mRNA in kidney is the same size as in liver, and is present to an extent proportional to the relative amount of enzyme.

KINASE-RELATED GENE PRODUCTS REQUIRED FOR YEAST CELL-TYPE
L 229 SPECIALIZATION, B. Errede, M. Teague, and L. Connell, University of North Carolina, Chapel Hill, NC 27514.

The unicellular eukaryote, *Saccharomyces cerevisiae* is a model system for the study of cell-type specialization. The **a** and α cell-types of this yeast are haploid; they are specialized for mating with each other. The **a**/α cell-type is diploid; it is unable to mate but is capable of meiosis and sporulation. With the exception of the mating type locus, *MAT*, all cell-types contain the same genetic information. The presence of either the **a** or the α allele at *MAT* determines whether cells will express the respective **a**-specific or α-specific genes and corresponding functions. The presence of both **a** and α alleles in diploid cells heterozygous at *MAT* causes repression of haploid specific genes. Expression of haploid specific genes depends on several regulatory determinants in addition to those encoded at *MAT*. Some of these have been identified by sterile (*ste*) mutations that prevent mating in both **a** and α haploid cells. The *STE7* and *STE11* genes, which are among this group, have been found to control the abundance of steady state mRNA from several known haploid specific genes. We have sequenced the *STE7* and *STE11* genes. The predicted amino acid sequence of each gene product has significant homology to the catalytic domain of protein kinases. Site-directed mutagenesis was applied to the *STE7* gene. Several substitution mutations at three distinct regions in the predicted kinase catalytic domain were isolated. Mutation of residues within the kinase-like domain causes a nonmating phenotype in yeast. This finding is consistent with the hypothesis that protein phosphorylation may be one function of the *STE7* gene product. The observation that the predicted *STE11* gene product also has homology to protein kinases suggests that a regulatory cascade involving multiple phosphoproteins may underlie cell-type specialization in yeast.

L 230 DELINEATING CIS-ACTING ELEMENTS RESPONSIBLE FOR GROWTH AND CELL CYCLE REGULATION OF THE MURINE DHFR GENE USING EPISOMAL VECTORS, John N. Feder and Robert T. Schimke, Stanford University, Stanford, CA, 94305.

Autonomously replicating EBV derived plasmids have been constructed containing various portions of the murine DHFR gene. Included in the constructs are deletions of 5′ end sequences, variations in intron number as well as variations in the length of the 3′ end. Human 293 cells have been transfected with these constructs and clones isolated. We plan to delineate what portions of the murine DHFR gene are involved in it's transcriptional response to serum factors as well as those sequences necessary for cell cycle regulation. This will be carried out by assaying steady state mRNA levels in serum starvation experiments and in cell cycle stage specific populations of cells as generated by centrifugal elutriation. These data should help in determining if the two processes are mechanistically coupled or disparate.

L 231 TWO PATHWAYS OF HUMAN PROINTERLEUKIN 1 GENE REGULATION. M. Fenton, B. Clark, K. Collins[1], A. Webb[1], and P. Auron[2], M.I.T., Wellesley College[1], and Tufts Univ.-NEMC[2]. Interleukin 1 (IL-1) is synthesized by monocytes as a polypeptide precursor (proIL-1) in response to various stimuli such as bacterial endotoxin (LPS) or phorbol myristic acetate (PMA). Two distinct human IL-1 genes (IL-1 α and IL-1 β) have been identified and sequenced. IL-1 β transcription is rapidly and transiently induced in THP-1 human monocytic leukemia cells stimulated with LPS. Cycloheximide (CH) treatment does not alter the initial kinetics of message production, but does result in a 2-fold message superinduction. PMA stimulation of IL-1 β expression in THP-1 cells is neither transient nor affected by CH treatment. Following initial induction with LPS, THP-1 cells are refractory to further LPS stimulation but can be re-induced with either PMA or CH. Similarly, THP-1 cells treated for 24 hr with PMA, to down-regulate protein kinase C (PKC), can still be induced to express IL-1 β mRNA with LPS. Thus, the proIL-1β gene may require two distinct factors for proper regulation; a pre-existing transcriptional activator and a newly synthesized repressor. Also, LPS and PMA appear to activate the proIL-1 β gene by different pathways, one of which acts via PKC. THP-1 cells treated with both LPS and PMA undergo a morphological transition from spherical non-adherant cells to macrophage-like flattened and attached cells. Therfore, PMA and LPS may induce cellular differentiation in coordination with IL-1 β expression.

L 232 STUDIES ON THE GENERAL FACTORS REQUIRED FOR INITIATION OF TRANSCRIPTION AT TATA SEQUENCE CONTAINING PROMOTERS, Osvaldo Flores and Danny Reinberg, Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School at Rutgers, Piscataway, NJ 08854-5635.

Initiation of transcription at various class II promoters composed only with the minimum sequences (TATA box and CAP site), required five transcription factors (IIA, IIB, IID, IIE and IIF) in addition to RNA polymerase II. These factors were required for the formation of a preinitiation complex that could initiate transcription, upon addition of the ribonucleoside triphosphates, in the presence of heparin or Sarkosyl concentrations that inhibited an unbound factor.

Previous studies have indicated that initiation of transcription required the hydrolysis of the $\beta-\gamma$ bond of ATP or dATP. Furthermore it was also suggested that TFIIE contained a DNA dependent ATPase activity. Our studies on the purification of TFIIE resulted in the isolation of two transcription factors, TFIIE and TFIIF, both free of any detectable ATPase activity; however initiation of transcription at various promoters with the reconstituted system still required the hydrolysis of the $\beta-\gamma$ bond of ATP. The role of ATP hydrolysis and of the above-mentioned factor in transcription of different TATA sequence containing promoter will be discussed.

L 233 GENE TRANSFER MEDIATED BY PROTEOLIPOSOMES, Susan Gould-Fogerite, Joseph E. Mazurkiewicz and Raphael J. Mannino, Albany Medical College, Albany, NY, 12208. Recently, a system for delivering significant quantities of molecules to the cytoplasm of essentially 100% of a population of animal cells in culture has been developed in our laboratory. The system utilizes large, unilameller, phospholipid vesicles which have the biologically active envelope glycoproteins of either influenza or Sendai virus integrated in the lipid bilayer. Analogous to their functions in the viruses, these proteins mediate binding to animal cells and fusion with cellular membranes, resulting in the introduction of vesicle contents directly into the cytoplasm. Sendai vesicles fuse gradually, without manipulation, whereas influenza vesicles can be activated to fuse essentially simultaneously by brief exposure of cell-bound vesicles to low pH. High or low molecular weight molecules have been encapsulated within the vesicles at high efficiencies; including nearly 40% of an added solution of large plasmid DNA. Fluorescently labeled antibodies, a protein (Phalloidin) which bound to its intracellular target (actin), and enzymes, have been delivered to cells in quantities similar to those achieved with microinjection. Sendai vesicle-mediated delivery of a bovine papilloma virus-based plasmid resulted in stable expression at 100,000 times greater efficiency than calcium phosphate precipitation, with respect to the quantity of DNA needed to achieve maximum transfection. Preliminary experiments indicate transient expression in at least 20% of the population. Current studies include 1) maximizing in vitro delivery of naked DNA 2) delivery DNA-protein complexes and 3) in vivo gene transfer.

L 234 DEVELOPMENT OF DNA AFFINITY TECHNIQUES TO PURIFY RNA POLYMERASE II TRANSCRIPTION FACTORS. Susan Garfinkel, Therese Brendler, Brian Safer, Roger B. Cohen, and John A. Thompson. Section on Protein Biosynthesis, LMH, NHLBI, NIH, Bethesda, MD, 20892.
Affinity adsoprtion, precipitation and partitioning techniques have been developed in order to purify and characterize RNA polymerase II transcription components from whole cell extracts (HeLa) and nuclear extracts (K562, regenerating rat liver). Using multicopy constructs of specific promoter elements of the Ad2 major late promoter (MLP), transcription factors can be efficiently isolated by affinity precipitation and transcriptional activity of the extracts can be efficiently depleted. By DNase I footprinting, gel electrophoresis mobility shift assays, and affinity labeling, two proteins which bind to the upstream promoter sequence (UPS) of the MLP (M_r=40,000 and 116,000) have been identified. Addition of partially purified preparations of either the 40 kilodalton or the 116 kilodalton proteins does not restore transcriptional activity to the depleted extracts. Alternatively, extracts can be depleted of activity using α-amanitin affinity chromatography to remove RNA polymerase II and associated factors. Highly purified RNA polymerase II is also not sufficient to restore transcriptional activity. However, RNA polymerase II and associated factors co-purified from active extracts were found to restore activity to the depleted extracts. The potential of UPS binding proteins and RNA polymerase II associated factors to modulate transcriptional activity is being further investigated using techniques of affinity sequestration of specific proteins with sequence-specific synthetic oligonucleotides.

L 235 RAINBOW TROUT METALLOTHIONEINS: CHARACTERIZATION OF TWO DISTINCT cDNA SEQUENCES. L. Gedamu, M. Zafarullah and K. Bonham. Department of Biology, University of Calgary, Calgary, Alberta, Canada

The rainbow trout hepatoma (RTH) cell line responds to heavy metals such as zinc and cadmium by synthesizing the ubiquitous thiol-rich protein metallothionein (MT). From this cell line we have isolated two full length cDNA clones, tMT-A and tMT-B, which encode two similar but distinct trout metallothioneins. The clones were isolated by virtue of the cross-homologies between the trout-MT mRNAs and a human MT riboprobe. Clones tMT-A and tMT-B code for proteins 61 and 60 amino acids in length respectively, the one extra amino acid in tMT-A is accounted for by an apparent insertion at position 31 of the protein. In addition, there are two other amino acid changes between the two iso-forms. Overall, the coding regions show extensive homologies to mammalian MT's, especially at the cysteine residues and at a core sequence at the boundary of the two domains. However, closer examination reveals a number of significant differences in positions usually invariant in the mammalian MTs. Northern blot analysis of RNA from metal treated RTH cells demonstrates MT-mRNA is induced to high levels by zinc, low levels by cadmium and minimally by copper. In contrast, intraperitoneal injections of rainbow trout demonstrated that all three metals induce MT-mRNA to comparable levels in the liver. Southern blot analysis of trout DNA cleaved with a variety of restriction enzymes suggests that the trout family of metallothionein genes is probably limited to these two members.
(Supported by NSERC and AHFMR)

L 236 REGULATION OF THE HUMAN DIHYDROFOLATE REDUCTASE GENE, Merrill E. Goldsmith and Kenneth H. Cowan, Clinical Pharmacology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

Dihydrofolate reductase (DHFR) levels are induced by conditions which affect cell growth. Among these are growth modulation following release from serum or amino acid deprivation, viral infection, hormonal induction, and incubation in methotrexate under noncytotoxic conditions. Two expression systems were developed to study regulation of the human gene. In one system, cells stably transfected with a DHFR minigene were used to study DHFR induction in response to methotrexate as well as our previously described growth modulation following release from either serum or amino acid deprivation. Previous studies indicated that the growth modulators apparently utilized both transcriptional and post-transcriptional control mechanisms. DHFR levels increased 6 fold after 24 hr incubation in 2 μM methotrexate. Minigene deletion analysis suggested that methotrexate modulated DHFR levels transcriptionally. In the second expression system, DHFR promoter sequences were coupled to the chloramphenicol acetyl transferase (CAT) gene protein coding sequence. The effects of SV40 T antigen on DHFR regulation were studied by transfecting DHFR-CAT into T antigen expressing (COS) and non-expressing (CV-1) cells. Expression of DHFR-CAT was 6 fold higher in T antigen expressing cells. Incubation of hormone-responsive human breast cancer cells transfected with DHFR-CAT for 24 hr in 1 nM estradiol resulted in a 10 fold increase in DHFR-CAT expression. Thus, estrogen and SV40 T antigen appeared to modulate DHFR at the level of transcription. Deletion analysis of the promoter has been initiated in order to determine the critical nucleotide sequences.

L 237 GENE TRANSFER MEDIATED BY PROTEOLIPOSOMES, Susan Gould-Fogerite, Joseph E. Mazurkiewicz and Raphael J. Mannino, Albany Medical College, Albany, NY, 12208.

Recently, a system for delivering significant quantities of molecules to the cytoplasm of essentially 100% of a population of animal cells in culture has been developed in our laboratory. The system utilizes large, unilameller, phospholipid vesicles which have the biologically active envelope glycoproteins of either influenza or Sendai virus integrated in the lipid bilayer. Analogous to their functions in the viruses, these proteins mediate binding to animal cells and fusion with cellular membranes, resulting in the introduction of vesicle contents directly into the cytoplasm. Sendai vesicles fuse gradually, without manipulation, whereas influenza vesicles can be activated to fuse essentially simultaneously by brief exposure of cell-bound vesicles to low pH. High or low molecular weight molecules have been encapsulated within the vesicles at high efficiencies; including nearly 40% of an added solution of large plasmid DNA. Fluorescently labeled antibodies, a protein (Phalloidin) which bound to its intracellular target (actin), and enzymes, have been delivered to cells in quantities similar to those achieved with microinjection. Sendai vesicle-mediated delivery of a bovine papilloma virus-based plasmid resulted in stable expression at 100,000 times greater efficiency than calcium phosphate precipitation, with respect to the quantity of DNA needed to achieve maximum transfection. Preliminary experiments indicate transient expression in at least 20% of the population. Current studies include 1) maximizing in vitro delivery of naked DNA 2) delivery DNA-protein complexes and 3) in vivo gene transfer.

L 238 TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL CONTROL OF RETROVIRAL GENE EXPRESSION IN EMBRYONAL CARCINOMA CELLS, Manuel Grez, Frank Hilberg and Wolfram Ostertag, Heinrich-Pette-Institut, Abteilung Zellbiologie, Martinistrasse 52, 2000 Hamburg 20, Federal Republic of Germany.

A retroviral host-range mutant of the myeloproliferative sarcoma virus (MPSV) which is expressed in the embryonal carcinoma cell lines F9 and PCC4 has been molecularly cloned and analyzed. This new virus, named PCMV, differs from MPSV by two major deletions one of which spans exactly one of the two 75 base pair repeats of the long terminal repeat (LTR). Functional analysis of the PCMV LTR showed that this region is essential for the increase transfer of neomycin resistance to PCC4 cells. Recombinant viruses containing the U3 region of PCMV and the body of MPSV are functional in PCC4 cells. Moreover, a chimeric LTR containing PCMV sequences upstream from position -150 joined to the promotor region of the moloney murine leukemia virus (Mo-MuLV) is fully active in embryonal carcinoma cells. However, recombinant viruses containing the U3 region of PCMV and the body of Mo-MuLV are only weekly expressed in EC cells suggesting that additional mutations outside from the U3 region of the LTR are require for the efficient expression of retroviral genomes in EC cells.

L 239 THE REGULATION OF EXPRESSION OF THE HUMAN β-GLOBIN GENES. F. Grosveld, M. Antoniou, E. deBoer, G. Habets, J. Hurst, G. Kollias, F. MacFarlane and N. Wrighton. National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

The human β-like globin genes are a cluster of five active genes. During development the ε-gene is expressed in the yolk sac, the foetal γ^G and γ^A genes in the foetal liver and δ and β primarily in adult bone marrow. We are studying the mechanism responsible for this pattern of expression by the introduction of globin gene constructs in cultured erythroid cells or transgenic mice. The analysis of a series of transformed cells and transgenic mice has shown: 1) a tissue-specific enhancer element downstream from the β-globin gene. This element can act on other genes in a tissue and developmental specific pattern in either orientation. 2) A tissue-specific promoter element that confers inducibility of the promoter in differentiating MEL cells. In addition, we have preliminary evidence for a DNA element that regulates the complete β-globin locus at all stages of development. We are presently characterizing the factors acting on these elements by in vitro binding and transcription assays.

L 240 CELL-TYPE SPECIFIC EXPRESSION OF THE RAT GROWTH HORMONE GENE IS MEDIATED BY A CIS-ACTING NEGATIVE REGULATORY SEQUENCE, Sylvain L. Guerin and David D. Moore, Dept. Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

Large fragments containing the rat growth hormone promoter and 5' flanking sequences direct strongly pituitary cell-type specific expression when transiently transfected into a variety of cell types. Removal of 5' flanking sequences generates a 236 base pair minimal rGH promoter which is active in both pituitary and non-pituitary cell types. Reinsertion of a -526 to -237 rGH 5' flanking fragment in either orientation immediately upstream represses the activity of the minimal rGH promoter 8 to 20 fold in non-pituitary cell types, but has no repressive effect on expression in pituitary cells. Insertion of the rGH fragment 1.6 kilobases downstream from the promoter has no significant effect on expression in either type of cell. The cell-type specific repression conferred by the rGH fragment acts only in cis. However, introduction of a high copy number plasmid containing the rGH 5' flanking fragment as a specific competitor in non-pituitary cells causes significant increases in expression of otherwise repressed rGH promoter constructs. This strongly suggests that in non-pituitary cells a trans-acting negative regulatory factor, absent or modified in pituitary cells, binds to the cis-acting sequence.

L 241 RAPID INDUCTION OF NEW TRANSCRIPTS FOLLOWING PLANT DEHYDRATION, Felix D. Guerrero and John Mullet, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843-2128.

Excised pea plants were rapidly dehydrated and then incubated for up to 4 hrs and the level of the hormone abscisic acid (ABA) quantified. After a 30 minute lag, ABA levels rapidly increased with time to a level 50 to 100 times higher than in non-stressed plants. Pretreatment with transcription inhibitors actinomycin D or cordycepin inhibited the induction of ABA, suggesting that increased ABA synthesis in dehydrated plants requires nuclear gene transcription.

Polyadenylated RNA was isolated, translated in vitro using a wheat germ extract system and translation products separated by two-dimensional gel electrophoresis. New translation products were selected within 30 minutes after the onset of dehydration. These products were found to differ from changes induced by heat shock or pretreatment of non-dehydrated plants with exogenous ABA. Plant nuclei were isolated from control and dehydrated plants. Transcription in the isolated nuclei was optimized for RNA polymerase II and ^{32}P-RNA synthesized in vitro was used as a probe for differential screening of cDNA libraries from dehydrated plant polyA$^+$ RNA.

DNASE I HYPERSENSITIVE SITES WITHIN LYT2 AND T CELL RECEPTOR GENES.
L 242 Ann Haberman, John Krawiec, and Yasuhiro Hashimoto, Department of Pathology, University of Pennsylvania, PA 19104
We have observed a pair of hypersensitive sites within the J-C intron of the beta 2 gene of the T cell receptor that differ in their sensitivity to DNase I digestion. These sites occur within T cells but not B cells or fibroblasts and are therefore likely to be tissue specific. Both B and T cells however, display a very strong hypersenitive site mapping to the first intron of the alpha chain constant region. Similiar studies of the upstream regulatory sequences of the Lyt2 gene are underway. We are currently investigating potential correlations of discrete developmental stages with the expression of hypersensitive sites within pre-T cell tumors. Comparison of those sequences comprising the hypersenitive regions to each other and to other lymphoid specific regulatory sequences such as immunoglobulin enhancers will also be presented.

ORGANIZATION OF A MAMMALIAN REPLICON, J. L. Hamlin, J. E. Looney, and P.
L 243 Dijkwel, University of Virginia School of Medicine, Charlottesville, VA 22908.
Our laboratory is interested in aspects of mammalian chromosome organization that facilitate DNA replication and transcription. We have isolated a series of overlapping recombinant cosmids that represents the entire amplified dihydrofolate reductase (DHFR) domain from a methotrexate-resistant CHO cell (J. E.Looney and J. L. Hamlin, Mol. Cell. Biol., in press). We believe that this 260 kb amplified unit (amplicon) is equivalent to a chromosomal replicon, since it contains a single origin of replication in its center, and is the initial unit of amplification in virtually all methotrexate-resistant Chinese hamster cell lines that we have examined. Our aim is to determine whether the DHFR amplicon represents a chromosomal loop in the parental drug-sensitive CHO cell, and, if so, what structural rearrangements occur in this loop during active transcription and replication processes. The outline of the map of functional sequences within the amplicon so far suggests that the DNA is attached at several places along its length, notably in the body of the DHFR gene, near the origin of replication, and, based on preliminary experiments, close to the ends of the amplified unit. Preferred topoisomerase II cutting sites are also observed at several positions in the chromatin of the amplicon; some are transient and some appear to be permanent throughout the cell cycle. Our working hypothesis is that chromosomal loops are relatively fixed sequence domains that are defined by permanent anchorage sites to the matrix (possibly through the agency of topoisomerase II), but that other elements such as origins of replication and genes become transiently associated with the matrix to facilitate interaction with the multiprotein complexes of transcription and replication.

Growth regulation of the transcription of ribosomal RNA precursor and upstream spacer sequences in rat rDNA

Christina A. Harrington and Dona M. Chikaraishi

L 244 Transcription of the rat ribosomal (rDNA) gene and adjacent flanking regions of the spacer was studied in actively growing vs. slowly growing or growth arrested hepatoma tissue culture cells. The number of active polymerase I molecules associated wth the DNA template was decreased in cells whose growth was arrested by removal of serum from the medium or whose growth was slowed by high cell density. The RNA chain elongation per polymerase I molecule in isolated nuclei was also lower in slowly growing or arrested cells. The distribution of active polymerase I molecules along the rDNA gene was assayed by hybridizing nascent RNA chains labeled in vitro with M13-rDNA recombinants either in solution or in dot blots. We have previously shown that sequences upstream of the 45S precursor rRNA gene are transcriptionally active both in vivo and in vitro (C. Harrington and D. Chikaraishi, in press). Surprisingly, transcripts from a region between -1.76 and -.168 in the spacer vary in transcription levels in a manner different than precursor rDNA regions in response to growth state alterations. In addition, the relative proportion of spacer transcription is higher in cells arrested by serum removal than in cells whose growth is slowed by high density. These results suggest that upstream spacer transcription may correlate with the growth state of the cells. It is possible that these spacer regions are involved in the regulation of rDNA transcription in response to the cellular environment.

L 245 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 pairwise 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. Factors present in the nuclei of mouse cells interact with one of the enhancer elements (β). The other elements do not interact with factors under the conditions employed and they do not compete with the β element for factor binding. We are currently fractionating nuclear extracts to purify these factors and we are studying their interaction with DNA at the nucleotide level.

L 246 ALPHA ADRENERGIC REGULATION OF CARDIAC GENE TRANSCRIPTION DURING HYPERTROPHY IN CULTURED MYOCARDIAL CELLS, S. A. Henderson, H. R. Lee, M. N. Sills, R. S. Meidell, D. Yuan, K. R. Chien, University of Texas Health Science Center, Dallas, TX 75235.
Alpha adrenergic (A) stimulation of neonatal rat myocardial cells (RMC) produces hypertrophy and in increase in myofibrillar protein content. To examine if this increase is due to alterations in RMC gene expression, we measured the steady state levels of myosin light chain-2 (MLC-2) mRNA by northern and slot blot hybridization, MLC-2 gene transcription by nuclear run-on analysis, and MLC-2 mRNA stability by ^{3}H-uridine pulse-chase studies. Following stimulation with phenylephrine (0.1mM), there was a 2-5 fold increase in MLC-2 mRNA, a >2 fold increase in ^{32}P-UTP incorporation into total nascent transcripts following in vitro pulse labeling of nuclei isolated from control (C) and A treated RMC ($6{:}27\times10^{6}$ cpm; A: 58×10^{6}; n=8, $p<0.01$), and a 2-4 fold increase in MLC-2 gene transcription, while MLC-2 mRNA stability remained unchanged. These increases were dose dependent, inhibited by prazosin, and not affected by propranolol or yohimbine. Non-myocardial cells display no increase in transcription following A treatment. Thus, A stimulation of RMC hypertrophy may be largely regulated through increases in gene transcription.

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FORMATION OF THE 3' END OF U1 snRNA. Nouria Hernandez, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

U1 and U2 snRNAs are thought to be transcribed by RNA polymerase II, because the synthesis of these RNAs is sensitive to low concentrations of α-amanitin. The RNAs are not polyadenylated, and the formation of their 3' ends requires a short conserved sequence, the "3' box", located a few nucleotides downstream from the RNA-coding region of the genes. We have shown that, in addition, 3' end formation of U1 requires a compatible U1 or U2 snRNA promoter, and that polymerase II promoters from mRNA-encoding genes cannot substitute for the snRNA promoters. This observation suggests that snRNA genes are transcribed by a specialized transcription complex, which somehow differs from the transcription complexes that synthesize mRNAs. It also suggests that the 3' ends of U1 and U2 snRNAs are formed by termination of transcription or by a processing event intimately coupled to transcription. We are now defining the precise promoter sequences required for the 3' end formation of U1. We find that a snRNA enhancer is necessary; by introducing point mutations in the enhancer region, we are examining whether we can define separate domains involved either in initiation of transcription or in 3' end formation. In addition, we are modifying the proximal region of the promoter by linker scanning mutations to determine whether additional promoter elements are involved in the formation of the 3' end of U1.

L 248 A STRUCTURE AND FUNCTION STUDY OF THE C-TERMINAL DOMAIN OF THE LARGEST SUBUNIT OF EUKARYOTIC RNA POLYMERASE II, C.J. Ingles, L.A. Allison, M. Moyle and D. Fitzpatrick. Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

DNA sequencing studies of the gene, RPO21, encoding the largest subunit of RNA polymerase II in yeast, Drosophila and hamster cells have indicated that this eukaryotic RNA polymerase II subunit differs from the analogous subunit B' of prokaryotic RNA polymerase by the addition of an unusual C-terminal domain. Each RPO21 polypeptide has a tandemly repeated heptapeptide sequence at its C-terminus. The yeast polypeptide has 26 repeats, the Drosophila 42, and the hamster 52 repeats of the same consensus Tyr Ser Pro Thr Ser Pro Ser sequence. We have suggested that this domain could provide an array of contact sites for interaction between RNA polymerase II and proteins that bind DNA and regulate transcription from adjacent start sites. Using integrative transformation we have now constructed a series of yeast strains containing altered RPO21 alleles. By progressively removing DNA encoding the heptapeptide repeats and transforming haploid and diploid yeast strains we have established the minimum number of repeats that is necessary for RNA polymerase II function. By precisely exchanging yeast and Drosophila C-terminal domains we have shown that this domain functions in a species-dependent fashion.

L 249 STRUCTURE AND EXPRESSION OF THE MOUSE THYMIDYLATE SYNTHASE GENE, Lee F. Johnson, James DeWille, Tiliang Deng, Dawei Li and Chung-Her Jenh, The Ohio State Univ., Columbus, Ohio 43210.

We are studying the structure and expression of the thymidylate synthase (TS) gene in cultured mouse fibroblasts. When serum-arrested cells are stimulated to proliferate, the rate of synthesis of TS and the content of TS mRNA increase at least 9-fold as the cells traverse S phase. This is due to an increase in the rate of transcription of the TS gene and to more efficient polyadenylation of TS mRNA in S phase cells than in G1 phase cells [Jenh, Geyer and Johnson, Mol. Cell. Biol. 5, 2527 (1985)]. Analysis of the sequence of TS cDNA revealed that the UAA stop codon is followed immediately by a poly(A) sequence, indicating that mouse TS mRNA lacks a 3' untranslated region. This was confirmed by comparing the genomic and cDNA sequences as well as by S1 and northern blot analyses. The entire TS gene has been cloned and sequenced to determine the intron positions as well as the flanking regions of the gene. S1 assays showed that TS gene transcription initiates from several locations in a 100 nucleotide region. Although there are no TATAA or CCAAT boxes in this region, there are consensus binding sequences for the transcriptional regulatory proteins Sp1 and USF (or MLTF). TS minigenes that have normal 5' and 3' flanking regions but lack introns have been constructed and transfected into TS(-) hamster cells. The minigenes are regulated properly if they contain at least 1 kb of 5' flanking DNA, but improperly if they contain only 0.25 kb of 5' flanking DNA. Present studies are directed toward identifying the sequences that are responsible for proper regulation of the TS gene as well as determining the of the roles of Sp1 and USF in the transcription of this gene.

L 250 Negative Control of Bovine Papillomavirus Transcription
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Treatment of Bovine Papillomavirus (BPV) 1 transformed mousefibroblasts with cycloheximide or puromycin led to a 10-fold increase in the amount of viral transcripts after as little as 1 h of protein synthesis inhibition. Nuclear run-on experiments showed an about 7-fold increase in specific transcriptional activity after cycloheximide treatment. The half-life of BPV 1 mRNAs was twice as long as in untreated controls.
Two differentially spliced mRNAs were previously shown to be transcribed from the BPV 1 open reading frame E2, which mediate trans-activation and repression of viral transcription, respectively. We inserted linkers with stop codons at the NcoI and KpnI site, which should knock-out one or the other function. Cycloheximide stimulation turned out to be unaffected by both mutations. Cycloheximide treatment had no effect on the amount of viral transcripts in cervical carcinoma derived HeLa, C4-1 and SiHa cells, containing Human Papillomavirus (HPV) 18 and HPV 16, respectively.

L 251 **CIS- ACTING SEQUENCES AND TRANS- ACTING FACTORS REGULATE THE EXPRESSION OF THE RAT α - AND β - MHC GENES.** **G. Koren, B. Nadal-Ginard and V. Mahdavi,** Howard Hughes Medical Institute, Children's Hospital and Harvard Medical School, Boston. Expression of the α -myosin heavy chain (MHC) gene is restricted to ventricular and atrial cardiac muscles while expression of the β-MHC gene is specific for ventricular cardiac and slow skeletal muscles. Induction of the expression of the α-MHC gene by thyroid hormone (T_3) accompanied by de-induction of the β-MHC gene is observed in the ventricles as well as in primary cardiocytes maintained in serum-free culture conditions. To determine the gene sequences required for the muscle specific expression and T_3 regulation of these genes, a series of MHC minigene and reporter (CAT) genes were constructed with successive deletion of 5' flanking DNA sequences and used in transient gene expression assays. Expression of β -MHC gene constructs carring 1500 to 650Bp of upstream sequences was observed in differentiated C_2 mouse muscle cells where the endogenous β-MHC gene is expressed at low level as well, but not in myoblasts or non-muscle cells. Progressive deletions of sequences upstream of position -320 abolished expression of the transfected β -MHC gene indicating that DNA sequences between -650 to -320 are required for the developmental and muscle-specific expression of this gene. Expression of α -MHC gene constructs with 1800Bp of upstream sequences was also detected, albeit to a much lower level, in C_2 myotubes where the α-MHC endogenous gene is not expressed. The level of expression of the transfected and endogenous α - and β -MHC genes was not affected by T_3 in the C_2 myotubes. However, T_3 dramaticaly increased the level of expression of both transfected and endogenous α -MHC genes in rat primary cardiocytes. These results show that muscle-specific expression of the α - and β -MHC genes and hormonal regulation of the α -MHC gene by T_3 involves interaction of 5' cis- regulatory sequences with tissue- specific trans- acting factors.

L 252 **GENE REGULATION IN THE DOPA DECARBOXYLASE SUBCLUSTER OF DROSOPHILA MELANOGASTER**

John Kullman and Theodore R.F. Wright, Department of Biology, University of Virginia, Charlottesville, Virginia 22901

The Dopa Decarboxylase (Ddc) region of Drosophila melanogaster is a cluster of 18 functionally related genes involved in catecholamine metabolism, cuticle formation and female fertility. The cluster can be divided into two distinct subclusters and we have focused our studies on the six "C" genes located within the Ddc subcluster. Using P-element transformation we have examined the role that clustering plays in controlling the expression of these genes. We have found that these genes are expressed in ectopic positions as individual genetic units allowing us to conclude that the expression of a specific gene's activity is not dependent on its inclusion in a chromosomal domain or the result of complex post-transcriptional processing events. We have determined the order of these genes making it possible to assign cDNAs and mRNAs to particular lethal complementation groups as well as assess the amount of 5'flanking sequence necessary to express five of the six "C" genes. Preliminary evidence exists that a cis-acting regulatory element (CRE) may control the expression of one gene pair while a different CRE contols a second gene pair.

Gene Regulation in Eukaryotes - B

L 300 TRANSCRIPTIONAL ACTIVATION AND DNASE I HYPERSENSITIVITY OF PLANT DEFENSE GENES

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Activation of plant defense genes was examined by analysis of transcripts completed in vitro in isolated nuclei. Elicitor treatment of suspension-cultured bean cells caused a marked stimulation of transcription of genes encoding cell wall hydroxy-proline-rich glycoproteins (HRGP) and the phenylpropanoid biosynthetic enzymes phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS). Increased transcription was observed 5 min after elicitor treatment in the case of PAL and CHS and 2 hr after elicitor treatment in the case of HRGP. For all three genes, increased transcription coincided with the onset of rapid accumulation of the corresponding mRNAs and hence with the expression of the phytoalexin (PAL, CHS) and lignin (HRGP) defense responses. Increased transcription of these genes was also observed in hypocotyls infected with races of the fungal pathogen Colletotrichum lindemuthianum. Transcriptional activation was observed in directly infected and uninfected tissue indicating the transmission of an intercellular signal for defense gene activation. Multiple DNAseI hypersensitive sites have been found in the regions surrounding the CHS genes in elicitor-treated cells. We are presently mapping these sites and analysing their regulation in individual members of the CHS gene family. We have also started to identify proteins that bind to these putative regulatory regions.

L 301 CIS- AND TRANS-ACTING FACTORS REQUIRED FOR TISSUE SPECIFIC EXPRESSION OF THE PRO-OPIOMELANOCORTIN GENE, J.L.M. Lebouille, L. Jeannotte, R.K. Plante, and J. Drouin, Institut de Recherches Cliniques de Montréal, Montréal,, Canada. H2W 1R7.

The gene encoding pro-opiomelanocortin (POMC), the precursor to ACTH, ß-endorphin and the melanotropins, is expressed in specific cells of the pituitary, in certain brains areas, in the testes, ovaries and placenta. We are characterizing cis- and trans-acting factors required for anterior pituitary specific expression of the POMC gene. POMC promoter utilization was assessed in a focus formation assay. Hybrid transcription units constituted of 5'-flanking POMC sequences fused to sequences coding for neomycin resistance (neo) were constructed. The pPOMCneo plasmids are about 50 times more efficiently utilized in AtT-20 cells, a mouse pituitary tumor cell line expressing POMC, than in fibroblasts, L cells. These plasmids exhibit partial activity in a growth hormone (GH)-expressing pituitary cell line GH_3. Deletion analysis of POMC upstream sequences suggest the presence of two DNA sequence elements required for promoter activity. We are currently characterizing nuclear extracts for the presence of factors which specifically bind the POMC promoter. Using extracts from AtT-20, GH_3 and L cells in gel retardation assays and footprinting experiments, we are investigating DNA binding activities which have the tissue distribution expected of trans-acting factors responsible for pituitary specific utilization of the POMC promoter. It will be particularly interesting to examine the binding of these trans-acting factors to POMC upstream sequences which were recently shown in this laboratory to bind purified glucocorticoid receptor and which are required for glucocorticoid inhibition of POMC transcription.

L 302 EVOLUTION OF MURINE IMMUNOGLOBULIN λ2 AND λ4 GENES. Fathia Mami and Thomas J. Kindt, Laboratory of Immunogenetics, NIAID, NIH, Bethesda, MD 20892

A genomic clone containing the λ constant (C) region genes, λ2 and λ4, has been isolated from a genomic library from the mouse strain SPE. SPE is an inbred strain derived from progenitors trapped near Grenada in Spain and has been classified as mus 3 or mus spretus. The λ chains of SPE are under investigation because this strain does not produce λ light chains of the Cλ1 type. The structure of the Cλ2-Cλ4 gene cluster in SPE has been conserved relative to laboratory mouse strains. Sequence comparison of SPE Cλ2 and Cλ4 genes indicate that these genes have evolved under different selective pressures. The Cλ2 genes are highly conserved (99% sequence identity) and the SPE gene appears to be functional. By contrast, the Cλ4 genes have significantly diverged in sequence and the SPE Cλ4 contains a large deletion relative to the BALB/c counterpart. This gene, which is not expressed in laboratory mouse strains presumably because of a fault in the joining (J) λ4 sequence, appears to have been under less stringent evolutionary pressure and is certainly a pseudogene in the SPE strain by virtue of the observed C region defects. The SPE Jλ4 gene includes all sequences required for a functional J gene. It appears that the Cλ4 gene became nonfunctional in an ancestral mouse species and that evolutionary drift may account for the accumulation of additional defects in this pseudogene.

L 303 NEURON-SPECIFIC EXPRESSION OF THE GnRH GENE IN TRANSGENIC ANIMALS
ANTHONY J. MASON, TIMOTHY A. STEWART AND PETER H. SEEBURG. GENENTECH INC., SOUTH SAN FRANCISCO, CA 94080.

The hypogonadal (hpg) mouse lacks a complete GnRH gene and consequently cannot reproduce (1). By introducing an intact GnRH gene into the genome of these mutant mice we obtained complete reversal of the hypogonadal phenotype. Transgenic hpg/hpg homozygotes of both sexes were capable of mating and producing offspring. Pituitary and serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin were retored to normal levels. Immunocytochemistry and in situ hybridization showed restoration of GnRH expression in the appropriate hypothalamic neurons of the transgenic hpg animals, demonstrating neural specific expression of the introduced gene. This system now gives us an opportunity to define regulatory DNA elements involved in neural-specific expression.

(1) A.J. Mason et al. Science (in press) 1986.

L 304 EVIDENCE FOR THE INTRINSIC REGULATION OF THE EXPRESSION OF THE ß-TUBULIN GENE ASSOCIATED WITH OPTIC NERVE OUTGROWTH IN THE CHICK, Jean-Marc Matter, Lidia Matter-Sadzinski and Isaac Bakst, The Salk Institute, San Diego, CA 92138.

The outgrowth of neuronal processes is dependent, among other things, upon the assembly of microtubules from their constituent tubulin subunits. Microtubules are heterodimers consisting of one α- and one ß-tubulin polypeptide and in the chick five functional ß-tubulin genes have been identified (Cleveland, D.W. & Sullivan, K.F., Ann. Rev. Biochem. 54:331-365, 1985). We have previously shown that in the chick retina there is a transient increase in the expression of the $ß_1$-tubulin gene that coincides closely with the outgrowth of the axons of the retinal ganglion cells. None of the other ß-tubulin genes shows a comparable increase in expression during this period. In situ hybridization has established that the 3.9 kb transcript encoded by the $ß_1$-tubulin gene is present almost exclusively within the ganglion cells. In cultured retinal explants, we have found that the $ß_1$-tubulin gene is only expressed when the culture conditions are adequate for the survival of ganglion cells and when neurite outgrowth occurs. A similar relationship between axonal outgrowth and expression of the $ß_1$-tubulin gene has been found during development of the chick optic tectum. We have recently used grafting experiments to determine whether the factors responsible for the regulated expression of the the $ß_1$-tubulin gene are intrinsic to the eye or are present in the surrounding embryonic tissues. In eyes grafted from 4 day old donor embryos into 7 day hosts, the $ß_1$-tubulin gene is expressed during the same time period as in normal eyes and appears not to be influenced by the age of the host. Taken together with our previous findings this suggests that the eye is largely autonomous in stimulating the expression of the $ß_1$-tubulin gene.

L 305 FUNCTIONAL ANALYSIS OF THE HUMAN γ-CRYSTALLIN GENE FAMILY

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The γ-crystallins of the human lens are encoded by a multigene family comprising 7 closely related members. Sequence analysis of five of these genes revealed that 3 correspond to potentially active gene members (G3,G4,G5) while the other 2 are closely related pseudogenes (G1ψ,G2ψ). The 5' flanking sequences from G3,G4,G5 were shown by gene transfer studies to direct transcription of the bacterial CAT gene in both primary chick lens epithelia and a rabbit lens cell line, but not in cultures of non-lens origin. In contrast, no transcriptional activity was detected from comparable flanking regions of G1ψ,G2ψ. Detailed sequence comparisons of the 5' regulatory regions revealed that active gene members contain several highly conserved domains which may constitute functional elements of a lens- specific γ-crystallin promoter. Expression of the coding sequences of G3,G4 and G5 from the human metallothionein IIA promoter in L cells followed by analysis of the encoded polypeptides allowed assignment of the individual genes to their corresponding translation products in the lens. These studies provide a framework for future investigations on the relationships between hereditary cataracts and putative lesions in the the human γ-crystallin gene family.

L 306 QUANTITATIVE TWO-DIMENSIONAL GEL ANALYSIS OF PROTEINS SYNTHESIZED IN SUBCLONES OF A RAT HEPATOMA CELL LINE. Mark J. Miller and Snorri S. Thorgeirsson, Laboratory of Experimental Carcinogenesis, NCI, Bethesda, MD 20892.

To examine the degree of clonal heterogeneity in the synthesis of polypeptides in neoplastic cells, single-cell subclones from the rat hepatoma cell line H4-II-E were isolated. Polypeptides from the clones were resolved on high resolution two-dimensional polyacrylamide gels, and quantitatively analyzed with a computerized two-dimensional gel analysis system developed in this laboratory. Only four qualitatively different spots were found which appear to be synthesized exclusively in one of the subclones. In contrast, 9.5% of the spots showed statistically significant quantitative differences when any one subclone was compared to any other. Most of the differences were less than 50%, though variations up to fourfold were observed. Different aged cultures of the same subclone showed similar numbers of quantitative differences, indicating that this variability was primarily associated with the cultures rather than the subclones. The distribution of quantitatively variable spots indicates that about 50% of the polypeptides in the same subclone may demonstrate such variability. Similar results were obtained with a second set of subclones derived from these primary ones. These results indicate that independent clonal cell lines quickly develop detectable quantitative differences in the expression of up to 50% of their polypeptides. These differences cannot, at present, be associated with the observable biology of the cells and probably reflects normal variations in the balance of cellular macromolecular synthesis.

L 307 IDENTIFICATION OF A SPECIFIC BINDING SITE FOR THYROID HORMONE RECEPTOR IN THE RAT GROWTH HORMONE PROMOTER, David D. Moore*, Ronald J. Koenig# and P. Reed Larsen#, *Dept. Molecular Biology, Mass General Hospital, Boston, MA 02114, and #Howard Hughes Medical Institute Laboratory, Dept. of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

Thyroid hormone (T_3) levels regulate the expression of a variety of genes in different tissues. In general, T_3 is thought to work by binding to a soluble intranuclear receptor protein, which then binds to DNA and influences the expression of nearby genes. We have partially purified T_3 receptor from rat liver, and examined its interaction with sequences in and around the rat growth hormone (rGH) gene, which is transcriptionally induced by T_3. Using electrophoretic band shift assays and footprinting techniques we have shown that the T_3/receptor complex binds specifically to a site within the rGH promoter. Analysis of appropriate deletion mutants of the promoter shows that this site is absolutely required for T_3 response of rGH. We conclude that T_3 receptor is a transcriptional regulatory protein which is activated by binding of T_3.

L 308 HNRNP CORE PROTEINS TYPE A1 ARE ENCODED BY A GENE FAMILY, C. Morandi, S. Riva, P. Tsoulfas, M. Buvoli, G. Biamonti, M.T. Bassi, A. Ghetti, Dipartimento di Genetica, Università di Pavia and IGBE CNR 27100 Pavia ITALY.

We have recently shown that some mammalian single stranded DNA binding proteins derive from hnRNP core proteins by specific proteolysis. Northern blotting experiments, using A1 cDNA as probe, reveal two major species of A1-specific mRNA at 1.4 and 1.8 Kb. These 2 mRNAs constitute about 1% of the total poly(A)$^+$ RNA. The difference in length of the two mRNAs is probably due to the use of two differential polyadenylation sites evidenced by the nucleotide sequence of A1 cDNAs. cDNA molecules corresponding to the 1.4 and 1.8 Kb mRNA were isolated from human liver and fibroblast cDNA libraries respectively. A direct comparison of the cDNA sequences also shows few differences both in the coding and in the non coding region. We can therefore conclude that there are at least two human genes encoding at least two different isotypes of the hnRNP A1 protein. These genes are highly conserved among different species (human, rat). Interestingly, the sequence conservation extendes also to the 3'-untranslated region. Southern blotting experiments and the restriction analysis of a human genomic DNA library in λ Charon 4A revealed the existence of numerous processed pseudogenes in addition to the expressed genes mentioned above.

L 309 c-myc EXPRESSION IN ADULT SKELETAL MUSCLE REGROWING FROM ATROPHY, Paul R. Morrison and Frank W. Booth, University of Texas Medical School, Houston, TX 77225

We have examined the expression of c-myc proto-oncogene in atrophied and regrowing fast-twitch skeletal muscle of adult female rats. Total cellular RNA was isolated by LiCl/urea procedure and dotted onto nitrocellulose. A c-myc DNA probe labelled with ^{32}P using the M13 hybridization procedure was used to determine the relative levels of c-myc in the RNA samples. c-myc mRNA levels were determined to be 39% higher in muscles after 2 days of recovery from atrophy, and 72% higher in muscles after 4 days of recovery. The role of c-myc in muscle recovering from atrophy is not known. However, we have previously noted a 26% increase in cytochrome c mRNA levels and a 28% increase in α skeletal muscle actin mRNA levels at the 4th day of recovery. (Supported by NIH grant AM 19393)

L 310 THE HUMAN α-SKELETAL ACTIN GENE ; EVIDENCE FOR THE ROLE OF MULTIPLE 5' FLANKING REGIONS IN TISSUE SPECIFIC EXPRESSION AND REGULATION George Muscat and Larry Kedes,The MEDIGEN Project, Department of Medicine, Stanford University School of Medicine And Veterans Administration Medical Center Palo Alto, CA 94304 .

Transfection into the cultured myogenic L8 and C2C12, and fibroblastic L.Tk$^-$ and NIH3T3 cell lines was used to investigate the transcriptional regulation of the human α-skeletal actin gene. We demonstrated that 2000 bp of the 5' flanking region directed high level transient expression of the bacterial chloramphenicol acetyl transferase (CAT) gene in differentiated mouse C2C12 and rat L8 myotubes, but not in mouse L.Tk$^-$ or NIH3T3 cells. Unidirectional 5' deletion analysis was used to delimit the boundary of cis-acting transcriptional elements. A region (–153 to –87) was shown to be an integral part of the promoter with respect to tissue specific developmental regulation during myogenesis. A cis-domain 3' of –90 was shown to interact with factors present in both myogenic and fibroblastic cells. Multiple regulatory upstream subdomains from –2000 to –726 and –626 to –153 were shown to respond to muscle specific factors with respect to positive modulation of transcription. Subcloning of these cis-domains (–2000 to –726,–726 to –87, –2000 to –1300,–1300 to –726, –726 to –36, –626 to –153,–153 to –87 and –153 to –36) into an enhancerless CAT vector (pA10CAT.2N) indicated all these elements encode the potential to modulate transcription independently .These sequences appear to be differentially utilized with respect to maximal expression in different myogenic cells. These observations are compatible with the possibility that the particular combination of domains used is dependent on the availability and/or relative levels of factors possessed by each cell type.

L 311 EVIDENCE FOR TRANSCRIPTIONAL ACTIVATION OF THE PLASMINOGEN ACTIVATOR GENE BY THE CATALYTIC SUBUNIT OF cAMP-DEPENDENT PROTEIN KINASE.

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Though gene regulatory effects of cAMP are well established, it remains uncertain, how cAMP signals are transmitted to responsive genes. In contrast to proposals invoking a role for regulatory subunits (RI/RII) of cAMP-dependent protein kinases (PK-A), our data strongly suggest that transcriptional activation may involve phosphorylation of trans-acting elements by the catalytic subunit (C) of PK-A. In particular, we have recently demonstrated that cAMP elevations produce rapid and reversible translocations of C but not RII subunits from the Golgi area to the nucleus of MDBK cells (1,2). We now have extended these immunocytochemical studies to a number of cell lines that respond to cAMP by transcriptional activation of the plasminogen activator gene. Data will be presented comparing levels of gene activation with the properties and subcellular distributions of PK-A subunits in wild-type and kinase-mutant LLC-PK$_1$ cell lines.

(1) Nigg, E.A. et al. (1985) Cell 41, 1039-1051.
(2) Nigg, E.A. et al. (1985) EMBO J. 4, 2801-2806.

L 312 A CIS-ACTING 18 BASE PAIR SEQUENCE MEDIATES cAMP-STIMULATED TRANSCRIPTION OF THE HUMAN GLYCOPROTEIN HORMONE α-SUBUNIT GENE, J. Nilson, B. Silver, J. Bokar, J. Virgin, E. Vallen, and A. Milsted, Case Western Reserve University, Cleveland, OH 44106

Cyclic AMP regulates transcription of the gene encoding the α-subunit of human chorionic gonadotropin in choriocarcinoma cells (BeWo). To define the sequences required for regulation by cAMP, we inserted fragments from the 5' flanking region of the α-subunit gene into a test vector containing the SV40 early promoter (devoid of its enhancer) linked to the bacterial chloramphenicol acetyltransferase gene. Results from transient expression assays in BeWo cells indicated that a 1500 bp fragment conferred cAMP-responsiveness to the CAT gene regardless of position or orientation of the insert relative to the viral promoter. A subfragment extending from -169 to -100 had the same effect on cAMP-induced expression. Furthermore, the entire stimulatory effect could be achieved with an 18 bp synthetic oligodeoxynucleotide corresponding to a direct repeat between positions -146 and -111. In the absence of cAMP, the α-subunit 5' flanking sequence also enhanced transcription from the SV40 early promoter. We localized this enhancer activity to the same -169/-100 fragment containing the cAMP response element. The 18 bp element alone, however, had no effect on basal expression. Thus, this short DNA sequence serves as a cAMP response element and also functions independently of other promoter-regulatory elements located in the 5' flanking sequence of the α-subunit gene.

L 313 THE UBX DOMAINOF THE BITHORAX COMPLEX; PRODUCTS AND REGULATION, Michael O'Connor, Mark Peifer, Rich Binari and Welcome Bender, Harvard Medical School, Boston.

The *Ubx* domain of the bithorax complex is responsible for determining the identities of the 3rd thoracic (T3) and first abdominal (A1) segments of the fly *Drosophila melanogaster*. Examination of mutations has shown that approximately 150 kb of DNA is required for normal *Ubx* function. Characterization of cDNA's has revealed that less than 6 kb is contained in spliced products. Most mutations do not map within known exons. In particular, deletions or mobile element insertions in an intron 30 to 50 kb downstream of the *Ubx* transcriptional start site cause partial transformation of T3 to T2 (*abx* and *bx* mutations), while deletions and insertions 10 to 50 kb upstream of the 5'end cause partial transformation of A1 to T3 (*bxd* and *pbx* mutants). Our current working hypothesis is that there are only a few proteins coded for by the *Ubx* domain, but these products are exquisitely controlled in a cell be cell manner by a complex array of cis-acting regulatory elements. The putative regulatory elements map up to 50kb from the Ubx 5' end and may account for much of the noncoding DNA. We have developed a method for cloning the entire 150kb *Ubx* domain as a contiguous unit so that we can examine directly what role particular sequences play in development. The scheme utilizes homologous and site-specific recombination to permit us to build plasmids with large DNA seqments from smaller ones. The building vectors are constructed such that the newly joined DNA sequences are contained within a P element, allowing them to be reintroduced into flies. We have used this strategy to reconstruct two large (30 kb) putative regulatory regions in a special version of the builing vector which contains an in frame fusion of the 5' *Ubx* exon to *lacZ*. One segment encompasses the sites of all known *abx* and *bx* mutations while the other contains DNA from the *bxd* region. We believe the former is responsible for directing *Ubx* expression in a portion of T3 while the latter directs expression in a part of A1. These constructs, as well as controls lacking the presumptive regulatory regions, have been injected into embryos and are being examined for tissue or cell-specific production of B-galactosidase.

L 314 SEQUENCE-SPECIFIC INTERACTIONS OF NUCLEAR FACTORS WITH THE INSULIN GENE ENHANCER. Helena Ohlsson and Thomas Edlund, Dept. of Microbiology, University of Umeå, S90187 Umeå, Sweden.

Cell-specific expression of insulin genes is controlled by at least two distinct 5' flanking elements. A transcriptional enhancer is located in the distal portion of 5' flanking DNA and another distinct regulatory element devoid of enhancer activity is located more proximal to the transcription start site. The activity of both elements is restricted to pancreatic B cells. We have shown that factors in nuclear extracts derived from an insulin-secreting cell-line, interact with at least 5 distinct regions within the insulin gene enhancer. Mutations which abolish these interactions also affect transcription of the insulin gene. Some of these interactions can only be seen in extracts prepared from insulin-producing cells, while others are observed in extracts prepared from a variety of cells. Fractionation of these extracts have shown that distinct proteins interact with different regions within the insulin 5' flanking DNA.

L 315 COORDINATE REGULATION OF THE MURINE ENDO A AND ENDO B CYTOKERATINS, Robert G. Oshima, Katrina Trevor and Elwood Linney, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Antisense Endo B cytokeratin RNA encoded by the first 625 bp of the cDNA in a MSV retrovirus vector was expressed in a derivative of the F9 embryonal carcinoma cell line (FOT5 cells). Two G418-resistant clones containing approximately 10 and 4 copies of the vector were selected which expressed a colinear transcript containing both neomycin and antisense Endo B sequences. Expression of a 5-fold excess of antisense Endo B RNA over endogenous, retinoic acid-induced Endo B RNA resulted in suppression of Endo B protein expression. In addition, the normal induction of Endo A, the type II cytokeratin which polymerizes with Endo B, was suppressed at the RNA and protein levels. Revertant clones, which synthesize little if any neo or antisense Endo B RNA, regain the ability to express the affected gene products in response to retinoic acid. These results indicate that the suppression of Endo B protein synthesis influences the stable levels of Endo A mRNA. Preliminary experiments suggest that Endo A mRNA may be suppressed at the transcriptional level.

L 316 CIS-ACTING ELEMENTS INVOLVED IN THE TISSUE SPECIFIC EXPRESSION OF THE RAT ALBUMIN GENE, M.O. Ott, P. Herbomel, J.M. Heard and A. Rollier-Mottura, Département de Biologie Moléculaire, Institut Pasteur, Paris, France.

A minimal fragment (-150/+16 bp) of the 5' end of the rat albumin gene is sufficient to ensure its tissue specific expression. Promoter activity is assayed at the RNA level (Sp6 analysis) and CAT protein produced in transiently transfected rat hepatoma cells of different phenotypes but of the same origin. Further deletions lead to differential steps of decrease in promoter activity depending on albumin positive or negative hepatoma cell line. Two domains (between -120 and -80) appear crucial for the tissue specific expression; their sequences are strongly conserved between the rat, mouse and human albumin genes. These domains seem to be binding sites for two distinct liver specific proteic factors, as shown by Cereghini et al., submitted. From internal deletions, we suggest involvement of more regulatory factors in the region downstream the CAAT box. This region appears necessary for the maintenance of the tissue specificity, not by itself but within the -150/+16 promoter, which is at least 250 times more efficient in albumin positive than in albumin negative hepatoma cells. In contrast, a -155/-55 fragment (and subpieces of it) behaves like an efficient enhancer, independent of its orientation and location "vis à vis" the HSV tk gene promoter and of the cell line used: albumin positive or negative hepatoma clone or rat fibroblast cell line. Linker scanning studies of this -150/+16 fragment of the rat albumin gene are carried out, in parallel with liver specific DNA binding proteins analysis, in order to understand the mechanisms of the albumin gene expression.

L 317 MAPPING OF THE GENE ENCODING THE 65 KILODALTON DNA BINDING PROTEIN OF HERPES SIMPLEX VIRUS TYPE 1. D.S. Parris[1], A. Cross[2], L. Haarr[3], D.J. McGeoch[2] and H.S. Marsden[2]. Ohio State University, Columbus, Ohio[1], MRC Virology Unit, Glasgow, Scotland[2], University of Bergen, Norway[3].

Herpes simplex virus type 1 (HSV-1) encodes a protein of molecular weight 65,000 which binds to double stranded DNA ($65K_{DBP}$). A monoclonal antibody (MAB 6898) directed against $65K_{DBP}$ has been isolated and used to demonstrate an association with the HSV DNA polymerase, suggesting a role for $65K_{DBP}$ in virus DNA replication. The monoclonal antibody does not react with any HSV-2 protein. This serotype-specificity enabled the antibody to be used together with a set of well-characterised HSV-1 x HSV-2 intertypic recombinants to map the gene encoding $65K_{DBP}$ to between coordinates 0.574-0.682 on the HSV-1 genome. This region is distinct from that encoding the DNA polymerase. The region encoding $65K_{DBP}$ was narrowed to coordinates 0.584-0.640 by hybrid arrest of translation experiments using the HindIII 1 fragment. We have determined the sequence of this 8.8Kbp HindIII 1 fragment and have identified three genes as candidates for encoding $65K_{DBP}$. Present efforts are directed towards determining which of these genes encodes the protein.

L 318 CHARACTERIZATION OF THE CIS-ACTING REGIONS THAT CONTROL TISSUE SPECIFIC EXPRESSION OR REPRESSION OF THE ACTINS AND LIGHT CHAIN GENES DURING MYOGENESIS, Bruce M. Paterson, Wolfgang Quitschke, Barbara Winter, Liliana DePonti, Ze-Yu Lin and Juanita Eldridge, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20892.

We have isolated the alpha cardiac and beta cytoplasmic actin genes, and the fast LC1/LC3 gene complex from the chicken in order to study gene regulation during myogenesis in vitro. Cis-acting regions from the actins and the LC1/LC3 genes that control tissue specific transcription and modulate levels of RNA expression have been defined. In vitro mutagenesis has been used to change specific sequence elements within these cis-acting regions to further define sequence elements involved in gene regulation. We are in the process of characterizing proteins that bind specifically to these regions in the hopes of defining tissue specific activators and repressors.

L 319 **A NON-FUSING, PROLIFERATING MUSCLE CELL LINE EXHIBITS NORMAL PATTERNS OF MUSCLE GENE EXPRESSION.** Kimber L. Poffenberger, Robert Wade[1], Larry Kedes[1], and Helen M. Blau. Departments of Pharmacology and Medicine[1], Stanford University School of Medicine, Stanford, CA 94305 and Veterans Administration Medical Center[1], Palo Alto, CA 94304.

Gene expression during myogenesis *in vitro* is characterized by a concurrent increase of muscle-specific transcripts and a decrease of many non-muscle transcripts. This pattern of regulation is observed *in vitro* in mouse muscle (C2C12) cells which characteristically withdraw from the cell cycle upon becoming confluent and which in fusion media (FM) fuse to form multinucleate myotubes.

We have isolated a C2C12-derived variant cell line which no longer fuses, but still expresses muscle-specific transcripts. Induction of muscle transcripts is delayed in the variant cells, but follows a similar pattern to that of parental cells. This demonstration that fusion is not essential for muscle differentiation has been shown by others with cell variants. The novel and striking property of our variant cells is that they are no longer contact inhibited and continue to proliferate in FM even as they are expressing muscle-specific differentiated functions. Thus, this cell line offers a unique opportunity to explore the relation between cell cycle control and the decision to differentiate. In addition, unlike other muscle cells, these variants are not lost as they differentiate and may therefore be particularly advantageous for studies of factors regulating the expression of muscle-specific genes.

L 320 UBIQUITOUS AND TISSUE-SPECIFIC EXPRESSION OF DIFFERENT RABBIT CLASS I MHC GENES. Marie-Christine Rebiere, Patrice N. Marche and Thomas J. Kindt, Laboratory of Immunogenetics, NIAID, NIH, Bethesda, Maryland 20892.

Studies of the major histocompatibility complex of the rabbit have indicated that they are 8 to 13 class I genes and a complement of class II genes similar in type and number of those of man. cDNA clones corresponding to transcripts of 4 different class I genes were obtained from a T-cell cDNA library and these have been structurally characterized. Genomic clones for 3 of the genes have been studied. Using Northern analyses and S1 mapping experiments, expression studies in various cells and tissues have begun using probes specific for these genes. Differential patterns of expression have been observed with 2 of them being ubiquitously expressed (although at varying levels) and another being limited to T cells, thymus, spleen and appendix. Constructs including the 5' region of these genes are being used to locate regulatory factors that bind in specific and non-specific fashions to sequences involved in differential control of the expression of the genes.

L 321 EXPRESSION OF T-PA IN C127 CELLS USING DIFFERENT POLYADENYLATION SIGNALS, Vemuri B. Reddy, Anthony J. Garramone and Nancy Hsiung, Integrated Genetics, 31 New York Avenue, Framingham, Massachusetts 10701.

BPV expression vectors were constructed for human t-PA cDNA using mouse metallothionein promoter and different 3' ends containing polyadenylation signals. We have used metallothionein, SV40 early and bovine growth hormone polyadenylation signals and found substantial difference in t-PA production levels in mouse cells. Both the SV40 early and bovine growth hormone 3' ends gave significantly greater t-PA expression levels (100-200 fold) than the metallothionein 3' end. The difference in protein levels correlated with the difference in mRNA abundance indicating that t-PA mRNA with metallothionein 3' end may be less stable. Actinomycin D procedure for mRNA half-life indicated that t-PA mRNA with SV40 early poly A sequences has a half-life of 8-10 hours similar to that of the cellular ß actin mRNA.

L 322 ISOLATION AND CHARACTERIZATION OF THE HUMAN TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE GENE. Lela K. Riley, Jennifer K. Morrow, Mary Jo Danton and Mary Sue Coleman. Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40536-0084.

Terminal deoxynucleotidyl transferase (TdT), a template-independent DNA polymerase, is normally expressed only in a subpopulation of pre-lymphocytes in the thymus and bone marrow. Malignant transformation of these cells often leads to over-expression of the enzyme. While the precise in vivo function of TdT is unknown, its expression has been correlated with gene rearrangements in pre-lymphocytes. In order to investigate the molecular basis for this selective expression of TdT, genomic clones containing the human gene have been isolated and characterized. Initially, full-length cDNA clones for TdT were isolated from a library generated from the human lymphoblastoid cell line, MOLT4R and confirmed using DNA sequence analysis. The cDNA was used to screen 6 x 10^5 isolates of a human genomic library. Fourteen overlapping genomic clones were identified which encompassed 35 KB of contiguous DNA. The clones contain 6.2 KB of 5' flanking and 9.4 KB of 3' flanking sequences. The gene contains at least 4 exons. Restriction maps have been constructed and exon/intron boundaries have been established. The 5' flanking regions of the gene have been sequenced to identify regulatory elements involved in tissue specific expression of TdT. This work was supported by a grant from the National Cancer Institute (CA19492).

L 323 INDUCTION OF ASYNCHRONOUS REPLICATION OF POLYOMA DNA BY ULTRAVIOLET IRRADIATION (UV) IS MEDIATED BY A TRANS ACTING FACTOR. Zeev Ronai, Esther Okin, Vincent DeLeo and I. Bernard Weinstein, Columbia University Cancer Center, New York, N.Y. 10032.

We have previously obtained evidence that induction of polyoma DNA replication by certain chemical carcinogens in the polyoma-transformed rat fibroblast cell line H3 is mediated by a trans acting factor (Lambert et. al., Carcinogenesis 7, 849, 1986). We have now extended this finding to the effects of UV irradiation. The action spectrum of UV light was first determined by exposing H3 cells to increasing doses of UV C (254nM), UV B (275-303nM) or UV A (308 - 400nM). The optimum dose for induction of polyoma DNA replication was 2 joules/m^2 of UV C. For UV B the optimum dose was 45 joules/m^2, and UV A was ineffective up to doses of 54 joules/cm^2. When normal rat 6 fibroblasts lacking polyoma DNA sequences were exposed to UV C (2 joules/m^2) and then fused to unirradiated H3 cells, using polyethylene glycol (PEG), this also led to induction of polyoma DNA synthesis by the H3 cells. A procedure was developed by which proteins obtained from Rat 6 cells could be packaged into red blood cells and then introduced into unirradiated H3 cells by PEG fusion. Using this procedure, we found that a protein fraction obtained from UV C irradiated rat 6 cells also induced polyoma DNA replication, whereas proteins obtained from unirradiated Rat 6 cells were inactive. This procedure is being used to further characterize the putative trans acting factor(s) produced by cells in response to DNA damage which enhances polyoma DNA replication. This system may serve as a useful model for elucidating various factors induced in mammalian cells in response to DNA damage.

L 324 REGULATION OF RAT MYOSIN LIGHT CHAIN GENES. Nadia Rosenthal and Maria Donoghue, Howard Hughes Medical Institute, Children's Hospital and Harvard Medical School, Boston, MA 02115.

In adult rat fast skeletal muscle, two myosin light chain proteins, LC_1 and LC_3, are produced from a single genetic unit. Although the two proteins are identical for the last carboxy terminal 141 amino acids, their amino termini differ in both length and sequence. The unique structures of the LC_1 and LC_3 proteins are generated by differential transcription from two promoters separated by 10 kilobases of DNA, accompanied by two alternate splicing pathways. In fetal muscle development, accumulation of the LC_1 mRNA precedes that of LC_3, suggesting that transcription and splicing of LC_1 and LC_3 messages is developmentally regulated. We have analysed the transcription of LC_1 and LC_3 mRNAs in the mouse myogenic cell line C2, and have shown that during differentiation, sequential accumulation of the two messages seen in vivo is retained in the C2 culture system. Expression vectors in which segments of cloned rat LC_1 and LC_3 promoter regions were linked to a CAT reporter gene were tested for expression in differentiating C2 cells. Although 1.5kb and 2.6kb upstream of LC_1 and LC_3 promoters respectively were included in the constructs, no CAT gene induction was observed. Addition of an SV40 enhancer to the constructs resulted in high level expression in muscle and non-muscle cells, indicating that the promoters are not tissue-specific. Results from current experiments testing the entire 25 kb light chain locus and adjoining sequences for distal enhancer activity will be presented.

L 325 A TRANSCRIPTIONAL ENHANCER IN THE FIRST INTRON OF THE MOUSE α_2(I) COLLAGEN GENE, Pellegrino Rossi and Benoit de Crombrugghe, Laboratory of Molecular Biology, National Institutes of Health, National Cancer Institute, Bethesda MD 20892.
We have identified an enhancer in the first intron of the α_2(I) collagen gene by cloning several fragments of the mouse α_2(I) collagen gene in a vector in which the early promoter of SV40, minus the SV40 enhancer, drives the gene for chloramphenicol transacetylase. The enhancer is localized between +400 bp and +1400 bp downstream of the start of transcription of the α_2(I) collagen gene. As for other enhancers, its activity is both orientation and position independent. The sequence of this segment contains several direct and inverted repeats and a segment with homology to the segment around the SphI site in the SV40 enhancer. The α_2(I) collagen gene enhancer stimulates the SV40 early promoter several fold more than the SV40 enhancer in NIH 3T3 cells. Subcloning of smaller fragments suggests it is composed of several elements. This enhancer also stimulates its cognate promoter by increasing at least tenfold the levels of correctly initiated transcripts. Studies to examine the interaction between the elements of this enhancer and several elements of the α_2(I) collagen promoter are in progress.

L 326 STUDY OF THE RAT TYROSINE AMINOTRANSFERASE GENE. CONTROL REGION INVOLVED IN TISSUE SPECIFIC REPRESSION. J. Roux, T. Grange, G. Majmudar and R. Pictet. Institut J. Monod Tour 43 - 2 Place Jussieu 75005 Paris, France.
The tyrosine aminotransferase (TAT) gene is expressed specifically in liver where its transcription is increased by glucocorticoids. In fibroblasts, the lack of expression of this gene has been shown to be mediated by a locus (Tse-1) located in mouse and human on a single chromosome (Killary and Fournier, 1984, Cell 38, 523). We have cloned 36 kb of the rat TAT genomic domain. We have studied some aspects of the regulation of the expression of the TAT gene by placing marker genes under the control of 9 kb of the TAT gene 5' untranscribed region and transfecting the constructions into cells in culture. We have obtained stably transfected hepatoma cells which express the bacterial xanthine guanine phosphoribosyl transferase (Ecogpt) gene under the control of the TAT gene regulatory elements. In these cells the Ecogpt activity is glucocorticoid inducible like the TAT activity. Both activities are extinguished in hybrids between these transfected hepatoma cells and fibroblasts. Moreover in fibroblasts which were stably transfected with the same hybrid gene, but not selected on its expression, the Ecogpt activity was not detected in 12 out of 13 analysed clones. When these non expressing fibroblasts were cultured in a medium containing xanthine and mycophenolic acid, Ecogpt expressing cells were selected with a frequency of 10^{-4}, but the Ecogpt activity was not modified by the presence of glucocorticoids. It has been reported that 2,5 kb of TAT gene 5' upstream region confer glucocorticoid inducibility not only in hepatoma cells but in fibroblasts as well (Schutz et al., 1986, Biol. Chem. 367 supp. p. 84). In contrast, using a transient expression assay, we found that in fibroblasts the chloramphenicol acetyl transferase gene placed under the control of the 9 kb of TAT 5' upstream region is not inducible by glucocorticoids.

L 327 DIRECT IDENTIFICATION OF SEQUENCE SPECIFIC DNA BINDING PROTEINS BY AFFINITY LABELING. Brian Safer, John A. Thompson, and Roger B. Cohen. Section on Protein Biosynthesis, LMH, NHLBI, NIH, Bethesda, MD 20892
A rapid affinity labeling procedure has been developed to directly identify sequence specific DNA binding proteins in crude nuclear extracts. Following the introduction of ^{32}P at specific phosphodiester bonds within a known DNA recognition sequence, crosslinking of specific bases to amino acid residues of proteins in direct contact with that site allows the transfer of radiolabel to the DNA binding protein. Detection of non-specific protein-DNA interactions and non-crosslinked ^{32}P probe are eliminated by nuclease digestion. The M_r of specific DNA binding proteins can then be determined by exclusion chromatography, of SDS PAGE followed by autoradiography. By this procedure, specific radiolabeling and identification of proteins binding to specific promoter sequences are readily achieved in crude extracts and can serve as the basis of their subsequent purification. The technique is also rapid in contrast to DNase I footprinting and gel electrophoresis mobility shift assays, both of which depend on titration of probe:protein:competitor DNA ratios. Using this approach with the upstream promoter sequence (UPS) of the Adenovirus 2 major late promoter (Ad 2 MLP) radiolabeled at specific bases, the RNA Pol II transcription factor which protects this site has been identified as a M_r =40,000 polypeptide. In addition, a second M_r = 116,000 polypeptide which competes for binding to the UPS has been identified using cloned Ad 2 MLP DNA as well as synthetic oligonucleotides radiolabeled at a unique pyrimidine residue within the UPS. Other factors involved in transcriptional regulation are currently being characterized by this procedure.

L 328 A NEGATIVE REGULATORY ELEMENT WITH FEATURES SIMILAR TO ENHANCERS, Jeffrey D. Saffer, The Jackson Laboratory, Bar Harbor, ME 04609. Study of the regulation of a cellular SV40-like bidirectional promoter has revealed the presence of a negative regulatory element with characteristics similar to those of enhancers. This element functions independently of orientation and position; but rather than enhance transcription initiation, it reduces transcription. This "reducer" element shows a lack of promoter specificity by decreasing expression in both directions from its natural promoter and by decreasing expression from the SV40 and human β-globin promoters and a polymerase III transcribed Alu family member. At least part of the negative element is contained in a 40 bp fragment that contains 20 bp of potential Z-DNA forming sequence. Such reducer elements may be involved in fine tuning the expression from a variety of promoters.

L 329 MULTIPLE CIS-ACTING ELEMENTS CONTROL EXPRESSION OF THE RAT CYTOCHROME C GENE, Richard C. Scarpulla and Mark J. Evans, Northwestern Medical School, Chicago, IL 60611 The mitochondria of animal cells are comprised of over 400 different proteins nearly all of which are encoded in the nuclear genome. Cytochrome c is a mitrochondrial respiratory component whose expression can vary over a 50-fold range in the cells of mammalian tissues and whose biosynthesis is hormonally controlled. To initiate the investigation of nuclear regulatory events that control mitrochondrial function, deletion mutants of the rat cytochrome c gene were constructed and their activities monitored upon introduction into animal cells. Several distinct cis-acting regions in the 5'flanking DNA influenced the basal level of gene activity in transfected CV-1 cells. Two discrete sites in the far upstream region (-491/-326) and (-215/-159) contributed equally to a 4-fold enhancement of gene expression. A proximal upstream region (-146/-66) contained two conserved CCAAT sequences and had a 10-fold effect on promoter function. The transfected gene displayed a 5 to 10-fold greater dependence upon the -215/-159 promoter element when introduced into COS-1 cells, a rapidly proliferating, noncontact inhibited derivative of CV-1. Co-transfection experiments with pRSV-T, a T antigen producing vector, demonstrated that this site was not directly trans-activated by the SV40 T antigen expressed in COS-1 cells. Thus, at least one of the multiple cis-acting regions in the cytochrome c promoter can mediate a cell-specific difference in cytochrome gene expression. Further analysis of these promoter elements should ultimately lead to the elucidation of molecular mechanisms governing the coordinate regulation of nuclear encoded mitochondrial genes in animal cells.

L 330 DNase I SENSITIVITY OF THE OVOMUCOID - OVOINHIBITOR GENE COMPLEX IN OVIDUCT NUCLEI AND RELATIVE LOCATION OF CR1 REPETITIVE SEQUENCES, Maxwell J. Scott, Ming-Jer Tsai and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

We have examined the relationship of CR1 repetitive sequences and DNase I sensitivity in oviduct nuclei of sequences in a 47 kilobase (kb) region that contains five CR1 sequences and the related and transcribed ovomucoid and ovoinhibitor genes. In addition, we investigated whether, for each of several DNA fragments in this 47 kb region, the coding and non-coding strands of a sequence were equally DNase I sensitive. When hen oviduct nuclei were treated with DNase I until 15-20% of the DNA was rendered perchloric acid soluble, the coding strand of the ovomucoid gene, part of the ovoinhibitor gene and the intergenic region were preferentially depleted in concentration 2-3 fold as determined by solution hybridization analysis using single-stranded RNA probes. Downstream of the ovomucoid gene there is a broad transition over about 6-8 kb from a DNase I sensitive to resistant configuration. Three CR1 sequences occur within this transition region. Another CR1 is in a region of intermediate DNase I sensitivity within the ovoinhibitor gene. The fifth CR1 is in the DNase I sensitive intergenic region. Thus, the position of most of the CR1 sequences is consistent with the proposal that CR1s have a role in defining transition regions of DNase I sensitivity. Unexpectedly, the non-coding strand of sequences within and immediately adjacent to the 5' end of the actively transcribed ovomucoid and ovalbumin genes were less sensitive to DNase I digestion than their respective coding strands. The DNA strands of other sequences in the 47 kb ovomucoid gene region were equally susceptible to DNase I digestion.

L 331 REGULATION OF EXPRESSION OF THE MOUSE BETA2-MICROGLOBULIN GENE, Rho H. Seong and Jane R. Parnes, Stanford University, Stanford, CA 94305.

We have been studying control sequences involved in the regulation of expression of the mouse B2-microglobulin (B2M) gene. We have placed the 5' flanking sequence of the B2M gene in front of the bacterial chloramphenicol acetyl transferase (CAT) gene and then constructed a series of 5' deletion mutants with progressively less B2M sequence driving CAT expression. We have also created a series of deletion mutants from the 3' end of the B2M 5' flanking sequence to remove the B2M promoter. In these latter constructs, the CAT gene is driven by the SV40 promoter and B2M upstream sequences (without the B2M TATA box). All of these constructs were assayed by short-term transfection into mouse fibroblasts followed by enzymatic assay for CAT activity. We have been able to separate the B2M promoter from upstream enhancing activity in this way. We have found that transcriptional enhancing activity is almost maximal with less than 200 bp. The 5' and 3' borders of this activity have been approximately mapped. Our data suggest that, in this system, the B2M "enhancer" is at least as active as the SV40 enhancer when comparing both in front of the SV40 promoter. We analyzed the DNA sequence of the B2M "enhancer" and have found a similarity to a crucial core element of SV40 enhancer (TGGAAAGTCCC). The significance of this similarity to the SV40 enhancer core sequence is now being investigated by introducing site-directed mutations. We have also examined the ability of gamma-interferon to stimulate CAT activity driven by the B2M promoter and upstream sequence. We could detect no alteration in CAT activity with as much as 2kb of B2M upstream sequence, despite the fact that gamma-interferon receptors are clearly present on these fibroblasts, as evidenced by an increase in cell surface expression of H-2K. It therefore seems likely that the sequence(s) responsive to gamma-interferon induction lie either 5' or 3' of the promoter region (i.e., within or downstream of the gene).

L 332 HUMAN GLYCOPHORIN A AND B ARE ENCODED BY DISTINCT AND SINGLE GENES NEGATIVELY AND COORDINATELY REGULATED BY THE TUMOR-PROMOTING PHORBOL ESTER, 12-O-TETRADECANOYL-PHORBOL 13-ACETATE (TPA), P.D. Siebert and M. Fukuda, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

The major sialoglycoprotein of the human erythrocyte membrane termed glycophorin A belongs to a family of related proteins that include two or more members termed glycophorin B and C. We have previously shown that treatment of the human erythroleukemic cell line, K562, with the tumor-promoting phorbol ester, TPA, results in a reduction of the cell surface expression of glycophorin A and levels of de novo biosynthesis. We have recently isolated several glycophorin A-cDNAs and have examined both the structural relationships of the glycophorin genes and their regulation by TPA. By Southern and in situ gel hybridization of genomic DNA utilizing cDNA and synthetic oligonucleotide probes we find that glycophorin A and B are encoded by distinct and single copy genes with a strong region of homology in the 5'-portion of the coding region. By northern and RNA dot blot experiments we find that the level of glycophorin A and B transcripts are negatively and coordinately regulated by TPA. A time course showed that the effect is rapid, within one hour following treatment levels of glycophorin transcripts drop to approximately 50% of control levels. Glycophorin transcripts were not present in human promyelocytic HL-60 cells suggesting that the exclusive expression of the glycophorin in erythroid cells is due to mechanisms preceding protein biosynthesis. (Supported by NIH grant CA-33000.)

L 333 REGULATION OF EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I GENES IN CELL LINES DERIVED FROM MIDSOMITE STAGE MOUSE EMBRYOS, Toby Silverman, Bonnie Orrison, John Langloss,* Gary Bratthauer,* Jun-ichi Miyazaki, Keiko Ozato and Alan Rein**, LDMI, NICHD, NIH, Bethesda, MD, *AFIP, Washington, D.C., and ** NCI-Frederick, Frederick, MD

Expression of MHC class I genes is developmentally regulated; both class I mRNA and surface proteins begin to be expressed at low levels after the midsomite stage of mouse embryogenesis. We have established a series of cell lines from gestational day 8 and day 11 mouse embryos and have examined the expression of class I genes. These cells exhibit characteristics of midgestational embryos and are negative for SSEA-1, a marker for early embryos and EC cells. On the basis of the patterns of class I gene expression, 4 different classes of cells can be defined: 1) no class I mRNA, no surface expression, 2) low class I mRNA, low surface expression, 3) moderate class I mRNA, moderate β2 microglobulin, no surface expression, and 4) moderate class I mRNA, moderate surface expression. We have examined the transcriptional regulation of MHC class I genes in these cells by using CAT fusion genes connected with the class I promoter region. Previously we found that transcription of class I genes is negatively regulated in F9 cells via a regulatory element (Class I Regulatory Element, CRE) in the 5' flanking region of the genes. The CRE, however, enhances class I gene expression in differentiated cells showing constitutive class I expression. We show that in some day 11 cells, the CRE exerts neither enhancer nor inhibitory activity, suggesting the existence of an intermediate stage in the developmental regulation of class I genes. Data for day 8 cells will also be presented.

L 334 An Approach for the Molecular Cloning of Genes Encoding Sequence-Specific DNA Binding Proteins. Harinder Singh, Jonathan H. LeBowitz and Phillip A. Sharp, Center for Cancer Research and Department of Biology, 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 on 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 cognate DNA substrate. We have established conditions for the specific detection of E. coli clones expressing high levels of either the bacteriophage λ O protein or the EBV nuclear antigen EBNA-1 using labeled DNAs containing the appropriate high affinity binding sites. This methodology has been extended to the isolation of two distinct mammalian cDNA clones that appear to encode the same sequence-specific DNA binding protein. Analysis of these clones will be presented.

L 335 GENE REGULATION DURING EARLY DEVELOPMENT OF DICTYOSTELIUM DISCOIDIUM, Charles Singleton and Suzanne Manning, Vanderbilt University, Nashville, TN 37235

We have identified twenty genes which are deactivated upon the onset of development in Dictyostelium. The rates of loss of each corresponding mRNA are similar to one another but distinct, and the overall decreases range from five to 1000 fold during the first eight hours of development. Several detailed characterizations of the deactivation events place the genes into three groups. The majority of the genes fall into group A: deactivation is dependent upon progress through the developmental program and upon developmental conditions (shaking the cells so as not to allow normal cell to cell interactions abolishes the decrease); it is independent of protein synthesis; and it is not seen in a group of aggregation deficient mutants apparently blocked during early development. One gene has been identified for group B. Its proper regulation is also independent of protein synthesis and dependent on progress through development but is not affected by developmental conditions. In almost all aggregation deficient mutants, regulation of this gene is normal. However, in one strain a novel form of misregulation occurs: the gene is not expressed at any time, even in vegetatively growing cells. Group C is also represented by one gene. Proper expression is dependent on the absolute time after initiation of development instead of progress through development. In the absence of protein synthesis, the mRNA level increases with time in development instead of the normal decrease. Finally, no aggregation deficient mutants have been found which misregulate this gene. Collectively, these characterizations reveal differences in behaviour which suggest the existence of at least three mechanisms for deactivation of gene expression upon the onset of development in Dictyostelium.

L 336 TRANSCRIPTIONAL REGULATORY ELEMENTS OF U4 SMALL NUCLEAR RNA GENES, William E. Stumph, Kathleen J. McNamara, Gina M. Korf and Michael L. Hoffman, San Diego State University, San Diego CA 92182.

The haploid genome of the American domestic chicken contains only two genes that code for U4 small nuclear RNA (Hoffman et al., Mol. Cell. Biol., in press). These two genes are closely linked within 465 bp of each other and are organized with the same transcriptional orientation in the genome. The downstream gene codes for chicken U4B RNA, whereas the cloned upstream gene (denoted the U4X gene) codes for a variant U4 RNA that differs at 7 base positions. Like U1 and U2 RNA genes, the U4B gene is transcribed by RNA polymerase II and contains distal and proximal regions in the 5'-flanking DNA (located approximately 200 and 55 base pairs upstream of the RNA cap site respectively) that are required for efficient U4B gene expression. Deletion of the distal element reduces U4B gene expression in microinjected oocytes to approximately 25% of wild type, whereas a further deletion encompassing the proximal region completely eliminates detectable U4B gene expression. The U4X gene, although not efficiently expressed in injected oocytes, appears to be expressed in chicken cells even though it contains potentially inactivating point mutations in both the distal and proximal control elements. Interestingly, a GC-rich 16 base pair perfect inverted repeat is present at positions -72 to -57 upstream of the U4X gene. This sequence (AGCGCGCCGGCGCGCT), which is a good candidate for a regulatory element, is not present upstream of other cloned snRNA genes. Thus, the expression of the U4X gene may be governed by a novel regulatory mechanism compared to the chicken U4B gene and other snRNA genes that have been studied.

L 337 TISSUE SPECIFIC METHYLATION OF THE HUMAN THYMIDINE KINASE GENE, Vicki L. Traina-Dorge, Melanie Ehrlich, and Prescott Deininger, L.S.U. Medical Center, New Orleans, LA 70112.

DNA methylation patterns of the human thymidine kinase (tk) gene were characterized in normal human tissues. Methylation patterns were tissue-specific. Low levels of methylation were observed at the 5' end of tk, and high levels at the 3' gene end in germ line tissue (sperm), and extraembryonic tissue (placenta and chorion). The converse pattern of hypermethylated 5' terminus and hypomethylated 3' terminus was demonstrated in differentiated somatic tissues (brain, heart, and lymphocyte). Major differences observed were due to specific sites flanking the gene both 3' and 5'. Specific 5' tk sequences (through the first exon) were generally hypomethylated in all tissues tested. Increased hypermethylation of the last 67% of the gene was characteristic of sperm, while methylation extended only 36% in the brain and heart. Placental DNA showed an intermediate pattern. These data suggest that hypomethylation of the 5' flanking sequence may allow the rapid expression of tk during gametogenesis and embryogenesis. De novo methylation of these specific sites both 5' and 3' of the gene may be responsible for the final loss or decrease of expression or a marking of tk for future regulation during cell differentiation and the change to nonproliferation.

L 338 A NOVEL TRANSCRIPTION FACTOR WHICH BINDS TO THE CHICKEN OVALBUMIN UPSTREAM PROMOTER SEQUENCE, Sophia Y. Tsai, Heng Wang, Lee-Ho Wang, Ikuko Sagami, Ming-Jer Tsai and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

A transcription factor which binds to the chicken ovalbumin promoter sequence (COUP) spanning between -70 and -90 is required for efficient transcription of the ovalbumin gene. This COUP transcription factor has been purified from HeLa cells using both conventional column chromatographic techniques and a specific DNA affinity column. It binds specifically to the ovalbumin promoter and its binding cannot be competed by the CCAAT containing DNA fragments of the chicken β-globin and the HSV-thymidine kinase genes. In contrast, the binding of the CCAAT binding protein to the chicken β-globin promoter is totally abolished in the presence of HSV-tk promoter but not affected by the presence of the COUP sequence. Furthermore, the COUP and the CCAAT binding proteins can be separated in an S300 column. Thus the COUP and the CCAAT binding proteins are different. Using methylation and ethylation interference assays we have defined the contact sites for purine bases and phosphate backbone and deduced a rotational symmetric sequences important for the binding of COUP transcription factor. In addition, another factor S300-2 is also required for the transcription of the ovalbumin gene. Kinetic analysis suggests that S300-2 may stabilize the COUP transcription factor - DNA complexes.

L 339 JC VIRUS GENOME EXPRESSION IN GLIAL CELLS OF THE HUMAN BRAIN IS NOT LIMITED TO A SPECIFIC SUBSET OF SUCH CELLS, Dominick Vacante, Kei Amemiya, and Eugene Major, NINCDS, NIH, Bethesda, Md. 20892

The human polyoma virus, JCV, causes demyelination in the human brain by lytic infection of the myelin producing oligodendrocyte. In cell culture, JCV propagation is generally restricted to cells derived from human fetal brain, principally glial cells. These cultures are comprised of a heterogeneous population of cells containing both astroglial as well as oligodendroglial cell types and their fetal precursors. To examine the cell specific nature of JCV gene expression in glial cells more accurately, we have used JC virions for infection and JCV DNA and recombinant DNA constructions for transfections and microinjections into separate populations of glial cells. These cells were "differentiated" on the basis of their phenotypic characteristics of reactivity to monoclonal antibodies against galactocerebroside (Gal c-oligo marker) and glial fibrillary acidic protein (GFAP-astrocyte marker). Data accumulated from these experiments indicate that JCV gene expression is most efficient in glial cells of the human brain but is not restricted to only the oligodendroglial class of cells. These data are also substantiated by our observations of biopsy brain tissue from JCV infected patients showing JCV DNA replication and virion antigen present in astrocytes. It would appear that those cell factors which recognize JCV DNA sequences to allow its expression are found in both the oligodendrocyte and the astrocyte.

L 340 REGULATION OF GENE EXPRESSION FOR THE 20kD GLYCOPROTEIN OF THE T3/T CELL RECEPTOR COMPLEX (T3-δ chain).

Peter van den Elsen*, Katia Georgopoulos and Cox Terhorst.
Lab. of Molecular Immunology, Dana-Farber Cancer Institute, Boston, MA 02115.

Transcription of the gene coding for the 20kD glycoprotein of the T3/T cell receptor complex is evident only in T-cells as assessed by Northern blotting experiments and not in other cell types. In order to study the mechanism of this apparent tissue-specific expression we have isolated and characterized the genes encoding both the human and murine T3-δ chain. Both genes have an identical organization and comprise 5 exons. Interestingly they do not contain the so-called TATA and CAAT boxes found in many eukaryotic promoters. DNA fragments comprising the putative promoter sequence have been isolated and fused to the gene coding for chloroamphenicol transferase (CAT). These hybrid plasmids were introduced into a variety of cells and tested for the production of CAT enzyme in a transcient assay. The results of these analysis have shown that the activity of the T3-δ promoter is not restricted to T-cells only. Nuclear run-off assays have indicated that initiation of transcription also occurs in non T-cells. These results indicate that events post the initiation of transcription are responsible for the tissue-specific appearance of the T3-δ mRNA.

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L 341 ANATOMY OF THE REGULATORY REGION OF THE HUMAN uPA GENE. P. Verde, S. Boast, A. Franzé, A. Lania and F. Blasi. - International Institute of Genetics and Biophysics, Via Marconi 10, 80125 Naples, Italy.

The human urinary type plasminogen activator gene (uPA) represents a model system for the transcriptional regulation of gene expression. Its activity is differentially modulated by various hormones and growth factors and the transformed neoplastic phenotype has been associated with a drastic increase in the level of uPA transcription. To identify the DNA sequences implicated in the control of uPA gene expression and in order to study the function of trans-acting factors which bind to the DNA sequences upstream to the TATA box, we have used the bacterial CAT gene as a reporter gene to assay the activity of the uPA promoter. DNA segments representing various extensions of the 5' end of the uPA gene have been fused to the CAT gene and the CAT activity, reflecting the relative efficiency of the promoter, has been measured in transiently transfected cells. This analysis demonstrates the presence of a DNA element required for full expression of the uPA gene. This element is located between -1900 and -2150 from the transcription initiation site. Fusion of these sequences to a heterologous promoter in both orientations strongly stimulates CAT activity, showing typical properties of a transcriptional enhancer. Preliminary results suggest that possible target sequences for induction by phorbol esters can be localized between -400 and the TATA box.

L 342 Derepression of the NAD-specific Glutamate Dehydrogenase of Neurospora crassa

P.J. Vierula and M. Kapoor, University of Calgary, Calgary, Canada

The catabolic, NAD-specific glutamate dehydrogenase (NAD-GDH) of Neurospora crassa is one of a multitude of enzymes controlled by catabolite repression. As assessed by in vitro translation of total poly(A+) RNA and immunoprecipitation with anti-GDH IgG, derepression is primarily under transcriptional control. NAD-GDH derepression begins within 30 min. of a shift of cells from a carbon rich medium to a minimal salts medium supplemented with glutamate. Transcript levels peak at about 60 min. after transfer and decline to maintenance levels of approximately 20% of peak levels.

The rapid NAD-GDH derepression response of N. crassa cells has suggested that a membrane associated component may be involved in maintenance of catabolite repression. To explore this possibility, repressed N. crassa cells were treated with sublethal concentrations of the membrane active antibiotics polymixin B and amphotericin B. Both treatments resulted in derepression of NAD-GDH at the transcriptional level but the kinetics of derepression by these two drugs are not identical. Whereas NAD-GDH activity in polymixin B-treated cells reaches a plateau within 2 hrs. of the start of treatment, activity continues to derepress at high rates for at least 4 hrs. after addition of amphotericin B. Experiments are currently in progress to delineate the molecular events associated with antibiotic induced derepression.

L 343 **DISTINCTIVE PATTERNS OF MUSCLE-SPECIFIC MRNA ACCUMULATION DURING HUMAN MYOGENESIS.** Robert Wade, Peter Gunning, Edna Hardeman[1], Reinhold Gahlmann, Helen Blau[1], & Larry Kedes. Departments of Medicine and Pharmacology[1], Stanford University School of Medicine, Stanford, CA 94305, and Veterans Administration Medical Center, Palo Alto, CA 94304.
We have isolated cDNA clones representing major muscle-specific mRNAs and have used them to monitor the degree to which transcripts accumulate in concert during the *in vitro* differentiation of cloned human primary myoblasts. The accumulation of transcripts encoding multiple isoforms of the major constituents of the muscle sarcomere (actins, myosin heavy chains, myosin light chains 1/3 & 2, troponins T, C, & I, alpha and beta tropomyosins), and several non-structural proteins (myoglobin, carbonic anhydrase III, ATP-ADP translocase, glyceraldehyde-3-phosphate dehydrogenase, & the heat shock protein HSP83) was examined over a 15 day myogenic time course as well as in fetal and adult human muscle. Differences were noted in both the time of appearance and the modulation of the absolute levels of a number of these transcripts. While several of the transcripts encoding slow-twitch sarcomeric proteins exhibited a degree of coordinate expression, a comparison of all transcripts demonstrated little if any identity in the patterns of mRNA accumulation. In addition, the pattern of transcript accumulation in human primary myoblasts differs substantially from that seen with rodent myogenic cell lines. These data suggest that each of these co-expressed muscle genes may follow its own myogenic program. These observations have important implications for the understanding of myofibrillar assembly and the role of common trans-acting reguatory factors in muscle development.

L 344 **TWO CELL-TYPE SPECIFIC FACTORS COMPETE FOR BINDING TO THE SKELETAL MUSCLE ACTIN PROMOTER.** Kenneth Walsh and Paul Schimmel, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
DNA fragments, which span the skeletal alpha actin promoter, form specific complexes with proteins from nuclear extracts of 11 different cultured cell types. These include two myocyte stage specific cell types—myoblast and myotube. We find that the myocyte nuclear extracts are distinguished from all of the others by the electrophoretic mobility of the predominant DNA complex. This complex is greatly diminished in the nine non-myocyte cell types, where a different complex predominates. The major myocyte and non-myocyte complexes are caused by distinct binding activities that compete for the same DNA sequence element. This element is located 78 nucleotides upstream of the transcription start site, within a region that is essential for expression and developmental regulation.

L 345 **Tn5 MUTAGENESIS OF THE SHORT UNIQUE REGION OF THE HERPES SIMPLEX VIRUS TYPE 1 GENOME,** Peter C. Weber, Myron Levine and Joseph C. Glorioso, The University of Michigan Medical School, Ann Arbor, MI 48109.
The short unique region (Us) of the herpes simplex virus type 1 (HSV-1) genome is suspected to encode 12 genes, based on nucleotide sequence and RNA transcript analysis. These include genes for two immediate early proteins, US1 (ICP22) and US12 (ICP47), and at least three glycoproteins, US4 (gG), US6 (gD), and US8 (gE). Currently, none of the Us gene products have well characterized functions and, with the exception of US1 and US8 through US12, no conditional lethal or deletion mutants for any of these genes are available. To explore the role of these genes in virus replication, we have employed a simple procedure for random mutagenesis of each putative Us gene. Cloned restriction fragments of Us were mutagenized in E. coli by random insertion of a bacterial transposon, Tn5, which is carried on a defective λ phage vector. The insertion of Tn5 into any coding region results in truncation of its corresponding gene product, as Tn5 carries stop codons in its terminal sequences. Accurate mapping of the positions of over 50 Tn5 insertions within the cloned HSV-1 sequences was performed by BamHI and HindIII digestions on the plasmid DNA. Insertions which are near the 5' end of the individual coding sequences were selected for cotransfection experiments in which mutant Us genes were recombined back into the genome. Recombinant mutant viruses were detected by hybridization of plaques blotted onto nitrocellulose filters using two 1.0 kb PstI Tn5 fragments as a radiolabeled probe. Numerous mutants have been isolated, suggesting that most of the Us functions are nonessential for HSV-1 growth in Vero cells, and that this procedure should be applicable for examining even larger regions of the HSV-1 genome.

L 346 TRANSCRIPTION OF SEA URCHIN HISTONE GENES IN EMBRYONIC NUCLEAR EXTRACTS, Eric Weinberg, Ling Tung, Gil Morris, and Insong Lee, University of Pennsylvania, Philadelphia, PA 19104.

Sea urchin H4 histone genes can be accurately transcribed in extracts prepared from embryonic nuclei. The extracts, prepared essentially by the methods of Morris et al., sustain transcription for at least 90 min. Both early and late embryonic histone genes can be transcribed in extracts from 9, 18, 30, and 36 hr embryos so the transcriptional regulation of these genes seen in vivo cannot at this time be reproduced in vitro. Nevertheless, the system has been valuable for determining essential cis-acting sequences and for testing shared factors by a competition assay. A set of 5' deletion mutants of the early H4 gene, when tested in the extract, indicate the presence of an essential sequence just upstream from the TATA box. This sequence has been found at this position in every early and late sea urchin H4 histone gene investigated thus far. Deletion analysis of the late H4 gene reveals a region essential for maximal transcription between -100 and -170. This region contains a sequence present in every late H4 gene but not present in early H4 genes. A two step competition assay shows that when an H4 gene is added to the extract in sufficient amounts, it sequesters essential factors needed to transcribe an H4 gene added subsequently. Early and late H4 genes both form stable transcription initiation complexes and share at least one factor essential for transcription. The transcription assay and gel-retardation methods are now being used to monitor fractionation of the extract to identify essential transcription factors.

L 347 TRANSCRIPTION OF THE Cμ GENE IN IgMIgG - BEARING B LYMPHOCYTES, Elizabeth A. Weiss and Dorothy Yuan. University of Texas Health Science Center, Dallas, Texas 75235

While resting B lymphocytes express IgM and IgD, a population of more differentiated B cells expresses IgM and IgG_3. Simultaneous IgM and IgD expression occurs by alternate processing of a 26 kb RNA transcript, Whether a similar mechanism allows concomitant IgM and IgG_3 expression is unclear since this would require an 82 kb transcript. DNA rearrangement in an $IgM^+IgG_3^+$ cell might delete the Cμ and Cδ genes, so that μ protein is a translation product of long-lived μ mRNA. To address this question, we examined splenic B cells which were activated by culturing with the mitogen, lipopolysaccharide. The IgG_3-bearing ($mIgG_3^+$) cells were isolated on a FACS on the basis of staining with biotinylated Protein A and FITC-Avidin. Double staining of sorted cells showed that 90% of the IgG_3^+ cells were also IgM^+. Surface iodination of cells demonstrated that the IgG_3 1) is the intrinsic membrane form and 2) is enriched in the sorted population. Biosynthetic labelling demonstrated active secretion of both IgM and IgG_3 in the sorted population. By in vitro pulse-labelling of nascent RNA, Cμ gene transcription, in addition to $C\gamma_3$ transcription, was demonstrated in a portion of the sorted cells at a level significantly higher that that which could be derived from contaminating $IgM^+IgG_3^-$ cells. Thus, alternate processing of a transcript encoding both Cμ and $C\gamma_3$ sequences must be the mechanism for concomitant μ and γ_3 heavy chain production in these cells. If an 82 kb transcript is made, one would now expect to see extension of transcription in $IgM^+IgG_3^+$ cells beyond the termination site of δ transcription in resting B cells which we have recently mapped to more than 4 kb 3' to $C\delta_{M2}$.

L 348 REGULATION OF β-GLOBIN GENE EXPRESSION BY 5' DNA SEQUENCES, Donna M. Williams[1], Rou-Lan Qian[2], Yu Gong[2], Alan N. Schechter[2], and Patricia E. Berg[2], [1]NHLBI and [2]NIDDK, National Institutes of Health, Bethesda MD 20892.

In an attempt to understand regulation of expression of the human β-globin gene by its 5' DNA sequences, we are studying fusions of the 5' flanking region to a heterologous gene, chloramphenicol acetyl transferase (CAT). Deletions were generated in the 5' flanking β-globin DNA using exonuclease Bal 31 and the fusion genes were introduced by calcium phosphate transfection into non-induced K562 cells, a human erythroleukemia cell line that normally cannot express β-globin. In transient assay studies, we observed no CAT activity with fusion genes retaining 639 or 610 bp 5' to the Cap site but did detect activity for deletions retaining 487 bp 5' to the Cap site. This suggests that negative regulatory factors may interact with this region of the flanking sequence. RNA analysis of cells expressing CAT after transfection indicates that the majority of βCAT message is correctly initiated. A deletion between -230 and -182 caused a loss of CAT expression in K562 cells, suggesting the loss of DNA needed for positive regulation. In contrast, this deletion did not cause decreased CAT expression in either Chinese hamster fibroblast cells or mouse erythroleukemia cells, which implies that the positive regulation may be specific for K562 cells. In parallel studies with hemin induced K562 cells either an increase or turning on of CAT activity was seen for all fusion genes except for the βCAT gene retaining only 182 bp 5' to the Cap site, which still showed no activity. These studies should facilitate the identification of the regulatory factors which modulate the expression of the human β-globin gene.

L 349 REGULATION OF SERUM AMYLOID A (SAA) EXPRESSION IN FIBROBLASTS TRANSFECTED WITH HUMAN SAA GENOMIC DNA. P. Woo,* M. Edbrooke,* H.R. Colten+, *Clinical Research Centre, Harrow, UK and +Department of Paediatrics, Washington University, St. Louis, USA.

Serum amyloid A, the precursor of amyloid A fibrils, is an acute phase reactant. Previous in vivo studies showed that interleukin 1 (IL1) and tumour necrosis factor (TNF) induce mouse SAA expression. In order to analyse the mechanism of cytokine control of SAA gene expression, an 18 Kb human genomic clone bearing the SAA gene was co-transfected with the herpes simplex thymidine kinase gene into murine L-cells using the calcium phosphate method. The SAA gene was constitutively expressed in three stable cell lines. Addition of recombinant human IL1 and tumour necrosis factor for 10-20 hrs induced a dose dependent increase in SAA specific mRNA. A corresponding increase in SAA synthesis and secretion was detected in transformants pulsed with ^{35}S-methionine. Addition of cyclohexamide abolished the cytokine-dependent increase in SAA mRNA. Therefore, the mechanism of induction of SAA expression is dependent on de novo synthesis of protein factor(s). Nucleotide sequences likely to be affected by these factors will be discussed.

L 350 AN IN VIVO ASSAY SYSTEM FOR TRANSCRIPTION FACTORS REGULATING THE HUMAN β-GLOBIN GENE N. C. Wrighton and F. Grosveld. National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

The five active human β-like-globin genes form a cluster extending over 60kb of DNA of order 5'-ε-^{G}γ-^{A}γ-δ-β-3'. During development differential globin gene expression occurs in a programme of changing tissue-specificity: the embryonic (ε) gene is expressed in embryonic yolk sac, foetal (^{G}γ and ^{A}γ) genes are active in foetal liver and adult (δ and β) gene transcription occurs in bone marrow. Globin gene transfer experiments into cell lines and transgenic mice have demonstrated the involvement of trans-acting regulatory factors in this process. This is further supported by cell fusion experiments in which normally silent globin genes are activated in MEL/K562 cell hybrids prior to nuclear fusion.
In order to construct a tester cell line capable of responding senstitively to factors regulating the β gene, a cDNA coding for human tissue-type plasminogen activator (t-PA) was inserted at the translation initiation codon of the β-gene. t-PA protein is detectable at extremely low levels using the fibrin-agar plaque assay. In addition, SV40 splice and poly-adenlyation signals were included to produce a mature transcript coding only for t-PA. The hybrid gene was introduced into L-cells and clones were fused to various erythroid and non-erythroid lines. The βt-PA construct in one clone, T1, could be substantially activated in heterokaryons by fusion to both induced and uninduced MEL cells, but not by fusion to HeLa or monkey kidney cells, nor when mixed but not fused to MEL cells. This tester cell line is currently being used to assay erythroid nuclear extracts for β-globin trans-acting factors by microinjection.

L 351 TRANSIENT EXPRESSION OF THE INTERFERON PROMOTER IN HUMAN CELLS. Steven Xanthoudakis, Deborah Alper, Adele Marshall and John Hiscott. Lady Davis Med. Res. Inst. and Dept. of Microbiology and Immunology, McGill University, Montreal, Quebec.

A human transient expression assay has been utilized to examine the inducible transcriptional activation of IFN-β and IFN-α promoters in a homologous cellular environment. Human 293 cells permitted Sendai virus or poly (rI-rC) inducible expression of IFNβ-CAT hybrid genes. Introduction of the SV40 enhancer 5' or 3' to the IFN-CAT gene increased basal (uninduced) levels of CAT activity; in one construct the SV40 enhancer-IFNβ regulatory region combination increased the inducibility of CAT activity 50-100 fold but in an orientation and position dependent manner. No expression from the IFNα-CAT hybrid gene was detected in 293 or HeLa cells, either at the level of CAT enzyme activity or mRNA production, indicating that human fibroblasts lack a factor required for expression of the IFN-α promoter. *In vivo* competition experiments were performed between IFN-CAT test genes and different transcriptional domains to examine the common and unique factors that interact with the IFN promoter. Nuclear protein extracts were prepared from myeloid leukemic KG-1 cells and analyzed for interferon specific DNA interactions by a gel electrophoresis DNA binding assay. On the basis of competition binding studies a protein was detected that bound specifically to the region -135 to -202 relative to the IFN-β mRNA start site. This factor may represent a nuclear protein responsible for repression of IFNβ transcription in the uninduced state.

L 352 TRANSIENT EXPRESSION OF THE INTERFERON PROMOTER IN HUMAN CELLS. Steven Xanthoudakis, Deborah Alper, Adele Marshall and John Hiscott. Lady Davis Med. Res. Inst. and Dept. of Microbiology and Immunology, McGill University, Montreal, Quebec.
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L 353 ISOLATION AND CHARACTERIZATION OF cDNA CLONES FOR HUMAN STEROID SULFATASE. P.H. Yen, E. Allen, B. Marsh, T. Mohandas & L.J. Shapiro, Harbor-UCLA Medical Center, Torrance, CA 90509. Steroid sulfatase (STS) is a microsomal enzyme that catalyzes the hydrolysis of a variety of 3β-hydroxysteroid sulfates. Deficiency of STS activity in humans results in X-linked ichthyosis. STS has been mapped to the distal end of the short arm of the human X-chromosome (Xp22.3-Xpter) and has been shown to escape X inactivation. We have isolated STS cDNA clones from a λgt11 placental expression library using antiserum against purified human placental STS. Southern hybridization with DNA from patients and somatic cell hybrids containing a number of deleted and translocated X chromosomes, indicated that the cDNA's correspond to sequences derived from the Xp22.3-Xpter region. There was complete concordance between expression of STS activity and the presence of these sequences. Comparison of the DNA sequences and the available amino acid sequence of STS confirms the authenticity of the isolated clone. Northern blot analysis showed that the STS cDNA sequences hybridized to 2.7kb and 5.2kb mRNA species in placenta and to 5.2kb and 7.0kb RNA's in fibroblasts. Sequences homologous to STS are also found on the long arm of the Y chromosome. Seven out of nine STS deficient patients studied to date have their entire STS gene deleted. The high prevalence of STS deficiency and the high frequency of deletion among STS patients suggests X/Y recombination as a possible mechanism for the defect. These STS clones should facilitate studies of the molecular etiology of X-linked ichthyosis, X chromosome inactivation, X/Y homology and recombination, and sex chromosome evolution.

L 354 USE OF RAT ALKALINE PHOSPHATASE AS A REPORTER GENE, Kyonggeun Yoon, Mark Thiede, Robert Buenaga and Gideon A. Rodan, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.
We constructed an expression vector which contains a rat alkaline phosphatase cDNA under the control of SV 40 early promoter and explored its use as a reporter molecule. Alkaline phosphatase offers the following advantages for this application: (1) spectroscopic assay of AP by paranitrophenyl phosphate is fast, inexpensive, non-radioactive and can be automated; (2) AP is an extremely stable enzyme with low turnover in situ, which yields reproducible measurements of AP activity ; (3) mammalian AP is a membrane protein and several sensitive and easy histochemical staining methods (chemical and antibody) can be applied for the visualization and selection of cells. AP expression vector (pSV AP) was constructed as follows. A blunt ended fragment of a rat cDNA (unpublished results) containing 152 bp of 5' untranslated sequences, 1572 bp of the complete coding region and 325 bp of 5' untranslated region was ligated into the Sma I site of PECE which contains the SV 40 early promoter, enhancer and polylinker. To assess the usefulness of the AP vector, equal amounts of pSV CAT and pSV AP were cotransfected and both AP and CAT activities were measured simultaneously in cell extracts. Cell extract dilutions which gave a 20% conversion to acetylated chloramphenicol showed a 20-fold increase in AP activity, relative to background by colorimetric measurement (about 1 OD at 410 nm after 60 min incubation). Histochemical staining with β-Naphtol phosphate showed that approximately 5% of cells were transfected with AP vector.

Control of Gene Transcription

L 400 SV40 LATE TRANSCRIPTION UNDER THE CONTROL OF A DROSOPHILA HSP70 REGULATORY UNIT
Jahanshah Amin and Walter A. Scott, Dept. of Biochemistry, University of Miami Medical School, Miami, FL 33101

In order to obtain SV40 late gene expression under the control of a conditional regulatory unit, we have constructed hybrid promoters in which the Drosophila hsp70 regulatory unit is fused to the SV40 late genes. The SV40 sequences (between nucleotide positions 200 and 310) were fused either to the Drosophila hsp70 regulatory unit (positions -4 to -194) or to a deletion mutant of this regulatory unit lacking the TATA box. These hybrid constructs were transfected into COS 7 cells and tested for expression by an immunofluorescence assay using monoclonal antibody against SV40 VP1 or by S1 nuclease protection. Constructs containing the hsp70 TATA box were heat inducible for transcription and VP1 expression, and the TATA box determined the sites for transcription initiation. In some constructs the TATA box was deleted in order to determine whether the hsp70 regulatory unit could utilize the authentic SV40 start sites upon heat induction. In these mutants, transcription was reduced, but expression of VP1 was still heat inducible. Locations of transcription start sites and mRNA levels for each of these constructs will be presented. Constructs with the intact hsp70 regulatory unit will be used to construct cell lines in which SV40 late proteins are synthesized in response to heat induction.

supported by State of Florida High Technology Council

L 401 IN-VITRO TRANSCRIPTION OF A HUMAN hsp 70 HEAT SHOCK GENE BY EXTRACTS PREPARED FROM HEAT-SHOCKED AND NON-HEAT-SHOCKED HUMAN CELLS.
Bernd-Joachim Benecke,Birgit Drabent and Annemarie Genthe, Department of Biochemistry, Ruhr-University, D-463 Bochum, Fed.Rep.Germany.
A cDNA clone homologous to HeLa cell mRNA encoding the 70 kDa human heat shock protein (hsp 70) was used to isolate a recombinant phage containing an entire human hsp 70 gene. Sequence analysis of the 5` and 3` flanking regions revealed the structural integrity of the regulatory elements. The functional integrity of this hsp 70 gene was substantiated by in-vitro transcription studies with nuclear extracts. Specific run-off transcripts, synthesized by RNA polymerase II, were obtained with extracts prepared from heat-shocked HeLa cells. Extracts from non-heat-shocked cells, though highly active in transcription of the adenoviral Sma F fragment, were found inactive in hsp 70 gene transcription. However, 5` deletion mutants lacking upstream sequences and the heat shock element (HSE) were transcribed by both heat shock and nonheat shock extracts with comparable efficiency. Therefore, it appears that a negative control is regulating the hsp 70 gene expression in the non-shocked-system.

L401A STRUCTURAL AND FUNCTIONAL ANALYSIS OF TWO HUMAN 7S RNA GENES
Bernd-Joachim Benecke and Winfried Krüger, Department of Biochem. Ruhr-University,D-463 Bochum, Fed.Rep.Germany.
Using purified RNA from HeLa cells, we have synthesized and cloned a cDNA encoding an almost entire 7S K RNA. This cDNA probe was used to isolate 7S K RNA gene sequences from a human genomic library by high-stringency colony hybridization. In order to differentiate between functional genes and related sequences we have used a rapid in-vitro transcription assay of purified phage DNA. With this additional screening criterion applied to selected clones, we have obtained one recombinant phage which contained a complete 7S K RNA gene and - immediately adjacent to its 3` end - a truncated pseudogene. The nucleotide sequence of both genes including the flanking regions has been determined. The functional integrity of the isolated 7S K RNA gene was verified by in-vitro transcription studies with cell-free extracts and by fingerprint analysis of the specific transcripts with ribonuclease T1. Essential sequence elements required for in-vitro transcription have been identified within and outside the coding region of the gene and will be presented in comparison to those found in a functional 7S L RNA gene, cloned in our laboratory as well.

L 402 REGULATON OF THE PROMOTER REGION OF THE δ1-CRYSTALLIN GENE. T. Borras, E. Wawrousek, D. Parker and J. Piatigorsky, NIH, NEI, Bethesda, MD 20892.

δ-Crystallin is the major structural protein of the lens of birds and reptiles. The δ-crystallin genes are developmentally regulated and expressed in the embryonic lens. δ-mRNA is first detected a few hours after lens induction, accumulates during development and is 80% of the total poly A^+ RNA in the 15 day-old embryonic chicken lens. No δ-mRNA is detected three months after hatching. Although there are two similar linked genes, δ1 and δ2, most, if not all, of the mature RNA is derived from the δ1 gene. We studied the transcriptional efficiency of the δ1 promoter in the pSVOCAT vector and assayed the constructs in an homologous cell system. Surprisingly, the activity of the δ1 promoter is very low when assayed at a stage in development when δ-mRNA levels are near maximal. Sequences between -344 and -43 are required for δ1 expression and show tissue-preference. Sequences between -601 and -344 reduce the activity of the δ1 promoter by 72%; this negative effect was observed preferentially in lens cells and did not reduce the activity of three viral promoters. The reduced promoter activity is mostly restored by competition in vivo with avian retroviral vectors but not with four non-replicating vectors. We suggest that the high level of δ1-mRNA in the chicken lens may be due to post-transcriptional accumulation rather than to a high rate of δ-crystallin transcription. Furthermore, the data raise the possibility that there is a tissue-related negative factor that could be involved in the developmental regulation of the δ1 gene.

L 403 FACTOR INTERACTIONS AT SV40 GC-BOX PROMOTER ELEMENTS IN INTACT NUCLEI, Robert L. Buchanan and Jay D. Gralla, Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, CA 90024

A primer extension footprinting procedure was used to probe simian virus 40 (SV40) regulatory elements at high resolution in intact nuclei isolated from infected monkey cells late in the viral lytic cycle. Very strong and specific protection from nuclease digestion was observed over the viral "GC-box" elements which are known to be important DNA regulatory sequences. Significant protection was also found over an adjacent region that includes the "late-early" transcription initiation sites expressed at this time. The region of strongest protection is coincident with that protected in vitro by partially purified transcription factors. Approximately 10% of the nuclear SV40 DNA has this GC-box region protected. This amount is comparable to estimates of the amount of "open" or non-nucleosomal SV40 chromatin, but much greater than estimates of complexes actively engaged in transcription or replication. Therefore, the interactions with this region in nuclei could be sufficient to promote formation of an "open" configuration of promoter chromatin, but most bound templates are not likely to be engaged in gene expression. At earlier times in the late lytic cycle the late proximal GC-boxes are less well protected, which might bring their occupancy closer to esimates of the low amount of transcriptionally active template.

L 404 PURIFICATION AND FUNCTIONAL ANALYSIS OF THE ADENOVIRUS MAJOR LATE PROMOTER UPSTREAM FACTOR (MLTF). Leonard Buckbinder and Danny Reinberg, Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School at Rutgers, Piscataway, NJ 08854-5635.

We have purified to homogeneity the adenovirus major late promoter upstream factor. The purification included two steps to remove endogenous nucleic acid and chromatography on a streptavidin agarose column containing multiple MLTF binding sites. Functional analysis indicated that the factor stimulated ten-fold the transcription reaction, this stimulation was due to both an increase on the number of template molecules able to form a committed complex resistant to low concentration of Sarkosyl and to an increase on the number of RNA polymerase II molecules able to transcribe a template committed to transcription. Our results are consistent with the observation that MLTF affected the binding stability of the TATA binding protein (TFIID) that serves as an entry site for the RNA polymerase II.

L 405 TRANSCRIPTION OF THE HUMAN ALBUMIN GENE. Jeannie Chan, Kazutada Watanabe, Kazuyuki Sawadaishi, and Taiki Tamaoki, University of Calgary, Calgary, Alberta, Canada.

The human albumin gene is separated by an intergenic distance of 14.5 kb from the alpha-fetoprotein gene on chromosome 4. Apart from their physical proximity, these two genes show distinct pattern of expression during normal development and malignant growth. To study cis-acting elements in the 5' flanking DNA of the albumin gene, a number of recombinant plasmids were constructed in which various lengths of albumin 5' flanking DNA were linked to the bacterial chloramphenicol acetyltransferase (CAT) gene. These plasmids were transfected into an albumin-producing cell line, HuH-7, or a non-producing cell line, HeLa, by the calcium phosphate precipitation method. A plasmid containing 275 bp of albumin 5' flanking DNA from the cap site could direct the expression of the CAT gene in HuH-7, but not in HeLa. This level of CAT expression in HuH-7 was not enhanced with CAT plasmids carrying progressively longer albumin 5' flanking DNA, up to 12 kb. Our results suggest that the promoter determines cell-specific expression of albumin in hepatoma cells. Furthermore, an enhancer is not present within 12 kb from the albumin cap site. This is in contrast to the alpha-fetoprotein gene where an enhancer is present between 3.3 and 5.1 kb upstream from the cap site. To study trans-acting factors that bind to the albumin promoter using the electrophoretic mobility shift assay, an end-labeled fragment containing 275 bp of albumin 5' flanking DNA was incubated with nuclear extract from HuH-7 or HeLa. Putative DNA-protein complexes were detected with both HuH-7 and HeLa nuclear extract. However, these complexes migrated with different mobilities, implying proteins that bound to the albumin promoter DNA in HuH-7 were different from those in HeLa.

L 406 HELA CELL FACTORS INVOLVED IN THE IN VITRO TRANSCRIPTION OF CRYSTALLIN GENES. Gokul C. Das[1,2] and Joram Piatigorsky[2]. [1] Division of Molecular Biology and Biophysics, School of Basic Life Sciences, University of Missouri-Kansas City, MO 64110 and [2] Laboratory of Molecular and Developmental Biology, National Eye Institute, NIH, Bethesda, MD 20892.

We have analyzed the 2 linked δ-crystallin genes (5' δ1-δ2 3') and the βB1-crystallin gene expressed in the chicken lens by in vitro transcription in a Hela cell extract. δ-Crystallin is the first crystallin synthesized and appears in the epithelial and fiber cells of the embryonic lens; by contrast, βB1-crystallin is synthesized specifically in the elongating cells later during lens development. Our previous competition experiments indicated that the δ1 promoter requires an Spl-like factor for transcription and is several-fold more efficient than the δ2 promoter in vitro. We now show the following by competition experiments. First, the δ1 and δ2 promoters require similar transcription factors in the Hela cell extract. Second, although the βB1 promoter has a similar efficiency as the δ1 promoter in vitro, the δ1 promoter is only partially competitive with the βB1 promoter. Moreover, the GC boxes of the SV40 early promoter eliminate transcription of the δ1 promoter but do not compete with the βB1 promoter. The δ1 promoter has 1 Spl binding site (CCGCCC) associated with a CCAAT box, while the βB1 promoter has several GC-rich clusters and lacks a CCAAT box. Interestingly, the PvuII-4 fragment of polyoma virus, which is necessary for cell-type specificity of viral growth, competes effectively with β1 transcription in the Hela cell extract. Our data suggest regulatory differences between the fiber cell-specific βB1-and the δ1-crystallin promoters.

L 407 PURIFICATION AND PROPERTIES OF NUCLEAR FACTOR I. C. Donath, R. I. Gander, A. Mack, A. Marschall, M. Meisterernst, U. Müller, G. Stelzer, K. Thalmeier and E.L. Winnacker, Institut für Biochemie, Universität München, Karlstrasse 23, D-8000 München 2, FRG.

We have developed a novel purification scheme for nuclear factor I from porcine liver involving preparative gel retention. The purified factor had a molecular weight of 72 kDa. Partial amino acid sequences from tryptic digests were obtained by gas phase sequencing. Screening of a porcine liver cDNA library in Lambda gt10 with appropriate oligonucleotides yielded a variety of clones the characteristics of which will be discussed. Additional clones were obtained by screening a Lambda gt11 library directly with corresponding antibodies or with an oligonucleotide carrying a nuclear factor I binding site.
In addition, we will report on the purification of a nuclear factor I-like preparation from S. cerevisiae. Finally it was observed that partially enriched nuclear factor I-like preparations from yeast bind to the -CAAT-region of the human c-myc gene promoter and corresponding regions from the yeast PGK- and actin genes. Similar binding constants were observed for the CAAT-sites containing oligonucleotides from yeast as well as from the NFI binding site from the adenovirus type 2 terminal repetition. (This work was supported by the Deutsche Forschungsgemeinschaft through SFB 304).

L 408 TRANSCRIPTIONAL REGULATION OF THE HUMAN RENIN GENE, Keith G. Duncan, John D. Baxter and Timothy L. Reudelhuber, University of California, San Francisco, CA 94143. Renin is an aspartyl protease whose synthesis and secretion by the kidney is the rate limiting step of the renin-angiotensin system controlling blood pressure homeostasis. Control of human renin gene expression has been studied in primary cultures of renin producing human chorion trophoblasts. A chimeric gene consisting of the human renin promoter from position -602 to +11 linked to the bacterial chloramphenicol acetyl transferase (CAT) gene has been constructed and transfected into HeLa cells and chorion cell primary cultures. The human renin CAT construct was expressed at a 6.2 fold greater level in chorion cells than in HeLa cells. This chimeric gene has been used to study regulation of renin gene expression. Calcium ionophore or forskolin treatment alone stimulated expression about 1.5 fold over control levels while phorbol ester alone had little effect on expression. Calcium ionophore and forskolin together stimulated expression 4 fold and forskolin and phorbol ester together stimulated expression 6 fold. Northern blotting has shown the endogenous renin mRNA of these chorion cells to be similarly regulated. These data indicate that the human renin gene promoter fragment from -602 to +11 contains elements responsible, in part, for the tissue specific expression as well as forskolin and phorbol ester mediated stimulation of renin gene expression.

L 409 TRANSCRIPTIONAL REGULATION OF THE DIHYDROFOLATE REDUCTASE GENE
Peggy J. Farnham and Robert T. Schimke, Stanford University, Stanford CA, 94305
We have undertaken a biochemical characterization of the transcriptional control mechanisms involved in cell cycle regulation using the murine dihydrofolate reductase (dhfr) gene as a model system. Nuclear extracts prepared from cells in different stages of the cell cycle display differential activity on the dhfr promoter in an in vitro transcription system, suggesting that dhfr transcription is modulated by a factor whose specific activity changes as cells progress through the cycle. This factor could function via protein-DNA and/or protein-protein interactions. We have begun to examine these possibilities using protein blotting, centrifugal fractionation, and gel retardation assays. A 166 base pair promoter fragment consisting of four 48 basepair repeats, each of which contains one SP1 binding site, can detect several proteins in the nuclear extract. At least four of these proteins (~95, 105, 110, and 130 kD) are co-precipitated with a stable transcription complex. This complex can be quantitatively separated from 95% of the proteins in the nuclear extract while retaining the ability to exclude transcription of a second promoter (either homologous or heterologous). A fragment which includes only 51 base pairs 5' of the major transcription start site and extends into the cDNA (and does not overlap the SP1 binding sites) has also been shown to bind protein in a gel retardation assay. Purification of these factors and characterization of their binding sites is now in progress.

L 410 SPECIFIC DNA-BINDING PROTEINS INTERACT WITH REGULATORY ELEMENTS OF THE ALPHA GLYCOPROTEIN HORMONE GENE PROMOTER. Louis H. Ferland and Pamela L. Mellon, Regulatory Biology Laboratory, The Salk Institute, La Jolla, CA 92037.
The single α subunit gene of the glycoprotein hormones is expressed as a component of four hormones (LH, CG, TSH and FSH). Each of these hormones contains a distinct β subunit, is expressed in distinct cell types and is differently regulated by other hormones. Thus, regulation of the α subunit gene is quite complex. Using gene transfer of the α promoter linked to a reporter gene, Delegeane and Mellon (abstract this volume) have identified regions of the 5' flanking DNA which are important for tissue-specific and cAMP-regulated expression in a placental cell line (JEG-3). We have detected the presence of DNA-binding activities in nuclear extracts which appear to correlate with these biologically active regions by DNase I protection experiments (footprinting). The region covered by one of these footprints (-109 to -148) contains a direct, exact 18 bp repeat and confers cAMP regulation. This footprint appears using both extracts from the placental cell line (JEG-3) and a nonexpressing cell line (CV-1). The deletion of one copy of the repeat reduces the footprint to half of its original size and the insertion of a linker in the remaining copy destroys both the cAMP response and the footprint. A second footprint is detected only with JEG-3 extracts in a region which has been demonstrated to participate in tissue-specificity (-159 to -179). A third footprint appears over a reverse CAAT consensus (-72 to -92) using either extract and is presumed to be a ubiquitous transcription factor. Thus, we have identified several DNA-binding factors whose binding sites appear to correlate with the tissue-specific and cAMP-inducible enhancers which regulate the alpha subunit gene of the glycoprotein hormones.

L 411 Analysis of the promoter of the cytoskeletal beta actin gene. Nevis Fregien* and Norman Davidson. California Institute of Technology, Department of Chemistry, Pasadena, Ca, 91125. *University of Miami, School of Medicine, Department of Anatomy and Cell Biology, Miami, Fl. 33101.

The cytoplasmic beta actin gene is expressed at very high levels in most cell types. We have compared the promoter for this gene to other promoters and find it to be one of the most active. We have tested various fragments of this gene for enhance activity in both stable and transient expression assays. The results show that sequences near the promoter can function as an enhances in stable assays but have little enhances activity in transient assays. The reason for the differences in the two assays is not clear.

We have attempted to identify the sequences in the beta actin gene which are responsible for the strong promoter activity observed in the transient assays. Deletion analysis has shown that sequences in the first intro are not necessary, but the splicing consensus sequences must be present for proper processing. Furthermore, sequences 5′ of the CAAT box can be deleted without any decrease in promoter strength.

L 412 POINT MUTATION IN THE POLYOMA ENHANCER ALTERS LOCAL DNA CONFORMATION, Frank K. Fujimura, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

A point mutation located within the enhancer of polyoma virus DNA permits productive infection of the murine embryonal carcinoma cell line, F9, which is refractory to infection by wild-type polyoma. This A:T to G:C transition results in a local conformational change in naked polyoma DNA that can be detected by limited digestion with either DNase I or copper-phenanthroline. Polyacrylamide gel electrophoresis of DNA fragments resulting from ligation of synthetic oligonucleotides having either the wild-type or mutant sequence showed that both sequences, when propagated in phase with the helix repeat by ligation of 10-mers, exhibited anomalously low electrophoretic mobilities relative to restriction fragments of known sizes. Ligation of 12-mers of these sequences resulted in fragment ladders having more normal electrophoretic mobilities. This phase-dependence coupled with the observation that the anomalous electrophoretic behavior was more pronounced by electrophoresis at lower temperature, strongly suggest that these polyoma enhancer sequences affect DNA bending. Although both wild-type and mutant 10-mer ladders showed anomalous electrophoretic mobilities, the wild-type fragments migrated significantly more slowly than those of the mutant, indicating a greater effect of the wild-type sequence on DNA curvature.

L 413 DNA-BINDING PROTEINS SPECIFIC FOR SV 40 ENHANCER
Claire Gaillard, Michèle Weber, and François Strauss
Institut Jacques Monod, 75251 Paris 05, France

We have purified and characterized several proteins that bind to the enhancer of SV 40 specifically.

Nuclear extracts of uninfected monkey CV1 cells were fractionated on various ion-exchange columns. Specific enhancer-binding proteins in the fractions were then detected by electrophoresis of the complexes formed with labeled enhancer DNA in the presence of unlabeled competitor DNA (gel retardation assay).

The binding sites of these proteins on the enhancer sequence were determined by three methods :
1. binding to different restriction fragments from the enhancer region
2. DNAse footprinting
3. interference of chemical modifications of DNA with protein binding.

L 414 The Purification and DNA-binding Activity of an *Ultrabithorax* Protein

Elizabeth Gavis, Mark Krasnow, Philip Beachy and David Hogness, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

The *Ultrabithorax* (*Ubx*) transcription unit from the bithorax complex is required for the proper specification of identity of parasegments five (PS5) and six (PS6) in *Drosophila*. Developmentally regulated mRNAs from the *Ubx* unit are differentially spliced to encode a family of at least five closely related proteins, one or more of which are likely to be required for *Ubx* function. We have overproduced two of these proteins in *E. coli* and purified one of them to homogenity. To elucidate the role of the *Ubx* proteins in development, we have begun biochemical characterization of the purified protein and have initiated similar studies on the other forms.

One function of *Ubx* is to prevent inappropriate expression of other homeotic loci in PS5 and PS6; in *Ubx* mutants *Antennapedia* (*Antp*) transcripts are elevated in this region. Therefore, we used filter binding and DNase I protection experiments to search for an interaction between the purified *Ubx* protein and sequences around one of the two *Antp* promoters. We have identified for one *Ubx* protein high affinity binding sites 6 kb upstream and 300 bp downstream of the *Antp* P1 transcription start site. Additional sites were found 50-300 bp downstream of the start of the *Ubx* transcript. DNase I protection spanned 25-80 bp in each region and the protected regions were comprised of one or more copies of the consensus $(TAA)_5$. A 26 bp sequence containing a single consensus from one of the large binding sites was sufficient for *Ubx* protein binding. *Ubx* showed similar affinity for a 20 bp region in the isolated consensus and the larger natural site.

L 415 MULTIPLE SEQUENCE ELEMENTS REQUIRED FOR BASAL TRANSCRIPTION OF THE HUMAN hsp70 GENE DIFFER IN RODENT AND HUMAN CELL LINES. **John M. Greene, Zoia Larin, Ian C.A. Taylor, Holly Prentice, and Robert E. Kingston. Department of Molecular Biology, Massachusetts General Hospital, Boston MA 02114 and Department of Genetics, Harvard Medical School.** **We have investigated sequences required for basal transcription of the human hsp70 gene in immortalized and primary cell lines of both rodents and humans. No difference in upstream sequence requirement is apparent between immortalized and primary lines of either species. However, deletion analysis reveal that much more sequence is required in human cells than in rodent cells. Fine analysis of the region downstream of -75 by a modified linker scanning protocol indicates three regions important for basal transcription: CCAATC in inverted orientation at -65, an SP1 binding site at -45, and the TATAA box at -25. Sequences between -84 and -1250 can partially compensate for these down mutants in both rodent and human cells. This suggests that human cells require these upstream elements, whereas in rodent lines these upstream elements can compensate for the loss of downstream element function but are not necessary for full basal transcription. We have also identified proteins binding to the downstream elements as well as discovering a protein bound adjacent to the TATA box in a region where mutants have no effect on in vivo transcription. Work is also in progress on investigating the interaction of the heat shock element at -105 with these downstream basal elements. To date we have demonstrated that the TATA box is necessary for inducibility of hsp70 transcription after heat shock, and we are investigating the role of other basal elements.**

L 416 IDENTIFICATION OF STRUCTURAL REQUIREMENTS FOR TRANSCRIPTION ACTIVATION OF THE CHICKEN ALPHA-SKELETAL ACTIN GENE PROMOTER

James M. Grichnik, Robert J. Schwartz, Baylor College of Medicine, Houston, Tx 77030

We have shown that nucleotides -200 to -11 upstream of chicken α-skeletal actin gene cap site are sufficient for stage-specific activation of transcription in transiently transfected chicken primary myoblast cultures. This fragment is specifically retarded by crude muscle nuclei extract on gel retention analysis (col. L. Gossett). In addition to other potential regulatory sequences, this region includes a pair of highly conserved sequence elements of the consensus CCAAATA(A/T)GG in which the upstream element is oriented in reverse. 5′ deletion analysis indicated that disruption of the upstream element (-127 3′ CCAAAgAAGG 5′ -136) resulted in a 7-fold decrease in activity. The chicken α-sk actin promoter displays an partial dyad symmetry about nucleotide -108. This promoter region, when flipped -11 to -318, is also able to drive stage-specific transcription. Data from 3′ serial deletions indicate that a significant drop in divergent transcription is noted when the distal element (-91 5′ CCAAATATGG 3′ -82) is disrupted. Both copies of these elements are required for transcriptional activation and may act in a cooperative manner. These elements are conserved in sarcomeric actin genes throughout vertebrate evolution. Preliminary data from injected developing Xenopus embryos suggests that the chicken α-sk actin promoter is activated at the appropriate stage (col. with J. Pelika and D. Wright) and transcriptional activation of the cardiac and skeletal α-actin gene promoters in transient transfection assay of developing myoblasts is indistinguishable. It seems possible that the sarcomeric actin genes may possess a conserved transcriptional control mechanism.

L 417 MECHANISMS OF METALLOTHIONEIN GENE REGULATION, Dean H. Hamer, National Cancer Institute, Bethesda, Maryland 20892

The transcription of mammalian metallothionein genes is inducible by a wide variety of environmental and developmental signals. We have identified and partially purified mouse nuclear factors that specifically bind to an upstream DNA sequence involved in heavy metal regulation of the mouse metallothionein-I gene. Binding of the nuclear factors to this site can be induced in vitro by the addition of ionic cadmium or cadmium - metallothionein. Complex formation is rapid, reversible by a metal chelator and appears to involve multiple proteins. Metallothionein gene expression also varies during development and in response to transformation by the ras oncogene. Attempts to isolate factors involved in these form of regulation are in progress.

L 418 MULTIPLE CELLULAR PROTEINS SPECIFICALLY BIND THE 21 BP REPEAT ELEMENT OF THE SV40 EARLY AND LATE PROMOTERS, ACTIVATING TRANSCRIPTION IN STRIKINGLY DIFFERENT WAYS, Ulla Hansen, Chung Han Kim, Catherine Heath, Alison Bertuch and David O'Brien, Dana-Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, MA 02115.

Using a specific DNA-binding assay, we have partially purified three proteins from uninfected HeLa cells which bind the SV40 21 bp repeat promoter element. The proteins separate chromatographically on a Heparin column, but all elute as approximately 230,000 daltons on a sizing column. Their DNA-binding and transcriptional properties have been analyzed in vitro. 1) The protein eluting at intermediate salt concentrations from the Heparin column seems identical to Spl. It dramatically stimulates transcription from the early-early initiation site of the SV40 early promoter (the major site used early in an SV40 lytic infection, pre-DNA replication). Five- to ten-fold less efficiently, a minor upstream late initiation site (L167) is also stimulated. 2) The protein eluting at lower salt concentrations binds SV40 DNA identically to Spl, both by footprinting and gel mobility shift assays. However, it is inert transcriptionally on the SV40 promoters. Thus, it may allow us to separate DNA-binding alone from transcriptional activation. 3) The protein eluting at higher salt concentrations has been named LSF (late SV40 transcription factor). It specifically stimulates transcription from all the initiation sites used late in an SV40 lytic infection, post-DNA replication. Thus, we hypothesize that LSF is involved in regulation of early to late SV40 transcription. Although LSF also binds the SV40 21 bp repeats, the resulting complexes migrate with different mobilities through a nondenaturing polyacrylamide gel that those of Spl. LSF seems also to bind the GC-motifs with a different order of affinities from Spl.

L 419 SPECIFIC FACTORS BINDING TO THE E2A LATE PROMOTER REGION OF ADENOVIRUS TYPE 2 DNA: EFFECT OF 5'-CCGG-3' METHYLATIONS ? Arnd Hoeveler and Walter Doerfler, Institute of Genetics, University of Cologne, Cologne, Germany.

It was shown previously that the in vitro methylation of three 5'-CCGG-3' sequences in the late E2A promoter of adenovirus type 2 (Ad2) DNA led to the inactivation of this promoter both after microinjection into Xenopus laevis oocytes and after transfection into mammalian cells (Langner et al., PNAS 81, 2950, 1984; PNAS 83, 1598, 1986). The inactivating effect of site specific methylations in the late E2A promoter of Ad2 could be overcome by trans-activation via E1 functions of Ad2 or Ad5 (Langner et al., PNAS 83, 1598, 1986). Current investigations have been directed towards the binding of specific factors to sequences in the E2A late promoter and upon the influence that the three methylated 5'-CC^{m}GG-3' sequences have on these DNA-protein interactions. The techniques of gel retardation and footprinting have been employed. Footprinting analyses have revealed six sites of protein binding in the E2A promoter region ranging from positions -30 to -250. Protein binding at these sites has not been drastically affected by 5'-CCGG-3' methylations. An area surrounding the non-canonical TATA box seems to be of particular interest. Here, minor differences in DNaseI protection patterns with methylated versus unmethylated DNA have been observed. Further work will be carried out to purify some of the factors binding to the E2A late promoter and to study the effects of promoter methylations on the binding of these factors. The possibility has to be considered that, in spite of binding to a specific promoter site, the functionality of a DNA-associated factor might be impeded by specific promoter methylations.
(Aided by DFG, SFB74-C1).

L 420 CHARACTERIZATION OF A MOUSE HSP70 GENE PROMOTER REVEALS EXTENSIVE NUCLEOTIDE HOMOLOGY TO TRANSCRIPTION ELEMENTS IN THE HUMAN HSP70 GENE. Clayton Hunt, Dana-Farber Cancer Institute, Boston, Ma. 02115. The mouse gene encoding the major HSP70 transcript produced in heat shocked mouse cells has been isolated and characterized by nucleotide sequencing and blot analysis. Structurally, the transcription unit has two unusual features not found previously in eucaryotic HSP70 genes. Northern and nucleotide sequence analysis indicates a primary transcript of approximately 3 kb, with 650 bp of 3' sequence, significantly larger than HSP70 transcripts studied to date. Secondly, while containing the long 5' nontranslated RNA leader found in most HSP70 mRNAs, the 225 bp mouse leader also contains tandemly repeated 33 bp elements.

The promoter region of the mouse gene has been localized by gene fusion studies to a region 580 bp upstream of the transcription start site. Alignment of the nucleotide sequence of this region to that of the previously characterized human HSP70 promoter revealed a highly conserved block of nucleotides, 23/28 matching, which spans an inverted CAAT box and a heat shock element (HSE) beginning 60 bp upstream of the mRNA start site for both genes. Interestingly, there is little homology in the mouse gene promoter to a region of the human gene implicated in serum regulated expression of HSP70. Northern blot analysis of RNA from growing mouse cells confirmed that there was little HSP70 synthesis under non-stressed conditions as opposed to the case with HSP70 expression in human HeLa cells.

L 421 CIS AND TRANS-ACTING ELEMENTS IN THE REGULATION OF MOUSE MHC CLASS I GENE EXPRESSION. A. Israël, A. Kimura, O. Yano, J. Kanellopoulos, O. Le Bail, M. Kieran & P. Kourilsky Biologie Moléculaire du Gène, INSTITUT PASTEUR : 75724 Paris Cédex 15, France.

Several important elements have been characterized in the promoter of the mouse H-2K^b gene, including two enhancer-like sequences and an interferon response sequence (IRS) which overlaps one of the enhancer sequences (sequence A) (Cell (1986) 44, 261-272). The IRS is necessary for induction by interferon α,β and γ, and requires the presence of sequence A in order to be functional (Nature (1986) 322, 743-746).

We have characterized by gel retardation and MPE-footprinting the binding of one (or several) protein factor(s) to the IRS-enhancer A region, and we have undergone purification of these factors. The relevance of these cis and trans-acting elements to the regulation of class I gene expression (including genes derived from the Qa and TI regions) will be discussed.

L 422 THE MURINE IGH ENHANCER CONTAINS AT LEAST TWO FUNCTIONAL DOMAINS, Tom Kadesch, Mike Kiledjian, Diane Ruezinsky and Li-Kuo Su, University of Pennsylvania, Philadelphia, PA 19104

We have employed transient transfections of CAT-based vectors into both lymphoid and non-lymphoid cells to delineate the functional elements within the murine IgH (heavy chain) enhancer. Earlier deletion analyses which failed to define a distinct border for the enhancer suggested that it may be composed of a number of functionally redundant elements. We thus attempted to construct artificial enhancers by multimerizing defined segments of the enhancer head to tail. Using this technique, we have shown that the enhancer contains at least two distinct functional domains whose enhancer activity is revealed upon multimerization. Similar to their unmodified parent, the novel enhancers constructed from each domain remain active only in lymphoid cells.

L 423 ROLE OF THE TRANSCRIPTION FACTOR E2F IN E1A MEDIATED COORDINATE GENE CONTROL, I. Kovesdi, R. Reichel and J. Nevins, Rockefeller University, New York, NY 10021.
We have previously detected a HeLa cell factor, termed E2F, that appears to mediate E1A promoter (Cell 45, 219). The factor binds to E2 promoter sequences critical for transcription and E1A induction and the factor increases markedly as a function of E1A. In addition to the E2 gene, four other early viral promoters are activated by the E1A gene product during a lytic viral infection. To address the mechanism for coordinate control of viral transcription by E1A we have now determined if the E2F factor recognizes any of the other E1A inducible promoters. Competition experiments revealed that the E2 factor did not recognize and bind to the E1B, E3, E4 or major late promoter sequences indicating that E1A stimulation of these promoters must involve additional factors. In addition, two cellular promoters stimulated by E1A, hsp70 and β globin, do not bind the E2F factor. In contrast, the E2F factor does recognize sequences in the E1A enhancer. Within the E1A enhancer are duplicated binding sites for the E2F factor. Finally, the binding of the factor to the enhancer is responsible for increased transcription, as a result of E1A protein, of a chimeric gene whose activity is dependent on the presence of the enhancer. We propose that the autostimulation of E1A transcription by the E1A gene product as previously observed in virus infection is mediated by the E2F factor.

L 424 PROMOTER BINDING OF RNA POLYMERASE I IS DIRECTED BY TRANSCRIPTION INITIATION FACTOR AND APPEARS DNA SEQUENCE-INDEPENDENT, Preecha Kownin and Marvin R. Paule, Dept. of Biochemistry, Colorado State University, Fort Collins, Colorado 80523.
DNase I footprinting of the Acanthamoeba castellanii rRNA promoter revealed two adjacent protected regions on the coding strand: between -67 and -14 by transcription initiation factor (TIF) and from -14 to +20 by RNA polymerase I (RNAP 1). To examine the role of TIF-RNAP 1 (protein-protein) interaction and nucleotide sequences surrounding the transcription start site (RNAP-DNA) in directing accurate transcription, a series of insertions and deletions were constructed between these two DNA regions. Insertions or deletions from 1 to 5 nucleotides long decreased the transcription efficiency. The transcripts from deleted mutants were shorter and from the inserted spacer mutants were longer than from the wild type promoter. Analysis of the transcripts from the spacer mutants by primer extension suggested that RNAP 1 always initiated RNA synthesis at a nearly constant distance from the TIF binding site. Spacer constructs of greater than ±5 nucleotide pairs completely abolished the ability of the DNA template to promote specific transcription. Since the AT-rich region surrounding the start site was replaced by insertion of GC-rich DNA, this result suggests that an AT-rich region between -10 and +3 is important for efficient initiation. However, this sequence is not required for RNAP 1 binding because footprinting of RNAP 1 was observed on the transcriptionally inactive constructs despite the change of nucleotide sequence. This data suggests that polymerase mainly recognizes the presence of TIF on the promoter by protein-protein interactions and relies only marginally, if at all, on protein-DNA contacts for promoter recognition. Supported by USPHS #GM26059.

L 425 TARGETS OF INTERACTION BETWEEN CELLULAR TRANSCRIPTION FACTORS AND THE Ad12 E1A PROMOTER. Inge Kruczek, Inst. Biochem. Munich, FRG.
Regions involved in the regulation of the Ad12 E1a promoter have been studied by transient expression assays and gel retention analysis. Two important positions have been detected:
1. Deletion of the leftmost 152 bp of the Ad12 DNA reduces E1a promoter activity 5 to 10fold, the deletion of the leftmost 170 bp at least 10fold. The deleted DNA includes the ITR (-164 bp) and is needed for transcription from both TATA boxes. ITR fragments at the 3'end of the CAT gene in either orientation restrict the main control element to DNA sequences between positions 144 and 170 and reveal that they serve as a transcriptional enhancer. Gel retention analysis demonstrates that cellular factors bind to these sequences.
2. Transfection of increasing amounts of DNA of the CAT constructs containing a promoter fragment from position 0-525 results in maximal CAT activity at 15 µg transfected DNA followed by a rapid promoter inactivation. Smaller promoter fragments yield increasing CAT activity according to increasing amounts of transfected DNA. Northern analysis suggests that this control mechanism occurs at the level of transcription initiation. Two in limited amountd occurring nuclear proteins of BHK cells bind between position 400 and 494. These factors are stimulated in an Ad12 transformed hamster cell line. The target for at least one of these factors could be mapped to the E1a CAP site.

L 426 IDENTIFICATION OF A CIS-ACTING DNA SEQUENCE(s) INVOLVED IN THE PANCREAS-SPECIFIC EXPRESSION OF ELASTASE I. Fred Kruse and Raymond J. MacDonald, Univ. of TX Health Science Center-Dallas, Texas 75235-9038.

A DNA sequence sufficient to direct pancreas-specific expression in transgenic mice has been localized to the region -205 to +8 of the rat elastase I gene (Ornitz, D.M., Palmiter, R.D., Hammer, R.E., Brinster, R.L., Swift, G.H., and MacDonald, R.J., Nature 313:600 (1985). In an attempt to map more precisely the cis-acting DNA sequence(s) responsible for pancreas-specific expression of elastase I, we have utilized site-directed mutagenesis to introduce 10 base pair (bp) substitutions into the rat elastase I 5'-flanking sequence between -205 and +8. The base segments starting at -180 and ending at -90 were changed, resulting in nine independent substitution mutants. Each altered elastase I fragment as well as the unmutagenized 213 bp fragment was tested for its ability to direct the expression of a chloramphenicol acetyltransferase (CAT) reporter gene when transfected into either a mouse pancreatic cell line or into mouse NIH 3T3 cells. Preliminary results indicate that the wild-type elastase I fragment directs the expression of CAT to a level approximately 1000-fold greater in the pancreas cells than in the 3T3 cells. In addition, the substitution analysis has revealed an area within the elastase I fragment that may be required for the pancreas-specific expression of this gene.

L 427 **PROTEIN-NUCLEOTIDE CONTACTS IN IMMUNOGLOBULIN V_H PROMOTER REGIONS.** Nicholas F. Landolfi, J. Donald Capra and Philip W. Tucker. The University of Texas Health Science Center, Dallas, TX 75235. All immunoglobulin (Ig) heavy chain variable region (V_H) genes contain the octamer ATGCAAAT 70-90 nucleotides upstream from the transcription initiation site. The complement of this sequence is found at an identical position in the reverse orientation in all light chain genes. Sequences containing this element have been shown to be involved in the lymphoid-specific expression of Ig genes. Analysis of nuclear extracts in a gel migration inhibition assay using an Ig V_H promoter region fragment containing the conserved octameric sequence detects several migration retarded species which represent specific DNA-protein complexes. The number and relative level of these complexes varies with the source of the nuclear extract. Some complexes are detected in all cell extracts, while one complex is lymphoid-specific and may be involved in the lymphoid-restricted expression of Ig genes. The level of this particular complex is increased in mitogen stimulated cells. We have examined each of the complexes detected in the gel migration inhibition assay by DNAse I protection ("footprinting"). Our analysis has revealed that all of the DNA-protein complexes detected involve contacts with the nucleotides of the octamer. Furthermore, one complex, present in both lymphoid and non-lymphoid extracts, also displays a DNA-protein contact adjacent (upstream) to the octamer. Our results also indicate that the interaction of proteins with the octamer can cause a local alteration in the structure of the DNA helix.

L 428 **5'-Flanking Regions Modulate Drosophila tRNA Gene Transcription by Regulating Active Complex Formation.** Alan Lofquist, Alonzo Garcia, and Stephen Sharp. UC Irvine, California College of Medicine, Irvine, CA 92717.

In vitro transcription of three identical Drosophila $tRNA^{Asn}$ genes in a Drosophila Schneider S3 cell-free extract showed the $tRNA^{Asn}$ genes had different transcription efficiencies. Exchanging the 5' flanking regions of these tRNA genes revealed that the differences in transcription activities were directly attributable to the corresponding 5'-flanking region. Stable complex competition assays suggested that the modulatory effect of 5'-flanking regions in these $tRNA^{Asn}$ genes was due to differences in the ability of their 5'-flanking sequences to form an active complex with RNA polymerase III. To localize and identify the transcription control mechanism responsible for the modulation of RNA polymerase III activity on Drosophila tRNA genes, we have constructed linker scanning mutations in the 5'-flanking regions of two of these genes. The effects of these mutations on gene function were studied by in vitro transcription and stable complex formation assays. The ability of different 5'-flanking sequences to activate or inhibit open complex formation and initiation of transcription by RNA polymerase III will be discussed.

L 429 MODULATION OF α-TIF DEPENDENT INDUCTION OF HSV-1 α GENES Jennifer L.C. McKnight, Phillip E. Pellett, Frank J. Jenkins and Bernard Roizman, University of Chicago, Chicago, IL. 60637. The induction of herpes simplex virus 1 α genes is mediated at the transcriptional level by the action of a structural component of the virion (α-trans induction factor; α-TIF). The domain of the α-TIF gene was mapped to the BamHI F fragment of the viral genome in transient expression assays with α regulated thymidine kinase (α-TK) chimeric indicator genes. These studies also demonstrated that sequences located 3' of the α-TIF gene were able to modulate the α-TIF dependent induction of α-TK. Nucleotide sequence and S_1 nuclease analyses confirmed the presence, within the 3' sequences, of two open reading frames (ORF) A and B, capable of specifying polypeptides with translated molecular weights of 70,527 and 77,357 respectively. Cotransfections of a construct containing both ORFs A and B with equimolar amounts of the α-TIF gene and a fixed concentration of the α-TK indicator gene, in transient expression assays, resulted in an increase in the level of α-TK induction two-fold over the levels observed using the α-TIF gene alone. Analyses of the functions specified by ORF A and B resulted the following: 1) Interruption of ORF A by 14 base pair linkers containing termination signals in all three reading frames increased the levels of the α-TIF dependent α-TK induction a minimum of two-fold over the levels observed in the presence of α-TIF alone; 2) A similar interruption of ORF B decreased the levels of α-TK induction; 3) Interruption of both ORFs A and B abolished any effect on the α-TIF dependent α-TK induction. Thus the effect of ORFs A and B in transient expression assays results from the expression of these genes, and is not due to sequence competition for transcriptional factors.

L 430 PROTEIN INTERACTIONS AND STRUCTURAL ALTERATIONS AT THE TRANSFERRIN RECEPTOR PROMOTER, W. Keith Miskimins, Margo Roberts, Michael P. Roberts and Frank H. Ruddle, Yale University, New Haven, CT 06511.

We are attempting to define the mechanisms that control expression of the transferrin receptor (TR) during cell growth. We have identified and characterized the promoter for the human TR gene. Using DNase I and EDTA-iron footprinting techniques, we have found multiple protein binding sites within the 100 bp just upstream of the TATA box. The site nearest the TATA box is centered by a 10 bp high affinity Sp1 consensus recognition sequence. Proximal to this site is a protein-binding sequence which is homologous to the cAMP and TPA responsive elements which have been described for the somatostatin and proenkephalin genes. Two additional binding sites are found just upstream. Three of the protein binding domains are conserved in the promoter region of human DHFR which, like TR, is expressed at high levels in mitogen stimulated cells. We have determined the helical periodicity within the TR promoter and have found that the upstream protein binding regions are separated by approximately 1 helical twist, suggesting that protein-protein interactions may be involved. Using a set of overlapping synthetic oligonucleotides and a protein blotting procedure, we have identified several proteins which bind to the promoter sequences. We have partially purified the proteins which recognize these domains and are determining their effects on in vitro transcription from the TR promoter. These studies should allow us to define the roles of these proteins in TR expression and to determine their relationship to cell growth control.

L 431 **$CC(A/T)_6GG$ has a regulatory role in the expression of the human α-cardiac actin gene in muscle cells.** Takeshi Miwa, Linda Boxer & Larry Kedes, Stanford Medical School and Veterans Administration Medical Center, Palo Alto, CA 940304

An upstream region from -177 bp to the transcription initiation site of the human α-cardiac actin gene directs transient expression of a bacterial chloramphenicol acetyltransferase (CAT) gene in muscle cells, but not in fibroblast cells (Minty & Kedes, Mol. Cell. Biol.; 6 2125 (1986)). We have modified this promoter region by 5'-deletion and linker-scanning mutations. The results showed that the sequences in and adjacent to two $CC(A/T)_6GG$ sequences located at -150 and -110 play an important positive role in transcription. Additional insertion and deletion mutants as well as linker-scanning mutants demonstrated that those two $CC(A/T)_6GG$ sequences can replace each other as the positive regulatory site. An *in vivo* competition assay was carried out, in which the promoter linked to the CAT gene (test gene) was transfected into the muscle cells together with small DNA fragments of the α-cardiac actin promoter region (competitor DNA). A DNA fragment (-113 to -84) that includes the $CC(A/T)_6GG$ sequence has significant competitive activity not only to the α-cardiac actin promoter but also to a promoter of α-skeletal actin, which co-expresses with cardiac actin in early stage of muscle development. Our results suggest that the tissue specific *trans* acting modulation factors present in muscle cells and common between cardiac and skeletal actins can be titrated out by this sequence. This result was confirmed by a gel retardation assay using the same DNA fragments.

L 432 TRANSCRIPTION AND FOOTPRINTING OF THE HUMAN U1snRNA GENE, James T. Murphy, Samuel I. Gunderson, Thomas H. Steinberg and Richard R. Burgess, University of Wisconsin, Madison WI 53706.

The promoter for the human U1 snRNA gene contains sequences homologous to the binding sites for the transcription factors USF, Sp1, and the ATGCAAAT octamer binding factor. These homologous sites are located within regions of the U1 promoter that we have shown to be required for efficient transcription. A combination of a gel retardation assay and DNA footprinting were used to show that these homologous sequences are protected by factors found in HeLa cell transcription extracts. The Ad2 major late promoter upstream factor, USF, gave a footprint between positions -68 and -57 on a U1 promoter DNA fragment. Factors present in an extract of HeLa nuclei protect a putative Sp1 binding between -74 and -65 and a likely octamer binding site site between -219 and -212. We suggest that USF, together with an Sp1-like factor and an octamer binding factor, are part of the U1 snRNA transcription complex, although we have not yet been able to show an effect of any of these partially purified factors on U1 expression. In addition to these more common transcription factors, we have evidence that suggests that other U-type snRNA specific transcription factors are required for proper U1 expression. An attempt to purify these snRNA specific transcription factors is underway.

L 433 **Involvement of Transcription Factors in Drosophila 5S RNA Gene Transcription.** Anita O'Connell, Alonzo Garcia, and Stephen Sharp. UC Irvine, California College of Medicine, Irvine, CA 92717.

The A-Box sequence motif of Drosophila tDNA is essential for faithful transcription and appears to be a site for TFIIIC activity. TFIIIC binding to tDNA however is absolutely dependent on the presence of the B-Box sequence motif. In conjunction with TFIIIB activity, TFIIIC forms a stable transcription complex on tDNA. Since Drosophila tDNA and 5S DNA share a requirement for TFIIIC, the question was raised whether 5S DNA contained sequences equivalent to the A-Box and B-Box of tDNA. The approach taken to solve this question involved independently "walking" A-Box and B-Box sequences across the 5S DNA using 5S DNA Linker-Scanning mutations as parent templates. Homologous in vitro transcription assays and stable complex formation assays revealed no A-Box or B-Box-equivalent sequences in the 5S RNA gene. It was expected that there may have been an A-Box like region since the sequence between nucleotides 22 to 31, although not identified as an ICR, has a 7-of-10, and the sequence 50 to 59 has a 9-of-10, homology to the A-Box consensus sequence of tDNA. It thus appears that TFIIIC functions differently in 5S DNA transcription compared to tDNA transcription. The mechanism of using this factor in the formation of the 5S DNA transcription complex does not appear to require sequestration of TFIIIC into a stable complex as required for tRNA gene transcription. The 5S RNA gene transcriptional unit is complex and cannot be considered to be a tRNA gene having a requirement for TFIIIA binding. Aspects of TFIIIC function will be discussed in view of our previous work which defined four control regions for Drosophila 5S RNA gene transcription.

L 434 CHARACTERIZATION OF PROMOTER REGIONS OF THE XENOPUS LAEVIS RIBOSOMAL RNA GENE THAT ARE REQUIRED FOR TRANSCRIPTIONAL ENHANCEMENT.
Louise Pape, Jolene Windle and Barbara Sollner-Webb,
The Johns Hopkins University School of Medicine, Baltimore MD 21205

Regions of the upstream spacer of the Xenopus laevis ribosomal RNA gene (the '60/81' bp repeats) direct transcriptional enhancement by RNA polymerase I (Reeder, Cell 38:349, 1984). Since these enhancer repeats are homologous to the central portion of the rDNA promoter, we asked which regions of the promoter are necessary to mediate the enhancement. To this end, the '60/81' bp repeats were cloned upstream of a series of rDNA promoters bearing linker scanning mutations. The linker scanning mutants with lesions in the essential promoter region from -140 to -128 and from -36 to +10 are not rescued by the enhancer, as shown by two independent assays for enhancer activity. Virtually all of the other linker scanning mutations do not interfere with enhancer activity. However, two linker scanning mutants in the 'enhancer cognate' region of the promoter (LS-106/-97 and LS-94/-89) which are wild type in their ability to direct transcriptional initiation are defective in their ability to mediate enhancement. Curiously, a mutant with polylinker sequences replacing virtually the entire enhancer cognate region from -115 to -77 is able to mediate enhancement, although LS-115/-77 contains mutations in all of the positions that LS-106/-97 does, the changes are to different nucleotides. We conclude that the precise sequence arrangement within the enhancer cognate region of the promoter is important in mediating rDNA transcriptional enhancement.

L 435 IDENTIFICATION, CHARACTERIZATION, AND PURIFICATION OF PROTEINS BINDING TO THE MOUSE IMMUNOGLOBULIN HEAVY CHAIN ENHANCER, Craig L. Peterson, Xiao-Fan Wang and Kathyrn L. Calame, Molecular Biology Institute and Department of Biological Chemistry, UCLA, Los Angeles, CA. 90024.

Eight protein binding sites on the immunoglobulin heavy chain (IgH) enhancer element have been identified by exonuclease III protection, gel retardation, and DNase I footprinting (Peterson et al. MCB Dec.1986). Site-directed mutagenesis is being used to determine the functional significance of six of these binding sites. Preliminary results indicate that deletion of sites B, C2 and C3 results in substantial decreases in enhancer function in vivo, but none of these individual mutations completely abolish enhancer function. These data are consistent with the hypothesis that enhancers are composed of multiple independent components that additively contribute to enhancer function. Binding site C2 binds a protein, designated IgHeIII, that is specific for the IgH enhancer. IgHeIII has been purified to near homogeneity from nuclear extracts of mouse plasmacytoma cells by heat treatment and sequential chromatography through DEAE sephacel, heparin agarose, FPLC mono Q, and FPLC mono S. Preliminary kinetic analyses has revealed that interactions occur between IgHeIII and proteins binding to adjacent sites. Such protein-protein interactions may allow unique mechanisms for regulation of enhancer function and allow the same proteins to function differently in different enhancers, depending on how the binding sites are positioned.

L 436 CLASS II GENES OF THE MHC IN MINIATURE SWINE, Karen Pratt, Sharon Germana, Mona El-Gamil, Francois Hirsch, and David H. Sachs, Transplantation Biology Section, Immunology Branch, National Cancer Institute, Bethesda, MD 20892.

The number, orientation, and order of the class II genes of the SLA are being studied through the analyses of cloned genomic DNA. These genes code for at least 6 different sets of α and β chains. Class II genes must be coordinately expressed in pairs since, in vivo, a specific α chain will complex only with its cognate β chain but, in vitro, can be experimentally induced to pair with other β chains (Germain, R.N. and Quill, H. 1986. Nature 320:72). Class II genes are highly conserved among mammals and, in fact, we have found analogs in the swine genome to the major and probably to the minor α and β genes in the human. We are in the process of fine genomic mapping and sequencing of this region of the swine genome. We will determine if DNA sequence homologies exist in the regulatory regions of the class II complex in swine similar to those found already in the mouse (Flavell et al. 1986. Science, 233:437; Gillies et al. 1984. Nature 310:594) and compare sequences in these regions to comparable regions in other mammals. This research will also determine if unique homologies are present in the regulatory regions of the various αβ pairs. These data will advance our understanding of the genetic basis of coordinate expression of class II α and β genes.

L 437 DNA-PROTEIN BINDING STUDIES OF THE PROMOTER/REGULATORY ELEMENT OF THE 78,000 DALTON GLUCOSE/CALCIUM IONOPHORE REGULATED PROTEIN. Elpidio Resendez, Jr., Augustine Y. Lin, Shin C. Chang, Yong K. Kim, Amy S. Lee. Department of Biochemistry, School of Medicine, University of Southern California, Los Angeles, CA 90033.

In many cell types, the synthesis of a group of proteins known as the glucose regulated proteins (GRPs) is enhanced when the cells are placed under several stressful conditions such as glucose starvation, or calcium ionophore treatment. The synthesis of GRPs is also increased when a Ts mutant K12 is cultured at 40°C. We have isolated the rat gene encoding the 78,000 dalton GRP. A 292 base pair (bp) region located 5' to the structural gene possesses enhancer-like properties when fused to a heterologous gene. This enhancer element confers calcium ionophore induction and can compete for trans-acting factors in vivo. Using the DNA-protein complex mobility shift gel assay, a 262 bp fragment that contains part of the enhancer element was found to bind to factor(s) isolated from human and hamster cell lines. By varying the amount of protein extract and salt concentration used in the binding reactions, we optimized the conditions for the formation of complexes. In addition, we tested the effectiveness of two non-specific competitors, poly (dI-dC) and E. coli DNA. Under optimum conditions we detected three complexes. One of these complexes shows relatively tight binding since it can only be dissociated at very high concentrations of non-specific competitor DNA. Also, this complex can be competed with lower concentrations of the unlabeled 262 bp fragment than non-specific DNA, showing that the binding is sequence specific. This complex is also observed using extracts from K12 cells under inducing (A23187 and 40° C) and non-inducing treatments. We are presently investigating if the concentration or affinity of the binding factor changes under the different treatment conditions.

L 438 ANALYSIS OF THE HUMAN VIMENTIN PROMOTER AND 5' FLANKING REGIONS
Rittling, S.R., and Baserga, R. Temple University School of Medicine Department of Pathology Philadelphia, PA 19140
We have isolated a genomic clone from a human library containing the first exon and 1.5 kb of 5' flanking sequences of the vimentin gene. The cDNA for this gene was isolated in our laboratory, and the steady-state levels of the vimentin mRNA are growth regulated. The vimentin promoter region contains CAAT and TATA boxes which by themselves support limited transcription of a reporter gene (CAT) placed downstream of the cap site. 5' deletion analysis of the promoter region indicates that there are two regions one at about -300, the other at about -1000, which enhance the promoter activity. In addition, a region at around -600 seems to exert negative control over the action of the enhanced promoter. Supported by Grant AG-00378 from the National Institutes of Health.

L 439 MOLECULAR ANALYSIS OF DEFECT IN LIGHT CHAIN GENE EXPRESSION IN HUMAN HEAVY CHAIN DISEASE, Steven M. Rosen, Mei-hui Teng, Joel N. Buxbaum, New York University Medical Center, NY, NY 10010.
Human Heavy Chain Disease (HCD) is a lymphoproliferative disorder of B cells that is characterized by the synthesis of an internally deleted heavy chain and in most cases the absence of light chain synthesis. The loss of light chain synthesis is associated with only certain types of heavy chain protein deletions, implying a regulatory role for those particular structures or the loci encoding them. We have undertaken molecular studies of the human HCD line Omm, which expresses an internally deleted protein and no light chain protein, to determine whether the absence of light chain protein synthesis in these cells is due to a regulatory phenomenon or a structural defect in the light chain gene. We have determined that Omm cells have deleted both kappa alleles and possess a single rearranged lambda gene. The L/H mRNA ratio the Omm cells is lower than that of normal immunoglobulin producing cells, thus implying a defect in the coordinate expression of heavy and light chain immunoglobulin genes. Sequence analysis of the cloned rearranged lambda gene shows that the gene possesses two intronic deletions; however, all exon structures are normal. Transfection of the rearranged lambda gene from the HCD cells into J558L, a line capable of expressing immunoglobulin light chain genes, allowed the transfected gene to be expressed at the protein level and secreted. These data indicate that the defect in light chain protein synthesis in the Omm HCD cells represents a regulatory defect that is intrinsic to the cellular environment of the HCD cells and may not be due to any defect within the gene.

L 440 UNUSUAL TRANSCRIPTION OF A CLASS II α GENE IN THE HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX (MHC). Sandra Rosen-Bronson and Eric O. Long, Laboratory of Immunogenetics, NIAID, NIH, Bethesda, Maryland 20892.
The human class II MHC α gene DZα produces two transcripts of ~1.2 kb and ~3.5 kb. In contrast, other class II MHC genes encoding the α and β chains of the HLA-DR, -DQ and -DP antigens are transcribed and processed into ~1.3 kb mRNAs. cDNA clones corresponding to the 3.5 kb DZα transcript show that it is not an unspliced precursor of the shorter transcript. The 3.5 kb RNA has a longer 3' untranslated region and the splicing pattern of the coding region does not give rise to the typical class II α chain exons predicted from the genomic DNA sequence. Nucleotide sequencing, primer extension and in vitro translation studies are in progress to define the splicing patterns of the two DZα transcripts and to determine the coding potential of this unusual immune response gene.

L 441 FOOTPRINT ANALYSIS OF AVIAN RETROVIRAL LTR'S AND GAG ENHANCER, Thomas A. Ryden, Kristen Carlberg, Elizabeth Mansfield, and Karen Beemon, Johns Hopkins University, Baltimore, MD 21218.

Transcriptional enhancers have been identified within avian retroviral LTR's and gag genes. In an attempt to further localize important sequences within these regions, we have performed DNase I footprint analysis using proteins from nuclear extracts. The Rous sarcoma virus LTR binds protein on an imperfect direct repeat at the 5' end of U3. The end-points of the footprint correspond to those of enhancer activity defined by deletion analysis. We have further shown that this binding activity fractionates in highly purified samples of an SV40 enhancer core binding protein, EBP-20/CBP (P. Johnson and S. McKnight, manuscript in preparation). We have also analyzed two less actively transcribed LTR's from Fujinami sarcoma virus and the endogenous virus RAV-0. Neither of these bind the highly purified EBP-20.

This laboratory has previously identified an enhancer within the gag protein coding region (Arrigo et al., Mol. Cell. Biol., Jan. 1987). The nuclear extract protects several sequences in this region. Strikingly, EBP-20 fractions protect sequences within a region shown by deletion analysis to be required for enhancement.

L 442 AFFINITY-COMPETITION CHROMATOGRAPHY OF SEQUENCE SPECIFIC DNA-BINDING PROTEINS, Michael G. Sargent, NIMR, London, U.K.

A method of purifying sequence specific DNA-binding proteins using affinity-competition, based on the ideas of Bearden (J. Biochem. Biophys. Methods, 2, 37, 1980) and Sanzo et al. (Biochem. 23, 6491, 1984) will be described. High salt extracts containing proteins of interest are continuously circulated through chromatographic columns containing specific and non-specific DNA sequences and a dialysis unit that allows the salt to diffuse out of the mobile phase. As the salt concentration is diluted to the point where interactions between proteins and DNA are favoured, the proteins are partitioned between the columns according to their relative affinity for the immobilised DNA. By using a large excess of heterologous DNA, relative to specific DNA considerable enrichment for specific sequences can be obtained. The technique has been investigated in detail with respect to (i) design of apparatus, (ii) choice of matrix and attachment of DNA, (iii) optimal operating conditions, (iv) optimal sample preparation. This has lead to the development of an apparatus that can be used to fractionate a single extract using a large number of columns in series, on which different DNAs are immobilised. The technique has been used to purify (i) factors that activate transcription of specific genes, (ii) phosphotyrosine containing proteins with sequence specific DNA-binding properties in transformed cells, (iii) other proteins that bind to sequence specific DNA.

L 443 ASPECTS OF VIMENTIN GENE REGULATION, [1]Christina M. Sax, [1]Francis X. Farrell, [2]Janet A. Tobian, [1]Zendra E. Zehner, [1]Virginia Commonwealth Univ., Richmond, VA 23298, and [2]NICHD, NIH, Bethesda MD 20205. Vimentin is one member of the intermediate filament multigene family which exhibits both tissue- and developmental-specific expression. In vivo, vimentin is expressed in cells of mesenchymal origin, and is down-regulated during myogenesis. The chicken vimentin 5' flanking region contains 5 GC boxes and an inversely oriented CCAAT box, while lacking a TATA box. In vitro and in vivo analysis indicates that the transcription factor Sp1 plays a role in vimentin expression. Both gel mobility shift and footprint analysis indicate that Sp1-enriched HeLa nuclear extracts specifically interact with the three most proximal GC boxes. When fused to the bacterial CAT gene, a fragment containing 4 GC boxes and the CCAAT box (+1 to -290) promotes transcription 10-fold when transfected into mouse L-cells (fibroblasts) or microinjected into Xenopus oocytes. Co-injection of the SV40 enhancer and its GC boxes inhibits this promoter activity by 82%. However, 3 CAT-fusions containing a single GC box do not act as efficient promoters in either system, suggesting that multiple GC boxes might be required for Sp1 to act efficiently in vimentin expression. Interestingly, these same 4 constructs do not promote CAT activity when transfected into a rat hepatoma cell line (MH1C1) which does not express vimentin. The nature of this inactivity is under investigation. Elements required for vimentin regulation during myogenesis have initially been defined by transfection into a rat myogenic cell line (L6E9). Both whole- and mini-gene constructs containing 3.5kb and 0.8kb, respectively, of 5' flanking sequence exhibit proper down-regulation of vimentin during differentiation. Analysis of these cis- and trans-regulatory elements is in progress.

L 444 DIFFERENTIAL INTERACTION OF IMMUNOGLOBULIN ENHANCER BINDING FACTORS WITH OTHER ENHANCER ELEMENTS, Uwe Schlokat and Peter Gruss, ZMBH, INF 282, 6900 Heidelberg, FRG.

The immunoglobulin heavy chain gene (IgH) enhancer represents a cis-essential control element that was shown to confer lymphoid-specific gene expression. In vivo and in vitro competition transcription experiments as well as genomic footprinting data have provided evidence for the involvement of trans-acting factors in this cell-type restricted enhancer-mediated transcriptional activation.
It has previously been suggested that the major enhancing activity of the IgH enhancer (a 1 kb Xba I fragment termed "Xba E") resides in the central Pst I-Eco R I fragment. Using this fragment (and subfragments thereof) for footprinting and band shifting experiments, we have identified at least three distinct factors (IgPE-1 to IgPE-3) which interact with the IgH enhancer in B-cell nuclear extract. Surprisingly, corresponding factors seem to present also in cell lines where the IgH enhancer is unable to potentiate transcription. Furthermore, IgPE-1 also binds to Moloney Murine Sarcoma Virus and Polyoma Virus enhancer sequences, whereas IgPE-2 binding can be competed by a homologous sequence motif of the Lymphotropic Papovavirus enhancer. Neither of these enhancers, all of which exhibit certain cell-type preferences, is able to interact with IgPE-3. Therefore, a combination of shared factors and factors, which are unique to a given enhancer, might be necessary for enhancer-mediated, cell-specific transcription. Experimental shuffling of the respective binding sites might lead to new combinations and, consequently, create enhancers with altered cell-type specificity. Transfection results are discussed in light of this hypothesis.

L 445 PROBING FOR SUPERCOILED-INDUCED DNA CONFORMATIONAL CHANGES, Petra M. Scholten, R.E. Herrera, L. Runkel and A. Nordheim, ZMBH, INF 282, 6900 Heidelberg, FRG.

Supercoiling is postulated to influence many cellular functions of DNA, i.e. replication, recombination, transposition and transcription. Several cases of supercoil-dependent modulations of transcription have been reported in prokaryotic and eukaryotic systems. Supercoiling has also been shown to induce in vitro secondary and tertiary structural changes in the DNA helix, e.g. cruziforms and Z-DNA.
Z-DNA structures have been detected in vitro in the 5' control regions of several eukaryotic transcription units, e.g. the YP2 and Cyt c genes of yeast, the human c-fos gene and the chicken β-globin gene. This conformational change was demonstrated by enhanced reactivity to the chemical diethyl pyrocarbonate (DEP) which preferentially reacts with the N-7 atoms of purine residues located in regions of Z-DNA. Presumably due to its hydrophobic character DEP easily passes into cells (analogously to DMS) and, therefore, can be potentially employed to probe for the formation of Z-DNA in vivo. In order to study the formation of Z-DNA in vivo, we have used an inducible bacterial system in which the superhelical state of DNA can be modulated. Results of these in vivo chemical mapping experiments will be discussed.

L 446 APOLIPOPROTEIN B GENE: STRUCTURE AND REGULATION, James Scott*, Richard J. Pease*, Lyn M. Powell*, Simon C. Wallis*, Brian Blackhart+, Brian J. McCarthy+, Robert W. Mahley+, Beatriz Levy-Wilson+ and Timothy J. Knott*, *MRC Clinical Research Centre, Harrow, England and +Gladstone Foundation Laboratories for Cardiovascular Disease, San Francisco, USA.

Apolipoprotein (apo) B is the sole protein component of low density lipoprotein (LDL) and is the ligand responsible for the receptor-mediated uptake and clearance of LDL from the circulation. We have determined the complete 4563 amino acid (MW 514 kDa) sequence of apo B precursor from cDNA clones. Numerous lipid binding structures are distributed throughout the extraordinary length of apo B and must serve its special functions as a nucleus for lipoprotein assembly and in maintaining plasma lipoprotein integrity. A domain enriched in basic amino acid residues has been identified as important for the cellular uptake of cholesterol by the LDL receptor pathway. A single copy gene for human apo B has been localised to chromosome 2 in the region p24. The human gene extends over 43 kb and comprises 29 exons and 28 introns. Most of the introns occur in the amino terminal one-third of the gene. A number of DNA polymorphisms have been detected which affect plasma lipid levels. We are currently studying the regulation of the gene.

L 447 METHYLATION OF 5' SEQUENCES INFLUENCES CHROMATIN STRUCTURE AND EXPRESSION OF THE THY-1 GENE; Michael Sneller and Kurt Gunter, NIAID/NIH, Bethesda, MD 20892. Thy-1 exhibits marked differences in expression in various tissues in many species; therefore, it is of interest to define possible mechanisms which may regulate Thy-1 expression. We produced Thy-1 negative variants of the murine T cell lymphoma EL-4 by mutagenesis with ethyl methanesulfonate (EMS), negative selection with anti-Thy-1 monoclonal antibodies (mAbs) plus complement (C) and fluorescence activated cell sorting (FACS). Thy-1 surface negative ($Thy\text{-}1^-$) mutants produced in this manner were shown to produce no detectable Thy-1 mRNA, but contained an intact Thy-1 gene as determined by Southern blotting. 5' CG sequences, which had been demethylated in the parent EL-4 clone, were completely methylated in the EMS-induced $Thy\text{-}1^-$ varient. In addition, a DNAase I hypersensitive site which mapped to the 5' end of the Thy-1 gene in EL-4 was absent in the $Thy\text{-}1^-$ varient. Treatment of this $Thy\text{-}1^-$ clone with 5-Azadeoxycytidine (5-dAZA) resulted in re-expression of surface Thy-1, demethylation of the 5' CG sequences, and regeneration of the DNAase I hypersensitive site. These studies indicate that methylation of certain critical DNA sequences in the 5' region of the Thy-1 gene can alter local chromatin structure and regulate expression of this gene.

L 448 Transcriptional Regulation of a Pair of Silkmoth Chorion Genes in Flies. N.A.Spoerel, S.A.Mitsialis and F.C.Kafatos. Biological Laboratories, Harvard University, Cambridge MA 02138.
Recent P-element mediated germline transformation studies have shown that the Bombyx mori chorion gene pair A/B.L12 is expressed in Drosophila with tissue and temporal specificity resembling the endogenous Drosophila chorion gene, s-15-1. The short common 5′ flanking region (271 bp) was fused to the bacterial chloramphenicol acetyltransferase (CAT) gene and shown to contain all the cis-regulatory elements essential for regulated transcription upon transfer into the Drosophila germ line. A series of deletion and linker-substitution derivatives of this promoter region are being tested to identify and further delineate the cis-regulatory elements. Removal of a hexanucleotide sequence (TCACGT), found in most insect chorion genes sequenced to date, abolished expression of the CAT-marker gene completely (less than 1/1000). Mutations targeting multiple inexact repeats of the sequence TTGNGAAA, variants of which can be found in several Drosophila chorion genes, changed the proper temporal expression profile during choriogenesis. The silkmoth promoter has also properties of a tissue-specific enhancer, and is able to confer tissue- and temporally-regulated expression onto a heterologous promoter (Alcohol Dehydrogenase).

L 449 DNA-PROTEIN INTERACTIONS WITHIN FUNCTIONAL ELEMENTS OF THE SV40 ENHANCER. Richard Sturm, Brian Ondek, and Winship Herr, Cold Spring Harbor Lab., Cold Spring Harbor, N.Y. 11724
Genetic revertant analysis has shown that the SV40 enhancer consists of at least three independent enhancer modules, 15 to 22 bp in length, designated A, B, and C [Herr and Clarke, Cell, 45, 461-470 (1986)]. Multiple tandem copies of either the B or C elements created from 17 bp synthetic oligonucleotides can enhance transcription of a human β-globin gene but not analogous B and C oligomers containing point mutations known to inactivate SV40 enhancer function. To examine the nature of trans-acting factors which may interact with these three elements we have performed gel retardation experiments using partially fractionated HeLa cell extracts. An SV40 enhancer fragment gives a major retarding activity that can be inhibited by competition with the wild type enhancer itself and the wild type and mutant B oligomers, but not the C oligomers indicating an interaction with the B element. The B domain contains a sequence homologous (boxed below) to sequences found in the Ig enhancer and promoter regions, histone H2B genes and several snRNA genes.

SV40 17 bp B domain AGTATGCAAAGCATGCA
dpm7→TT A G ←dpm2

The dpm2 point mutations, which inactivate the SV40 enhancer B domain but do not apparently affect the interactions detected by gel retardation, map just outside this region. To analyze the significance of the octamer sequence we have created a further mutant called dpm7. The effect of the dpm7 mutant on viral viability is significantly less than the dpm2 mutations. We are presently analyzing the effects of the dpm7 mutations on enhancer function and DNA-protein interactions.

L 450 THE CHICKEN αA-CRYSTALLIN GENE: STRUCTURE AND FUNCTION. Mark A. Thompson, John F. Klement, James W. Hawkins, Ana B. Chepelinsky and Joram Piatigorsky, Laboratory of Molecular and Developmental Biology, National Eye Institute, NIH, Bethesda, MD 20892.

The single chicken αA-crystallin gene and 2.5 kb of its 5' flanking sequence have been isolated and sequenced. The gene is 3.4 kb long, contains 3 exons and lacks the insert exon found in rodents. RNA from the insert exon is alternatively spliced into mRNA in rodent lenses . The gene structures and coding sequences in the αA-crystallin are highly conserved between chicken, mice and hamsters. The 5' flanking sequences are less similar between rodents and chickens. The best homology between the 5' flanking sequences of the αA-crystallin genes of hamster, mouse and chicken exist between the TATA box and position -65. Our experiments on the mouse and those of Okazaki et al., (EMBO J. 4, 2589-2595, 1986) on the chicken αA-crystallin gene suggest that different 5' flanking sequences are critical for function in these 2 species. As different assays and experimental designs were used in these previous experiments, we are now comparing the chicken and mouse αA-crystallin promoters using the pSVO-CAT expression vector in explanted embryonic chicken lens epithelia. A fragment comprising -111 to +46 of the mouse promoter is very efficient in this system; initial experiments indicate that -279 to +10 of the chicken promoter functions in the chicken lens epithelia, although less efficiently than the mouse promoter. Further deletions of the chicken promoter are in progress.

L 451 THE REGULATION OF EXPRESSION OF THE TISSUE-SPECIFIC CHICKEN ERYTHROCYTE HISTONE H5, Cecelia D. Trainor and James Douglas Engel, Northwestern University, Evanston IL 60201.

Chicken erythrocyte histone H5 is a tissue-specific protein found only in nucleated erythroid cells. Histone H5 is a replacement variant histone which gradually substitutes for linker histone H1 during erythroid cellular maturation. Its function is unknown but it is thought to participate in inducing nuclear condensation and the suppression of continued transcription which mark this lineage. Histone H5 is the earliest differentiation marker yet identified in the chicken erythroid lineage. We are studying the regulation of expression of this gene and protein and have determined that H5 messenger RNA accumulates only in erythroid tissues. We have employed a transient expression assay to determine that H5 mRNA is expressed in a tissue-specific manner when the H5 gene is introduced into erythroid (Avian Erythroblastosis Virus transformed erythroid precursor cells) versus non-erythroid (chicken embryo fibroblasts) cells by transfection. We have constructed a series of deletion mutants of the histone H5 gene for the transfection assay, and have localized regions of the locus which are involved in the tissue-specific expression of this gene. We have also utilized histone H5/chloramphenicol acetyl transferase hybrid genes to define the function of some segments of the locus. We hope to use these regulatory elements to identify proteins important to the erythroid differentiation process.

L 452 REGULATION OF PLASMINOGEN ACTIVATOR GENE EXPRESSION IN PIG KIDNEY CELLS. D. von der Ahe, D. Pearson, J. Nakagawa and Y. Nagamine, Friedrich Miescher-Institut, P.O.Box 2543, CH-4002 Basel, Switzerland.

Urokinase-type plasminogen activator (uPA) gene expression in a pig kidney cell line, LLC-PK$_1$ is induced by the cAMP-elevating hormones calcitonin and vasopressin and by phorbol esters acting through a cAMP-independent pathway. uPA mRNA accumulation is not reduced by protein synthesis inhibitors indicating that uPA gene activation is a primary transcriptional event. To understand the basis of hormone regulation we have been investigating the promoter region and flanking sequences of the uPA gene. Using constructions with the CAT gene as a reporter gene we found that uPA-CAT is not expressed in HeLa cells, but in LLC-PK$_1$ and COS cells uPA-CAT is well expressed. By deleting the 5'-flanking region of the hybrid gene to -82, basal CAT expression and hormone-induced expression changed only slightly. To identify transacting factors involved in the regulation of uPA gene expression we studied protein-DNA interactions with a variety of methods. In gel shift and in DNase I footprinting analyses we showed specific interactions between nuclear proteins from LLC-PK$_1$ cells and 5'-flanking sequences of the uPA gene. We are investigating the effect of cAMP and phorbol esters in protein-DNA interactions.

L 453 SEQUENCE REQUIREMENTS FOR THE REGULATED EXPRESSION OF A HERPESVIRUS LATE GENE, Jerry P. Weir, Department of Microbiology, The University of Tennessee, Knoxville, TN 37996-0845.

The gene that codes for the Herpes Simplex Virus 1 (HSV-1) glycoprotein C (gC) gene is classified as a late herpesvirus gene by virtue of its stringent requirement of viral DNA replication for expression. To examine the DNA sequences that regulate expression of the gC gene, the region of DNA that includes the mRNA start site and sequences upstream from it, was cloned 5' to the coding sequences for the bacterial enzyme, β-galactosidase (β-gal), such that the gC promoter directed transcription of β-gal. Although this chimeric gene was expressed when transfected into mammalian cells that were also infected with HSV-1, it did not appear to be regulated as a late viral gene. When the chimeric gene was introduced into the viral genome at the thymidine kinase (TK) locus by homologous recombination, β-gal was expressed by the recombinant virus as a true late gene. Recombination into the TK gene was facilitated by addition of a β-gal indicator into the agar overlay of a plaque assay that selected for TK^- virus. To define the sequences that are involved in regulation of gC as a late gene, a series of deletions were made in the gC promoter-regulatory region. The effect of these deletions on the expression of β-gal in recombinant viruses was used to define the boundaries of the late gene regulatory region.

L 454 TRANSCRIPTIONAL CONTROL OF MOUSE MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II GENES, Jerold G. Woodward and Michael Kern.

The major histocompatibility complex (MHC) class II genes code for cell surface Ia molecules which are differentially expressed on different tissues. In addition, Ia expression is induced on certain cells upon exposure to gamma interferon. Using nuclear run-off analysis, we have shown that induction of cell surface Ia expression by gamma interferon is largely the result of transcriptional rate increases in the class II genes. Increased transcription is detected in as little as 4 hours after the addition of IFN-gamma and is not inhibited by cyclohexamide. In order to evaluate the presence of trans-acting factors, we performed DNA binding assays with crude nuclear extracts and a 542 bp fragment of DNA encompassing the A beta promoter region. A high affinity DNA binding protein was detected in nuclear extracts from both expressing and non-expressing cells. This region contains the class II consensus sequence, CTGATTGGTT. The relationship of this DNA binding protein to the tissue specific transcription of the class II genes is currently being investigated.

L 455 TRANS-ACTING FACTOR(S) INTERACTING WITH RAT SKELETAL EMBRYONIC MYOSIN HEAVY CHAIN PROMOTER IS (ARE) FOUND IN BOTH HELA AND FUSED MOUSE C2 CELLS. Yie-Teh Yu and Bernardo Nadal-Ginard, Howard Hughes Medical Institute, Children's Hospital, and Harvard Medical School, Boston, MA 02115. Transient expression studies on rat skeletal embryonic myosin heavy chain (MHC_{Emb}) promoter indicated that the nucleotide sequence downstream from -280 (transcription start site, +1) is sufficient for the tissue-specific expression in fused mouse C2 cells. This DNA segment of MHC_{Emb} promoter has been further dissected to identify the nucleotide sequence which is required for promoter activity. A series of upstream nucleotide sequence deletion mutants of MHC_{Emb} promoter were constructed (D1, D2, D3, and D4) which contain upstream sequence to approximately -1450, -279,-208, and -110, respectively. The promoter activities of these mutants were assayed in an *in vitro* transcription system utilizing nuclear extract prepared from HeLa cells. All mutants showed similar level of transcriptional activity, indicating that the MHC_{Emb} promoter is located downstream from -110. The presence of trans-acting factors in HeLa nuclear extract, which interact with MHC_{Emb} promoter was demonstrated by competition studies in the *in vitro* transcription system. The transcriptional activity of mutant D2 was reduced only when a DNA fragment (P, -21 to -110) was added to the *in vitro* transcription assay, suggesting competition for the trans-acting factors which interact with fragment P. The interaction of fragment P with trans-acting factors in the nuclear extracts prepared from HeLa and fused C2 cells was examined in a protein-DNA binding mobility shift assay in the presence of poly(dI-dC). Both nuclear extracts demonstrated specific binding of fragment P. This protein-DNA binding in both nuclear extracts was unstable at higher concentration of mg^{+2} (> 1mM), but was relatively stable at various concentration of NaCl (up to 150mM). Although the exact binding site has not yet been mapped, the trans-acting factors appear not to bind to the CAAT box of MHC_{Emb} promoter. The identification of the binding sites is now in progress.

Post-Transcriptional Control of Gene Expression

L 500 CELL-TYPE-SPECIFIC COLLAGEN GENE EXPRESSION IS DETERMINED BY POST-TRANSCRIPTIONAL MECHANISMS. Sherrill L. Adams, Ingrid M. Weiss, Julie A. Gubernick, John Choi, Ji-hou Xin, Kim M. Pallante and Vickie D. Bennett. University of Pennsylvania, PA 19104.

Type I collagen is found in the extracellular matrix of nearly all connective tissues except cartilage, but displays dramatic quantitative variation. Type II collagen, in contrast, is found almost exclusively in cartilage. When we examined the molecular basis for this cell-type-specificity, we found that transcription rates of both the types I and II collagen genes are relatively constant, but that there are discrepancies between transcription rates and steady-state RNA levels in some cell types, suggesting that cell-type-specific differences in expression of these genes are due in part to differences in RNA stability, rather than to differences in transcription rates. Furthermore, the gene encoding the α2 subunit of type I collagen displays cell-type-specific utilization of transcription start sites, giving rise to RNAs with different 5' untranslated regions. We are currently investigating the effects of alternate transcription start sites on stability and translational efficiency of the α2(I) collagen RNA. This is of particular interest, since in cartilage-producing cells the type II collagen RNA is translated preferentially, while the α2(I) collagen RNA is subjected to a severe elongation block, perhaps due to the altered 5' untranslated region of the RNA in these cells. Thus we believe that the cell-type-specificity of types I and II collagen gene expression are determined by a variety of post-transcriptional mechanisms.

L 501 SIMIAN VIRUS 40 T ANTIGEN MEDIATES TRANS-ACTIVATION INDIRECTLY THROUGH THE ACTIVATION OF EXISTING CELLULAR TRANSCRIPTION FACTORS, James C. Alwine and Gregory Gallo, Dept. of Micro., Univ. of Penna., Phila., PA 19104-6076.

Our laboratory has previously demonstrated that SV40 T antigen mediates the activation, in trans, of the SV40 late promoter as well as a variety of other viral and cellular promoters. Several experiments suggest that this activation does not require the direct interaction of T antigen with DNA. T antigen may mediate trans-activation indirectly through the induction or activation of cellular trans-acting factors. Using the viral late promoter we have tested this model. The sequence elements of the late promoter necessary for T antigen mediated trans-activation, have been previously defined in this laboratory by in vivo expression analyses. The elements are denoted tau (SV40 nucs. 154-200) and delta (SV40 nucs. 200-294), both colinear with the enhancer region, and omega, which includes sequences within the origin of replication. Using gel retardation analyses, we have determined that specific cellular factors bind to these elements. These factors are present in CV-1 cells (permissive monkey cells which do not contain T antigen) in approximately the same amount as in COS cells (CV-1 cells which produce T antigen and can activate the late promoter). However, the factors form more stable complexes with the promoter elements when derived from COS cells. The data suggest that T antigen affects existing cellular transcription factors allowing them to activate the late promoter through increased stability of binding to the tau, delta and omega promoter elements. This may explain how cellular gene expression can be altered by T antigen; the activated factors would bind more stably to their normal cellular target promoters, as well as the late promoter.

L 502 SPLICING OF MUTUALLY EXCLUSIVE EXONS OF TROPONIN T IN TRANSFECTED CELLS. Athena Andreadis and Bernardo Nadal-Ginard, Howard Hughes Medical Institute, Children's Hospital and Harvard Medical School, Boston, MA. 02115.

The rat skeletal troponin T gene gives rise to a single primary transcript, yet could code for up to 64 isoforms via alternative splicing. Two exons in its 3'end are incorporated in a mutually exclusive manner in the mature mRNA. Of these two exons, the 5' proximal (α) is adult-specific, whereas the distal one (β) is embryo- and muscle cell line-specific. Scanning of the sequence within and around these exons has revealed no significant deviation of their donor/acceptor sites from the consensus sequences, nor any obvious binding sites for trans regulatory factors, although hairpins could potentially form. Experiments are in progress to clarify the mechanism responsible for the differential processing of the two exons. Minigenes containing genomic subfragments that include α and β have been constructed and transfected into non-muscle (HeLa, cos) and muscle (YB30, C2) cells. Analysis of the minigene transcripts by S1 nuclease mapping has shown that, in non-muscle and growing muscle cells, the α(adult) exon is always absent, while the β(embryonic) is present. As in the native gene, the two exons are never spliced together. We have constructed deletions of β together with increasing lengths of its surrounding introns and are examining the resulting transcripts for inclusion of α. Deletion of β and its immediately flanking region does not promote incorporation of α; therefore, the exclusion of α is not a simple matter of donor/acceptor relative affinities. The results imply that both cis and trans factors are involved in this selection mechanism.

L 503 TRANSLATIONAL REGULATION OF THE 35S RNA OF CAULIFLOWER MOSAIC VIRUS
Gail Baughman and Stephen Howell, Univ. of Calif., San Diego CA

Cauliflower mosaic virus (CaMV) is a circular, double stranded plant DNA virus of approximately 8 kb in length. Two mRNAs are synthesized in infected plants. 35S RNA, transcribed by a strong promoter serves as template for replication by reverse transcription and encodes 6 potential protein products. The 19S RNA is transcribed from a weaker promoter and codes for the inclusion body protein. *In vitro*, 35S RNA is not efficiently translated compared to the 19S RNA. In order to examine features of the RNA that influence translational efficiency, RNA corresponding to the 5' region of 35S RNA, including the 600 b leader region and the first two potential ORFs (VII and I), was synthesized using the T7 transcription system and translated *in vitro*. Deletion analysis of this RNA identified a small region (68b) encompassing the tRNA primer site for DNA replication by reverse transcription and the initiation site for ORFVII translation that when removed, stimulates ORFI synthesis. The 68b deletion destroys the ability of the RNA to synthesize ORFVII gene product, therefore we tested if this protein normally acts as a translational repressor to modulate ORFI synthesis. RNA from a contruct containing an in-frame internal deletion that removes one-half of ORFVII failed to synthesize ORFI efficiently, indicating that the ORFVII protein itself is not responsible for the decreased expression of ORFI *in vitro*.

L 504 USE OF ANTISENSE RNA IN THE POST-TRANSCRIPTIONAL ALTERATION OF EXPRESSION OF A MOUSE DEVELOPMENTALLY REGULATED GENE, B-GLUCURONIDASE, Arturo Bevilacqua and Robert P. Erickson, University of Michigan, Ann Arbor, MI 48109

The study of gene function in most eucaryotes is difficult to analyze by standard mutational techniques. A valid alternative appears to be the use of antisense RNA. The application of this technique to a developmental system has previously been successful in Drosophila, where phenocopies of the Kruppel mutation were produced by injecting embryos with an antisense RNA of the Kruppel transcript. We have studied the possibility of inhibiting the expression of mouse B-glucuronidase by antisense RNA. This enzyme can be measured in single embryo and increases 100 fold its activity in the preimplantation stage of development. It is also of interest because its complete deficiency in humans results in Mucopolysaccharidosis VII. Thus, a successful application of the antisense technique would provide a new tool for in vivo genetic analysis in mammals and an animal model of a human disease. We have utilized an antisense RNA synthesized in vitro on a 1.4 kb cDNA clone of mouse B-glucuronidase gene, which is complementary to most of the coding sequence, but misses the 5', ribosome binding region (p Gus-2). Mouse zygotes were injected with 20-30 pg of such RNA (approximately 1/5 of the toal amount of RNA of the embryo at that stage), cultured to blastocyst and subjected to microfluorimetric assay for B-glucuronidase activity. We obtained activities varying from 50% to 60% that of control embryos. Injection of a control, unrelated RNA did not affect the enzyme activity. Limiting factors in RNA injection in embryos are dilution and degradation of the RNA using ^{35}S-labeled antisense RNA. 40 hours after injection, when embryos are at 4 cell/8 cell stages of development, the amount of injected RNA is reduced to 10%. In order to overcome the difficulties of the RNA injection and 5' missing construct, we are going to use expression vectors carrying both the construct already tested, and other constructs including the whole 5' region obtained from a genomic clone of the gene.

L 505 CIS AND TRANS FACTORS CONTROL MUSCLE-SPECIFIC DIFFERENTIAL SPLICING OF BOTH ALTERNATIVE AND CONSTITUTIVE EXONS IN TROPONIN T MINIGENE CONSTRUCTS, Roger E. Breitbart & Bernardo Nadal-Ginard, The Children's Hosp. & Harvard Medical School, Boston, MA

The rat fast skeletal muscle troponin T (TnT) gene constitutes a remarkable system in which to study the mechanisms of alternative RNA splicing. The differential incorporation of seven of its exons, subject to developmental and tissue-specific controls, generates as many as 64 TnT isoforms from the single gene (Medford et al, Cell 38:409-421, 1984; Breitbart et al, Cell 41:67-82, 1985; Breitbart & Nadal-Ginard, J. Mol. Biol. 188:313-324, 1986). We have undertaken experiments designed to analyze the mechanisms responsible for the differential splicing of five exons near the 5' end of the TnT gene. Minigene constructs containing genomic subfragments bearing these exons have been engineered in plasmid vectors and transfected into a variety of cells in culture. The RNAs spliced from the minigene transcripts have been characterized by S1 nuclease mapping. Remarkably, several different RNAs are produced in non-muscle (HeLa, COS, L) cells by the alternative incorporation of the TnT exons. An identical pattern of splicing is seen in (C2) myoblasts, which do not express the endogenous TnT gene. As these transfected muscle cells differentiate to form myotubes, however, additional combinations of the alternative exons are generated, ultimately resembling those of the endogenous TnT mRNAs. Of particular interest is the finding that certain constitutive TnT exons are also spliced out of the transcripts in non-muscle cells and myoblasts, while they are uniformly incorporated in myotubes. The exclusion of certain exons, which are flanked by conventional donor and acceptor sequences, from some spliced RNAs in non-muscle cells implicates cis regulatory elements in the control of this alternative processing. The appearance of muscle-specific splicing patterns in myotubes, including the appropriate incorporation of constitutive exons, must derive from trans-acting factors which are presumably specific to myogenic differentiation.

L 506 CONTROL OF A FIBROIN GENE EXPRESSION BY tRNA. Graciela C. Candelas, Raquel Dompenciel, Gerardo Arroyo, Anselmo Ortiz, Teresa Candelas and Clara E. Carrasco, Department of Biology, University of Puerto Rico, Rio Piedras, PR 00931.
During the analysis of fibroin production by the large ampullate glands of the spider, Nephila clavipes, we have found the process to be limited by tRNAs. In the cell-free translations of the fibroin's mRNA, both the size of the fibroin product (approximately 320,000 daltons) and the efficiency of elongation depend on tRNA populations present in the system. Increased translational efficiency and the full-size product are obtained upon supplementation with the tRNA complement from actively secreting glands. Stimulated glands in culture display functional adaptation of tRNA population through two waves of de novo tRNA synthesis and this has been found to be exclusive in the gland tissues. Within the time sequence of events elicited through stimulation for fibroin synthesis, the first peak of small RNA synthesis precedes that of the template . It is of rather large magnitude and contains small RNAs and several tRNAs, of which the major components are two alanine isoacceptor species. Of these, one turns out to be tissue- specific and to correlate quantitatively with active fibroin synthesis. The other wave is of lesser magnitude, and appears subsequent to the messenger and prior to the fibroin. This wave contains alanine and glycine tRNA isoacceptors, among others, in the same ratio as the corresponding amino acids appear in the fibroin. These findings add to the list of homologies displayed between the fibroin production strategies of the silkglands of Bombyx and the large ampullates of Nephila clavipes which we have discovered through our previous studies.
Supported by NSF DCB 8510244 and NIH RR 08102 Grants.

L 507 KINETICS OF pre-mRNA SPLICING IN VIVO: EVIDENCE FOR MULTIPLE LARIAT BRANCHPOINTS. Paul Cizdziel and Edwin C. Murphy, Jr. Dept. of Tumor Biology, UTSCC, Houston TX.
We have taken advantage of a novel temperature sensitive splicing event to study splicing of a viral pre-mRNA in intact cells. It was previously shown that MuSVts110 viral transcripts can be spliced only at growth temperatures of 33°C or lower: no splicing occurs at 39°C. Accordingly, MuSVts110-infected cells (6m2 cells) growing at 39°C, were shifted to 33°C and successive events which occur during splicing of the viral transcript were studied by S1 nuclease and primer extension analysis. No splicing intermediates were observed in 6m2 cells at 39°C, and only began to accumulate after a 60 minute lag period following the shift to 33°C, at which time excision at the 5' splice site and lariat containing intermediates could first be discerned. During the following 30 minutes, ligated exons began to accumulate. There appeared to be four separate branchpoints, defined as temperature dependent blocks to primer extension, at positions 26,44,62, and 75 bases upstream from the 3' splice site. These branch points were not observed in cells infected with a splicing-defective MuSVts110 revertant lacking 5 bases (AG/TGT) at the 3' splice site. Additional studies with this revertant suggest that the temperature dependent block to splicing is at an early step in the splicing process since excision at the 5' splice site uncoupled to exon ligation was observed in the revertant, but only at normally permissive temperatures for splicing. This result implies that an intact 3' splice site is not necessary for 5' splice site cleavage. Conversely, lariat intermediates do not form in the revertant RNAs cut at the 5' splice site, suggesting that all or part of the missing AG/TGT sequence at the 3' splice site was required for lariat formation.

L 508 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 (Labieniec-Pintel, L., and Pintel, D., J. Virol., 1986, Vol. 57, No. 3, pp. 1163). Retroviral shuttle vectors possessing MVM DNA which includes the P39 transcription unit are being used to analyze individual MVM capsid gene products resulting from cDNA's representing differentially spliced MVM 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.

L 509 ANALYSIS OF THE PROCESSING AND POLYADENYLATION REGION OF THE HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE IN VIVO AND IN VITRO IN HELA CELL NUCLEAR EXTRACTS.

Charles N. Cole, Fang Zhang, Roger Denome and Catherine Heath, Department of Biochemistry and The Molecular Genetics Center, Dartmouth Medical School, Hanover, NH 03756.

By using linker scanning, internal deletion and small insertion mutations, we have defined the sequences required for efficient 3′ end RNA processing of the HSV-tk gene. Efficient processing requires the proximal AATAAA hexanucleotide and the GT-cluster located downstream of the processing site. Replacement of the second AATAAA with a linker had no effect on 3′ end processing, while removal of the proximal AATAAA abolished 3′ end processing. Replacement of portions of the GT-cluster with a linker reduced the efficiency of 3′ end processing. Experiments are in progress to define the optimal spatial arrangement for efficient processing. Various mutants were transferred to T7/SP6 vectors and substrates prepared for in vitro analysis. These mutants showed a pattern of processing sites and efficiencies in vitro that were almost identical to that seen in vivo. Assembly of substrate into a complex whose mobility is retarded on polyacrylamide gels requires ATP, Mg^{++}, the presence of a polyadenylation signal, and incubation at 30°C. Competition assays are in progress to define the sequences required for complex formation. In related experiments, we are examining the pattern of poly(A) site selection in constructs containing multiple poly(A) signals.

The sequence of the processing and polyadenylation signal region of the HSV-tk gene is:
TATGACGGC**AATAAA**AAGACAG**AATAAA**ACCCAC**GGGTGTTGGGTCGTTTGTT**CATAAACCCGGG

L 510 STEROID-DEPENDENT BINDING OF TRANSCRIPTION FACTORS TO MMTV CHROMATIN IN VIVO.

Michael G. Cordingley, Anna Tate Riegel and Gordon L. Hager, Laboratory of Experimental Carcinogenesis, NCI, Bethesda, MD 20892.

We have employed an exonuclease III-resistance assay to detect the interaction of factors with the glucocorticoid-inducible promoter of MMTV in vivo. The promoter was amplified on bovine papilloma virus minichromosomes to approximately 200 copies per cell, permitting high resolution analysis of ExoIII-resistant boundaries on MMTV sequences. Two separate factors are shown to bind to the promoter in response to induction. Binding of the receptor at the glucocorticoid response element (GRE) therefore mediates the recruitment of at least two putative transcription factors to the MMTV promoter (Cell, in press). For both factors, apparent binding affinity and concentration in nuclear extracts is unaffected by hormone treatment of cells prior to extract preparation. Nucleosomes are positioned in a phased array on the MMTV LTR. The second member of this array, which is associated with sequences containing the GRE, is selectively lost during transcriptional activation. We discuss the ramifications of these observations for the mechanism of trans-activation of the GRE by the glucocorticoid receptor.

L 511 THE ROLE OF SPLICE SITE SEQUENCES IN ALTERNATIVE SPLICING OF EUKARYOTIC PRE-mRNA,

I.C. Eperon, L.P. Eperon and I.M. Wheatley, Department of Biochemistry, University of Leicester, Leicester LE1 7RH, UK.

We are studying the importance of the many individual sequences that contribute to the 5' splice site consensus (1). In particular, we are interested in whether these sequences are significant in modulating the usage of sites where alternatives exist. We have begun a series of experiments to show whether splice site sequences can be ranked in a hierarchy of preferential use when the sequences are placed in a constant environment and act in competition with a reference splice site (see abstract by L.P. Eperon et al.). Our results showed that of the two sequences used as alternative 5' splice sites in the E1a gene of adenovirus, one (the downstream site) ranked far above the other in our hierarchy (2) and would be expected to be used to the exclusion of the upstream site in the E1a gene. Since this is not the case, we have begun to test the relative importance of sequence and position in this gene by exchanging the two sequences in an E1a gene. The results of splicing in vivo and in vitro will be presented. We are also engaged in extending the hierarchy of preferential use to sequences from several examples of complex natural splicing patterns.

References

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L 512 THE EFFECT OF SECONDARY STRUCTURE ON 5' SPLICE SITE USE IN EUKARYOTIC PRE-mRNA, L.P. Eperon, A.J. Else, A.D. Griffiths and I.C. Eperon, Department of Biochemistry, University of Leicester, Leicester LE1 7RH, UK.

We have devised an assay for testing sequence preferences amongst 5' splice sites of eukaryotic pre-mRNA. In the assay "test" sequences are inserted 25 nucleotides upstream of a "reference" site; the 5' splice site of IVS-2 of rabbit β-globin (Eperon et al., Nature 324 (1986) in press). We have observed an interesting effect of secondary structure on site use in vivo: with a plasmid with a single β-globin test sequence (glo) transfected into HeLa cells, 64% of the spliced products have used the test site; in contrast, when a complementary sense oligonucleotide is positioned just upstream of that test site then only the reference site is used. A small hairpin thus sequesters a 5' splice site. We have proposed a model to explain the effect of secondary structure on site use. It is being tested by the insertion of various length sequences between the two oligonucleotides that form the stem of the hairpin. Our first results show that beyond a certain loop length the test site is released for splicing. The results of further experiments will be reported.

L 513 A NOVEL SCHEME OF ALTERNATIVE SPLICING IN THE TROPONIN T FAMILY REVEALED BY THE CLONING AND SEQUENCING OF TWO DIFFERENT HUMAN SLOW ISOFORM cDNAS, Reinhold Gahlmann, Anthony B. Troutt, Robert P. Wade, Peter Gunning, and Larry Kedes, MEDIGEN Project, Department of Medicine, Stanford Medical School and Veterans Administration Medical Center, Palo Alto, CA 94305. Adult cardiac muscle, slow skeletal muscle and fast skeletal muscle contain different isoforms of TnT. Two different cardiac-TnT proteins are derived from a single gene by alternative RNA splicing. More than 40 and potentially 64 distinct variants of the fast-TnT isoform are derived from a single gene by an even more complex scheme of alternative splicing. We provide the first sequence information for the slow isoform of TnT. Our analysis reveals that a single slow-TnT gene present in the human genome gives rise to at least two different slow-TnT variants by yet a third scheme of alternative splicing. Thus, the transcripts of all three members of the TnT gene family undergo alternative splicing but the splicing patterns for each isoform is unique. Furthermore, the observed variations in slow-TnT splicing generate major structural differences between the two corresponding proteins and are likely to effect their functions. These features raise important questions of why and how all the individual members of the closely related TnT gene family developed such complex but different schemes of alternative splicing to create sets of variant proteins.

L 514 ALTERNATIVE SPLICING OF MYOSIN LIGHT CHAIN EXONS IS CIS REGULATED. M.E. Gallego and B. Nadal-Ginard. Howard Hughes Medical Institute, Department of Cardiology, Children's Hospital. Harvard Medical School. Myosin light chain 1 (MLC1) and myosin light chain 3 (MLC 3) isoforms are produce from a single gene by the use of two different promoters and alternative splicing of the respective RNA transcripts. The two promotors are separated by a 10kb intron. Therefore, this gene produces two different precursor mRNAs of significantly different primary structure, both in size and sequence. In this gene there are two miniexons coding for MLC 3 (exon 3) and MLC 1 (exon 4) that are differentially spliced and used in a mutually exclusive manner in MLC 3 (exon 3) and MLC 1 (exon 4) mRNAs. When these two miniexons are introduced into the second intron of the insulin gene, driven by the SV40 promoter/enhancer, they are not spliced together independently of the cells in which the construct has been introduced, either myogenic or non-myogenic. Exon 3 is only included in the mRNA when exon 4 has been deleted from the DNA constructs. Other plasmids containing different combinations of MLC gene exons 1 to 5 have been made. Results obtained with these constructs demonstrate the mutually exclusive use of exons 3 and 4 is not dependent on the secondary structure of MLC1 and MLC3 pre-mRNAs. Therefore, the pattern of splicing is cis regulated with no need for muscle specific trans acting factors. The different affinities of the donor sites of exons 1 and 2 for the aceptor sites of exons 3 and 4 determine which of those will be included. These results demonstrate that not all the donor and aceptor sites are equivalent.

A snRNP ASSOCIATED PROTEIN BINDS THE 3' SPLICE SITE OF PRE MESSENGER RNA

L 515 Volker Gerke and Joan A. Steitz, Yale University, New Haven, CT 06510

Efficient splicing of mammalian mRNA precursors (pre-mRNAs) requires three important regions in the pre-mRNA molecule, the 5' and 3' splice sites as well as the site of lariat formation. Two different small nuclear ribonucleoprotein particles (snRNPs) of the Sm class have been shown to interact with two of these important pre-mRNA regions: U1 snRNPs recognize the 5' splice site most likely by RNA basepairing, and U2 snRNPs associate with the branch point (for review see Padgett et al., Ann. Rev. Biochem. 55, 1119).

Here, we demonstrate that recognition of the 3' splice site is very likely to be carried out by a protein and not an RNA component. Using RNAse T1 protection experiments to assay for 3' splice site binding activity (Chabot et al., Science 230, 1344) we show that biochemical fractionation of a HeLa cell nuclear extract in the presence of 15 mM $MgCl_2$ results in the separation of all major snRNPs from the 3' splice site binding activity. In low Mg^{2+} (1 mM), however, this activity is associated with snRNPs as judged by copurification and immunoprecipitation with snRNA specific anti-2,2,7-trimethylguanosine antibodies. The 3' splice site binding activity copurifies with a protein of molecular weight 70 Kd and responds to mutations in the 3' splice site region. Protein blots probed with labeled pre-mRNAs reveal that the 70 Kd protein can bind RNA directly showing a preference for regions between the branch point and the 3' splice site. The 70 Kd protein also represents a previously unrecognized Sm antigen as it is immunoblotted as well as immunoprecipitated with anti-Sm antibodies.

GENE EXPRESSION DURING DIFFERENTIATION OF PREADIPOCYTE CELL LINES,

L 516 Paul Grimaldi,Christian Dani,Alain Doglio and Gérard Ailhaud,Biochimie(CNRS),Nice,France

The adipose conversion of Ob17 cells (established from mouse adipose tissue) and 3T3-F442A cells involves the expression of at least two separate sets of markers which are differently regulated (Doglio et al., 1986, Biochem.J., 238, 123). The expression of unidentified pOb24 mRNA (confined to adipose tissue) is an early event independent of growth hormone (GH), triiodothyronine (T3) and insulin supplementation, all within a physiological range of concentrations. The expression of pOb24 mRNA (undetectable in 3T3-C2 control cells) is tightly coupled to growth arrest induced by confluence, thymidine block or serum deprivation. Runoff transcription experiments reveal that the regulation of pOb24 gene expression occurs at a transcriptional level. In contrast to pOb24 mRNA, the later emergence in Ob17 cells of mRNAs encoding for glycerophosphate dehydrogenase (GPDH), a myelin-P2 homologue (aP2) and a homologue of serine proteases (adipsin) requires GH and T3. Transcription of aP2 and GPDH genes is low or non existing in GH-deprived cells and increases markedly in GH-treated cells. A specific increase in spermidine intracellular concentrations (3-5 fold at the nuclear level) is highly correlated with the expression of aP2 and GPDH genes in GH-treated cells. Active transcription of aP2 and GPDH genes occurs in nuclei of T3-deprived cells whereas primary and/or mature transcripts remain undetectable, indicating a critical stabilization of mRNAs mediated by T3. A similar but less potent stabilization of aP2 and GPDH transcripts is also mediated by insulin (detected already in the nucleus). Thus, i) the multihormonal control of gene expression is limited to late markers of adipose conversion and ii) the multihormonal regulation of the expression of a single gene (aP2 or GPDH gene) is controlled both at a transcriptional and post-transcriptional level.

TRANSCRIPTIONAL TERMINATION WITHIN THE MOUSE rRNA GENE PROMOTER,

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The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The mouse rRNA gene promoter consists of a core domain (residues -39 to +5) that is necessary and sufficient for transcription in vitro as well as several domains that extend upstream to residue ~-140 and augment the level of transcription (MCB 5, 554-562). We have identified another promoter domain that surrounds residue -168. Its unique feature is that it evidently acts by directing transcriptional termination. We assessed activity of cloned 5' deletion mutants of mouse rDNA both after transient transfection ('in vivo') and in a mouse S-100 cell extract. In both systems, cloned genes that lack the -168 promoter domain exhibit a high level of transcription that reads into the initiation region from upstream, while templates that contain this region effectively prevent upstream read-in, suggesting that the -168 region directs a termination event. Templates in which this 'terminator' signal is cloned downstream of a full promoter produce a distinct transcript that ends at residue -182 and is released from the transcription apparatus. We hypothesize that this termination event may be part of a mechanism by which RNA polymerases remain associated with the rRNA gene at the end of each 47S coding region and are channeled directly to the next promoter.

L 518 MODULATION OF EXPRESSION OF TRANSFECTED IMMUNOGLOBULIN GENES BY DNA INSERTS IN THE 5' UNTRANSLATED REGION: A RELATIONSHIP BETWEEN RNA ACCUMULATION AND SIZE OF THE 5' PROXIMAL EXON, Marvin B. Hendricks and Michael J. Banker, Integrated Genetics Inc., Framingham MA 01701

We show that expression of transfected immunoglobulin (Ig) genes can be modulated by the insertion of DNA fragments of varying sizes into the 5' untranslated region. Run-off transcription experiments performed with nuclei from stable transformants of myeloma cells indicated that this modulation occurs at a post-transcriptional level. RNA levels programmed by these mutant Ig genes in stable transformants range from less than 1% to 100% of wild type. We found that the size of the 5' proximal exon generated by these mutant genes rather than the size of the DNA insert itself correlates with expression levels. The generation of 5' proximal exons shorter than about 430 base pairs appears to be conducive to high mRNA levels. These results imply that processing events at the 5' end of Ig transcripts can influence the level of stable mRNA accumulation in B-lymphocytes.

L 519 ANALYSIS OF LTR SEQUENCES CONTROLLING TRANSCRIPTION AND TRANSLATION OF VISNA VIRUS
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Visna virus is a pathogenic lentivirus of sheep that shares morphologic features and sequence homology with Human Immunodeficiency Virus (HIV). Like HIV, expression directed by the visna virus LTR is activated in trans over one hundred-fold by viral infection. Our laboratory is studying what sequences regulate viral transcription and translation in both uninfected and infected cells by making deletions and linker scanner mutations in LTRs expressing the bacterial gene chloramphenicol acetyltransferase (CAT). The results of CAT assays to date indicate that the specific sequences required for efficient expression vary depending on the cell type. Sequences between approximately -67 and -5 must be present for significant activity in uninfected cells, and for responsiveness to trans-activation in visna virus-infected cells. Inclusion of sequences between -5 and +90 increases the basal activity of the visna virus LTR moderately, and increases the magnitude of trans-activation by five-fold. Analysis of RNA transcripts and CAT activities suggests that trans-activation is mediated by enhancement of both transcriptional and post-transcriptional events.

L 520 REGIONS WITHIN THE ADENOVIRUS MAJOR-LATE TRIPARTITE LEADER RESTRICT EXPRESSION OF GENE SEQUENCES UNDER CONTROL OF THE SV40 PROMOTER. Manley T.F. Huang and Cornelia M. Gorman, Genentech, S.S.F.,CA. An expression vector for factor VIII cDNA was constructed that contains the SV40 early promoter, a splice/donor sequence from the adenovirus major late (AML) leader, a synthetic IgE acceptor sequence, and the SV40 polyadenlyation signal (SIS). Transient expression of factor VIII was examined upon transfection into six mammalian cell lines and found to be restricted to COS cells. A homologous vector that differs only in the splice/donor sequences has been found to transiently express factor VIII in these six cell lines. Therefore, restricted expression of the SIS vector must be due to sequences within the AML leader (+13 to +198 relative to the start of transcription). Initiation of transcription from the AML promoter appears to depend upon AML leader sequences +33 to +190 plus trans-acting factors encoded or induced by adenovirus (Mansour et al.,MCB 6(7):2684-2694.1986). Since it is known that SV40 antigen, which is expressed in COS cells, can complement adenovirus mutations in E1A and E1B, we are examining the role of adenoviral early gene products in overcoming restricted expression. The CAT gene fused to the SIS regulatory sequences is being transfected into KB cell lines already expressing genes for E1A and/or E1B. The ability to express CAT in these cell lines will determine the involvement of E1A, E1B, or both in the restricted expression of AML leader sequences and whether this effect occurs at the level of (1) initiation of transcription; (2) transport of mRNA to the cytoplasm; or (3) increased stability of mRNA.

L 521 PARTIAL PURIFICATION AND CHARACTERIZATION OF A SERUM FACTOR WHICH MODULATES HEPATOCYTE SPECIFIC mRNAs HALF-LIFE, Douglas M. Jefferson and David E. Johnston, Tufts University School of Med. and New England Medical Center, Boston MA 02111.

Reports of posttranscriptional regulation of gene expression by alterations in cytosolic mRNA half-life have increased dramatically. We originally reported (Jefferson, Mol. Cell. Biol. 4:1929,1984) that when adult rat hepatocytes are placed in primary culture their transcription rate decreased to approximatly 8% within 24hrs. Cells maintained in hormonally defined medium (serum-free) (HDM) were able to maintain near in vivo levels of their steady state mRNA for a number of tissue specific genes over the 5 days of culture. However, those maintained in 10% serum supplemented medium (SSM) or HDM+SSM exhibited barely detectable levels of tissue specific mRNAs due to increased degradation (hepatoma cell lines mRNA levels are unaffected by SSM). We have used this culture system to monitor the purification of the factor in fetal calf serum that causes mRNA destabilization (FCS-DF). The FCS-DF is stable at 60oC for 30 min. but loses some activity when it was heated at 80oC for 30 min. This activity can not be extracted by organic solutions. The levels of FCS-DF are the same in serum and plasma. Ammonium sulfate at 70% saturation but not 55% precipitated most of the activity. This 70% ammonium sulfate pellet was further fractionated on a sephacyl S-200 molecular sieving column which gave a FCS-DF peak corresponding to a Mw of 60,000-70,000. The addition of serum albumin to the cultures was shown to have little FCS-DF activity. The final purification of this factor will provide an important tool with which to delineate the cascade of events which culminate in changes in mRNA metabolism. This project was supported by NIH P30 AM34928.

L 522 BIOCHEMICAL AND GENETIC ANALYSES OF AN AVIAN SARCOMA VIRUS SPLICING MUTANT AND REVERTANTS. R. A. Katz and A.M. Skalka, Department of Molecular Oncology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

Avian sarcoma virus (retrovirus) RNA splicing is regulated such that approximately one-half of the full-length RNA transcript (5'L gag-pol-env-src3') remains unspliced to serve as gag-pol mRNA and progeny virus genomic RNA. Env subgenomic mRNA (5'L-env-src3') is formed by the joining of a short leader segment (L) to the downstream env coding region thereby removing the gag-pol coding intron. Using an infectious viral DNA clone, we introduced a short insertion into the gag-pol intron adjacent to the env splice acceptor site. The resultant mutant was replication defective. Genetic analysis indicated that the mutant could efficiently provide env in trans. However, S1 nuclease analysis of the RNA which it produced revealed a severe reduction in the amount of the unspliced form with a concomitant increase in the amount of the spliced form as compared to a wild type control. Thus, the replication defect in the mutant can be explained by the absence of progeny genomic RNA, and the mutation appears to increase the efficiency of splicing. Analysis of two independent revertants, which grow like the wild type virus, suggests that minor nucleotide changes in the intron or exon near the env splice acceptor site are sufficient to restore infectivity. Further study of these and additional revertants should provide clues to the nature and location of sequences which are important in the control of mRNA splicing.

L 523 Restricted Specificity of Translational Control Mediated by Eukaryotic Initiation Factor 2 Alpha in Transfected Cells. Randal J. Kaufman, Genetics Institute Inc., Cambridge, MA 02140.

The efficient translation of adenovirus late mRNAs is dependent on the presence of the adenovirus virus associated (VA) RNAs I and II. These VA RNAs are abundant RNA polymerase III transcripts found late in adenovirus infection. They may achieve an increase in late mRNA translation by preventing the activation of the double stranded RNA activated (DAI) protein kinase, thereby preventing the phosphorylation of the alpha subunit of eukaryotic initiation factor-2. The translational efficiency of mRNAs transcribed from plasmid DNAs introduced into COS-1 monkey kidney cells, can also be increased 10-20 fold by the co-expression of adenovirus VA RNA. It is demonstrated here that the addition of 2-aminopurine, a specific inhibitor of DAI protein kinase, to the culture medium, can also increase the translational efficiency of plasmid-derived mRNAs in COS-1 cells. Furthermore, it has been found that this increase, mediated by VA RNA or 2-aminopurine, is specific to these plasmid-derived mRNAs, and there is no qualitative or quantitative alteration in host protein synthesis. These observations are consistent with a model invoking localized activation of DAI protein kinase leading to a translational block.

L 524 EFFECTS OF EXON SEQUENCES ON PRE-MRNA SPLICING IN VITRO. Ryszard Kole and Paul J. Furdon. University of North Carolina, Chapel Hill, N.C. 27514

We have shown previously that truncation of the human beta-globin pre-mRNA in the second exon, 14 nucleotides downstream from the 3′ splice site leads to inhibition of cleavage at the 3′ splice site and exon ligation without affecting cleavage at the 5′ splice site and lariat formation (Furdon and Kole, PNAS 83,927 (1986)). This finding suggested that specific exon sequences may play a role in splicing. We now show that: 1) Several non-globin sequences (e.g. polylinker sequence, fragments of pBR322) substituted 14 nucleotides downstream from the 3′ splice site restore efficient splicing. 2) In the above cases the efficiency of the splicing reaction depends on the length of the second exon and not on specific sequence. 3) Pre-mRNA transcripts containing 5′ exon truncations leaving only 20 nucleotides are spliced as efficiently as transcripts with the full length first exon. 4) Substitution of an intron sequence at 14 nucleotides downstream from the 3′ splice site inhibits both cleavage at the 5′ splice site and subsequent steps of the reaction suggesting that the substituted fragment carries a "poison sequence". The significance of the latter finding is now under investigation.

L 525 **THE CYTOPLASMIC TRANSLATION INHIBITORY RNA & RNP OF CHICK EMBRYONIC MUSCLE: POSSIBLE ROLE IN MYOGENESIS AS "ANTI MESSENGER RNA".**

Q.T. Kong, Q.L. Wu, M. Raychowdhury, and S. Sarkar, Dept. Muscle Res., Boston Biomed. Res. Inst., Boston, MA 02114, U.S.A.

We have previously isolated and characterized a novel cytoplasmic translation inhibitory 10 S RNP (iRNP) containing a 4 S RNA (iRNA) species from chick embryonic muscle (JBC, 256, 5077, 1981). Both iRNA and iRNP are potent inhibitors of in vitro translation of a variety of mRNAs. The inhibition is due to a specific effect on mRNA binding to the 43 S preinitiation complex (JBC, 258, 15141, 1983). The biochemical properties of iRNA and iRNP indicate that they represent a novel class of cytoplasmic macromolecules unrelated to mRNP and SNRNP particles (ABB, 248, 89, 1986). The iRNA has no effect on the phosphorylation of the alpha subunit of eIF2 in an in vitro assay system indicating that its activity is not mediated through the eIF2 phosphorylation pathway. The iRNA consists of several subspecies in the 65-140 nucleotide size range. Several subspecies have been resolved as biologically active forms. One iRNA subspecies shows differential inhibition of in vitro translation of different mRNAs. The iRNA subspecies hybridize with muscle mRNAs in "dot blots". Several iRNA subspecies contain 5′-ppp termini and show microheterogeneity at the 3′ and 5′ region. These results suggest that iRNA may act as a cellular "anti-messenger RNA" and its role in myogenesis is to regulate efficiently the cytoplasmic mRNA levels, thus augmenting the transcriptional control(s) which operate during myogenesis.

L 526 ANALYSIS OF THE INVOLVEMENT OF PROTEIN FACTORS AND snRNPs IN pre-mRNA SPLICING IN VITRO. A. Kramer, D. Frendewey and W. Keller, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, FRG.

We have fractionated extracts from HeLa cell nuclei to identify protein factors that are required in conjunction with UsnRNPs for the splicing of a synthetic pre-mRNA carrying part of the adenovirus-2 major late transcription unit. So far, four distinct activities could be detected which are all required to generate spliced RNA in an in vitro reaction. Currently we are investigating at which stage of the splicing pathway the factors act. One of the activities (SF1) cofractionates through several steps with proteins containing an αSm-antigenic determinant. However, none of the known UsnRNAs can be detected in the most purified fractions. In conjunction with U2snRNP and another as yet unidentified splicing activity (SF0), SF1 is required for the assembly of the pre-mRNA into a large RNase T1 resistant complex. The region of the pre-mRNA which is protected from RNase T1 digestion includes the $(Py)_n$ directly adjacent to the 3′ splice site, the branchsite and sequences further upstream within the intron. By itself SF1 binds to pre-mRNAs carrying a 3′ splice site, forming a smaller complex which is sensitve to RNase T1 digestion. Since U2snRNP has been shown to bind to the branchsite region (Black et al., Cell 42, 737-750, 1985) it is likely that SF1 represents the activity that interacts with the $(Py)_n$ at a very early stage of the splicing reaction and is required for an interaction of U2snRNP with the branchsite region.

L 527 GENETIC ANALYSIS OF THE HUMAN THYMIDINE KINASE PROMOTER. Jordan A. Kreidberg and Thomas J. Kelly. Johns Hopkins Univ. Sch. of Medicine. Baltimore. Md. 21205. The promoter of the human thymidine kinase gene has been defined by DNA sequence and genetic analyses. These analyses have delimited the promoter to an 83 base pair region immediately upstream of the mRNA start site. This promoter region contains sequences common to other eukaryotic promoters including G/C rich hexanucleotides, a CAAT box, and an AT rich region. Selection of stable TK^+ cell lines after transfection of Ltk^- cells with the human and herpes simplex virus TK genes has facilitated the study of the regulation of TK genes. TK mRNA and enzyme activity levels regulate normally in TK^+ cells derived with human TK genes containing only the 83 base pair promoter region. When these growth arrested TK^+ cell lines are stimulated with serum, both TK mRNA and enzyme activity levels increase. In addition, in cell lines derived with the HSV-TK gene, the levels of HSV-TK mRNA also increase upon stimulation, although activity levels do not change. Placement of the SV40 early promoter upstream of the human TK gene results in increased human TK mRNA levels in non-growing cells, however TK activity regulates normally in these cells. The regulation of TK enzymatic activity is therefore at least partially independent of the levels of TK mRNA.

L 528 **STRUCTURE AND FUNCTION OF MAMMALIAN SPLICEOSOMES.** **Angus I. Lamond; Paula J. Grabowski; M. Magda Konarska and Phillip A. Sharp.** Center for Cancer Research, M.I.T., Cambridge, MA 02139.

We have studied *in vitro* the binding of mammalian spliceosomes to β-globin and adenovirus preRNAs using Hela cell nuclear extracts. Analysis of splicing complexes by native gel electrophoresis reveals three, ATP-dependent complexes, α,β and γ, that form on preRNAs. The three complexes are kinetically related and form in the order α-β-γ. The γ complex contains predominantly the splicing intermediates, i.e. free 5' exon and intron lariat+3' exon; γ subsequently converts into separate, smaller complexes which contain the intron lariat and spliced exons respectively. Deletions or point mutations at the 5' splice site (SS) allow formation of α, β and γ complexes, but at a reduced rate. Point mutations at the 3' intron-exon junction severely inhibit spliceosome assembly and prevent γ complex formation. The 3' SS is thus the primary target for spliceosome assembly. Affinity purification and Northern blot analysis have been used to study spliceosome composition. Both methods show that U2, U4, U5 and U6 snRNAs are present in the spliceosome. Surprisingly, U1 snRNA is not detected. The rapidly formed α complex contains only U2 snRNA. Formation of all the ATP-dependent complexes can be specifically inhibited with monoclonal antibodies directed against snRNP antigens.

L 529 SECONDARY STRUCTURAL FEATURES IN THE PATHWAY OF THE TENTATIVE ALTERNATIVE SPLICING OF HUMAN α-LIKE GLOBIN mRNA, S.-Y. LE AND JACOB V. MAIZEL, Jr., Laboratory of Mathematical Biology, National Cancer Institute, NIH Frederick, MD. 21701.
The primary transport of human α-like globin gene contains 2 intervening sequences that are removed by RNA splicing. Two tentative splicing mechanisms have been simulated: in mechanism A the first step in the in vitro splicing reaction is a cut at the 5' splice site of intron 1 and in mechanism B of intron 2, of α1, α2 and ζ globin pre-mRNA. The secondary structures of RNA species generated in Mechanisms A and B are computed by using a new approach in which the current dynamic algorithm is combined with a statistical analysis of conserved substructures in the RNA folding process. The tree graphs of those secondary structures are compared and the transferable ratios of secondary structures between RNA species generated during in vitro splicing are calculated. The total transferable ratios of secondary structure between α1 and ζ globin precursors and their mature mRNAs are 5.3 and 2.7 times more in Mechanism B than in Mechanism A but for α2 globin 3.5 times more in Mechanism A than in Mechanism B. The structural features of conserved sequence elements of introns in the secondary structures are also analyzed. According to both the structural features of conserved sequence elements and the transferable ratios of secondary structure of mature mRNA we suggest that there are different orders of intron excision from α globin precursors with multiple intervening sequences, and that intron 2 is excised before intron 1 from α1 and ζ globin pre-mRNA, while intron 1 is excised before intron 2 for 2 globin.

L 530 TISSUE-SPECIFIC EXPRESSION OF THE HUMAN ALPHA-1-ANTITRYPSIN GENE, Yi Li, Rong-Fong Shen, Rick N. Sifers, Heng Wang, Sophia Y. Tsai and Savio L.C. Woo, Howard Hughes Medical Institute, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Alpha-1-antitrypsin (α1-AT) is the major plasma serine-protease inhibitor synthesized in the liver. Its physiological function is to inhibit neutrophil elastase in the lung, and genetic deficiency of α1-AT predisposes affected individuals to development of chronic obstructive pulmonary emphysema. The normal human α1-AT gene was cloned and the entire gene has been sequenced. To identify cis-acting elements controlling liver specific expression of the α1-AT gene, we constructed recombinants containing various regions of the 5' flanking sequence of the human α1-AT gene linked to the bacterial chloramphenicol acetyltransferase (CAT) gene and used them to transfect human hepatoma cell lines as well as non-hepatic cell lines. The sequence from -345 to -30 of the gene was found to be able to confer sepcificity of CAT expression in human hepatoma cell lines HepG2 and PLC/PRT/5, but not in HeLa and NIH 3T3 cells. Gel retardation assay was performed using hepatoma nuclear extracts and the end-labeled 5' flanking sequence. Bands with reduced electrophoretic mobility were observed in the gel with poly dI-dC as competitor. These data suggest the presence of trans-acting factors in the hepatoma cells that direct the expression of the human α1-AT gene by interacting with its cis-acting elements in a tissue-specific manner.

L 531 MOLECULAR BIOLOGY OF NEUROTRANSMITTER EXPRESSION. Jacques J.B MALLET Neurobiology, CNRS, 91190 Gif-sur-Yvette

Increasing evidence indicates that neurotransmitter expression and metabo -lism is a dynamic process regulated both in the developing and adult orga -nism by a variety of external factors. For example, certain neurons have the ability to change their neurotransmitter phenotype, a process which represent an integral part of development. Also, in some instances, it has been shown that increased neuronal stimuli are followed by long-term changes of the enzyme activities of neurotransmitter synthesizing enzymes.

To analyse these regulations and the interactions between various neurotrans -mitters systems, we have isolated several genes encoding enzymes that are involved in the expression of the catecholamines, serotonine and GABA. Using several methods including in situ hybridization on tissues sections, the SP6 system, S1 mapping experiments, transfection in established cell lines etc, we have investigated the various mechanisms controlling the expression of theses genes and have shown that alternative splicing play an important role in neurotransmitter expression. Finally, we will present evidence indicating that mutations in the gene encoding tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis may be important in the aetiology of an autosomal dominant form of manic-depression psychosis.

L 532 TISSUE SPECIFIC EXPRESSION AND cDNA CLONING OF NUCLEAR RIBONUCLEOPROTEIN ANTIGENS. George McAllister and Michael R. Lerner, Yale University School of Medicine, New Haven, CT 06510.

Patients with systemic lupus erythamatosus (SLE) and other autoimmune disorders have circulating antibodies to small ribonucleoprotein particles (snRNPs) among other antigens. There is growing evidence that these snRNPs are involved in RNA processing reactions. Although much is known about the sequence and conservation of the RNA components of these particles, less is known about their corresponding proteins. We used the technique of Western blotting and SLE sera to investigate the tissue distribution of various snRNP polypeptides in rats. Most of the polypeptides examined (68K, A, B and D) are expressed at fairly constant levels in all tissues. However, B' is much more prominent in neural tissues than in non-neural tissues. B' in rats exhibits a different electrophoretic mobility compared to B' in HeLa cells and a monoclonal antibody that recognizes both B and B' in HeLa cells only recognizes B in rats. It is possible that such variations in snRNPs in different tissues may play a role in tissue specific splicing events.

We used an antiserum which recognizes predominantly Sm antigens to probe a λ gt11 rat brain cDNA library and isolated a clone containing a 500bp insert. A fusion protein made by this clone is recognized by Sm antisera but not by non-Sm antisera. Antibodies eluted from this fusion protein also recognize B, B' and A polypeptides in purified snRNP preparations and tissues. This result suggests that B, B' and A have a shared epitope.

L 533 ASSEMBLY OF PRECURSOR RNA CONTAINING THE L3 POLY(A) SITE INTO RNA-PROTEIN COMPLEXES, Claire L. Moore * Hagit Scolnik-David[#], and Phillip A. Sharp,[#] Dept. of Molecular Biology, Tufts University Medical School, Boston, MA 02111 (#) and Center for Cancer Research and Dept. of Biology, M.I.T., Cambridge, MA 02139 (#)

In the presence of HeLa nuclear extract at 30°C, precursor RNA containing the L3 poly(A) site is found in complexes with characteristic sedimentation values when analyzed by centrifugation through 10-30% glycerol gradients. In the absence of ATP, or at 5 minutes in the presence of ATP, these complexes are approximately 35S in size. Over time, there is a progressive shift to 50S complexes, which parallels the appearance of polyadenylated RNA. By 30 to 60 minutes most of the RNA appears in the 50S region. The 50S particle contains both unreacted precursor and RNA which has been cleaved and polyadenylated at the L3 site. The 50S complexes also form on precursor RNA in the presence of AMP(CH_2)P_2, an analog of ATP with a nonhydrolyzable α-β bond which blocks polyadenylation but not cleavage at L3. When incubated with ATP and nuclear extract, RNA without known polyadenylation signals sediments only in the 35S region of the gradient. These results suggest that the 50S complex may contain factors for recognition and processing of precursor. Complexes which form on precursor have also been examined by their electrophoretic mobility in nondenaturing gels. In the presence of ATP, a very slowly migrating complex rapdily assembles onto precursor RNA. In contrast to the 50S particle, most of the precursor is found in this gel complex by 5 minutes of incubation. This complex also forms in the presence of heparin, which dissociates the 50S particle. Mutation of the upstream AATAAA polyadenylation signal to AAGAAA or deletion of sequences 5n. beyond the L3 poly(A) site abolishes complex formation. Experiments are in progress to determine the relationship of the complexes observed by the two methods, as well as the potential involvement or snRNPs in their formation.

L 534 A NONDEFECTIVE RECOMBINANT ADENOVIRUS INDUCES SEROCOVERSION TO HEPATITIS B SURFACE ANTIGEN

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Global eradication of Hepatitis B may require universal immunization with a safe, effective vaccine that is easily administered. Presently, adenovirus vaccine administered orally as live virus tablets prevents respiratory adenovirus infections. Nondefective recombinant adenoviruses carrying the hepatitis B virus (HBV) surface antigen gene in the E3 region of the adenoviral genome represents a novel approach to immunization against HBV. Human cells infected with these recombinant adenoviruses secreted immunoreactive HBV surface antigen. Hamsters inoculated intranasally with these live recombinant adenoviruses consistently produced antibody against both adenovirus and HBV surface antigen.

L 535 MONITORING GENE ACTIVITY WITH LUCIFERASE GENE FUSIONS.

David W. Ow*, Keith V. Wood, Jeffrey R. de Wet, Jerry Jacobs, Marlene Deluca, Donald R. Helinski and Stephen H. Howell. Department of Biology and Chemistry, University of California San Diego, La Jolla, CA 92093. *Present address: USDA/UCB Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710.

Firefly luciferase catalyzes a light emitting reaction in the presence of Mg-ATP, oxygen, and the small organic substrate luciferin. A cDNA clone, which encodes biologically active luciferase (de Wet et al., PNAS 82:7870), was fused to various plant promoters. These constructs were used to transform plant cells and to produce transgenic plants. Transgenic plants and cell lines produce luciferase and can glow when watered with a luciferin solution (Ow et al., Science 234). The emitted light can be detected in situ by x-ray or color film, or image-intensifying video equipment. The pattern of light emission is used to follow the expression of viral (CaMV 35S) and leaf-specific (RUBP carboxylase) promoters during plant development. In addition, since the in vitro assay for luciferase is more sensitive and rapid than a conventional CAT assay, we have used this reporter gene to define functional domains of plant promoters in transient expression studies.

L 536 **A NOVEL HYBRID α-TROPOMYOSIN IN FIBROBLASTS IS PRODUCED BY ALTERNATIVE SPLICING OF TRANSCRIPTS FROM THE SKELETAL MUSCLE α-TROPOMYOSIN GENE** Sonia H. Pearson-White and Charles P. Emerson, Jr., University of Virginia, Charlottesville, VA 22901.

A single α-tropomyosin gene in the Japanese quail exhibits the tightly controlled expression of various isoforms by an alternative splicing mechanism. Two full length cDNA clones were isolated from embryonic myofibers which differentiated in culture. These cDNAs were characterized by nucleotide sequence analysis and their differential expression patterns were analyzed. cC402 is full length at 1214 nucleotides long and encodes the major skeletal muscle α-tropomyosin expressed in adult skeletal muscle and in embryonic muscle culture as myoblasts fuse and begin to express the set of contractile proteins. cC401 is 1816 nucleotides long, and the nucleotide sequence is identical with the skeletal muscle form until an abrupt point of divergence which corresponds to the splice junction between amino acid residues 257 and 258. From this point through the C-terminus at amino acid residue 284, the derived amino acid sequence corresponds exactly to the chick smooth muscle γ-tropomyosin C-terminal exon. Thus this cDNA exhibits a novel hybrid primary structure, 90% skeletal muscle sequence, plus smooth muscle structure for the C-terminal 10% of the molecule. cC401-type mRNA is expressed at low levels in cultured embryonic myoblasts and myofibers and at high levels in cultured skin fibroblasts, but not in adult muscle. S1 mapping and Northern blot experiments using cC402 and cC401-specific probes revealed a simple pattern of α-tropomyosin gene expression in skeletal muscle, and a complex combinatorial processing pattern in smooth muscle and cultured skin fibroblasts. Thus the expression of the single skeletal muscle α-tropomyosin gene is tightly regulated both transcriptionally and post-transcriptionally, producing specific quantities of products with both constant and variable structural features in different tissues.

L 537 **TRANSLATIONAL REGULATION OF RIBOSOME SYNTHESIS: EVIDENCE FOR HORMONAL CONTROL,** Maria Pellegrini, Karen Yamamoto and Aline Chadarevian, Molecular Biology, University of Southern California, Los Angeles, CA 90089.

Following stimulation of a secretory gland in Drosophila, both a loss of ribosomal material and the need for new secretory protein synthesis induces a 2-3 fold increase in the translation of ribosomal protein (r-protein) mRNAs. This is due both to an increase in utilization of the r-protein mRNAs and to a general increase in protein synthesis rates. Previously synthesized r-protein mRNAs are found both on and off polysomes prior to stimulation of the glands and only on polysomes following stimulation. The level of the r-protein mRNAs does not change during the induction period. The accumulation of r-proteins clearly precedes the induction of new rRNA synthesis. In vitro, the stimulation of new secretory protein and ribosome synthesis can be accurately reproduced by 10^{-9} M juvenile hormone (JH). JH stimulation requires a source of external calcium or the release of internal calcium stores by the ionophore A23187. In addition, phorbol esters can mimic the upregulation of ribosome synthesis in the glands. These results suggest that increased protein synthesis and greater utilization of r-protein mRNAs can be effected by JH through a mechanism involving calcium and kinase C. None of these events are growth-related in that these secretory cells are nonmitotic.

L 538 **AUTOGENOUS REGULATION OF HISTONE mRNA DECAY.** S.W. Peltz, G. Brewer, and J. Ross, McArdle Lab. for Cancer Research, Univ. of Wisconsin Madison, WI 53706.

We have established an in vitro mRNA decay system that reflects the rates of mRNA decay found in whole cells (Ross, J. and Kobs, G., J. Mol. Biol., 188, 579-593). The relative decay rates for gamma globin, delta globin, c-myc, and histone mRNAs in vitro are the same as those in whole cells. Histone H4 mRNA is degraded in a 3' to 5' direction both in vitro and in whole cells, probably by an exonuclease.

Using the in vitro system, we have investigated the control of expression of that class of histone proteins that are cell cycle regulated. The mRNAs for these proteins accumulate to high levels during S-phase, and rapidly disappear after DNA synthesis stops. We and others have suggested that the histone proteins autoregulate the turnover of their own mRNAs. To investigate this hypothesis, we added a cytoplasmic supernatant fraction and various concentrations of histone core proteins to the in vitro decay system. Histone proteins, at concentrations of $1x10^{-6}$ M and above, increased the decay of histone H4 mRNA by approximately 5 fold. The histone proteins specifically accelerated histone mRNA decay, since they had no effect on the decay rates of c-myc and gamma globin mRNAs. Other single stranded nucleic acid binding proteins, recA and SSB, did not increase the decay rate of histone H4 mRNA. This autogenous regulatory mechanism could account for the repid disappearance of histone mRNA after S phase. When DNA synthesis is completed, newly synthesized histone proteins accumulate in the cytoplasm. The free histone protein pool size in the cytoplasm increases, leading to enhanced decay of histone mRNA, assuring that histone proteins are made in abundance only when histone proteins are required to bind newly synthesized DNA.

L 539 **DNA SEQUENCES REQUIRED FOR THE REGULATION OF A HUMAN GENE BY INTERFERON.** *Andrew C.G. Porter, Yuti Chernajovsky, George R. Stark and Ian M. Kerr, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.*
The gene, 6-16, coding for an interferon inducible mRNA has been isolated from a human genomic library and characterised (*EMBO. J.* **5**: 1601-1606 (1986)). A 6.7 kb genomic DNA fragment, beginning 217 bp upstream of the first exon and ending 100 bp downstream of the polyA site, is interferon inducible (at least twenty-fold) after its stable transfection into mouse Ltk$^-$ cells. The inclusion of additional flanking sequences does not significantly alter the degree of inducibility. In addition, proximal 5'- flanking sequences confer some interferon inducibility (greater than five-fold) upon an indicator gene (chloramphenicol acetyl transferase) in stably transfected cells. Regulation is therefore at least partly at the level of transcription and involves some or all of the 217 bp of DNA immediately upstream of the first exon. The importance of a 41 bp direct repeat in this region in confering interferon inducibility is under investigation.

L 540 Transient elevation in the rate of type I procollagen and fibronectin biosynthesis in bleomycin-treated cells is posttranscriptionally regulated. Rajendra Raghow, Suguru Osawa, Pati Irish and Andrew H. Kang, VA Medical Center and the University of Tennessee, Memphis, TN 38104.

Bleomycin is known to produce lung parenchymal injury and interstitial fibrosis. Increased rates of synthesis of both type I collagen and fibronectin have been observed in the bleomycin-treated lungs in several species. The relationhip between the bleomycin-induced cell damage and the altered metabolism of extracellular matrix involving cellular and humoral factors is not fully understood. To explore if bleomycin could directly alter the expression of extracellular matrix genes, we treated cultured rat fibroblasts with $1x10^{-3}$ units/ml of bleomycin for increasing durations and measured the rates of biosynthesis of type I procollagen and fibronectin. Bleomycin rapidly shut off DNA synthesis (60-90% inhibition) in fibroblasts after 15 min treatment. The rate of synthesis of type I procollagen and fibronectin was selectively elevated 3-4 fold compared to control cells. The elevation in the rate of synthesis of type I procollagen and fibronectin was only transient, since the rates fell to the control levels at 24 h following bleomycin treatment. The steady-state levels of mRNAs specifying procollagen type I, fibronectin and β-actin did not change significantly during the course of bleomycin treatment. The evidence presented here strongly suggests that the transient elevation in the rates of the two extracellular proteins in bleomycin-treated cells occurs by posttranscriptional mechanisms.

L 541 A CONSERVED AU SEQUENCE FROM THE 3' UNTRANSLATED REGION OF GM-CSF mRNA MEDIATES SELECTIVE mRNA DEGRADATION, Gray Shaw and Robert Kamen, Genetics Institute, Cambridge, MA 02140.
The mRNAs of transiently expressed genes frequently contain an AU-rich sequence in the 3' untranslated region. We introduced a 51 nucleotide AT sequence from a human lymphokine gene, GM-CSF, into the 3' untranslated region of the rabbit B-globin gene. Our experiments demonstrate that this caused the otherwise stable B-globin mRNA to become highly unstable in vivo. The instability conferred by the AU sequence in the mRNA was partially alleviated by treatment of the cells with cycloheximide. We propose that the AU sequences are the recognition signal for an mRNA processing pathway which specifically degrades the mRNAs for certain lymphokines, cytokines, and proto-oncogene.

L 542 MODIFIED NUCLEAR PROCESING OF ALPHA$_1$-ACID GLYCOPROTEIN RNA FOLLOWING INFLAMMATORY INDUCTION. Brian R. Shiels, Wolfgang Northemann, Michael R. Gehring, Georg H. Fey
Research Institute of Scripps Clinic, La Jolla, CA 92037

Rat alpha$_1$-acid glycoprotein (AGP) is an acute phase reactant which shows a marked elevation in mRNA level following inflammatory induction. It has been reported that a post-transcriptional mechanism, involving RNA processing, regulates the expression of this gene. We have studied the processing of the AGP primary transcript during an inflammatory response. Nuclear RNA was purified and analyzed by sequential hybridization of Northern blots with intron-specific oligonucleotides. This analysis showed that the introns are removed in the preferred order intron 1, intron 4, intron 5, intron 3 and intron 2. The splicing pathway was not found to be altered during inflammation. However, the final nuclear precursor detected by an exon oligonucleotide exhibited size changes, manifest as a quantitative shift from the final precursor observed at 3, 6 and 9 hours to a second progressively shorter form at 12, 18, and 24 hours. Deadenylation of nuclear RNA showed that the difference in size of the precursor is due to a change in poly A tail length, which occurs after the splicing out of the last intron. The size change of nuclear RNA reflects alterations observed in the cytoplasmic mRNA, but evidence suggests that our nuclear preparations were not contaminated with cytoplasmic RNA. Moreover, we have observed that at 24 hours large poly A tails cannot be detected on cytoplasmic RNA, even though novel transcripts, which have long tails, continue to be produced We conclude that a) reduction in poly A tail size is a rapid event, b) this event occurs in the nuclear compartment and c) poly A tail processing is modified, following inflammatory stimulation.

L 543 POLYMERASE SPECIFICITY OF mRNA MATURATION, Sangram S. Sisodia, Barbara Sollner-Webb and Don W. Cleveland. Johns Hopkins University School of Medicine, Baltimore, Md. 21205.

Each of the RNA polymerases in eukaryotic nuclei transcribe a distinct set of genes. The resulting transcripts are processed through specific pathways to yield their respective mature RNA species. Although some of the mechanisms of these complex RNA maturation pathways have been elucidated, it remains unclear whether these processes are, in fact, polymerase specific. A priori, nascent RNAs could be channeled through their appropriate processing pathway as a consequence of a tight (perhaps obligatory) coupling of the polymerase and the processing machineries. On the other hand, specificity in RNA maturation could be achieved solely by the nucleotide sequences of the transcript and could be independent of the polymerase complex involved in the actual transcription event. We have approached this question by transient transfection of a hybrid gene comprised of a polymerase III promoter (derived from a Xenopus 5S gene) fused to a fragment of a chicken β tubulin gene which contains a complete intron segment including 5' and 3' splice junctions. Using an assay involving RNase H and S1 nuclease, we demonstrate that the chimeric RNAs synthesized in vivo by RNA polymerase III can be accurately spliced. Although the splicing efficiency of these RNAs is low (<20 %) at early times post-transfection, it rises to >90% at longer times after transfection. The latter result suggests that the splicing efficiency of chimeric molecules may be temporally regulated by some, as yet unknown, mechanism. These results rule out the scenario that in vivo transcription and splicing complexes are obligatorily coupled or co-compartmentalized.

L 544 ISOLATION OF cDNA CLONES ENCODING THE HUMAN RNP ANTIGEN, Richard A. Spritz*, Kathleen M. Strunk*, George E. Hoganson, Jr.*, and Sallie O. Hoch+, *Laboratory of Genetics, University of Wisconsin, Madison, WI and +Agouron Institute, La Jolla, CA

The U class of small nuclear ribonucleoprotein complexes (snRNPs) are a group of stable and discrete small RNA-protein complexes that are ubiquitous among higher eukaryotes. At least three of the U snRNPs (U1, U2, and U4/U6) are involved in pre-mRNA splicing in animals. U1 snRNP, the best characterized, binds to 5' splice sites and to U2 snRNP during splicing. Each U RNP contains a specific low molecular weight "U" snRNA species plus a common core of several small polypeptides, the Sm antigens. In addition, U1 snRNP contains a specific 77 kd polypeptide, the RNP antigen. We have used an anti-human RNP monoclonal antibody as probe to isolate multiple independent cross-hybridizing cDNA clones that encode the human RNP antigen. The frequency of RNP cDNA clones is very low, suggesting that RNP mRNA comprises only about 1/250,000 of total cellular mRNA. Studies of the structure of the RNP cDNA, protein, and gene are in progress.

L 545 ALTERNATIVE SPLICING WITHIN THE MAJOR OPEN READING FRAME OF ANTENNAPEDIA OF DROSOPHILA MELANOGASTER. Virginia L. Stroeher and Richard L. Garber, University of Washington, Seattle WA 98195.

We have previously identified four RNAs transcribed from the homeotic gene Antennapedia which differ with respect to their 5' and 3' untranslated sequences but share a single large open reading frame. Recently, we have detected splice pattern variations within this open reading frame which result in a diversity of RNA species. If translated, these would encode a family of related proteins containing the homeobox domain but differing in the sequences preceding this domain. We present data on the temporal and spatial restrictions of these different RNA forms and discuss how each correlates with the genetically defined functions of the Antennapedia gene.

L 546 ALTERNATIVE SPLICING OF THE αA_2-CRYSTALLIN RNA OF THE RODENT LENS IS NEITHER TISSUE- NOR SPECIES-SPECIFIC. G. Thomas and J. Piatigorsky, National Eye Institute, NIH, Bethesda, MD 20892.

Crystallins are the major structural proteins of the transparent eye lens of vertebrates. αA_2-Crystallin of the mouse and its variant form, αA^{ins}-crystallin, which has an additional 23 amino acids inserted between amino acids 63 and 64, are derived from a single gene and are produced by alternative splicing of its RNA transcript. This alternatively spliced product is found only in rodents. The splicing which leads to the production of the αA^{ins}-crystallin mRNA is inefficient and occurs in only 10-20% of the primary transcripts, although the requirements for lariat formation are satisfied. One possible explanation for this low frequency is the presence of an altered 3' splice junction in the insert exon (AG:GC instead of the more common AG:GT). We investigated whether the alternative splicing in this lens gene of rodents involves some tissue- or species-specific mechanism or whether it is a 'leaky' event. We have transfected lens and non-lens cells from different species with a hybrid gene construct including an RSV LTR, exon 1, the alternatively spliced exon and part of exon 2 of the αA-crystallin gene and the bacterial chloramphenicol acetyltransferase gene. We found that the alternative splicing event occurs in different cell types with the same efficiency as in the mouse lens and that this alternative splicing is neither tissue- nor species-specific.

L 547 IN VITRO SPLICING OF KAPPA IMMUNOGLOBULIN PRECURSOR mRNA. Brian Van Ness and David Lowery, Department of Biochemistry, The University of Iowa, Iowa City, IA 52242.

The *in vitro* splicng of kappa immunoglobulin precursor mRNA was studied as a naturally occurring mRNA possessing multiple 5'-splice sites. Several kappa mRNAs have been generated *in vitro* utilizing an SP6 transcription system and spliced in nuclear extracts derived from HeLa cells. Products and intermediates arising from *in vitro* splicing were identified. Spliced products and branchpoints were identified by primer extension analysis, and accurate splicing was verified by sequencing the spliced products. In contrast to the *in vivo* situation where only the 5'-most splice donor site is apparently used, all of the 5' splice sites were used *in vitro* with equal frequency. The presence or absence of variable region sequences or the deletion of intron sequences had no effect on splice site selection *in vitro*.

TWO δ-CRYSTALLIN POLYPEPTIDES ARE DERIVED FROM A CLONED δ1-CRYSTALLIN cDNA

L 548 Eric F. Wawrousek, Teresa Borrás, Diana S. Parker and Joram Piatigorsky, Laboratory of Molecular and Developmental Biology, N.E.I., N.I.H., Bethesda, MD. 20892

δ-Crystallin, the major water soluble protein in the transparent eye lens of birds and reptiles, is a tetrameric protein consisting of subunits with molecular masses near 48,000 Da (48K) and 50,000 Da (50K). The ratio of synthesis of the 48K to 50K polypeptides is 3:1 in the normal chicken lens, but can be significantly altered by changes in the intracellular ionic composition. The two polypeptides are immunologically similar and yield similar tryptic peptides. Although there are two tandemly linked δ-crystallin genes in the chicken genome, only the 5' most gene (δ1) appears to be expressed at levels sufficient to account for δ-crystallin polypeptide synthesis. In vitro transcription and translation of a cloned δ1-crystallin cDNA produces both of the polypeptides in a salt dependent manner similar to that observed for poly[A]$^+$ lens RNA. Premature translation termination shows that the difference between the two polypeptides resides in the N-terminal 1/3 of the protein, but point mutations of the first two in-phase AUG codons have eliminated alternative translation initiation at two AUG codons as a possible cause of the observed protein heterogeneity. Immunoblotting with antisera to synthetic peptides has shown that the N- and C-terminal regions of the 48K and 50K polypeptides are similar, and that translation initiation upstream of the first AUG codon does not occur. Since previous pulse-chase experiments have shown that δ-crystallin is not post-translationally processed, we suggest that a co-translational protein modification generates the observed heterogeneity in δ-crystallin.

L 549 **SIGNAL REQUIRED FOR POST-TRANSCRIPTIONAL, AUTOREGULATORY CONTROL OF β TUBULIN EXPRESSION LIES WITHIN THE FIRST 16 CODONS OF THE mRNA. Tim J. Yen, David A. Gay and Don W. Cleveland, Johns Hopkins University School of Medicine, Baltimore, MD 21205.**

Most animal cells rapidly and specifically depress synthesis of α and β tubulin polypeptides upon increases in the intracellular pool of free subunits either by treatment with microtubule destabilizing drugs or by direct microinjection of tubulin subunits. This autoregulatory control event has been shown to be due to the rapid, specific loss of tubulin mRNAs within the cytoplasm. Experiments measuring the transcription rates of tubulin genes revealed no significant changes when tubulin subunit pools were altered. Utilizing a transient transfection assay, various hybrid tubulin/thymidine kinase gene constructs have been tested to identify the regions responsible for autoregulation. The results indicate that only the first 48 nucleotides of the coding portion of exon 1 in mouse β tubulin mRNA are required to confer regulation. Furthermore, similar regions from chicken, human, and yeast β tubulin genes when fused to thymidine kinase also conferred regulation. However, when the same sequences were fused to tk so that they were out of frame with the tk polypeptide, regulation by the pool of tubulin subunits was lost. This supports our previous observation that only tubulin mRNAs that are polysome bound are subject to the autoregulation. Thus, the post-transcriptional, autoregulatory control of tubulin mRNA levels is tightly coupled to translation.

L 550 DIRECT MEASUREMENT OF STABILITY OF mRNA FOR μs VS μm. POLYPEPTIDE CHAINS. Dorothy Yuan. Univ. of Texas Health Science Center, Dallas, TX 75235.

Polyclonal stimulation (lipopolysaccharide, LPS) of B lymphocytes results in a dramatic increase (10 to 20 fold) in steady state levels of mRNA for μ chain of secreted IgM. Previous studies have shown that part of this increase is due to enhanced transcription (3 to 5 fold) of the μ chain gene. Inhibition of mRNA synthesis in vivo followed by measurement of the relative steady state level of μ_m mRNA vs. μ_smRNA have revealed that the latter is approximately 30 percent more stable than the former. The BCL1 B cell tumor line can be also stimulated by LPS to produce higher levels of both μ_s and μ_m mRNA. However, the total level of increase is only 3 to 5 fold and no enhanced transcription can be demonstrated. Therefore, in contrast to non-transformed cells, tumor cells can regulated mRNA levels for μ chains only by posttranscriptional mechanisms. The greater stability of μ_smRNA may be related to the observation that μ_s polypeptide chains are translated at a higher rate even in cells with much lower amounts of μ_smRNA.

Oncogene Proteins and Control of Gene Expression

L 600 HEMANGIOMA DEVELOPMENT IN TRANSGENIC MICE CARRYING POLYOMA MIDDLE T ANTIGEN, Victoria Bautch[1], John Hassell[2], Sachiko Toda[1] and Douglas Hanahan[1], [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y., 11724, and [2]McGill University, Montreal, Canada.

Transgenic mice carrying a modified polyoma (Py) genome that is origin-defective, late region-defective, and encodes only Py middle T antigen (MT Ag) have been generated. These animals heritably develop hemangiomas, which are a proliferation of the vascular endothelial cells. The hemangiomas are fatal to the animals at 1-4 mos. of age and are found at numerous body sites at autopsy. These tumors are positive for the expression of Py MT Ag by the immune-complex kinase assay, and the autophosphorylation of Py MT Ag in this assay indicates that the protein is associated with a cellular kinase in vivo. At least some of the cells contributing to the primary tumor are tumorigenic because the hemangiomas induced by Py MT Ag can be passaged in syngeneic mice. We are currently investigating the contributions to the specificity of this phenotype by (i) the specificity of gene expression from the Py promoter/enhancer linked to the MT Ag gene, and (ii) the requirements for in vivo transformation of cells by Py MT Ag.

L 601 ISOLATION OF CARCINOEMBRYONIC ANTIGEN (CEA) cDNA CLONES, N. Beauchemin, S. Benchimol, D. Cournoyer, A. Fuks and C.P. Stanners, McGill University, Department of Biochemistry and McGill Cancer Centre, Montreal, Quebec, Canada H3G 1Y6.

Carcinoembryonic antigen (CEA) expression is perhaps the most prevalent of phenotypic changes observed in human cancer cells as it is produced in nearly all tumours but specially in colon, breast and lung tumours. CEA is also produced during human embryogenesis, while a related, but distinctly different series of antigens termed CEA-cross-reactive antigens can be produced by a variety of adult normal cells. CEA is a large membrane glycoprotein (M.W. 180,000, 60% carbohydrate); its normal function, role in carcinogenesis and molecular basis for expression in tumours are completely unknown. We have observed extreme clonal variability in CEA production by cultured colon carcinoma cell lines and have used this to develop a set of cell clones with a 10,000-fold range in the production of this glycoprotein. We constructed a large size-selected λgt10 cDNA library from mRNA extracted from a high-producer cell line and, using oligonucleotide probes representing the CEA amino acid sequence, have isolated 30 CEA cDNA clones, many of which are full length. The nucleotide sequence of these clones consists of: a 5' untranslated leader sequence, a region coding for a hydrophobic leader polypeptide of 34 amino acids, a region representing the exact amino acid sequence of the NH_2-terminus of CEA, a variable number (1 to 5) of surprisingly similar repeats of 534 bp each, a short region coding for the C-terminus of the protein and a long 3' untranslated sequence which includes an Alu repetitive sequence. The cDNA clones were used as probes to demonstrate that the large variation in CEA production in the cell clones is precisely reflected in the levels of CEA mRNA. (Supported by MRC & NCIC).

L 602 POSITIVE AND NEGATIVE REGULATION OF k LIGHT CHAIN GENE ENHANCER BY E1A AND p53 PROTEINS, Yehudit Bergman, The Hebrew University, Jerusalem, ISRAEL.

The adenovirus type 2 (Ad2) E1A proteins are known to be transcriptional regulators of eukaryotic gene transcription. They can activate or repress a large number of viral and cellular promoters and enhancers. We have studied the effect of Ad2 E1A proteins on the activity of the mouse k light chain gene enhancer. It was found that the E1A proteins have a pleitropic effect on the regulation of k chain gene transcription. In lymphoid cells, the mouse k chain gene enhancer is repressed by the E1A products, whereas in fibroblasts, where the k enhancer is not fully active, the E1A proteins activate it.

Similar to the E1A proteins, the p53 cellular tumor antigen can complement Ha-ras in transforming rat embryo fibroblasts into tumorogenic cells. This similarity prompted us to investigate the ability of p53 product to regulate k chain transcription. We have found that the p53 antigen highly activates the k chain gene enhancer in non-lymphoid cells. Since two different oncogenes, that specify nuclear proteins, enhance the transcription of k chain gene enhancer in fibroblasts, it seems possible that the gene products of these oncogenes are turning a second messenger, which is acting on the k chain gene enhancer. Identifying these cellular genes which are activated or repressed by the E1A and p53 gene products, would be of great interest.

L 603 THE EXPRESSION OF THE HUMAN HA-RAS ONCOGENE IS MODULATED BY DNA METHYLATION. Maria G. Borrello, Marco A. Pierotti, Rosangela Donghi, Maria R. Cattadori, Italia Bongarzone, Piera Mondellini, Catia Traversari and Giuseppe Della Porta. Division of Experimental Oncology A, Istituto Nazionale Tumori, Via G. Venezian 1, 20133 Milan, Italy.

The oncogenes of the ras family display promoter regions containing CG clusters similar to those found in eukaryotic house-keeping genes, like hprt, whose expression has been found to be modulated by DNA methylation. Therefore, we have investigated the effect of DNA methylation on the expression of a cloned human Ha-ras oncogene, activated by a point mutation at its 12th codon. By co-transfection with a plasmid carrying a selectable gene we have inserted fully methylated Ha-ras oncogene in NIH-3T3 cells which remained morphologically normal and not tumorigenic. A cloned NIH-3T3 cell line, carrying the silenced methylated Ha-ras gene, was treated with the demethylating analog 5'-azacytidine. After the treatment: a) the morphology of the cells turned from flat to reflactile spindle shape, b) the cells acquired the property to growth in 0.3% agar and to form tumor when injected in nude mice, c) the promotor region (0.8 SacI fragment) of the Ha-ras gene was specifically demethylated, d) the cells were found to produce Ha-ras associated mRNA and p21. These data show that DNA methylation can modulate the expression of a genetically altered human ras oncogene.

L 604 DECAY OF HUMAN C-MYC MESSENGER RNA IN CELL-FREE EXTRACTS: G. Brewer, S. Peltz and J. Ross, McArdle Laboratory for Cancer Research and Dept. of Pathology, Univ. of Wisconsin, Madison, WI 53706.

We have developed a cell-free system to investigate the decay of mRNA. The components include ATP, mono- and divalent cations, a non-specific RNase inhibitor, and polysomes from a human erythroleukemia cell line, K562. Two properties of this system indicate that it is a valid model for studying mRNA decay: 1. Relative decay rates of mRNAs in cells and in vitro are similar. Thus, rRNA and γ-globin mRNA are stable, while c-myc, α-globin and histone mRNAs are unstable. 2. Histone mRNA is degraded exonucleolytically 3' to 5', both in cells and in vitro.

The experiments described here concern the mechanism of c-myc mRNA degradation. As determined by S1 nuclease mapping and Northern blotting, c-myc mRNA is also degraded 3' to 5' by a divalent cation-dependent ribonuclease activity. The decay rate is ATP-dependent because c-myc mRNA is degraded significantly faster without than with ATP. In contrast, the absence of ATP has little or no effect on γ-globin, α-globin or H4 histone mRNA decay. Deproteinized c-myc mRNA is very labile, whereas γ-globin mRNA is stable. Therefore, in vitro synthesized, 32P-labeled RNAs can be exploited as substrates in this system. The use of these substrates should permit us to characterize the enzymes and cofactors involved in c-myc mRNA decay and to map sequences which determine its decay rate.

L 605 STUDIES ON THE INITIATION OF TRANSCRIPTION AT DIFFERENT CLASS II PROMOTERS. Leonard Buckbinder, Juan Carcamo, Patricia Cortez, Osvaldo Flores and Danny Reinberg, Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School at Rutgers, Piscataway, NJ 08854-5635.

We have analyzed the factors required for specific initiation of transcription at different class II promoters (adenovirus major late, EIV, IX, IVa2, mouse β globin and cMyc). Five general factors (IIA, IIB, IID, IIE, and IIF) operating via minimal promoter sequences (TATA box or equivalent and CAP site) were required in addition to RNA polymerase II for the formation of a preinitiation complex that could initiate transcription, upon addition of the ribonucleoside triphosphates, in the presence of heparin or Sarkosyl concentrations that inhibited an unbound factor. We have also purified the EIV and the adenovirus major late promoters upstream specific factors and one of the proteins that recognized the EIV enhancer region and studied the mechanism by which they stimulated transcription.

A model for the ordered assembly of the general factors into a preinitiation complex and a general mechanism by which gene specific factor affected the transcription reaction will be presented.

L 606 CHARACTERIZATION OF CELL LINES ALTERED IN THE ABILITY TO EXPRESS SV40 T-AG, Vera Byrnes and Warren Maltzman, Waksman Institute of Microbiology, Rutgers University, Piscataway, N.J. 08854.

Expression of the Simian virus 40 (SV40) early gene product T-antigen, (T-Ag), has been extensively utilized as a model system for studying the regulation of eukaryotic gene expression. We have isolated spontaneously transformed cell lines, (NT12's), by carrying subclones of NIH3T3's without dilution for an average of 15 passages. Upon SV40 infection of NT12's the level of T-Ag, as detected by immunoprecipitating metabolically labelled protein, is reduced 10 to 300 fold compared to NIH3T3's, depending on the cell line examined. The goal of this study is to characterize the level at which T-Ag expression is blocked, which will ultimately allow the NT12's to be utilized as a system for characterizing factors involved in the regulation of gene expression.

We have studied the block to T-Ag expression as possibly being due to an inhibition of SV40 infection, transcription, translation, or post translational events. We have shown that the decrease in T-Ag is not due to the inability SV40 to penetrate the cell, since SV40 specific DNA sequences can be detected in the nuclei of infected cells. However, in three out of five NT12 lines T-Ag specific RNA sequences, as detected by slot blot analysis, are decreased eight to fifteen fold compared to parental NIH3T3 cells, indicating a depression of T-Ag transcription. In the other two NT12 lines, the T-Ag RNA levels were essentially the same as in NIH3T3's. Furthermore, Western blot analysis and studies of T-Ag stability indicate that whatever T-Ag is present at the protein level is not any less stable than that in NIH3T3's, implicating an altered post-transcriptional regulation.

L 607 CARDIAC C-MYC mRNA LEVELS MARKEDLY INCREASE AFTER INSULIN ADMINISTRATION TO DIABETIC RATS, Enrico Cagliero, Alice Barrieux and Wolfgang H. Dillmann, University of California, San Diego CA 92103.

Adult cardiac myocytes are nondividing cells whose size,RNA/DNA and protein/DNA ratios are decreased in diabetes;these abnormalities are reverted by insulin. The proto-oncogene c-myc is primarily involved in cell growth but was also shown to influence gene expression. In order to determine if the stimulatory effect of insulin on RNA transcription and protein synthesis in diabetic hearts is accompanied by increases in c-myc levels,we administered 2 U of regular insulin/100 g body weight i.v. to streptozotocin diabetic and control rats at time 0. Controls also received glucose 500 mg i.v. and 1.5 g i.p. to avoid hypoglycemia. At 10,30,60,180 and 300 minutes the animals were sacrificed,the hearts excised and total RNA extracted with the guanidine thiocyanate method. Twenty µg of RNA were hybridized to an excess of ^{32}P labelled RNA probe complementary to human c-myc exon 2 in 80% formamide at 45°C for 12 hours. Single stranded RNA was digested with RNAase T_1 (4 µg/ml) and RNAase A (80 µg/ml) and the hybrids run on a denaturing gel and visualized by autoradiography. Control and diabetic hearts showed very low baseline levels of c-myc. Insulin rapidly increased c-myc mRNA levels in diabetic hearts (10 fold increment at 30',20 fold at 60' and 22 fold at 300') but did not induce a significant change in control hearts. In conclusion c-myc mRNA is rapidly responsive to insulin,showing a marked increase in diabetic hearts. Since myocytes, which comprise 75% of heart mass do not proliferate,these data suggest a role for c-myc in mediating the insulin stimulatory effect on protein synthesis independent of cell growth.

L 608 EXPRESSION OF CELL CYCLE REGULATED GENES IN SERUM STIMULATED AND SV40 INFECTED CELLS. Susan E. Conrad , Moriko Ito, Hyung-Il Lee and Christine Stewart. Dept. of Microbiology, Michigan State University, East Lansing, Michigan 48824. We have studied the regulation of two cell cycle regulated genes in resting cells stimulated to re-enter the cell cycle by the addition of either fresh serum or SV40 virus. In one set of experiments, we have examined the regulation of the cellular thymidine kinase (TK) gene. TK mRNA levels increase approximately 20 fold during S phase in cells induced by either treatment. In serum stimulated cells the induction is due to both transcriptional and post-transcriptional control mechanisms, while in SV40 infected cells we see no evidence for transcriptional induction although the TK mRNA accumulates to high levels during S phase. The mechanisms of induction by these two mitogenic agents may therefore differ in important ways. In a second set of experiments we have examined the expression of the c-myc gene, which has previously been shown to be induced very rapidly after serum treatment. As expected, addition of fresh serum causes a rapid increase in the level of c-myc mRNA. Surprisingly, infection with SV40 gives rise to a complicated pattern of expression. The c-myc mRNA is induced within 1 hr of infection, the level returns to that seen in resting cells by 4 hours, is increased again by 12 hours, and the RNA is gone by 24 hours. Further experiments to analyze the basis of this complicated pattern of expression will be described.

L 609 ROLE FOR A GTP-BINDING PROTEIN IN THE MITOGENIC RESPONSES TO SEROTONIN AND BOMBESIN. Shaun R. Coughlin, John J. Letterio, and Lewis T. Williams, Howard Hughes Medical Institute, UCSF, San Francisco, CA 94131
The mechanisms by which activation of growth factor receptors elicits mitogenic responses are unknown. One mechanism of signal transduction important in the action of many neurotransmitters and peptide hormones utilizes guanine nucleotide binding proteins to couple receptors to effector molecules. An important feature of a subset of guanine nucleotide binding proteins is sensitivity to specific ADP-ribosylation and hence inactivation by pertussis toxin. For a number of hormone-induced responses, inhibition by pertussis toxin has been used to indicate involvement of a guanine nucleotide binding protein in signal transduction. We assessed the ability of pertussis toxin to inhibit mitogenic responses to serotonin, phorbol ester, and platelet-derived growth factor(PDGF) in vascular smooth muscle cell cultures, and to bombesin, phorbol ester, or PDGF in Swiss 3T3 cultures. Pertussis toxin (5ng/ml,2h,37°C) abolished serotonin-induced increases in ^{3}H-thymidine uptake in smooth muscle cell cultures and bombesin-induced increases in c-myc mRNA levels, ^{3}H-thymidine uptake, and cell number in Swiss 3T3 cultures, but had no significant effect on responses to phorbol or PDGF. The pertussis sensitivity of the mitogenic responses to serotonin and bombesin suggests involvement of a guanine nucleotide binding protein in transducing responses to these agents.

L 610 CHARACTERIZATION OF cDNA CLONES FROM THE REGULATORY REGION TRANSCRIPTS OF THE VISNA VIRUS GENOME. J.L.Davis and J.E. Clements,Johns Hopkins University, Baltimore,MD.21205.
Visna Virus is a lentivirus which causes a chronic, progressive disease in sheep involving the brain, lungs, and lymph nodes. Previous studies in our laboratory have demonstrated that retricted viral replication and gene expression in vivo involve complex and multifaceted phenomena. One or more aspects of genetic regulation may be a consequence of the expression of two novel open reading frames, Q and S, located between the pol and env genes. Five viral mRNA species are present in infected cells: a 9.4 kb species containing the genomic RNA as well as the gag-pol message; a 5.0kb species which may represent a Q specific transcript; a doublet at 4.3kb - one species which may be an S specific transcript, and one species which most likely encodes the env protein; and most interestingly, two small multiply spliced transcripts of 1.8kb and 1.5kb which contain sequences from a portion of the S ORF spliced to sequences from the 3' part of the env gene. To study any regulatory nature of such proteins in detail, we have isolated cDNA clones of these transcripts. The structure and function of some of these clones will be presented.

L 611 TRANSFORMATION BY V-MOS AND V-RAS DECREASES THE ACTIVITY OF A CCAAT DNA BINDING FACTOR AND ANOTHER SPECIFIC DNA BINDING FACTOR IN FIBROBLASTS,
Benoit de Crombrugghe, Atsushi Hatamochi, Pellegrino Rossi, Gerard Karsenty, Emile Van Schaftingen, and Paul Golumbek, Laboratory of Molecular Biology, National Institutes of Health, National Cancer Institute, Bethesda MD 20892.
In many different cells, transformation by oncogenes inhibits the expression of differentiation-specific genes. In fibroblasts, transformation by oncogenes such as v-mos, v-ras and v-src inhibits the synthesis of type I collagen, one of the major specialized biosynthetic products of these cells. This inhibition is mediated by a transcriptional mechanism. We have focused our studies on the gene for the α_2 chain of type I collagen and have identified several trans-acting factors in nuclear extracts of NIH 3T3 cells which bind to defined sequences in the promoter of this gene. After transformation of fibroblasts by either v-mos or v-ras, there is a decrease in the activity of both a CCAAT binding factor and a second specific DNA binding factor, whereas the activity of nuclear factor I, another DNA binding protein, is unchanged. Similar results were obtained after treatment of NIH 3T3 cells with phorbol myristate acetate, a condition which also inhibits the synthesis of the type I collagen chains and their transcripts in these cells. Since these factors bind to regions of the promoter that are needed for optimal transcription of the α_2(I) collagen gene, the decrease in their binding activity is likely to participate in the down regulation of this gene by oncogenes and by phorbol esters.

L 612 EXPRESSION OF c-fos ANTISENSE SEQUENCES IN F9 EMBRYONAL CARCINOMA CELLS, S. A. Edwards and E. D. Adamson, La Jolla Cancer Research Foundation, La Jolla, CA 92037.
It has been suggested that c-fos expression is a necessary if not sufficient condition in the progression of F9 cell differentiation (Ruther et al., EMBO J. 4:1775). We have attempted to test this proposition by inhibiting c-fos expression in these cells with an SV40-based expression vector, pSVneosof, which programs expression of c-fos antisense sequences as a 3' extension of a neo mRNA transcript. We have partially characterized seven G418 resistant clones isolated in transfection experiments. Five expressed hybrid neo-sof transcripts: two (ns-2 and ns-3) were of the expected size; two (ns-1 and ns-5) were smaller than expected; and one (ns-4) had larger than expected transcripts. A sixth clone, (ns-6), expressed a neo mRNA in which fos sequences were deleted. Of the clones so far tested, ns-1 and ns-3 appear to be inhibited in the induction of laminin, type IV collagen, and proteoglycan 19 RNA transcripts, in response to retinoic acid and cAMP whereas ns-4 and ns-6 appear to be normal.

The seventh clone (nr7) expressed no neo transcripts, had no detectable integrated plasmid sequences or aminoglycoside phosphotransferase activity, and therefore appears to be the first reported case of natural conversion to G418 resistance.

L 613 TRANSCRIPTIONAL (RUN-ON) ACTIVITY OF THE C-MYC GENE LOCUS IN RESTING AND ACTIVATED PRIMARY LYMPHOCYTES VERSUS DMSO INDUCED AND UNINDUCED HL60 AND BURKITT LYMPHOMA CELLS.
Dirk Eick, Rudolf Berger, Axel Polack and Georg W.Bornkamm, Institut für Virologie, Zentrum für Hygiene der Universität Freiburg, Hermann-Herder-Str. 11, 7800 Freiburg, FRG.

We have analysed the nuclear run-on activity of the c-myc gene in resting primary lymphocytes and at different times after stimulation with Pokeweed mitogen. The transcriptional run-on activity of c-myc exon 1 was comparably high in resting and stimulated cells. High nuclear transcription of c-myc exon 2 and 3, however, was only detected in stimulated lymphocytes. C-myc activation in resting lymphocytes is therefore, at least in part, regulated at the transcriptional level. A model system for transcriptional down-regulation of c-myc gene expression are HL60 cells after addition of dimethylsulfoxide (DMSO). Nuclear run-on transcription of the c-myc locus in DMSO induced HL60 cells is similar to that of resting lymphocytes with an actively transcribed exon 1. Finally,we have analysed a number of Burkitt lymphoma cell lines with t(8;14) and variant t(2;8) or t(8;22) translocations and describe the transcriptional responsivness of mutated or truncated c-myc genes, harboured by these cells, to DMSO.
Supported by Deutsche Forschungsgemeinschaft (SFB 31).

L 614 **SV40 T ANTIGEN BUT NOT POLYOMA VIRUS LARGE T ANTIGEN INHIBITS MYOGENIC DIFFERENTIATION AND INDUCES DNA SYNTHESIS IN TERMINALLY DIFFERENTIATED MYOTUBES: THEY DO NOT DEINDUCE MUSCLE-SPECIFIC GENES,** **Takeshi Endo and Bernardo Nadal-Ginard,** Howard Hughes Medical Institute, Children's Hospital, and Harvard Medical School, Boston, MA 02115.
Cultured muscle cell lines provide a remarkable system to study the mechanisms of commitment and terminal differentiation. Some of the viral or cellular oncogenes have been shown to regulate cellular growth and differentiation. To address whether myogenic cell growth and differentiation are controlled by DNA tumor virus oncogenes, SV40 T antigen (SV T) and polyoma virus large T antigen (Py T), we transfected mouse C2 and rat L6E9-8 myogenic cells with recombinant DNA molecules encoding SV T tsA58 under the control of metallothionein promoter (pMtSVTts) (a gift of K. Peden) and Py T tsa (pLTtsa) [Rassoulzadegan et al., PNAS 80 :4354 (1983)] and obtained stably transformed cells. The SV T-transfected cells differentiated to form myotubes at nonpermissive temperature (40°C) but did not at permissive temperature (33°C), whereas the Py T-transfected cells differentiated at either temperature. This suggests that tumor-causing oncogene but not "cell establishment/immortalization" gene inhibits myogenic differentiation. However, the induction of SV T in the myotubes did not deinduce the muscle-specific genes, consistent with our previous report that c-myc induced in myotubes does not suppress the differentiated phenotype [Endo & Nadal-Ginard, Mol. Cell. Biol. 6: 1412 (1986)]
Of particular interest is the finding that the induction of SV T but not that of Py T in the myotubes caused DNA synthesis in the committed cell nuclei as demonstrated by the high level of incorporation of [3H] thymidine. These results imply that DNA synthesis in terminally differentiated myotubes is not irreversibly repressed since it can be reinduced by the viral oncogene. Furthermore, the concomitance of muscle-specific gene expression and DNA replication demonstrates that muscle-specific genes are also expressed in other phase(s) than GO/G1.

L 615 PRO-1 PROMOTION SENSITIVITY mRNA IDENTIFIED IN MOUSE JB6 AND HUMAN TUMOR CELL LINES. Robert R. Garrity[1], Glenn C. Hegamyer[2], and Nancy H. Colburn[2], [1]Program Resources, Inc., NCI-FCRF, Frederick, MD 21701, [2]Cell Biology Section, Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD 21701-1013.
Promotion sensitive (P^+) mouse JB6 variants show a 100-fold greater sensitivity to promotion of neoplastic transformation by TPA than do their insensitive (P^-) counterparts. The nucleotide sequence of activated mouse promotion sensitivity gene pro-1 predicts an open reading frame, and signal sequences expected for a transcriptionally active RNA polymerase II gene. A single stranded oligomer, synthesized antisense to pro-1's predicted sequence, as well as double stranded pro-1 restriction fragments, hybridize to a unique polyadenylated transcript. Northern analysis also indicates that unique pro-1 hybridizable transcripts are found in cytoplasmic RNAs of certain human tumors whose DNA contains activated pro-1. P^- variants show reduced levels of pro-1 hybridizable RNA when compared to P^+ and neoplastically transformed clonal lines. Apparent differences in the levels of messages encoded by activated and nonactivated pro-1 genes (i.e., in P^+ and P^- cells) suggest that the mode of activation involves transcriptional or post-transcriptional control. To investigate pro-1's mode of activation we are currently screening carcinoma, P^+, and P^- cDNA libraries. Comparison of the sequences of active and inactive pro-1 cDNAs may clarify critical differences that account for biological activity.

L 616 INDUCTION OF c-*FOS* EXPRESSION BY EXTRACELLULAR SIGNALS
Michael Z. Gilman, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

We have previously identified several intracellular signalling pathways and nuclear DNA-binding activities that appear to participate in the communication of extracellular signals to the c-*fos* gene. Among the DNA binding activities, we have identified a protein that binds to a short sequence in the mouse c-*fos* gene required for induction of the gene by serum in fibroblasts. Current experiments address two issues. First, we have begun to purify this protein to learn how it functions in mediating induction of c-*fos* transcription. Second, we are addressing the role of this protein and its binding site in the response of the c-*fos* gene to the many different extracellular signals that induce c-*fos* expression. The strategy has been to introduce point mutations into this site and to test these mutant sites for binding *in vitro* and for inducibility *in vivo*. Initial results suggest that mutations that affect protein binding *in vitro* also affect the activity of the site *in vivo*. Furthermore, these mutations affect in parallel induction by serum and by phorbol esters.

L 617 TRANSCRIPTIONAL CONTROL AND STRUCTURE OF THE EGF RECEPTOR GENE. John Haley, Paul Bennett, Lynn Wilson, Mark Berger and Mike Waterfield, Ludwig Institute for Cancer Research in the Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX.
The EGF receptor has been cloned and mapped by restriction analysis and electron microscopy of genomic cDNA heteroduplexs. The coding sequences span 110 kb and are divided into 26 exons. The EGF receptor gene 5' flanking region was cloned from A431 DNA. RNA levels for the EGF receptor are increased by TPA and serum stimulation in both A431 and human foreskin fibroblasts. The EGF receptor promoter contains no TATA box, no CAAT element is GC rich and includes seven potential Sp1 binding sites (CCGCCC; three of which are in the first intron) and four TCCTCCTCCTCC repeats. Three major clusters of transcription initiation sites are seen in vivo. Transcriptional activity of the 5' flanking region and GC rich regions within the first intron was investigated by construction of fusion plasmids. Chimeric constructs were transiently and stably transfected into host lines (CV1, Hela, CHO, HE3, A431, MDA468, MDA 231 and SVK14), CAT actively quantitated, and Hirt supernatant plasmid concentration and RNA starts determined. Deletion of the upstream region (-450 to -1100) had little effect on transcription freom linear or circular templates and did not alter RNA initiation sites. Deletion of the distal GC box slightly reduced activity, while deltion of the two distal GC boxes and intervening four TCC type repeats disrupted normal RNA start sites yet maintained activity. The promoter is negatively competed by the SV40 21 bp repeats, yet is strongly activated by adenovirus E1A protein. Latest results on protein binding and modulation of expression will be discussed.

L 618 TRANSCRIPTION FACTORS AND ENHANCER DNA SEQUENCES MEDIATING DIFFERENTIAL EXPRESSION IN LYMPHOCYTES FROM THE RETROVIRUS SL3-3, Bengt Hallberg, Anders Thornell and Thomas Grundström, University of Umeå, Umeå, Sweden.
The T lymphocyte specificity of the mouse retrovirus SL3-3 is at least partially due to an enhancer preferentially expressed in T lymphocytes. We have constructed an extensive set of mutants and analysed them in vivo by a transcient expression assay and in vitro by 'footprinting' and electrophoretic mobility shift techniques to examine the enhancer DNA sequences and transcription factors interacting to mediate the differential expression in lymphocytes. The in vivo results limit the DNA sequences essential for T lymphocyte specific stimulation of transcription to a short segment of the U3 region of the LTR. A larger segment of the U3 region was neccesary for the lower level of stimulation of transcription found in B lymphocytes. The 'footprinting' analysis of the U3 region revealed several different DNA segments interacting with nuclear protein extracts from the T and B lymphocyte cell lines studied. Electrophoretic mobility shift experiments identified several protein-DNA complexes characteristic of each of the different lymphocyte cell lines. The complexes are compeatable with a small excess of the homologous DNA sequence but not with other DNA sequences. Both methylation and point mutation of specific nucleotides interfere with complex formation. Protein purification data as well as a comparition with the effect of point mutations in vivo will be discussed.

L 619 DETECTION OF TRANS-ACTING PROTEINS INTERACTING WITH RETROVIRAL LTR SEQUENCES IN NORMAL AND TRANSFORMED CELLS. W.L. Wendy Hsiao, Martin Begemann and I. Bernard Weinstein, Columbia University Cancer Center, New York, N.Y. 10032.
High levels of poly A^+ RNAs homologous to endogenous retrovirus-related DNA sequences are expressed in carcinogen-transformed rodent cells. A possible explanation is that the tumor cells synthesize a trans-acting factor binds sequences present in the endogenous retroviral-LTR DNA sequences, and activates their expression. The CAT transient expression system was utilized to determine whether transformed cells differ from their normal counterparts in terms of their ability to transcribe the CAT gene when it is linked to different promoter sequences, including two independently isolated endogenous IAP-LTRs (prcm CAT, pIAP-CAT). The ability of each construct to express CAT activity, displayed the following order: promCAT > pRSV-CAT> pSV2-CAT > pIAP-CAT > pSVO-CAT, in both the normal and transformed cells. However, CAT activity was 3-10 fold higher in the transformed than in the normal cells. Studies with ^{32}P-labelled DNA indicated that this difference was not due to greater uptake of the transfected DNAs by the transformed cells. Competition studies also provide evidence that factors required for the expression of IAP-LTR-linked sequences are present in limited amounts in normal 10T1/2 cells, but are present in excess amounts in transformed cells. Studies using a band shift gel-electrophoresis method indicate that the transformed cells contain large amounts of specific proteins that bind to both IAP-LTR and MoMuLV-LTR sequences.

L 620 TURNOVER OF HUMAN C-MYC DELETION AND HYBRID CYTOPLASMIC RNA SPECIES. Thomas R. Jones and Michael D. Cole, Department of Molecular Biology, Princeton University, Princeton NJ 08544.
Expression of the normal 3 exon c-myc gene correlates with the growth state of the cell; modulations in c-myc RNA expression are due to both transcriptional and post-transcriptional processes. The half-life of normal c-myc RNA is about 30 minutes. However, the half-life of c-myc RNAs lacking exon 1 in plasmacytoma or Burkitt's lymphoma cells are increased 4- to 10-fold (Rabbitts et al., EMBO J. 4:3727-3733 [1985]; Piechaczyk et al., Cell 42:589- 597 [1985]). We investigated which sequences of the human c-myc gene were necessary to impart a short half-life by making various deletion and c-myc/neo (hybrid) constructs and analyzing the cytoplasmic stability of their transcripts in stably transformed NIH3T3 cells through the use of actinomycin D. The normal human gene has the expected short half-life in mouse cells. A gene lacking only exon 2 also has a short half-life, while a gene lacking only exon 1 has a long half-life (3-4 hr). When the first exon is present on a neo transcript (normal half-life >4 hr), no effect is observed. The message derived from a c-myc gene lacking most of exon 3 is very stable. Transcripts from hybrid genes containing most of exons 1 and 3 separated by either 320b or 1450b of neo coding sequences have a short half-life, similar to the normal c-myc message. The results suggest that sequences from both exons 1 and 3 must be present on a transcript to impart cytoplasmic instability.

L 621 ACTIVATION AND REPRESSION OF MAMMALIAN GENE EXPRESSION BY THE C-MYC PROTEIN, Rima F. Kaddurah-Daouk*, John M. Greene*, Albert S. Baldwin, Jr+. and Robert E. Kingston*, *Department of Genetics, Harvard Medical School and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, +Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
One mechanism by which nuclear localized oncogenes might transform cells is through an ability to regulate gene expression. We show that the c-myc protein stimulates the level of appropriately initiated expression from the human heat shock protein 70 promoter. Sequences required for full activation lie upstream of the transcription initiation site and are distinct from sequences necessary for basal expression. These sequences are also distinct from promoter sequences necessary for heat induction, serum induction and induction by the papovavirus T antigens. The c-myc protein inhibits appropriately initiated expression from the mouse metallothionein I promoter. This inhibition is not the result of promoter competition, but instead appears to result from specific inhibition of elements of the mouse metallothionein I promoter. Expression from the adenovirus EII promoter is slightly inhibited, while expression from the SV40 early promoter is minimally affected by the c-myc protein. Both the spectrum of promoters regulated by the c-myc protein and the sequence requirements for that regulation differ from those of previously characterized viral trans-activating proteins. The data suggest that the c-myc protein can both stimulate and inhibit transcription from mammalian promoters in a novel manner.

L 622 EBV Transactivates the HIV Promoter
Shannon C. Kenney and Joseph S. Pagano
Lineberger Cancer Research Center, University of North Carolina at Chapel Hill.

HIV replicates well in EBV-positive B cell lines but not in EBV-negative B cell lines, suggesting that EBV may provide an essential "helper" function for HIV in B cells. We placed the HIV LTR upstream of the CAT gene, and confirmed that the HIV promoter can be transactivated by the HIV TAT gene. We then tested whether the HIV promoter could also be transactivated by an EBV gene. The EBV BamHl M LFl immediate early gene product produces a transactivation of the HIV LTR comparable to that seen with TAT. The combined effect of the EBV immediate early gene product and TAT is additive. The CMV IE gene product produces a similar transactivation of the HIV LTR (done in collaboration with M. Davis and E.S. Huang). Preliminary RNA analysis suggests that both the EBV IE gene product and the HIV TAT increase the level of RNA detected from the HIV promoter. We are currently analyzing deletion mutants of the HIV promoter to determine if the promoter sequences necessary for EBV transactivation are different from those necessary for TAT transactivation. We are also investigating whether an HIV deletion mutant defective in TAT expression (which requires the presence of TAT in trans to replicate) can be complemented by the presence of the EBV immediate-early gene product.

L 623 THE EXPRESSION OF GENES RESPONSIVE TO TUMOR NECROSIS FACTOR (TNF). Martin Krönke, Stefan Schütze, Peter Scheurich, Carsten Schlüter, Ugur Ücer, and Klaus Pfizenmaier, Clinical Research Group, Max-Planck-Society, 3400 Göttingen, FR Germany
Both recombinant tumor necrosis factor (TNF) and recombinant interferon-gamma (IFN-gamma) induce differentiation of a human histiocytic, leukemic cell line, U937, which is associated with inhibition of cell proliferation. In these cells, TNF induces e. g. c-fos mRNA and 2'-5' oligo A synthetase (2'-5'-OASE) mRNA accumulation. In contrast, the expression of c-myc and the histone genes is markedly reduced in TNF treated cells. We have established two subclones of U937, U937.C27 and U937.G3, which proved to be resistant to the cytostatic action of TNF despite expression of high affinity TNF receptors. In distinction to the parental U937 cell line, the subclones C27 and G3 both express Fc receptors at the cell surface, indicating that they apparently have progressed to a higher state of differentiation. In these resistant clones, TNF neither induces 2'-5'OASE gene expression nor reduces c-myc mRNA levels. Upon TNF treatment, the pattern of phosphorylated proteins in the TNF resistant subclones lacks some of the typical changes observed with TNF sensitive U937 cells, indicating that signal transduction may result in the activation or inactivation of different enzymes, which in turn may explain the different pattern of responding genes. Interestingly, rIFN-gamma treatment, which on its own had no effects, can restore TNF sensitivity. The data presented indicate that the TNF inducible gene pattern varies with the status of U937 cell differentiation and can be modulated by IFN-gamma.

L 624 THE EFFECTS OF c-fos ANTISENSE ON THE INDUCTION OF c-fos AFTER INTERFERON TREATMENT, Ben-Zion Levi, Yechiel Shai,* Chaiken M. Irwin* and Keiko Ozato, LDMI, NICHD and *NIDDK, NIH, Bethesda, MD, 20892

The proto-oncogene c-fos is expressed in normal embryonic and adult tissues. Its expression is induced in a variety of in vitro cells by growth factors and other agents. In addition, we have found that murine interferons (both IFN-α/β and IFN-γ) induce c-fos gene expression in NIH 3T3, embryonal teratocarcinoma F_9 cells and many other cells. c-fos expression is rapid and transient, similar to the inductions seen by other stimuli. To assess functional significance of c-fos gene expression by interferons and other stimuli, we adopted the approach of antisense RNA for blocking the induction of c-fos gene. Hinc II and Hinc II-Pvu II fragments of the murine c-fos containing the promoter region as well as most of the first exon were cloned in the reverse orientation into the mammalian expression vectors pRSV-2 and pRSV-3. Stable transformants of F_9 cells constitutively expressing c-fos antisense RNA were isolated. These cells maintain the undifferentiated morphology but can be induced with retinoic acid to express major histocompatibility complex (MHC) class I antigens on cell surface and to lose the embryonal antigen SSEA-1. The effect of c-fos antisense on c-fos induction by interferon and other stimuli is tested at the level of mRNA and protein. Data will be discussed in terms of possible roles of c-fos gene expression in eliciting interferon effects and cellular differentiation.

L 625 DIFFERENTIAL REGULATION OF GENE EXPRESSION IN AD2- AND SV40-TRANSFORMED HAMSTER CELLS. A. Levine, K. Akagi, K. Murai, M. Haddada and C.Patch, NICHD, Bethesda, MD. Ad2- and SV40-transformed hamster embryo cells differ markedly in their phenotypic properties including the potential for inducing tumors in hamsters. Both Ad2- and SV40-transformed cells are immortalized and readily induce tumors in newborn syngeneic hamsters, but only SV40-transformed cells are highly oncogenic in syngeneic and allogeneic adult hamsters. The reasons for this difference in oncogenic potential remain elusive. However, recent studies with transforming growth factors (TGFs) indicate that these factors play an important role in determining many characteristics of transformed cells, and could play a significant role in determining their oncogenic potential. To determine whether TGFs secreted by Ad2- or SV40-transformed cells differ, we have examined the ability of media conditioned by these two phenotypes to modulate thymidine uptake in quiescent untransformed cells. We found that both transformed phenotypes secrete very similar TGF α-like mitogenic factors which inhibit binding of [^{125}I]-EGF to its receptor. However, SV40-transformed cells, but not Ad2-transformed cells, also secrete a powerful mitogenic inhibitor (MI). The MI secreted by SV40-transformed cells is inhibitory for several transformed and normal cell types, with a cytostatic, not cytolytic, action. MI elutes from HPLC columns with a molecular weight of 24 Kd, but differs from TGF β in several properties. The MI secreted by SV40-transformed cells also inhibits thymidine uptake by con A-stimulated spleen lymphocytes. This finding suggests that MI might contribute to the extreme oncogenicity of SV40-transformed cells by inhibiting mobilization of immune effector cells at the site of tumor cell proliferation.

L 626 PRODUCTION OF RECOMBINANT HTLV-III ENVELOPE PROTEIN IN INSECT CELLS AND ITS POTENTIAL USE IN A SUBUNIT VACCINE

Debra L. Lynn[1], James R. Rusche[1], Scott D. Putney[1], Helen Carson[1], Thomas J. Matthews[2], Marjorie Robert-Guroff[3], Kai Khron[3], Dani P. Bolognesi[2], and Robert C. Gallo[3]. [1]Repligen Corporation, Cambridge, MA., [2]Department of Surgery, Duke University Medical School, Durham, NC, [3]Laboratory of Tumor Cell Biology, NCI/NIH, Bethesda, MD.

The entire HTLV-III envelope gene, gpl60, was cloned into a baculovirus vector and expressed in an insect cell line. The gpl60 remained uncleaved and was shown to be glycosylated and inserted in the cell membrane. Sera obtained from animals immunized with whole infected insect cells expressing gpl60 or with purified gpl60 can neutralize HTLV-III infection of human T cells in culture. Further analysis of the immune response to these and other HTLV-III constructions expressed in insect cells will be presented.

L 627 OVER-EXPRESSION OF ENDOGENOUS AND HETEROLOGOUS ORNITHINE DECARBOXYLASE
Lisa McConlogue and Philip Coffino, Cetus Corp., Emeryville ,CA 94608, and Univ. of Calif. at San Francisco, San Francisco, CA 90401.
We selected and characterized a series of animal cell variants that overproduce Ornithine Decarboxylase (ODC), an enzyme that is regulated with the growth state of the cell. S49 mouse cells that are resistant to the ODC inhibitor difluromethyl ornithine (DFMO) have overproduced the endogenous ODC gene by three different mechanisms; 1) overproduction of ODC mRNA without gene amplification, 2) increased translatibility of the ODC mRNA and 3) gene amplification. If one separates the ODC cDNA from its regulatory elements, however, the only mechanism by which cells overproduce the heterologous ODC is by amplification of the transfected gene. ODC-negative CHO cells were transfected with a plasmid, pdhOD1, that expresses the trimmed ODC cDNA from the SV40 early promoter, and dhfr from the MMTV ltr. Selection of DFMO resistant variants gave rise to cells that overexpress ODC, have amplified both the heterologous ODC gene and the linked dhfr gene at least 700 fold, and over-express the unselected, linked dhfr gene 1000 fold. Therefore, by separating the ODC cDNA from its regulatory sequences, we have developed an amplifiable vector based on ODC that can be used to select animal cells that over-express an unselected, linked gene 1000 fold.

L 628 SOMATIC MUTATIONS IN HUMAN C-MYC GENES, Kenneth F. Mitchell and Carol A. Vallone, E. I. du Pont, Glenolden, PA 19036.

Sequence analysis of Burkitt lymphoma c-myc genes has shown that mutations are common within, the presumed regulatory, first exon of the gene. We have found alterations as minimal as 2 single base changes or as great as multiple changes and deletions in genes isolated from, so called, variant tumors with 8:22 translocations.

Deletions appear to arise from recombinations involving small regions of homology whereas base changes appear to be randomly located. Overall, the changes indicate that secondary structures can form in different regions of this exon and suggest that these may provide binding sites for, as yet unknown, proteins.

In one case the mutational events are focused in the first exon but the chromosomal translocation site is greater than 40 Kb away, 3 prime of the gene, and involves an exchange of DNA between a region of chromosome 8 with no known function and gene 3 of the immunoglobulin lambda locus.

L 629 POST-TRANSCRIPTIONAL STUDIES OF C-MYC IN DIFFERENTIATING HL60 CELLS, Lynn S. Mitchell, George D. Birnie, Keith Vass, Rosemary Neill, Sheila V. Graham, Mary Harper, Lorna Love, The Beatson Institute for Cancer Research, Glasgow G61 1BD, Scotland.
We have used the promyelocytic cell line HL60 as a model for studying gene expression during differentiation. This cell line can be induced to differentiate along the granulocytic pathway by Retinoic Acid (RA) or Dimethylsulphoxide (DMSO) treatment, or along the monocytic pathway by TPA. Studies have previously shown that following induction c-myc expression is lost in the HL60 system. We have studied the abundances of c-myc transcripts at short time intervals following induction along both pathways. After only 1 hour exposure to DMSO c-myc transcripts drop to almost undetectable levels However during a RA or a TPA induction a biphasic response is observed, with c-myc transcripts decreasing early after induction followed by a slight increase before the RNA becomes undetectable. It has been observed that most c-myc transcripts are nuclear-confined when HL60 is in the uninduced state. Following induction we observe a marked decrease in c-myc RNA. To discover how this decrease is brought about nuclear run-off assays are being carried out at early times of induction to deduce whether this is due to transcriptional control. The stability of c-myc mRNA during differentiation is being estimated by actinomycin D studies. We also observe a movement of c-myc transcripts on to the polysomes after 1½ hours TPA or DMSO treatment. The mechanisms behind these translational changes are being investigated.

L 630 EFFECTS OF HTLV-I ENCODED pX ON EXPRESSION OF IL-3 AND GM-CSF GENES, Shoichiro Miyatake, *Rene De Waal Malefyt, Yutaka Takebe, Takashi Yokota, Takeshi Otsuka, *Jan De Vries, Naoko Arai, Frank Lee, #Motoharu Seiki, #Mitsuaki Yoshida and Ken-ichi Arai, DNAX Research Institute, Palo Alto, CA 94304, *UNICET Laboratory, 69572 Dardilly, France, #Cancer Institute, Tokyo 170, Japan.

IL-3 and GM-CSF are lymphokines produced by antigen or lectin activated T cells. To study the regulation of the expression of lymphokine genes during T cell activation, plasmids carrying the CAT gene downstream of the 5' flanking region of the mouse IL-3 gene and the mouse or human GM-CSF genes were introduced into various T cell clones. Lectin-inducible CAT expression was observed in several cell lines following transfection of the hybrid genes.

It is known that the IL-2 receptor and certain cellular genes are expressed in HTLV-I infected T cells, and the pX encoded by the HTLV-I genome stimulates transcription from the LTR of HTLV-I. To test the possibility that pX activates several lymphokine genes, cotransfection of plasmids carrying the pX gene and lymphokine-CAT fusion gene were performed. In various cell lines, pX enhanced the expression of CAT activity 10-50 fold. The region of IL-3 and GM-CSF genes required for induction by lectin and for activation by HTLV-I encoded pX will be presented.

L 631 E1A GENE-REGULATING ACTIVITIES INVOLVED IN THE INDUCTION OF PRIMARY CELL PROLIFERATION, Elizabeth Moran[1], Ned Lamb[1], Michael B. Mathews[1] and Brad Zerler[2], [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 and [2]Molecular Therapeutics Inc., West Haven, CT 06516.

The adenovirus 12S E1A cDNA product is able to immortalize quiescent primary baby rat kidney (BRK) cells as efficiently as the 13S E1A product, but is much less efficient than the 13S product in transactivating virus early genes. To determine whether the 12S product has a gene activating function involved in the induction of cell proliferation, separate from the activity in the 13S product required for efficient activation of virus early genes, we have tested the ability of the 12S product to activate expression of cellular products required for proliferation. We have identified a cellular protein, proliferating cell nuclear antigen (PCNA) whose synthesis is stimulated as efficiently by the 12S product as by the 13S product. Using in vitro mutagenesis procedures, we have identified two separate domains within the 12S and 13S common sequences that are required for the initiation of cell division and transformation in quiescent BRK cells. The first is required for the induction of DNA synthesis in infected cells, while the second is dispensable for the induction of DNA synthesis and the activation of PCNA expression, but required for the cells to progress through mitosis. Either of these functions can be eliminated without impairing E1A-dependent transactivation of virus early genes.

L 632 ANTISENSE RNA OF PROTO-ONCOGENE c-fos BLOCKS RENEWED GROWTH OF QUIESCENT 3T3 CELLS, Kazuko Nishikura* and John M. Murray†, The Wistar Institute of Anatomy and Biology* and University of Pennsylvania†, Philadelphia, PA 19104.

The c-fos gene product was hypothesized to be important for the transition from quiescence to renewed cell growth because high levels of c-fos expression are observed during this transition, but direct proof is lacking. The possibility still remains that c-fos expression, though normally accompanying the response to mitogens, is not itself a prerequisite for the subsequent DNA replication and cell division.

Recently a series of experiments has shown that introduction of an antisense RNA complementary to a specific mRNA into cells can effectively create or mimic the null-mutant phenotype, which should be quite useful to investigate gene products of unknown physiological function. In this study, we have used antisense RNA to elucidate the physiological function of c-fos gene products. In NIH3T3 fibroblast cells transformed with multiple copies of antisense c-fos DNA fused to mouse mammary tumor virus (MMTV) promoter, antisense c-fos RNA is expressed, while the sense c-fos mRNA and protein levels are greatly decreased in the presence of dexamethasone. Although the transformant grows normally in the exponentially growing phase, a large fraction of cells synchronized in the quiescent state could not reenter S phase or, in other words, restart the cell cycle, in the presence of dexamethasone. Our results prove for the first time that c-fos proto-oncogene expression is a prerequisite for the transition from G_0 into renewed cell growth.

L 633 **FELINE LEUKEMIA VIRUS ENVELOPE PROTEIN EXPRESSION ENCODED BY A RECOMBINANT VACCINIA VIRUS: APPARENT LACK OF IMMUNOGENICITY IN VACCINATED ANIMALS.** Jack H. Nunberg, James H. Gilbert, and Niels C. Pedersen*. Cetus Corporation, Emeryville, CA 94608, and *University of California School of Veterinary Medicine, Davis, CA 95616

We have constructed a recombinant vaccinia virus encoding the expression of the Feline Leukemia virus (FeLV) envelope gene of the Gardner-Arnstein strain of FeLV subgroup B. Cells infected with the recombinant virus (vFeLVenv) express and process the FeLV envelope precursor protein similarly to cells infected with authentic FeLV. The mature gp70 protein is transported to and accumulates on the surface of vFeLVenv-infected cells. Vaccinia virus replication and FeLV gp70 accumulation was also observed in cells of feline and murine origin, albeit at levels somewhat reduced from those in human cells. Toward the goal of developing a recombinant vaccinia virus as a live vaccine for feline leukemia disease in cats, immunogenicity studies were performed in cats and mice. These experiments yielded surprising results: although animals mounted a typical virus-neutralizing antibody response to the vaccinia virus vector, we were unable to detect antibodies against FeLV gp70 in any of the vaccinated animals. These results may point to complexities involved in the development of vaccines to protect against retrovirus infection.

L 634 ENHANCED EXPRESSION OF THE HUMAN IMMUNODEFECIENCY VIRUS (HIV) LONG TERMINAL REPEAT SEQUENCE BY HERPESVIRUSES, Jeffrey M. Ostrove, Howard E. Gendelman and John M. Leonard, NIAID, NIH, Bethesda MD 20892.

In order to determine whether Herpes Simplex Virus type 1 (KOS) has an effect on Human Immunodeficiency Virus (HIV) gene expression, the HIV long terminal repeats (HIV-LTR) were linked to the indicator gene chloramphenicol acetyltransferase (CAT). When Vero cells were transfected with HIV-LTR-CAT and then infected with HSV-1 there was a 25-fold stimulation in the expression of CAT. Vero cells cotransfected with HIV-LTR-CAT plus a clone containing the tat gene showed an 18-fold stimulation of CAT activity; when these cotransfected cells were superinfected with HSV-1 a 70-fold stimulation of CAT activity was detected. To investigate which HSV genes were responsible for this stimulatory effect, clones containing ICP27, ICP4 and ICP0 were cotransfected with HIV-LTR-CAT plus tat. ICP4 and ICP0 both showed a stimulation of CAT expression while ICP27 had no effect. These results varied slightly when HeLa cells or SW480 cells were used. The level of CAT mRNA was shown to correlate well with the stimulatory effects seen, implying transcriptional activation of the HIV-LTR-CAT by Herpesvirus gene products.

L 635 REGULATION OF EXPRESSION OF THE c-mos PROTO-ONCOGENE, Richard S. Paules[1], Friedrich Propst[1], Donald G. Blair[2] and George F. Vande Woude[1], [1]BRI-Basic Research Program; [2]Laboratory of Molecular Oncology; NCI-Frederick Cancer Research Facility, Frederick, MD 21701.

The cellular homolog of the transforming gene of Moloney murine sarcoma virus, mos, exhibits novel patterns of expression in various tissues. While c-mos expression is not detectable in most tissues, relatively abundant amounts of c-mos transcripts have been detected in gonadal tissues of rodents and primates. In mice, the testicular and ovarian c-mos transcripts are 3' co-terminal but have different tissue-specific transcription start sites. The level of testicular and ovarian c-mos expression is developmentally regulated. We will show that c-mos expression is also tissue-specific in primates and appears to be order-specific as well.

The nucleotide sequences of the c-mos locus of both humans and African green monkeys reveal the presence of additional open reading frames that begin about 100 bases upstream of the conserved c-mos ATG and continue for more than 100 bases, overlapping the c-mos open reading frame. We have demonstrated that the presence of these upstream sequences can reduce the transforming activity of primate c-mos in NIH/3T3 transfection assays. Patas monkey c-mos transcripts in ovaries start just upstream of the first c-mos ATG and do not appear to contain the ATG of the upstream overlapping open reading frame. Start sites of c-mos transcripts in both human and patas monkey testes occur within the c-mos open reading frame, just upstream of a second ATG codon. The product expressed from these transcripts would lack the N-terminal ATP-binding domain of the predicted c-mos protein. Research sponsored by the National Cancer Institute, DHHS, under Contract NO. NO1-CO-23909 with Bionetics Research, Inc.

L 636 MODULATION AND CHARACTERIZATION OF THE C-FOS ENHANCER BINDING FACTOR. Ron Prywes, Tobe Fisch, and Robert G. Roeder. The Rockefeller University, New York, N.Y. 10021.

We have analyzed a nuclear factor which binds specifically to the c-fos enhancer. Treatment of A431 cells with epidermal growth factor results in a rapid increase in the level of c-fos transcription and a concomitant increase in binding of the factor to the enhancer. Surprisingly, as transcription decreases rapidly the enhancer activity remains elevated. In addition, although HeLa cells exhibit no detectable transcription of the c-fos gene, they contain significant amounts of binding activity comparable to those in induced A431 cells. These results suggest that regulation of c-fos transcription involves more than simply increased enhancer-binding activity. Transcription of c-fos in A431 cells is also markedly induced by the tumor promoter TPA and by the calcium ionophore A23187, yet neither induced an increased level of the enhancer-binding activity. These agents thus appear to activate c-fos transcription via a mechanism distinct from that utilized by EGF. We are testing which sequences are responsible for TPA and A23187 induced transcription in vivo. In addition, in order to better understand regulation via the enhancer, we have extensively purified the c-fos enhancer binding factor by specific DNA affinity chromatography.

L 637 ISOLATION AND ASSIGNMENT OF DNA MARKERS POTENTIALLY ASSOCIATED WITH MALIGNANT GROWTH AND OTHER HUMAN DISEASES, Shizhen Qin*, Lisa Davis, Hiroshi Nikai and Thomas B. Shows, Roswell Park Memorial Institute, Buffalo NY 14263; *Fudan University, Shanghai, China

The WARG locus is characterized by Wilms' tumor,aniridia,urogenital disorders and mental retardation. This disorder is inherited and can be associated with chromosome 11 deletions ranging from 11p11.2-11p15. The smallest region of overlap is 11p13. Isolate and assign DNA markers in this region is a critical step of characterizing the structures and functions of genes or sequences related to the anti-oncogenic process or the development of the iris and urogenital tissue and mental functions. Using the strategy of fusion human WAGR fibroblasts heterozygous for an 11p deletion with mouse cells,human-mouse cell hybrids retaining a deleted chromosome 11 were obtained. These hubrid cell lines have different deletion patterns and the smallest common deletion is 11p13. Results of DNA hybridization indicated that the follicle stimulating hormone(beta polypeptide) and catalase genes are located within 11p13. Undefined DNA fragments isolated from a Los Alamos chromosome 11 library,D11RP245 and D11RP69, are located at the proximal p12 boundary and p11.2,respectively,which are close to the break-points of WARG deletions. 11q13 region participates in nonrandom chromosomal rearrangement with chromosome 14 resulting in malignant transformation of the cells. Markers located at this region presents the opportunity to study the genetic mechanisms of malignant transformation. A cluster of pepsinogen genes(PGA1,PGA2 and PGA3) have been assigned to 11q13. A DNA fragment isolated from the chromosome 11 library is also assigned to this region.

L 638 DELETION OF AN mRNA DESTABILIZING ELEMENT IS RESPONSIBLE FOR THE INCREASED ONCOGENICITY OF PROTO-ONCOGENE FOS. Vincent Raymond and Inder M. Verma, The Salk Institute, San Diego, CA 92138

In numerous cell lines, the induced expression of the proto-oncogene fos (c-fos) is characterized by transient mRNA accumulation. The c-fos gene can also transform fibroblasts provided two manipulations are carried out: linkage of LTR sequences, and removal of an A+T rich stretch of 67 nucleotides in the 3' untranslated region. Recently, highly conserved A+T rich tracts, with an ATTTA consensus motif, have also been identified in the 3' non-coding region of several genes expressed in a transient fashion.

To assess for a possible function of the 67 base pair (bp) fragment at the post-transcriptional level, deletion mutants in the 3'untranslated region and in the COOH terminal coding domain of the c-fos gene under the control of the v-fos LTR were transfected in NIH 3T3 cells. Quantitative analysis of the mRNA after treating the cells with actinomycin D revealed that the 67 bp fragment was responsible for reduced levels of stable mRNA. The rate of accelerated degradation of the RNA containing the 67 bp sequence was estimated to be at least 10-fold. Co-transfected control DNA indicated that there was no effect of the constructs on the efficiency of transfection nor the rate of transcription. These results strongly suggest that the repeated AUUUA motif in the 3'untranslated region of c-fos mRNA is part of a destabilizing element recognized by a specific RNase involved in rapid messenger degradation, as is also the case for the hGM-CSF mRNA. They support the notion that oncogenicity of the v-fos gene, where the destabilizer was deleted during transduction, is partly due to increased mRNA half-life.

L 639 c-Ha-ras Activation: Non-linkage with Spontaneous Murine Hepatocellular Carcinogenesis, Lewis V. Rodriguez[1], Jagannadha Sastry [1], Gary E. Gallick[1], and James E. Womack[2], University of Texas System Cancer Center[1], Houston, Texas 77030 and Texas A&M University[2], College Station, Tx. 77840

The potential role of proto-oncogene activation in induction of spontaneous hepatocarcinogenesis is under study in a mouse model system comprised of seven Recombinant Inbred (RI) Mouse Strains that were constructed from a segregating B6C3F2 generation of the progenitor cross C57BL/6N X C3H/HeN mice. Recently, activated c-Ha-ras was identified in NIH3T3 transfection assays performed with DNA from chemically induced and spontaneous hepatic B6C3 mouse tumors. From transfection analysis it was postulated that a mutation in c-Ha-ras was responsible for activation and that this is an early event in murine hepatocarcinogenesis. The present study however, demonstrates constitutive c-Ha-ras expression in livers of aging RI strains as well as in C57Bl/6N, the progenitor strain with no history of spontaneous hepatomas in our laboratories. Further, no qualitative or quantitative differences were evident between c-Ha-ras Northern blot resolved transcripts from tumor and normal livers of C3H, B6C3 and RI mice. Lastly, immunoprecipitation and gel analysis of the c-Ha-ras p21 gene product in tumor and normal livers did not detect any electrophoretically altered forms of $p21^{ras}$ which are characteristic of codon 12 or 61 ras point mutations that putatively "activate" this proto-oncogene.
(supported by NCI/NIH Grants CA33305 (LVR) CA39803 (GEG) and funds from Exxon Company, USA, LVR).

L 640 THE INDUCTION OF ORNITHINE DECARBOXYLASE (ODC) BY THE TUMOR PROMOTER 12-O-TETRADECANOYLPHORBOLACETATE (TPA) IS MAINLY CONTROLLED AT THE POST-TRANSCRIPTIONAL LEVEL, Stefan Rose-John, Gabriele Rincke and Friedrich Marks, Dept. of Biochemistry, German Cancer Research Center, P101949, 6900 Heidelberg, FRG.
The expression of ODC-mRNA following treatment with the tumor promoter TPA in murine Swiss 3T3 cells has been studied. The induction of ODC-mRNA by TPA (0.1 µM) is clearly detectable after 20-40 min, peaks after 60-120 min and declines to control levels within 24 hrs. The time course of ODC-mRNA induction by TPA is identical in 0.5% and 10% fetal calf serum (in DMEM). Using an in vitro nuclear transcription assay, we find that the polymerase II density on the ODC gene is constant before and during TPA stimulation. This leads us to the interpretation that TPA induces ODC at the post-transcriptional level. In agreement with this interpretation, we are able to detect stable ODC transcripts after treatment with cycloheximide (CHI). TPA slightly enhances CHI induction of ODC-mRNA, indicating that besides the main regulation at the post-transcriptional level there exists a minor component of transcriptional regulation of ODC expression in 3T3 cells.

L 641 cDNA CLONING AND PRELIMINARY CHARACTERIZATION OF IL2-INDUCED mRNA IN CLONED T HELPER LYMPHOCYTES Daniel E. Sabath and Michael B. Prystowsky, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104.
A cDNA library in lambda gt10 was created using polyadenylated RNA from the cloned murine T helper line, L2, which had been stimulated with human recombinant IL2 for 20 hours. At this time, the cells were at the G1 to S phase transition of the cell cycle. The library was screened by differential hybridization: clones were selected by their ability to hybridize to radiolabeled cDNA from IL2-stimulated L2 cells, but not from unstimulated cells. Approximately 40,000 plaques from the amplified library were screened in the primary screening, which yielded 82 positive plaques. Of these, 28 were rescreened. Of these 28, 5 yielded two distinct positive clones, 22 yielded a single positive clone, and one of these was negative, for a total of 32 positive clones from this secondary screening. Four were selected for further analysis. When used as probes on RNA blots, these four clones hybridized to mRNAs from L2 cells that showed higher levels upon IL2 stimulation. Three of the clones, B7, B11, and B18 contained inserts that cross-hybridized on Southern blots, while the fourth clone, B4, did not hybridize to any of these. The RNA complimentary to clone B4 was between 18S and 28S in size and increased 10-fold, while the RNA for clone B7 was less than 18S in size and increased 5-fold, relative to the total increase in RNA content induced by IL2. In addition to being induced by IL2 in cloned T cells, the RNAs for clones B4 and B7 were induced in murine bone marrow cells by GM-CSF, with different time courses of expression.

L 642 SV40 ENHANCER ACTIVITY IN MOUSE EMBRYONAL CARCINOMA CELLS, Merilyn Sleigh, CSIRO Division of Molecular Biology, North Ryde, 2113, Australia.

The block to viral gene expression in F9 mouse embryonal carcinoma cells disappears as the cells differentiate in the presence of retinoic acid. Increased expression from the SV40 early promoter is correlated with increased activity of viral enhancer sequences. Competition experiments were carried out using homologous (SV40) or heterologous (polyoma) enhancer fragments. These suggested that low enhancer activity in undifferentiated cells was at least partly due to the presence of a repressor molecule. This bound to a subsection of the polyoma enhancer, in a region overlapping that involved in binding enhancer activating molecules. Preliminary studies indicate a repressor which is stable in undifferentiated cells, but rapidly turning over in differentiated F9 cells. The decrease in repressor stability may partly or wholly explain SV40 enhancer activation during F9 cell differentiation.

L 643 SEPARATION OF FUNCTIONAL DOMAINS WITHIN THE HUMAN CYTOMEGALOVIRUS MAJOR IMMEDIATE EARLY 72kd PROTEIN. R.M. Stenberg. Department of Microbiology, West Virginia University, Morgantown, West Virginia 26506.

Immediate early (IE) region 1 of human cytomegalovirus (CMV) codes for the 72kd major IE protein. To address 72 kd protein function, the CMV region 2 early promoter was cloned into pSVOCAT and its activity examined in the presence and absence of plasmids coding for the 72 kd protein. The data demonstrates that the 72 kd protein is both necessary and sufficient to activate the region 2 promoter (pR2CAT) in COS cells. In order to examine the domain responsible for the positive activation of early gene expression, deletion mutations were constructed within the coding region of the 72 kd protein. The S12 mutation, which deletes the carboxyl-terminal 145 amino acids and has been shown to be defective for autoregulation, is capable of transactivating the early promoter. This demonstrates that the domains responsible for positive and negative regulation by the 72 kd protein reside within different portions of the protein and can be separated. Another mutation E24, which deletes 143 amino acids from the middle of the protein, consistantly activates the promoter to only 50 percent of the level of the wild type. The plasmid P240, which deletes the amino-terminal 38 amino acids, functions as well as the wild type. These data indicate that the functional domain involved in positive activation of early genes may reside between amino acids 39 and 130. Deletion and point mutations within this domain are currently being utilized to test this hypothesis.

L 644 EFFECT OF UPSTREAM OPEN READING FRAMES IN THE 5' LEADER OF THE ROUS SARCOMA VIRUS *v-src* mRNA ON TRANSLATION AND EFFICIENCY OF TRANSFORMATION, C. M. Stoltzfus, J. B. Knight, and L.-J. Chang, Department of Microbiology, University of Iowa, Iowa City, IA 52242

The 5' leader of Rous sarcoma virus (RSV) *v-src* gene mRNA has four upstream AUG codons and the 5'-distal upstream AUG is the initiation codon for the viral *gag* and *env* products. This upstream AUG was removed from an intronless *v-src* expression plasmid by point mutagenesis. Less than two-fold increases were observed in the efficiency of *in vitro* translation of $pp60^{src}$; similar results were obtained in *in vivo* transient expression assays. This suggests that reinitiation of protein synthesis at the *src* AUG occurs with an efficiency greater than 50%. Similar transformation efficiencies to that of the wild-type plasmid were obtained in chick fibroblasts when the mutant plasmid was cotransfected with helper RAV-1 DNA suggesting that the presence of the upstream *gag* AUG does not affect the transformation function of the downstream *v-src* gene. Several 5'-leader deletion mutants, both containing and lacking the *gag* AUG, demonstrated similar levels of protein expression and steady state levels of RNA compared to the wild-type plasmids and AUG point mutants when they were transfected into cells but the transformation efficiencies were 100-fold lower. The deleted region includes one of the two *cis*-acting packaging signals in the RSV *v-src* mRNA. We propose that the *v-src* mRNA must be packaged and spread to adjacent cells to efficiently transform chicken fibroblasts. The properties of the viral genomes arising from such transfection experiments is under investigation.

L 645 ALTERED REGULATION OF THE EXPRESSION OF THE PROTO-ONCOGENES C-FOS AND C-MYC IN SCLERODERMA FIBROBLASTS, Maria E. Trojanowska, Cynthia A. Grotendorst, Gary R. Grotendorst and E. Carwile LeRoy, Medical University of South Carolina, Charleston, SC 29425.

The expression of two proto-oncogenes, c-myc and c-fos, differs in vitro in control human adult fibroblasts and fibroblasts from individuals with systemic sclerosis (scleroderma). The level of c-myc RNA in confluent serum-starved control fibroblasts is very low and increases 30 fold after stimulation with growth factors. In contrast, c-myc RNA level in confluent, serum-starved scleroderma fibroblasts is 15 fold higher than in control cells and increases only 2-3 fold after growth factor stimulation. The level of c-fos mRNA is very low in confluent control and scleroderma fibroblasts, but can be strongly induced by poly(I):poly(C) in control fibroblasts. Scleroderma fibroblasts fail to express high levels of c-fos in response to poly(I):poly(C). Altered regulation of expression of c-myc and c-fos in scleroderma fibroblasts provides an interesting system to study the relationship between abnormal proto-oncogene expression and cell proliferation in non-transformed cells.

L 646 ENHANCER-LIKE SEQUENCES IN THE C-MYC GENE, C. A. Vallone and K. F. Mitchell, E. I. du Pont, Glenolden, PA 19036.

Two elements within the second intron of the c-myc gene are highly conserved between mouse and human. Further investigation of the 100 bp. sequence revealed extensive homology with the immunoglobulin heavy chain enhancer, and with core viral enhancer sequences. To study the possible regulatory function of this element, we constructed a set of plasmids in which this area was deleted from a normal human c-myc gene and from an aberrant human c-myc from a Burkitts lymphoma patient. Plasmids were transfected into mouse myeloma cell lines. Transcriptional activity was assessed by cytoplasmic RNA dot blot comparison after 30h. A 2-fold decrease of c-myc message was produced from deletion plasmids as compared to the parent plasmids, with both normal and Burkitts c-myc. Transfection into pre-B cell lines is currently being studied.

In addition, plasmids are being constructed to contain the putative c-myc enhancer with the chloramphenicol acetyltransferase (CAT) gene and driven with the SV40 early promoter, the c-myc protomer region and the aberrant c-myc promoter.

L 647 REGULATION OF GENE EXPRESSION BY V-ABL TYROSINE KINASE, Jean Y.J. Wang and Edward T. Kipreos, Department of Biology, University of California, San Diego, La Jolla, CA 92093.

Oncogene v-abl transforms fibroblasts and lymphoid cells by its tyrosine kinase activity. It is known the expression of several cellular genes, e.g., TGF-alpha, and p53, is elevated in v-abl transformed cells. To probe the role of the transforming tyrosine kinase in the regulation of these genes, we have isolated temperature-sensitive tyrosine kinase mutants of the v-abl oncogene. These mutants were obtained by a direct screening of defects in tyrosine kinase using antibody for phosphotyrosine. The v-abl oncogene was mutagenized in vitro and then expressed in E. coli. Bacterial colonies expressing temperature-sensitive tyrosine kinases were identified by immunoblotting with anti-phosphotyrosine antibody. Two independent mutants were obtained and they retained ts kinase phenotype in mammalian cells. These mutant v-abl genes exhibit temperature-sensitive transforming activity. The role of tyrosine kinase in the regulation of gene expression will be examined using these ts kinase mutants. Cells expressing ts tyrosine kinase will be established at the nonpermissive temperature so that they are not transformed. Correlation between the activation of tyrosine kinase and the activation of gene expression will be investigated. These studies will show if the tyrosine kinase activity has a direct effect on the regulation of gene expression.

L 648 **REGULATION OF ADENOVIRUS EARLY GENE EXPRESSION BY THE E1B 19K TUMOR ANTIGEN**, Eileen White, Amy Denton and Bruce Stillman, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Expression of adenovirus E1A and E1B genes is required for the regulation of early gene expression and cellular transformation. The E1A gene products possess the ability to trans-activate transcription of viral and cellular genes and to repress transcription from enhancer-dependent promoters. We have demonstrated, by the characterization of adenovirus E1B 19K gene mutant viruses, that the E1B 19K tumor antigen plays an important role in negatively regulating E1A-dependent trans-activation. Viruses which lack the ability to synthesize a functional E1B 19K protein cause the overexpression of E1A gene products during infection. This leads to over-stimulation of early gene transcription and to an accelerated viral infection. The ability of the E1B 19K protein to negatively regulate early gene expression was dependent on expression of the E1A proteins. Expression of either E1A 12S or 13S gene products was sufficient for E1B 19K protein mediated negative regulation, where expression of neither 12S or 13S E1A gene products eliminated the requirement for E1B 19K gene function. We therefore conclude that a function of the E1B 19K tumor antigen is to down-regulate the levels of E1A proteins during adenovirus infection. The absence of this important regulatory phenomonon disturbs the temporal regulation of transcription during productive infection and impares the ability of adenovirus to transform rodent cells. Possible mechanisms by which the E1B 19K protein regulates gene expression will be discussed.

L 649 REGULATORY DOMAINS ADJACENT TO THE HUMAN C-MYC GENE, N.M. Wilkie, B. Whitelaw and J. Lang, Beatson Institute for Cancer Research, Glasgow G1, Scotland, and J. Katonaga and R.Tjian, Dept. of Biochemistry, U.C. Berkeley, Cal.

Previous studies have identified several regions in the human c-myc 5' flanking DNA which act as cis-acting positive (PRE) or negative (NRE) regulatory elements for the c-myc and other heterologous promoters. These regions correlate with previously identified in vivo DNAse hypersensitive sites I, IIa and IIb (Siebenlist et al, Cell 37; 381, 1984). We have shown that one such negative -acting region (NRE2, Whitelaw et al, 1986, Symposium on Growth Factors, Tumour Promoters and Cancer Genes, in press), contains binding sites for the specific DNA-binding proteins SP1 and CTF/NF-1. The role of these proteins in modulating negative regulation has been studied by site directed mutagenesis of the binding sites combined by DNA footprinting analysis and functional analysis using DNA-mediated gene transfer.

L 650 TRANSCRIPTIONAL CONTROL OF HUMAN C-MYC PROTO-ONCOGENE BY ANTI-SENSE RNA PLASMIDS. Kazushige Yokoyama. RIKEN(The Institute of Physical and Chemical Research) Koyadai-3-1-1,Yatabe,Tsukuba,Ibaragi 305,JAPAN.

Anti-sense human c-myc DNA with E.coli xanthine-guanine phosphoribosyl transferase(Ecogpt) gene was introdused into HL60 human promyelocytic leukemia cell line by the protoplast fusion at a transformation frequency of $1x10^{-7}$.High level expression of anti-sense RNA was obtained by selection of cells resistant to progressively higher level of mycophenolic acid over a period more than 6 months.Examination of genomic DNA isolated from the anti-sense clones showed that the progressive effect of generating higher levels of Ecogpt was gene-amplification of anti-sense DNA genome which resulted in approximately 100 to 250 copies.The stable anti-sense c-myc clones showed a 15 to 25 fold greater number of anti-sense transcripts than the endogenous c-myc mRNA. The slow-est growth rate of anti-sense clones is demonstrated only in HL60 cells transfected by anti-sense c-myc gene(Growth rate:95-140 h/cell cycle vs 30-36 h/cell cycle).
Cytochemical studies for α-naphtyl acetate esterase,naphtol AS-D Chloroacetate estarase and the immunofluoresense staining studies by monocyte specific monoclonal antibodies(OKM-1,-5) showed the close correlation between the decrease of c-myc protein and the increasing number of HL60 with a monocytic phenotype. In vitro run-on assay and the CAT assay of c-myc promoter region demonstrated that the expression of endogenous c-myc gene by anti-sense RNA plasmid is regulated not only at translational but also at transcriptional level.
PstI-PvuII fragment of 5' leder sequence of c-myc gene (920 bp) is the transcriptional target of anti-sense RNA plasmid and this suppresion decreases the cell proliferation and trigger the cell differentiation of HL60 into the monocyte.The mechanism of this anti-sense RNA induced suppression is studied in progress.

Late Additions

L 651 THE HA-RAS ONCOGENE DIRECTED BY A MILK PROTEIN GENE PROMOTER: EXPRESSION AND TUMOR INDUCTION IN TRANSGENIC MICE: A. Andres, C. Schoenenberger, E. Lucassen, M. LeMeur*, P. Gerlinger* and B. Groner. Ludwig Institute for Cancer Research, Inselspital, 3010 Bern, Switzerland, *CNRS, 11, rue Humann, 67085 Strasbourg, France

Five transgenic mouse lines have been established which stably acquired the activated human Ha-ras gene subjected to the control of the murine Whey Acidic Protein (WAP) gene promoter. In two lines the expression pattern of the transgene was comparable to the endogenous WAP gene. In a third line (line 69) the transgene integrated into the Y chromosome. In this line we find constitutive WAP-ras expression in the parotid gland. The incidence of tumor formation is different for the mammary gland and the parotid gland. Whereas only one female developed a mammary tumor at the age of 10 months, all males of line 69 developed adenocarcinomas of the parotid gland between the ages of 7 and 10 months. There is a greater abundance of WAP-ras RNA in the tumors than in the corresponding normal tissue of the same individual. In non-transgenic control animals no tumors are observed. Currently the expression of the transgene in normal tissue and tumors is compared by in situ hybridization and run-on experiments. In addition we intend to increase the tumor incidence in the female lines either by maintenance of lactogenic stimulation or by introduction of a second oncogene, the mouse c-myc gene subjected to the WAP promoter, into their germline.

L 652 EXPRESSION STUDIES OF THREE NEUROPEPTIDE HORMONE GENES, O. Andrisani, C. Minth, R. Haun, and J. Dixon, Department of Biochemisty, Purdue University, West Lafayette, IN 47907. To understand the expression and regulation of the neuropeptide hormone genes somatostatin, NPY and CCK, we have utilized the approach of cloning the promoter region of these genes 5′ to the structural gene encoding the bacterial chloramphenicol acetyl transferase. 5′ deletion of the promoter regions of these hybrid genes were constructed in order to define the segments involved in the transcriptional activity of these promoters. The activity of these deletion constructs was examined by introducting the plasmids into cell lines of neuronal and non-neuronal origin. In the case of the rat somatostatin promoter, the deletion analysis revealed that only sequences 60 bp 5′ to the transcriptional start sites are necessary for the expression of the reporter CAT gene. A deletion terminating at position -43, results in loss of the transcriptional activity of the promoter. The somatostatin-CAT constructs are selectively expressed when introduced in the neuronal cell line CA-77, a somatostatin producing cell lines, but not in Hela, BSC40 or CHO cells. The somatostatin promoter fragment containing the segments between -60 and -43 was utilized in band shift assays, using cell extracts from CA-77 cells and HeLa cells. Based on these assays, we have identified factor(s) which specifically recognized the somatostatin promoter fragment. Similar analyses were performed on the 5′ sequences of the human NPY gene. The NPY-CAT fusions were expressed in the neuronal PC-12 and CA-77 cell lines. However, this promoter is not utilized in HeLa, BSC40, or CHO cells. Transient assays performed using the rat CCK promoter show a high level of expression in COS, HeLa, and WE cell lines. However, primer extension analysis indicates that these cell lines are initiating transcription at a site 5′ to the expected site of initiation.

L 653 CARDIAC C-MYC mRNA LEVELS MARKEDLY INCREASE AFTER INSULIN ADMINISTRATION TO DIABETIC RATS, Enrico Cagliero, Alice Barrieux and Wolfgang H. Dillmann, University of California, San Diego CA 92103.
Adult cardiac myocytes are nondividing cells whose size,RNA/DNA and protein/DNA ratios are decreased in diabetes;these abnormalities are reverted by insulin. The proto-oncogene c-myc is primarily involved in cell growth but was also shown to influence gene expression. In order to determine if the stimulatory effect of insulin on RNA transcription and protein synthesis in diabetic hearts is accompanied by increases in c-myc levels,we administered 2 U of regular insulin/100 g body weight i.v. to streptozotocin diabetic and control rats at time 0. Controls also received glucose 500 mg i.v. and 1.5 g i.p. to avoid hypoglycemia. At 10,30,60,180 and 300 minutes the animals were sacrificed,the hearts excised and total RNA extracted with the guanidine thiocyanate method. Twenty µg of RNA were hybridized to an excess of ^{32}P labelled RNA probe complementary to human c-myc exon 2 in 80% formamide at 45°C for 12 hours. Single stranded RNA was digested with RNAase T_1 (4 µg/ml) and RNAase A (80 µg/ml) and the hybrids run on a denaturing gel and visualized by autoradiography. Control and diabetic hearts showed very low baseline levels of c-myc. Insulin rapidly increased c-myc mRNA levels in diabetic hearts (10 fold increment at 30',20 fold at 60' and 22 fold at 300') but did not induce a significant change in control hearts. In conclusion c-myc mRNA is rapidly responsive to insulin,showing a marked increase in diabetic hearts. Since myocytes, which comprise 75% of heart mass do not proliferate,these data suggest a role for c-myc in mediating the insulin stimulatory effect on protein synthesis independent of cell growth.

L 654 **CHARACTERIZATION OF THE INTERACTION OF A HEPATOCYTE-SPECIFIC TRANSCRIPTIONAL FACTOR WITH THE PROMOTER REGION OF THE GENE FOR THE BETA CHAIN OF FIBRINOGEN.** Gilles Courtois, John G. Morgan, Genevieve Fourel and Gerald R. Crabtree. Stanford University Medical School, Stanford, CA 94305.

We have previously reported that deletion of sequences between 117 and 78 bp 5' to the transcription initiation site of the gene for the beta chain of fibrinogen results in a 25-fold decrease in the transcriptional efficiency of the beta fibrinogen promoter. Using DNAse I footprinting, we find that a protein present in a NaCl nuclear extract of the rat hepatocyte cell line FAZA binds to this essential region and produces a strong footprint over sequences between 101 and 82 bp upstream of the promoter. A weaker footprint is seen directly adjacent to this site and covers an additional 8 bp 3' to the major footprint. A 43 bp restriction fragment which includes this sequence is specifically retarded on low ionic strength gels after incubation with nuclear extracts from either rat liver, the human hepatocyte cell line HepG2, or the rat FAZA hepatocyte line. However, nuclear extracts from HeLa cells, L cells, the myoblast cell line C_2C_{12}, Jurkat human T cells, or TEPC 3660 mouse B cells produce no detactable retardation of the end-labeled restriction fragment. These same extracts, however, did contain SP1, the CAAT-binding protein, and the adenovirus major late promoter transcriptional factor (MLTF). This hepatocyte-specific nuclear factor has a sequence selectivity which is unlike any previously reported transcriptional factor in that while it competes with itself at equal molar ratios, binding of the factor is not competed by a 100-fold molar excess of the CAAT-binding site from the alpha globin gene, the adenovirus late major promoter, the SV40 enhancer or the SV40 21 bp repeats. Judged by band intensity on polyacrylamide gels, the factor is present at a concentration in liver cell nuclei similar to MLTF or SP1 and thus it should be possible to characterize this factor by direct biochemical approaches.

L 655 ACTIN AND MYOSIN GENES IN THE MOUSE. P. Daubas, S. Alonso, P. Barton, I. Garner, B. Robert and M. Buckingham, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris CEDEX 15 France. We have isolated a number of muscle type actin and myosin genes in the mouse. Examination of the promoter regions indicates that some of these genes have a sequence homologous to the E1A enhancer core sequence, with an adjacent sequence which is conserved between some muscle genes. In order to test the importance of the 5' upstream sequences for developmental and tissue-specific expression of these genes, transient expression experiments, using different muscle promoters fused to the CAT gene, are in progress. The cardiac actin gene, expressed in adult heart, is co-expressed in foetal muscle with the skeletal actin gene. In BALB/c mice, there is a direct duplication of the 5' end of the cardiac actin gene including the promoter and the first 3 coding exons. Both promoters are active in adult cardiac tissue but the level of mature cardiac actin mRNA is low. In this situation, transcripts of the skeletal actin gene accumulate to abnormally high levels in the hearts of such mutant mice. This suggests tight regulatory coupling for this actin gene pair.

L 656 ON THE MECHANISM OF PROMOTER SELECTION IN MAMMALIAN MITOCHONDRIA, Robert P. Fisher and David A. Clayton, Stanford University School of Medicine, Stanford CA 94305.

Mitochondrial transcription factor (mtTF), isolated from mitochondria of human KB cells, activates transcription from either promoter (HSP or LSP) of human mtDNA through sequence-specific binding to upstream regulatory elements. The factor has been purified, apparently nearly to homogeneity, by conventional ion exchange chromatography. Its purity, based on an electrophoretic mobility-shift assay of DNA-binding, is greater than 50%, while SDS-PAGE reveals a single major polypetide of approximate M_r 25 kd upon silver staining. This polypeptide, moreover, copurifies with activity-- measured by transcriptional stimulation, DNase footprinting, and DNA fragment retardation-- through several purification steps. The factor sediments between 2S and 3S in glycerol gradients, consistent with its being a 25 kd monomeric protein. Although a single common factor appears to specify both HSP- and LSP-selective transcription, sequence comparisons reveal that the two mtTF-responsive elements are in opposite orientations with respect to the direction of major transcription at the two promoters. In addition, mtTF-LSP complexes dissociate slowly in vitro, whereas mtTF-HSP complexes dissociate nearly as rapidly as complexes formed by mtTF and nonpromoter DNA fragments.

L 657 IDENTIFICATION OF A RETROVIRAL PROMOTER BINDING PROTEIN IN UNINFECTED ENBRYONAL CARCINOMA CELLS. Frederic Flamant and Joseph Sorge. Scripps Clinic and Research Foundation, La Jolla, California 92037.

Transcript of stably integrated retrovirus is repressed in mouse embryonal carcinoma cells. In the same way, transient expression of transfected genes is inhibited in these cells when their transcription is controlled by a retroviral large terminal repeat promoter (LTR promoter).

When an LTR enhancer is replaced by a mutant polyoma virus enhancer, transient expression is restored but viral growth is not. This suggests the existence of two levels of inhibition of retroviral expression in embryonal cells (Linney *et al.*, 1984).

In an attempt to identify putative repressor proteins in F9 and PCC4 embryonal cells, we have studied DNA binding proteins in a high salt crude nuclear protein extract. Using a gel retention assay, we have been able to detect different proteins binding to the LTR of the amphotropic 4070A retrovirus.

We focused our studies on the most abundant of these proteins which we did not find in 3T3 and 3T6 fibroblast nuclear extracts.

The DNA binding site has been precisely mapped between nucleotides -150 and -180 (before cap site). This region has no previously described function and does not show homology with known protein binding sites.

L 658 COMPETITION BETWEEN VIRAL ENHANCERS IN MOUSE L CELLS INDICATES THE PRESENCE OF DIFFERENT FACTORS TRANSREGULATING ENHANCEMENT OF TRANSCRIPTION, Antonia Martin Gallardo, Francois Pasleau, Fred C. Leung, Bruce Kelder and John Kopchick. Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065

We have examined the degree of competition of various enhancer/promoters in mouse L cells, using bovine growth hormone as test gene. The enhancer/promoter were those derived from the cytomegalovirus immediate early gene (CMV IE), the Rous sarcoma virus (RSV), and the Moloney-murine leukemia virus (MLV) LTRs. The CMV IE and the MLV LTR enhancers competed with the enhancer from RSV, but they did not compete with each other. Both CMV IE and MLV LTR enhancer/promoters directed high levels of bGH expression in mouse L cells. One class bound to the regulatory DNA region from the CMV IE gene, and the other class bound to the MLV LTR. The involvement of these binding factors in promoter strength will be discussed.

L 659 SELF-SPLICING OF THE TERMINAL INTRON OF THE YEAST CYTOCHROME *b* GENE. Alexandra Gampel and Alexander Tzagoloff, Columbia University, New York NY 10027.

A region of the yeast mitochondrial cytochrome *b* gene encompassing the entire terminal intron plus flanking exonic sequences has been cloned into an SP6 vector. The corresponding SP6 transcript as well as the native cytochrome *b* pre-mRNA containing the terminal intervening sequence were found to act as substrates for the autocatalytic excision of the intervening sequence *in vitro*. This reaction proceeds under conditions previously shown by Cech and coworkers to be suitable for protein-independent excision of the *Tetrahymena* rRNA intervening sequence. The same region of the cytochrome *b* gene from a yeast mutant, defective in splicing due to a mutation in a critical sequence inside the terminal intron, has also been cloned into an SP6 vector. The corresponding mutant transcript fails to self-splice in the *in vitro* assay. These observations provide strong presumptive evidence that *in vivo* processing of the terminal intervening sequence of the cytochrome *b* pre-mRNA occurs by an autocatalytic mechanism analagous to that shown for other class I introns. *In vivo* processing of the terminal intervening sequence, however, exhibits complete dependence on a protein factor previously shown to be encoded by the nuclear gene CBP2. We are currently studying the role of the CBP2 protein in the *in vivo* splicing reaction.

L 660 REGULATION OF PROTO-ONCOGENE EXPRESSION IN LPS-TREATED MURINE MACROPHAGES, Thomas A. Hamilton, Martino Introna, Charles S. Tannenbaum, Robert J. Bast, Jr., and Dolph O. Adams, Duke University Medical Center, Durham, NC 27710.
mRNAs encoding the PDGF inducible competence genes c-myc, c-fos, JE and KC are induced in macrophages following treatment with trace amounts of lipopolysaccharide (LPS). The r-fos gene was not detectably induced by LPS under the experimental conditions employed in this study. The induction of all four genes was dependent upon the dose of LPS but they exhibited different time dependencies. All mRNAs were induced within 30 min from the initiation of treatment. While JE mRNA continued to accumulate for up to 12 hrs, c-myc, c-fos and KC mRNAs were only transiently expressed. c-fos, JE and KC were all inducible by a variety of structurally diverse but functionally similar agents (e.g., heat killed Listeria monocytogenes, maleyl-BSA, and fucoidan). Interferon-gamma, a potent but functionally distinct stimulus of macrophage activation did not effect their expression. The expression of c-fos and JE mRNA could be induced by treatment of macrophages with phorbol myristate acetate and/or the Ca^{++} ionophore A23187 while mRNA for KC was largely unaffected by these agents. These results suggest the possibility that expression of the c-fos and JE genes are regulated by products of polyphosphoinositide hydrolysis. The mechanism controlling KC expression remains unknown. Taken together the data suggest that LPS stimulates at least two independent routes of early gene expression which may be related to the acquisition of enhanced function.

L 661 FACTORS AFFECTING GENE EXPRESSION IN RETROVIRAL VECTORS
[~]D.J.Jolly, [+]J.-K.Yee, [+]J.C.Moores, [α]A.D.Miller & [+]T.Friedmann, [~] Inserm U33, Bicêtre 94275, France; [+]UCSD, CA 92093; [α]Hutchinson Cancer Ctr. WA 98104. We have previously shown that the HPRT gene introduced as a retroviral vector into HPRT[-] Epstein-Barr transformed human lymphoblasts gives levels of between 5 - 23% wild type HPRT activity (Willis et al. J.Biol.Chem. 259, 7842). Since the Moloney murine leukemia virus LTR, which drives the HPRT gene transcription, is normally thought of as a powerful promotor, we have asked what factors reliably increase gene expression, as measured by HPRT expression. This was assayed by constructing various modifications of the original vector, transiently transfecting various packaging lines and assaying titres on fibroblasts and lymphoblasts. HPRT levels were then determined for the bulk infectants descended from the various constructs. In the lymphoblasts this value is very steady and the HPRT activity is easily and quantitatively measured using cytosols.
The largest reproducible changes in activity (2 - 3 fold) were seen with a) deletion of a poly G.polyC stretch, b) addition of a 6 amino acid tail at tha C terminus. These modifications presumably affect the rate of RNA chain elongation and protein stability respectively. This suggests that significant increases in activity can be seen in such systems by manipulating factors other than promotors and enhancers.

L 662 REGULATION OF TWO DISTINCT GLUTAMATE PERMEASES, K. E. Kempsell and I.R. Booth, Department of Genetics and Microbiology, University of Aberdeen, Marischal College, Broad Street, Aberdeen
Growth of E.coli on glutamate requires a mutation at either the gltS or the gltR locus which results in enhanced levels of glutamate transport. Evidence will be presented that these two loci affect separate transport systems. The regulation of the gltS locus has been investigated using lacZ fusions and evidence will be presented that the locus is under positive control. The effect of the rpoA(phs) locus which cause a deficiency in growth on glutamate on the activity of the two glutamate transport systems will reported.

L 663 TRANSIENT CHANGES IN THE PROTEIN PATTERN CAUSED BY THE ACTIVATED RAS GENE PRODUCT
R. Klemenz, Ludwig Institute for Cancer Research, Inselspital, 3010 Bern, Switzerland

Expression of the activated human H-ras gene leads to cell transformation. Some biochemical changes, provoked by the activated ras gene product (p21), are of a transient nature and no longer detectable in fully transformed cells. To study such effects we employed cell lines containing the activated and normal ras genes fused to the inducible promotors derived from MMTV or the hsp 70 gene. Following the induction of ras gene transcription we investigated the alteration in the patterns of newly synthesized proteins using 2D gel analysis. Expression of both, the normal and the mutated p21, results in numerous changes. In many cases the mutated p21 has a considerably stronger effect despite its much lower accumulation. Some changes are soley attributable to the mutated p21. Most changes are of a transient nature. Enhanced synthesis predominates while suppressed expression is rare.

L 664 REGULATION OF GENE EXPRESSION IN MACROPHAGE BY TWO IMMUNOMODULATORS, INTERFERON-γ AND LIPOPOLYSACCHARIDE, T.J. Koerner, Thomas A. Hamilton, and Dolph O. Adams, Duke University Medical Center, Durham, NC 27710.
Interferon-(IFNγ) and lipopolysaccharide (LPS) are known to be potent immunoregulatory signals. These agents are both necessary and sufficient to activate macrophages to a tumoricidal capability while they have antagonistic effects on the induction of macrophages antigen-presentation capabilities. Accumulation of I-A mRNA is caused by treatment with IFNγ. The response is seen to begin within a few hours and to continue for 24 h. Co-treatment of macrophages with IFN and LPS results in suppressed levels of I-A mRNA. This suppression is blocked by cycloheximide. The expression of tumor necrosis factor (TNF), also known as cachetin, is induced by treating macrophages with LPS. This is correlated to induction of TNF mRNA by LPS. This accumulation of TNF mRNA is augmented by simultaneous or previous exposure to IFNγ. The mechanisms of IFN potentiating the TNF response to LPS and LPS suppressing the IFNγ - induced I-A expression are being investigated.

L 665 EVIDENCE THAT PAPILLOMAVIRUSES MAY ENCODE A TRANSCRIPTIONAL REPRESSOR,
Paul F. Lambert, Barbara A. Spalholz and Peter M. Howley, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, Maryland 20892
We have obtained evidence that BPV-1 encodes a negative transcriptional regulatory function. This finding is based upon the functional analysis of a BPV-1 cDNA. Cotransfection of this cDNA with wild type BPV-1 DNA results in the marked inhibition of BPV-1 induced transformation of mouse C127 cells. The cDNA does not inhibit transformation, however, if the BPV-1 transforming region is placed under heterologous promoter control, indicating that the inhibition is manifested through an intact BPV-1 regulatory region. This cDNA was also found to repress BPV-1 E2 transactivation of the BPV Long Control Region (LCR) enhancer in pA1ØCAT. The cDNA, however, does not repress expression of SV4Ø enhancer dependent transcription in pSV2CAT. We have mapped the region within the LCR required for repression to coincide with the minimal E2 conditional enhancer. These results suggest that this cDNA encodes a negative transcriptional regulatory factor specific to the BPV-1 transcriptional control region. Based upon genetic studies of the cDNA, we preliminarily map the repressor function to the carboxyl portion of the E2 ORF. At this time we are investigating the mechanism of repression and the role of the repressor function in BPV-1 transformed cells.

L 666 EXPRESSION OF HUMAN SARCOMA PHENOTYPE IN NIH/3T3 AND HUMAN FIBROBLAST CELLS FOLLOWING TRANSFECTION OF HUMAN SARCOMA DNA, Teresa A. Lehman, H. Lalitha Kumari, Charles F. Shuller, Soldano Ferrone* and George E. Milo, The Ohio State University, Columbus, OH 43210 and *New York Medical College, Valhalla, NY 10595.
DNA was isolated from human leiomyosarcoma cells and transfected into both NIH/3T3 cells and human neonatal fibroblasts. Transfected NIH/3T3 cells formed morphologically transformed foci in 3 weeks. Cells from these foci were isolated, repopulated *in vitro* and injected into nude mice to assay tumorigenicity. The cells induced progressively growing fibrosarcomas in mice but did not react with a monoclonal antibody (MoAb) 345.134S which recognizes a cell surface antigen associated with sarcoma cells. Transfected human fibroblasts formed morphologically transformed foci and expressed anchorage independent growth in soft agar. Colonies isolated from soft agar and repopulated *in vitro* induced fibrosarcomas in nude mice, and were positive for MoAb 345.134S. Hybridization of leiomyosarcoma DNA with ras oncogene probes revealed a 4-fold amplification of the N-ras oncogene. Although human repetitive sequences were present in transfected NIH/3T3 cell DNA, no unique DNA fragments were detected in this DNA when hybridized to N-ras, K-ras and H-ras probes as compared to untransfected NIH/3T3 cell DNA. This work was supported in part by NIH-NCI CA 25907-04 (GEM) and NIH-NCI T32 CA 09498-02 (TAL).

L 667 CONTROL OF YEAST GAL1-10 EXPRESSION BY ITS REGULATORY PROTEINS: A NUCLEAR FOOTPRINTING STUDY, D. Lohr (Arizona State, Tempe, AZ 85287), T. Torchia and J. Hopper (Penn State, Hershey, PA 17033).

The positive, essential regulatory protein GAL4 binds to the major GAL1-10 regulatory DNA sequence, the Upstream Activator Sequence (UAS) to promote expression of the genes in wild type cells grown in galactose. We find that GAL4 also binds to the UAS under conditions in which the genes are not expressed (glycerol/ethanol grown cells) or not even inducible (80^s, super repressor cells). Thus, GAL4 binding is necessary but not sufficient for gene expression.

The negative regulatory protein GAL80 plays a role in control steps subsequent to GAL4 binding. In wild type cells, it is involved in the protection of the TATA box regions 5' of both GAL1 and 10 and of a region very close to the UAS, 5' of GAL1. The former protection occurs whether or not the genes are expressed, while the latter protection is expression dependent. These DNA/protein interactions play some role in controlling GAL expression, but comparison of gene expression levels to the structural changes and some indirect footprint data suggest that protein-protein interactions between GAL4 and GAL80 also play a major role in control.

L 668 HOST CELL POLYPEPTIDES SYNTHESISED INCREASINGLY AFTER HSV INFECTION ARE ALSO SYNTHESISED IN INCREASED AMOUNTS IN TRANSFORMED CELLS, J.C.M. Macnab, R.E.P. Hewitt, A Orr and N B LaThangue* MRC Institute of Virology, University of Glasgow, Church Street, Glasgow G11 5JR. *National Institute for Medical Research, Mill Hill, London.
Transformation by HSV results in oncogenic cells in which the retention of HSV DNA coding for a transforming protein is not detected (Cameron *et al*, 1985). However, infection with HSV induces the accumulation of cellular polypeptides whose induction is a prominent feature of cells which have been immortalised or transformed by several viruses eg adeno and RSV (Macnab *et al*, 1985). Further characterisation of these polypeptides which are induced to accumulate upon HSV infection shows that they are similar to those which stimulate immune responses in the sera of tumour bearing animals. Peptide mapping has shown that although hsp90 can be increased on HSV infection and in transformation, its presence is distinct from other polypeptides of this molecular weight which are also identified. Using a monoclonal antibody generated by Dr. N.B. LaThangue against cellular polypeptides which are induced on HSV infection. We also identify cellular polypeptides of MWs 90,000, 40,000 and 35,000.

The analysis of clones isolated from our cDNA libraries will help us to characterise the genes involved. These studies will be discussed within the concept of a role for HSV in oncogenesis.

L 669 DOMINANT REGULATORS OF THE DROSOPHILA WHITE LOCUS, Leonard Rabinow and James Birchler, Harvard Biological Laboratories, 16 Divinity Ave., Cambridge MA. 02138. Autosomal second-site mutations altering expression of the Drosophila white locus have been isolated in hybrid dysgenic screens. One of these, Inr-a, increases pigment levels of wild-type white alleles, as well as those with lesions in the structural gene. white alleles with defects 5' to the presumed structural region block its action. Inr-a effects all adult tissues in which white is expressed, i.e. the eyes, testes, and malpighian tubules. The Inr-a mutant is homozygous lethal, and two EMS induced alleles complement neither the lethality nor the eye-color phenotypes of the original mutation. Over 20 kb. of genomic sequences from 48E6-7 on the polytene chromosomes have been cloned by P-element tagging. A revertant of Inr-a lacks an insertion found in the original allele, confirming that the appropriate sequences have been cloned. Polyadenylated transcripts expressed throughout development have been detected from both strands of the cloned region.

L 670 EARLY TRANSCRIPTION BY PARTIALLY PURIFIED ENZYMES FROM VACCINIA VIRIONS: ISOLATION OF A TRANS-ACTING TRANSCRIPTION TERMINATION FACTOR. Stewart Shuman, Steven Broyles and Bernard Moss, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892. An RNA polymerase fraction which transcribes vaccinia virus early genes has been partially purified from virus cores by deoxycholate extraction, removal of endogenous DNA, and chromatography on DEAE-cellulose. Accurately initiated and terminated RNAs are synthesized by this enzyme in the presence of a linear duplex DNA template. A molar excess of RNA product to template DNA is observed. Transcription is abolished in the presence of 2 μg/ml heparin.

Glycerol gradient sedimentation resolves the transcription system into two components: (I) a rapidly sedimenting DNA-dependent RNA polymerase fraction capable of initiation at the early promoter with elongation of RNA beyond the site of in vivo termination to yield a runoff transcript, and (II) a more slowly sedimenting fraction, itself devoid of RNA polymerase, which restores efficient termination when added back to fraction (I). Termination by this trans-acting factor does not occur via endonucleolytic processing of RNA. Termination factor is heat-labile and resistant to N-ethyl maleimide. Termination by the factor requires specific sequence information upstream of the site of termination. RNA transcripts synthesized by the reconstituted system do not remain associated with the DNA template.

L 671 REGULATION OF THE C-MYC ONCOGENE DURING DIFFERENTIATION OF HL60 CELLS, Ulrich Siebenlist, Peter Braasler, Kathleen Kelly National Institutes of Health, NIAID, Bethesda, Md. 20892

We have studied the downregulation of the c-myc gene during differentiation of HL60 with DMSO. We can distinguish at least two modes of regulation, one in which transcription is initiated but no complete transcript is made and one in which there appears to be a downmodulation of transcription initiation. Early on during differentiation, transcription through the first exon of myc remains at high levels as judged by nuclear run - on experiments. On the other hand, no transcriptional activity can be measured through the third exon within the nucleus and consequently no message appears in the cytoplasm. This state is rapidly reversible: upon withdrawal of DMSO predifferentiation levels of cytoplasmic mature myc message are observed. Later during the course of differentiation transcription through the first exon falls off dramatically and is not rapidly reversible. Interestingly, this coincides with major changes within the chromatin structure of the regulatory region of this gene. Several DNAase I hypersensitive sites disappear. This new chromatin structure resembels that seen on the transcriptionally silent myc allele in Burkitt's lymphomas. The implications of this data for the role of c-myc in tumor formation will be discussed.

L 672 THE REPRESSOR GENE OF STREPTOMYCES PHAGE ϕC31

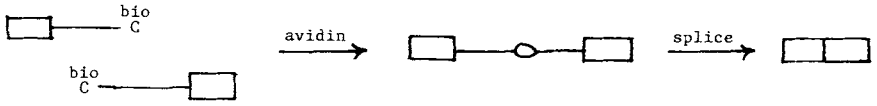
R.B.Sinclair and M.J.Bibb, John Innes Institute,Norwich,UK.

The nucleotide sequence of 3.4kb of DNA containing the c (repressor) gene of the Streptomyces phage ϕc31 has been determined. Sequence changes in c gene point and deletion mutants and computer analysis have identified the relevant open reading frame. The deduced protein product of this gene contains a strongly predicted DNA binding motif. In vitro and in vivo studies have been used to demonstrate the transcriptional start site and in vitro studies have confirmed the size of the protein to be c.74,000 as predicted from the analysis of the DNA and inferred protein sequence.

L 673 INTRON TRACKING IS NOT REQUIRED TO BRING PRE-mRNA SPLICE SITES TOGETHER

David Solnick, Sloan-Kettering Institute, New York, N.Y. 10021

Several mechanisms have been proposed to explain how the pre-mRNA splicing apparatus selects and juxtaposes splice sites in preparation for their cleavage and ligation. One model argues that a component of the splicing apparatus binds to a splice site and then tracks along the intron until the opposing splice site is encountered. I have tested this model by introducing protein 'roadblocks' into the intron. In the first experiment, a transcriptional template was constructed which contained just one cytosine residue,located in the middle of a 400 nt intron flanked by two small exons. The template and biotinylated CTP were used to introduce a biotin group into the intron. Bound avidin was able to block reverse transcriptase at the C residue, but did not inhibit splicing of the substrate in a cell-free system. In the second experiment (see below), I tested the ability of avidin (which has multiple sites for biotin) to mediate trans splicing between two half-substrates. Even with its intron interrupted by a protein bridge, the RNA was spliced almost as efficiently as a unimolecular control RNA. These results argue that the juxtapositioning of splice sites does not require tracking between them.



L 674 RNA EXPRESSION PATTERNS IN THE RAT L8 MYOGENIC CELL LINE

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Quantitative changes in cytoplasmic mRNA levels for several muscle specific transcripts were investigated by Northern blot analysis during in vitro differentiation of the rat L8 myogenic cell line. The myogenic time course extended from preconfluent myoblasts to 8-day myotubes and produced a wide range of temporal expression patterns for the various muscle transcripts. The transcript levels for genes such as myosin heavy chain, α-skeletal actin, α-cardiac actin, troponin T *fast*, and myosin light chain 2 *fast* rose from undetectable to near adult levels during this time course. In contrast, the β- and γ-cytoplasmic actin transcripts were down regulated to undetectable levels in the late stage myotubes. Other transcripts such as myosin light chain1 *slow* appeared only in late stage myotubes, while others such as carbonic anhydrase III, myosin light chain 3 *fast*, troponin I *fast*, troponin I *slow*, and troponin T *slow* were rare or not detectable. The regulation of these genes in L8 cells differs from that seen during human primary myoblast differentiation in two important aspects. First, there is less temporal coordination in human compared with the rat L8 cultures. Second, a number of transcripts observed in human myogenic cultures are not seen in L8 cells. These differences suggest that L8 cell differentiation may not perfectly reflect the behavior of primary myogenic cultures. In order to investigate the relationship between the steady-state cytoplasmic mRNA levels and the transcriptional activity of the various genes, nuclear run-on analysis was performed on isolated nuclei at various times during L8 myogenesis. Suprisingly, most muscle-specific genes demonstrated active transcription even at times when no cytoplamsic mRNA was detectable suggests a post transcriptional mechanism contributing to the regulation of these genes. However, considerations such as strand-specificity and localization of the transcribed regions are currently being investigated in order to address the biological significance of these observations.

L 675 PROPERTIES OF MAMMALIAN HEAT SHOCK ACTIVATOR PROTEIN, Charles Tsai, Vincenzo Zimarino and Carl Wu, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Heat shock genes from yeast to human possess one or more copies of a cis-acting heat shock control DNA element (HSE), consensus CT_GAA__TTC_AG. The strong evolutionary conservation of the HSE and the functional expression of heat shock genes in heterologous systems suggest a similar conservation in the binding domain(s) of the heat shock activator protein (originally identified and recently purified in our laboratory to homogeneity from Drosophila cells) that binds specifically to the HSE. We show that nuclear extracts of heat shocked mouse L cells and human HeLa cells possess an activity that footprints on the Drosophila HSE. The mammalian and Drosophila footprints are similar but not identical. We are currently purifying the mammalian heat shock activator protein and performing a comparative footprint analysis in order to identify conserved and divergent contact sites on the HSE.

Refs: C. Wu, Nature 309 229-234, 1984.
311 81-84, 1985
317 84-87, 1985

L 676 PURIFICATION OF A DEVELOPMENTALLY REGULATED, SEQUENCE-SPECIFIC DNA-BINDING PROTEIN FOR THE DROSOPHILA FUSHI TARAZU GENE, Hitoshi Ueda and Carl Wu, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

The Drosophila fushi tarazu (ftz) segmentation gene is expressed in a pattern of 7 stripes in embryonic blastoderm at about 2-4 hr of embryogenesis, and later in the ventral nervous system at about 5-10 hr of development. In order to study the regulatory mechanisms of spatial expression of the ftz gene we have looked for DNA-binding protein factors in nuclear extracts of 1-4 hr, 5-9 hr, and 12-24 hr Drosophila embryos by means of an exonuclease protection (footprint) assay. We have found at least 9 footprints in a region encompassing positions -900 to +390 near the transcriptional start site. One of these footprint activities, located at -223 to -243, increases significantly after 9 hr of embryogenesis, when the ftz gene becomes inactive. We have purified this factor to near homogeneity from 1 kg of Drosophila embryos using conventional and affinity chromatography, in which the ligand is the binding site synthesized and coupled to CNBr-activated Sepharose as a double strand oligonucleotide. The purified protein protects the -223 to -243 region when either exoIII or DNaseI is used as nuclease probe. Chromatin mapping experiments suggest that this binding site is occupied in vivo.

L 677 REPETITIVE INDUCTION OF SPECIFIC DNA-BINDING ACTIVITY OF DROSOPHILA HEAT SHOCK ACTIVATOR PROTEIN IN ABSENCE OF PROTEIN SYNTHESIS, Vincenzo Zimarino and Carl Wu, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20892.

Drosophila tissue culture cells stimulated by heat shock contain high levels of heat shock activator protein which binds specifically to the heat shock control DNA element. In contrast, nonshocked cells possess low basal levels of binding activity. By means of an exonuclease protection assay, we show that within 30 sec of heat shock of intact cells the sequence-specific binding activity in whole cell extracts increases detectably, reaching a plateau level by 5 min of shock. Removal of the heat stimulus returns the activity to basal levels within minutes. Known chemical inducers of heat shock genes elicit a similar pattern of specific binding activity, as does stimulation by heat or chemicals in the presence of protein synthesis inhibitors, even if the stimulus-relaxation is repeated sequentially through 5 cycles. Our results are inconsistent with models which propose proteolysis as the chief means of mediating heat shock transcriptional control. Rather, they suggest that heat shock activator pre-exists in nonshocked cells in a non high-affinity) binding form (I), which is converted upon cell stimulus to a high affinity, sequence-specific binding form (II), probably through post-translational modification. This conversion may be the critical switch that leads to transcriptional activation of heat shock genes.