DNA-PROTEIN INTERACTIONS IN TRANSCRIPTION

Organizer: Jay Gralla
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Structure of DNA-Protein Complexes

O 001 CRYSTALLOGRAPHY OF THE *trp* OPERATOR/REPRESSOR SYSTEM: MOLECULAR FLEXIBILITY IN GENETIC REGULATION, C. L. Lawson, R.-g. Zhang, Z. Otwinowski, R. Q. Marmorstein, R. W. Schevitz, B. Luisi, A. Joachimiak and P. B. Sigler. Department of Biochemistry and Molecular Biology, The University of Chicago, 920 East 58th Street, Chicago, Illinois 60637

Two crystal forms of *trp* repressor and one of the inactive unliganded aporepressor have been refined to atomic resolution. Comparison of these structures reveals that the extensive inter-subunit interface of four intertwined α-helices from each subunit forms a rigid unified domain that resists tertiary structural changes. This 'central core' is flanked symmetrically by two flexible 'reading heads' that contain a bihelical motif. Two tryptophan molecules bind symmetrically to sites each wedged between the core and reading heads and force the reading heads apart so that they can penetrate successive major grooves of B-DNA. When unliganded, the reading heads collapse *en bloc* toward the dyad, diminishing their separation to the point where they cannot complement the contours of B-DNA. Comparison of the two repressor crystal structures showed an unexpected degree of flexibility within the reading heads, i.e., the binding of the corepressor ligand does *not* create a unique and rigid complementary surface for the operator, but rather appears designed to allow repressor to adjust to the variable helical parameters and molecular contours in its 'one dimensional' search for the operator site.

The functional consequences of L-tryptophan binding have been further clarified by the refined crystal structure of the pseudorepressor, an adduct formed by competing desamino analogs of L-tryptophan (e.g., indole propanoic acid) that produce a structure that is nearly isomorphous to repressor but cannot bind DNA. Progress in the high resolution structure determination of a crystalline *trp* repressor/operator complex will be discussed.

Zhang, R.-g., Joachimiak, A., Lawson, C. L., Schevitz, R. W., Otwinowski, Z. and Sigler, P. B. (1987) *Nature* **327**, 591.

Lawson, C. L., Zhang, R.-g., Schevitz, R. W., Otwinowski, Z., Joachimiak, A. and Sigler, P. B. (1987). *Proteins* (in press).

O 002 STRUCTURES OF KLENOW FRAGMENT COMPLEXED WITH DNA AND γδ RESOLVASE, Thomas A. Steitz, Jonathan M. Friedman, Lorena S. Beese, Paul S. Freemont, Mark R. Sanderson, Graham Hatfull and Nigel F. Grindley, Howard Hughes Medical Institute at Yale University, New Haven, CT 06511.

High resolution crystal structures of E. coli DNA polymerase I large fragment (Klenow) co-crystallized with duplex DNA or complexed with single stranded DNA show four nucleotides of single stranded DNA bound to the 3'-5' exonuclease active site. The 3' terminal nucleotide of DNA binds nearly identically as dNMP binds. Two divalent metal ions (which can be Mg^{++}, Mn^{++} or Zn^{++}) interact with the 5' phosphate of bound dNMP and are 4.3 Å apart. These metal ions are in a position to interact with the DNA phosphodiester to be cleaved and are proposed to catalyze the exonuclease reaction by a mechanism that may be related to mechanisms of other enzymes that catalyze phosphoryl transfer, including RNA enzymes. The fact that a 3' terminal mismatched base destabilizes duplex DNA would favor its: 1. sliding from the duplex binding polymerase active site, 2. melting, and 3. binding and being excised at the single stranded DNA binding 3'-5' exonuclease active site 30 Å away. Large crystals of a Klenow fragment mutant protein lacking exonuclease activity have been grown at low ionic strength and neutral pH in the presence of DNA, a dNTP analog and Mg^{++}. These crystals are trigonal space group $P3_2$ with a = b = 112.0 Å, c = 138 Å.

The structure of the large fragment of γδ resolvase (a site specific recombinase) has been derived from a 3 Å resolution electron density map. This structure has implications for the recombination reaction.

DNA-Protein Interactions in Transcription

DNA Recognition and Flexibility

O 003 **DNA BENDING AND TRANSCRIPTION ACTIVATION BY CAP PROTEIN**, Donald M. Crothers, Marc R. Gartenberg, David C. Straney, Susan B. Straney, and Sandra S. Zinkel, Department of Chemistry, Yale University, New Haven, CT. We have studied the sequence requirements for protein-induced bending, using the *E. coli* CAP protein as a model and examining about 200 mutants of the DNA sequence to which the protein binds. We find that DNA sequence determinants spanning at least 34 bp modulate the extent of CAP-induced bending and protein binding affinity, demonstrating that a regulatory domain substantially larger than the consensus sequence determines the structure and stability of this protein-DNA complex. Least squares analysis of the data indicates that the results can be accounted for using a model that attributes bend properties to dinucleotides; models that use mononucleotides, or which consider complementary dinucleotides to be equivalent, are significantly less satisfactory. Small sequence changes that lead to less favorable bending parameters in the non-consensus region can decrease the binding affinity by as much as an order of magnitude, and decrease the angle of the induced bend by up to ~30°. In regions outside the consensus sequence, bend centers coincide with positions where DNA grooves face the protein; a quantitative ranking of all 16 dinucleotides reveals the generalization that A-T rich sequences favor bending and binding where the minor groove faces the protein, whereas G-C rich sequences do so where the major groove faces the protein. We find that Pu-Pu dinucleotides are not equivalent to their Py-Py complements, implying an appreciable tilt component in such cases. This leads to a systematic preference for Pu-Pu dinucleotides over Py-Py on the 5′ side of a bend toward the minor groove, with the reverse preference on the 3′ side.

We have studied the fate of the CAP-induced DNA bend during transcription initiation by using constructs which combine A-tract bends with the *lac* promoter in variable helical phasing. Gel electrophoresis separates closed, open and initiated polymerase complexes which also contain CAP protein. The dependence of relative gel mobility on phasing between the two bends allows us to characterize the magnitude and direction of the promoter bend in these complexes. Footprinting studies of ternary complexes resolved by electrophoresis reveals retention of the characteristic CAP footprint, and shows strong stabilization of CAP binding by the presence of polymerase in the open complex. This stabilization effect is lost when half a helical turn of DNA is inserted between polymerase and CAP, and partially recovered when the insert is a full helical turn. The primary effect of CAP on transcription initiation is to accelerate formation of the open complex, with a smaller effect on stability of the closed complex.

O 004 THE RESOLVASE-res INTERACTION: DISTORTION OF DNA BY A SITE-SPECIFIC RECOMBINASE, Nigel D.F. Grindley, Graham F. Hatfull, Joseph J. Salvo and Vichien Rimphanitchayakit, Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06510

Bacterial transposons of the Tn3 family encode a conservative site-specific recombination system that consists of the resolvase protein and its DNA substrate, the res site. The γδ res site is a 120 bp DNA segment which contains three binding sites for resolvase. Each binding site consists of inverted copies of a conserved 9 bp sequence separated by a spacer of variable length (10 bp in site I, 16 bp in site II and 7 bp in site III). Binding of the γδ resolvase to each individual binding site induces a bend in the DNA. In addition, the DNA between sites I and II in the fully complexed res site is also bent. This intersite bending requires the presence of site III and suggests that there is a protein-mediated connection between sites I and III (and/or II) which forms res into a topologically closed structure.

Recombination occurs at the center of site I. When footprinting a resolvase-site I complex with the intercalating agent MPE.Fe(II), we observed enhanced cleavage at the center of site I and proposed that the central dinucleotide (ApT) was distorted (kinked) with the minor groove opened up and exposed on the outside of the resolvase induced bend (1). To investigate the sequence requirements of this structural deformation, we have constructed site I analogues with all possible dinucleotides substituted at the center. Many mutations significantly reduce the binding of resolvase in vitro; efficient binding requires that one of the base pairs be wild-type (i.e., 5′-A or 3′-T), and neither of the 5′ positions be T. The data are not compatible with a specific pattern of resolvase-base pair contacts. Indeed, resolvase binds better to site I if bases of the central dinucleotide are removed. We suggest that the energy cost of forming the kink at the center of site I is relatively low only for certain dinucleotide combinations. Although we see normal (wild-type) enhancement of MPE.Fe(II) cleavage with all the dinucleotide mutants that bind with normal affinity, only the wild-type ApT provides an efficient recombination substrate. The basis for the recombinational deficiency of the mutants will be discussed.

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O 005 THE BENDING OF DNA IN NUCLEOSOMES AND OTHER NUCLEOPROTEIN COMPLEXES, Andrew A. Travers, William G. Turnell and Sandra C. Satchwell, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, England.

The sequence dependent conformational flexibility of DNA is a major determinant of specificity in the formation of protein-DNA complexes. This variation in conformation is reflected both in the axial flexibility, or bendability, of a DNA sequence and also in its torsional flexibility.

The precise positioning of nucleosomes on a defined DNA sequence is correlated with the preferred periodic occurrence of certain short sequence elements at particular rotational orientations (1). An additional constraint which specifies particular translation positions, is a departure from a regular periodicity in the neighbourhood of the nucleosome dyad. We conclude that nucleosome positioning is largely determined by sequence dependent variation in DNA flexibility (2). Analysis of DNA sequences associated with dinucleosomes further suggests that this same property facilitates the formation of higher order chromatin structures.

Sequence dependent variation in the external structure of DNA could allow recognition of specific DNA structures - as opposed to sequences - by proteins. We describe a protein sequence motif for DNA binding which could interact preferentially with a particular class of external features of DNA.

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Prokaryotic Activators and RNA Polymerase

O 006 HOW SIGMA RECOGNIZES DNA, Carol A. Gross, Deborah A. Siegele, James C. Hu and Deborah W. Cowing University of Wisconsin-Madison 53706.

We have systematically assayed the *in vivo* promoter recognition properties of 13 mutations in *rpoD*, the gene that encodes the σ70 subunit of *E. coli* RNA polymerase, using transcriptional fusions to 38 mutant and wild-type promoters. We found three classes of *rpoD* mutations: 1) Mutations that suggest contacts between amino acid side chains of σ70 and specific bases in the promoter, 2) Mutations that appear to affect either sequence independent contacts to promoter DNA or isomerization of the polymerase, and 3) Mutations that have little or no effect on recognition. We suggest that a sequence near the C-terminus of σ70, which is similar to the helix-turn-helix DNA binding motif of phage and bacterial DNA binding proteins, is responsible for recognition of the –35 region, while a sequence internal to σ70, in a region which is highly conserved among σ factors, recognizes the –10 region of the promoter.

O 007 **HOW SIGMA FACTORS TOUCH PROMOTERS: GENETIC EVIDENCE FOR AN AMINO ACID-BASE PAIR CONTACT SITE** Peter Zuber, David Daniels, Judy Healy, H. Luke Carter III, Simon Cutting, Charles P. Moran, Jr., and Richard Losick. Department of Cellular and Developmental Biology, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, Department of Botany and Microbiology, Oklahoma State University, Stillwater, Oklahoma 74078, Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta Georgia 30322

We present genetic evidence for a contact site between an amino acid residue in the NH_2-terminal region of high amino acid conservation among RNA polymerase sigma factors and a base pair in the "-10" region of a cognate promoter. The product of the sporulation regulatory gene *spo0H* is an RNA polymerase sigma factor called σ^H, which governs the transcription of a *B. subtilis* sporulation gene known as *spoVG*. We have found that a threoinine to isoleucine substitution at residue 100 in σ^H changes the specificity of σ^H-RNA polymerase in such a way as to decrease its capacity to use the wild-type *spoVG* promoter while increasing its capacity to use a mutant form of the promoter. The mutant promoter contains a G:C to A:T nucleotide subtitution at position -13, which is a strong down mutation for RNA polymerase containing wild-type σ^H (that is, σ^H with threonine at position 100). We interpret these observations as indicating that amino acid 100 is the site of contact with base pair -13, such that threoine contacts the G:C base pair of the wild-type promoter and isoleucine contacts the A:T base pair of the mutant promoter. In support of this inference, substitution of threonine at position 100 with an amino acid residue (alanine) lacking a bulky side chain is found to cause a striking loss in the specificity of σ^H-polymerase such that promoters having either G:C or A:T at position -13 are utilized with similar efficiencies. We propose that sigma factors contact the -10 region of their cognate promoters by means of amino acid residues embedded within the NH_2-terminal region of high amino acid conservation.

O 008 ACTIVATORS AND THE EFFECTS OF DNA SUPERCOILING ON TRANSCRIPTION INITIATION IN E. COLI, William McClure, Michael Schwartz, James Goodrich, and Xiaoyong Li, Dept. Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA 15213.

1. The pattern of CAP*cAMP activation at the lac promoter (P1) has been investigated on a series of supercoiled templates. The templates were prepared so that the initial superhelical density ( ) varied between 0 and -0.08. Control experiments showed that the effective varied during the kinetic experiments, which were designed to evaluate the contribution of CAP*cAMP on the rates of open complex formation. When appropriate corrections were made, we found that DNA supercoiling had its largest effect on the isomerization step of open complex formation. 2. The activation of gal P1 by CAP*cAMP was found to be greater than that recently reported by Herbert et al. (1986) PNAS 83: 2807. We found that the partitioning of RNA polymerase between gal P1 and gal P2 greatly favors gal P2 in the absence of CAP*cAMP. Thus, CAP*cAMP is found to block gal P2 occupancy and to increase greatly the promoter strength of gal P1. The experiments were undertaken originally in order to assess the effect of gal repressor on transcription initiation at the gal promoters. 3. Closed complexes have been quantified at the P_{RM} promoter using several enzymatic and chemical protection protocols. The patterns of protection were evaluated under several experimental conditions including different temperatures and different salt concentrations. The overall patterns were comparable with those observed for open complexes. The most important difference was that the extent of protection at any location depended on the concentration of RNA polymerase. Moreover, a quantitative treatment showed that the binding constant determined from the extents of protection agreed well with the K_B value determined for this promoter using standard kinetic assays (TAU plots) under the same experimental conditions. Thus, in the scheme shown below, the identity of the closed complex (RPc) is supported by both functional (activity assays) and physical (protection assays) evidence.

$$R + P \overset{K_B}{\rightleftharpoons} RP_c \overset{k_2}{\rightleftharpoons} RP_o \xrightarrow{} \xrightarrow{NTPs} \cdots \rightarrow RNA$$

O 009 DNA LOOPS AND CONTACTS BY ARA-C PROTEIN, Robert Schleif, Biochemistry Department, Brandeis University, Waltham, Massachusetts 02254.
AraC protein positively and negatively regulates expression of the L-arabinose operon araBAD of Escherichia coli and negatively regulates the araC gene. DNA loops formed by AraC protein bound to two sites separated by 210 base pairs and by 120 base pairs participate in the regulation. One loop predominates in the absence of arabinose, and a different loop predominates in the presence of arabinose. Looping the DNA interferes with activity of a promoter contained within the loop. The shift from one loop form to the other depends upon the presence or absence of arabinose and the sequence of the DNA to which AraC protein is bound.

We have adapted the Maxam-Gilbert and "band shift" gel binding assay technologies to locate specific amino acid-base interactions made by DNA-binding proteins. Site-specific mutagenesis is first used to change a residue to glycine or alanine, and then the resulting missing contact, if any, in the DNA binding site is displayed by the method. This approach correctly found the contact lost by lambda phage repressor when a lysine was changed to a glycine. This approach is being applied to AraC protein to locate the regions of the protein that contact DNA and to probe for arabinose-dependent effects on its DNA contacts.

Recognition of Eukaryotic Promoter Elements

O 010 PURIFICATION AND PROPERTIES OF RNA POLYMERASE I- SPECIFIC TRANSCRIPTION FACTORS
Ingrid Grummt, Joachim Clos and Ingrid Bartsch, Institut für Biochemie, Röntgenring 11, 8700 Würzburg, FRG

We have investigated the cis-acting elements and trans-acting factors involved in initiation and termination of mouse ribosomal gene transcription.
INITIATION : The specificity of transcription initiation by pol I is brought about by the interaction of a species-specific DNA-binding protein (factor TIF-IB) with defined sequences in the core promoter (from -21 to -16).Interestingly, upstream sequences (between -150 and -112) strongly affect the binding of TIF-IB to the core.The stable binding of TIF-IB to the rDNA promoter is the first step in the assembly of transcription initiation complexes. Functional initiation complexes contain in addition to factor TIF-IB at least another essential factor (TIF-IC) and a growth - regulated activity, termed TIF-IA. Analysis of a series of deletion, linker scanning and spacing change mutants demonstrate that the spatial array of both the core and the upstream control element is crucial for both the stability of transcription initiation complexes and transcriptional efficiency. We have purified TIF-IB to apparent molecular homogeneity and show that both DNA binding and transcriptional activity reside within a ~220 kD protein which appears to bind to DNA as a dimer.

TERMINATION : Termination of mouse rDNA transcription is mediated by the interaction of a nuclear factor with a conserved sequence element AGGTCGACCAG $\begin{matrix}T\\A\end{matrix}$ NTCCG (the "Sal box") in the 3'terminal spacer.We have purified the "Sal box" binding protein and show that the 100 kD polypeptide functions as a transcription termination factor.Although the 18 bp "Sal box" is sufficient and necessary to stop pol I movement and to release the nascent RNA from the template, sequences flanking the box contribute to the accuracy and efficiency of 3'end formation.

Enhancers

O 011 CONSTITUTIVE AND INDUCIBLE TRANSCRIPTIONAL ENHANCERS, P. Chambon, LGME/CNRS and U.184/INSERM, Faculté de Médecine, 67085-Strasbourg Cédex, France.
I will discuss the work currently performed in our laboratory on the SV40 enhancer and its cognate trans-acting factors (1-4), and on the inducible enhancer factors which belong to the nuclear receptor multigene family [oestrogen receptors (5 and refs therein), progesterone receptors (6 and refs therein), retinoid receptors (7 and refs therein). The observation that yeast trans-acting transcriptional factors (eg. GAL4, GCN4) stimulate transcription in HeLa cells from several class B (II) eukaryotic promoters containing the cognate UAS (8 and refs therein) will be discussed. I will also describe the use of chimeric proteins containing DNA-binding domains and activating regions coming from either yeast or nuclear receptor trans-activators in order to characterize the multiple functional domains responsible for the inducible enhancer activity of nuclear receptors.

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O 012 TRANSACTING FACTORS THAT CONTROL LIVER SPECIFIC GENE, Marta Blumenfeld*, Silvia Cereghini*, Tanguy Chouard*, Philippe Herbomel*, Anna Mottura-Rollier**, Michel Raymondjean*, Marie-Odile Ott**, François Tronche*, Mary Weiss** and Moshe Yaniv*, Unité des Virus oncogènes* and Unité de Génétique de la Différenciation**, UA 04-1149 du CNRS, Département de Biologie Moléculaire, Institut Pasteur, 75724 Paris Cedex 15, France.
In an attempt to understand the mechanisms that govern tissue specific gene expression, we have analyzed the DNA sequences of the rat albumin gene required for liver specific expression. By deletion and linker scan analysis, we showed that at least five elements are required for maximal promoter activity in transfected hepatoma cells: distal elements I, II and III (DEI, DEII and DEIII) located between -100 and -160 relative to the cap site, the CCAAT box homology at -80 and a proximal element (PE) located at -60. Deletion of DEIII caused a 2-3 fold decrease in activity, deletion or replacement of DEII or DEI has a down effect of 7 and 20-fold, respectively. Finally, replacement by linker of the CCAAT or of the PE element reduced the activity 50-100 fold. Several of these elements function as partially tissue specific enhancer when placed upstream or downstream of the tk promoter.
By DNase I footprinting and gel retardation assays, we identified five cellular factors interacting with the cis elements defined above. NFI/CTF is interacting with alb DEII. CBP(EBP) of McKnight et al. interacts with DEI element. The CCAAT sequences are recognized by a novel CCAAT box binding factor (ACF), probably identical to factor NFy. Finally, the proximal element is recognized by a factor present only in hepatocytes. It recognizes an imperfect palindrome present in several liver specific genes. In dedifferentiated rat hepatocarcinoma cells that do not express the albumin gene, APF is replaced by a different factor that recognizes the same purine residues, perhaps being the repressor or extinguishor previously defined by somatic cell genetics.
Our studies clearly show the existence of a hierarchy in the elements composing the albumin promoter. The contribution of proximal elements (PE, CCAAT) is more important than that of distal elements. The most proximal element seems to be a major component of the tissue specificity.

Repression and Activation Mechanisms

O 013 DNA SEQUENCES INVOLVED IN EXPRESSION AND REGULATION OF DEOR, CYTR AND cAMP/CRP CONTROLLED GENES IN ESCHERICIA COLI, Poul Valentin-Hansen, Department of Molecular Biology, Odense University, Denmark.

To increase our knowledge about protein-DNA interaction we have dissected a set of promoter regions that are regulated by the same components. Specifically, we have addressed 1) how proteins present in low concentration in the cell are able to find their specific targets on the chromosome and occupy these sites efficiently, and 2) how the interplay takes place between regulatory proteins and RNA polymerase. Our model system consists of genes coding for proteins involved in up-take and catabolism of ribo- and deoxyribonucleosides. Transcriptional initiation of these genes is negatively controlled by two regulatory proteins, the DeoR-and CytR repressors. The promoter regions can be classified into three groups regulated by a) CytR or b) DeoR alone, or c) by both CytR and DeoR.Furthermore,the cAMP/CRP complex is required for activation of all the CytR controlled transcriptional units. In previous papers we have described the structure of the deo operon and the long-range interaction between DeoR regulatory signals (1 ,2 ,3). In order to gain insight into the mechanism by which the CytR repressor functions we have analyzed the deoP2, cddP and tsxP2 promoter regions. These studies have led to the following conconclusions **i**) Two CRP binding sites are located around bp -45 (CRP1) and -95 (CRP2) in the promoter regions; **ii**) the CRP2 target is not needed for high expression but is involved in CytR binding; **iii**) CytR and CRP recognize very similar or identical targets;and **iiii**) introduction of additional CRP-targets at specific locations can abolish CytR regulation in an intact promoter and can reestablish regulation in a deoP2 promoter deleted for CRP2, respectively. Our results suggest,therefore, that the repressor and the activator are rivals and that CytR antagonizes CRP activation. This new version of negative regulation, showing a deal of similarity to the cI/cro story of phage λ, might well turn out to be a general way by which all organism can controll gene expression.

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Induction Mechanisms

O 014 Signal Transduction and Transcriptional Regulation by the Glucocorticoid Receptor. Paul J. Godowski, Dennis Sakai and Keith R. Yamamoto, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0448.

The glucocorticoid receptor protein, upon interaction with ligand, binds with high affinity to specific DNA sequences. Transcription is enhanced by receptor binding at one class of glucocorticoid response elements (GREs), whereas it is repressed by the binding of the receptor to a second class of sequences termed negative GREs (nGREs). A 20bp nGRE from the prolactin gene contains, in addition to a receptor binding site, an enhancer activity conferred by binding of a nonreceptor factor. In the absence of bound receptor, the nGRE enhances transcription from linked promoters; this effect is reversed upon hormone addition. Previous studies defined discrete portions of the 795 aa rat glucocorticoid receptor required for DNA binding and hormone binding. The hormone binding domain, located at the C-terminus of the receptor, represses receptor activity prior to ligand binding; deletion of this domain results in a constitutively active protein. The DNA binding domain, located between aa 440 and aa 525, is sufficient both for transcriptional activation and for repression. These activities can be genetically uncoupled. One mutant has been isolated that remains fully competent for induction but is unable to repress transcription; a second mutant can repress transcription but is defective for enhancement, even though it retains GRE binding activity in vitro.

To determine whether additional regions of the receptor may also function directly in transcriptional regulation, we have fused portions of the receptor to the DNA binding domain of the bacterial lexA protein. Some of these fusion proteins, when expressed in mammalian cells, enhance transcription from promoters linked to lex operator sequences. Interestingly, this activity is still fully hormone-dependent, and deletion of the hormone binding domain again yields constitutive activators. These studies have identified a second transcriptional activation domain in the amino terminal portion of the receptor.

O 015 DNA-BINDING AND TRANSCRIPTIONAL ACTIVATION BY THE YEAST ACTIVATORS HAP1, HAP2, AND HAP3, Steve Hahn, Jim Olesen, Susan Forsburg, Karl Pfeifer, Lewis Chodosh and Leonard Guarente, Massachusetts Institute of Technology, Cambridge, MA 02139.

The yeast *CYC1* gene (iso-1-cytochrome C) is activated by two distinct UASs, UAS1 and UAS2. UAS1 is activated by HAP1, which also activates the *CYC7* gene (iso-2-cytochrome c) while UAS2 is activated by the combined action of HAP2 and HAP3, which activates many genes involved in electron transport. Recent experiments have provided several insights into the molecular basis of HAP1, HAP2, and HAP3 activation.

1) HAP1 activates by binding to sequences in UAS1 and the CYC7UAS. The binding sites differ in sequence and HAP1 contains overlapping but distinct DNA binding domains that mediate the recognition.

2) Heme stimulates HAP1 binding to both sites. The heme domain of HAP1 lies just carboxyl to the DNA binding domain and acts by inhibiting DNA-binding in the absence of heme.

3) HAP2 and HAP3 form a complex in solution which binds to UAS2. The UAS2 UP1 site contains a CCAAT box.

4) A factor from HeLa cells that binds to the CCAAT box of adenovirus consists of two polypeptides which are functionally interchangable with HAP2/HAP3.

O 016 DYNAMICS OF TRANSCRIPTIONAL ACTIVATION AT HEAT SHOCK LOCI, John T. Lis, Cornell University, Ithaca, NY 14853.

A gene's transit from a transcriptionally dormant to a highly active state requires a large battery of specific molecular interactions and reactions. The heat shock genes can be induced several hundred-fold within minutes of a stress stimulus. The initiation of these events is dependent on the interaction of a protein factor with short stretches of DNA sequence, the heat shock regulatory elements. We are using the heat shock response as a model in Drosophila and yeast to investigate the mechanism of gene activation and the accompanying changes in chromatin structure. Our efforts have focused on interacting experimental approaches of reverse genetics (1), photo-crosslinking (2,3), and biochemical purification. Recent results obtained using these approaches will be presented and the implications on transcription initiation and elongation, and on the establishment of a domain of active chromatin will be discussed .

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The Structure of Transcribed Chromatin

O 017 DISTRIBUTION PATTERNS OF CHROMOSOMAL PROTEINS RELATED TO GENE ACTIVITY, S.C.R. Elgin, T.J. Dietz, J.C. Eissenberg*, D.S. Gilmour, T.C. James+, E. Siegfried, G.H. Thomas, A. van Daal, C. Wagner, Washington University, St. Louis, MO 63130; *St. Louis University School of Medicine, St. Louis, MO 63104; +Weslyan University, Middletown, CT 06157

Whether or not a gene is accessible to the activation process is likely to reflect its packaging into chromatin at several different levels. Using immunofluorescent staining of the polytene chromosomes of Drosophila, in conjunction with biochemical techniques, we have identified several distinct patterns of protein-DNA interaction pertinent to the activity state. C1A9, a protein of ca. 18 kD, is preferentially associated with the heterochromatin, and may play a specific role in heterochromatin condensation. Several specific antisera, including one directed against the histone H2A variant hv1 of Tetrahymena, stain a subset of the euchromatic loci, indicating an association of the antigen with loci activated under developmental control, regardless of the transcriptional state. (A gene encoding the Drosophila H2A variant has been cloned and mapped to locus 97CD.) In contrast, antibody staining indicates that RNA polymerase and topoisomerase I are prominently associated with a gene only when it is being actively transcribed (1). Using an inhibitor, camptothecin, we have shown that high levels of topoisomerase I are found over the regions of transcription of the heat shock genes (but not in the spacers) on both strands of the DNA. This coincides with the regions of perturbation of the nucleosome pattern following heat shock gene activation (2), suggesting that topoisomerase I might play a role in "unpackaging" the chromatin fiber.

Active/inducible genes are characterized by the presence of DNase I hypersensitive sites at or near the 5′ end. In the case of the inactive but inducible Drosophila heat shock genes, the regulatory heat shock consensus sequences (HSCS's) are observed to lie within these open regions (2). High resolution mapping of hsp 26 using the indirect end-labelling technique has demonstrated that TATA-box binding protein is present prior to heat shock; following heat shock, a new footprint is detected over the two HSCS's centered at positions -60 and -343. A large region (150 bp) of protected DNA characterized by 10 bp periodic DNase I cleavage sites lies between the two HSCS's, suggesting the presence of a nucleosome. We suggest that the binding of the TATA-box factor and of a specifically positioned nucleosome generates the DNase I hypersensitive sites, leaving the HSCS's accessible; the folding of the DNA by the nucleosome will bring these sites into proximity, perhaps facilitating gene activation.

1. Fleischmann et al., 1984, Proc. Natl. Acad. Sci. USA 81, 6958-62.
2. Cartwright and Elgin, 1986, Molec. Cell Biol. 6, 779-791.

O 018 INTERACTION OF THE E. COLI HU PROTEIN WITH DNA AND WITH THE E. COLI NUCLEOID, David E. Pettijohn, Steven S. Broyles, Yvonne Hodges-Garcia, and Vern L. Shellman, Dept. of Biochemistry, Biophysics/Genetics, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

Our previous studies of the HU-DNA complex have indicated that the bound DNA is restrained in negative DNA supercoils and that the DNA in this complex is cleaved by several different nucleases at a periodicity of 8.5 bases. One interpretation of the cleavage pattern is that the DNA helix is overwound to a helical repeat of 8.5 bp per turn. In this case the combined effects of HU on DNA twist and writhe would lead to much tighter DNA supercoiling than indicated from studies of DNA linking number alone. To test this possibility current studies are examining the HU-induced compaction of defined DNA fragments (in collaboration with P. Hagerman using birefringence rotational relaxation) and are also defining in more detail the restraint of negative DNA supercoils by HU using a cruciform transition assay. The latter studies show that the transition of a lac-operon inverted repeat DNA sequence from linear to cruciform in plasmid pocel2 normally occurs at a median linking number deficit of $\Delta\sigma$ = -0.050, while it occurs at a value more negative by $\Delta\sigma$ =0.032 when HU is bound at saturation (one HU dimer per 30 bp DNA). If this change is due exclusively to the restraint of negative torsional tension by the HU, the change is consistent with the previously proposed estimates of DNA supercoiling by HU.

In further experiments we are attempting to define the distribution of HU protein in living *E. coli* cells by examining via fluorescent microscopy the distribution *in vivo* of fluorescene labeled HU or fluorescene labelled anti-HU antibodies. The labelled HU is active in supercoiling DNA *in vitro* and can be introduced into living bacterial cells permeabilized by EDTA. The distribution of the added HU inferred from the fluorescence images is primarily into the bacterial nucleoid, but in an non-uniform manner favoring selected sites within the chromosome.

O 019 TRANSCRIPTION-DEPENDENT DNA SUPERCOILING IN YEAST DNA TOPOISOMERASE MUTANTS
Steven J. Brill and Rolf Sternglanz, SUNY, Stony Brook, NY 11794

Yeast strains with mutations in the genes for DNA topoisomerases I and II have been identified previously by us and by others. The topoisomerase II mutants (top2) are conditional-lethal temperature-sensitive mutants while the topoisomerase I mutants (top1) are viable and exhibit no significant growth defects. We report here the identification of a novel DNA supercoiling activity observable in top1 mutants and top1-top2 double mutants. Plasmids carrying transcriptionally active genes are found to be extremely negatively supercoiled when isolated from these mutant strains. In contrast, the same plasmids isolated from Top^+ cells have normal superhelicity, as do transcriptionally inactive plasmids such as the 2-micron circle isolated from mutant cells. Supercoiling of the plasmid takes place in the complete absense of topoisomerase I and is apparently not dependent on topoisomerase II since it can occur at the non-permissive temperature in a top1-top2 mutant, well after the inactivation of topoisomerase II relaxation activity. In fact, it is under these conditions that the supercoiling activity is most readily seen. Whether this activity is due to an unusual form of topoisomerase II that is not heat labile in a top2-1 temperature-sensitive mutant or whether it is really due to a new enzyme has yet to be determined. Several lines of evidence suggest that the supercoiling event is not related to transcriptional activation: 1) it requires functional RNA polymerase II, 2) it is coincident with RNA accumulation, and 3) the degree of supercoiling is related to transcript length. These results suggest that this topoisomerase activity operates during transcriptional elongation. A model will be discussed which considers the role of topoisomerase I and the new topoisomerase in the movement of RNA polymerase along the DNA template.

O 020 CHROMATIN ASSEMBLY *IN VITRO*,
Akiko Shimamura, David Tremethick and Abraham Worcel, Department of Biology, University of Rochester, Rochester, New York 14627

We have scaled up the chromatin assembly reaction catalyzed by the high speed supernatant of Xenopus oocytes (oocyte S-150, Glikin, Ruberti and Worcel, Cell 37, 33-41, 1984). The new protocols, which will be described, incorporate an ATP generating system that maintains a constant ATP concentration throughout the incubation. Under these conditions, the assembly is quantitative and it can also be performed in large volumes, which greatly facilitates the analysis by eliminating the requirement for ^{32}P-tagged DNA circles (Shimamura, Jessee and Worcel, Methods in Enzymology, 1987, in press).

We have followed the changes in DNA supercoiling, transcription, and chromatin structure, on a 5S RNA gene plasmid assembled into chromatin in this *in vitro* system. Under conditions of protein excess, formation of nucleosomal chromatin correlates with repression of 5S RNA transcription. 5S RNA transcription is observed at early times of chromatin assembly, before the nascent chromatin matures into nucleosomal chromatin. At these early stages of assembly the minichromosomes display a disrupted nucleosomal structure that is sensitive to MNase. In contrast, the transcriptionally inactive, mature minichromosomes are more resistant to MNase digestion and they display conventional nucleosomes regularly spaced at 170-190 bp intervals.

The scaled up preparation of chromatin has also allowed the purification of chemical amounts of *in vitro* assembled chromatin for the analysis of the proteins that are associated with the minichromosomes. Results of such studies will also be presented.

Poster Session 1

O 100 THE INTERACTION OF 434 REPRESSOR WITH DNA, A.K.Aggarwal, D.Rodgers, M.Drottar, J.E.Anderson, M.Ptashne and S.C.Harrison, Howard Hughes Medical Institute and Harvard University, Cambridge, MA 02138.

X-ray crystallography can reveal atomic details of protein-DNA interactions. Anderson et al(1) determined the structure of a complex between the DNA binding domain of 434 repressor(R1-69) and a 14 base pair synthetic operator("14mer"). The structure confirmed proposals for the interaction of a helix-turn-helix motif with the DNA major groove and demonstrated the importance of the way protein docks with DNA backbone. Anderson et al presented a scheme for these sequence specific and non-sequence specific contacts. To obtain more accurate and more detailed structural information, two further R1-69/DNA complexes have been crystallised. One incorporates a 14mer with a permuted sequence. It shows an altered protein dimer interaction, corresponding to an extra effective twist of 24 degrees. A model based on data to 3 A is currently being refined. The other new crystals contain a 20 base DNA segment with the authentic OR1 sequence and 1-base 5' overhangs. Two forms (space groups P21 and P212121) are found. They diffract to 2.5 A. We anticipate presenting a model based on these higher resolution crystals.

1) Anderson,J.E.,Ptashne,M. and Harrison,S.C. (1987) Nature 326, 846-852

O 101 Adenovirus Infection Alters Transcription Factor MLTF-UAS DNA Interactions. R. Albin and S.J. Flint, Dept. of Molecular Biology, Princeton University, Princeton, N.J. 08544

To understand the mechanism by which the adenovirus 289R E1A proteins transactivate transcription we have examined the interactions of cellular transcription factors with DNA control elements of the Ad2 mLP. Nuclear extracts prepared from cells harvested 8-9 hrs after Ad5 infection transcribe mL RNA in vitro 7-10 fold more actively than extracts prepared from uninfected cells, and this transcriptional stimulatory activity is dependent on both the synthesis and quantity of the viral 289R E1A proteins. Efficient transcription from the mL promoter in vitro requires at least two cellular factors, factor TFIID that interacts with sequences centered at the TATA box, and MLTF that recognizes the UAS element (-66 to -50). Extracts prepared from infected cells stimulated the specific binding of factor MLTF to its target sequence. Kinetic analyses established that the stimulation of MLTF binding was due to an acceleration in the rate of formation of MLTF-UAS DNA complexes in extracts of infected cells, and that the more rapid rate of MLTF-UAS DNA complex formation was not the result of a virus-induced increase in the concentration of this factor. Moreover, chromatographic separation of the E1A proteins from MLTF showed that the accelerated rate of MLTF binding in infected cells is dependent on the presence of E1A protein in the reaction mix. These analyses suggest a mechanism in which the 289R E1A proteins stimulate mL transcription by altering the kinetic pathway of MLTF binding due to direct interaction with MLTF, UAS DNA, or both.

O 102 **SIMIAN VIRUS 40 LARGE T ANTIGEN MEDIATES TRANS-ACTIVATION INDIRECTLY THROUGH ALTERATIONS IN THE DNA BINDING CHARACTERISTICS AND BINDING STABILITY OF SIMIAN TRANSCRIPTION FACTORS.** James C. Alwine, Gregory Gallo, Gwen Gilinger and Joe Manuppello. Dept. of Micro., Univ. of Pennsylvania, Phila., PA 19104-6076.

The late promoter of simian virus 40 is transcriptionally activated, in trans, by large T antigen, the viral early gene product. Data suggest that T antigen's trans-activation function does not require direct interaction with DNA. We demonstrate that late promoter elements (omega, tau and delta), necessary for T antigen mediated trans-activation, are binding sites for simian cellular factors. Two of the late promoter elements (omega and tau) bind the same factor or family of factors. These factors bind to a site similar to the Hela factor AP1. We refer to these factors as the simian AP1 sequence recognition proteins (sAP1-SRPs). Compared to normal simian CV-1P cells, the sAP1-SRPs from T antigen producing COS cells, or from 14 hour SV40-infected CV-1P cells, show altered binding patterns to both the omega and tau binding sites. The sAP1-SRPs from T antigen containing cells bind to the tau site more stably compared to analogous CV-1P factors. Overall, the data show: 1) that the activatable late promoter elements contain binding sites for simian cellular DNA binding proteins; 2) that the presence of T antigen causes alterations in the binding characteristics of specific simian cellular DNA binding factors; 3) that factors which bind to the late promoter elements have altered and more stable binding characteristics in the presence of T antigen. These points suggest that T antigen mediates trans-activation indirectly through the alteration of binding of at least one specific simian cellular factor, sAP1-SRP, or through the induction of a family of sAP1-SRP factors.

O 103 MECHANISMS INVOLVED IN THE REGULATION OF EUKARYOTIC rDNA TRANSCRIPTION : EFFECTS OF bFGF, François Amalric, Véronique Baldin, Pascale Belenguer, Gérard Bouche, M. Caizergues-Ferrer, Hervé Prats* and Jean-Pierre Tauber*, Centre de Recherche de Biochimie et de Génétique Cellulaires du C.N.R.S. and *Unité INSERM 168, Toulouse France.

Four classes of proteins have been shown to be involved in the transcription of rDNA : RNA polymerase I, polymerase I specific transcription factors, topoisomerase I and nucleolin. To investigate the regulation process of rDNA transcription, we have developed a cell system in which a growth factor regulates cell growth, cell differenciation and rDNA transcription. Basic Fibroblast Growth Factor (bFGF) strongly controls the growth of primary culture of Adult Bovine Aortic Endothelial (ABAE) cells. We have shown that following the addition of bFGF alone, G1 resting sparse cells undergo the G1⟶S transition and the transcription of ribosomal genes is stimulated. Moreover, the addition of bFGF to nuclei isolated from these sparse resting cells increases in vitro the transcription of rDNA genes and induces the phosphorylation of a set of nuclear proteins and among them nucleolin. We have shown that a protein kinase NII that is localized in the nucleolus plays a key role in the control of rDNA transcription. In vitro, it used as substrates, nucleolin, topoisomerase I and transcription factors or/and RNA polymerase I. We postulate that bFGF could act on rRNA synthesis through the activation of a protein kinase involved in the phosphorylation of most of the factors implicated in the transcription or ribosomal genes.

To valid these in vitro results, we had to demonstrate that bFGF was present in vivo in the nucleolus of sparse ABAE cells. This was shown by immunocytochemistry using a monospecific polyclonal serum raised against bFGF or adding iodinated bFGF to the medium culture.

O 104 MULTIPLE TRANS-ACTING REGULATORS OF HO GENE EXPRESSION IN YEAST. Brenda Andrews, Warren Kruger and Ira Herskowitz. UCSF, San Francisco CA 94143-0448.

The HO gene encodes a site-specific endonuclease which cuts the mating-type locus at a defined site to initiate the process of mating-type switching. Expression of the HO gene is subject to a number of controls mediated by a 1400 basepair upstream regulatory region. HO is expressed in mothers but not in daughters (cell lineage control) and is transcribed during late G1 (cell cycle control). Transcription of the HO gene requires activators encoded by the SWI genes. In addition, a number of suppressors of swi-mutants (SIN genes) have been isolated. The SIN genes may encode repressors of HO.

We have been defining those SWI and SIN genes which have sites of action within the region of the HO promoter that is necessary for cell-cycle control (URS2 or upstream regulatory region 2). It is clear from previous work that two of the SWI activators, SWI4 and SWI6, must act via repeated sequences (consensus CACGAAAA) within URS2 to mediate cell-cycle control (Breeden and Nasmyth, Cell 48, 389). By assaying segments of the HO promoter in a plasmid assay system, we have obtained evidence that SWI1 and SIN1 are involved in negative regulation within the URS2 region. We suggest that SWI1 and SIN1 act to silence the URS2 region for most of the cell cycle. We are testing this hypothesis using probes for the various regulators in the pathway. In addition, we are combining probes from the URS2 region with extracts from mutant and wild-type strains in a gel binding assay to obtain direct evidence for the involvement of SWI and SIN regulators in interactions with the HO promoter.

O 105 FACTOR(S) RECOGNIZING THE RAT SOMATOSTATIN PROMOTER, Ourania Andrisani, David Pot, Zhu Zheng and Jack Dixon, Purdue University, Department of Biochemistry, West Lafayette, IN 47907.

We have identified three sequence specific DNA-protein complexes which are formed after *in vitro* binding to nuclear CA-77 or HeLa extracts with the -70 to -29 region of the somatostatin promoter. The binding site includes the TGACGTCA module which is also present in cAMP-responsive and E1A inducible promoters. Deletions disrupting the TGACGTCA module disrupt complex formation *in vitro* and expression *in vivo*, suggesting that one of these complexes is involved in the activation of transcription of the somatostatin gene. The protein binding activities responsible for the formation of each of the three complexes have been fractionated from rat brain whole cell extract by DEAE Sepharose chromatography.

In order to evaluate the role of each of the three complexes, we are carrying out *in vitro* transcription assays. The results of the *in vitro* transcription assays will be presented.

We are also investigating the affect of single point mutations within the TGACGTCA module. We have obtained a set of point mutations and by *in vitro* competition experiments we show that the most critical residues are located within the center of the TGACGTCA module. The *in vivo* affect of these point mutants on the expression of the somatostatin promoter will be presented.

O 106 THE JUN ONCOPROTEIN IS A SEQUENCE-SPECIFIC TRANS-ACTIVATOR SIMILAR OR IDENTICAL TO TRANSCRIPTION FACTOR AP1, Peter Angel, Elizabeth Allegretto, Steve Okino, Kazue Hattori and Michael Karin, Dept. of Pharm. M-036, UCSD, La Jolla, CA 92093

The mammalian transcription factor AP1 has been shown to bind to specific sites in the regulatory region of different genes and activate their transcription. In vivo the AP1 binding site acts as a phorbol ester inducible enhancer element (TRE) and treatment of cells with the tumor promoter TPA leads to a rapid increase in AP1 binding activity. These results place AP1 at the receiving end of a complex pathway responsible for transmitting the effects of TPA acting at the plasma membrane to the transcriptional machinery. Interestingly the yeast transcriptional activator GCN4 recognizes a sequence which is highly similar to the recognition site of AP1. Accordingly the ideal GCN4 site is an efficient competitor for binding of AP1 to the TREs of TPA inducible genes. The DNA binding domain of GCN4 shows significant homology to a region of vjun, an oncogene product located in the nucleus, which was recently isolated from avian sarcoma virus 17. We used synthetic oligodeoxynucleotides derived from the putative DNA binding region of vjun to isolate the cDNA of the human cjun. Footprinting experiments demonstrate that the proteins encoded by the avian vjun and human cjun, expressed in E. coli, are sequence specific DNA binding proteins recognizing the same sequences as AP1. In addition, purified AP1 is recognized by two different anti-jun antisera. Overexpression of vjun in mammalian cells leads to up to a 100-fold enhanced expression of a cotransfected reporter gene driven by promoters which contain AP1 sites. Deletion of the AP1 sites leads to a considerable decrease in trans-activation. These experiments show that a nuclear acting oncogene product is able to trans-activate genes in an AP1 dependent manner.

O 107 SEQUENCE AND FACTOR COMPONENTS OF DIHYDROFOLATE REDUCTASE GENE TRANSCRIPTION IN METHOTREXATE RESISTANT CHINESE HAMSTER OVARY CELLS. Jane C. Azizkhan, Michael C. Blake, Andrew G. Swick, and Jeanne W. Kahn, Lineberger Cancer Research Center and the Departments of Pediatrics and Pharmacology, University of North Carolina, Chapel Hill, NC 27599-7295.

The minimal DHFR promoter sequence required for transcription in vitro includes 2 Sp1 binding sites 5' to the start of transcription. Transcription of a template that contains 2 additional sites further 5' to the gene is not different from that of the minimal promoter. Gel retardation and DNaseI footprinting studies reveal a complex pattern of protein DNA interactions in the 80 bp region from -100 to -180. The gel retardation and DNAseI footprinting pattern obtained with HeLa cell or methotrexate resistant CHO cells is the same. Transcription in vitro of the DHFR template is approximately one half that from the adenovirus major late promoter in an nuclear extract from Hela or methotrexate resistant CHO cells. In vitro mutagenesis experiments are underway to further define the sequence requiremenrts for binding and in vitro transcription and protein factors that specifically stimulate DHFR gene transcription have been partially purified.

O 108 CHARACTERIZATION OF THE UPSTREAM ENHANCER ELEMENTS OF THE RAT AND MOUSE ALBUMIN GENES, R. S. Herbst, N. Friedman, H. Isom, J. E. Darnell Jr., L. E. Babiss, Rockefeller University, New York, NY 10021.

We have previously analyzed the DNA-protein interactions which occur in the promoter proximal region (-300 to -50) of the rat albumin promoter that confer tissue specific transcription (Babiss et al., *Genes and Development 1*: 256-267). Using an adenovirus vector system and an SV40-transformed rat hepatocyte cell line, SV1, we have now identified DNA sequences which are responsible for maintaining the high rate of transcription from the rat and mouse albumin promoters. These sequences are located at -2.8kb for the rat albumin gene and -9.5kb for the mouse albumin gene, relative to the known start sites for albumin transcription. These enhancers do not appear to be related by sequence comparison, but both increase the rate of transcription and mRNA accumulation by 3-4 fold . Using gel retardation, exo III stop and DMS protection assays, we have identified at least 3 sites of DNA-protein interactions that appear to be tissue specific and developmentally regulated. One of the regions may interact with the ubiquitous protein NF1 since it is competed by a segment of DNA that is promoter proximal to the albumin gene cap site and known to interact with this protein. Additional DNA sequences have been identified which may also be responsible for the maintenance of tissue-specific expression of the endogenous albumin gene. When compared to the liver-specific genes alpha-1-antitrypsin and transthyretin, at least one region for both the mouse and rat albumin enhancers may utilize a shared AP-3 like liver specific factor. To begin to understand the complex DNA-protein interactions that lead to the formation of stable transcription complexes, we have begun to analyze the transcription of several liver-specific promoters in vitro. While no evidence for enhancer activity has been observed in our standard transcription system, we have begun to use histone assembled templates and fractionated liver extracts as a means of scoring enhancer function.

O 109 PHOTOAFFINITY LABELING OF RNA POLYMERASE III TRANSCRIPTION COMPLEXES, Blaine Bartholomew, Claude F. Meares, and Michael E. Dahmus, University of California, Davis 95616.

The proteins contacting nascent RNA transcripts in RNA polymerase III transcription complexes have been examined using photoaffinity labeling techniques. The photoaffinity analog 4-S-UTP was incorporated along with α-^{32}P CTP into VAI transcripts using a phosphocellulose fractionated HeLa S-100 extract and the adenovirus VAI gene cloned into pBR322 plasmid. The labeled photoreactive nascent RNA was crosslinked to proximal proteins in the transcription complex and then RNase T1 treated to reduce the size of the RNA crosslinked to the protein subunits. The proteins were resolved by SDS-PAGE and analyzed by autoradiography.

Two large proteins were specifically photoaffinity labeled. Labeling was dependent on irradiation, 4-S-UTP, exogenous DNA template, and inhibited by 200 µg/ml α-amanitin. Photoaffinity labeled transcription factor IIIC was then separated from other components by it binding stability to DNA in 1 M KCl. Two large subunits of IIIC (Mr ~138 kDa and ~155 kDa) appeared to contact nascent RNA transcripts. Analysis of samples by phosphocellulose chromatography using a linear KCl salt gradient indicates that factor IIIB may also be photoaffinity labeled.

O 110 SEQUENCES AND FACTORS INVOLVED IN TRANSCRIPTION TERMINATION BY POLI Ingrid Bartsch, Anne Kuhn, Christa Schoneberg, Margarete Hannappel and Ingrid Grummt, Inst. für Biochemie, Röntgenring 11, 8700 Würzburg, FRG.

An 18 bp conserved sequence AGGTCGACCAG$^{AT}_{TA}$NTCCG (the "Sal box"), present 8 times in the mouse 3'NTS, functions as transcription terminator for pol I. This motif is recognized by a specific DNA-binding protein, which functions as a transcription termination factor. We have purified this pol I-specific termination factor by chromatography on three conventional columns followed by specific affinity columns. The purified protein has a molecular weight of 95 $\pm$ 5 kD and contains both the specific DNA-binding activity and the capacity to terminate rDNA transcription. Limited proteolytic treatment of this protein yields a resistant core which is still able to specifically bind the "Sal box". However, the ability of the factor to mediate transcription termination is lost after protease treatment indicating that the DNA-binding and the functional domain can be physically separated. The binding of this factor to the "Sal box" is essential but not sufficient for the efficient formation of authentic 3'ends. The sequences immediately 3'to the "Sal box" contribute to the efficiency of the termination reaction, whereas the accuracy of 3'end formation is mediated by 5'flanking sequences. There are at least two different nuclear factors which specifically bind to the DNA sequences upstream and downstream of the "Sal box".

O 111 PLASMID STABILITY INDUCED BY PROMOTER ACTIVITIES TO PSC101 DERIVATIVES LACKING THE *PAR* LOCUS ; Serge L. Beaucage and Stanley N. Cohen Stanford University School of Medicine, Stanford, CA 94305.

The plasmid pSC101 encodes a genetic determinant termed partition or *par* that accomplishes stable inheritance of the plasmid in a population of dividing cells. Several partition related (PR) segments within the *par* region appear to be involved in the process. The role of the PR segments has been investigated by site-directed mutagenesis and a functional analysis of the requirements for plasmid stability has been initiated. In the absence of the *par* region, the stability of pSC101 derivatives can be restored in *cis* by the transcriptional activity of certain promoters. The relevance of the orientation and location of these transcriptional elements to plasmid stabilization is presented along with preliminary results suggesting that a mechanism by which these promoters stabilize *par*-deleted pSC101 derivatives is dependant on plasmid superhelicity. The potential relationship between transcription initiation and plasmid copy number has been evaluated for plasmids lacking the *par* region. Unlike promoter-stabilized pSC101 derivatives, the stability and superhelicity of pSC101 derivatives harboring the *par* locus are not altered under limiting amounts of gyrase *in vivo*. A model involving DNA supercoiling and the stability of pSC101 derivatives is proposed.

O 112 OPERATOR SPECIFICITY OF DOMINANT-NEGATIVE MUTANTS OF LAC REPRESSOR, Joan L. Betz and Majilinde Z. Fall, University of Colorado Medical School, Denver, CO 80262.

The specific binding of dominant-negative (I^{-d}) lactose (lac) repressors to wild-type as well as mutant (O^c) lac operators has been examined to explore the sequence-specific interaction of the lac repressor with its target. Twenty-two $lacI^{-d}$ genes encoding substitutions in the amino-terminal 60 amino acids were cloned in a derivative of plasmid pBR322. The mutant repressors were examined for polypeptide size and stability, and for binding to the inducer isopropyl-β-D-thiogalactoside (IPTG). Several of the I^{-d} repressors were shown to be partially degraded *in vivo*, and all bound IPTG with approximately the wild-type affinity. The mutant repressors' affinities for wild-type operator ranged from <1 to about 5% of wild-type repressor's affinity, and with only a few exceptions, showed no particular specificities for any of the single site O^c operators tested.

Site-directed mutagenesis is being used to investigate the importance of amino acid residues 17-25, the presumed 'recognition helix' making site-specific contacts with the operator. A synthetic 'doped' oligonucleotide (containing 91% of the correct base and 3% of each of the other bases at the positions in lacI encoding amino acid residues 17 to 25) was hybridized to lacI-M13 single strand phage DNA. Following infection of an appropriate host strain, $lacI^{-d}$ mutant phages were detected as blue rather than white plaques on XGal indicator plates. A number of novel substitutions have been identified and examined for their binding to wild-type and O^c lac operators.

O 113 ON THE ROLE OF NUCLEOSOMAL HISTONE ACETYLATION IN MODULATING GENE STRUCTURE AND ACTIVITY, Lidia C.Boffa, Maria Rita Mariani, IST, 16132 Genova, Italy; Janice Walker & Vincent G. Allfrey, Rockefeller University, New York, NY 10021.

We are studying how the structure of nucleosomes is altered during transcription on the assumption that changes depend, at least in part, on the post-synthetic modification of DNA-binding proteins. Recent developments provide evidence that the nucleosomes unfold during transcription. This change in topology can be monitored by availability of the SH-group of histone H3 to Hg-affinity chromatography. An important question is whether nucleosomal unfolding is coupled closely to RNA transcription. To investigate this problem we have studied the effects of RNA synthetic inhibition by α-amanitin on permeabilized HeLa cells. Comparison of nucleosomes from control cells and those exposed to the inhibitor showed that the retention on Hg-column was unchanged at 20' but greatly deminished following 40' incubation of the treated cells. These data suggest a fearly long recoiling time. RNA synthesis was inhibited by 50% within 20' and 80% in 40', as judged by the incorporation of ^{3}H-5'-UTP. We also cheked if the timing of nucleosomal recompaction, following inhibition of RNA synthesis, can be related to post-synthetic modification of histones. We have therefore tested the effects of Na-butyrate (an inhibitor of histone deacetylation) on nucleosomes exposed to α-amanitin: they appeared to be stabilized in the unfolded conformation. This result strongly imply that the open configuration of nucleosomes depend to some extent on the level of acetylation of the histone component. The question then arises to whether hyperacetylation is a sufficient condition for unfolding of the nucleosomal core. Since HeLa cells exposed to high level of butyrate, which generate a high percentage of multiacetylated forms of H3 and H4, showed no encrease in the proportion of nucleosomes binding to the Hg-column as compared to control preparations, we concluded that acetylation " per se " seams to be a prerequisite but can not fully account of changes in nucleosomes structure.

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O 114 PHYSICAL AND FUNCTIONAL STUDIES OF THE ENHANCER STIMULATING E2 PROTEINS OF PAPILLOMAVIRUSES. Thomas R. Broker, Hirohiko Hirochika, Rei Hirochika and Louise T. Chow. Biochemistry Dept., University of Rochester School of Medicine, Rochester, N.Y. 14642

The transcriptional enhancer sequences of the papillomaviruses are regulated by trans-acting factors encoded by the viral E2 open reading frame. Using the chloramphenicol acetyltransferase (CAT) assay in transiently transfected monkey CV-1 cells, the enhancer of human papillomavirus type 11 (HPV-11) has been localized to a 270 base pair tract immediately preceding the E6 ORF. It consists of two functional components. The first is a constitutive enhancer containing a number of different sequences homologous to the recognition motifs of cellular transcription factors. The second domain, E2-RS, is an inducible enhancer with several copies of a consensus sequence $ACCN_6GGT$ responsive to the E2 protein. Tandem copies of portions of the constitutive domain function as an E2-independent enhancer, whereas multiple copies of a synthetic oligonucleotide containing E2-RS is necessary and sufficient for transcriptional activation by the E2 protein encoded by HPV-1, HPV-11 or bovine papillomavirus type 1. We have also expressed all three E2 proteins in Escherichia coli. Filter-binding analyses with crude E. coli lysates show that the E2 proteins bind to the E2-RS with an affinity proportional to the copy number, but do not bind to mutated motifs. The E2 proteins purified by DNA affinity chromatography protect a DNA segment containing the E2-RS in pancreatic DNase I footprinting analyses. We conclude that E2 proteins activate the enhancer by binding directly to the E2-RS and interacting with other transcriptional factors.

O 115 SUG1, A NEW TRANSCRIPTIONAL FACTOR?, Jacqueline Bromberg, Genetics Curriculum, Univ. North Carolina, Chapel Hill, NC. and Stephen Johnston. Dept. of Botany, Duke Univ., Durham, NC.

We have cloned a suppressor of a defective GAL4 protein. The yeast GAL4 protein is a positive acting transcriptional factor. Its Zn^{2+} finger containing amino terminus binds to a DNA consensus sequence UAS_G while the acidic carboxyl end is required to activate transcription. We engineered an altered GAL4 protein, gal4-62 Bg, which is missing its acidic terminus and therefore Gal-. An extragenic suppressor, sug1, was isolated as a Gal^+ revertant and found to be allele specific for gal4-62 Bg. Thus, sug1 protein may interact with the aberrant activating domain of the gal4-62 protein. We have cloned SUG1 by complementation. This gene encodes a 1.8kb transcript of medium to low abundance and maps by OFAGE gel analysis to chromosome VII or XV. Further characterization of SUG1 will allow us to determine its role in transcription.

O 116 STUDIES ON ELA TRANSACTIVATED PROMOTERS.ISOLATION OF A 76,000 DA FACTOR REQUIRED FOR TRANSCRIPTION FROM THE ADENOVIRUS ENCODED ELA,EII,EIII AND EIV PROMOTERS.Patricia Cortes,Leonard Buckbinder,Nancy Rak,Vincente Mirales and Danny Reinberg.Department of Biochemistry.University of Medicine & Dentistry of New Jersey-Robert Wood Johnson Medical School.

We have purified from HeLa cells, using a functional transcription assay,a 76,000 Da protein (EivF)specifically required for transcription from the adenovirus Eiv promoter and other early viral promoters.Purified EivF bound to the Eiv promoter,other adenovirus promoters known to be Ela transactivated and the inverted terminal repeats (ITR) of the virus.DNAse I footprinting experiments demonstrated that EivF bound to the sequence GT(G/T)ACG(T/A).Consistent with the observation that EivF was absolutely required to initiate transcription from the Eiv promoter and that two EivF sites mapped in the ITR sequences,we show that the ITR sequences were able to promote transcription from the Eiv CAP site in an Ela dependent fashion.Nuclear extracts prepared from HeLa cells contained more than one factor capable of binding to the GTGACGT sequence;however only one of these factors was able to promote transcription from the EIa,EII,EIII and EIV promoters.The purified and transcriptionally active EivF bound to sequence elements present in the somatostatin and gonadotropin promoters that had been previously shown to be responsive to cyclic AMP;however EivF was not able to drive transcription from the cyclic AMP inducible promoters.

O 117 RAP30/74: A GENERAL INITIATION FACTOR THAT BINDS TO RNA POLYMERASE II. *Zachary F. Burton, +Mary Sopta, +Marie Killeen, Loris G. Ortolan and +Jack Greenblatt, C.H. Best Institute of Medical Research and +Department of Medical Genetics, University of Toronto, Toronto, Ontario, CANADA M5G 1L6. *Present address: Department of Biochemistry, Michigan State University, East Lansing, MI 48824.

RNA polymerase II-associating proteins (RAPs) were initially isolated and identified by chromatography of mammalian cell extracts on immobilized calf thymus RNA polymerase II (Sopta et al., J. Biol. Chem. 260:10353-10361, 1985). We subsequently showed that at least one of the RAPs is a transcriptional initiation factor (Burton et al., EMBO J. 5:2923-2930, 1986). Human RAP30 was isolated by affinity chromatography and injected into a rabbit to produce anti-RAP30 antiserum. This antiserum was then used to screen a lambda gt11 library made with mRNA derived from HL-60 cells, and a human cDNA encoding RAP30 was isolated. This clone was used to produce RAP30-β-galactosidase fusion protein, which in turn was used to affinity-purify anti-RAP30 immunoglobulin from crude serum. This monospecific anti-RAP30 antibody was used to show that: 1) RAP30 binds to RAP74; and 2) the RAP30/74 complex is required for accurate initiation by RNA polymerase II. RAP30/74 was shown to be required for accurate transcription from four mammalian promoters that have "TATA" boxes and from one promoter that lacks a "TATA" box. RAP30/74 is not required for initiation by RNA polymerase III at the adenovirus VA promoters. Therefore, RAP30/74 is a general initiation factor that binds to RNA polymerase II. (Z.F.B. and M.S. are equal contributors to this work.)

O 118 AT LEAST THREE DIFFERENT RNA POLYMERASE HOLOENZYMES DIRECT TRANSCRIPTION OF THE AGARASE GENE (dagA) OF STREPTOMYCES COELICOLOR A3(2), Mark J. Buttner, Alison M. Smith and Mervyn J. Bibb, AFRC Institute of Plant Science Research, John Innes Institute, Colney Lane, Norwich NR4 7UH, UK.
The dagA gene of S. coelicolor encodes an extracellular enzyme which is involved in the catabolism of agar. Analysis of the transcription of the dagA gene identified four promoters which initiate transcription approximately 32, 77, 125 and 220 bp upstream of the coding sequence. Using a combination of FPLC gel filtration and anion exchange chromatography three different RNA polymerase holoenzymes were separated, each of which transcribes from only one of the dagA promoters. Holoenzyme reconstitution experiments identified the sigma factors responsible for recognition of two of the promoters. The previously identified $E\sigma^{49}$ transcribes from the dagAp3 promoter while a novel species, $E\sigma^{28}$, recognises the dagAp2 promoter. Circumstantial evidence suggests that the third holoenzyme, which transcribes from the dagAp4 promoter, is the previously identified $E\sigma^{35}$.

O 119 DIFFERENTIATION BETWEEN ELONGATION-ACTIVE AND ELONGATION-INACTIVE RNA POLYMERASE MOLECULES. James J. Butzow, Lisa L. Oehrl and Gunther L. Eichhorn, Gerontology Research Center, NIH/NIA, Baltimore, MD 21224.
RNA polymerase (E. coli) purified to gross physical homogeneity still exhibits heterogeneity in its activity. Commonly some 30-50% of the core polymerase in extensively purified preparations appears to be competent in processive elongation on T7 DNA. The status of the remaining 50-70% of the cores is not well understood, either as to activity at different stages in the transcription process or possible defects in the protein. We have examined the population of enzyme molecules by introducing competition by heparin-sepharose matrix during the elongation phase on T7 DNA, under standard T7 assay conditions. Elongation continues unimpaired after physical removal from the reaction of a sizeable proportion of the cores by extraction onto heparin-sepharose. Up to 70% of the putatively inactive cores are extracted with fresh preparations, less with preparations showing diminished activity after extensive storage. Competition by heparin appears to be differentiating between enzyme molecules involved in elongation and other enzyme molecules that are inactive toward elongation.

O 120 *IN VITRO* TRANSCRIPTION OF THE P4 PHAGE LATE PROMOTER,
Emily C. Dale[1], John Keener[2,3], Sydney Kustu[2,3], and Richard Calendar[1], Departments of Molecular Biology1 and Microbiology2, University of California, Berkeley, California 94720, and Department of Bacteriology3, University of California, Davis, California 95616
The late genes of satellite bacteriophage P4 are cotranscribed from a single promoter which shares little homology with known classes of *E. coli* promoters (E. Dale, G. Christie and R. Calendar, J. Mol. Biol. 192:793-803, 1986). P4 late transcription is stimulated *in vivo* by the *ogr* gene product of P4's helper phage P2 and by the P4 transactivation (δ) gene product, which is homologous to the *ogr* gene product (B. Kalionis, M. Pritchard, and J. B. Egan, J. Mol. Biol. 191:211-220, 1986). In a coupled transcription-translation system, the P4 late gene promoter can be activated by either the δ protein or by the *ogr* protein in the absence of any other phage-encoded factors. Antibodies against the σ^{70} subunit of *E. coli* RNA polymerase inhibit *in vitro* expression of the P4 late promoter, suggesting that the *ogr* and δ proteins act as positive regulators, rather than as σ factors.

O 122 REGULATION OF A TRANS-ACTING FACTOR BY PHOSPHORYLATION
Anthony R. Cashmore and Neeraj Datta, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104

We have isolated and partially purified a trans-acting factor from pea nuclei which binds specifically to the pea Rubisco SS3.6 promotor. The binding activity of this factor is reversibly regulated by phosphorylation. It is phosphorylated by NII kinase and is inactive in the phosphorylated form. Reconstitution experiments with purified phosphorylating enzymes are in progress.

O 123 IDENTIFICATION OF NUCLEAR FACTORS (NF) THAT BINDS TO TWO CONSERVED SEQUENCES OF THE I-A_β GENE. Antonio Celada and Richard A. Maki, La Jolla Cancer Research Foundation, La Jolla, CA 92037.
Human and murine class II genes of the MHC show a striking homology at 50-120 bp upstream of the transcription start site. This area is composed of two conserved sequences (a 13-mer and an 8-mer separated by 19-20 bp), identified as *cis*-acting transcriptional elements. We have shown by application of a modified gel electrophoresis DNA binding assay that a NF binds to the 8-mer conserved sequence. This NF is present in murine B and T lymphocytes, macrophages, mastocytes, fibroblasts and human B lymphocytes and macrophages. No modifications of the NF have been found after treatment of the cells with interferon-γ or cycloheximide. The binding site was defined by using DNase I and dimethyl sulfate protection assays. The putative binding sequence is closely related to the sequence, CCAAT, which is often found associated with the promoter of a gene and is recognized by transcriptional factors CTF and NF-I. Oligonucleotides that contain the binding site sequences for NF-I and the α-globin CCAAT element, however, do not compete for binding of the NF to the 8-mer conserved sequence of I-A_β described here, suggesting that, in spite of the similarity of the binding sequence, the NF identified in this report may be different. Using the dimethyl sulfate protection assays and large amounts of nuclear proteins, we found a second NF that binds to the 13-mer conserved sequence. These nuclear proteins may be *trans*-acting factors that mediate the specific transcription of I-A.

O 124 PHOSPHORYLATION MODULATES THE ACTIVITY OF THE TRANSCRIPTIONAL ACTIVATOR ADR1,
J.R. Cherry, T. Johnson, K. Dollard, C.L. Denis, University of New Hampshire, Durham, NH.

ADR1 is the primary activator of transcription of the glucose-repressible alcohol dehydrogenase II gene (*ADH2*) of *S. cerevisiae*. Three mutations which all cause a partial escape from glucose repression occur in a sequence of five consecutive amino acids which can serve as a site of phosphorylation by cAMP-dependent protein kinase. The reversible phosphorylation of ADR1 may be affected by these mutations and thus be disrupting a crucial step in the glucose repression of ADHII.

In vitro phosphorylation of mutant and wild-type ADR1 proteins expressed in *E. coli* have been performed and found to support the above model. The mutations decrease or eliminate phosphorylation at the serine in the phosphorylation consensus sequence. In addition, yeast lacking the regulatory subunit of cAMP-dependent protein kinase (*bcy1*) show decreased ADHII expression. These results indicate that phosphorylation diminishes ADR1-mediated transcriptional activation of *ADH2*.

O 125 MODULATION OF TRANSCRIPTIONAL ACTIVITY VIA PROTEIN-PROTEIN INTERACTIONS, Robert H. Chiu and Michael Karin, University of California, San Diego, La Jolla, CA 92093

Phorbol esters (TPA) treatment of cells lead to induction of various proto-oncogenes, growth factor genes, and genes encoding secreted proteases. In addition, TPA increase the activity of viral enhancer elements. To identify trans-acting factors that mediate the transcriptional response to TPA we used the SV40 enhancer as a model, because it was known to be composed of several discrete cis-acting elements which are recognized by multiple trans acting factors(1). At least four different TPA responsive elements were observed and responsive activities were depend on cell type. The induction response was likely to involve at least two distinct post-translational steps which modulate the activity of the proteins that recognize these elements. Furthermore, one of the transcriptional factor AP_2 appears to mediate transcriptional activation in response to two different signal-transduction pathway(2), one involving the phorbol-ester and diacylglycerol activated protein kinase C, the other involving cAMP-dependent protein kinase A. To examine whether the interactions of trans-acting factors induced by different inducers are required for maximal transcriptional activation will be discussed.

(1)Robert Chiu et al, Nature 329, 648 1987
(2)Masayoshi Imagawa et al, Cell 51, 251 1987

O 126 CHARACTERIZATION OF THE XENOPUS XlHbox 1 HOMEOBOX GENE, Ken W.Y. Cho, Christopher Wright, Guillermo Oliver, Andreas Fritz and Eddy DeRobertis, University of California, Los Angeles, CA 90024.

We describe the characterization of the XlHbox 1 Xenopus homeobox gene, previously reported as AC-1 (Cell 37, 1984). cDNA isolation and transcriptional analysis of this gene reveal that differential utilization of two promoters produces two transcripts which are differentially expressed during embryogenesis. A novel feature of these events is that alternative transcription leads to the production of a new domain in the N-terminal end of the homeobox protein. Immuno-staining of the sectioned Xenopus and mice embryos has demonstrated that these two homeodomain proteins are differentially distributed spatially; one being expressed more posterior than the other in a variety of different tissues.

To analyze functions of the homeotic genes, we have tested their ability to bind to specific DNA sequences. The β-galactosidase-XlHbox 1 homeobox fusion protein recognizes a CAATTAAA sequence motif. Whether this sequence has any functional relevance in transcription is currently being investigated.

O 127 "THE GENOME IS DIVIDED INTO DOMAINS BY A/T-RICH DNA SEGMENTS THAT ASSOCIATE WITH THE NUCLEAR MATRIX" Peter Cockerill, Walter and Eliza Hall Institute of Medical Research, 3050 Australia

The genome is divided into domains of about 50kb of DNA in length. These domains can be visualised as loops of DNA when histones are extracted from either nuclei or metaphase chromosomes. I have identified a very heterogeneous family of A/T-rich DNA elements which act to anchor domains of DNA to either the nuclear matrix or the chromosome scaffold (Cell 44, 273-282 (1986). These elements have been termed MARs (matrix associations regions) and probably define DNA domains in the genome. A comparison of various MARs reveals that they are typically 75% A+T, and the strength of binding increases with the percentage A+T and the length of the A/T-rich region. MARs usually contain topoisomerase II binding sites and appear to bind to the nuclear matrix via multiple interactions occurring over a stretch of about 300 bp of DNA. I have shown that the topoisomerase II consensus sequence is all the sequence information that is required to achieve in vitro binding to the nuclear matrix. An efficient MAR can be synthesized by polymerising copies of an oligonucleotide containing a topoisomerase II sequence. When 12 or more copies of the oligonucleotide are included then a MAR is obtained that binds more efficiently than the mouse kappa Ig gene MAR. The kappa MAR had been used here as a model to predict features necessary to synthesize a MAR. I have assayed a 200kb stretch of the mouse heavy chain immunoglobulin gene for MARs using an in vitro DNA binding assay. I have found blocks of MARs in four intergenic regions which divide the locus into domains having an average size of about 50kb (as predicted for the genome as a whole). While MARs appear to be structural elements, they may also play roles in DNA replication and transcription, by interacting with topoisomerase II.

O 128 **THE ZINC FINGER DNA BINDING DOMAINS FROM TWO YEAST TRANSCRIPTIONAL ACTIVATOR PROTEINS ARE FUNCTIONALLY IDENTICAL,** J. Christopher Corton and Stephen Johnston, Dept. of Botany, Duke Univ., Durham, NC.

A number of transcriptional activator proteins from yeast including GAL4, which activates the GAL genes and PPR1, which activates genes in the pyrimidine pathway, have Zn finger domains within defined DNA binding regions. An earlier model proposed that the finger of GAL4 interacts nonspecifically with the chromatin and that the protein sequences immediately adjacent to the finger are involved in specific recognition of the GAL upstream activating sequence (UAS) (Salmeron, J. and Johnston, S. 1986 Nucleic Acids Res. 14, 7767). To test this model we replaced the GAL4 finger region with increasing portions of the finger region from PPR1. The 4PPR1-1 fusion contained all but five amino acids of the finger from PPR1; 4PPR1-2 contained all of the PPR1 finger plus 8 amino acids on the C-terminal side; and 4PPR1-3 contains all the PPR1 finger plus 23 amino acids of PPR1 sequence. All fusions cloned into YEp351 were analyzed in a GAL4 deleted, MEL1 strain. The 4PPR1-1 and 4PPR1-2 fusions grew on galactose, were able to activate MEL1 expression and bound to the GAL UAS. The 4PPR1-3 fusion on the other hand, did none of these things. Our results show that the zinc finger domain of PPR1 can functionally substitute for that of GAL4, and indicates that the zinc finger in these proteins is involved in nonspecific interactions with the chromatin.

O 129 REQUIREMENT OF POLYOMA PROTEIN-INTERACTIVE SEQUENCES FOR EXPRESSION ON PCC4-aza1 CELLS, L. Couture and J.M. Lehman, Albany Medical College, Albany NY 12208.

Recently, DNA-protein interactions have been reported (PNAS,83:8550,1986; Cancer Cells 4, p106, 1986) in the polyoma (py) enhancer region involving the 6bp inverted repeat 'C-element' as well as the adjacent 9bp G-C rich inverted repeat. Both of these regions are maintained in pyPCC4 embryonal carcinoma cell host range mutants including a non- selected py strain (LPT) characterized in this lab which expresses large T antigen in 30-40% of infected PCC4-aza1 cells. These mutants are characterized as essentially having substituted a span of DNA, encompassing minimally the A enhancer core, for a variable deletion, often beginning immediately 3' to the G-C rich inverted repeat, of the Pvu-II-4 fragment typically containing the B-enhancer element. We have set out to determine the requirement for these binding events *in vivo* by creating a number of py enhancer region constructs containing various deletions of this region and/or the second A-enhancer and replacing them into the wild type strain A3 in order to test for their ability to express Tag as well as replication efficiency in both PCC4-aza1 and primary mouse fibroblast cells. Deletions of various portions of the C-element and G-C repeat but containing the second A-enhancer and deletion of the remainder of the Pvu-II-4 fragment express in PCC4's as well as constructs containing the C-element and G-C repeat. A construct with two adjacent A-enhancer elements and no Pvu-II-4 fragment grows poorly but significantly better than the Pvu-II-4 containing wild type strain (P16) from which it was derived. A construct with only one A-enhancer and no Pvu-II-4 fragment grows only to the same background level as does A3. Constructs with further deletions into the 60 bp separating the two A enhancers as well as constructs maintaining the separation but with exogenous spacer DNA will be presented. A mechanism for py enhancer function in PCC4 cells will be discussed.

O 130 ENDO A, DEVELOPMENTALY REGULATED GENE IS DEREPRESSED IN EMBRYONAL CARCINOMA (EC) CELLS BY THE PRODUCTS OF C-MYC AND E1A GENES - Chantal Cremisi, Rosine Onclercq and Agnes Lavenu, Institut Pasteur, 25, rue du Docteur Roux - Paris - 75015 - France.

We previously showed that Polyoma large T (PyT) antigen is negatively regulated in mouse embryonal carcinoma cells (EC cells) and that a labile inhibitor blocks transcription of the gene, endo A. We recall that these genes are very early markers of embryogenesis, as both genes start to be expressed in the earliest differentiated cells, i.e. the trophectoderm layer of the blastocyst stage.

Here we demonstrate that endo A gene is negatively regulated in EC cells and shares negative regulatory trans-acting factors with PyT antigen. The product of the human proto-oncogene c-myc exerts a regulatory effect on these genes ; it is able to derepress them at the transcriptional level.

The E1A gene of Adenovirus 5 also activates endo A gene expression in EC cells, but it exerts an opposit effect on Py early promotor.

Thus, the proto-oncogene c-myc acts as a transcriptional regulator and derepresses expression of an endogenous gene. The fact that E1A gene also activates endo A gene in EC cells, raises the possibility, that c-myc gene product is a functional homologuein the cell of E1A protein.

O 131 THE RAT ALPHA-FOETOPROTEIN PROMOTER GOVERNS THE LIVER-SPECIFIC EXPRESSION AND BINDS SEVERAL NUCLEAR PROTEINS, J.L. Danan, M. Jose and A. Poliard. Laboratoire d'Enzymologie du CNRS, 91190 Gif-sur-Yvette, France

Alpha-foetoprotein (AFP) is the major plasma protein during fetal life. In the course of development, the transcription of this gene decreases to reach very low levels in the adult liver. We have tested the promoter region of the rat AFP gene for its ability to govern the expression of this gene. CAT transient expression experiments were performed. The results indicate that the proximal flanking region of the rat AFP (-300 to -10) is able to direct liver specific expression. Efficient expression however required the presence of an upstream element whose precise location within 2.5 kb of the 5' extragenic region is now under study. In order to look for proteins putatively involved in the control of the rat AFP gene expression, we have analyzed the binding of proteins from liver nuclear extracts to the rat AFP promoter region (-200 to + 40). This was first done by using a gel retention assay. Several stable and specific high affinity complexes were detected with extracts from adult and fetal liver. One of these complexes appeared to be liver specific since it is not detected with extracts from spleen, kidney or brain. This complex is due to the binding of a liver factor to the region -65 to -40 of the AFP promoter as shown by footprinting experiments and confirmed by gel gift competition experiments using an oligonucleotide covering this region. This region has been shown to be of crucial importance for the liver specific expression of the mouse AFP by the group of S. Tilghman. We were also able to show by competition experiments that a widely distributed factor, likely NF_1, binds in the region -125 to -100 of the rat AFP promoter.

O 132 SYNERGISM BETWEEN ENHANCER ACTIVITIES OF THE GLYCOPROTEIN HORMONE α-SUBUNIT GENE. Angelo M. Delegeane and Pamela L. Mellon, The Salk Institute, La Jolla, CA. 92037

The 5'-flanking region of the human glycoprotein hormone α-subunit gene contains at least two enhancer activities, cAMP reponsiveness and placental tissue specificity. Using a transient expression assay, the cAMP enhancer activity was localized to a duplicated 18-bp region which can confer responsiveness to a heterologous promoter in a distance- and orientation-independent manner. The tissue specific element (TSE) resides in the sequences 5' to the cAMP response element (CRE) and is dependent upon the presence of the CRE for activity, as the TSE alone does not confer tissue specificity to a heterologous promoter or to the α-subunit gene promoter in placental cells (Mol.Cell.Biol. 7, 3994, 1987). Elimination of one copy of the CRE by an internal deletion of 18-bp does not affect the cAMP reponse but reduces the tissue-specific activity by six-fold and elimination of both copies destroys both activities. The α-subunit gene CRE can be replaced with the somatostatin CRE and still confer tissue specificity, albeit at a lower level, while cAMP responsiveness remains unchanged. To examine how the interaction of these elements is involved in the generation of tissue specificity, a variety of rearranged α-subunit gene 5'-flanking control regions were analyzed. The TSE and CRE were able to interact and generate tissue specificity regardless of their orientation with respect to each other, although activity was decreased by two-fold when the TSE was moved 300-bp upstream of the CRE and lost with further distances. Thus, the synergistic interaction between these two elements, though independent of orientation, is enhanced by their proximity to each other.

O 134 Analysis of the requirements for a super-high expression phenomenon in a thermotolerant Bacillus species

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Methylotrophs, when grown at a low growth rate and supplied with limiting amounts of methanol, generally synthesize high amounts of the first dissimilatory enzyme, e.g. methanol oxidase in certain yeast strains (1) and methanol dehydrogenase in methylotrophic prokaryotes (2). Recently a thermotolerant Bacillus species was isolated in our laboratory. This strain synthesizes an unusual NAD-dependent alcohol dehydrogenase enzyme, well in excess of 30% of total cellular protein when grown with methanol. A study is made of the regulation of transcription, the role of sigma factor(s) and copy number of the gene, coding for this protein.

(1) van Dijken et. al. Arch. Microbiol **111**,137. (2) Harms et. al. J. Bacteriol. **169**,3969

O 135 **CRYSTAL STRUCTURE OF KINETOPLAST DNA BENDING LOCUS**

Anna D. DiGabriele, Mark R. Sanderson, Thomas A. Steitz, Yale University, New Haven, CT 06511.

Studies of the abnormal gel mobilities of a series of synthetic DNA oligonucleotides have led to the hypothesis that certain DNA sequences are inherently bent. The site of bending consists of four or five adjacent adenine residues flanked on the 5' and 3' ends by DNA of random sequence (Koo, et al., Nature 320, 501, 1985). In order to determine the molecular basis of DNA bending, the sequence dCGCAAAAATGCG has been crystallized and its structure has been determined at 2.8Å. The structure was solved by applying the phases of the structure dCGCGAATTCGCG, which crystallizes with the same cell dimensions as our dodecamer (Wing,et al., Nature 287,755,1980). Preliminary results suggest that a bend of approximately 20 degrees exists in the direction of base pair roll. Our current model also suggests the presence of bifurcated hydrogen bonds between adjacent steps of the adenine tract. Refinement using both NUCLSQ (Westhof,et al.,JMB 184,119,1985) and XPLOR (Brunger,et al.,Science 235,458,1987) is in progress.

O 136 THE PRO-OPIOMELANOCORTIN GENE: A MODEL FOR NEGATIVE REGULATION OF TRANSCRIPTION BY GLUCOCORTICOIDS

Jacques Drouin, Mona Nemer, Richard K. Plante, Mark Trifiro and Orjan Wrange*, Clinical Research Institute of Montreal, Montreal, Canada, H2W 1R7, *Karolinska Institute, Stockholm, Sweden.

The gene encoding pro-opiomelanocortin (POMC) offers an interesting model system to study negative control of transcription in eucaryotes. Indeed, glucocorticoid hormones specifically inhibit transcription of the POMC gene in the anterior pituitary.

In order to identify DNA sequence elements responsible for transcription inhibition by glucocorticoids, chimaeric genes constituted of POMC promoter fragments fused to bacterial sequences encoding neomycin resistance (neo) were tested by electroporation into POMC-expressing AtT-20 cells of pituitary origin. The POMCneo hybrid gene is transcribed efficiently from the correct start site in these cells. Deletion analysis indicates that sequences up to position -478 bp are required for tissue-specific transcription. Similar deletion analyses suggest that sequences within a promoter fragment extending from positions -34 to -132 bp are essential for glucocorticoid inhibition of transcription. This region contains one of the three *in vitro* binding sites for purified glucocorticoid receptor identified in POMC upstream sequences. Since this binding site overlaps a putative "CCAAT" box sequence, glucocorticoid inhibition of POMC transcription may result from receptor binding in the "CCAAT" box region and impaired "CCAAT" box function.

O 137 *IN VIVO* FOOTPRINTING OF TRANSCRIPTION COMPLEXES AT THE *LAC* AND *GLNALG* OPERONS OF *E. COLI*

Selina Sasse-Dwight and Jay D. Gralla, University of California, Los Angeles, CA 90024.

In vivo footprinting using primer extension analysis was used to examine transcription complexes at the *lac* and *glnALG* operons of *E. coli*. Plots of operator occupancy versus active repressor concentration *in vivo* for the *lac* primary (O1) and pseudo (O3) operators were obtained by inducing the cells with various amounts of IPTG and probing repressor binding with dimethyl sulfate. These plots demonstrate that O1 binds repressor 10.3-fold more tightly than O3 *in vivo*. Deletion of O1 drastically reduced O3's ability to bind repressor, demonstrating that the two operators bind repressor cooperatively *in vivo*. Addition of the drug coumermycin *in vivo* suggested that this cooperativity may require DNA supercoiling.

Using the same *in vivo* footprinting techniques to study the *glnALG* operon, we found that the NRI activator protein binds to the upstream NRI sites 1 and 2 on the same helix face when the cells are deprived of nitrogen. Occupancy of these two sites was reduced when σ^{54} was missing from the background strain. Eσ^{54} was found to bind to the opposite helix face from NRI at the *glnAp2* promoter, even under conditions of nitrogen excess or in the absence of NRI from the background strain where there is little or no transcription from this promoter. Potassium permanganate, which we found detects open complexes at the *lac* promoter, was used to examine open complex formation at *glnAp2* in various background strains. An open complex was detected *in vivo* only under conditions of nitrogen deprivation when both NRI and σ^{54} were present in the cell.

O 138 **CHARACTERIZATION OF FACTORS BINDING TO THE UPSTREAM REGION OF THE MUSCLE CREATINE KINASE GENE**, Cathleen Earhart-Ohlendorf, Pamela A. Benfield, and Mark L. Pearson, E. I. du Pont de Nemours & Co., Inc., Experimental Station, Wilmington, DE 19898

Creatine kinase is a dimeric protein which can be composed of two different monomers, brain or muscle. The B monomer is the primary form in brain and other nonmyogenic tissue, while the M form is found in skeletal muscle and cardiac tissue. Expression of the creatine kinase genes is developmentally regulated. The early stages of skeletal muscle development are characterized by myoblast fusion and an accompanying replacement of brain creatine kinase in the myoblast by muscle creatine kinases in the myotube. We are interested in studying the factors responsible for selective expression of the creatine kinase gene in skeletal muscle development. The upstream region of the muscle creatine kinase gene contains three homologous sites for a sequence specific binding activity present in nuclear extracts from myogenic cells (L6 and C2). The characteristics of the binding activities found in differentiated myotube extracts appear to differ from those found in undifferentiated myoblast extracts. The relative affinity of the factor for the three sites has been determined by equilibrium competitions studies. Nuclear extracts from the nonmyogenic L cell line contain a sequence specific binding activity with different properties. The exact sites of binding have been determined by DNAaseI and premethylation interference footprinting.

O 139 TWO NEW FINGER-LIKE PROTEINS FROM MOUSE, Michele Ernoult-Lange and Dean H. Hamer, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

The Zn finger protein family appears to be involved in general regulatory functions in several eukaryotic organisms such as Xenopus (TFIIIA), Drosophila (Kruppel) and yeast (ADR1). We report here the characterization of two mouse genes that encode potential Zn-finger proteins. These clones were isolated from a cDNA library on the basis of DNA homology with two different finger-coding probes: 1) a synthetic oligonucleotide corresponding to a consensus sequence of Kruppel finger-coding region; and 2) the EcoRI fragment of λmkrl (Chowdhury et al., Cell 48:771-778, 1987) containing only the finger sequences from a previously isolated mouse gene. The amino acid sequences which have been deduced from the nucleotide sequence determination revealed that these two cDNA encode for two distinct proteins which contain from 4 to 7 finger motifs. In one of these, the potential DNA-binding as well as Zn liganding amino acids are highly repetitive, raising the possibility that the binding site is a repetitive DNA sequence. Studies on the DNA binding and tissue distribution of these proteins are in progress.

O 140 **DNA BINDING PROTEINS INTERACT WITH THE YEAST COPPER-METALLOTHIONEIN PROMOTER** Tina Etcheverry and Wayne Forrester Genentech, Inc., South San Francisco, CA 97080

The yeast CUP1 gene encodes copper-metallothionein and is regulated by copper ions in the media. DNA sequences required for copper regulation have been identified by studying the effects of various promoter deletions *in vivo*. There are six repeated elements within this region that have the consensus sequence TCTTTTTGCT. Deletion of some of these repeated elements reduce or eliminate gene expression suggesting that these sequences are required for positive regulation of the gene.

DNA binding proteins which interact with this upstream regulatory region have been partially purified using heparin Sepharose chromatography and gel mobility shift assays. DNase I footprint analysis revealed the presence of one factor which recognizes the repeated element. At least two other DNA binding activities are seen for non-homologous sequences. Surprisingly the binding activities are not affected by the presence of copper or treatment with the copper chelator diethyldithiocarbamic acid. We are in the process of purifying these proteins by affinity chromatography. Through this process, we hope to further characterize these proteins and identify their role in mediating the copper response in yeast.

O 141 MOLECULAR ANALYSIS OF A COMPLEX TRANSCRIPTIONAL CONTROL REGION IN YEAST, Philip Farabaugh, Xiao-bei Liao and James Kapakos, Department of Microbiology, U. of Conn. Health Center, Farmington, Connecticut 06032.

We have defined the sites which modulate the level of transcription of the retroviral-like transposon Ty917 of the yeast *Saccharomyces cerevisiae*. The sites which establish the level of transcription initiation map both upstream and downstream of the transcriptional initiation site. The upstream sites, a UAS-like site and a TATA-box, are necessary but not sufficient to promote high level initiation. Maximal expression requires the presence of two downstream enhancer-like sites, located between 140 and 320 bp into the transcribed portion of the element. These together act to stimulate transcription about 1200-fold. A site located about 480 bp into the transcribed region when present decreases upstream transcription about 3-fold; this capacity is similar to that of negative enhancers or silencers.

Ty elements can also act to stimulate the expression of genes located immediately upstream of their promoter end. Adjacent gene transcription also requires sites located downstream of the transcription initiation site. A site located about 430 bp into the element (between the enhancer and silencer-like sites) is required only for adjacent gene expression. We are investigating whether this site is sufficient for this stimulation or whether adjacent gene transcription also requires the enhancer-like sites. Adjacent gene transcription is depressed 2 to 3-fold by the silencer-like site.

It is probable that the sites which we have identified are the binding site of transcriptional initiation factors. We are using a combination of genetic and biochemical techniques to determine if this is true and to attempt to determine the way in which interaction between the multiple putative factors regulates initiation. Preliminary genetic results suggest that the UAS may be complex, containing two independent domains, and that each enhancer-like site specifically interacts with a portion of the UAS. Gel mobility shift experiments show that protein factors bind specifically to both the upstream and downstream regions. Partial purification suggests that at least two factors bind to each region.

O 142 REGULATION OF OM2 EXPRESSION IN VIBRIO ANGUILLARUM, David H. Farrell, Luis A. Actis, and Jorge H. Crosa, Oregon Health Sciences University, Portland, OR 97201.

Vibrio anguillarum, the etiologic agent of the disease vibriosis, harbors a 65kbp plasmid, pJM1, which is required for virulence. pJM1 encodes a high-affinity iron-uptake system which synthesizes an iron-binding siderophore, anguibactin, and an outer membrane protein required for uptake of ferric-anguibactin complexes, OM2. The expression of OM2 is regulated at the transcriptional level by the concentration of iron in the medium, such that at high iron concentrations, OM2 mRNA synthesis is repressed. The region of pJM1 containing the iron transport genes, including those for OM2, has been cloned as a 10kbp fragment in pBR325 and conjugated into a plasmidless strain of V. anguillarum. In this transconjugant, OM2 expression is regulated by iron as in the wild type. However, cells harboring a 6kbp derivative, which is deleted downstream of the OM2 gene, do not show repression of OM2 synthesis at high iron concentrations. DNA sequence analysis of the deleted region shows the presence of an open-reading frame immediately downstream of the OM2 gene. In addition, the deleted 6kbp clone shows the presence of a new 12.5kd protein in coupled transcription/translation reactions which is likely to be the truncated N-terminus of the protein encoded by the open-reading frame. Our evidence suggests that this protein is responsible for the transcriptional regulation of OM2 by iron.

O 143 THE HISTONE OCTAMER: A CONFORMATIONALLY FLEXIBLE STRUCTURE. G.D. FASMAN AND K. PARK, Department of Biochemistry, Brandeis University, Waltham, MA 02254

The literature contains conflicting data concerning the conformation of the histone octamer in both the crystal and in solution, as well as in the nucleosome. The conformation of the histone octamer complex has been shown herein, by circular dichroism (CD) studies, to be highly dependent on the nature and concentration of the salt milieu. Two different salts were used, NaCl and $(NH_4)_2SO_4$, to study the dependency of the conformation of the histone octamer on the salt concentration. With increasing concentrations of NaCl, the α-helical content of the octamer increased, with a concomitant decrease in the β-sheet content (0.2M to 4.0M). At 2.0M NaCl, the concentration at which Uberbacher et al.[Science 232,]147-1249 (1986)] used small-angle neutron scattering to study the octamer's size and shape, the analysis of the CD curve yielded 44% α-helix, 16% β-sheet and 40% random structure for the octamer. In a relatively narrow range of the concentration of $(NH_4)_2SO_4$, from 2.0M to 2.5M, there was a transition from α-helix to random coil with no significant change in β-sheet content. At 2.3M, $(NH_4)_2SO_4$, the concentration at which Burlingame et al. [Science 233, 413-414 (1984)] used to obtain histone octamer crystals, a higher α-helical content was found by CD, with 49% α-helix and 51% β-sheet. Even though a direct comparison between the nucleosome core particle and histone octamer cannot be made, these results would indicate that the histone core and nucleosome can be viewed as very pliable structures whose conformation can be altered by a change in local solvent milieu, partially resolving the contradictory claims concerning the X-ray analysis of crystals obtained under varying conditions.

O 144 STUDIES ON TRANSCRIPTION FACTORS IIB, IIE AND IIF. THREE RNA POLYMERASE II ASSOCIATED PROTEINS, Osvaldo Flores, Edio Maldonado, Zachary Burton*, Jack Greenblatt* and Danny Reinberg, Departament of Biochemistry, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School.

Transcription from class II promoters required five general transcription factors (IIA, IIB, IID, IIE, and IIF) in addition to RNA polymerase II. Factors IIB, IIE, and IIF interacted with a preinitiation complex via protein:protein interactions. The purification of TFIIE resulted in the isolation of a new general transcription factor, TFIIF. This factor has been purified to homogeneity and shown, by renaturation of proteins from polyacrylamide SDS gels and antibody studies, to be a single polypeptide with a mass of 29,000 daltons. Purified TFIIF interacted with RNA polymerase II. TFIIF corresponded to one of the RNA Polymerase II associated factors (Rap 30) which was isolated by RNA Polymerase II affinity chromatography. Studies with antibodies against TFIIE/Rap 30 indicated that the factor was absolutely required for transcription of different class II promoters.

O 145 A Complex of Three Cellular Factors Activate the Polyomavirus Enhancer In Mouse Fibroblasts. William R. Folk*, Mark E. Martin*, Jacques Piette†, Moshe Yaniv† and Wei Jen Tang*. *Dept. of Microbiology, Univ. of Texas, Austin, TX 78712. †Pasteur Institute, 25 rue du Dr. Roux, Paris, France.

The polyomavirus enhancer is composed of multiple functionally redundant elements whose activities vary with cell type. Polyomavirus DNA replication and transcription in mouse fibroblasts is dependent upon the enhancer located between nts 5046-5295.

To better understand the role of cellular factors in activation of individual enhancer elements, we have isolated several polyomavirus mutants and revertants with multiple point mutations which inactivate most or all of the enhancer elements. (Tang et al, 1987. Mol. Cell. Biol. 7:1681-1690.) Analysis of protein binding to mutant enhancer sequences indicate that three cellular factors, PEA1, PEA2 and PEA3, are capable of binding to sequences between nts 5130-5155 *in vitro*. Formation of a complex of these proteins is required for maximal enhancer activity and induces a DNAseI hypersensitive site at approximately nt 5125 (HS-1) in viral chromatin. Mutations in PEA1 or PEA3 target sequences reduce enhancer function and decreases HS-1 in viral chromatin. Duplication of other enhancer elements can compensate for mutations in the PEA1 binding site suggesting possible interactions between the protein complexes associated with the duplicated sequences.

O 146 **FUNCTIONAL ANALYSIS OF TWO LINKED METALLOTHIONEIN-I GENE PROMOTERS,** Randy Foster and Lashitew Gedamu, University of Calgary, Calgary, Canada T2N 1N4

Human metallothioneins(MTs) exist as a multigene family consisting of at least five clustered functional MT-I and one functional MT-IIA genes. We have isolated two linked human MT-I genes, MT-IF and MT-IG, organized in a head to head fashion from a genomic library. S1 nuclease studies have shown that these two genes are functional and that they are expressed in a cell type specific pattern. Although both genes are coordinately induced by heavy metals in a hepatoblastoma derived cell line, Hep G2, the level of expression of the MT-IG gene exceeds that of the MT-IF gene. In order to investigate the possible differences in the transcriptional activity of the MT-IF and MT-IG promoters we have fused the proximal portions of their respective promoters to the chloramphenicol acetyl transferase (CAT) gene. The transient expression of the MT/CAT fusion genes were analyzed in transfected Hep G2 cells by CAT assays and S1 nuclease protection analysis. These studies indicate that in Hep G2 cells the MT-IG promoter is 5X transcriptionally more active than that of the MT-IF gene. Deletion analysis of the MT-IF and MT-IG promoters has revealed that at least 160 bp of upstream sequence is sufficient to elicit coordinate heavy metal induction and differential promoter activity of these two genes. Electrophoretic mobility shift experiments using 160 bp of proximal 5' flanking sequence indicates that the two genes exhibit differenial affinity for trans-acting factors. The regulation of the expression of these genes in Hep G2 cells may be effected by differential affinity of trans-acting factors for their respective cis-acting promoter elements. Supported by AHFMR and MRC.

O 147 PURIFICATION AND CHARACTERIZATION OF HUMAN HISTONE H1 GENE PROMOTER TRANSCRIPTION FACTORS, Paola Gallinari, Franca LaBella and Nathaniel Heintz, Rockefeller University, New York, NY 10021

Our interest is to elucidate mechanisms regulating human histone H1 gene transcription during the cell cycle, by isolating and biochemically characterizing the trans-acting factors that bind to specific DNA sequences on H1 gene promoter. Two distinct DNA binding activities have been identified in nuclear extracts from HeLa cells and mapped within a 170bp region of H1 gene promoter. H1 distal factor binds to an H1 specific AC-rich region, 100bp upstream of the CAP site; H1 proximal factor, binds to the specific consensus sequence between postitions -55 and -39. This proximal region (H1 subtype specific domain) harbors a CCAAT box on the coding strand, but the homology between different H1 genes extends well beyond this element, so that the additional conserved flanking nucleotides might result in subtype specific utilization. Deletion of the CCAAT box reduces in vitro transcription to 15% whereas deletion of the downstream homology further depresses transcription to 5% of the wild type level. The factors were partially co-purified from HeLa nuclear extracts using classical chromatographic fractionation procedures, and then separated by specific oligo DNA affinity chromatography. Analysis of chromatographic fractions on SDS-PAGE indicates that a protein with a M.W. of 45kd co-elutes with H1 proximal binding activity in gel electrophoresis retardation assays. This protein protects from DNAse I digestion a sequence of 20 nucleotides coincident with the H1 specific domain and the methylation of both G residues complementary to the two C's of the CCAAT motif abolish binding.Sequencing data of the HPLC purified protein are in progress.

O 148 A CCAAT DNA BINDING FACTOR CONSISTING OF TWO DIFFERENT COMPONENTS THAT ARE BOTH REQUIRED FOR DNA BINDING, P. Golumbek, A. Hatamochi, E. Van Schaftingen, G. Karsenty, S. Maity, and B. de Crombrugghe, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

We have identified in nuclear extracts of NIH 3T3 fibroblasts and rat liver a protein which binds to the CCAAT motif of several promoters including the Long Terminal Repeat of Rous Sarcoma Virus, the β-actin, and the mouse α2 (I) collagen promoters. The factor which was purified using as assay DNA binding to a segment of the mouse α2 (I) collagen promoter, consists of two components, designated A and B, which are separable by ion exchange chromatography. In crude extracts, factor A is heat labile, whereas factor B is heat resistant. Both factors are required for DNA binding and are present in the DNA-protein complex. Both factors have been purified to homogeneity using sequence specific affinity chromatography. Renaturation of purified material from an SDS-PAGE gel, yields Mr values of 39,000 and 41,000 for factors A and B respectively. Competition experiments indicate that this CCAAT binding complex has a different binding specificity from other factors such as Nuclear Factor 1, COUP and CBP which recognize an analogous motif. Furthermore, the biochemical characteristics and molecular weights of each of the two components of this factor indicate it is different from these other factors. Point mutations, within the CCAAT motif of the α2 (I) collagen promoter, which lower the binding affinity of the purified CCAAT protein, also cause a decrease in the activity of the promoter as assayed by DNA transfection. This suggests that the CCAAT factor which we have purified plays a role in the expression of the α2 (I) collagen gene as well as several other genes.

O 149 CREB REQUIRES DIMERIZATION FOR TRANSCRIPTIONAL ACTIVITY, Gustavo A. Gonzalez, William H. Biggs, III, Karen K. Yamamoto and Marc R. Montminy, The Salk Institute, La Jolla, CA 92037.

Although numerous eukaryotic DNA binding proteins have been characterized, the mechanisms by which these regulatory proteins form transcriptionally active protein-DNA complexes are unknown. We have previously characterized and purified a 43 Kd DNA-binding phosphoprotein (CREB) which mediates induction of somatostatin gene transcription by cAMP. CREB binds with high affinity to the cAMP response elements (CREs) of numerous neuropeptide and growth factor related genes. Most of these CREs contain a conserved core palindrome-TGACGTCA-prompting us to ask whether CREB binds to DNA as a dimer. Methylation interference experiments indicated that each CREB "monomer" would recognize a 5 bp motif-CGTCA-within the core. Gel retardation assays using purified CREB and a 28 bp CRE oligonucleotide revealed two protein-DNA complexes. The high molecular weight (HMW) complex formed exponentially as a function of CREB concentration whereas the low molecular weight (LMW) complex increased linearly. These results suggested that the LMW complex represents CREB monomer whereas the HMW complex emerges from the cooperative interaction of two CREB monomers to form a dimer. Two copies of the motif-CGTCA- appear to be necessary for this dimer to form as removal of one motif from the CRE oligonucleotide abolished the HMW complex. Additionally, this single-motif oligonucleotide was unable to induce transcription in response to cAMP when placed upstream of a CAT fusion gene. Although both monomer and dimer bind with high affinity, it appears that the dimer form is the transcriptionally active form of CREB.

O 150 THE DNA BINDING SPECIFICITY OF α2 IS ALTERED BY *a*1 PROTEIN, Caroline Goutte and Alexander D. Johnson, University of California, San Francisco, 94143.

The α2 repressor protein of Saccharomyces cerevisiae is a sequence-specific DNA-binding protein involved in cell-type determination. α2 represses a set of cell-type specific genes (the *a*-specific genes) by binding to an operator sequence located upstream of each target gene. We have found that the binding specificity of α2 is altered by a second yeast regulatory protein, the *a*1 protein. Under the influence of *a*1, α2 no longer recognizes the sequence upstream of *a*-specific genes, but instead acquires the ability to recognize a different DNA sequence found upstream of the haploid-specific genes. The α2 repressor thus has two distinct binding activities: the α2 binding activity and the *a*1-α2 binding activity. Both the α2 binding site and the *a*1-α2 binding site function *in vivo* to mediate cell-type specific repression of a test promoter. Thus, the α2 repressor turns off two sets of genes by virtue of its two binding activities.

O 152 CHARACTERIZATION OF LACTOTROPH-SPECIFIC IN VITRO TRANSCRIPTION OF THE RAT PROLACTIN GENE, Jennifer F. Krupp and Arthur Gutierrez-Hartmann, Univ. of Colorado Health Sciences Center, Denver, CO 80262

Expression of the rat prolactin (PRL) gene is restricted to the lactotroph cells of the anterior pituitary. To study the mechanisms that regulate rat PRL gene expression, we have established a cell-free transcription system from cultured GH_3 rat pituitary tumor cells that faithfully recapitulate the tissue-specific transcription of the rat PRL gene and we have documented the presence of GH_3 cell-specific factors that selectively interact with the rat PRL promoter. Here, we characterize the template, KCl, $MgCl_2$, and GH_3 whole cell extract concentrations required for optimal in vitro transcription of the rat PRL gene, using the Rous sarcoma virus (RSV) promoter as an internal control. The data demonstrate the differential salt, factor, and template requirements of the rat PRL and RSV promoters for efficient in vitro transcription, and reveal the importance of optimizing the conditions of each promoter being investigated. An optimized lactotroph-specific in vitro transcription assay, as described here, provides a particularly powerful biochemical tool to further address the molecular mechanism involved in tissue-specific expression.

O 153 EVIDENCE FOR INDEPENDENT REGULATION OF LAMBDA AND KAPPA IMMUNOGLOBULIN GENES, James Hagman and Ursula Storb, Dept. Mol. Gen. & Cell Biol., University of Chicago, Chicago, IL 60637

The murine lambda immunoglobulin light chain gene cluster shares many features with kappa light chain genes, yet the identification of the kappa enhancer and trans-acting factors has not revealed a similar control for lambda. Correct expression of stably transfected rearranged lambda genes is achieved in kappa- or lambda-producing myeloma cells, but only at 0.2-1% the level of endogenous expression. Addition of the immunoglobulin heavy, kappa, or SV40 enhancers increases expression to the level seen in the same cells with transfected kappa genes. An enhancer-like region is present in the lambda J-C intron, but appears to be non-functional in these late-stage cells. We are currently seeking additional cis-acting sequences by enlarging existing clones with DNA derived from murine genomic clones covering a total of 144kb. Alternatively, cell lines which represent earlier stages of B cell development are being transfected in light of recent data supporting the existence of separate kappa and lambda B cell lineages.

O 154 OVEREXPRESSION OF THE TRANSACTIVATION GENE OF SATELLITE PHAGE P4.

Conrad Halling and Richard Calendar, University of California, Berkeley, CA 94720.

Satellite bacteriophage P4 is able to grow lytically only in the presence of a helper bacteriophage, P2. P2 supplies P4 with the products of the P2 late genes, which are required to make the P4 phage particle and lyse the bacterial host, *E. coli*. P4 facilitates its lytic growth by positively regulating the transcription of the P2 late genes, a process termed transactivation. Transactivation is controlled by the P4 δ gene, which codes for a transcription factor that acts at the late promoters of P2. During infection by P2 alone, P2 late gene expression requires (1) the product of the P2 *ogr* gene, which activates transcription of the P2 late genes, and (2) concurrent replication of P2 DNA, which is controlled by the P2 early *A* and *B* genes. δgp (166 amino acid residues) contains two regions similar to *ogr*gp (72 amino acid residues), but δgp can function in the absence of *ogr*gp and can activate transcription of the late genes of a replication-deficient P2 helper. Genetic evidence suggests that δgp and *ogr*gp interact with α-subunit of RNA polymerase. We have cloned δ into a λ P_L overproduction vector; after induction for 3 hr at 42°C, δgp makes up about 1% of total cellular protein. We have initiated the purification of δgp from cells in which it has been overproduced in order to determine the mechanism by which δgp activates transcription from the P2 late promoters.

O 155 THE POLYOMAVIRUS ENHANCER COMPRISES MULTIPLE FUNCTIONAL ELEMENTS, John A. Hassell, William J. Muller and Daniel Dufort, 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 trascription, 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 the enhancer elements. We are currently fractionating nuclear extracts to purify these factors and we are studying their interaction with DNA at the nucleotide level.

O 156 CHARACTERIZATION OF THE UPSTREAM ENHANCER ELEMENTS OF THE RAT AND MOUSE ALBUMIN GENES, R. S. Herbst, N. Friedman, H. Isom, J. E. Darnell Jr., L. E. Babiss, Rockefeller University, New York, NY 10021.

We have previously analyzed the DNA-protein interactions which occur in the promoter proximal region (-300 to -50) of the rat albumin promoter that confer tissue specific transcription (Babiss et al., *Genes and Development 1*: 256-267). Using an adenovirus vector system and an SV40-transformed rat hepatocyte cell line, SV1, we have now identified DNA sequences which are responsible for maintaining the high rate of transcription from the rat and mouse albumin promoters. These sequences are located at -2.8kb for the rat albumin gene and -9.5kb for the mouse albumin gene, relative to the known start sites for albumin transcription. These enhancers do not appear to be related by sequence comparison, but both increase the rate of transcription and mRNA accumulation by 3-4 fold . Using gel retardation, exo III stop and DMS protection assays, we have identified at least 3 sites of DNA-protein interactions that appear to be tissue specific and developmentally regulated. One of the regions may interact with the ubiquitous protein NF1 since it is competed by a segment of DNA that is promoter proximal to the albumin gene cap site and known to interact with this protein. Additional DNA sequences have been identified which may also be responsible for the maintenance of tissue-specific expression of the endogenous albumin gene. When compared to the liver-specific genes alpha-1-antitrypsin and transthyretin, at least one region for both the mouse and rat albumin enhancers may utilize a shared AP-3 like liver specific factor. To begin to understand the complex DNA-protein interactions that lead to the formation of stable transcription complexes, we have begun to analyze the transcription of several liver-specific promoters in vitro. While no evidence for enhancer activity has been observed in our standard transcription system, we have begun to use histone assembled templates and fractionated liver extracts as a means of scoring enhancer function.

O 157 INTERACTION OF THE BACTERIOPHAGE Ø29 PROTEIN p6 WITH DOUBLE-STRANDED DNA.
J.M. Hermoso, M. Serrano, I. Prieto and M. Salas. Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma de Madrid, Canto Blanco, 28049 Madrid, Spain.

The B. subtilis phage Ø29 contains a linear double-stranded DNA with a terminal protein covalently linked to both ends and replicates by a protein-priming mechanism. The viral protein p6, needed for Ø29 DNA synthesis in vivo, stimulates replication in an in vitro system with purified proteins. Furthermore, protein p6 inhibits transcription in vitro from the Ø29 early promoter C2 located near the right end of the genome. Binding of protein p6 to double-stranded DNA has been detected by gel retardation assay and induces positive supercoiling to relaxed circular DNA. By electron microscopy, protein p6 was shown to cover the DNA. DNase I footprint experiments of protein p6 performed with Ø29 DNA terminal fragments show a regular pattern of hypersensitive bands spaced about 24 nucleotides along the fragment, flanking protected regions. A regular pattern is also obtained by hydroxyl radical footprinting, in which 3-4 nucleotides long protected regions are observed along the fragment, spaced about 12 nucleotides. The protected regions are displaced in one strand with respect to the other in such a way that they lay opposite each other in the minor groove. These results suggest a helical binding of protein p6 around the Ø29 DNA terminal sequences.

O 158 A THR TO ALA EXCHANGE IN THE RECOGNITION α-HELIX OF TET REPRESSOR DISTINGUISHES BETWEEN A GC AND AN AT BASE PAIR AT POSITION SIX OF TET OPERATOR, Wolfgang Hillen, Lothar Altschmied, Ralf Baumeister, and Klaus Pfleiderer, Lehrstuhl für Mikrobiologie, FAU Erlangen, Staudtstr. 5, 8520 Erlangen, FRG.

The Tn1721- and Tn10-encoded Tet repressor proteins are able to distinguish efficiently between the respective tet operator sequences despite of the fact that they differ only by an AT to GC transition in the sixth base pair. In order to define the sections of the repressor proteins recognizing this transition, in vitro recombinants of both tetR genes were constructed. Firstly, common restriction sites were introduced in both genes and secondly, recombinant genes consisting of sequences mixed from both wild type tetR genes were constructed in vitro. The resulting mixed genes, in which replacements ranged from the entire N-terminus of the Tn10 tetR gene by the Tn1721 borne tetR gene to single amino acid exchanges, were expressed and their products studied in vivo and, after purification, in vitro for tet operator recognition. A single amino acid exchange from Ala to Thr at position 43 in the recognition α-helix of Tet repressor is able to distinguish between the AT and GC base pairs at position six of tet operator. However, exchanging this amino acid along with the entire recognition α-helix3 or with the entire positioning α-helix2 or with both sequences improves the operator distinction ability of these Tet repressor variants. It is assumed that direct contacts of the amino acid 43 and the fine positioning of this structure mediated by the entire helix-turn-helix structural motif contribute to recognition at base pair six of tet operator.

O 159 INACTIVATION BY SEQUENCE-SPECIFIC METHYLATION OF THE LATE E2A PROMOTER OF AD2 DNA IN A CELL-FREE SYSTEM. Arnd Hoeveler, Pawel Dobrzanski, and Walter Doerfler, Institute of Genetics, University of Cologne, Cologne, Germany.

In the long-term inactivation of viral and non-viral eukaryotic genes, sequence-specific promoter methylations play an important role. The modification of the three 5'-CCGG-3' sequences at positions +23, +5 and -215, relative to the cap site of the late E2A promoter of adenovirus type 2 (Ad2), sufficed to silence the gene in transient expression (Langner et al., PNAS 81, 2950, 1984), or in mammalian cells (Langner et al., PNAS 83, 1598, 1986) and after fixation of the E2A promoter-CAT gene construct in the genome of hamster cells (Müller and Doerfler, Virology 61, 0000, 1987). The mechanisms responsible for the methylation-inactivation of promoters are not yet understood. It was, therefore, necessary to develop a cell-free transcription system that would respond to a methylated eukaryotic promoter. In nuclear extracts from HeLa cells the late E2A promoter of Ad2, which was in vitro methylated at the three 5'-CCGG-3' sequences, exhibited strikingly lower activity than the unmethylated construct. Circular templates had to be used and the DNA concentration had to be kept at a critical level. This system may enable us to determine cellular factors that play a role in responding to methylated nucleotides at specific sites in a promoter and in turning off or reducing the activity of such a promoter. In work on the direct effect of sequence-specific promoter methylations on protein binding to the late E2A promoter, we have failed to detect differences between the unmethylated and the 5'-CCGG-3' methylated constructs (Hoeveler and Doerfler, DNA 6, 449, 1987). It will be the aim of further studies to identify and isolate such factors and add them to the in vitro system to elucidate their functions directly.

O 160 Mutations within conserved regions of the GAL4 binding site.
Melvyn Hollis and Mark Ptashne, Department of Biochemistry and Molecular biology, Harvard University, 7 Divinity Ave, Cambridge, MA 02138.

The yeast activator protein GAL4 stimulates transcription of five enzymes involved in galactose catabolism by binding to sites found upstream of each gene (1). Comparison of the naturally occurring GAL4 binding sites (2,3) reveals two regions of striking sequence conservation within the 17 base-pair sequences. Firstly the outer three bases of each half-site (reading 5' to 3') are almost exclusively C-G-G, and secondly the central base is almost always a T or an A. Using a near-consensus reference GAL4 binding site, we have made all possible single and double-symmetric changes in the conserved regions. The mutant sites have been assayed for their ability to bind GAL4 by in vitro DNase footprinting using an amino terminal fragment of GAL4 (amino acids 1 to 147) purified from E. coli (H. Kakidani and M. Ptashne, unpublished). A number of the sites have been assayed for UAS activity in yeast and these data will also be presented.

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O 161 AN UPSTREAM REGULATORY FACTOR (ATF) FACILITATES INTERACTIONS OF THE TATA FACTOR (TFIID) AND OTHER BASIC TRANSCRIPTION COMPONENTS WITH THE ADENOVIRUS E4 PROMOTER.
M. Horikoshi[1], T.-Y. Hai[2], Y.-S. Lin[2], M. Green[2], and R.G. Roeder[1]. [1]Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021, [2]Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

Transcription from the E4 promoter is stimulated markedly, both in vivo and in vitro, by upstream promoter and enhancer elements (K. Lee and M. Green, EMBO J 6:1345, 1987) which recognize a common cellular transcription factor designated ATF. Both highly purfied ATF and partially purified TFIID (TATA recognition factor; N. Nakajima, M. Horikoshi, and R. Roeder, unpublished) bind independently to the E4 promoter. However, cooperative ineractions between ATF and TFIID are observed (cf. M. Sawadogo and R. Roeder, Cell 43:165, 1985) when both are present simultaneously, as evidenced both by altered interactions of TFIID downstream of the initiation site and by increased ATF interactions at one of the upstream sites. These ATF-TFIID interactions in turn facilitate recognition by RNA polymerase II and factors IIB and IIE, as evidenced by further alterations in DNase footprint patterns when these factors are present. Visualization of these latter interactions depends on the presence of multiple ATF sites and purified ATF. These results suggest that ATF stimulates transcription from the E4 promoter by alterations in the TFIID interactions, which in turn facilitate productive interactions with the remainder of the basic transcriptional machinery, and they provide a general model for the action of other upstream regulatory proteins in stimulating promoter activity (transcription).

O 162 CIS- AND TRANS-ACTING FACTORS ESSENTIAL FOR RAT INSULIN II GENE EXPRESSION,
Young-Ping Hwung, David T. Crowe, Lee-Ho Wang and Ming-Jer Tsai, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

Three regions, -260 to -253 , -101 to -92 and -53 to -46 have been determined as essential for the expression of the rat insulin II gene by transfecting linker-scanning mutants into HIT cells, a hamster insulinoma cell line. Band-shifting and DNase I footprinting assays were employed to detect factors binding to the critical promoter element from -53 to -46. A binding activity which footprints the region between -60 and -40 was found in both HIT and HeLa cells. The mutation in this region which greatly decreased the promoter activity in transfection experiments also significantly reduced the binding activity, suggesting that this binding factor may play a functional role in the expression of the rat insulin II gene by binding to the critical region. This binding activity has been purified from HeLa cells. It co-purified with the COUP (chicken ovalbumin upstream promoter) transcription factor, a DNA binding protein which is required for efficient tanscription of the chicken ovalbumin gene in vitro. The footprint sequences of the COUP transcription factor in the insulin and ovalbumin promoters share limited similarity, but competition and renaturation analyses demonstrated that the insulin promoter binding factor is the same as the COUP transcription factor. The modes of binding revealed by the purine and phosphate contacts indicate that the COUP transcription factor binds to the two promoters in generally similar manners, but there are some major differences. The most striking difference is that the COUP transcription factor wraps around the ovalbumin promoter, whereas it binds to only one face of the DNA duplex on the insulin promoter.

O 163 MULTIPLE DNA-PROTEIN BINDING DOMAINS INTERACT TO MODULATE CELL-SPECIFIC AND c-AMPRESPONSIVE EXPRESSION OF THE GLYCOPROTEIN HORMONE α-GENE, J. Larry Jameson, J. Paul J. Deutsch, Alvin C. Powers, and Joel F. Habener, Thyroid Unit and Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Boston, MA 02114.
The human glycoprotein hormone α-gene is a cAMP-responsive gene expressed specifically in pituitary and placenta. Control sequences from the α-gene were characterized in transient expression assays using deletions of the 5'-flanking sequence linked to the chloramphenicol acetyl transferase (CAT) coding sequence. The α-gene sequence between -236 and -100 confers both tissue-specific expression and cAMP-responsiveness to the α-promoter and to several heterologous promoters (glucagon, somatostatin, SV40, chorionic gonadotropin-β). The α-gene and somatostatin promoters were most receptive to modulation by the α-gene enhancer. The cAMP-response element (CRE) (-146 to -111) corresponds to an 18 bp repeated sequence containing a palindrome, TGACGTCA. An up- stream enhancer sequence (UES; -169 to -147) adjacent to the CRE confers cell-specific expression, but requires the CRE for function with heterologous promoters. DNase I footprinting of the α-gene sequence between -236 and -100 demonstrates two major pro- tected regions: -178 to -156 corresponding to the upstream enhancer sequence (UES) and -146 to -112 corresponding to the 18 bp repeated sequences that contain cAMP-responsive enhancer (CRE). Binding to a DNA fragment (-236 to -100) that contains both the UES and CRE domains was 10-fold more effective than that using either fragment alone. In summary, there are multiple adjacent DNA-protein binding domains in the human α-gene that interact to facilitate protein binding and to synergistically stimulate transcription.

O 164 PHORBOL ESTER REGULATION OF MURINE INTERLEUKIN-2 TRANSCRIPTION, David Jensen, Bart Kleyn and Julianne Sando, University of Virginia, Charlottesville, VA 22908.
Phorbol Esters (PE) cause multiple effects in sensitive mouse EL4 thymoma cells. Among these is the accumulation of mRNA for several lymphokines including Interleukin 2 (IL2) and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF). Past work on the PE signal transduction pathway has identified early events associated with activation and translocation of Protein Kinase C to membrane, and intermediate steps blocked by inhibitors at specific times (eg. a pretranscriptional protein synthesis-sensitive step occurs between 1 and 2 hours after PE addition [Harrison et. al, J. Biol. Chem., 262, 234, 1987]). Investigation of mechanisms for the signal transduction to nuclei have yielded evidence against existence of PKC in nuclei of EL4 cells or translocation of the enzyme to nuclei following exposure of the cells to PE [Jensen and Sando, Cancer Res. 47, 3686, 1987]. This suggests, instead, that a substrate(s) may be necessary for the signaling process. Because the PKC pathway diverges at the level of substrates, it is difficult to identify and follow only those substrates involved in the PE signal to the nucleus.

Thus, the identification of factors involved in the distal end of the PE signaling pathway is being pursued. Nuclear run-on experiments have confirmed that the increase in IL2 and GM-CSF mRNA seen in these cells is due to an increase in the transcription rate. Current work is addressed at identifying factors present in phorbol ester-treated (not control) cells which can stimulate transcription of IL2 and GM-CSF in vitro.

O 165 **THE S1-SENSITIVE FORM OF $d(C\text{-}T)_{18}{\cdot}d(A\text{-}G)_{18}$ IN PLASMIDS: CHEMICAL PROBING RESULTS SUPPORT A TRIPLE-HELICAL MODEL.** Brian Johnston, Dept. of Biology, MIT, Cambridge, MA 02139.

Homopurine/homopyrimidine (Pu/Py) sequences are frequently found in flanking regions of eukaryotic genes, often near or within promoter regions. Some of these sequences are sensitive to S1 nuclease both in vivo and, in supercoiled plasmids, in vitro. One Pu/Py sequence derived from the 3' flanking region of a human U1 gene, $d(C\text{-}T)_{18}{\cdot}d(A\text{-}G)_{18}$, undergoes a transition to an altered, underwound structure which is sensitive to a variety of single-strand-specific nucleases under the influence of mildly acid pH and/or supercoiling (Htun et al., PNAS 81, 7288-7292, 1984). I have used chemical probes which are sensitive to unusual DNA conformations (Johnston and Rich, Cell 42, 713-724, 1985) to examine the region of the U1 gene containing this sequence in a plasmid provided by H. Htun and J.E. Dahlberg. The $d(C\text{-}T)_{18}{\cdot}d(A\text{-}G)_{18}$ sequence exhibits hyperreactivity to hydroxylamine, methoxylamine, osmium tetroxide and diethyl pyrocarbonate (DEP) under conditions of pH 6 or lower and a negative superhelical density of 0.05 or higher. The patterns of reactivity in the purine and pyrimidine strands are distinctly different. On the CT strand there is a region of hypersensitivity in the middle and at one end of the CT tract, while the AG strand shows hyperreactivity to DEP from one end up to and including the middle of the AG tract. Under some conditions there are nearby regions of hypersensitivity indicative of other secondary structures as well. These results provide considerable support for the model proposed by Lyamichev *et al.* (J. Biomol. Struct. Dynam. 3, 667-669, 1986) for CT/AG sequences, in which the C-T double-helix disproportionates into a triple-helical region and a single-stranded region. In the case of the U1 CT/AG sequence, I find that only one of the two possible isomers of this structure is formed at significant levels.

O 166 INTERACTIONS OF GAL4 PROTEIN WITH DNA AND OTHER PROTEINS. Stephen A. Johnston, John M. Salmeron, Scott Langdon, Jackie Bromberg and Chris Corton. Duke University, Durham, N.C. 27706.

We are studying three activities of the positive regulatory protein, GAL4, from yeast: 1) How does GAL4 bind DNA, 2) How does GAL4 interact with the negative regulator GAL80 and 3) With what transcriptional factors does GAL4 interact. We have proposed that the DNA binding specificty of GAL4 protein is not conferred by the cysteine finger motif but by the region immediately downstream. We present experiments that test this hypothesis. We have developed an in-vitro assay of GAL4-GAL80 protein interaction. We have used this assay to demonstrate that the binding of GAL80 to GAL4 and its repression of GAL4-mediated transcriptional activation are separable functions. We have used a genetic selection for genes which encode transcriptional factors interacting with GAL4. We present the characterization of these factors.

O 167 SEQUENCE SPECIFIC DNA-BINDING PROTEINS IN DEVELOPING SEEDS OF BRASSICA NAPUS. L-G Josefsson, M.L. Ericson, M. Ellerström, H-O Gustafsson, M. Lenman, E. Murén, J. Rödin, K. Ståhlberg and L. Rask.
Dept of Cell Research, Swedish University of Agricultural Sciences, Uppsala Biomedical Center, Box 596, S-75124 Uppsala, Sweden

We have detected several DNA-binding proteins present in developing seeds and capable of interacting with short ds oligonucleotides in a sequence specific manner. The different oligo used contain sequences found in the upstream (promotor) region as well as downstream from a gene encoding a major rapeseed storage protein, Napin. We are presently performing correlative studies (between expression and presence/activity of the binding proteins) and are also trying to establish a functional assay system (in vitro initiation/transcription) in order to assertain a role for these proteins in the regulation of the storage protein genes. Compared to other plant gene systems, the above is the first description of a gene which binds several distinct factors, the potential function of which are to regulate its expression.

O 168 BOTH PROMOTER AND ENHANCER OF IMMUNOGLOBULIN GENES ARE TARGETS FOR SUPPRESSION MECHANISM IN SOMATIC CELL HYBRIDS BETWEEN MOUSE MYELOMA CELLS AND MOUSE FIBROBLASTS. Steffen Junker, Viggo Nielsen, Didier Picard, and Patrick Matthias, Institute of Human Genetics, University of Aarhus, DK-8000 Aarhus C, DENMARK.

When immunoglobulin-producing B-cells are fused with fibroblastic cells, expression of immunoglobulins gets suppressed by a mechanism that selectively abolishes transcription of immunoglobulin genes. We have used gene transfection followed by cell fusion to define regulatory elements within the immunoglobulin transcriptional unit involved in that down-regulation. The transcriptional contributions of the heavy chain enhancer element and the Kappa light chain promoter were tested individually by fusing cloned, stably transformed mouse myeloma cells with mouse cl. 1D cells. To avoid significant loss of chromosomes, hybrid cells were isolated shortly after fusion by fluorescence-activated cell sorting and harvested within 2-3 weeks thereafter. On the basis of RNase protection mapping and of nuclear run-on assays, we show that both the kappa light chain promoter and the Ig heavy chain enhancer contain regulatory information that is made redundant or suppressed in the hybrid environment.

O 169 ISOLATION OF A FUNCTIONAL HUMAN GENE FOR BRAIN CREATINE KINASE, Ghaleb H. Daouk, Rima Kaddurah-Daouk*, Robert Kingston* and Paul Schimmel, Massachusetts Institute of Technology, Cambridge, MA 02139, and *Massachusetts General Hospital/Harvard Medical School, Boston, MA 02114.
There is evidence that the gene for the B-isozyme of creatine kinase is regulated during cell differentiation, is under hormonal control, and is activated in a small cell lung carcinoma. In order to investigate further the mechanisms of these processes, the human gene was isolated and the structure of the promoter region was determined. A human DNA fragment of 8 kbp was shown to encompass the entire coding region and 850 bp of 5'-flanking sequence. This fragment was transfected into 3 cell lines and shown to express functional enzyme. The 5'-end of the gene is split by a 230 bp intron that is located 12 bp upstream of the initiator ATG codon. Transcription initiation occurs at a site that is approximately 69 bp upstream of the 5'-end of this intron. The DNA sequence in the region upstream of the 5'-end of the mRNA is suggestive of two superimposed promoters that contain additional sequence elements that are known to regulate expression of other eukaryote genes. The 5'-region also has a remarkable homology to the overlapping promoters of the adenovirus EIIaE gene. These elements collectively form the basis for initial investigations of this gene's control mechanism.

O 170 SITE-SPECIFIC INTERACTION OF FIS PROTEIN WITH DNA AND CLONING OF THE *fis* GENE, Christian Koch, Falko Rudt and Regine Kahmann, Institut für Genbiologische Forschung Berlin GmbH, D-1000 Berlin 33 (West).
Host range switching in phage Mu is controlled by the site-specific inversion of the G segment. Inversion is catalyzed by the phage coded DNA invertase Gin which interacts specifically with the two recombination sites (IR). Recombination is greatly stimulated by a *cis* acting sequence that acts relatively independent of distance and orientation with respect to IR and has been termed recombinational enhancer. The stimulatory effect is mediated by a host protein termed FIS. FIS interacts with three sites within the enhancer, the binding is non-cooperative. Binding induces a conformational change in DNA and we demonstrate that a specific type of bending is prerequisite for enhancer function. Using synthetic oligonucleotides we have cloned the *fis* gene of *E. coli*. The protein is structurally unrelated to class II DNA binding proteins like HU and IHF. We propose a model for the interaction of FIS with the enhancer and speculate on possible roles for FIS in *E. coli*.

O 171 INDIVIDUAL INSULIN ENHANCER MODULES INTERACT WITH A β-CELL SPECIFIC PROTEIN AND MEDIATE POSITIVE β-CELL SPECIFIC REGULATION OF TRANSCRIPTION, Olof Karlsson[1], Helena Ohlsson[1], Michael D. Walker[2] and Thomas Edlund[1,2], [1]Dept. of Microbiology, University of UMEA, UMEA S90187, SWEDEN, [2]Dept. of Biochemistry, Weizmann Institute of Science, Rehovot 76100, ISRAEL.

The rat insulin enhancer has been shown to be of a mosaic structure and the cis-acting elements of importance for the enhancer activity have been defined. Two short 9bp sequences designated IEB1 and IEB2 are of crucial importance for the enhancer activity since mutation of either sequence leads to a 10-fold decrease in activity and the double mutant eliminates all enhancer activity. We demonstrate that synthetic oligonucleotides carrying either IEB1 or IEB2 can confer β-cell specificity on a heterologous promoter and when combined restore a significant part of the insulin enhancer activity. In addition we have shown that these two sequences interact with an identical or very similar β-cell specific nuclear protein designated IEF1. We propose that the interaction of IEF1 with IEB1 and IEB2 control the β-cell specific activity of the insulin gene enhancer.

O 172 MUTATIONS IN THREE DIFFERENT UPSTREAM ELEMENTS INHIBIT THE ACTIVITY OF THE MOUSE α2 (I) COLLAGEN PROMOTER, G. Karsenty, P. Golumbek, A. Hatamochi and B. de Crombrugghe, The University of Texas M. D. Anderson Hospital and Tumor Institute.

The promoter of the mouse α2 (I) collagen gene contains at least three different positive cis-acting elements in addition to the TATA box between +1 and -350. A cell specific enhancer element is also present in the first intron of this gene. The first of these promoter elements contains a CCAAT sequence between -80 and -84. This element binds a factor consisting of two different components which are both needed for DNA binding. Four different point mutations in the CCAAT motif, which strongly inhibit binding of this CCAAT binding complex, each decreases the activity of the promoter at least 8-fold when assayed by DNA transfection of NIH 3T3 fibroblasts. The second cis-acting element is located around -250. Two mutations in this element, one a 3 bp and another a 4 bp substitution decrease the activity of the promoter in DNA transfection experiments by about 8-fold. A fragment which contains one of these mutations does not compete for binding of a protein to the wild type sequence around -250. The third element is located between -315 and -295 and binds NF1. Both one bp and two bp mutations in this site decrease the activity of the promoter 8- to 10-fold in DNA transfection assays and inhibit binding of NF1. This element also mediates the activation of the α2 (I) collagen promoter which is produced by treatment of fibroblasts by TGF-β. Our results indicate that the integrity of each of these three cis-acting elements is required for optimal activity of this promoter and suggest cooperativity between the three different promoter DNA binding factors in the formation of a transcription initiation complex.

O 173 INDUCTION OF THE C-FOS GENE AT BIRTH IS ASSOCIATED WITH AN INDUCIBLE NUCLEAR BINDING PROTEIN(S). John Kasik, Ben-Zion Levi, Peter Burke and Keiko Ozato, NICHD, NIH, Bethesda, MD, 20892.

c-fos, the cellular homologue of the viral oncogene v-fos, codes for a 55 kD nuclear binding protein. In mice,there is low constitutive expression of the gene during the perinatal period with a 10- to 40-fold induction occuring on the day of birth. To further define the events involved in the induction of this gene, nuclear extracts were prepared from mouse liver according to the method of Gorski, et al. (Cell 47:467). Tissue was collected at 19 days gestation, on the day of birth, at 7 days of age and from adult mice. A radiolabeled 105 bp Ava II - Nae I fragment probe containing murine c-fos enhancer elements was prepared and gel retardation experiments were performed. Three regions were identified which could be competed away using an unlabeled 34 bp synthetic oligonucleotide which corresponded to the serum response element. Region one was the uppermost set of bands. These were most intense in adult tissue and of diminished intensity in 19 day, day of birth and 7 day tissues. Region two consisted of multiple bands which were expresssed with equal intensity at all four ages. Region three bands were weakest in adult tissue and of low intensity in day 19 and day 7 tissue. These bands were very intense on the day of birth. No regions were identified which could be competed away with unlabeled nonspecific synthetic oligonucleotide or with synthetic oligonucleotide specific for sequences of the 5' regulatory region of c-fos gene. The prominence of region three on the day of birth suggests that this protein is involved with the induction of c-fos gene.

O 174 REGULATORY EFFECT OF Ap4A AND ITS BINDING PROTEIN ON THE INITIATION OF DNA REPLICATION, K. Kuratomi and Y. Kobayashi, Tokyo Medical College, Tokyo 160, JAPAN

It was shown in our experimental result (1) that the exsistence of low concentration(10^{-7} to 10^{-9}M) of diadenosine 5',5'''-P^1,P^4-tetraphosphate (Ap4A) stimulated the initiation of DNA replication on pSY317 DNA containing oriC (2) in the presence of fraction II (3). Basing on this result, the analysis of a DEAE-cellulose column chromatogram fraction from fraction II with preparative isoelectric focusing electrophoresis indicated that the Ap4A-binding activity occurs in both acidic and basic fractions (pI=4 and 8). Further fractionation of the acidic fraction revealed that three proteins [fraction A (64KDa), fraction B (40KDa) and fraction C (14KDa)] have Ap4A-binding activity in the presence of Mn^{2+} or Fe^{2+} and DTT or gluthathione. The Ap4A-binding activity of the fraction B was enhanced in the presence of dnaA protein or cAMP while DNA polymerase I and cGMP were inhibitory and GMP had no effect. On the other hand, the fraction B also stimulated the conversion of the superhelical pSY317 to relaxed form with topoisomerase I. The addition of Ap4A further increased this activity. Both topoisomerase I and RNase H were found to be the factors which determine the specificity of the initiation of DNA replication from oriC (4). Since the coexistence of Ap4A, Fe^{2+} and Ap4A-binding fraction B in the oriC-initiation assay system stimulated the initiation activity, the above results may suggest that Ap4A-binding protein and Ap4A participate in the regulatory mechanisms of the initiation of DNA replication.

(1) Kobayashi & Kuratomi (1985) 13th Intl. Cong. Biochem., TU-021, p.235. (2) Yasuda & Hirota (1977) PNAS 74, 5458. (3) Fuller, Kaguni & Kornberg (1981) PNAS 78, 7370. (4) Fuller et al. (1983) Machanisms of DNA Replication and Recombination p.275, Alan R. Liss, Inc., NY.

O 175 SCREENING FOR NOVEL TYPE II RESTRICTION ENDONUCLEASES, Christoph Kessler, Bryan J. Bolton, Klaus Kaluza, Gabriela Herz, Michael Jarsch, Gerhard Berger and Gudrun Schmitz. Boehringer Mannheim GmbH, Biochemica Forschung, Nonnenwald 2, D-8122 Penzberg, FRG.

We have screened 760 different non-pathogenic bacteria of the groups listed below for the presence of potentially new type II restriction endonucleases. Among all Enterobacteriaceae and Lactobacillaceae screened we could detect only few site-specific activities, whereas in all other groups we found a high number of species producing different type II restriction endonucleases: Actinomycetes: 99/171 (58%); Bacillaceae: 37/64 (58%); Cytophagaceae: 17/18 (94%); Enterobacteriaceae: 2/83 (2%); Lactobacillaceae: 0/31 (0%); Pseudomonaceae: 5/38 (13%); Rhizobiaceae: 9/18 (50%); Streptococcaceae: 16/34 (47%); Acinetobacter: 11/26 (42%); Achromobacter: 93/221 (42%); Flavobacteria: 4/15 (27%). Data on new specificities are presented (1,2). In more detail a new class IIS restriction endonuclease, Ksp632I, with novel sequence specificity, discovered in Kluyvera species is described. Ksp632I recognizes the following assymmetric hexanucleotide sequence and cleaves 1 and 4 nucleotides, respectively, distal from the recognition sequence: 5'-GTCTTCN/NNN-N-3'
3'-CAGAAGN-NNN/N-5'.

Ksp632I may complement class IIS enzymes in the universal restriction approach and as a tool for generating defined unidirectional deletions or insertions.

1. Kessler, C., Neumaier, P.S. and Wolf, W. Gene 33 (1985) 1-102.
2. Kessler, C. and Höltke, H.-J. Gene 47 (1986) 1-153.

O 176 IDENTIFICATION OF KINASE C REGULATED TRANS-ACTING FACTORS CONTROLLING C-MYC GENE EXPRESSION, A. Christie King, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709. Phorbol esters and growth factors that stimulate phosphoinositide metabolism and generate the second messenger diacylglycerol (DG) stimulate transcriptional activation of a class of cellular oncogenes encoding for DNA binding proteins. Both phorbol esters and DG activate the calcium and phospholipid-dependent protein kinase C, an enzyme implicated to regulate c-myc gene expression. Analysis of the primary sequence of Kinase C predicts the presence of a putative DNA binding domain. In this study, we have purified Kinase C to apparent homogeneity and studied whether this enzyme is capable of binding directly to DNA in vitro. The purified enzyme has both calcium, phospholipid-dependent kinase activity and nonspecific DNA binding measured using DNA cellulose chromatography and a nitrocellulose filter assay. Protein blots of purified Kinase C hybridized with DNA probes support the notion that the holokinase (M_r 76 kD) is a DNA binding protein. The kinetics of inactivation of both activities by heat are identical. These data are among the first to suggest a nuclear location for Kinase C. Evidence that Kinase C is translocated to a nuclear site resistant to nonionic detergents after activation with phorbol esters will be presented. Using a nitrocellulose filter absorption assay, gel electrophoresis, and DNAse protection we have evident that Kinase C cooperates with other cellular factors to bind selectively to regulatory sequences in c-myc.

O 177 A CCAAT BINDING PROTEIN MEDIATES THE TRANSCRIPTIONAL RESPONSE OF THE HUMAN THYMIDINE KINASE PROMOTER DURING THE CELL CYCLE. G.B. Knight, J.M. Gudas, and A.B. Pardee. Dana Farber Cancer Institute, Boston, MA 02115.

Thymidine kinase (TK) expression parallels the onset of DNA synthesis. Both transcriptional and posttranscriptional controls appear to be involved in the more than 20 fold increase in TK mRNA observed at the beginning of S phase. Sequences in the 5' flanking region of the human TK gene have been shown to be important for transcription. An electrophoretic mobility shift assay was used to detect nuclear protein binding to the TK promoter. Throughout the G_0 to S phase transition, multiple specific nucleoprotein complexes were observed, although their abundance changed temporally. At the G_1/S boundary, the pattern of bound complexes found during G_1 changes dramatically to complexes of smaller apparent size. This change correlates well with the previously observed increase in TK mRNA transcription at this time. Using methylation interference assays, the binding site was determined to be an inverted CCAAT sequence. A pair of inverted CCAAT boxes located at -67 and -36 are contained within 19 bp repeats. To demonstrate the functional importance of the inverted CCAAT boxes to the cell cycle regulated transcription of TK mRNA, normal and mutant TK promoter/chloramphenicol acetyltransferase (CAT) gene chimeras were constructed and tested.

O 178 HOMOPURINE-HOMOPYRIMIDINE SEQUENCES: FORMATION OF THE TRIPLE-HELICAL STRUCTURE IN SUPERCOILED PLASMID DNA AND EFFECTS ON THE LEVEL OF GENE EXPRESSION.

Yoshinori Kohwi and Terumi Kohwi-Shigematsu, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Homopurine-homopyrimidine sequences found in promoter-regulatory regions of various eukaryotic genes are known to adopt a non-B DNA structure in supercoiled plasmid DNA. With the use of bromoacetaldehyde (BAA), a chemical specific for unpaired DNA bases, and subsequent use of S1 nuclease to cut the BAA-modified DNA, we previously reported that poly(dG)-poly(dC) sequences not only adopt BAA-reactive structure, but also induce their neighboring sequences to adopt such non-B structure. We have devised a chemical method to cleave DNA at the BAA-modified DNA sites and those of its analogue, chloroacetaldehyde (CAA), without the subsequent use of S1 nuclease. Using this alternative method, we found that, under torsional stress, a poly(dG)-poly(dC) sequence can form a dynamic structure containing triple strands. A specific triplex, either poly(dG)-poly(dG)-poly(dC) or poly(dC)-poly(dG)-poly(dC), is formed depending on pH and the presence or absence of magnesium ions. Interestingly, the formation of either triplex structure also depends on which DNA strand (top or bottom) is homopurine or homopyrimidine. A transfection experiment using the chloramphenicol transferase (CAT) gene fused to homopurine-homopyrimidine sequences revealed that this type of sequence had a dramatic enhancement effect on the level of CAT gene expression.

O 179 POSITIVE INTERACTIONS BETWEEN CAP AND RNA POLYMERASE ON BINDING AT THE *LAC* PROMOTER, Annie Kolb, Malcolm Buckle and Henri Buc, INSTITUT PASTEUR, 75724 PARIS CEDEX 15, France

Activation of transcription by the CAP protein in the presence of cAMP is a multistep process. *In vitro* we have found that activation already occurs at the level of closed complex formation for the *lac* and *gal* promoters, but can extend until the escape of the enzyme from the open complex in the case of the *malT* promoter. In order to determine whether CAP protein moves along with RNA polymerase during the early stages of transcription, we have analysed using specific antibodies the protein content of initiation and elongation complexes observed in gel retardation assays.

The approach allows a quantification of the positive interactions between CAP and polymerase at those various steps.

O 180 MULTIPLE TRANS-ACTING FACTORS BIND TO THE RAT ELASTASE I ENHANCER AND PROMOTER. Cynthia T. Komro, Carolyn H. Michnoff, Fred Kruse and Raymond J. MacDonald. University of Texas Southwestern Medical Center, Dallas, Texas 75235.

Elastase I (EI) is specifically expressed in the acinar cells of the exocrine pancreas. At least three distinct cis-acting elements are responsible for EI expression in pancreatic 266-6 cells (Kruse, F., Komro, C.T., Michnoff, C.H., and MacDonald, R.J., Mol. Cell. Biol., in press). Two of these elements lie within the tissue-specific EI enhancer and the third is located at the enhancer/promoter boundary. DNase I protection studies with nuclear extracts from pancreatic cells showed that factors bind to at least 5 discrete regions in the three elements. Two regions within 50 base pairs upstream of the transcriptional start site were also protected. Some mutations within the three cis-acting elements reduced protection of the corresponding regions from DNase I. Thus, we propose that specific proteins bound to the EI enhancer and promoter are involved in the transcriptional regulation of the EI gene. To investigate the presence of tissue-specific factors, liver extracts were compared to pancreatic extracts. All of the DNase I footprints seen with pancreatic extracts were also detected with liver extracts. A factor detected in liver but not pancreatic extracts bound to a region in the enhancer which does not appear to be involved in transcriptional regulation.

O 181 TISSUE SPECIFIC DNA-PROTEIN INTERACTIONS AT THE D-J-REGION OF THE MURINE IMMUNOGLOBULIN HEAVY CHAIN LOCUS, Andreas H. Kottmann, Hermann Eibel and Georges Köhler, Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, Federal Republic of Germany.
We are interested in the identification of (pre-B-) lymphocyte specific DNA-binding proteins involved in the ordered assembly of Ig-genes. In a first step we analyzed their potential binding sites at the Ig-heavy chain locus by screening the entire 3600 bp long region between the most proximal diversity element DQ_{52} and the heavy chain enhancer for tissue specific DNA-protein interactions. The complete region was subcloned as restriction fragments of about 220 bp in length and subjected to gel retardation- and DNA-foot print analysis using nuclear extracts from pre-B- and liver cell origin. The surprising result shows that all but two fragments interact with non-histone nuclear proteins. Out of a total of 16 fragments two carry (pre-B) lymphocyte and four liver cell specific protein binding sites. Eight fragments show binding to nuclear proteins of lymphocytes as well as of liver cells. The lymphocyte specific interactions, however, take place at sites located 5' to D- and J-elements, whereas the liver cell specific interactions employ sites located 3' to the J-segments. We are now in the process of purifying (pre-B-) lymphocyte specific factors and of cloning their genes.

O 182 DNA TWISTABILITY AFFECTS THE AFFINITY OF 434 OPERATOR FOR 434 REPRESSOR, G.B. Koudelka, P. Harbury, S. Harrison, M. Ptashne, Harvard Univ. Cambridge MA. 02138.
When 434 operator is bound to 434 repressor, the DNA, near the center of the operator, is overwound and the minor groove is compressed[1]. Additionally, the operator is gently bent around the protein. Evidently, on or all of these DNA deformations are required so that the ''recognition helix'', present in each monomer of the bound dimer, can make optimal contacts within each half-site of the dyad symmetric binding site. We have shown that the central four bp of the operator are not contacted by repressor, nevertheless, the operator's affinity for repressor is determined (in part) by the composition of these base pairs[2]. We have suggested that the base compostions near the center of the operator affects the operator's affinity for repressor by altering the ease with which the operator can be overtwisted and/or bent. We show here that both DNA flexibility and repressor flexibility influence the strength of the repressor-operator interaction: an operator with a single-stranded nick at its center has a higher affinity for repressor than does the intact operator: and a repressor bearing a mutation that presumably results in a relaxed dimer interaction is less sensitive than is wild-type to changes in the flexibility of the operator. We show that the central base pairs effect on operator affinity is independent of the slight overall bend of the operator seen in the repressor-operator complex. The effects on affinity that result from changes at the central operator positions are independent of the neighboring sequences, suggesting that the structure of the base pairs, not interactions between them, are responsible for the different twistabilities of different operators.
References 1. Anderson, J.E., et al. (1987) Nature 326, 846-852. 2. Koudelka, G.B., et al. (1987) Nature 326, 886-888.

O 183 ACTIVATION OF TRANSCRIPTION FACTOR IIIC BY THE ADENOVIRUS E1A PROTEIN, Robert Kovelman, Warren K. Hoeffler, and Robert G. Roeder, The Rockefeller University, New York, NY 10021.
The factor(s) responsible for the adenovirus E1A-stimulated transcription of RNA polymerase III genes was localized previously in a chromatographic fraction containing transcription factor IIIC (TFIIIC). In further studies of HeLa cell extracts, two distinct forms of TFIIIC, which were chromatographically separable, generated VA_1 RNA gene-protein complexes that were distinguished by electrophoretic mobility shift assays. The form of TFIIIC which generated the more slowly migrating promoter complex had greater transcriptional activity in vitro. DNase I protection and methylation interference assays did not reveal any differences in the binding sites of the two forms, but the transcriptionally more active form associated more rapidly with the promoter and formed a more salt resistant complex. A dramatic increase in the amount of the more active form of TFIIIC resulted from either E1A expression during infection or growth of the cells in a higher concentration of serum, and correlated with an increase in transcription in the extracts, whereas template commitment assays indicated that overall TFIIIC concentrations remained unchanged during viral infection.

O 184 OVEREXPRESSION OF THE HUMAN ESTROGEN RECEPTOR IN CHO CELLS. Peter J. Kushner[1], John Shine[1], John D. Baxter[1], and Geoffrey L. Greene[2], 1. U.C.S.F, San Francisco, CA 94143, and 2. University of Chicago, Chicago, ILL, 60637.
In order to prepare large amounts of the human estrogen receptor (ER) for biochemical and physical studies we have employed the cloned ER sequences to construct Chinese Hamster Ovary (CHO) cell line derivatives that overexpress the ER. The human metallothionein IIA promoter augmented with the SV40 enhancer was employed to drive transcription of the ER cDNA. Initial pools of neo^r CHO cells were derived by co-transfection with the ER vector along with pSV2neo and a plasmid spanning the complete human metallothionein IIA gene as a potential marker for gene amplification associated with selection for cadmium resistance. Cells from the initial pools contained up to 3 pM of receptor per mg cell protein, several fold more than MCF7 breast tumor cells, and responded to selection for cadmium resistance and subsequent stepwise amplification with increases in ER expression to levels greater than 100 pM per mg protein, equivalent to 0.66 % of cell protein or 8.5 million receptors per cell. The receptor from CHO cells has a Kd. of binding for estradiol of .5 nM, similar to that of MCF7 cells, and is active in conferring estrogen dependant stimulation of expression to a reporter gene bearing an estrogen responsive element. It appears, therefore, that these cells will be a good source of functional biosynthetic ER.

O 185 TRANSCRIPTIONAL REGULATION OF INTRACISTERNAL A-PARTICLE (IAP) GENES, Bruce T. Lamb, and Chin C. Howe, The Wistar Institute, Philadelphia, PA 19104.
IAP is an endogenous retrovirus-like element present in about 1000 copies in the mouse genome. Unlike infectious exogenous retroviruses, endogenous retroviruses in general appear to be non-pathogenic, perhaps due to the restricted expression of the IAP gene. However, in plasmacytoma cells, the IAP gene integrates into the flanking region of the c-mos oncogene and activates it. Thus, the study of regulation of IAP transcriptional repression and activation should help in understanding the transcriptional regulation of neoplasia. Using mouse embryonal carcinoma (EC) F9 cells, which differentiate to parietal endoderm (PE)-like cells when induced with retinoic acid, we showed that the IAP gene is efficiently expressed in PE-like cells but not in F9 cells. We have localized the promoter and enhancer regions of the gene and have demonstrated that one cellular factor in PE-like cells binds to the enhancer region, while in F9 cells, two factors, one apparently similar to the factor present in PE-like cells, bind to the enhancer sequence. Since the IAP gene is not efficiently expressed in F9 cells, the F9-specific protein may be a repressor and the PE-specific protein an activator. We plan to characterize these three trans-acting factors.

O 186 THE RATE OF COMPLEX FORMATION BETWEEN RNA POLYMERASE (RNAP) AND PROMOTER DETERMINES THE LEVEL OF REPRESSION BY E.COLI LAC REPRESSOR, Michael Lanzer and Hermann Bujard, Zentrum für Molekulare Biologie Heidelberg, 6900 Heidelberg, FRG.
Repressors of various operons bind within promoter sequences thereby preventing the association between RNAP and promoter. Direct competition between RNAP and repressor for their respective binding sites may therefore determine the level of repression in such systems. This suggests that changes in the rate of complex formation between RNAP and promoter would influence repression. We have therefore constructed several promoter - operator fusions which harbor the lac operator in a position corresponding exactly to the wt - situation. The analysis of these constructs in vivo shows a clear correlation between the level of repression and the rate of complex formation between RNAP and the promoter. The repressor - operator interactions remain unchanged.
When operators are placed into different regions of the 70bp promoter sequence levels of repression far exceeding the P_{lac} situation can be achieved. A model explaining these data will be discussed.

O 187 THE THYROID HORMONE RECEPTOR DOES NOT RECOGNIZE A CLASSIC CONSENSUS SEQUENCE. Thomas N. Lavin, Mark F. Norman, Peter Kushner, Brian West, James Apriletti and Daniel Catanzaro. Univ. of Calif. at San Francisco Med. Ctr., San Francisco, CA, USA and Univ. of Sydney, Sydney, Australia.

Multiple thyroid hormone receptor binding sites have been identified on the 5' flanking regions of three thyroid hormone responsive genes. Four such sites have been footprinted by methylation interfernce or MPE footprinting techniques. Other sites have been identified by direct binding studies with 24bp oligonucleotides. Sequences which bind the receptor have little sequence similarity and protected residues are spaced differently in different binding regions. Underlying periods of specific nucleotides have not been recognized. In one such binding region, DNA bending is induced by receptor binding. Destruction of the bending region destroys thyroid hormone response. Thus the thyroid hormone receptor binds to DNA in a unique and highly specific manner utilizing novel mechanisms of recognition which depend on the ability to induce a similar three dimensional structures of DNA.

O 188 ENHANCEMENT OF EXPRESSION OF ilvGp2 BY BINDING OF IHF TO ilvGp1, Robert P. Lawther, University of South Carolina, Columbia, SC, 29208.
Transcription in vitro of the ilvGMEDA operon regulatory region indicates two promoter-like sequences, ilvGp1 and ilvGp2, with the second promoter residing closest to the structural genes. Analysis of cellular RNA indicates that transcription in vivo initiates solely from ilvGp2. Our previous analysis indicated that transcription in vivo does not initiate from ilvGp1 because of the binding of an unknown factor that precludes the binding of DNA dependent RNA polymerase. Using polyacrylamide gel electrophoresis, immunity host factor (IHF) has been identified as the factor binding to ilvGp1. This analysis has included using both (a) extracts of strains defective for IHF formation, and (b) binding of ilvGp1 containing restriction fragments with purified IHF. The prescence of IHF represses transcription in vitro of ilvGp1 while enhancing transcription in vitro from ilvGp2.

O 189 ARABINOSE-DEPENDENT ARAC-BINDING TO DIFFERENT DNA SITES CONTROL TRANSCRIPTION OF ARABAD AND ARAC, Nancy L. Lee, Eileen P. Hamilton, & Christopher S. Franklyn, Univ. of California Santa Barbara, Santa Barbara, CA 93106. Transcription of araBAD is regulated both positively and negatively by AraC, while the divergent araC gene is down-regulated 10-fold by its own product. AraC protein binds four sites on the DNA: $araI_1$,$araI_2$,$araO_1$,& $araO_2$. The $araI_1$ & $araI_2$ sites are contiguous and next to the polymerase binding site of araBAD promoter; $araO_1$ is congruent with the polymerase binding site at the araC promoter, and $araO_2$ lies within the leader region of araC. It is known that, in the absence of arabinose, AraC binds $araO_2$ and araI to produce repression of araBAD (Dunn et al.,Martin et al.). We found that the araI site consists of two regions, $araI_1$ & $araI_2$; the binding to the downstream $araI_2$ site is inducer-dependent and requires simultaneous binding to $araI_1$. We propose that araBAD activation results from $araI_2$ binding, and that repression-loop formation involves $araI_1$ but not $araI_2$; the function of $araI_1$ is that of a switch. We also found that, in the absence of inducer, the binding of AraC to $araI_1/araO_2$ is responsible for not only repression of araBAD but also the autoregulation of araC. Upon inducer addition, autoregulation is maintained by switching AraC occupancy from $araI_1/araO_2$ to $araO_1/araO_2$. We propose that AraC carries out its selective and diversified control of the araBAD and araC promoters by alternating between two states of cooperative bindings to its cognate DNA sites. The above model is based on in vitro binding studies and in vivo assays of promoter activites in mutants carrying selectively inactivated DNA sites.

O 190 CLONING OF THE PHAGE P4 psu GENE AND DEMONSTRATION OF **Psu** ACTIVITY, Nora A. Linderoth and Richard Calendar, Dept. of Molecular Biology, University of California, Berkeley, CA 94720.

The product of the P4 psu (polarity suppression) gene can suppress polarity in the late operons of helper phage P2 by antitermination. Two possibilities are that **Psu** interacts directly with termination factor **Rho** to prevent its action or, that **Psu** interacts with RNA polymerase causing it to ignore termination signals. We have cloned the P4 psu gene into plasmid expression vectors which overproduce **Psu** protein upon induction. We have determined that plasmid-encoded **Psu** protein is biologically active *in vivo* by observing the effects of polarity suppression in P2. We demonstrate that **Psu** is the only P4 protein required for polarity suppression in phage P2. Northern blot analysis has been used to moniter termination and readthrough by *E. coli* RNA polymerase at specific termination sites *in vivo* in the presence and absence of **Psu**. We observe increased readthrough of the **Rho**-dependent terminator, λ t_{R1}, in the presence of **Psu**. **Psu** does not affect termination at three **Rho**-independent sites tested -- phage T7 TE, the *E. coli* trp attenuator, and rrnB T1 (*E. coli*). **Psu**-mediated antitermination does not require that transcription be initiated from a phage promoter, nor is there a requirement for an auxiliary transcription factor such as the P2 ogr or P4 delta proteins. We are currently investigating whether **Psu** possesses a preference to mediate antitermination through **Rho**-dependent versus **Rho**-independent termination sites.

O 191 DNA-PROTEIN INTERACTIONS ON THE YEAST GAL 1-10 PROMOTER: MECHANISMS FOR CONTROL OF GENE EXPRESSION, Dennis Lohr, Chemistry Dept., Arizona State University, Tempe, AZ, 85287-1604 and Tim Torchia and Jim Hopper, Dept. of Biological Chemistry, Penn State University, Hershey, PA, 17033.

GAL 1 and 10 are divergently transcribed genes involved in the utilization of galactose as a carbon source in yeast. Their expression is strongly carbon source dependent: highly expressed in galactose; not expressed but readily inducible in glycerol/ethanol or lactate; repressed in glucose. The major DNA sequence element for induction, the Upstream Activator Sequence (UAS), has been identified. An essential positive (GAL4) and a negative (GAL80) regulatory protein are known. These genes have been cloned and single and double disruption strains of yeast have been made: a 4^D strain which lacks 4 function; an 80^D strain which lacks 80 function; a $4^D/80^D$ strain which lacks both functions. Nuclei were isolated from these various strains and DNA/protein interactions on the GAL1-10 control region were assessed by DNase I and MPE-Fe (II) footprinting, using an indirect end label approach. This analysis was done on wild type cells grown in various carbon sources and in the various regulatory protein disruption strains. This produces a mechanistic picture of the events associated with gene regulation at this locus. We find a hierarchy of DNA protein interactions differing in strength and in location. For example there is a very strong GAL4/UAS interaction in expressed and in inducible cells. There is a weaker GAL80 dependent interaction at other locations which is less dependent on expression. A model for control of expression at this locus incorporating these and other known features of the system will be presented.

O 192 DNA FLEXIBILITY AND T-ANTIGEN OCCUPANCY IN THE SV40 TRANSCRIPTION COMPLEX. L.C. Lutter, B. Petryniak, K.G. Hadlock, M.W. Quasney, and N. Franken, Molecular Biology Dept., Henry Ford Hospital, Detroit, MI 48202.

We have characterized the degree of rotational flexibility of the DNA in the SV40 late transcription complex and total SV40 minichromosomes. Isolated total minichromosomes as well as transcription complexes each unwind by about one turn when the temperature is raised from 0° to 37°C, in the presence of topoisomerase, while free SV40 DNA unwinds by about 5 turns. Thus, the transcription complex differs very little from the bulk minichromosome population: each contains the equivalent of about 20% rotationally "free" DNA with the remainder of the DNA being constrained. This result differs significantly from that of the yeast 2 micron plasmid, where about 70% of the DNA behaves as if it were free [Saavedra and Huberman, Cell 45 65 (1986)]. These findings indicate there is a substantial difference between yeast and mammalian chromatin structure.

We have also characterized the presence of T-antigen on SV40 late transcription complexes. We find that approximately a quarter of the in vitro SV40 transcription activity in nuclear extract or in gradient-isolated minichromosomes can be specifically precipitated by polyclonal and certain monoclonal antibodies against T-antigen. Immunoprecipitation enriches for transcription complexes relative to bulk minichromosomes and selectively precipitates high-salt-active [300mM $(NH_4)_2SO_4$)] rather than low-salt-active [30mM $(NH_4)SO_4$)] in vitro activity. These results provide the first direct evidence for the presence of T-antigen on the late transcription complex and further indicate that T-antigen is present on a specific subset of that population.

O 193 PROCESSIVITY IN EARLY STAGES OF TRANSCRIPTION BY T7 RNA POLYMERASE, Craig T. Martin, Daniel K. Muller, & Joseph E. Coleman, Yale University, New Haven, CT 06510.
Immediately following initiation of transcription, T7 RNA polymerase enters a phase in which dissociation of the enzyme-DNA-RNA ternary complex significantly competes with elongation, a process referred to in the *E. coli* enzyme as abortive cycling [Carpousis, A. J. & Gralla, J. D. (1980) *Biochemistry 19*, 3245-3253]. Characterization of this process in the T7 RNA polymerase system under various reaction conditions and on templates with differing message sequences reveals that conversion to a highly processive ternary complex occurs after incorporation of 8 bases and that the relative competition between dissociation and elongation up to this point is influenced by several different forces. In particular, the sequence dependence of abortive fall-off suggests that dissociation is favored immediately following incorporation of UMP, and is less likely following incorporation of GMP into the RNA message. Abortive cycling is unchanged in transcription from a synthetic oligonucleotide template which is double-stranded in the promoter region, but single-stranded throughout the entire message region. This result proves that melting and reannealing of the DNA duplex in the coding region do not contribute to abortive cycling. Furthermore, weakening of promoter binding by an order of magnitude affects abortive cycling only slightly, suggesting that strong interactions with the promoter are not the major cause of abortive cycling. Kinetic analyses show that conversion to a highly processive ternary complex after the incorporation of 8 bases may reflect a large decrease in the unimolecular rate of dissociation of the complex due to increased contacts between the nascent RNA and the DNA template and between RNA and enzyme. Finally it is shown that when transcribing a message of initial sequence GGG..., in the presence of GTP as the sole nucleotide, T7 RNA polymerase synthesizes a ladder of poly-r(G) transcripts due to a slipping of the RNA message along the DNA template. This ladder is largely truncated at a length of 14 bases, providing information on steric constraints in the ternary complex positioned at +3 on the DNA template.

Poster Session 2

O 194 POLYAMINE BINDING SITES ON NUCLEOSOME CORE PARTICLES, Harry R. Matthews and James E. Morgan, University of California, Davis.
Polyamines and acetylpolyamines are naturally-occurring cations that bind to DNA and chromatin *in vitro* and probably *in vivo*. Thermal denaturation and circular dichroism studies show that polyamines and acetylpolyamines increase the stability and change the conformation of DNA in the nucleosome core particle (J.E. Morgan, J.W. Blankenship and H.R. Matthews, 1987, Biochemistry 26 3643-3649). These effects are primarily charge-dependent, although secondary effects are also observed. Acetylation of polyamines partially reverses these effects, mainly through loss of charge. Thermal denaturation and circular dichroism effects display saturation at approximately 30 to 40 spermidine molecules added per core particle. Determination of the location of the preferred binding sites on nucleosome core particles is being approached by the use of a photoaffinity polyamine analogue (Azido-Nitro-Benzoyl-spermine, ANB-spermine). ANB-spermine appears to stabilize nucleosome core particles similarly to the naturally occurring polyamine, acetylspermine. Photoaffinity labeling studies with ANB-spermine show covalent attachment of ANB-spermine to both protein and DNA in the nucleosome core particle. The locations on the DNA have been determined by ^{32}P end-labeling and an exonuclease protection technique. There are 6 to 8 regions of preferential binding, all in areas where the two turns of the DNA helix are closely apposed. Evidently there are quite specific features of the core particle which determine polyamine binding sites. Further studies of these sites are in progress. Supported by the National Science Foundation.

O 195 STUDIES ON PROTEIN BINDING TO THE κE2 SITE OF THE HUMAN IMMUNOGLOBULIN κ ENHANCER, Edward E. Max, Jeffrey M. Gimble, James R. Flanagan and David Recker, NIAID, NIH, Bethesda, MD 28092
We have been analyzing the in vitro binding of nuclear proteins to the the human κ enhancer using an exonuclease protection assay. Protected domains demonstrated with λ exonuclease and T7 gene 6 exonuclease are not identical. Both exonucleases detect a protected domain in the 5' region of the enhancer that includes sequences homologous to the κB and κE1 motifs described in the mouse enhancer. T7 exonuclease also detects a protected domain in the 3' region that includes κE2. Competition experiments with several oligonucleotides demonstrate that this binding is sequence specific. Proteins with κE2 binding activity producing identical exonuclease protection bands have been found in nuclear extracts of several tissues and species. A κE2 binding protein (NF-κE2) from pig spleen has been purified approximately 5000-fold by ion exchange and oligonucleotide affinity chromatography. Evidence suggests that it may also bind to kE3, but less strongly. Partially purified NF-κE2 produces a 27 bp DNase I footprint centered over the kE2 site.

O 196 IDENTIFICATION OF A DOMAIN IN THE PHAGE T3 AND T7 RNA POLYMERASES THAT IS RESPONSIBLE FOR SPECIFIC PROMOTER RECOGNITION, William T. McAllister and Keith Joho, SUNY-Health Science Center at Brooklyn, Brooklyn, New York 11203.
Bacteriophages T3 and T7 encode single subunit DNA-dependent RNA polymerases of ca 100 kd (883 amino acids). Despite a high degree of structural similarity between the two enzymes (their amino acid sequences are 82% identical), neither enzyme will utilize promoters that are recognized by the other. The promoter sequences for the two phage polymerases, which are closely related to a highly conserved 23 bp consensus sequence differ significantly only over the 3 bp region from -10 to -12. We have synthesized promoter variants that differ at only 1 position and have found that the primary determinant of promoter specificity is the bp position -11.

Fine structure restriction mapping of T3/T7 recombinant phage indicates that the specificity-determining region of the polymerase lies in the carboxy terminal portion of the protein distal to amino acid 616. To more closely define the region of promoter specificity, a series of hybrid T3/T7 RNA polymerase genes was generated in vitro using cloned genes. Analysis of the resulting hybrid enzymes demonstrates that the region responsible for promoter specificity lies between amino acids 672-754. This region does not appear to be related to the helix-turn-helix motif found in other sequence-specific DNA binding proteins. Within the 80 bp region there are only 11 amino acid substitutions between T3 and T7. Site directed mutagenesis to assess the importance of each of these amino acid residues to specificity is being carried out.

O 197 INDUCIBLE AND CONSTITUITIVELY BOUND TRANS FACTORS INTERACT WITH a CYCLIC AMP RESPONSIVE ELEMENT OF THE HUMAN GONADOTROPIN ALPHA GENE. Craig E. McKnight*, Jerome F. Strauss III, Alfredo Ulloa-Aguirre, Davor Solter*. University of Pennsylvania and Wistar Institute*, Philadelphia, Pa 19103.
Human chorionic gonadotropin (HCG) is a glycoprotein hormone composed of an alpha and a beta subunit both of which are expressed in the placenta. The alpha protein is encoded by a single copy gene and is the same alpha subunit found in the anterior pituitary heterodimeric proteins luteinizing hormone, follicle-stimulating hormone and thyroid stimulating hormone. The actual physiologic trigger responsible for the induction of HCG is, as of yet, undefined, however it is well accepted that cAMP plays a role in increasing the transcription of the alpha subunit. We have shown that a trans-acting factor is present in the nucleus of primary placental trophoblasts and that this factor appears to bind constituitively to a cAMP responsive cis element (CRE) of the alpha gene. In addition, we have found that a cAMP inducible binding factor is also present in these cells and interacts with the CRE region. We have determined the sequences protected by these factors within the CRE by methylation interference and are in the process of trying to characterize these proteins.

O 198 FORMATION OF A PRE-INITIATION COMPLEX AT THE ADENOVIRUS MAJOR LATE PROMOTER.ROLE OF MLTF, Alejandro Merino, Leonard Buckbinder, Juan Carcamo and Danny Reinberg. Department of Biochemistry, University of Medicine & Dentistry of N.J.-Robert Wood Johnson Medical School. Initiation of transcription from the Ad-MLP required five general factors (IIA,IIB,IID, IIE,IIF) in addition to RNA polymerase II.These factors associated into a complex in the absence of ribonucleoside triphosphates when incubated with DNA containing a promoter.The addition of MLTF to reactions reconstituted with the general factors and the MLP resulted in a 10 to 20 fold stimulation of the overall transcription reaction; however, when the reaction was limited to one round of transcription,MLTF stimulated five-fold.This stimulation was due to an increase in the amount of preinitiation complex formed. The first step in the formation of a preinitiation complex,the committed complex is slow and requires transcription factors IIA and IID.The second step involved the binding of RNA polymeraseII which required a TFIID bound to the TATA sequence.The third step involved the association of transcription factors IIB, IIE and IIF; these factors associated with the complex by interacting with the RNA polymerase II. The MLTF stimulation was due to an increase in the number of template DNA molecules that formed a committed complex. The direct effect of MLTF was to establish a more stable committed complex. Committed complexes formed in the presence of MLTF remained bound to the DNA after transcription by SP6 or T7 RNA polymerases through the major late promoter. Reactions carried out in the presence of MLTF allowed multiple RNA polymerase molecules to enter into the transcription cycle.

O 199 MODULATION OF EGF RECEPTOR GENE EXPRESSION VIA A S1 NUCLEASE-SENSITIVE DIRECT REPEAT SEQUENCE, Glenn Merlino, Yoshihiro Jinno, Alfred Johnson and Ira Pastan. Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD 20892.

Regulation of expression of the epidermal growth factor (EGF) receptor gene, the cellular counterpart to the avian erythroblastosis virus erbB oncogene, is not well understood. Previously, we reported the isolation and partial characterization of the EGF receptor promoter. The promoter is highly GC rich, contains no TATA box or CAAT box, and has multiple transcriptional start sites. An S1 nuclease-sensitive site has now been found approximately 100 bp upstream of the major in vivo transcriptional initiation site. Two sites of direct repeat sequences are found in this area: one contains 14 out of 15 identical base pairs, the other 10 out of 10 base pairs. Both direct repeat sequences conform to the motif TCCTCCTCC. When deletional mutations are made in this region using either Bal31 or S1 nuclease, in vivo activity drops 3-5 fold, based on transfection analysis in monkey kidney CV-1 cells and human epidermoid carcinoma KB cells using promoter-CAT constructions. Primer extension analysis of RNA isolated from CV-1 cells transfected with normal or mutated EGF receptor promoter-CAT plasmids confirmed this change in gene expression. Examination of nuclear protein binding to normal and deleted promoters by gel retardation analysis revealed that at least one specific factor which binds to the S1 nuclease-sensitive region cannot bind to the mutated promoter. This binding site was confirmed by DNase I footprinting. These results suggest that a nuclear DNA binding protein interacts with the direct repeat region of the EGF receptor promoter and either directly or indirectly stimulates transcription.

O 200 INTERACTIONS BETWEEN THE HOX 2.3 PROMOTER REGION AND REGULATORY PROTEINS PRESENT IN NUCLEAR EXTRACTS. Frits Meijlink, Peter Verrijzer, Wim de Graaff and Jacqueline Deschamps, Hubrecht Laboratory, Uppsalalaan 8, 3584CT Utrecht, The Netherlands.

Homeobox containing genes of the mouse are believed to have an important function in embryogenesis, possibly as essential as that of their Drosophila homologs. Previously, we cloned and characterized the mouse Hox 2.3 gene which is located on chromosome 11. Its sequence shows a high degree of similarity to that of the Hoxl.1 gene on chromosome 6, extending upstream from the protein coding region. High expression of Hox 2.3 is detected in specific regions in the spinal cord of mid to late gestational embryos and in embryonal carcinoma cells only after retinoic acid treatment. We are interested not only in the way these genes may control differentiation by regulating expression of other genes, but also in the regulation of of expression of Hox genes themselves. We attempt to identify protein factors, and eventually the genes encoding these proteins, by studying interactions between putative control regions of the mouse Hox2.3 gene and specific components of nuclear extracts from embryonal carcinoma and other cells.

O 201 MOLECULAR MODELING OF ZINC BINDING DOMAINS FROM XENOPUS TRANSCRIPTION FACTOR IIIA. George S. Michaels, Bernard R. Brooks and Richard J. Feldmann, National Institutes of Health, Bethesda, Md. 20894.

The zinc binding repeat was first described for the *Xenopus* transcription factor IIIa (TFIIIa). This protein contain nine tandem repeats of the 30 amino acid domain consisting of two cysteine and two histidine residues that can coordinate zinc ions in a tetraheadral fashion. This zinc binding motif has observed in the sequences of several other proteins that are also suspected to be involved in transcriptional regulation. In order to better understand the structural characteristics of these zinc binding domains, we analyzed each of the "Zinc Finger" domains from TFIIIa by molecular dynamics.

The results of the molecular dynamics and minimizations revealed that two of the "Zinc Fingers" may fold to form stable α helical regions, while the other domains appeared to have a more extended structure. The ability of these zinc binding domains to form α helices suggests an alternate mechanism for the formation of a divergent helix-turn-helix DNA binding region that would facilitate the site specific interaction with the internal control regions of *Xenopus* 5s RNA genes.

O 202 MECHANISM OF LYMPHOKINE GENE ACTIVATION BY ANTIGEN AND HTLV-I ENCODED pX IN T CELLS. Shoichiro Miyatake, *Rene De Waal Malefyt, Yutaka Takabe, Takashi Yokota, Joseph Shlomai, *Jan De Vries, Naoko Arai, #Motoharu Seiki, #Mitsuaki Yoshida and Ken-ichi Arai. DNAX Research Institute, Palo Alto, CA 94304, USA; *UNICET Laboratory for Immunological Research, 27 Chemin des Peupliers, 69573 Dardilly, FRANCE; #Department of Viral Oncology, Cancer Institute, Kami-Ikebukuro, Toshima-ku, Tokyo 170, JAPAN.

Transcription of various lymphokine genes in T cells can be induced by antigenic stimulation by treatment of T cells with lectins, phorbol esters (TPA and Ca ionophore (A23187). Many HTLV I transformed T cell lines produce various lymphokine genes constitutively, and it is suggested that the p40x gene product encoded by the HTLV I genome may act as a transcriptional activator of various lymphokine genes. To study the mechanism of lymphokine gene activation by different stimulatory signals or by HTLV I infection, we have constructed fusions between the bacterial CAT gene and the 5' flanking regions of several lymphokine genes. A GM-CSF CAT fusion transfected into the Jurkat human T cell line could be activated by treatment with TPA and PHA or PHA and A23187. The GM-CSF and IL-3 promoters could also be activated when co-transfected with a constitutive p40x plasmid in Jurkat cells, Hela and L cells. Deletion studies of the GM-CSF promoter revealed that the region 73 to 95 bp upstream of the transcription initiation site is required for stimulation by either TPA/PHA or p40x in Jurkat cells. Interestingly, the SV40 enhancer also responds to TPA/PHA or p40x, although there are no significant sequence homology between the SV40 enhancer and the GM-CSF 5' region.

O 203 THE HIGH RESOLUTION STRUCTURE OF PHAGE 434 REPRESSOR AMINO TERMINAL DOMAIN AND CRO PROTEINS. A.Mondragon, S.Subbiah, S.Almo, C.Wolberger, M.Drottar, M.Ptashne and S.C.Harrison, Harvard University and Howard Hughes Medical Institute, Cambridge MA 02138.

The repressor and cro proteins of phage 434, a close relative of phage lambda, bind to a set of six related 14 base-pair operator sites in the phage genome. The binding affinity for these site differs from site to site for the two proteins. This differential binding affinity creates a regulatory switch.

The crystal structures of both the repressor amino terminal domain and cro proteins have been determined and refined to 2.0 A and 2.35 A respectively. The two proteins are very similar, having an identical fold mwith five alpha helices. The 'helix-turn-helix' motif is almost identical in both proteins and similar to that found in other DNA binding proteins. The main differences occur in a region known to interact directly with the DNA phosphate backbone. These differences may affect the way both proteins bind to DNA.

O 204 IDENTIFICATION OF TWO YEAST rRNA ENHANCER BINDING PROTEINS, REB1 AND REB2, Bernice E. Morrow and Jonathan R. Warner, Albert Einstein College of Medicine, Bronx, NY 10461.

There are approximately 200 tandem repeats of rRNA genes transcribed by RNA polymerase I in the yeast, Saccharomyces cerevisiae. We have localized an enhancer of rRNA transcription to a 190 bp fragment near the site of termination of the upstream gene, 2.2 kb from the initiation site of the downstream gene (Elion and Warner, MCB, 6, 2089 (1986). We have identified two factors (REB1 and REB2) that bind to independent sites within the 5' region of the enhancer. The predominant complex in band shift assays results from the binding of REB1 to the enhancer. REB1 protects approximately 10 nucleotides on duplex DNA from DNase 1 cleavage. REB2 binds to a different sequence, 20 bp downstream from the REB1 site. Synthetic double stranded oligonucleotides containing the REB1 site were ligated to form concatamers and used in competitive band shift assays. The binding of REB1, but not of REB2, to the enhancer DNA fragment was inhibited by the synthetic oligomer. Purification of REB1 and REB2 is underway. Neither the synthetic oligomer nor the rRNA enhancer was found to compete with a protein which binds specifically to the HOMOL 1 and RPG sequences of the ribosomal protein gene, CYH2 suggesting that the synthesis of ribosomal RNA and ribosomal proteins are not coordinately regulated by the same DNA binding protein.

O 205 THE EFFECT OF NUCLEOSOME POSITION ON IN VITRO TRANSCRIPTION OF 5S RNA BY RNA POLYMERASE III. Randall H. Morse & Robert T. Simpson, National Institutes of Health, Bethesda, MD 20892.

The effect on transcription of incorporating portions of the 5S RNA gene into nucleosomes was determined by transcribing reconstituted chromatin in an activated frog egg extract. Nucleosomes were assembled with chicken erythrocyte histones and the plasmid pXBS201, which bears the 5S RNA gene. Treatment of these reconstituted plasmids with a restriction endonuclease whose recognition sequence lies within the transcribed region results in cleavage of all those molecules in which the recognition sequence is not protected by histone proteins. When such a sample is transcribed, any full length transcripts observed must arise from templates bearing a nucleosome at the given restriction endonuclease recognition sequence. By using this protocol, we have found that nucleosomes on or in the near vicinity of the TFIIIA binding domain completely inhibit transcription. To look at the effect of nucleosomes downstream of the TFIIIA binding site, we constructed 5S maxigenes by placing DNA inserts between the TFIIIA binding site and the transcription termination signal. Experiments with these constructs show that RNA polymerase III is not impeded by downstream nucleosomes. We are presently attempting to determine whether or not these nucleosomes are displaced during transcription.

O 206 FUNCTIONAL ELEMENTS OF THE STEROID HORMONE-RESPONSIVE PROMOTER OF MOUSE MAMMARY TUMOR VIRUS. David O. Peterson, Mark G. Toohey, Mike Huang, and Jae Lee, Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843.

Transcription of the proviral genes of mouse mammary tumor virus (MMTV) is induced by several classes of steroid hormones. A transcriptional enhancer whose activity is modulated by steroid hormone-receptor complexes, as well as a regulatory element that mediates a negative effect on the basal rate of MMTV transcription, are present within the proviral long terminal repeat. In order to understand the parameters that determine the basal activity of the MMTV promoter and the molecular mechanisms by which this basal activity is regulated both positively and negatively, the functional elements of the promoter have been characterized by construction of a set of 20 linker scanning (LS) mutations. Analysis of these mutant promoters with a transient transfection assay revealed that sequences containing a binding site for the transcription factor nuclear factor 1 (NF-1), a TATA box, and sequences related to the element known as the octamer (ATGCAAAT) were all sensitive to mutation, while alterations in other promoter sequences were without significant effect. Additional mutant promoters have also been constructed by oligonucleotide directed, site-specific mutagenesis. Gel electrophoresis mobility shift assays and other techniques have been used to identify nuclear proteins that recognize elements of the MMTV promoter and to begin to quantitate their affinity for wild-type and mutant promoter sequences.

O 207 HEPATITIS B VIRUS DNA INTEGRATION IN A SEQUENCE WHICH IS HIGHLY SIMILAR TO A RETINOIC ACID RECEPTOR, Magnus Pfahl and Doris Benbrook, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Steroid hormone receptor genes belong to a multigene family that encodes transcriptional regulatory proteins including the v-erbA oncogene product and the retinoic acid receptor. Recently, a hepatitis B virus DNA integration was found next to a liver cell sequence bearing a striking resemblance to the supposed DNA-binding domain of steroid hormone receptors. In order to identify the normal functions of this unknown receptor, we isolated and sequenced cDNA clones which hybridized with oligonucleotide probes from the reported sequence. A cDNA clone isolated in this manner from a human placental library was found to indeed encode a steroid hormone receptor-type molecule. This new receptor appears to be a member of a new receptor subclass which includes the vitamin A receptor.

O 208 BIOCHEMICAL CHARACTERIZATION OF THE VIRD ENDONUCLEASE FROM AGROBACTERIUM TUMEFACIENS, Sandra G. Porter, and Eugene Nester, University of Washington, Seattle, WA 98195.

The virD endonuclease is instrumental in processing the T-DNA for transfer from Agrobacterium tumefaciens to a recipient plant cell. In vivo, virD endonuclease activity leads to the production of site as well as strand-specific nicks in both of the 25bp direct repeats which border the T-DNA. The processing events initiated by virD activity lead to the production of a single-stranded T-DNA molecule which has been proposed to be an intermediate in T-DNA transfer. Two proteins(VirD1 and VirD2) are required for the site-specific endonuclease activity in vivo although the interaction between them is not understood. In this study, we demonstrate a physical interaction between VirD1 and VirD2 by SDS-polyacrylamide gel analysis. In addition, we have been able to show that the endonuclease activity resides in VirD2 by in vitro nicking assays.

O 209 IDENTIFICATION OF BASAL AND cAMP REGULATORY ELEMENTS IN THE PROMOTER OF THE PEPCK GENE, Patrick G. Quinn, Tai W. Wong, Mark A. Magnuson, John B. Shabb and Daryl K. Granner, Vanderbilt University, Nashville, TN 37232.

Promoter elements, which are important for basal and cAMP-regulated expression of the PEPCK gene, have been identified by analysis of a series of PEPCK promoter internal deletion mutants in transfection and DNase I protection experiments. The internal deletion mutants examined spanned the region from -129 bp to -18 bp within the PEPCK promoter. All contained PEPCK DNA from -600 to +69 bp except for specific bases that were replaced with a Sal I linker. CV1 cells were transfected with wild type and mutant CAT plasmids, whose expression was normalized by determining the expression of contransfected SV2-βgal. In addition, wild type and mutant DNA templates were used as probes in DNase I protection experiments to determine sites of protein-DNA interaction.

Deletion of the 5' recognition sequence for NF1/CAAT had no effect on promoter strength, but mutation of the 3' recognition sequence for NF1/CAAT resulted in a 50% decrease in promoter strength. DNase I protection experiments showed that deletion of the 5' end of the NF1/CAAT binding site resulted in a loss of the NF1 footprint and appearance of a CAAT footprint, whereas mutation of the 3' end of the binding site resulted in loss of both NF1 and CAAT footprints. Two linker scanner mutants (internal deletions that maintain helical spacing) which disrupt core sequences of the cAMP regulatory element (CRE) resulted in loss of cAMP responsiveness and an 85% decrease in basal promoter strength, indicating that the CRE is associated with a basal stimulatory element (BSE). Mutation of the core sequence of CRE resulted in the loss of the DNase I footprint over the CRE. Internal deletions flanking the CRE showed no loss of induction by cAMP but did have reduced promoter strength. This delimits the CRE to an 18 bp region between nucleotides -99 and -82. Analysis of internal deletions between the CRE and the TATA homology centered at -27 identified a basal inhibitory element (BIE) adjacent to a basal stimulatory element (BSE) as well as a BSE coincident with the TATA homology.

O 210 SEQUENCE-SPECIFIC DNA-BINDING OF THE FOS PROTEIN COMPLEX.
Frank J. Rauscher III[1], B. Robert Franza Jr.[2] and Tom Curran[1], [1]Department of Molecular Oncology, Roche Institute of Molecular Biology, Nutley. NJ 07110; [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

The c-fos proto-oncogene was identified as the transforming gene transduced by the FBJ and FBR murine osteogenic sarcoma viruses. c-fos encodes a nuclear phosphoprotein which forms a complex with other cellular proteins, is associated with chromatin and binds to DNA in vitro. Transcription of c-fos is rapidly and transiently induced by a diverse set of extracellular stimuli including mitogens, differentiation inducers and agents which depolarize neurons both in vitro and in vivo. We have proposed that c-fos functions as a cellular immediate early gene that couples short-term stimuli occurring at the membrane to long-term adaptive responses of the cell. It is thought that the long-term adaptive changes require alterations in gene expression mediated by c-fos. Studies of an adipocyte differentiation system have provided evidence that the Fos complex binds to regulatory regions of specific genes (Distel et al. Cell 49:835, 1987). We have now identified several different transcriptional regulatory regions including a negative regulatory element in the human immunodeficiency virus LTR and the Gibbon ape leukemia virus enhancer region that bind to the Fos protein complex and several Fos-related antigens. Binding proteins have been identified by gel shift assays and by using a rapid DNA affinity precipitation assay coupled with high resolution two-dimensional gel analysis. The common sequence motif among these elements is identical to the consensus binding site for the HeLa cell activator protein (AP-1). These results imply that biochemically distinct transcriptional regulators recognize similar sequence elements and suggest the Fos protein complex might play a direct role in the regulation of gene expression, perhaps at the AP-1 binding site.

O 211 A DISTAL TATA SEQUENCE REQUIRED FOR TRANSCRIPTION OF THE ADENOVIRUS IVa2 PROMOTER. A "NON TATA SEQUENCE PROMOTER", Sergio Lobos, Juan Carcamo, Leonard Buckbinder and Danny Reinberg. Department of Biochemistry, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School.

The initiation site of transcription from the adenovirus encoded IVa 2 promoter is located 210 nucleotides upstream from the CAP site of the major late promoter. Transcription from these promoters is divergent.

In order to gain insights into the mechanism of initiation of transcription of non TATA sequence containing promoters we studied the IVa 2 promoters. Reconstitution experiments indicated that transcription from the IVa 2 promoter required all the previously defined transcription factors, including the TATA binding protein. The requirement for TFIID was suggested by the following results: 1) reconstitution experiments indicated that TFIID and the activity required for IVa 2 transcription co-purified over 8 different chromatographic steps. 2) deletion of the MLP TATA sequence abolished transcription from the IVa 2 promoter. Previous studies have indicated that transcription from the MLP was stimulated by MLTF, a site specific DNA binding protein that binds to a symmetric element present upstream of the TATA box. The addition of MLTF to a IVa 2 in vitro transcription reaction resulted in 12-fold stimulation. MLTF did not overcome the requirements of TFIID in IVa 2 transcription. Point mutations in the MLTF binding site that resulted in a reduced binding of MLTF tothe promoter produced low levels of stimulation of IVa 2 transcription.

O 212 TRANSCRIPTIONAL REGULATORS OF ADRENAL STEROID 21-HYDROXYLASE. Douglas A. Rice, Mark S. Kronenberg, Bernard P. Schimmer, and Keith L. Parker, Duke University, Durham, NC 27710. Adrenal steroid 21-hydroxylase (21-OH) is required for the synthesis of mineralocorticoids and glucocorticoids, and is expressed primarily in the adrenal cortex. Expression of 21-OH is transcriptionally regulated by ACTH via increased cAMP levels. We have previously analyzed expression of the murine 21-OH gene following stable transfections into the mouse adrenocortical tumor cell line Y1. Expression was stimulated up to 10-fold by ACTH, and deletion of 5' flanking sequences beyond -230 resulted in markedly decreased levels of expression. More recent experiments utilizing human growth hormone as a reporter gene in transient transfections have shown that sequences from -330 to -150 function as a tissue-specific enhancer element in Y1 cells. We have continued analyses of this promoter region using DNAase I footprinting and methylation interference to identify sequences in this region which interact with DNA-binding proteins present in nuclear extracts from steroidogenic and non-steroidogenic cell lines. These studies have defined several sequences in the 5' flanking region of the 21-OH gene that interact with DNA-binding proteins; at least one of these footprints is only seen using nuclear extracts from steroidogeneic cells. Currently, we are performing functional studies to determine the role of these sequences in the expression of 21-OH in Y1 cells.

O 213 THE REGULATION OF CELLULAR DIFFERENTIATION IN DICTYOSTELIUM DISCOIDEUM BY cAMP AND DIF. Jeffrey Williams, Keith Jermyn, Anne Early and Karen Duffy, Imperial Cancer Research Fund, Clare Hall, S. Mimms, Herts, EN6 3LD U.K. Cyclic AMP and DIF, a low molecular weight chlorinated compound of known structure, act antagonistically to regulate cellular differentiation in Dictyostelium. Cyclic AMP induces the expression of prespore genes and DIF induces prestalk gene expression. One important role of DIF may be to repress prespore gene expression in a proportion of cells in the aggregate, and we have shown that DIF acts as a transcriptional repressor of the PsA gene, a slug extracellular matrix protein which is encoded by a prespore-specific mRNA. Prestalk cells synthesize two extracellular matrix proteins, ST310 and ST430, and transcription of the cognate genes is rapidly induced by DIF (Williams et al., 1987 Cell 49, 185-192). The upstream regions of the two prestalk genes, and that of the PsA prespore gene, have been shown to direct correctly regulated expression after transformation into Dictyostelium. We are now analyzing the cis-acting regulatory elements and the trans-acting factors responsible for their induction.

O 300 INSULIN REGULATION OF A DNA BINDING PROTEIN SPECIFIC FOR THE HUMAN GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE PROMOTER.

M. Alexander-Bridges, N. Nasrin, I. Kang, M. Denaro, C. Ramaika and X. Kong, Massachusetts General Hospital, Boston, MA 02114

Insulin stimulates the transcription of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene in 3T3 adipocytes. A 500 BP fragment of DNA containing the 5'-flanking sequences of the gene fused to the chloramphenicol acetyl transferase (CAT) gene is induced 10-fold by insulin in hepatoma cells and 2 to 3-fold by insulin in 3T3 adipocytes. Using a series of BAL31 deletion mutants, we have mapped sequences that mediate this effect to a fragment 80 nucleotides in length. Nuclear extracts of 3T3 adipocytes contain an insulin-induced DNA binding protein that specifically retards the migration of labelled DNA in a non-denaturing gel. We are in the process of isolating and characterizing this DNA binding activity.

O 301 A DNA BINDING PROTEIN IS INVOLVED IN REGULATION OF *ILVIH* OPERON TRANSCRIPTION Debra A. Aker, Ezio Ricca, Wang Qing, and Joseph M. Calvo, Cornell University, Ithaca, NY 14853

Efficient transcription of the *ilvIH* operon of *E.coli* requires the promoter region and additional DNA located several hundred base pairs upstream. Transcription is repressed 5-fold when cells are grown in the presence of leucine. We have partially purified a protein (called IHB for ilvIH Binding Protein) that binds specifically to the *ilvIH* promoter/regulatory region. Two binding sites have been detected: from -310 to -200 and from -99 to -41. The following evidence suggests that the IHB protein is involved in leucine regulation of *ilvIH* transcription : 1) Leucine inhibits binding of IHB to DNA when it is added to the binding reaction *in vitro* . 2) IHB from a mutant strain that lacks leucine repression (mutation unlinked to *ilvIH*) binds to DNA but the mobility of the resulting complex is altered. Leucine does not inhibit formation of this complex.

Finally, naked DNA from this region migrates anomalously on acrylamide gels, suggesting that it has a natural bend. The center of this bend is located between the two IHB binding sites.

O 302 BAND MOBILITY SHIFT ASSAY IDENTIFIES A COMPONENT OF THE RAT METALLOTHIONEIN-1 (MT-1) METAL RESPONSIVE ELEMENT (MRE) TRANSCRIPTION FACTOR. Robert D. Andersen, Susan J. Taplitz, Anita M. Oberbauer and Harvey R. Herschman, Department of Biological Chemistry and Laboratory of Biomedical and Environmental Sciences, UCLA, CA 90024.

Using nuclear extracts from Fao cells, a rat hepatoma cell line, we have performed a band mobility shift assay to identify proteins binding to rat MT-1 promoter sequences. With labeled DNA fragments which contain MRE sequences from the MT-1 promoter we identify a shifted band which appears in extracts isolated from cells pre-treated with 3-5 μM $CdCl_2$, a dosage which leads to full induction of MT-1 gene transcription. This same band is seen in control extracts in the presence of $CdCl_2$. This inducible mobility shift band is only seen when we use a Tris-glycine-buffered acrylamide gel. Tris-borate-EDTA-buffered gels show no difference between control and induced extracts. UV cross-linking analysis indicates a single labeled polypeptide which, on SDS-PAGE, migrates with an apparent size of 39 kD. We are using the mobility shift assay on nuclear extracts derived from rat liver to purify this protein. We believe it to be a component of the transcription factor(s) which we have previously identified by dimethyl sulfate footprinting analysis in vivo and which binds specifically to MRE sequences in the rat MT-1 promoter in the presence of cadmium ion (Andersen et al., Mol. Cell. Biol. 7, 3574-81 [1987]).

O 303 THE DNA BINDING DOMAIN OF SV40 LARGE T ANTIGEN AS AN IN VIVO REPRESSOR IN E.COLI.: A MODEL SYSTEM TO ANALYSE EUKARYOTIC DNA BINDING PROTEINS. Helge Bastian, Avril Arthur and Ellen Fanning, Institute for Biochemistry, University of Munich, Munich, F.R.G.

The large T antigen of SV40 mediates both control of SV40 gene expression and initiation of SV40 DNA replication by specific DNA binding to two major sites, I and II, in the SV40 origin. Thus it is a particularly interesiting model protein for studying eukaryotic DNA/protein interactions. The genomic coding sequence of large T antigen was clones into an E.coli expression vector via new restriction sites introduced at the intron boundaries. In vitro DNA binding tests of wild type protein plus a range of mutants allowed the positive identification of the essential DNA binding domain for both sites I and II between residues 131-259 (ref. 1). No obvious DNA binding motif (eg. helix-turn-helix, zinc finger) is located in this region. Thus an alternative, genetical approach was developed to analyse DNA binding mutants in an analogous way to the prokaryotic repressor systems(eg. cro, lac I). The E. coli reporter gene, gal K was cloned under the control of the tac promoter where the operator sequence has been replaced by wild type and mutant SV40 DNA binding site(s). In conjunction with a "T antigen" expression vector, repression of gal K can be detected in vivo. Such a repressor system provides the means to rapidly analyse a range of point mutants, both amino acid and nucleotide changes, for altered DNA binding profiles.

1. Arthur, A.K., Höß , A. and Fanning, E. J. Virol. (in press)

O 304 THE USE OF LOOP FORMATION BETWEEN lac OPERATORS TO STUDY THE PHYSICAL PROPERTIES OF DNA in vivo. Gregory Bellomy and M. Thomas Record, Jr., Departments of Chemistry and Biochemistry, University of Wisconsin, Madison, WI 53706.

It has been shown previously for several systems that DNA loop formation is the mechanism of gene regulation via action-at-a-distance. In the case of *lac* operator binding we present evidence that looping is the mechanism by which an upstream operator can aid in repression at a primary constitutive operator (O^c) site *in vivo*. Twelve plasmids were constructed with varying interoperator spacings. These were used in an *in vivo* assay of repression using galactokinase production in the presence and absence of IPTG (Isopropyl-β-D-Galactopyranoside) as a measure of operator occupancy. The results show an oscillatory pattern of repression as interoperator spacing is increased, as expected if operators must be torsionally aligned for loop formation. In addition to demonstrating loop formation *in vivo* this data can be used to infer the physical properties of DNA *in vivo*. Specifically, the period of the oscillations in repression can be interpreted as the local helical repeat of DNA. The repression data are not consistent with a helical repeat of 10.5 bp/turn, but are better fit by assuming a helical repeat of 9.0 or 11.7 bp/turn. The amplitudes of the oscillations show that the torsional rigidity of DNA *in vivo* is similar to the value *in vitro* , but that the apparent lateral rigidity is approximately a factor of five lower *in vivo* than *in vitro*.

This decrease in the apparent persistence length may well be due to CAP (Catabolite Activator Protein)-induced DNA bending or DNA supercoiling in our *in vivo* system. Analogous experiments with a CAP^- strain will help clarify the role of CAP bending in loop formation.

O 305 TRANSCRIPTION REGULATION OF THE HOUSEKEEPING GENE FOR HUMAN TRIOSEPHOSPHATE ISOMERASE, Thomas Boyer and Lynne Maquat, Roswell Park Mem. Inst., Bflo., NY 14263.

To examine the functional organization of the triosephosphate isomerase (TPI) promoter, deletion, insertion, and linker scanning mutations were introduced into the TPI promoter of hybrid TPI/β-globin genes. These genes were transiently expressed in mouse L and human HeLa cells, and the effects of each mutation on the frequency and position of transcription initiation were assayed by S_1 nuclease transcript mapping and primer extension. Our results indicate that the TPI promoter, while effectively housekeeping, is distinctively facultative-like in its modular functional organization. At least three spatially discrete regulatory elements residing between positions -589 and +1 in L cells and -920 and -7 in HeLa cells coordinately promote maximum hybrid gene transcription. Two of these elements reside downstream of position -64 and include a putative Sp1 binding site and a TATA box. These elements cooperatively control both the frequency and position of transcription initiation. An additional element(s) resides between positions -589 and -73 in L cells and -920 and -73 in HeLa cells and regulates only the frequency of transcription initiation. Minimal promoter function is achieved through the interaction of the TATA box and one of the other two regulatory elements. With all three elements, transcription is stimulated at least 4- and 15-fold in L and HeLa cells, respectively, above the level of transcription promoted by the TATA box and only one additional element. Finally, sequences between position -7200 and either -2800 in L cells or -920 in HeLa cells reduce transcription approximately 7-fold relative to the level of transcription achieved with the maximally active promoter.

O 306 JURKAT T CELL NUCLEAR FACTOR BINDS THE INTERLEUKIN-2 PROMOTER REGION AND IS IMPORTANT FOR IN VITRO TRANSCRIPTION. M. Brunvand, U. Siebenlist, National Institute of Allergy and Infectious Diseases, LIR, Bethesda, MD 20892

Interleukin-2 (IL-2) is a lymphokine secreted by T lymphocytes after mitogen or antigen stimulation. This lymphokine is important for the coordinated activation of the immune response in humans. Control of expression of this important lymphokine is modulated at the level of transcription. Previously we documented the acquisition of a DNase I hypersensitive site 5' of the start site of transcription. Fujita,et al have indicated this same area is important for maximal gene induction. Using the mobility retardation assay we have documented a nuclear factor from resting and mitogen stimulated Jurkat T cells which binds to the region of the hypersensitive site. The DNase I footprint generated with these extracts spans a 22 base stretch of DNA 5' to the transcription start site. We are assessing the functional significance of regions within the IL-2 promoter with in vitro transcription.

O 307 TRANSCRIPTION ACTIVATION OF THE KLEBSIELLA PNEUMONIAE NIFH PROMOTER BY UPSTREAM-BOUND NIFA PROTEIN, Martin Buck and Enrique Morett, AFRC - IPSR Nitrogen Fixation Laboratory, Sussex University, Brighton BN1 9RQ, England.

Sequences located 100 to 200 bp upstream of the start of the transcription have been shown to be necessary for transcriptional activation of the Klebsiella pneumoniae nifH, U, B and F promoters by the nif-specific transcriptional activator NifA. By using in vivo dimethyl-sulphate protection experiments we have shown that NifA binds specifically to the nifH UAS and by directed mutagenesis about the C-terminal of NifA is the DNA binding domain of this protein. Results show that mutant nifH UAS's which produce a promoter-down phenotype do not bind NifA well. Similarly mutations in the proposed DNA recognition helix of NifA which prevented transcriptional activation were shown to result in reduced binding of NifA to the nifH UAS. No evidence for strong binding of NifA to the downstream nifH promoter element was obtained. Together with previous results demonstrating that the UAS must be located on the correct face of the DNA helix to participate in efficient transcriptional activation,[1] these results indicate that NifA is bound upstream and activates transcription by interacting with downstream-bound transcription factors (RNA polymerase and the ntrA encoded sigma factor) by DNA loop formation.

Ref. 1. Buck, Cannon and Woodcock (1987) Molecular Microbiology, 1, 243-249.

O 308 DEVELOPMENTAL AND TISSUE SPECIFICITY OF DNA BINDING PROTEIN TO THE MHC CLASS I PROMOTER. Peter A. Burke, Yasuaki Shirayoshi, John Kasik, Kazushige Hamada, Ettore Appella and Keiko Ozato. NICHD, NIH, Bethesda, MD, 20892.

A 35 base pair segment of the promoter region of the mouse class I gene (the CRE) has been identified as a positive regulator of transcription in differentiated cells as well as a down regulatory element in embryonic carcinoma cells. Using gel retardation and methylation interference, nuclear extracts (NE) from various cell lines produce three distinct bands that correspond to three regions (I, II, III) within the CRE. NE from several adult tissue and extracts taken from different points in the perinatal period were tested for the presence of these specific DNA binding proteins. NE from adult liver and spleen showed specific binding to Reg. I and Reg. II of the CRE, brain however showed specific binding to Reg. II but no evidence of a Reg. I binding protein. Liver NE from progressive stages of perinatal development showed the constitutive presence of Reg. II binding protein but Reg. I binding protein was not present till the day of birth and appeared to increase in the adult. The presence or absence of Reg. I binding is directly correlated with that of class I mRNA levels in the developing embryo and neonate. The absence of Reg. I binding protein in brain is consistant with the lack of class I mRNA. The tissue specificity and developmental modulation of Reg. I binding protein indicate its important in vivo role in transcriptional control of the class I gene. The functional significance of Reg. I binding is presently be evaluated using mutant CAT constructs.

O 309 DNA REPLICATION-INDEPENDENT AND MEIOSIS-SPECIFIC H2B GENE HAS A REPRESSED S PHASE-SPECIFIC TRANSCRIPTION REGULATORY ELEMENT, Inhwan Hwang and Chi-Bom Chae, Dept. of Biochemistry, Univ. of North Carolina, Chapel Hill, N.C. 27599.

During mammalian spermatogenesis somatic H2B histones are replaced by a testis-specific H2B variant (TH2B). Expression of rat TH2B gene is confined to pachytene spermatocytes of meiotic prophase I and independent of DNA replication. However, cloned upstream sequence of rat TH2B gene directs expression of the fused CAT gene during S phase of the cell cycle. This result suggests that the TH2B gene has an S phase-specific regulatory element (SPRE) and the element is repressed in the cells. We have identified the SPRE by systematic deletion analysis and DNase foot-printing assay, and the SPRE is localized immediately upstream of the octamer sequence. The synthesis of the SPRE-specific trans-acting nuclear factor also appears to be S phase-specific. An H2A histone gene is localized 230 bp upstream of the TH2B gene, and the gene is transcribed in an apposite direction. It is possible that the neighboring genes share the same SPRE and other regulatory elements. The DNA containing somatic H2B gene has similar sequence organization as the DNA containing TH2B including SPRE and H2A histone gene. Work is in progress to elucidate the mechanism of repression of the TH2B SPRE and activation of the TH2B regulatory elements specific for pachytene spermatocytes.

O 310 SEQUENCES REQUIRED FOR EXPRESSION FROM A BACTERIOPHAGE P2 LATE GENE PROMOTER. Nancy Grambow, Nancy Stokes and Gail E. Christie, Virginia Commonwealth University, Richmond, VA 23298.

The four late operons of temperate coliphage P2 are transcribed from promoters which do not resemble those normally recognized by E. coli σ^{70} RNA polymerase. Transcription from these late promoters requires trans-acting factors encoded by P2 or satellite phage P4. Cloned P2 late promoters can be activated by infection with the replication-defective mutants P2 Aam81 or Aam127, but not by wild-type P2. High levels of expression from cloned late promoters can also be obtained by supplying the P4 δ (transactivation) protein in trans. We have fused the P2 late promoter for the FETUD operon to the cat gene in order to study the promoter's functional domains. Sequential 5'-end promoter deletions were generated using BAL-31 nuclease and assayed for levels of cat expression following infection with P2 Aam81 or induction of P4 δ protein synthesis from a compatible plasmid. Enzyme production was abolished by a deletion between nucleotides -69 and -64 from the start of transcription. This area contains a region of DNA sequence homology which is conserved among the four P2 late gene promoters, and includes a small dyad symmetry element. Using synthetic oligonucleotides, we have also generated a collection of lac fusion plasmids carrying mutations in late promoter sequences upstream of -25, and analyzed these for activation of lacZ expression in the presence of phage-encoded factors.

O 311 STRUCTURAL CHARACTERIZATION OF THE 5′ REGION OF THE HUMAN INSULIN RECEPTOR GENE D. M. Cook, D. S. Tewari and R. Taub. HHMI U. Pennsylvania. School Med., Philadelphia, PA. 19104

The insulin receptor is a tyrosine-kinase receptor that mediates primarily metabolic effects. The insulin receptor protein is present in most cell types but is expressed at a higher level in certain tissues. Little is known about the regulation of expression of this gene. We have isolated the 5′ region of the human insulin receptor gene from a genomic cosmid library and examined its structural properties. Restriction mapping and sequencing analysis of this cosmid have revealed the boundaries of exons 1 and 2 which are separated by about 25 kb. Primer extension and S1 nuclease mapping of the promoter region have provided evidence for the presence of multiple transcriptional start sites with no apparent TATA or CCAAT box sequences. Sequence analysis of this upstream region of the gene has revealed sequence with extremely high G-C content of approximately 80% comprising an HTF island. The insulin receptor promoter region shows close homology to the promoters of other housekeeping genes including EGF receptor and c-ras. This region contains several G-C repeats that are potential binding sites for the cellular transcription factor Sp1. Potential binding sites for the transcription factors AP1 and AP2 are also evident. Studies are in progress to analyze this 5′ region for promoter and enhancer activity.

O 312 ANALYSIS OF SALT STRESS INDUCIBLE PROMOTERS FOR PEPCase GENES IN *MESEMBRYANTHEMUM CRYSTALLINUM*. John C. Cushman, Gabriele Meyer, Jürgen M. Schmitt, and Hans J. Bohnert. Department of Biochemisty. University of Arizona, Tucson, AZ 85721 USA.

The desert plant *Mesembryanthemum crystallinum* undergoes a shift in carbon metabolism from C3 to CAM (Crassulacean Acid Metabolism) upon exposure to salt/drought stress. The major enzyme of CAM, phosphoenolpyruvate carboxylase (PEPCase; E.C. 4.1.1.31), increases in activity and protein level over 50 fold in fully salt stressed plants (i.e. 500 mM NaCl for approximately 8 days). This induction is paralleled by a corresponding increase in PEPCase mRNA levels. Biochemical studies suggest that C3- and CAM-specific forms of PEPCase exist. We have isolated PEPCase cDNA clones and are presently characterizing genes from genomic libraries established in Lambda FIX. PEPCase in *M. crystallinum* is encoded by a small family of 2 to 3 genes. Induction of specific members of the PEPCase gene family during stress is regulated at the level of transcription and/or mRNA stability. The analysis will include a study of transcription control regions of the C3- and CAM-specific forms of the PEPCase genes. These studies provide information about the structure of plant promoters which respond to salt stress. Supported in part by grants from the NSF, DFG, and USDA.

O 313 COOPERATIVE REPRESSION OF TRANSCRIPTION INITIATION OF THE *DEO* OPERON OF *E.COLI*, Gert Dandanell and Karin Hammer, Institute of Biological Chemistry B, University of Copenhagen, Sølvgade 83, 1307 Copenhagen K, Denmark. The *deo* operon of *E.coli* which encodes genes that are involved in nucleoside catabolism is regulated by at least three DNA-binding proteins. The regulatory region contains two promoters. *deoP1* is regulated by the *deoR* repressor while *deoP2* is regulated by both the *deoR* and the *cytR* repressors and by the CRP•cAMP complex. Thus both promoters are regulated by the *deoR* repressor. We have found *in vivo* that both of these operator sites are required in order to obtain efficient repression of the *deo* operon. This cooperative binding of *deoR* to two operator sites has been shown to occur when the two sites are separated by 200-1000 bp (1) and 1000-5000 bp (2). We have now studied the regulation when the two sites are very close to each other (50-200 bp) as well as the regulatory effects of mutations in one of the operator sites.

The activity and regulation of the *deoP2* promoter has been studied in *cya*$^-$ strains (cAMP deficient) and we found that the *deoR* regulated activity is independent of cAMP while the *cytR* regulated activity strictly depends on the precense of cAMP.

1) Gert Dandanell and Karin Hammer (1985) Two operator sites separated by 599 base pairs are required for *deoR* repression of the *deo* operon of *Escherichia coli*. EMBO J. **4**: 3333-3338.
2) G. Dandanell, P. Valentin-Hansen, J.E.L. Larsen and K. Hammer (1987) Long-range cooperativity between gene regulatory sequences in a prokaryote. Nature **325**: 823-826.

O 314 RNA POLYMERASE: A PROFILE OF RIFAMPICIN INHIBITION EXAMINED AT DISCRETE BOND FORMING STEPS DURING TRANSCRIPTION, Suzanne M. Kelly and Don Dennis, Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716.
Rifampicin is a noncompetitive inhibitor of phosphodiester bond formation over the entire range of transcription examined (i.e. bonds 1, 2, 3, 4-10, 10-20, 10-N, 20-n) for the E. coli RNA polymerase enzyme.

The inhibition by rifampicin was determined (K_i=14 μM) for several different discrete bonds by measuring the formation of a single phosphodiester bond between a preformed cognate primer and one additional nucleotide monomer (i.e. N_2, N_3; N_3, N_4) or a series of bonds formed between a precision assembled bound transcript and nucleotide monomers sufficient to elongate to a defined length (i.e. $N_4 \rightarrow N_{10}$; $N_{10} \rightarrow N_{20}$; $N_{20} \rightarrow n$). The product identification was done by HPLC (short products up to tetranucleotides) or by PAGE (dinucleotides up to duodecanucleotides), and the quantitation by scintilation counting of collected fractions or excised gel bands or glass filters (in the case of products longer than decamers). In the presence of rifampicin, the apparent V_m' values examined over this large range of transcript lengths displayed a minimum coincident with the formation of a tetranucleotide transcript and increased sharply for the formation of transcripts of either shorter or longer lengths. A simple scheme is presented which consolidates and is consistent with known rifampicin studies.

O 315 THE BACILLUS SUBTILIS PHAGE ϕ105 REPRESSOR-OPERATOR INTERACTION: MUTATIONAL ANALYSIS AND IN VITRO BINDING STUDIES, Patrick Dhaese, Luc Van Kaer, Rebecca De Clercq and Marc Van Montagu, Laboratorium voor Genetica, RUG, B-9000 Gent (Belgium)

Early gene regulation in the temperate B. subtilis phage ϕ105 involves the specific binding of a repressor [1] to at least three separate operator sites, each of which consists of an identical, directly repeated "core" of 14 bp [2]. An unusual feature of this 14-bp sequence (5'-GACGGAAATACAAG-3') is the absence of twofold rotational symmetry. A large number of repressor mutants deficient in binding to a single wild-type operator element was isolated. Their positions together define the DNA-binding helix-turn-helix domain, of which the second "recognition" helix extends from Arg-29 to Arg-37. By correlating this information with the previously isolated ϕ105 operator-constitutive mutations [2], is was possible to propose some specific amino acid-base pair contacts, e.g. Arg-29 → G1, Ser-30 → C3, Asp-34 → G4. In a second, more systematic approach, several chemically synthesized variant operator sequences were tested for their ability to compete with the wild-type sequence in binding to highly purified repressor, using nitrocellulose filter retention assays. Repressor-operator binding was also analyzed by DNase footprinting, by gel retardation assays, and electron microscopical observation of the complexes formed. The results with respect to sequence specificity, affinity, and cooperativity determinants will be discussed.

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O 316 ISOLATION AND CHARACTERIZATION OF A REGULATORY PROTEIN OF THE INCF TRANSFER SYSTEM, L. Di Laurenzio , B. B. Finlay , L. Frost , W. Paranchych
Department of Biochemistry , University of Alberta , Edmonton , Alberta , Canada , T6G 2H7

The origin of transfer (*oriT*) of the IncF plasmids is organised into three regions as determined by DNA sequence analysis; 1) the site of nicking of the plasmid and initiation of transfer to the recipient cell , 2) the binding site of the nickase , a complex of the *tra*Y and *tra*Z gene products , 3) the binding site of the *tra*M protein which signals that a competent mating pair has formed and that DNA transfer should begin . This study involves the *tra*M/*ori*T system of the IncFV plasmid pED208 , the derepressed form of Fo *lac*.

The *tra*M gene was cloned into the overexpression vector T7-3 which employes a T7 RNA polymerase system. *tra*M is a 13 Kd protein that is normally expressed at low levels and is found associated with the inner membrane of the cell.When overexpressed , *tra*Mp was found in the cytoplasmic fraction and has been purified by $(NH_4)_2SO_4$ fractionation and FPLC gel exclusion chromatography. Amino acid determination and N-terminal analysis of small amounts of traMp purified by electroelution from SDS-PAGE confirmed the amino acid sequence obtained from DNA sequence analysis and revealed that the traMp protein does not contain a signal sequence . Examination of the *tra*Mp predicted amino acid sequence revealed several potential DNA binding domains with classical helix-bend-helix motif.

DNA retardation assays were performed using ^{32}P-end labelled *Taq*I and *Nla*III fragments from the *ori*T region of pED208 isolated from preparative polyacrylamide gels ; these showed that *tra*Mp protein bound strongly to a 190 bp *Taq*I fragment containing the proposed *tra*Mp binding site as determined by DNA sequence analysis of the four known alleles for *tra*M/*ori*T among IncF plasmids.

The proposed *tra*M binding site revealed a complex pattern of direct and inverted repeats characterized by 12 *Hin*fI like sites of sequence GAT/ATC . We are currently trying to identify the minimum DNA sequence for full binding activity and suspect that it involves the *Hin*fI rich region which corresponds to the *tra*Mp binding site proposed for other IncF plasmids.

O 317 NUCLEOTIDE SEQUENCE OF THE *E. COLI FAD R* GENE, A MULTIFUNCTIONAL REGULATOR OF FATTY ACID METABOLISM, Concetta C. DiRusso, Universty of Tennessee, Memphis, Tn 38163.

In *Escherichia coli* fatty acid degradation, acetate degradation and fatty acid biosynthesis appear to be regulated in a complex manner by the product of the *fadR* gene. There is substantial genetic and biochemical evidence for negative transcriptional control by *fadR* of *fadL, fadD, fadE,* and *fadAB*. These genes encode proteins required for the transport, activation and *beta*-oxidation of long-chain fatty acids. *fadR* is also a negative transcriptional regulator of the *aceBAK* operon which encodes the enzymes of the glyoxylate shunt. Regulation of *aceBAK* is complex involving *fadR* and a second repressor, *iclR* . In addition, mutations in *fadR* result in reduced synthesis of fatty acids by a mechanism which is undefined at this time. In previous work I cloned the *fadR* gene and identified a 29,000MW polypeptide specifically associated with *fadR* activity. In the present work I have sequenced a 1294 base pair *Hin*DIII-*Eco*RV fragment carrying the *fadR* gene and identified the transcriptional start site. An open-reading frame capable of encoding a 238 amino acid polypeptide with a molecular weight of 26,972 was derived from the DNA sequence. Amino acids 34-54 of the derived peptide sequence show sequence homology to the helix-turn-helix binding motif believed to be important for DNA binding in other well-characterized transcriptional regulatory proteins. Subclones containing the entire coding sequence confer wild-type *fadR* activity on *fadR* mutants while subclones deleted within the predicted coding sequence eliminate *fadR* activity as measured by repression of *beta*-galactosidase activity of *fadA-lacZ fadR* ::Tn*10* strains. This sequence information is currently being used to isolate the *fadR* protein to begin a molecular analysis of *fadR* interaction with each of the *fadR*-responsive genes and operons. This will provide valuable information regarding the regulation of these biochemically interrelated pathways of fatty acid metabolism.

O 318 MECHANISM OF DNA-SEQUENCE RECOGNITION BY THE E. COLI CATABOLITE GENE ACTIVATOR PROTEIN (CAP) PROBED BY SITE-DIRECTED MUTAGENESIS. R.H. Ebright,[1,2] J. Beckwith,[2] T. Kunkel,[3] J. Krakow,[4] A. Kolb,[5] and H. Buc.[5] [1]Rutgers University, New Brunswick NJ 08855, [2]Harvard Medical School, Boston MA 02115, [3]NIH-NIEHS, Research Triangle Park NC 27709, [4]Hunter College, New York NY 10021, [5]Institut Pasteur, Paris F75724, France.

Detailed models for the structure of the CAP-DNA complex have been proposed independently by Weber and Steitz ("model I," PNAS 81:3973, 1984), and by Ebright and co-workers ("model II," PNAS 81:7274, 1984). The predicted amino acid-base pair contacts in the two models are similar but not identical. We have utilized site-directed mutagenesis to test the predictions that differ in the two models:

1. Model I predicts that Lys188 makes a direct contact with base pair 7 of the DNA half-site. In contrast, model II predicts that Lys188 makes no direct contact with a DNA base pair. We have substituted Lys188 by Asn--the side chain of which is too short, in either model I or model II, to make a direct contact with a DNA base pair. The Asn^{188} substitution produces no perturbation of specificity at base pairs 5, 6, 7, or 8. We conclude that model I is in error.
2. Model I predicts that Glu181 makes direct contacts with two DNA base pairs: base pair 6 and base pair 7. In contrast, model II predicts that Glu181 makes a direct contact with only one DNA base pair: base pair 7. We have substituted Glu181 by Gln--the side chain of which can make only one of the two direct contacts predicted by model I. The Gln^{181} substitution produces no perturbation of specificity at position 6 and only a partial reduction of specificity at position 7. We again conclude that model I is in error.

O 319 **REGULATORY FACTORS AND *CIS*-ACTING ELEMENTS INVOLVED IN HUMAN proIL-1β GENE EXPRESSION.** M.J. Fenton[1], B.D. Clark[1], A.C. Webb[3], and P.E. Auron[1,2]. [1] Massachusetts Institute of Technology, Cambridge, MA 02139; [2] Tufts Univ.-New England Medical Center, Boston, MA 02111; [3] Wellesley College, Wellesley, MA 02181.

Prointerleukin 1 β (proIL-1β) transcription is induced in monocytic cells is by agents such as lipopolysaccharide and phorbol myristic acetate. The proIL-1β gene is differentially regulated at the level of transcription and message stability. We have previously suggested that the transient kinetics of mRNA accumulation in THP-1 mononuclear cells should require specific *trans*-acting factors. Using a electrophoretic band-shift assay system and nuclear extracts isolated from cells at various stages of induction, we have located several regions which bind *trans*-acting factors, including an octamer (ATTTGCAT)-binding protein and a putative repressor factor. The binding of these factors correlates well with the kinetics of transcriptional modulation. Factors which recognize the promoter region are not present in several non-monocytic cell lines, or in leukemic T cell lines which express proIL-1α. We have also identified a region 200-500 bp upstream of the transcriptional start site which acts as a cell type-specific cellular enhancer element, as analyzed by transfection of expression vectors employing the chloramphenicol acetyl transferase reporter molecule.

O 320 **SPECIFIC BINDING OF PROTEINS FROM RHIZOBIUM MELILOTI CELL-FREE EXTRACTS CONTAINING NodD TO DNA SEQUENCES UPSTREAM OF INDUCIBLE NODULATION GENES,** **Robert F. Fisher, Thomas T. Egelhoff, John T. Mulligan and Sharon R. Long, Stanford University, Stanford, CA 94305.**

Nodulation (nod) genes in Rhizobium meliloti are transcriptionally induced by flavonoid signal molecules such as luteolin, which are produced by its symbiotic host plant, alfalfa. Expression of nodD is required to permit this flavone-mediated induction. Starting approximately 25 bp upstream of each set of inducible nod genes lies a highly conserved 50 bp segment (the nod box). We have used the gel retardation assay to demonstrate a specific interaction between DNA fragments which contain sequences upstream of the inducible nod genes, and extracts from R. meliloti which overproduce NodD, the nodD gene product. Specific retardation of electrophoretic mobility correlates with the presence and concentration of NodD in the extracts. Antibody directed against NodD removed the specific DNA binding activity from the extracts.

O 321 EFFECTS OF HIGH EXPRESSION OF THE ANTITERMINATION PROTEINS OF LAMBDOID PHAGES. N.C. Franklin & Jed Doelling, Biology Dept., Univ.of Utah, Salt Lake City 84112. Transcription of lambdoid bacteriophages is regulated post-initiation by phage-coded anti-termination proteins called "N". The N proteins of phages λ, Ø21 and P22 are homologous in their time of synthesis, role and character as proteins, but they differ widely in amino acid sequence (Franklin, J.Mol.Biol.181:75,85, 1985). During transcription N protein is found within a complex of host RNA polymerase and other host proteins (Greenblatt et al, Steenbock Symp. 1986; Barik et al, Cell 50:885, 1987). Normally N requires for its function a phage-coded site of recognition, nut, accounting for the type specificity of each N protein. It is likely that N recognizes this site in the RNA transcript or in transient RNA/DNA, rather than in DNA.

Cloned under ptac on a pBR-derived plasmid, N genes of λ, Ø21 and P22 are highly expressed when induced by IPTG. The type specificity of N proteins is found to be relaxed when they are overproduced. N may therefore become engaged in abnormal situations independent of nut recognition, a possible factor in the lethal effect of these plasmids under conditions of IPTG induction. An example is the N-stimulation of lacZ', downstream from N on the same plasmid but limited by transcription termination. We are purifying N protein from both the soluble and insoluble forms that it takes in hyperproducing cells. The availability of pure N protein in ample amounts will provide for structural studies as well as direct assays of its binding properties. For binding, we will test specific sequences and structures of DNA or RNA, in presence or absence of other host proteins.

O 322 METAL-RESPONSIVE CONVERSION OF MerR REPRESSOR TO ACTIVATOR; CHARACTERIZATION OF SPECIFIC MerR/RNAP/MER PROMOTER TERNARY COMPLEXES, Betsy Frantz, Diana Ralston, Mark Chael, Myung Shin and Thomas V. O'Halloran, Department of Chemistry & BMBCB, Northwestern University, Evanston, Illinois 60208.

We have shown by in vitro analysis that the MerR purified protein autoregulates its own expression and is converted to an activator of mercury resistance gene transcription in response to submicromolar levels of mercuric chloride. MerR protein interactions at overlapping and transcriptionally divergent merR and merTPAD promoters have been probed by DnaseI, DMS and hydroxyl radical protection and ethylation interference. The protein contacts primarily a single face of the DNA helix and specific contacts appear unchanged both in the presence and absence of Hg(II). The overall affinity of protein for DNA is reduced 2-10 fold in the presence of Hg(II). RNA Polymerase complexes of the two promoters were isolated by gel shift and footprinted as above. The complexes possess characteristic features of RNA Polymerase/promoter "open" complexes and the merTPAD complex contains some unique MerR footprint features as well. It appears that MerR acts as an activator by redirecting RNA Polymerase from merR promoter complexes to transcriptionally competent merTPAD promoter complexes.

O 323 CIS AND TRANS-ACTING MUTATIONS OF CUP1 GENE TRANSCRIPTION, Peter Furst, Rebecca Hawes Hackett, Dean Hamer. Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

The CUP1 gene of the yeast S. cerevisiae codes for a low molecular weight, copper-binding protein, denoted copper metallothionein, which is required to prevent copper toxicity. The CUP1 locus is transcriptionally activated by the addition of copper to the media. Most laboratory strains of S. cerevisiae contain multiple copies of CUP1 and are resistant to 0.3 M Cu^{++}. Ethyl methanesulfonate (EMS) mutagenesis of a copper-resistant strain containing an integrated CUP1-lacz gene fusion yielded several types of Cu-sensitive mutants. One class of mutants has a trans-acting defect in CUP1 gene induction. Using a "Southwestern" blotting technique it has been possible to show that crude extracts from these mutants fail to bind a labeled CUP1 promoter fragment that is bound by the wt extracts. In addition, the effects of various oligonucleotide-directed mutations in the CUP1 promoter region are being assayed. Extensive mutagenesis of a 40 bp control region has revealed intercalated positively and negatively acting regulatory sequences. Studies on the interactions between the cis and trans-acting mutations will be described.

O 324 CONSTRUCTION AND CHARACTERIZATION OF VARIANTS OF THE E. COLI rrnB P1 PROMOTER. Tamas Gaal[1], Herman A. deBoer[2], and Richard L. Gourse[1]. (1) Department of Genetics, University of Georgia, Athens, GA 30602 USA (2) Biochemistry Department, Gorlaeus Laboratories, University of Leiden, Wassenaarseweg 76, 2300 Leiden, The Netherlands

We have generated a collection of random mutants in the rrnB P1 promoter using oligonucleotide synthesis in the presence of low levels of contaminating nucleotides. Our collection should contain representatives of single base changes at every position from -88 to +1 with respect to the transcription start site. We have generated mutations using two fragments, one containing the upstream activator sequence (UAS) and one without the UAS. By making promoter - lacZ fusions in phage lambda, we have cloned the promoter variants into the chromosome of E. coli. From the phenotypes of the lambda plaques and lysogens we have been able to select for up mutations, representing derepressed or growth rate independent promoters, as well as for down mutations, representing changes in either the RNA polymerase binding site or in the UAS. We have also isolated silent mutations, which should be useful in identifying positions not important to the transcription initiation rate or to growth rate dependent control. We will discuss mutations which correspond to specific phenotypes in order to define the DNA determinants of upstream activation and growth rate dependence of rRNA synthesis.

O 325 THE DNA DETERMINANTS OF CAP-INDUCED BENDING, Marc R. Gartenberg and Donald M. Crothers, Department of Chemistry, Yale University, New Haven, CT 06511.

Catabolite activator protein (CAP) binds to and bends its DNA binding site in the *E. coli lac promoter*. We have examined the influence of DNA sequence on the extent of CAP-induced bending and protein binding affinity. A large CAP binding site mutant library was generated and the relative gel electrophoretic mobilities of CAP bound fragments were compared. Bending loci were identified by positions where sequence composition strongly influences complex mobility. In regions flanking the consensus sequence, bend centers coincide with positions where DNA grooves face the protein; A-T rich sequences favor bending where the minor groove faces the protein and G-C rich sequences favor bending where the major groove faces the protein. Further quantitative analysis of the data 1) verified that the dinucleotide is the smallest independent structural unit to describe accurately the properties of the CAP-induced bend and 2) ranked the dinucleotides at each position according to their influence on DNA bending. Competitive binding assays revealed that mutants which bend better, bind with greater affinity. The DNA sequence spanning at least 34 bp modulates induced bending and binding demonstrating that the regulatory domain is substantially larger than the consensus sequence. Subtle modulation of protein-DNA complex structure and stability is likely to be desirable for a protein like CAP that regulates multiple operons. Our data correlate with the observed statistical sequence preferences responsible for the rotational positioning of DNA in nucleosomes. We believe the sequence determinants which govern CAP-induced bending may represent general rules by which non-specific protein-DNA interactions lead to DNA bending.

O 326 THE TN10 ENCODED TET REPRESSOR-OPERATOR-INTERACTION IS FUNCTIONAL IN PLANT CELLS, Christiane Gatz, Institut für Genbiologische Forschung, Ihnestr 63, 1 Berlin 33 FRG.

The Tn10 encoded tet repressor-operator-system was used to regulate transcription from the 35S cauliflower mosaic virus (CaMV)-promoter in plant protoplasts. Expression was monitored in a transient expression system using electroporated tobacco protoplasts. The tet repressor, being expressed in the plant cells under the control of eucaryotic transcription signals, blocks transcription of a 35S-CaMV-promoter-chloramphenicol acetyltransferase (CAT) fusion gene when one of the operators is located between the CAAT-box and the TATA-box and the second beween the TATA-box and the transcription start site. In the presence of sublethal concentrations of the inducer tetracycline expression is restored to full activity. Location of the operators downstream of the transcription start site does not significantly affect transcription in the presence of the repressor. These experiments show for the first time that a procaryotic regulatory protein can function in plant cells. The tet repressor-operator-system may be useful as an on/off switch for the regulation of gene expression in gene transfer experiments.

O 327 ASSOCIATION STATES, RNA-BINDING, AND FUNCTION OF E. COLI TRANSCRIPTION TERMINATION PROTEIN RHO

Johannes Geiselmann, Thomas D. Yager, Stanley G. Gill, and P. H. von Hippel, Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, OR 97403

During rho-dependent transcription termination, rho protein of E. coli binds the nascent RNA transcript and releases it in a process that requires the hydrolysis of ATP. We have investigated the interaction of rho with various RNA cofactors and the concomitant structural changes in the protein.

Rho protein can form a stable hexamer under "near physiological" buffer conditions (molecular weight determined by sedimentation, light-scattering, neutron and x-ray scattering). The subunit arrangement is probably not 6-fold symmetrical, as indicated by a stable dimer intermediate on "semi-denaturing" gels. In the presence of oligo(rC) of length <20 nucleotide residues, the rho hexamer associates to form a homogeneous dodecamer. The oligo(rC) appears to be bound with a stoichiometry of six RNAs per dodecamer. With longer RNAs, the homogeneous 12-mer is no longer generated. Poly(rC) of lenth n=70 nucleotide residues binds with a 1:1 stoichiometry to the hexamer, consistent with the site size measured by fluorescence titrations (McSwiggen, Bear and von Hippel, JMB, in press). The apparent molecular weight of this species is between that of a hexamer and a dodecamer. A complex behavior is observed for RNAs of intermediate length.

Currently we are investigating how these structural changes are related to the binding and hydrolysis of ATP. Ultimately, we hope to integrate all these properties of rho to give a detailed molecular mechanism by which rho brings about transcript release.

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O 328 STRUCTURAL AND FUNCTIONAL ANALYSIS OF *cmp*, AN ENHANCER OF PLASMID REPLICATION.

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Replication of the staphylococcal plasmid pT181 requires the plasmid-encoded RepC protein. RepC, that is *trans*-acting and rate-limiting for replication, binds to the plasmid's origin of replication (*ori*) and introduces a site-specific nick that serves as a primer for 3' extension. Studies presented in this paper have shown that *cmp*, a plasmid sequence element located about 1 kilobase from *ori*, facilitates utilization of RepC by *ori*.

Genetic studies demonstrate that pT181 *ori* on a cmp^+ plasmid competes more efficiently for the initiator protein than the same *ori* on a co-resident cmp^- plasmid. *cmp* increases maximal replication rate in cmp^+ plasmids relative to cmp^-, as shown by analysis of the kinetics of plasmid repopulation. Taken together, these results indicate that the presence of *cmp* on the plasmid genome increases the frequency of initiation of plasmid replication.

cmp maps within a 250 base pair fragment, and contains a locus of DNA bending. *cmp* functions independently of the orientation of the element or its location in the plasmid genome.

Cmp^- plasmid mutants have a lower level of titratable negative supercoiling than Cmp^+ plasmids. No preferred gyrase cleavage site maps within the *cmp* region *in vivo*, nor does *cmp* appear to be a binding site for the initiator protein.

The structural features of *cmp* (*cis*-activity, distance from *ori*, orientation- and location-independence) and the role of the element in increasing initiation frequency make it reminiscent of eukaryotic enhancers. However, unlike these elements or the transcriptional activators described in prokaryotic systems, *cmp* has no primary effect on gene expression. The possible mechanisms by which *cmp* affects the site-specific RepC-*ori* interaction and plasmid topology will be discussed.

O 329 E. COLI NUSA AND SIGMA FACTORS: ASSOCIATION STATES AND BINDING INTERACTIONS WITH RNA POLYMERASE

S.C. Gill, T.D. Yager, J. Geiselmann, W.A. Rees and P.H. von Hippel
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In order to understand binding interactions between proteins, the initial association states must be known. We have determined the association states of both NusA and sigma proteins over a wide range of salt concentrations (0.01 to 1.5 M KCl) using dimethyl-suberimidate chemical crosslinking, velocity sedimentation, quasi-elastic laser light scattering, and equilibrium sedimentation. Results from all of these experiments have shown that both NusA and sigma proteins exist as homogeneous populations of monomers under all conditions tested.

Currently we are studying the competition between NusA and sigma for binding to core polymerase under conditions where core polymerase exists as a monomer (Shanner et al. *Biochemistry* 1982, 21, 5539-5551). Several techniques are currently being used. One involves competition between fluorescently labelled sigma factor and unlabelled NusA protein for binding to core polymerase. A second technique involves determining the effect of NusA on the ablity of holoenzyme to carry out abortive initiation on poly d(A-T). Preliminary results from these and other experiments suggest that sigma and NusA have fairly similar binding affinities for core polymerase. We have found no evidence to suggest simultaneous binding of both sigma and NusA to core polymerase.

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O 330 E. COLI RIBOSOMAL RNA PROMOTER-RNA POLYMERASE INTERACTIONS, Richard L. Gourse, Janet T. Newlands, and Ramona R. Dickson, University of Georgia, Athens, GA 30602 and Pieter L. deHaseth, Case Western Reserve University, Cleveland, Ohio 44106.

The rate of ribosome synthesis is determined primarily by the rate of transcription initiation at rRNA promoters. In order to provide a basis for understanding rRNA transcription, we have taken genetic and biochemical approaches to define the rRNA promoter-RNA polymerase interaction. Filter binding and footprinting analyses show that RNA polymerase forms specific, stable complexes with the rrnB P1 promoter, but only in low salt and when the initiating nucleotides are included in the reaction. The kinetics and stoichiometry of the interaction have been measured under the required conditions and show that the rate of binding is as fast as or faster than the rate of binding at any other E. coli promoter and that only one RNA polymerase molecule per promoter is required.

In DNAase I and hydroxyl radical footprinting experiments, the complex is characterized by protection slightly upstream of regions protected in typical E. coli promoters. These likely contacts correlate with a region defined in vivo, the UAS, which is required for maximal transcription rates and which has the characteristics of bent DNA. Furthermore, protection experiments with fragments containing or lacking the UAS indicate that the UAS alters the manner in which RNA polymerase interacts with the rRNA promoter.

Finally, we have found that changing the spacing between the -10 and -35 regions of the RNA polymerase recognition site has a striking effect on rRNA control and rRNA synthesis rates in vivo. These will be discussed in terms of the insights they provide on growth rate dependent control and activation of rRNA transcription initiation.

O 331 BINDING OF HOST- AND VIRAL-SPECIFIC FACTORS TO A BACULOVIRUS ENHANCER. Linda A. Guarino, Department of Entomology, Texas A&M University, College Station, TX 77843.

The delayed early 39K gene of *Autographa californica* nuclear polyhedrosis virus (AcNPV) is *trans*-activated by the immediate early IE-1 gene. Transcription of 39K was enhanced 1000-fold when the viral enhancer *hr*5 was cloned upstream of the 39K promoter. Enhancer activity was dependent upon the presence of IE-1, although the enhancer did not affect the expression of IE-1. To analyze the nature of the interaction between IE-1 and the enhancer, nuclear extracts were prepared from normal insect cells and cells which transiently expressed the IE-1 gene product. Gel retention assays indicated that normal cells contained a factor that bound to the enhancer. DNase footprint analysis demonstrated that the normal cell factor bound to the conserved 28 bp inverted repeat. Binding was increased by partial denaturation of the probe. Nuclease S1 sensitivity indicated that the binding site was located in the double-stranded region of a stem-loop structure in the palindrome. IE-1 transfected cells contained an additional binding factor. Competition experiments and DNase footprint analysis indicated that the two factors bound to different sequences in the 240 bp enhancer fragment.

O 332 λ P_{RM} MUTATIONS AFFECT ACTIVATION OF P_{RM} AND REPRESSION OF P_R. Gary Gussin, Jen-Jen Hwang, Julia Ferm and Susan Brown. University of Iowa, Iowa City, Iowa 52242.

The λ P_{RM} promoter controls expression of the cI gene, whose product (repressor) acts in two ways to maintain a prophage: (1) By repressing P_L and P_R, it prevents expression of genes necessary for lytic multiplication; (2) In binding to the rightward operator O_R, it activates P_{RM}. Mutations can affect both the intrinsic activity of P_{RM} and the degree to which it is stimulated by repressor. A double mutant that contains a prm$^-$ mutation (prmE37) at -14 and an "up" mutation (prmup-1) at -31 responds poorly, or not at all, to repressor both in vitro and in vivo. This effect is due at least in part to prmup-1. Although the mutation increases agreement of P_{RM} with the consensus promoter sequence, it may affect the conformation of P_{RM}-RNA polymerase (RNAP) complexes in such a way that their interaction with repressor bound to the adjacent operator segment, O_R2, is no longer optimal. This interaction was also probed by examining effects of mutations in P_{RM} on repression of P_R in vitro. The data indicate that the quantity of repressor necessary to repress P_R is inversely correlated with the promoter strength of various mutant derivatives of P_{RM}. This result supports the idea that open complex formation by RNAP at P_{RM} enhances repressor binding to O_R2. Moreover, mutations in the -35 region of P_{RM}, such as prmup-1, also alter the shape of the P_R-repressor titration curves. This appears to be due to alteration of the configuration of bound RNAP, which in turn affects the conformation of a repressor dimer bound at O_R2 in such a way that its cooperative interaction with a repressor dimer bound at O_R1 is also disrupted. Thus, a mutation in one promoter (P_{RM}) can affect regulation of a second promoter (P_R) through a series of protein-protein interactions.

O 333 REGULATORY MUTANTS OF THE MU MOM OPERON, Stanley Hattman and Laurel Wall, University of Rochester, Rochester, NY 14627.
The bacteriophage Mu mom gene encodes a DNA modification function. Expression of mom is regulated by the host DNA methyltransferase (Dam) and at least two phage genes, C and com. The C gene product is known to be required for the "turn on" of other late genes, as well. The com gene (whose product appears to be required for mom translation) partially overlaps mom in a different reading frame on the same mRNA transcript, with com being located proximal to the 5' end. To study transcriptional regulation of the mom operon, we constructed a mom-lacZ protein fusion plasmid that produces -galactosidase activity only when Com and C proteins are provided in dam^+ cells.

To obtain regulatory mutants, we subcloned a fragment containing the 5' flanking region plus a portion of com into plasmid vectors containing the M13 origin of replication. Single stranded DNAs were mutagenized in vitro with nitrous acid. After synthesizing the complementary strand and cutting with restriction nucleases, the regulatory region was cloned back into the original mom-lacZ fusion plasmid. C-independent constitutive mutants (tin) and mutants non-inducible by C (tin^O) were identified following transformation of appropriate hosts. The mutational alteration in each plasmid was identifed by DNA sequence analysis, and the results of these studies will be presented.

O 334 COMPARISON OF THE DNA-BINDING ACTIVITIES OF *Drosophila* HOMEO BOX PROTEINS. Timothy Hoey and Michael Levine, Columbia University, New York, NY 10027.

even-skipped (eve) is a member of a small group of genes in *Drosophila* that define the segmentation pattern of the early embryo. *eve* plays a key role in a network of interactions among segmentation genes; there is evidence that the *eve* protein controls morphogenesis by regulating the expression of the segmentation gene *engrailed (en)*, and by autoregulating its own expression. It is possible that these regulatory interactions occur at the level of transcription since *eve* contains a homeo box. We show that *eve* protein binds with high affinity to specific sequences near the *en* and *eve* transcription units. Other *Drosophila* homeo box proteins bind to different subsets of the *eve* protein binding sites with varying affinities. This supports the the proposal that cross-regulatory interactions among homeo box genes involve a competition for similar *cis* regulatory sequences.

O 335 THE REGULATION OF THE EXTERNALLY INDUCED PHOSPHOGLYCERATE TRANSPORT SYSTEM OF SALMONELLA TYPHIMURIUM. Jenshiang Hong, Dianne Goldrick and shu-qin Jiang, Boston Biomedical Research Institute, 20 Staniford Street, Boston, MA 02114.
S. typhimurium has a phosphoglycerate transport system that is induced only by inducer outside, but not inside, the cell. E. coli lacks this system. The system has been cloned; it consists of four genes, PCBA. The P gene encodes the transporter; the A the activator; and the B and C two regulatory proteins involved in the inducibility of the P gene. All four proteins are membrane-bound. The complete nucleotide sequence of the transport system has been determined. The transcription start point (T.S.P.) is at -43 from the translation start point. Although the pgtP expression appears to be positively regulated by the A gene, deletion analysis of the regulatory region indicates that the regulation of the pgtP gene expression is more complex than could be explained by the classical positive control model. Plasmids with short deletions in the region upstream from the P gene have been constructed, some of which exhibited constitutive transport phenotype while others exhibited nontransport phenotype. It appears that the pgtP gene expression is regulated through two sites located some distance from the T.S.P. The URS (upstream repression site) at -250 to -300 is required for repression because its removal renders a constitutive pgtP expression. The UAS (upstream activation site) at -311 to -395 is necessary for activation since its removal abolishes the pgtP expression, and is probably the site to which the activator binds since the activation requires the pgtA gene. These two sites act independently since the spacing between them can be shortened, by at least 11-bp, or lengthened, by 1,444-bp, without any effect on inducibility of the pgtP expression. (Supported by N.I.H. grant GM31836).

O 336 IDENTIFICATION AND PURIFICATION OF A HUMAN IMMUNOGLOBULIN ENHANCER BINDING PROTEIN NF-kB. Kiyoshi Kawakami, Claus Scheidereit and Robert G. Roeder, Rockefeller University, New York, NY 10021.

An enhancer binding factor NF-kB, which is found only in cells that transcribe immuno-globulin light chain genes, has been purified from a nuclear extract of Namalwa (a human Burkitt lymphoma) cells from a sequence specific DNA affinity column. The purified NF-kB has been identified as a 51 kd polypeptide by UV crosslinking analysis. Footprint and Methylation interference analysis have shown that purified NF-kB has a binding activity specific for k light chain enhancer sequence and for Human Immunodeficiency Virus-1 promoter sequence.

O 337 REGULATION OF TNF GENE EXPRESSION IN MACROPHAGES. Michael J. Klemsz, Antonio Celada and Richard A. Maki, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Stimulation of bone marrow-derived murine macrophages, or the macrophage-like cell lines P388D1 and WR19M.1 with IFNγ results in the increased expression of mRNA specific for tumor necrosis factor (TNF). This induction of increased TNF expression is at least in part due to the increased transcription of the TNF gene. Stimulation of murine macrophages or macrophage cell lines with cycloheximide or cycloheximide in combination with IFNγ also results in increased TNF mRNA accumulation. This increase, however, is due to an increased stabilization of the TNF message. To study the mechanism by which IFNγ regulates the transcription of the TNF loci, we have begun to define cis-acting 5′ regulatory DNA sequences and specific DNA binding proteins. All 1.4 kilobases of 5′ flanking sequences of the TNF gene have been cloned next to the assayable CAT gene. Deletion analysis of these sequences indicate that less than 500 basepairs are required for TNF expression, following transient transfection into the P388D1 cell line. We have sequenced this promoter region and show the existence of a TATA box approximately 50 basepairs 5′ to the transcription start site. Additionally, one Spl binding site in the opposite orientation is located 70 basepairs 5′ to the start site. Analysis of specific DNA binding proteins, isolated from the macrophage-like cell lines which may be involved in the regulation of TNF expression by IFNγ will be discussed.

O 338 DNA TOPOLOGY AND ACTIVE CHROMATIN ASSEMBLY *IN VITRO*. Eric B. Kmiec, Donna Gadbois, Lindsay Hinck, Timothy O'Neill, Jo Ann Sekiguchi, Richard Swank, U. of Cal. Davis, Davis, CA 95616. We have examined the energy requirements of *in vitro* chromatin assembly using relaxed, circular DNA templates and a cell-free extract from *Xenopus laevis* oocytes (S-150). The deposition of nucleosomes on circular DNA can occur with endogenous levels of ATP while extended chromatin assembly requires an additional energy source. The anti-trypanosomal drug, Berenil, prevents high levels of DNA supercoiling, but does not block chromatin assembly. This observation suggests that part of the supercoiled DNA resulting from the action of the S-150 may be an unconstrained topological state unassociated with chromatin structure. Consistent with this hypothesis is our successful isolation of a protein complex from the S-150 that can introduce unconstrained supercoils into circular DNA templates. The gyratory activity of this complex is completely abolished by 5 μM Berenil, suggesting that the enzyme which catalyzes the reaction is a Type I Topoisomerase. The addition of this protein complex to a transcription reaction stimulates the level of RNA Pol III gene expression 5-fold whereas purified *Xenopus laevis* Type I Topoisomerase diminishes the transcription signal. The role of DNA topology in the establishment of an active chromatin template may be to introduce subtle structural changes that lead to a more desirable or accessible DNA template to which factors can bind.

O 340 DNA SUPERCOILING INFLUENCES DNA LOOP FORMATION, Helmut Krämer, Michèle Amouyal*, Alfred Nordheim+ and Benno Müller-Hill, Institut für Genetik der Universtät zu Köln, Weyertal 121, 5000 Köln 41, FRG, *Institut Pasteur, Département de Biologie Moléculaire, 25, Rue du Dr Roux, 75724 Paris Cedex, France and +Zentrum für Molekulare Biologie der Universität Heidelberg, Im Neuenheimer Feld, 6900 Heidelberg, FRG.
Tetrameric lac repressor can simultaneously bind to two lac operators, forcing the intervening DNA to form a loop. We used a gel retardation assay to investigate the influence of DNA supercoiling on DNA loop formation in topoisomers. We constructed 15 DNA minicircles carrying two lac operators at distances ranging from 153 to 168 bp. Low DNA supercoiling (σ=-0.023) had little effect when operator spacing was optimal for loop formation in linear DNA (158 bp). In contrast, the stability of DNA loops was drastically increased at a operator spacing of 163 bp, where no loops could be found with relaxed minicircles. The altered spacing requirement for the formation of the most stable loops indicates an altered helical repeat of 10.7 bp for these DNA minicircles. Elevated DNA supercoiling (σ=-0.046) enhanced the stability of the complexes at all distances tested. These results hint, that alterations of DNA supercoiling in vivo might alter the capability of DNA bound proteins to interact at a distance.

O 341 E. COLI RNA POLYMERASE ABORTIVE INITIATION AND ELONGATING COMPLEX FORMATION: ROLES OF THE PROMOTER AND EARLY TRANSCRIBED SEQUENCES, Barbara Krummel and Michael J. Chamberlin, U. of California, Berkeley, CA 94720.
We are studying the properties and structure of E. coli RNA polymerase complexes formed at a variety of promoters during the early steps of initiation and chain elongation. The sites used include phage T7A1, λ P_L, or Tac promoters as well as hybrid sites at which the initially transcribed sequences have been altered. Previous studies have shown that initiation complexes possess sigma factor, have a DNase I footprint similar to an open promoter complex, and transcripts formed by such complexes can be released (abortive initiation). Transition of the initiation complex into an elongating complex leads to a decreased DNase I footprint, greatly increased stability of transcript association and, at some point, release of sigma factor. We have used nondenaturing PAGE and gel filtration columns to isolate ternary complexes which are assayed for sigma factor content and DNase I footprinted. Our results show that alteration of the transcribed sequence (from +1) can change the pattern of aborted transcripts and may alter the point of transition from an initiating to an elongating ternary complex as well as the probable point of sigma release. However, it is not the early transcribed sequence alone that is the determining factor. Two templates with different promoters, but identical transcribed sequences, each have unique abortive transcription patterns and potential transition points. Synthesis of a 6 nucleotide transcript is sufficient for isolation of a stable ternary complex on one of these templates while an 11 nucleotide transcript is required on the other. The unique combination of promoter and early transcribed regions control abortive initiation and may regulate the transition from initiation to elongation.

O 342 INHIBITION OF TRANSCRIPTION ELONGATION BY THE BACTERIOPHAGE T4 ALC GENE PRODUCT. E. Kutter, R. Drivdahl, K. d'Acci & B. Guttman, Evergreen, Olympia, WA 98505

The T4 alc gene product inhibits the transcription of cytosine-containing DNA. It is involved in T4's shutoff of transcription of its bacterial host and of other phage, working together with the T4 gpalt, which is injected with the phage DNA and ADP ribosylates the polymerase. Looking at hybridization of labeled RNA to specific cloned host genes, we find that the shutoff of host transcription is delayed by several minutes with (alc, endoII, endoIV) and (alc, alt, mod, endoIV) mutants but is still largely complete by 10 minutes.

The inhibitory effects can also be observed in vitro when the polymerase is partially purified through heparin agarose. Gpalt is responsible for a 50% drop in activity within the first minute after infection, while gpalc is responsible for a further 2-3 fold drop over the next 4 minutes when dC-DNA is used as the template. (This latter drop is not seen on dHMC-DNA templates.)

Rifampicin challenge, end labeling with gamma-^{32}P ATP, and selective initiation with a dinucleotide (using a T7 DNA template) all indicate that gpalc's inhibition of transcription is due solely to inhibition of elongation; initiation rates are unaffected. Binding of gpalc to the polymerase is weak. Wild-type (but not mutant) gpalc copurifies with the polymerase through heparin agarose but not in subsequent steps. Immunoprecipitation also indicates that gpalc is not tightly bound to RNA polymerase intracellularly. Gpalc also binds to dC-DNA (as was reported also by Snustad et al., J. Virol. 60:1185). The most likely model is that gpalc inhibits transcript elongation by interaction with actively transcribing core polymerase in conjunction with cytosine-rich sequences on the template.

O 343 IDENTIFICATION OF AN INTERGENIC TERMINATION SITE BETWEEN THE LAC Z AND Y GENES OF ESCHERICHIA COLI. Catherine Kwan, George J. Murakawa and Donald P. Nierlich, Mt. St. Mary's College and UCLA, L.A., CA 90024.

The lac Z, Y and A genes of E. coli span a region of over 5300 nucleotides. Surprisingly, we have demonstrated that the predominant lac mRNA species present in cells is the 3000 nucleotide lac Z transcript. We have also determined the 3' terminus of this transcript using S1 nuclease mapping. The 3' terminus is located 40 nucleotides after the last translated codon of lac Z. A G:C rich hairpin structure followed by a stretch of U's can be constructed at this site. This structure resembles a rho independent termination site.

In order to further establish that this site functions as a transcriptional termination we have carried out in vitro transcription experiments. A plasmid was constructured that has the λ P_R promotor fused to the distal protion of lac Z and the intergenic area between lac Z and Y. This plasmid was used in an in vitro transcription system with purified E. coli RNA polymerase. The RNA products were analyzed by gel electrophoresis. The results show clearly that termination occurs at this intergenic site.

O 344 TOPO I AND TOPO II CLEAVAGE SITES IN POLYOMA MINICHROMOSOMES. Marialuisa Lavitrano, Terumi Kohwi-Shigematsu, and Corrado Spadafora, La Jolla Cancer Research Foundation, La Jolla, CA 92037.
We have mapped the cleavage sites of Topoisomerases I and II on the polyoma minichromosomes in 3T6-infected cells by use of m-AMSA, VM 26, and Camptothecin. m-AMSA and VM 26 induce Topo II cleavage both in the coding and the regulatory regions. An almost identical pattern of five discrete bands is shared by both drugs in the enhancer region. In contrast to the cleavage pattern of the enhancer region, that of the coding region shows considerable differences with very few, if any, common sites between m-AMSA- and VM 26-treated cells. Such patterns remain almost unmodified between 22 and 36 hours after virus infection. We have found that some of these Topo II sites, especially those induced by m-AMSA, are matched by S1 hypersensitive sites determined on polyoma chromatin and on extracted DNA. Topoisomerase I inhibitor, Camptothecin, induces cleavage almost exclusively on transcribed regions while a major cleavage site was found in the enhancer region.

O 345 SITE SPECIFIC PERTURBATION ANALYSIS OF PROMOTERS. J.Lebowitz, J. Sullivan, B. Johnson and P. T. Chan. University of Alabama at Birmingham, Birmingham,Alabama 35294.
In order to gain further insight into the different sets of interactions that control the functional stages of transcription initiation we need to be able to probe changes in RNA polymerase-promoter contact interactions at different sites in the promoter and correlate these changes with the parameters that govern promoter activity. We are exploring the following strategy to accomplish this objective. The lac UV 5 promoter, as a model system, is being synthesized in such a way that a nucleotide gap exists at a specific site exposing a base for site directed chemical modification. This exposed base will then be modified with a reagent specific for this unpaired base. After modification a fully duplex structurally perturbed promoter is generated by further manipulation of the oligonucleotide strands. This structurally perturbed promoter can now be examined for changes in: 1. contact interactions; 2. RNA polymerase binding; 3. abortive initiation; and 4. abortive cycling vs. active transcription. By introducing unique modifications at other sites and repeating the analysis for perturbed functions we hope to develop an understanding of the molecular parameters that govern promoter activity.
Supported by American Cancer Society Grant NP-582.

O 346 EFFECTORS OF THE STEPS IN E. COLI RNA POLYMERASE TRANSCRIPTION INITIATION, S. Leirmo and M. T. Record, Jr., Depts. of Chemistry and Biochemistry, University of Wisconsin, Madison, Wisconsin, 53706

Roe and Record (1985 J. Mol. Biol. 185, 441) proposed a three-step mechanism for open complex formation of the λPr promoter: initial binding, protein isomerization, helix opening. We have examined the effects of cation and anion concentration and type and DNA supercoiling on both the bimolecular and isomerization association rate constants. Cation release in the initial binding step alone accounts for most of the salt dependence of the overall process. The nature of the anion dramatically affects both initial binding and subsequent isomerization steps, with rate constants increased 2-50 fold by substitution of the organic anions acetate and glutamate for chloride, yet the net number of anions involved is small. Anion effects are expected to be protein-based since under these conditions E. coli RNA polymerase conformational equilibria are anion dependent while DNA helix opening is not. Anion effects on the magnitude of the association rate constants correlate well with measurements of anion interactions with protein surface area (Arakawa and Timasheff, 1984, Biochemistry 23, 5912), and support the proposal that isomerization to the open complex involves burial of protein hydrophobic surface. Substitution of a supercoiled for a linear template also increases both bimolecular and isomerization rate constants.

O 347 INTERACTION OF LYMPHOID-SPECIFIC NUCLEAR PROTEINS WITH THE OCTAMER MOTIF OF THE HUMAN INTERLEUKIN-4 GENE, Min Li-Weber, Astrid Eder, Anke M.J. Peters and Peter H. Krammer, Institute for Immunology and Genetics, German Cancer Research Center, D-6900 Heidelberg, FRG.

B-cell stimulatory factor (BSF-1)/Interleukin-4 (IL-4), a lymphokine secreted by activated T cells, has multiple biological activities affecting growth and differentiation of B-cells, T-cells, and mast cells. The human IL-4 gene, like most eukaryotic genes, contains a TATA box 19-26 bp upstream from the 5' end and multiple transcriptional control elements, i.e. one octamer motif and two CCAAT boxes upstream of the TATA box. Three distinct nuclear proteins interacting specifically with the octamer motif of the human IL-4 promoter region have been detected. Among them two octamer-binding proteins (OBP) (OBP-1 and OBP-2) may be lymphoid-specific. OBP-1 and OBP-2 may regulate IL-4 gene activation. We demonstrated that at least one of these two proteins is distinct from the lymphoid-specific octamer binding factor NF-A2 previously studied (Staudt, L.M. et al., Nature 323, 640, 1986).

O 348 PHOTOAFFINITY LABELING OF *E. COLI* RNA POLYMERASE/POLY-dAT TRANSCRIPTION COMPLEXES, Claude F. Meares and Thomas M. Stackhouse, University of California, Davis, CA 95616.

To elucidate the molecular interactions during transcription by *E. coli* RNA polymerase, we have performed a quantitative analysis of the photoaffinity labeling produced by an aryl azide positioned at the leading (5') end of the nascent RNA. Macromolecular contacts on the path of RNA across the transcription complex containing the template poly[d(A-T)] are observed as a function of the length of the transcript. Quantitative analysis provides the percent yield of photoaffinity labeling in the transcription complex by each length of RNA. Significant yields are observed for DNA, the β/β' subunits (analyzed together), and the σ subunit. The α subunit is not labeled under these experimental conditions. The DNA template is labeled by the leading ends of RNA molecules 5-18 bases long, with yields ranging from 1-6%. Photoaffinity labeling of the poly[d(A-T)] is also observed for many transcript lengths longer than 18, but the yields are too low to quantitate. Labeling of the β/β' subunits occurs with ≈50% yields for transcripts of lengths ≥12; low but significant labeling yields of 1-8% by shorter RNAs (3-10 nucleotides) are observed. The RNAs most likely to be photoattached to the σ subunit are 9-12 nucleotides long, with a maximum photoaffinity labeling yield of 15% by the decanucleotide. Labeling of the σ subunit is low but still detectable for transcript lengths >12. These results modify the conclusions of previous work concerning the release of σ from an *E. coli* RNA polymerase/poly [d(A-T)] transcription complex (U. Hansen and W. R. McClure, J. Biol. Chem. **255**, 9564-9570 [1980]). The photoaffinity labeling of σ in poly[d(A-T)] transcription complexes differs from the results observed with DNA containing either the lambda P_R or the T7 A1 promoter (S. L. Bernhard and C. F. Meares, *Biochemistry* **25**, 5914-5919 [1986]), providing further evidence that the interaction between the nucleic acids and the σ subunit in the transcription complex depends on the nucleotide sequence.

O 349 GENE REGULATION OF SUCCINATE DEHYDROGENASE IN B. SUBTILIS. Lars Melin, Alex von Gabain and *Lars Rutberg. Dept. of Bact. Karolinska Inst. Box 60400, S-104 Stockholm. *Dept. of Microbiology, University of Lund, Solveg. 21 S-223 62 LUND.
Succinate dehydrogenase (SDH) in B. subtilis is a membrane-bound enzyme complex containing equimolar amounts of 3 subunits: cytochrome b_{558}, flavinprotein, and iron-sulfur protein. The structural genes for these subunits are arranged in an sdh operon; the direction of transcription is cytochrome-flavoprotein - iron-sulfur protein. The genes have been cloned and sequenced with John Guest, University of Sheffield. A sigma 43 promoter region is 90 bp upstream of the ATG initiation codon for the cytochrome gene. This region, TTGACG - 17 nt - TAAAAT is preceeded by an A-T rich region (80% A-T) of about 30 bp. sdh-115 which reduces SDH enzyme protein and activity to less than 0.5% of wild type is a G to A transition in the -35 region, correlated with a decreased production of sdh specific mRNA. Regulation of SDH is complex. When the bacteria reach the post-exponential growth phase, enzyme protein and activity increase 2 to 4-fold. SDH is also subjected to glucose repression. When 100 bp. upstream the -35 region of the promoter is deleted, the inducibility of the operon is lost. The sdh promoter region has been inserted upstream of a promoterless cat gene in a derivate of the high copy plasmid pPL603. No effect of glucose or growth phase on cat expression was seen with this construct in B. subtilis. A fragment of this plasmid containing the sdh promoter region and the cat gene was then inserted into the B. subtilis chromosome in tandem with the sdh operon. When present as single copy cat gene expression showed the same growth phase dependence and glucose sensitivity as the sdh operon.

O 350 DEVELOPMENTAL REGULATION OF AN RNA POLYMERASE SIGMA FACTOR, Charles P. Moran, Jr. and Teresa J. Kenney, Emory University School of Medicine, Atlanta, GA 30322.
Bacillus subtilis produces several RNA polymerase sigma factors. Two of the secondary sigmas are essential for endospore formation -- σ^H, which is present in vegetative cells, and σ^E, which is produced exclusively after the start of endospore formation. The structural gene that encodes σ^E is part of the spoIIG operon. Transcription of this operon is activated after the start of sporulation.

We have determined the start-point of transcription and nucleotide sequence of the spoIIG promoter. This promoter contains sequences that are similar to those found at the -10 and -35 regions of promoters that are used by $E\sigma^A$, the primary form of RNA polymerase in vegetative cells; however, the -35-like sequence is centered at position -40. Single base substitutions in these -10 and -35-like sequences reduced utilization of the spoIIG promoter in vivo. $E\sigma^A$, but not $E\sigma^H$ or other secondary forms of RNA polymerase, accurately initiated transcription from the spoIIG promoter in an in vitro assay; therefore, we suggest that $E\sigma^A$ transcribes the spoIIG operon. The effects of mutations in this promoter lead us to speculate that regulation of this sporulation specific transcription may involve a novel activator that binds near the -10 region of the promoter and compensates for the unusual spacing between the -10 region and the -35-like sequence.

O 351 SECONDARY STRUCTURES IN SUPERCOILED PLASMID DNA ARE LOCATED AT PROMOTERS AND REPLICATION ORIGIN IN VITRO AND IN VIVO. P.H. Noirot and R.P. Novick, Department of Plasmid Biology, Public Health Research Institute, New York, N.Y. 10016.
pT181 is a naturally occurring plasmid of *Staphylococcus aureus*, fully sequenced and genetically well characterized. The structure of its supercoiled DNA was investigated *in vitro* using nuclease S1 as a probe for single stranded regions. The major S1-sensitive sites mapped in two strong promoters of the plasmid. Minor sites mapped in the replication origin and in a third promoter. These sites correspond either to potential hairpins or to locally denatured A-T rich regions.

It is striking that in a plasmid which is 70% A-T rich, all the S1-sensitive sites detected *in vitro* are located in regions of biological significance (which are not the most A-T rich regions). This shows that DNA sequences having promoter or replication origin functions may have special physical properties at the level of DNA itself.

The presence of *in vivo* secondary structures was investigated by using bromoacetaldehyde (BAA), which can react with unpaired A and C residues *in vivo*. We found that all of the sites that we had characterized *in vitro* also reacted with BAA *in vivo* but with different frequencies. For instance, the promoter of the *pre* gene, encoding a site-specific recombinase (Pre) (M.L. Gennaro et al (1987) J. Bacteriol. **169**, 2601-2610), is the weakest S1-sensitive site *in vitro* and the strongest site *in vivo*.

The *pre* promoter sequences overlap the site at which Pre-mediated recombination occurs. We are currently testing the hypothesis that the increased sensitivity to BAA *in vivo* is a consequence of increased DNA unwinding due to the interaction between the Pre protein and its recombination site.

O 352 **A PUTATIVE NATURALLY OCCURRING "NULL RECEPTOR" MAY BEHAVE AS A REPRESSOR OF GLUCOCORTICOID INDUCIBLE GENES.** James S. Norris, S.L. MacLeod and L.E. Cornett, Departments of Medicine and Physiology/Biophysics, University of Arkansas Medical School, Little Rock, AR 72205.

The smooth muscle derived DDT_1 MF2 wild type (WT) cell line has a number of well characterized genes and pathways that are responsive to glucocorticoids. A nonresponsive variant of WT cells has been isolated (GR1). This cloned line has 60-70% of the WT glucocorticoid receptor but only 18% of the steroid binding capacity. Our data suggests the variant receptor is a "null receptor." To our surprise, glucocorticoid receptor expression vectors transfected into GR1 cells failed to reconstitute glucocorticoid inducible CAT activity suggesting the possibility of a receptor processing defect. To test this hypothesis, human estrogen receptor (HEO) and Vit-TK-CAT plasmids were introduced. Estradiol induced CAT activity in both variant and WT cells thus demonstrating that estrogen receptors were able to transactivate ERE's. From these and related data a second hypothesis was proposed which suggests the variant glucocorticoid receptor represses transcription of GRE containing enhancers in the presence or absence of glucocorticoid. This is being tested. The preliminary results demonstrate hMtIIa-CAT can be activated in WT cells by $1x10^{-8}$M glucocorticoid, aldosterone or 100 uM $ZnCl_2$. In the variant neither glucocorticoid, aldosterone nor $ZnCl_2$ activate the reporter. These data are consistent with our second hypothesis. Further work with deletion mutants will be presented. Supported by GM 30669.

O 353 PURIFICATION OF PROTEINS WHICH BIND TO THE PUTATIVE NUCLEAR FACTOR I BINDING SITE IN THE MOUSE α_2(I) COLLAGEN PROMOTER. Jouko Oikarinen and Jukka Ristiniemi, Collagen Research Unit and Biocenter, University of Oulu, Oulu, Finland

We have previously demonstrated that a factor that is identical or related to nuclear factor I (NF I) binds to a region from -315 to -295 with respect to the start of transcription in the mouse α_2(I) collagen promoter (J. Biol. Chem. 262, 11064-70, 1987). Based on functional deletion analysis, this region is needed for efficient expression of a chimeric gene driven by the promoter. Recently, we have purified a protein which binds to this site from rat liver using heparin-agarose chromatography and two successive DNA recognition site affinity chromatographies. About 1800-fold purification over nuclear extract was achieved. SDS-polyacrylamide gel electrophoresis and silver staining revealed a major band, 32,000 of molecular weight, and a faint 64,000-molecular-weight band. The preparation contains two activities which can be separated by gel filtration on a Sephacryl S-300 matrix. Using this purified factor and gel retardation assay or DNase I footprinting, several additional binding sites for this factor were found in the α_2(I) collagen promoter fragment spanning from -1340 to +1520. Dissociation constants for several promoter fragments were determined using nitrocellulose filter binding assay. They range from 20 nM to 3.5 nM, when the consensus sequence for NF I has a dissociation constant of 0.45 nM.

O 354 ZINC-DEPENDENT STRUCTURE AND DNA BINDING OF A SINGLE FINGER DOMAIN OF ADR1. Grace Párraga, Suzanna J. Horvath*, Arri Eisen, Wayne Taylor, Leroy Hood*, E.T. Young and Rachel E. Klevit. Department of Biochemistry, University of Washington, Seattle WA 98195 *Department of Biology, California Institute of Technology, Pasadena CA 91125

The 'zinc-finger' DNA-binding motif has been proposed on the basis of sequence analysis, partial proteolysis, and zinc content of Xenopus transcription factor IIIA. Other eukaryotic proteins have subsequently been found to contain contiguous repeat units of this postulated DNA-binding domain. The yeast protein ADR1, a positive regulator of transcription of the glucose-repressible alcohol dehydrogenase gene (ADH2) contains 2 adjacent finger domains. Point mutations in ADR1 showed that specific conserved residues within the finger domains were essential for function.

A 31 residue peptide corresponding to the second zinc finger domain of ADR1 was chemically synthesised by the step-wise solid phase method and purified by reverse-phase HPLC. Circular dichroism spectroscopy indicates that the peptide adopts a folded structure in the presence of zinc; the presence of 5mM $ZnCl_2$ resulted in an increase in negative ellipticity at 222 nm and 208 nm, suggestive of the formation of α-helical structure in the peptide. The peptide also binds Co^{2+} and the absorption spectrum of the peptide-Co^{2+} complex is consistent with tetrahedral coordination of the metal ion. Furthermore, ^{1}H NMR spectroscopy indicted that the peptide exists in a single major conformation in the presence of zinc. In addition, histidine residue resonances were shifted suggesting they are involved in liganding zinc. 2D ^{1}H NMR NOESY spectra have allowed an assignment of the residues involved in the α-helix suggested by the CD spectroscopy and a model has been proposed for the single 'zinc-finger'. Finally, data will be presented illustrating the zinc-dependent DNA-binding properties of the peptide, supporting the hypothesis that a single 'zinc-finger' is an independent structure sufficient for DNA binding.

O 355 FUNCTIONAL ANALYSIS OF THE HEPATITIS B VIRUS ENHANCER ELEMENT, N.U.Patel, S.Jameel and A. Siddiqui. Dept. of Microbiology/Immunology, University of Colorado Medical School, Denver.

We have previously shown by means of gene transfer experiments that the human hepatitis B viral (HBV) enhancer exhibits cell-type specificity for human hepatoma cells and that this is due to the presence of trans-acting factor(s) present in the liver cells. Using gel retardatiOn and Dnase 1 protection assays we show the binding of multiple proteins to the enhancer DNA in vitro.

Mutational studies at some of these Dnase I protected regions were carried out in order to determine not only , (1) the functional significance of these protein-DNA interactions but (2) to better understand the factor(s) involved in cell-specific interactions.

O 356 A DNA BINDING PROTEIN ACTS AS A TRANSCRIPTIONAL ROADBLOCK

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What results when an elongating RNA polymerase encounters DNA-bound protein is of general interest. To ask if a protein bound tightly to a specific sequence blocks further elongation by E. coli RNA polymerase, transcription on templates associated with a mutant of the EcoRI endonuclease has been studied in vitro. This protein (E111G) binds to the wild type recognition sequence with high affinity yet carries out no appreciable cleavage (1, 2). When a DNA template containing one EcoRI site is transcribed in the presence of E111G and rifampicin, two RNA products are seen: a full-length runoff transcript and a truncated RNA species whose 3' endpoint is immediately upstream of the EcoRI recognition sequence. Under conditions of complete binding by E111G, the shorter transcript is the major RNA species appearing. Blockage appears long-lived and not dependent on the DNA sequence context upstream of the EcoRI site. During steady state transcription an additional RNA 3' endpoint, due to a second RNA polymerase stalled behind the first, is seen. From analysis of the RNA 3' ends, the position of the 3' terminal ribonucleotide with respect to the leading edge of the RNA polymerase ternary complex has been located.

1. King, K., Wright, D. and Modrich, P. (1986) Fed. Proc. Abstracts 45, 1913.
2. King, K., Benkovic, S. J. and Modrich, P. (submitted).

O 357 THE TRANSCRIPTIONAL ACTIVATORS OF HTLV-I, HTLV-II AND BLV ACTIVATE EXPRESSION OF THE HUMAN c-fos PROMOTER. George N. Pavlakis, Nicholas Grammatikakis and Barbara K. Felber, NCI-Frederick Cancer Research Facility, BRI-Basic Research Program, Frederick, MD 21701.

The viruses belonging to the HTLV-I family (HTLV-I, HTLV-II and BLV) have similar genome organization and encode transcriptional activators of their LTR promoters. These activators act via an approximately 10 bp cis-element that has characteristics of an inducible enhancer. This element also exists within the human and mouse c-fos promoters and in an area shown to be important for PDGF stimulation. We have shown that the c-fos promoter is activated by the transactivators of HTLV-I, HTLV-II and BLV after transfections in mouse cells. Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-74101 with Bionetics Research, Inc.

O 358 CRYSTALLIZATION OF THE MET REPRESSOR FROM *E. coli*.
Simon E.V. Phillips[1], John B. Rafferty[1], Carlos Rojas[2], Ginette Boulot[3], Isabelle Saint-Girons[3], Yvonne Guillou[3] and Georges N. Cohen[3], (1) Astbury Department of Biophysics, University of Leeds, Leeds LS2 9JT UK, (2) Departamento de Fisica, Universidad Nacional de Colombia, Bogota, Colombia, (3) Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris, France.

The met repressor from E. *coli* is the product of the MetJ gene, and controls transcription of genes for the biosynthesis of methionine, with S-adenosylmethionine acting as corepressor. It contains a region of sequence related to the first part of the "helix-turn-helix" motif of other repressors, but lacking part of the second helix. It has been crystallized in space group $P2_1$, with unit cell dimensions a=35.6, b=62.6, c=44.5Å, ß=102.4° and one aporepressor dimer per asymmetric unit. Preliminary X-ray diffraction photographs show measurable intensities to beyond 1.5Å resolution, and low-resolution crystallographic analysis shows it to be a dimer with two-fold symmetry, consisting almost entirely of α-helices. High-resolution structural results will be presented if available.

O 359 ANALYSIS OF THE MOUSE α1-GLOBIN GENE PROMOTER, Mark Plumb, Kay MacLeod, Helen Wainwright, Mark Walker, Jonathan Frampton and Paul Harrison, The Beatson Institute for Cancer Research, Glasgow, U.K.
Using footprint analyses and functional assays using promoter sequences linked to the CAT gene in transient transfection assays, we have identified two erythroid-specific regulatory domains within 250bp 5' from the α1-globin gene CAP site. One contains the CCAAT and CACCC motifs. In some tissues, binding to the CCAAT box by the ubiquitous CCAAT-binding protein (CBP) is prevented by the binding of a displacement protein (αP2b) which binds to an overlapping sequence. The relative nuclear concentration of CBP and αP2b may determine the transcriptional activity of the promoter. A second domain contains a sequence (GATAAG) which is bound by an erythroid-specific protein conserved in chicken, mouse and human. The GATAAG motif is found in the 5' flanking promoter sequences of a variety of globin genes including the mouse β-major, and chicken α-pi, α-D and β-rho globin genes (Kemper et al (1987), Mol. Cell Biol. 7, 2059). Functional analyses suggest that the 1 and -maj GATAAG elements respectively confer a 2 and 8 fold erythroid-specific enhancement of CAT gene transcription in erythroleukemia cells compared to fibroblasts.

O 360 A PUTATIVE DNA-BINDING DOMAIN WITHIN THE *virG* LOCUS OF THE *A. tumefaciens* Ti PLASMIDS, Bradford S. Powell and Clarence I. Kado, Department of Plant Pathology, University of California, Davis, CA 95616. Positive regulation of virulence (*vir*) genes contained within tumor-inducing (Ti) plasmids of *Agrobacterium tumefaciens* is mediated by *virA* and *virG* in the presence of phenolic compounds exuded by wounded plant cells. A model for *vir*-gene regulation is based on homologies between amino acid sequences derived from *virA* and *virG* and those from a family of two-gene regulatory systems of various bacterial taxa. By similarity *virA* would encode a signal-transducer and *virG* a transcriptional activator. The nucleotide sequence of *virG* of the nopaline-type plasmid pTiC58 was determined and found to have 77% identity to *virG* of the octopine-type plasmids, and therefore is also homologous to the family of regulatory genes. The inferred VirG protein is most similar to its homologues in the carboxy-terminal region which is believed to contain the domain responsible for transcriptional activation. A plot of predicted secondary structure reveals within this region an alpha helix-turn-alpha helix which is conserved between *virG* genes of the two types of plasmids but not among the family of regulatory genes. A gene fusion has been constructed and hybrid protein missing the amino-terminal half of VirG has been isolated to test the possibility of a DNA-binding domain in the carboxy-terminal region of VirG.

O 361 THE E.COLI MALTOSE REGULON. Evelyne Richet, Dominique Vidal-Ingigliardi and Olivier Raibaud. Unité de Génétique Moléculaire, Institut Pasteur, Paris.

The expression of the E.coli maltose regulon is controlled by MalT, a transcriptional activator. The protein (Mr = 102,000) has recently been purified to homogeneity in an active form and shown to be an ATP-binding protein. In agreement with in vivo observations, the purified MalT protein alone stimulates initiation of transcription at malPp by the RNA polymerase holoenzyme, whereas both MalT and CRP (cAMP Receptor Protein) are required for activation of transcription at malEp and malKp. MalT binds promoter regions that contain the conserved hexanucleotide 5'-GGAT/GGA-3', a sequence genetically identified as the site of action of MalT. MalT binding to DNA depends strictly upon the presence of both ATP and maltotriose, the inducer of the regulon.

malEp and malKp, a pair of divergent promoters which control the synthesis of the transport system components, have been further characterized. Genetic experiments indicate that these promoters are functionally coupled; malKp activity strongly depends on the MalT binding site(s) proximal to malEp (at -240 upstream of malKp), and vice versa. The intergenic region contains four CRP-binding sites. malEp and malKp therefore appear to extend over $\sim$240 bp, encompassing several series of binding sites for both activators, CRP and MalT.These features are reminiscent of eukaryotic promoters. When present alone, MalT binding to the malEp/malKp region induces a DNase I cleavage pattern in the central region which is characteristic of a DNA loop or DNA wrapping on a protein core. In-phase insertions in the middle of this region do not inactivate these promoters whereas insertions which are out of phase inactivate them.

O 362 ACRIDINE-INDUCED FRAMESHIFT IN BACTERIOPHAGE T4, Lynn S. Ripley, University of Medicine and Dentistry of New Jersey, Newark, NJ 07103

The type II topoisomerase of bacteriophage T4 is a central determinant of the frequency and specificity of acridine-induced frameshift mutations. Acridine-induced frameshift mutagenesis is specifically reduced in a mutant defective in topoisomerase activity. The ability of an acridine to promote topoisomerase-dependent cleavage at specific DNA sites in vitro is correlated to its ability to produce frameshift mutations at those sites in vivo. The specific phosphodiester bonds cleaved in vivo. The cospecificity of in vitro cleavage and in vivo mutation implication acridine-induced, topoisomerase-mediated DNA cleavage as intermediates of acridine-induced mutagenesis in T4.

We have examined the effects of nucleotide sequence changes on topoisomerase-mediated cleavage and mutagenesis. The types of changes leading to altered activity of topoisomerase will be discussed.

O 363 CHROMOSOMAL LOOP/NUCLEAR MATRIX ORGANIZATION OF TRANSCRIPTIONALLY ACTIVE AND INACTIVE RNA POLYMERASES IN HELA NUCLEI, Michel Roberge and E. Morton Bradbury, University of California, Davis, CA 95616.

The relative distribution of transcriptionally active and inactive RNA polymerases I and II between the nuclear matrix/scaffold and chromosomal loops of HeLa cells was determined. Total RNA polymerase was assessed by immunoblotting and transcribing RNA polymerase by a photoaffinity labeling technique in isolated nuclei. Nuclear matrix/scaffold was isolated by three methods using high-intermediate- or low- salt extraction. The distributions of both transcribing and non-transcribing RNA polymerases differed considerably with the different methods. Intermediate-salt followed by high-salt treatment of DNAse I-digested nuclei released all transcribing and non-transcribing RNA polymerases. Either intermediate- or high-salt treatment showed significant association of RNA polymerases with the nuclear matrix which can be interpreted as artifacts. Nuclear scaffolds isolated with lithium diiodosalicylate (low-salt) contained very little RNA polymerase. This treatment, however, dissociated RNA polymerase II transcription complexes. These results show that RNA polymerases, both in their active and inactive forms, are not nuclear matrix proteins and suggest that they are not fixed but move around DNA loops during transcription.

O 364 GENETIC ANALYSIS OF MerR, THE POSITIVE/NEGATIVE REGULATORY PROTEIN OF THE TN21 MER OPERON. Wilma Ross, Soon Jung Park and Anne O. Summers, Department of Microbiology, University of Georgia, Athens, Georgia 30602.

The 16kd MerR protein regulates transcription from the Tn21 mer operon both negatively, in the absence of Hg(II), and positively in the presence of Hg(II). We have constructed a multicopy operon fusion of the mer promoter to lacZ, in which three levels of expression from the mer promoter can be clearly observed: repressed, unregulated or derepressed (MerR-), and Hg(II)-activated. This fusion plasmid was used to isolate hydroxylamine-generated mutants defective in MerR-mediated regulation. Several classes of mutants have been identified, including both MerR mutants and cis-acting mutants. Among the most interesting MerR mutants are those selectively altered in only one of the two MerR functions, either repression or induction. Members of one class are defective for induction, but retain full repression activity in the absence of Hg(II). A majority of these mutants map in the carboxy terminus of MerR. A subclass of the induction defective mutants remains fully repressed in the presence of Hg(II). These contain mutations in 3 of the 4 cysteine residues of MerR, indicating a critical role for these residues in MerR function, possibly as ligands for Hg(II). Other induction defective mutants show a slight response to Hg(II), resulting in partial or full derepression. This subclass includes a mutation in the second of two potential helix-turn-helix regions of MerR, regions possibly involved in DNA binding. An additional class of MerR mutants is derepressed in the absence of Hg(II), but fully Hg(II)-inducible. This class includes mutations resulting in partial activation of transcription in the absence of Hg(II).

O 365 TRANSCRIPTIONAL ACTIVATION BY SINGLE-STRANDED DNA BINDING PROTEINS. Nam Young Baek and Lucia B. Rothman-Denes University of Chicago, Chicago, Ill 60637.

Coliphage N4 utilizes three different RNA polymerases for transcription of its 72 kb double-stranded linear DNA genome. We have previously shown that early transcription is catalyzed by a phage-coded, virion encapsulated RNA polymerase. This enzyme requires supercoiled template and E. coli single-stranded DNA binding protein (SSB) for promoter recognition. Activation by SSB is specific, no other single-stranded DNA binding protein can substitute.
N4 late transcription is carried out by the E. coli RNA polymerase. Sites of late transcription initiation have been identified. There is no interhomology between these sequences at -10 and -35 and low homology to the E. coli sigma-70 consensus sequence. The sequence AGTCGGTT is found near all late start sites and is located primarily between +1 and -10. DNAs carrying these sequences are poor templates for in vitro transcription by E. coli RNA polymerase. In vivo utilization of late transcription initiation start sites is not dependent on N4 DNA replication but requires the activity of the N4-coded single-stranded DNA binding protein (N4 DBP). Experiments are in progress to determine the role, if any, of the conserve octanucleotide in promoter recognition and the mechanism of transcriptional activation by N4 DBP.

O 366 FUNCTIONAL ANALYSIS OF A MAMMALIAN TRANSCRIPTIONAL ACTIVATOR PROTEIN

Sandro Rusconi, Yvonne Severne, Stefan Wieland, Markus Thali, Institute for molecular biology, Univ. ZH, CH-8093 Zuerich Hoenggerber, Switzerland.

We used the glucocorticoid receptor (GR) and its target enhancer element (GRE) as a model system to develop improved tools for the systematic study of the phenomenon of trans-activation. We have developed three powerful techniques with this system. a) An extremely sensitive assay for trans-activation: this will become the basis for a genetic screening of revertant transcriptional activators, i.e. derived from a functionless GR. b) The construction of a mutagenesis "cassette" to this purpose, we entirely re-designed the c-DNA portion encoding the essential GR functions, in a manner that allows rapid generation and direct in vivo testing of specific mutants. c) The linkage to the GR of an additional DNA binding domain derived from an unrelated protein: this allows the generation of bifunctional transcriptional activators which can be used to better understand the phenotypes obtained by site-directed mutagenesis, with the help of a cis-trans or a trans-trans complementation assay. Perspectives: as the results indicate that it is possible to generate chimaeric GR molecules with the desired additional specificity, we plan to use this fact for the direct selection of further DNA binding factors from mammalian cells.

O 367 ALLELOCHEMICALS: INTERACTIONS WITH NUCLEAR MEMBRANE STRUCTURES, Matthew Ryuntyu, Department of Agronomy and Soil Science, University of New England, Armidale. N.S.W. 2351.

Various allelochemicals have been found to inhibit respiration of 0_2-consumption by mitochondria and uncouple oxidative phosphorylation from respiration (Lovett and Ryuntyu, Primary Effect of Benzilamine: Mitochondrial Membranes. Proc. 1st Inter. Congress of Plant Tissue Culture Tropical Species, Bogota: 1987).

Thus, allelochemicals can decrease ATP production by inhibiting mitochondrial activities on the basis of destruction of the mitochondrial membrane, as well as nuclear membrane. Membrane of damaged nucleus can be observed TEM. A number of mitochondria increase may be followed by intense activity of the Golgi vesicles. Because mitochondrial reactions involved in ATP production take place on intermitochondrial membranes, it is quite possible that the manner by which allelochemicals inhibit mineral transport across the nuclear membrane and the tonplast and the manner by which they inhibit mitochondrial activities are similar. The ability of mitochondria to synthesize protein is perhaps not surprising, since it is well established that they contain DNA and so have some degree of genetic autonomy. A strong possibility is that allelochemicals alter permeability of membranes in both instances. It is possible that the conpounds solubilize into membranes, and thus cause a "loosening" of the membrane structure so that minerals can "leak" across the membrane.

O368 **A BIPARTITE MODEL FOR NEGATIVE REGULATION OF YEAST GAL4 PROTEIN FUNCTION.** J.M. Salmeron, Jr., S.D. Langdon, and S.A. Johnston, Duke University, Durham, NC.

The Saccharomyces cerevisiae GAL4 protein activates transcription of galactose metabolism enzyme genes. GAL4-mediated gene expression is blocked in the absence of galactose by the negative regulatory GAL80 protein, an interaction that requires the carboxy-terminal 28 amino acids of GAL4. To learn more about the mechanism of GAL80-mediated negative regulation, we are studying the GAL4 homolog from Kluyveromyces lactis, LAC9. LAC9 activates transcription in S. cerevisiae, but is not regulated by GAL80. We have eliminated overexpression of LAC9 as a possible cause for the lack of observable interaction with GAL80 protein. GAL4 and LAC9 carboxyl termini are very similar, but not identical. We have mutated the GAL4 carboxyl terminus, incorporating all features of LAC9, and find that no change affects the ability of GAL4 protein to interact with GAL80. Furthermore, no reciprocal change in the LAC9 carboxyl terminus conditions a GAL80-responsive LAC9 protein. Taken together with results demonstrating that carboxy-terminal deletions of GAL4 protein still bind GAL80 in vitro, we propose a model for negative regulation in which GAL80 acts by binding to the middle of GAL4 protein, and inhibits transcription by masking the GAL4 carboxyl terminus from cellular transcription factors.

Poster Session 3

O 369 RESOLVASE PROTEIN BENDS DNA TOWARDS THE MAJOR GROOVE AT THE SITE OF RECOMBINATION, Joseph J. Salvo*# and Nigel D.F. Grindley, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510.

The relative direction of DNA bends can be deduced from helical phasing experiments. If two DNA bends are linked by DNA spacer sequences of variable length, then the gel mobility of each phasing variant depends on the relative position of one bend with respect to the other. We coupled a segment of intrinsically bent kinetoplast DNA (K DNA) from Leishmania tarentolae with the protein-induced DNA bend locus that spans the site of resolvase-mediated recombination. The observed electrophoretic mobilities of the resolvase-complexed DNA fragments varies cyclically with spacer length, and the minima occur when the centers of the bend loci are separated by an integral number of helical turns. This implies that both bends are in the same relative orientation. Independent evidence concerning the absolute structure of K DNA allows us to deduce that the resolvase-induced bend is toward the major groove.

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O 370 MOLECULAR MODELING OF SPECIFIC INTERACTIONS IN THE λ REPRESSOR-OPERATOR COMPLEX. Akinori Sarai,[1] Frederick H. Hausheer[2] and Yoshinori Takeda.[3] [1] Lab. Math. Biol., DCBD, NCI, NIH, Bethesda, MD 20892, [2] Adv. Sci. Comp. Lab., and [3] Lab. Math. Biol., NCI-FCRF, PRI, Frederick, MD 21701.

We have studied the specific interactions involved in the λ repressor-operator complex by systematically substituting the bases within the operator and measuring the affinity change. The complete mapping of the binding free energy change along the 17-bp sequence of operator enabled us to identify the location of specific interactions. The results show that the specific interactions are concentrated in the non-α-helical regions (the N-terminal arms and the loop region between α_3 and α_4 helices) than in the α_3 helix region, and that λ repressor binds to the operator qdite asymmetrically with respect to the center of approximate two-fold symmetric sequence. In this report, we will present a model of specific molecular interactions based on experimental data and computer simulation of the λ repressor-operator complex. The structure of the λ-DNA complex is first energy-refined, followed by molecular dynamics simulation of equilibrium structure including counter ions and water molecules. Such simulations suggest that λ repressor binds to the operator asymmetrically, consistent with experimental results. The N-terminal arm in the consensus side interacts directly with base pairs, whereas the the other arm in the non-consensus side interacts mainly with phosphate backbone. The details of the molecular interactions between base pairs and amino acids and hydration structure in the complex will be discussed.

O 371 **REGULATION OF THE C-FOS PROTO-ONCOGENE TRANSCRIPTION,** Paolo Sassone-Corsi, Jane Visvader, Masahiro Fujii, John C. Sisson and Inder M. Verma, The Salk Institute, P.O. Box 85800, San Diego, California 92138.

Proto-oncogene c-fos is induced in response to a variety of agents. We have previously shown that c-fos gene expression is regulated by both negative and positive cellular factors. One type of positive regulation is induced by agonists of adenylate cyclase. We report that human c-fos-CAT recombinants transfected into PC12 rat pheochromocytoma cells and in JEG-3 human choriocarcinoma cells are induced by forskolin and that this induction is greatly reduced in the mutant PC12 cell line A126-1B2, which is deficient in cAMP-dependent protein kinase II. The cAMP induction of the c-fos promoter requires multiple regulatory elements bearing the consensus sequence TGACGTA. Using deletion analysis and DNase I footprinting assays we have defined one of these elements centered at position -60. The specific binding of a nuclear protein(s) to the c-fos CRE can be competed by the somatostatin and α-chorionic gonadotropin CREs. We also demonstrate that the affinity-purified 43 kd CRE nuclear binding protein (CREBP) from PC12 cells also bindseto the c-fos CRE. We suggest that c-fos activation by cAMP requires the post-translational modification of the specific CREBP. The c-fos promoter is also induced by tat-I gene products of the *human T-cell leukemia virus type I* (HTLV-I). The region of the c-fos promoter required for this trans-activation is between -362/-223; this region also contains the DSE (dyad symmetry element) which is involved in serum, TPA and EGF induction. The induction by the tat-I products on this c-fos promoter element is orientation independent. Furthermore, the expression of the endogenous c-fos gene is increased in HTLV-I infected cell line.

O 372 MOLECULAR CONTACTS BETWEEN POLY(ADP-RIBOSE) POLYMERASE AND DNA, Srinivas S Sastry and Ernest Kun University of California, San Fransisco, CA, 94143. The interaction between poly(ADP-ribose) polymerase and various uniquely end labeled restriction fragments from SV40 and pBR322 DNAs was studied employing nuclease protection experiments. DNaseI footprinting indicated that approximately 66-85 bp of DNA was protected by poly(ADP-ribose) polymerase from DNaseI attack, and that a segment of DNA probably lies outside on the surface of the polymerase protein in the polymerase-DNA complex. Of the four different restriction fragments assayed for polymerase binding, only the 209 bp EcoRI-PstI SV40 DNA fragment showed clear poly(ADP-ribose) polymerase footprints, indicating the specific binding of the polymerase to this DNA fragment. Since this 209 bp EcoRI-PstI SV40 DNA fragment is overall A+T rich and is intrinsically bent,we conclude that the specific binding of the polymerase to the internal length of DNA is dictated perhaps by A+T richness and DNA bending. The polymerase also binds to the 5' and 3' termini of other DNAs as assayed by digestion of the polymerase-DNA complexes with λ exo and exo III. Methylation protection experiments and the binding of the polymerase to glucosylated and nonglucosylated T4 DNA suggest that the polymerase makes major groove contacts. The SV40 209 bp fragment also contains unusual DNA conformations as indicated by its sensitivity to several single strand-specific probes. The polymerase specifically binds these unusual conformations.

O 373 DELIMITATION OF THE BINDING OF PUFF-SPECIFIC PROTEINS AT THE SALIVARY GLAND SECRETION GENE SGS-4 OF DROSOPHILA MELANOGASTER. H. Saumweber, M. Frasch . Institut für Entwicklungsphysiologie, Universität Köln, 5000 Cologne FRG. Max Planck Institut für Entwicklungsbiologie Tübingen, Abt.1

Two Puff-specific proteins of D. melanogaster (Bj6-ag, Bx42-ag) have been identified by the use of monoclonal antibodies. By biochemical criteria both proteins are associated with chromatin. Both proteins can be localized at a number of puffs on salivary gland polytene chromosomes. More detailed studies of one of these sites, at the salivary gland secretion gene sgs-4, showed a strong correlation of the presence of these proteins with gene activity. Using a number of transformed Drosophila stocks, the region necessary for binding of both proteins could be delimitated to a 2.5 kbp directly upstream the structural gene. Studies with deletion stocks suggest, that the binding of Bx42-ag may depend on a 40 bp region containing a consensus sequence found for ecdyson regulated genes of Drosophila.

O 374 **MOLECULAR GENETIC ANALYSIS OF A BACTERIOPHAGE CHROMATIN PROTEIN**
M. Sayre and E.P. Geiduschek, Biology Dept., University of California, San Diego, La Jolla, CA

The lytic *Bacillus subtilis* phage SP01 encodes an abundant 99-amino acid DNA-binding protein, transcription factor 1 (TF1). TF1 is a type II DNA binding protein; other well-known members of this family are *E. coli* HU, IHF and *Bacillus stearothermophilus* HU. Unlike HU, TF1 binds preferentially to hydroxymethyluracil-containing DNA, and exhibits both nonspecific and site-specific binding to this DNA. Some of its preferred sites on SP01 DNA overlap RNA polymerase binding sites at early promoters, and TF1 inhibits transcription of SPO1 DNA by bacterial RNA polymerase *in vitro*. We have made mutant TF1 genes and put them back into phage chromosomes to examine their effects *in vivo*. We have also purified mutant proteins from overproducing *E.coli* strains to study their DNA-binding properties. Segregation analyses with partially diploid phage heterozygous for TF1 show that TF1 is essential for plaque formation. A lethal amber mutation at codon 91 generates a truncated protein that appears to dimerize like normal TF1 but cannot bind to SPO1 DNA. The relationship of primary structure to the site-specific DNA-binding activity of TF1 is further dissected by analysis of purified TF1 missense mutant proteins. For example, substitution of Gln for Phe at codon 61 in the proposed DNA binding "arm" abolishes site-specific but not nonspecific binding to SPO1 DNA *in vitro*.

O 375 TRANSCRIPTIONAL REGULATION OF THE HUMAN INTERFERON-ß GENE
Christina Scharnhorst, Hansjörg Hauser, Willi Dirks, Klaus Hoffmann, Heide Reil, GBF, D-3300 Braunschweig, FRG

Induction of the human IFN-ß gene in many fibroblastoid cell lines in response to viral or dsRNA inducers occurs at the level of transcription activation. In some cell lines superinduction enhances the (induced) transcription of IFN-ß. The DNA-elements controlling the transcription activation process have been defined by construction and transfer of gene mutants and hybrids into mouse, hamster and primate cells: Several upstrem DNA-motifs act in a synergistic manner, even over long distances to achieve full induction activity. DNA sequences mediating the transcriptional activation by induction and superinduction are identical. The regulatory sequences interact with cellular factors, mediators in the induction processes. Promoter competition assays demonstrate the existence of repressing and activating factors. The action of activating factors can only be detected in the induced state. Superinduction is found to be an enhancement of the activation process. With these experiments the DNA sequences for binding or repressor and activator binding could be identified. The binding and action of cellular factors is also demonstrated by gel shifting assays, DNAse footprinting and transcription *in vitro*. Induction and superinduction of a mouse IFN-α-promoter is also observed in several cell lines. However, both promoters, human $ß_1$ and mouse α_4, do not compete for the same factors in transient *in vivo* assays.

O 376 COOPERATIVITY OF THE GLUCOCORTICOID RECEPTOR AND THE CACCC-BOX BINDING FACTOR

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The rat tryptophan oxygenase (TO) gene is expressed in liver and can be induced by glucocorticoid hormones. Both transient and stable expression of a transfected TO-CAT fusion gene were inducible by dexamethasone. Analysis of transcripts from 5'-deletion mutants identified two glucocorticoid responsive elements (GRE), located 450 bp and 1200 bp upstream of the RNA start site[1]. These experiments revealed a discrepancy between the results obtained by binding tests with purified glucocorticoid receptor and by functional assays of deletion mutants[1]: In addition to the footprint region covered by the steroid receptor a sequence further upstream is required for steroid induction. This sequence contains in antisense orientation a region of 12 nucleotides identical to the part of the ß globin promoter which harbors the CACCC-box element. Footprint experiments showed efficient competition with an oligonucleotide containing the ß globin upstream sequence, but showed no competition with mutants or GRE sequences. These results suggest the binding of the CACCC-factor immediately adjacent to the glucocorticoid receptor. To distinguish between TO specific effects and general mechanisms of steroid induction, we combined CACCC-box sequences with a minimal glucocorticoid receptor binding site of the mouse mammary tumor virus (MMTV) and cloned it in front of the thymidine kinase (tk) promoter, which in turn controlled the CAT gene. This construct was inducible by dexamethasone, whereas a corresponding construct without CACCC-element was not. In order to ascertain whether the effect of the CACCC-sequence is dependent on the distance from the GRE sequence we inserted a linker sequence between the CACCC and GRE sequences, which allowed the generation of a number of spacing mutants. Transient expression of these spacing mutants resulted in optimal inductions only for those mutants, whose linker sequences differed in length by multiples of about 10 bp. A control plasmid, which contained the very same sequences in identical order and distance as one of the inducible spacing mutants except that the GRE sequence was replaced by a linker sequence, was not inducible. These results show cooperativity between the CACCC-box and the GRE depending on the precise stereo-specific alignment of the two elements.

1) Danesch, U., Gloss, B., Schmid, W., Schütz, G., Schüle, R. and Renkawitz, R. (1987), EMBO J., 6: 625-630

O 378 IN VITRO TRANSCRIPTION BY NUCLEAR EXTRACTS FROM RAT MUSCLE TISSUE. Zahradka, P., Larson, D.E. & Sells, B.H. Dept. Molecular Biology & Genetics, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

A cell free transcription system has been developed using extracts prepared from rat L6 myoblasts and terminally differentiated myotubes. These extracts accurately transcribe cloned rRNA and ribosomal protein L-32 templates and display the same pattern of rRNA and ribosomal protein gene transcription that occur in vivo (Zahradka & Sells, 1988, Eur. J. Biochem. in press). To determine whether gene expression in mature muscle tissue is comparable to that found in L6 myotubes, transcriptionally active nuclear extracts from rat muscle tissue were prepared. This protocol requires rapid processing of the tissue following sacrifice of the animal and extensive purification of the nuclei prior to salt extraction. The ability of the muscle nuclear extract to transcribe several genes, including rRNA, r-protein and the adenovirus major late promoter, was assessed and compared with the activity present in extracts from myoblasts and myotubes. These studies show that gene expression by myotube and muscle extracts have a high degree of similarity thus suggesting that the myoblast cell culture system is a useful model for muscle development. Supported by MRC and JDF.

O 379 PHORBOL ESTER INDUCED ENHANCER BINDING FACTOR NF-κB CAN ACTIVATE TRANSCRIPTION IN NON-LYMPHOID CELLS, Barbara Nelsen*, Lars Hellman* and Ranjan Sen*, *Brandeis University, Waltham, Ma 02254.

NF-κB was originally identified as a B cell-specific nuclear factor that interacted with the immunoglobulin κ light chain gene enhancer. Subsequently it was shown that it could also be transiently induced in T cells and non-lymphoid cells by active phorbol esters (PMA). It was not clear however, whether the induced protein could activate transcription as well in non-lymphoid cells. To focus on the role of NF-κB as a transcriptional activator we constructed a recombinant plasmid that carried 3 copies of the κ-enhancer fragment (κ3) that binds NF-κB, upstream from the bacterial chloramphenicol acetyltransferase gene transcribed from the SV40 early promoter ($p(\kappa3)_3CAT$). We have shown that the κ3 trimer functions as a stage - specific B cell regulatory element. It is inactive in non-lymphoid cells and pre-B cells; and as active as the μ enhancer in B cells and plasma cells. To study induction in non-lymphoid cells we generated stable Hela cells transfectants carrying $p(\kappa3)_3CAT$. Upon treatment with PMA an 8-10 fold increase in CAT activity and correctly initiated RNA was observed. Furthermore, the kinetics of RNA induction followed closely the pattern observed for the induction of NF-κB with PMA. Thus active phorbol esters induce a factor in non-lymphoid cells with the sequence specificity and functional characteristics of stably expressed NF-κB in B cells.

O 380 INDUCTION OF EGR-1 EXPRESSION DURING ANTIGEN RECEPTOR DRIVEN B LYMPHOCYTE ACTIVATION.
Vicki L.Seyfert*, Vikas P. Sukhatme**, and John G. Monroe*,
*University of Pennsylvania, Phila. PA and **University of Chicago, Chicago, IL.

Egr-1 is a recently identified early growth response gene which is induced following mitogenic stimulation of fibroblasts, epithelial cells and lymphocytes with kinetics similar to that of the protooncogene c-fos. Recent studies and sequence information suggest that egr-1 may function as a transcription factor. In order to further characterize egr-1 we are studying its role in antigen receptor stimulated $G_o \rightarrow G_1$ transition in murine B lymphocytes. A transient increase in egr-1 mRNA levels was found at 0.5 hours following crosslinking of surface immunoglobulin (sIg) with anti-Ig antibodies. Previous studies have shown that transduction of signals through surface Ig is associated with 2 distinct components; activation of protein kinase C and elevation of $[Ca^{2+}]_i$. Using phorbol diester and Ca^{2+} ionophore to activate PKC and increase $[Ca^{++}]$, respectively we have found that upregulation of egr-1 mRNA levels is linked to the PKC but not the Ca^{2+} component of the sIg signal transduction pathway. Additional studies in progress are aimed at elucidating the relationship of egr-1 to $G_o \rightarrow G_1$ transition in response to other murine B lymphocyte stimulants that do not act through PKC.

O 381 ADENOVIRUS E1A PROTEIN BINDS AN AP-1-LIKE ACTIVITY. X. P. Shi*, R. Weinmann+, and M. Rosenberg*, Smith Kline and French Laboratories, King of Prussia, PA 19406*; Wistar Institute, Philadelphia, PA 19104+

Adenovirus E1A proteins are transacting factors which function in both transcriptional activation and repression of certain viral and cellular genes. Unlike many other transacting proteins, E1A does not bind DNA specifically and thus, its function is probably mediated through interaction with cellular factors. To identify these cellular factors, we isolated proteins from a hela whole cell extract which selectively bound to E1A 13S and 12S protein columns. We demonstrated that the protein eluents from these columns were required for specific transcription of adenovirus E1A responsive promoters. Using DNase I protection studies carried out on two different promoters known to respond either positively or negatively to E1A function (i.e., adenovirus E3 and SV40 early) and another promoter carrying a known AP-1 binding site (human MT IIA promoter), we detected an AP-1-like binding activity which was specifically retained by both E1A13S and 12S columns. Other factors which bind to these promoters were not retained on these columns. The protected AP-1 binding region defined in adenovirus E3 promoter is known to be critical for E1A transactivation. Moreover, the protected AP-1 region in the SV40 early promoter is likely to be important for E1A repression. Thus, we suggest that AP-1, or an AP-1-like protein, may be a common target for E1A interaction for both its activation and repression functions in these two promoters.

O 382 **INTERACTION OF T7 RNA POLYMERASE WITH DNA IN AN ELONGATION COMPLEX ARRESTED AT A SPECIFIC PSORALEN ADDUCT SITE**
Yun-bo Shi, Howard Gamper, and John E. Hearst, Department of Chemistry, University of California, Berkeley, CA 94720

We have probed the interaction of T7 RNA polymerase with DNA in an elongation complex arrested by a site specifically placed psoralen diadduct or furan-side monoadduct using DNase I footprinting techniques. The psoralen derivative HMT (4'-hydroxymethyl-4, 5', 8-trimethylpsoralen) was placed at a specific site in the middle of a chemically synthesized double-stranded DNA fragment containing a T7 RNA polymerase promoter at one end. The psoralen molecule was photochemically attached either to two adjacent thymidine residues on opposite strands as a diadduct or to only one thymidine residue on the coding strand as a furan-side monoadduct. Using these psoralen modified DNAs as templates for transcription, we found that T7 RNA polymerase was blocked at the psoralen adduct site and that the arrested elongation complex protected about 15 nucleotides upstream from the adduct on the coding strand and 20 nucleotides around the adduct on the noncoding strand from DNase I digestion. The two psoralen modified DNA templates yielded identical RNA transcripts and DNase I footprints. In contrast, T7 RNA polymerase protected only the coding strand from -20 to +8 in the initiation complex. These results suggest that the RNA polymerase undergoes a marked conformational change upon converting from an initiation complex to an elongation complex. (This work was supported by NIH Grant GM 11180.)

O 383 INVESTIGATION OF RSV LTR ENHANCER-BINDING PROTEINS, Mary L. Shimkus, Amy M. Boulden, and Linda J. Sealy, Vanderbilt University, Nashville, TN, 27232.
The mechanism whereby the Rous Sarcoma virus (RSV) long terminal repeat (LTR) enhancer stimulates the transcription of a cis-linked gene appears to be similar to other enhancers in that specific trans-acting protein factors are involved. Gel electrophoretic mobility shift assays have identified two factors - enhancer factors I (EFI) and II (EFII) - present in nuclear extracts prepared from quail cells and 12-14 day chick embryos, which specifically bind to two different regions in the 286 bp enhancer of RSV. Investigations are currently underway to demonstrate that the DNA-protein complexes formed in vitro are functionally significant in vivo. Combinations of the enhancer factor binding sites are being tested to determine the minimum sequences required for full enhancer activity. Enhancer factor binding to a minimum enhancer, and to the minimum enhancer which has been directly mutagenized, will be correlated with the abilities of such DNA's to enhance transcription in vivo after insertion into a construct containing the chloramphenicol acetyl *transferase* (CAT) gene transcription unit. Recently, a third factor - EFIII - has been identified which binds to the RSV enhancer in a region with homology to the serum response element (SRE). The binding of EFIII to this DNA in vitro is being correlated with the ability of this DNA in vivo to increase the activity of the RSV enhancer in response to serum. Concurrently, efforts are being made to purify the protein components of the enhancer factors via a combination of conventional column purification steps and by affinity chromatography with DNA-cellulose resins prepared with the minimum synthetic oligonucleotide to which the enhancer factors specifically bind.

O 384 IN VITRO ANALYSIS OF THE α2U GLOBULIN PROMOTER. PROTEIN SHARING BETWEEN TWO HIGHLY ACTIVE LIVER PROMOTERS, F. Sierra* and U. Schibler, Université de Genève, Dept. de Biologie Moleculaire Sciences II, 30 Quai E. Ansermet, CH-1214 Genève 4. *Current Adress: Nestlé Research Centre, Nestec Ltd., Vers-chez-les-Blanc, CH-1000 Lausanne 26 (Switzerland).
We have analyzed the protein: DNA interactions as well as the transcriptional activity of the rat α2U-globulin promoter in liver and spleen. In vitro transcription assays indicate that mutant templates containing at least 115 bp of upstream sequences are efficiently transcribed in liver but not in spleen nuclear extracts. In contrast, a mutant template containing 77 bp of upstream sequences is highly active in nuclear extracts from either tissue. A further deletion to -43 dramatically decreases the level of transcription observed in both tissues. Footprint analysis of this region indicates the binding of several proteins in both nuclear extracts, yielding a similar overall DNase I protection pattern for liver and spleen nuclear proteins. However, more detailed studies indicate that these cis- acting control elements bind different factors in the expressing and non-expressing tissues. More interestingly, these experiments also revealed that most proteins binding to the α2U-globulin promoter are also shared with the albumin promoter. Specifically, we have observed that both of these promoters bind factors that are highly enriched in liver extracts. One of these factors is heat stable and might correspond to EBP/CBP; the other is a member of the NF-1 family, which is enriched in liver (ΨNF-1). The two promoters share binding sites for yet another protein, which might correspond to HTF-1. Protein binding sites for apparently ubiquitous transcription factors are note shared by the two promoters.

O 385 THE STEROID-FREE GLUCOCORTICOID AND ESTROGEN RECEPTORS INTERACT WITH THE HORMONE REGULATORY ELEMENT OF MMTV IN VIVO, Emily P. Slater, Michael Schauer, Jutta Arnemann, Gerhard Posseckert and Miguel Beato, Philipps Universität, 3550 Marburg, F.R.G.
In a particular clone of the human mammary cell line T47D that exhibits very low levels of endogenous glucocorticoid receptor (GR), transfection of the GRcDNA in an expression vector confers glucocorticoid inducibility to a cotransfected reporter gene linked to the hormone regulatory element (HRE) of mouse mammary tumor virus (MMTV). In this cell line the same constructions are highly inducible by progestins, and we know that the progesterone receptor (PR) binds to the HRE of MMTV. At suboptimal levels of GRcDNA and hormones the effects of progestins and glucocorticoids are additive. Induction by progestins is, however, progressively inhibited when increasing amounts of GRcDNA are cotransfected with the reporter gene and no glucocorticoids are added. Mutants of the GRcDNA that yield receptor molecules without DNA binding activity, do not inhibit progestins response of cotransfected MMTV-containing constructions. The estrogen receptor (ER) also binds in vitro to the HRE of MMTV, although in vivo there is no stimulation of the MMTV constructs in response to estrogen. Interestingly, increasing amounts of transfected ERcDNA also progressively inhibit the induction of the transfected MMTV constructs by progestins in T47D cells. Thus, these results suggest that under the conditions of gene transfer the steroid-free glucocorticoid and estrogen receptors do interact with the HRE in the long terminal repeat region of MMTV.

O 386 TRUNCATED ESTROGEN RECEPTORS IN MOUSE MAMMARY TUMORS
Mels Sluyser, B. Moncharmont, A.W.M. Rijkers and C.C.J. de Goeij, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Tumors that initially respond to hormone therapy lose their hormone responsiveness after a time and then grow autonomously. We have proposed that this is due to the appearance of aberrant steroid receptors (mutated, truncated) that bind to DNA and give a constitutive growth signal even in the absence of hormone (M. Sluyser and J. Mester, Nature 315, 546, 1985). In order to investigate this hypothesis, we have studied estrogen receptors (ERs) in mammary tumors of GR mice. GR mammary tumors initially are estrogen and progesterone dependent, but lose this hormonal dependency during serial transplantations.
Hormone dependent GR mammary tumors were found to contain 6.5 kb ERmRNA transcripts that encode a 65 kDa receptor protein. By contrast, some hormone independent tumors contained shorter size ERmRNAs and lower molecular weight ER proteins. The interaction of these truncated ERs with estrogen responsive elements on genomic DNA is currently being investigated.

O 387 THE ESCHERICHIA COLI DAM GENE IS EXPRESSED AS A DISTAL GENE OF AN OPERON, Piotr Jonczyk, Russ Hines, and Douglas W. Smith, Dept Biology, UCSD, La Jolla, CA 92093.
DNA containing the Escherichia coli dam gene and sequences upstream from this gene were cloned from the Clarke-Carbon plasmids pLC29-47 and pLC13-42. Promoter activity was localized using pKO expression vectors and galactokinase assays to two regions, one 1650 to 2100 base pairs (bp) and the other beyond 2400 bp, upstream of the dam gene. No promoter activity was detected immediately in front of this gene; however, transcription through this region was enhanced about 3-fold at restrictive temperatures in E. coli dnaA46 cells. The nucleotide sequence upstream of dam has been determined. An open reading frame termed damX is present between the nearest promoter region and the dam gene. Codon usage and base frequency analysis indicate this is expressed as a protein, of predicted size 46 kD. A 46 kD protein is expressed from this ORF, detected using minicell analysis of appropriate derivative plasmids. Although no function has been determined for the damX gene product, significant homology exists between its amino acid sequence and that of the E. coli tonB protein. Sequence comparisons between the region upstream of damX and the aroB gene (FEBS letters 200:11-17; 1986) show that the aroB gene is immediately upstream of damX, and that the promoter activity nearest to dam is found within the aroB structural gene. This activity is relatively weak (about 10% that of the E. coli gal operon promoter). The promoter activity detected beyond 2400 bp upstream of dam is likely to be that of the aroB gene. An RNA transcript of about 3000 bases has been detected by Northern analysis using dam gene probes; transcripts expressing dam from the aroB promoter should be 4000 bases or larger. Restriction site comparisons show that the E. coli mrcA gene resides about 6 kb upstream of aroB.

O 388 TRANSCRIPTIONAL OF RIBOSOMAL RNA GENES Barbara Sollner-Webb, Louis Pape, Val Culotta, Sheryl Henderson, John Tower, and Josiane Eid
The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

We are studying transcription of rRNA genes, using mouse and frog as model systems. Over the last few years we have shown that the frog (MCB 6,1228; 4585) and mouse (MCB 5,554) rDNA promoters are large, multi-domain structures and that transcription can also be stimulated by polymerase I terminator sequences that reside just upstream of the rDNA promoter (Cell 47,891). Transcription requires two factors: 'D' which binds primarily to the upstream half of the core promoter domain and 'C' which binds to the rDNA/D complex (MCB 6,3451). C is an activated subform of RNA polymerase I, the decreased abundance of which evidently down-regulates rDNA transcription under various growth inhibitory conditions (Cell 50,873). Mouse C and D also transcribe the frog rDNA promoter (PNAS 84,7498) and a rodent rDNA spacer promoter (JT, SH, and BSW, in prep.). In other studies, we have recently developed an in vitro system responsive to the Xenopus rDNA enhancer and found that it acts at a preinitiation step in stable complex assembly (LP and BSW, in prep). Our data indicate that both the cis-stimulation and trans-competition of promoters by the rDNA enhancer are due to its interaction with the frog equivalent of factor D (LP et al., submitted).

We have also reinvestigated the chromatin structure of active Xenopus rRNA genes and demonstrated a regular 200 bp repeating organization, indicative of an array of active nucleosomes (VC and BSW, Cell, in press).

Finally, we have succeeded in transiently transfecting trypanosomes and having this DNA transcribed, opening the way to study gene expression in these organisms (PNAS 84,7812).

O 389 STUDIES ON THE REGULATION OF THY-1 GENE EXPRESSION. E. Spanopoulou, G. Kollias, V. Giguere and F. Grosveld, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

Detailed analysis of the mouse Thy-1 promoter revealed the presence of a methylation-free island in the 5' region and the absence of a conventional TATA and CAAT box. The presence of multiple CAP sites has been verified, some of them being brain-specific. Various regulatory elements in the 5' region have been analyzed by linker-scanning mutation and our studies are currently focused on brain and T-cell specific elements. Finally, the existence of two alternative promoters, as indicated by others, was excluded.

In an attempt to examine the developmental and tissue-specific expression of the Thy-1 gene, several constructs have been introduced into transgenic mice. Two of them showed inappropriate expression in peripheral T-cells, whereas a mouse-human hybrid coding for the human Thy1 protein, gave rise to kidney tumours.

O 390 CORRELATION BETWEEN THE CONFORMATION OF *E. coli* -10 HEXAMER SEQUENCES AND PROMOTER STRENGTH : USE OF ORTHOPHENANTHROLINE CUPROUS COMPLEX AS A STRUCTURAL INDEX, Annick Spassky1, Sylvie Rimsky, Henri Buc and Steve Busby[2], [1]Institut Pasteur, Département de Biologie Moléculaire, 25 rue du Docteur Roux, 75724 Paris Cedex 15 (France) ; [2]University of Birmingham, Department of Biochemistry, P.O.Box 363, Birmingham B15 2TT (U.K.)

We have used the artificial nuclease activity of 1-10 phenanthroline copper ion (1) to analyse conformational modifications induced by point mutations in the "-10 conserved region "of the lactose and galactose operon promoters (2 ,3,4) . In each case , we also determined the rate of formation of transcriptionnally actives complexes , by following the appearance of new $(OP)_2Cu^+$ hyperreactives bands. From these two analyses a correlation has been established between the conformationnal state of the promoter and the rate of formation of the kinetically competent transcription complex. As we have shown elsewhere that $(OP)_2$ Cu^+ symmetric reactivity across the minor groove is associated with high unstacking energy bases, the significance of such a correlation will be discussed in terms of the conformational dynamics of promoter "-10 region ".

1- Sigman D.S. 1986, Accounts of Chemical Research 19, 180-186.
2- Sigman D.S. ,Spassky A. , Rimsky S. and Buc H. 1985, Biopolymers 24, 183-197.
3- Spassky A. and Sigman D.S. 1985, Biochemistry 24, 8050-8056.
4- Spassky A., Rimsky S , Buc H. and Busby S (submitted).

O 391 PROTEIN-DNA CROSSLINKING IN SOLUBLE RAT LIVER CHROMATIN USING AN UV PULSE LASER, Heinz Staffelbach and Theo Koller, Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zürich, Switzerland.

We have established the crosslinking of histones to DNA in soluble rat liver chromatin using a 248nm-15nsec pulse laser.

The following experimental approaches have been used to test for UV-induced histone-DNA crosslinking:

1) In agarose gel electrophoresis under dissociating conditions, irradiated nucleosome cores give rise to a band shift in 20-30% of the ethidium bromide stained material.

2) 12-15% of the DNA of irradiated core particles are trapped in a nitrocellulose filter binding assay.

3) Up to 50% of the DNA of irradiated rat liver chromatin coprecipitate with proteins. The radical-scavenger beta-mercaptoethanol reduces the crosslinking efficiency by about 10%.

4) For the identification of the crosslinked histones, the protein components of isolated complexes were analyzed by SDS-PAGE. Mainly H3 was found to be bound, followed by H2B, H2A and H4 in decreasing order.

In all four approaches, the crosslinking efficiency was on the order of 1.5-3% at 100-200mJ. The method is now applied to the ribosomal genes of Dictyostelium discoideum to elucidate the arrangement of proteins in different gene regions and transcriptional states.

O 392 DETERMINANTS OF TOPOISOMERASE I CLEAVAGE IN VIVO, A. Francis Stewart, Uwe Strähle and Günther Schütz, German Cancer Research Center, Institute of Cell and Tumor Biology, Im Neuenheimer Feld 280, 6900 Heidelberg, F.R.G.

A role for topoisomerase I in transcriptional elongation in vivo has been suggested based upon results obtained with the topoisomerase I inhibitor, camptothecin (1). In both the Drosophila heat shock genes (2) and the rat tyrosine aminotransferase (TAT) gene, increases in transcriptional activity are reflected by increased levels of topoisomerase I cleavages trapped in the transcribed regions. Camptothecin concomitantly causes mRNA levels to fall and this effect is mediated at the level of transcriptional elongation as determined by nuclear run-on experiments. The sites of topoisomerase I cleavage within the transcribed region of the TAT gene are, however, invariant and primarily determined by the DNA sequence as demonstrated by the cleavage pattern obtained in vitro using purified topoisomerase I in the presence or absence of camptothecin. Outside of the transcribed region, in vivo-trapped cleavages occur only at a selected subset of sites observed in vitro. The discrepancy can be adequately explained by the position of nucleosomes and hypersensitive sites as determined by MPE. Fe(II) or DNase I digestions of nuclei. This suggests that mere accessibility to DNA is a major determinant of topoisomerase I activity in vivo. Consistent with this proposition is the fact that the in vivo cleavage pattern can be reproduced in isolated nuclei after incubation in the presence of camptothecin.

1. Stewart, A.F. and Schütz, G. (1987) Cell, 50, 1109.
2. Gilmour, D.S. and Elgin, S.C.R. (1987) Mol.Cell.Biol. 7, 141.

O 393 HLA CLASS II TRANSCRIPTION: B CELL OCTAMER BINDING IS NOT SUFFICIENT IN B CELL LINES OR NECESSARY IN GAMMA INTERFERON INDUCED OR T CELL LINES, Eva Stimac, Sue Lyons and Donald Pious, University of Washington, Seattle, WA 98195.

HLA DR and other human class II histocompatibility genes are transcribed in Epstein-Barr virus transformed B lymphocyte cell lines but not in most T cell leukemia lines. A protein factor in nuclear extracts from B cell lines but not from T cell lines binds to the octamer sequence ATTTGCAT 52 bp 5' of the cap site in the DRα gene. This binding factor is present in the class II negative B cell mutant 6.1.6, but its presence does not result in class II transcription. On the other hand, HUT-78, a T cell line which expresses class II mRNA constitutively, lacks detectable B cell specific binding factor, and gamma interferon does not induce any octamer binding factors during induction of class II mRNA in human fibroblast, HeLa, or melanoma cell lines. In addition, lipopolysaccharide stimulation of mouse pre-B cells increases binding to the octamer sequence without inducing class II mRNA.

O 394 ANALYSIS OF SEQUENCE RELATEDNESS OF STEROID RESPONSE ELEMENTS AND MODE OF ACTION IN INDUCTION OF TRANSCRIPTION, Uwe Strähle, Waltraud Ankenbauer, Gerd Klock, Wolfgang Schmid and Günther Schütz, German Cancer Research Center, Institute of Cell and Tumor Biology, Im Neuenheimer Feld 280, 6900 Heidelberg, F.R.G.

To define the minimal sequence required for glucocorticoid induction and its relationship with that of other steroid responsive elements, oligonucleotides derived from the regulatory region of the tyrosine aminotransferase gene were tested upstream of the thymidine kinase promoter. A DNA sequence of 15 base pairs having partial or perfect symmetry is sufficient to mediate glucocorticoid induction of gene expression. Surprisingly, the same oligonucleotide is also recognized by the progesterone receptor. A closely related, but distinct element confers inducibility by estrogens. To analyze the mechanism by which receptor bound to DNA activates transcription, the 15 bp glucocorticoid response element (GRE) was inserted upstream of a TATA box. RNAase protection analysis shows that the level of correctly initiated RNA is stimulated by hormone indicating that the TATA box is sufficient for mediating glucocorticoid inducibility. We previously showed that the two GREs of the tyrosine aminotransferase gene act in a synergistic manner. A 35 bp long sequence from the chicken vitellogenin gene, which harbors binding sites for the glucocorticoid and estradiol receptor in close proximity, also leads to synergistic induction by two different steroids. Analysis of the dose dependence demonstrates that this synergism is based on an increase in affinity of the receptors to their binding sites.

O 395 A SEQUENCE-SPECIFIC SINGLE STRAND BINDING PROTEIN FOR THE LATE-CODING STRAND OF THE SV40 CONTROL REGION.

Claire Gaillard, Michèle Weber, and François Strauss.
Institut Jacques Monod, 2 Place Jussieu, 75251 Paris 05, France.

We have detected and purified a nuclear protein from uninfected monkey CV1 cells that binds specifically in vitro to the late-coding SV40 DNA strand in the region of transcription control, without any detectable binding to the complementary single strand. Nuclease protection experiments detect two binding sites in the 21 base pair repeat region. The protein does not bind to this region in the double strand form, nor does it bind to the RNA synthesized in vitro using either DNA strand as template. The possibility that this protein plays a role in the control of transcription is being investigated.

O 396 G-GAMMA GLOBIN GENE SEQUENCES THAT INTERACT WITH NUCLEAR FACTORS IN A TISSUE SPECIFIC AND DEVELOPMENTALLY SPECIFIC PATTERN. Kathy Sykes and Russel Kaufman, Duke University Medical Center, Durham, NC.

Analysis of normal and mutant gamma globin genes suggest that the upstream region is composed of multiple regulatory elements which modulate expression of these genes. We have identified specific sequences that interact with nuclear factors from erythroid and non-erythroid cells in a tissue and developmentally specific pattern. We incubated end-labelled DNA fragments representing short segments of the human G-gamma globin gene upstream region with nuclear extracts of induced or uninduced K562 cells, MEL cells, and HeLa cells, and analyzed their interactions in band migration retardation assays. One of the DNA fragments extending from position -135 to -260 demonstrated the loss of a band in hemin induced K562 extracts that is seen in uninduced K562. We have localized the binding of a sequence specific factor to a 27 base pair region centered around -200. We determined that a 27 base pair DNA containing a single base mutation at -202 does not compete for factors binding to the wild type 27 base pair DNA. To further analyze this region, we tested the ability of other sequences, known to bind specific factors, to compete with the -200 region fragment for binding factors. We conclude that a 27 base pair sequence centered on position -202 binds to nuclear factors in a tissue and developmentally specific pattern. Furthermore, a fragment with a mutation at -202, associated with a condition known as Hereditary Persistance of Fetal Hemoglobin has an altered pattern of nuclear factor interactions.

O 397 PROPERTIES OF SV40 LARGE T ANTIGEN:P53 COMPLEXES. L. Tack[1], J. Wright[1], Ashok Srinivasan[2], J. Pipas[2], S. Deb[3] and P. Tegtmeyer[3]. [1]Salk Institute, San Diego, CA; [2]University of Pittsburgh, Pittsburgh, PA; [3]State University of New York, Stony Brook, NY.

SV40 large T antigen is a multifunctional protein required to initiate viral DNA replication. T associates with the cellular p53 protein in both SV40 infected and transformed cells. We have studied several properties of T+p53 complexes compared to T without p53 (To). T+p53 has both enhanced ATPase and GTPase activities as well as altered helicase activity. T bound to p53 is more phosphorylated *in vivo* than To; treatment of T+p53 with phosphatase reverses the enhanced ATPase activity. Analysis of the specific *in vivo* phosphorylation of T bound to p53 revealed an altered pattern of phosphotryptic peptides. We have also analyzed mutant T antigens unable to bind p53 (Peden et al, 1987). All are defective for viral DNA replication and also appear to have altered phosphorylation sites, are unable to oligomerize and are ATPase-inactive. In DNase footprinting assays, the DNA binding activity of T+p53 complexes to the SV40 *ori* was altered when performed under replication conditions; enhanced protection of site III was observed, in addition to sites I and II. Equivalent amounts of T_o protected only sites I and II. That T+p53 may interact with regulatory sequences at the SV40 *ori* suggests a role for this complex in modulating a *ori*-related activity such as the initiation of SV40 DNA replication and/or transcription. Our results using specific monoclonal antibodies, different biochemical assays and mutant T antigens are consistent with a structural and functional relationship between p53 binding, ATPase activity and phosphorylation of the T+p53 complex. Bound p53 also appears to alter other biochemical activities of SV40 T and may play an important role in productive infection.

O 398 HOW CRO REPRESSOR RECOGNIZES OPERATOR DNA SEQUENCES, *Takeda, Y. and **Sarai, A. *NCI-FCRF, PRI, Frederick, MD 21701 and **NCI, NIH, Bethesda, MD 20892.

We have studied the Cro repressor-operator DNA interaction by three approaches. (1) We chemically synthesized 21-mer of OR1, changed all the base pairs with three base substitutions at each base position, and measured quantitatively the affinities of Cro to these mutant operators by the filter binding assay. The results show that the sequence-specific interaction between Cro and the operator occur at the 1 to 7 and 11 to 17 base pairs of the 17 bp operator, and Cro binds to the pseudo-symmetrical operator almost symmetrically. (2) We isolated mutant cro's by changing the amino acids which form the sequence-specific interactions (such as Tyr 26, Gln 27, Ser 28, Lys 32 and Arg 38), and determined the base pair(s) each of these amino acids is interacting with by observing base specificity changes. (3) We analyzed the results obtained above by the computer graphics system. Our model of the Cro-operator DNA binding explains how Cro repressor recognizes specific operator DNA sequences, in particular, how Cro repressor binds to the six operators with the different affinities.

O 399 CIS- AND TRANS-ACTING FACTORS REQUIRED FOR TISSUE SPECIFIC EXPRESSION OF THE PRO-OPIOMELANOCORTIN GENE, Marc Therrien, Lucie Jeannotte, Yves Tremblay, Mark Trifiro, Mona Nemer and Jacques Drouin, Clinical Research Institute of Montreal, Montreal, Canada, H2W 1R7

The gene encoding pro-opiomelanocortin (POMC), the precursor to ACTH, β-endorphin and the melanotropins, is expressed in specific cells of the pituitary, in certain brain areas, testes, ovaries and placenta. We have characterized cis-acting factors required for anterior pituitary specific expression of the POMC gene. POMC promoter activity was assessed in a focus formation assay using hybrid genes constituted of 5'-flanking POMC sequences fused to sequences coding for neomycin resistance (neo). These POMCneo constructs are at least 50 times more active in AtT-20 cells, a mouse pituitary tumor cell line expressing POMC, than in fibroblasts, L cells. In agreement with these results transgenic mice which carry POMCneo constructs predominantly express the transgene in the pituitary.

Deletion analysis as well as fusion of POMC promoter fragments to an heterologous promoter ($pRSV\text{-}_{130}neo$) identified at least two domains within the first 480 bp of the promoter which are recognized and active specifically in AtT-20 cells. These fragments bind ubiquitous and AtT-20-specific factors as revealed by DNAse I footprinting experiments. Replacement mutagenesis is currently underway to further characterize binding sites for ubiquitous and AtT-20-specific factors which contribute to anterior pituitary-specific expression of the POMC gene.

O 400 CHARACTERIZATION OF CIS AND TRANS ELEMENTS REGULATING γ-CRYSTALLIN GENE EXPRESSION. Mark Tini[1,2], Si Lok[1,3], Daphne R. Goring[1,3], Susan O. Meakin[1,2], Lap-Chee Tsui[1,3], Martin L. Breitman[1,2]. (1) Department of Medical Genetics, University of Toronto, Toronto, Ontario, Canada, M5S 1A8. (2) Mount Sinai Hospital Research Institute, 600 University Avenue, Toronto, Ontario, Canada, M5G 1X5. (3) Department of Medical Genetics, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada, M5G 1X8.

The γ-crystallins of the mammalian eye lens are encoded in a multigene family whose individual members are differentially regulated during development. Previous work from our laboratory has demonstrated that the 5' flanking sequences of representative mouse and human γ-genes contain lens-specific promoter activity as well as sufficient information to direct appropriate developmental expression of reporter genes in the lenses of transgenic mice. This information has been localized to sequences -226 to +45 of the mouse γ_2 gene. Detailed mutation analysis of this interval has revealed that it comprises at least two regions which are essential for promoter function: a proximal region (-67 to +8) which spans a 44 bp segment that is highly conserved among different γ-genes, and a distal region (-189 to -162) which comprises part of an enhancer element. Using gel retardation assays, we have shown that DNA fragments spanning either the proximal or distal region detect specific binding activity in nuclear extracts prepared from lens cells of 14 day-old chick embryos. Moreover, binding to at least the distal segment could be effectively competed by the upstream sequences of the divergent mouse γ_4 gene. Thus, although mouse γ_2 and γ_4 are differentially regulated during development, our results suggest that common transcriptional factors interact with the upstream regulatory elements of both genes.

O 401 IDENTIFICATION OF A DNA BINDING PROTEIN WHICH MEDIATES THE BINDING OF PROGESTERONE RECEPTOR TO ITS TARGET SITE. Sophia Y. Tsai, Milan Bagchi, Ming-Jer Tsai, Nancy Weigel and Bert W. O'Malley. Baylor College of Medicine, Houston, Texas 77030.

A hormone responsive element has recently been defined in the rat tyrosine amino transferase gene. When this glucocorticoid/progesterone responsive element (GRE/PRE) was linked to a TK-CAT construct and transfected together with a progesterone receptor expression vector into CV-1 cells, we observed that the expression of TK promoter was regulated by progesterone. Recently, we have identified a protein which binds to a 15 base pair oligonucleotide containing the GRE/PRE and formed a protein DNA-complex (complex I). This binding protein is termed "PRE-B". The binding is specific since it can be competed by oligonucleotide containing GRE/PRE, but not by those containing ERE, COUP or GRE/PRE having two altered nucleotides. PRE-B is not cell specific and is found in HeLa, chicken oviduct, COS, and rat liver cell extracts. In the presence of progesterone receptor preparation, a slower migrating protein-DNA complex (complex II) in addition to complex I is formed. Antibody specific for progesterone receptor further shifted complex II to an even slower migrating complex indicating the involvement of receptor in formation of complex II. Taken together, these results suggest that complex II formation requires both receptor and PRE-B. Partially purified PRE-B footprints weakly on GRE/PRE region, yet the addition of receptor preparation which has no footprinting activity itself yielded a much more pronounced footprint. Thus, both receptor and PRE-B are required for high affinity binding to the GRE/PRE sequence. Our results are consistent with the hypothesis that PRE-B mediates steroid receptor function.

O 402 FOOTPRINTING ANALYSIS OF RNA POLYMERASE II -CONTAINING TRANSCRIPTION COMPLEXES
Michael W. Van Dyke, Michele Sawadogo and Robert G. Roeder
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We are investigating the physical interactions between various transcription factors and the major-late promoter of adenovirus-2 by both DNase I and methidiumpropyl-EDTA·Fe(II) [MPE] cleavage-protection methods. Preliminary results indicate that the optimal interaction of RNA polymerase II with the promoter requires the presence of all general transcription factors (TFIID, TFIIB, and TFIIE) together with the upstream stimulatory factor, USF. Subsequent addition of nucleotides causes the polymerase to clear the promoter, with low concentrations of α-amanitin inhibiting this effect. These studies should provide new insights into both the basic mechanism of class II gene transcription and the mechanism(s) of action for upstream stimulatory proteins.

O 403 SEQUENCE ANALYSIS OF MURINE CYTOKERATIN ENDO A (N° 8) cDNA REVEALS THE PRESENCE IN PCC4 EMBRYONAL CARCINOMA CELLS OF RARE mRNA SPECIES INITIATED UPSTREAM THE NORMAL 5' END, Marc Vasseur[1], Alix Sémat[2], Philippe Brûlet[1], and Yves Michel Darmon[2], 1) Institut Pasteur, 25 Rue du Dr Roux, 75624 Paris Cédex, France, and 2) Centre International de Recherches Dermatologiques (C.I.R.D.), Valbonne, France.

EndoA (keratin 8) is a cytoskeletal protein expressed in simple epithelia together with EndoB (keratin 18). Filaments formed by this keratin pair are the first cytoskeletal elements to be expressed during early mouse embryogenesis in trophectoderm and are induced by retinoic acid in embryonal carcinoma cells. We have isolated EndoA cDNA clones from libaries prepared with polyA+ RNA isolated from PCC4 embryonal carcinoma cells. Sequencing of overlapping cDNAs allowed us to determine the complete sequence of EndoA. During the course of these analyses, cDNA clones extending upstream the normal 5' end of the mRNA were found. These observations support other results (PNAS (1985) 82:8535) showing the presence of EndoA transcripts initiated upstream the normal 5' end in early mouse embryos (from 2-cell to blastocyst stage). The nature and function of an alternative promoter for the EndoA gene used in the mouse embryo before the first differentiation will be discussed.

O 404 THE EFFECTS OF MUTATIONS IN THE LEADER REGION OF THE rrnB OPERON FROM E. coli ON rRNA SYNTHESIS AND CELL GROWTH Martin Zacharias, Jürgen Eberle and Rolf Wagner, Institut für Physikalische Biologie, Universität Düsseldorf, D-4000 Düsseldorf 1 , FRG

The regions upstream to the rRNA genes in E. coli harbour important DNA elements for the regulation of the rRNA synthesis. It is known that activator regions, RNA polymerase pause sites as well as antitermination recognition sequences (box A, B and C) affect the transcription which is started either from the rRNA gene promoter P1 or P2. To investigate the functional significance of a potential transcription termination region (t_L) between the antitermination recognition sequence (box A) and the start of the 16S RNA gene we have constructed a series of deletion and transition mutations in that particular sequence. Cells containing the mutated sequences on multicopy plasmids were assayed for changes in their growth characteristics under different growth conditions and for differences in the rRNA synthesis rates. DNA fragments with base changes in the potential terminator structure t_L were cloned into the test plasmid pOL 6 and assayed for the capacity to terminate transcription. The results obtained with the deletion plasmids indicate that the t_L structure plays a specific role in the mechanism of antitermination in rRNA transcription. This can be inferred from the resulting polarity in the transcription of the promoter proximal 16S RNA versus the promoter distal 23S RNA gene, when the t_L structure is eliminated. The decrease in 23S RNA synthesis is very likely compensated for by an concomitant increase in total rRNA transcription. This is evident by an increase in the accumulation of the co-transcribed $tRNA^{Glu}_2$. Three of the base change mutations lead to an alteration in the phenotype of the cells, namely small colony formation. This change is temperature dependent.

O405 DIRECT CLONING OF 5' REGULATORY SEQUENCES AND SELECTION OF COORDINATELY CONTROLLED PROMOTERS R. Walter, P. Shaw, C. Mueller, and U. Schibler, U. of Geneva, CH-1211

We have characterized and purified (Mueller and Schibler) a transcription factor involved in the liver-specific expression of the albumin gene. Several lines of evidence indicate that this protein is EBP/CBP, a factor that has recently been described by Johnson et al. To test whether this protein has a more general role in liver-specific gene expression, we Searched for binding sites in other liver-specific promoters. To this end, we designed a protocol allowing direct screening of genomic recombinant libraries for promoter sequences that are highly active in hepatocytes. As hybridization probes, we used short fragments of poly A^+ RNA that had been chemically decapped and enzymatically recapped with ^{32}P GTP. Hybridizing clones were assumed to contain the 5' end of the gene. This was confirmed by S1 analysis of selected clones.Of the twelve clones that were chosen for further analysis, seven were highly liver-specific, while the others were members of the α2u-globulin gene family, which are also expressed in other secretory tissues. To test for EBP/CBP binding sites, we performed a filter-binding assay using heat-treated liver nuclear extracts. Members of the α2u-globulin gene family and another, as yet unidentified clone contained restriction fragments which were specifically retained. Footprint and competition analysis with purified protein and specific oligonucleotides confirmed the presence of binding sites within the promoter-proximal fragments, suggesting that the respective genes may be co-ordinately regulated in hepatocytes.

O 406 ANALYSIS OF SEA URCHIN H4 HISTONE GENE PROMOTERS, Eric Weinberg, Lin Tung, Insong Lee, and Paul Chamberlin, University of Pennsylvania, Philadelphia, PA 19104.

During sea urchin embryogenesis, there is a differential regulation of at least two sets of histone genes. The "early" and "late" H4 genes are regulated primarily on the transcriptional level. We have been using a nuclear extract (Morris et al., PNAS 83 3674; 1986) to transcribe sea urchin histone genes and study the sequence requirements for high levels of transcription. Maximal transcription of early and late H4 genes requires 150-200 bp of 5' flanking sequence. Within this region, Bal 31 generated deletions extending progressively towards the start site result in a gradual decrease in the level of transcription so that, for example, a -43 deletion of the early H4 gene retains only 5-10% transcriptional activity. A deletion of the early H4 gene extending from -139 to -102 results in a 10-fold decrease in activity. This sequence contains regions of homology with the region between -60 and -95 of the late H4 gene which is also required for high activity and which gives a strong DNAase I footprint. Both regions compete for a common factor in band retardation assays. For maximal transcription in vitro, a period of 10' preincubation of template with extract is required before addition of nucleoside triphosphates. This observation has allowed us to do commitment assays which show that the early and late H4 genes share at least one factor required for transcription which binds stably to form an efficient initiation complex. This factor is not required for efficient transcription of the early H2B gene. This factor binds upstream of position -102 5' of the early H4 gene.

O 407 INTERACTION OF *Bacillus licheniformis penI* REPRESSOR WITH ITS OPERATORS, Vaughan Wittman and Hing Wong, Cetus Corp., Emeryville, CA 94608.
The expression of the inducible enzyme penicillinase of *Bacillus licheniformis* is regulated by a repressor to which it is genetically closely linked (Dubnau and Pollock, 1965; Sherratt and Collins, 1973). The molecular organization of the chromosomal genes for penicillinase (*penP*) and repressor (*penI*) has recently been clarified (Himeno, Imanaka and Aiba, 1986) and indicates that the two genes are transcribed divergently from with a 364 nucleotide stretch between the coding sequences. We have cloned and sequenced the repressor gene ($penI^C$) from strain 749/C which constituatively produces penicillinase due to an inactive repressor. The $penI^C$ gene was mutated to wild-type and hyper-produced in *E. coli*. The wild-type repressor protein was extensively purified and used in gel-retardation and DNase I protection experiments to locate and define the operator sequences. The spacial arrangement of the operators and hierarchy in repressor binding seen in the protection experiments indicate that (a) the *penI* gene product represses the expression of the *penP* gene by physically blocking the RNA polymerase binding site and (b) the *penI* gene is autoregulated. Additional approaches for investigation of repressor-operator and repressor-repressor interactions will be discussed.

O 408 MECHANISM OF FORMATION OF TRANSCRIPTION MACHINERY, Wu, Guang-Jer, Emory University School of Medicine, Atlanta, Ga. 30322.
The adenovirus specific VARNA genes are transcribed by host RNA polymeraseIII. To explore the mechanism of formation of transcription machinery with the VA RNA genes, we applied the method of Culotta, et al., (Mol.Cell Biol.,(1985) 5, 1582-1590) to separate stable complexes from unbound proteins by differential centrifugation. We extended their observations to other Pol. III-genes, such as Ad2 VARNA genes, human tRNA genes, EBV EBER genes, and a human Alu-family RNA gene, suggesting that the TFIIIA is not required for formation of pelletable stable complexes. Further studies on the effects of deletion and linker-scanning mutations of the VARNA1 gene on formation of the pelletable complexes were similar to their effects on transcription, indicating that the pelletable complexes are actually the fully functional transcription machineries. The formation of non-pelletable complexes was a fast process, but formation of pelletable transcription complexes was a slower process, suggesting that this process is more complex. Formation of this pelletable transcription machinery was not dependent on the tertiary conformation of the template DNA. The optimal temperature for its formation was similar to that for optimal transcription. The machinery was stable at 4-10 C, less stable at 18-29 C, and unstable at 37 C. It was also unstable at KCl concentrations higher than 500 mM at 4 C. Further progress is in process to characterize proteins required for formation of this machinery and the dynamics of this process.

O 409 INTERACTION OF REGULATORY PROTEINS WITH THE HUMAN INTERFERON PROMOTER, S. Xanthoudakis and J. Hiscott, Lady Davis Research Inst. - Jewish General Hospital, and Dept. of Microbiology and Immunology, McGill University, Montreal, Canada.
Nuclear protein extracts were prepared from uninduced myeloid leukemic KG-1 cells and analyzed for interferon specific DNA interactions by the gel electrophoresis binding assay. On the basis of competition binding studies a protein was detected that bound specifically to the upstream region of the IFN-β promoter -135 to -202 relative to the IFN-β mRNA start site; no competition was observed with other eukaryotic and viral promoter/enhancer fragments, indicating specificity for the IFN promoter. This factor may represent a nuclear protein involved in the repression of IFN-β transcription in the uninduced state. Similarly, to examine IFN inducibility factors binding to the IFN-β regulatory element (IRE), high efficiency nuclear extracts were prepared from uninduced and IFN primed, Sendai virus-induced HeLa S3 cells. Extracts were probed with radiolabeled synthetic oligonucleotides corresponding to the IRE (-77 to -37), as well as smaller fragments which subdivided the IRE into a negative regulatory element (-63 to -37) and an inducibility element (-77 to -64). In both uninduced and induced extracts, proteins were detected that bound specifically to the IRE; when binding to the radiolabeled IRE was competed with different upstream IFN-β fragments, a 10^2-10^3 fold increase in factor binding to the IRE was observed. These results are consistent with a model in which a protein complex interacts with upstream IFN-β sequences and, in so doing, releases a protein with high affinity for IRE sequences.

O 410 TRANSCRIPTIONAL CONTROL OF THE ENDOGENOUS MYC PROTOONCOGENE BY ANTISENSE RNA. Kazushige Yokoyama. Tsukuba Life Science Center,RIKEN. Tsukuba,Ibaraki,Japan.

A plasmid carrying antisense human c-myc DNA and the gene encoding E.coli xanthine-guanine phosphoribosyl transferase(Ecogpt) was introduced into human promyelocytic leukemia cell line HL 60 by protoplast fusion. High-level expression of antisense MYC RNA was obtained by selecting cells resistant to progressively higher levels of mycophenolic acid over a period of more than 6 months. The constitutive production of MYC protein in clones producing high levels of antisense MYC RNA was reduced by 90 % compared to parental HL 60 cells.

Inhibition of MYC expression was observed not only at the translational but also at the transcriptional level,implying that antisense RNA can regulate transcription of the MYC gene. The PstI-PvuII fragment(approximately 920 bp) of the MYC leader sequence is the primary transcriptional target of the antisense RNA. Although the molecular mechanism of this repre -ssion of the MYC gene is now not known,the synthesis of trans-acting negative regulatory elements that bind to the enhancer-like region might be triggered by RNA-RNA base paring or by additional regulatory factors induced by the antisense RNA. We have identified 74 KDa protein in the nuclei of antisense clones that could be candidate for regulatory proteins of MYC gene expression. Foot print studies showed that the binding site of 74 KDa is a TCC-CACC box in the promotor region of the MYC gene. The suppression of endogenous MYC gene expression by antisense RNA decreases cell proliferation and triggers monocyte differentiation.

O 411 PURIFICATION AND CHARACTERISTICS OF THE RNA POLYMERASE III TRANSCRIPTION FACTOR TFIIIC2. Steven K. Yoshinaga and Arnold J. Berk, Molecular Biology Institute, U.C.L.A., Los Angeles, CA. 90024.

Human cells expressing adenovirus E1A proteins transcribe transfected tRNA and adenovirus VA1 genes at > 10 fold higher levels than uninfected HeLa cells. We have shown that the increased level of transcription observed *in vivo* is reflected in the *in vitro* transcriptional activity of cell extracts. Fractionation of these extracts by chromatography on phosphocellulose suggests that the higher activity of the extracts of adenovirus-infected cells was due to increased activity of the transcription factor which is the limiting component required for the specific initiation of tRNA and VA1 transcription, i.e. TFIIIC. In the effort to purify the TFIIIC component to homogeneity (in order to analyze the E1A effect), we separated TFIIIC into two functional components we called TFIIIC1 andTFIIIC2. Both TFIIIC1 andTFIIIC2 are required for *in vitro* transcription of the VA1 gene and exhibit DNA binding properties normally attributed to the "holo" TFIIIC enzyme.

We have utilized specific DNA binding chromatography and a combination of other chromatographies to purify the TFIIIC2 component thousands of fold. The most purified TFIIIC2 fraction is active in both *in vitro* transcription and DNA binding assays. The method of TFIIIC2 purification and analysis of TFIIIC2 polypeptide composition will be described. Characteristics of the purified TFIIIC2 component have been analyzed for its function in RNA polymerase III transcriptional initiation.

O 412 MODULATION OF HLA CLASS I EXPRESSION IN BURKITT LYMPHOMA LINES, Karen R. Zachow, Maria G. Masucci*, George Klein*, and Harry T. Orr, University of Minnesota, Minneapolis, MN 55455, *Karolinska Institute, S-10401 Stockholm, Sweden.

The polymorphic HLA class I molecules of the major histocompatability complex are expressed on nearly all somatic cells. Through their association with 'foreign' antigens on the cell surface, the class I heterodimers are responsible for cytotoxic T lymphocyte (CTL) recognition of virus-infected, and possibly malignant, cells. Studies of Burkitt lymphoma cell lines (BLs) with HLA-restricted and HLA-specific CTLs and with monoclonal antibodies directed against HLA molecules have revealed a decrease in expression of the class I gene HLA-A11.[1] This decrease was seen in Epstein-Barr Virus (EBV) carrying and EBV-negative BL but not in EBV transformed lymphoblastoid cell lines derived from the Burkitt lymphoma patients. Northern blots of total and nuclear RNA from these BLs probed with HLA-A11-specific oligonucleotide probes demonstrate a drastic reduction in HLA-A11 mRNA and strongly suggest that the reduced expression is due to a decrease in transcription. The down regulation of HLA-A11 appears to be specific as overall levels of class I RNA are not greatly decreased in the BLs. The involvement of a trans-acting factor is being assessed by measurement of HLA-A11 expression in BLs after they have been transfected with an intact HLA-A11 gene. The HLA-A11 sequences involved in this modulation will be determined using chimeric constructs of HLA-A11 and an HLA-A gene that is unaffected by the BL phenotype.

(1) Masucci, M.G., et al. (1987) PNAS 84:4567-4571.

O 500 NEGATIVE REGULATION BY GLUCOCORTICOIDS THROUGH INTERFERENCE WITH cAMP RESPONSIVE ENHANCERS, I. Akerblom*, E. Slater#, J. Baxter†, M. Beato#& P. Mellon*, *Salk Institute La Jolla CA 92037; #Institut Fur Molekularbiologie, Marburg, FRG; †UCSF, San Francisco CA 94143. Although the transactivation properties of steroid receptors and their enhancers are well-characterized for inducible genes, the mechanisms for negative regulation by steroids remain unknown. We demonstrate that the same glucocorticoid receptor (GR) which mediates positive activation can negatively regulate expression of the human glycoprotein hormone α-subunit gene. 168 bp of α5'-DNA linked to CAT was cotransfected with a GR expression plasmid into placental cells which produce the glycoprotein hormone, chorionic gonadotropin. Glucocorticoids produced a 90% decrease in CAT activity, in contrast to strong induction of MMTV-TKcat. DNaseI and DMS protection revealed GR-binding sites overlapping two sites (CREs) for a factor crucial for both cAMP response and placental specificity. Negative regulation could be conferred to a heterologous promoter; but inhibition was observed only when the CREs were induced to act as enhancers either by elevated cAMP or by a tissue-specific element which acts coordinately with the CREs to confer placental expression. Though the CREs are crucial for α-subunit expression in placental cells, they contribute little to the low basal expression in other cell types; thus, when α-cat and GR were cotransfected into GC or CV-1 cells, glucocorticoids slightly induced activity. Our results strongly support a model in which negative control is exerted through direct GR competition with CRE-binding proteins for binding to DNA with consequent blockage of CRE activity. This mechanism of steric hindrance between transcription factors may apply not only to negative regulation by steroid receptors but also more generally to negative regulation in other systems.

O 501 INDUCTION OF HEME OXYGENASE MESSENGER RNA BY HEME AND HEME-HEMOPEXIN. Jawed Alam, Shigeki Shibahara* and Ann Smith. Louisiana State University Medical Center, New Orleans, LA 70112 and *Friedrich Miescher-Institut, CH4002 Basel, Switzerland.
Hemopexin is a serum glycoprotein which transports heme to some tissues, including liver, *via a receptor-mediated process. Heme, taken into the cell, is catabolized by heme* oxygenase (HO) and the heme-iron released is stored on ferritin, while hemopexin recycles to the circulation. While this system is the physiological mechanism by which heme is transported into cells expressing the hemopexin receptor, previous in vitro studies have only utilized free heme. Treatment of minimal deviation mouse hepatoma (Hepa) cells and human promyelocytic HL-60 cells, with varying concentrations of either free heme or heme-hemopexin complexes caused an increase within 1-2 h in the steady-state level of HO mRNA. Maximum induction of 10-20 fold occurred within 4h with 10uM heme-hemopexin and 1-2uM free heme. The difference in concentrations of free heme and heme-hemopexin required for equivalent induction is due to differences in the rate of cellular accumulation of heme as determined by ^{55}Fe-heme or ^{55}Fe-heme-hemopexin uptake assays. The cellular content of HO mRNA correlates with the intracellular concentration of ^{55}Fe-heme. In these experiments, the steady state levels of mRNA for the control proteins, ribosomal protein L7 and beta-actin, were unaffected. Results from in vitro nuclear run-off transcription assays indicate that transcriptional activation is not responsible for substrate-induced accumulation of HO mRNA in these cell types. The effects of heme on the stability and turnover rate of HO mRNA is being examined. (Supported by NIH Grants DK 37463 and DK 27237).

O 502 ANALYSIS OF OVINE β-LACTOGLOBULIN GENE EXPRESSION DURING MAMMARY GLAND DEVELOPMENT, Simak Ali, C. Bruce A. Whitelaw, Stephen Harris, J. Paul Simons and A. John Clark, Institute of Physiology and Genetics Research, Edinburgh, Scotland, UK.

The β-lactoglobulin gene (BLG) encodes the major whey protein in the milk of ruminants. Ovine BLG transcripts comprise approximately 5% of polyA+ RNA in the lactating mammary gland. We have cloned and characterised the ovine BLG structural gene. The gene is 4.9 kb in length, comprised of seven exons and is a member of a large, highly divergent gene family encoding *tissue-specific secretory proteins. Milk protein genes are developmentally* controlled and our aim is to study the tissue specificity and hormonal regulation of BLG. Ovine BLG expression was analysed at various time points during pregnancy and lactation. Northern blot analysis of total mammary RNA indicates that BLG expression is occurs very early in pregnancy. We have generated transgenic mice carrying BLG, which express it appropriately in the mammary gland. The developmental pattern of BLG expression in the mice during pregnancy and lactation is similar to that observed in sheep. This shows that the sequences required for development- and mammary-specific expression are present in the injected DNA fragment.

O 503 REGULATION OF CHORIONIC GONADOTROPIN GENE EXPRESSION BY THE PROTEIN KINASE C PATHWAY. B. Andersen, G. Kennedy, and J. Nilson. Case Western Reserve University, Cleveland, OH 44106.

Cyclic AMP stimulates transcription of the α-subunit and chorionic gonadotropin (CG)β genes. To determine whether activators of protein kinase C also regulate these genes we treated choriocarcinoma cells with phorbol myristate acetate (PMA) and a cell permeable diacylglycerol analogue, dioctanoylglycerol. This resulted in a 3- to 4-fold induction of mRNAs encoding both the α and CGβ subunits. In nuclear run-off experiments PMA increased transcription of the α and CGβ genes 2- to 3-fold. To define the regulatory sequences required for the effect of PMA on the α subunit gene we used a transient expression assay in choriocarcinoma cells. We inserted fragments from the 5' flanking sequence of the α subunit gene upstream of a reporter gene, chloramphenicol acetyl transferase. These experiments localized the PMA response element to the proximal 170 bp of 5' flanking sequence. This same region also contains an 18 bp element that binds a transacting factor that mediates the cAMP response of the gene. We are now in the process of determining the boundaries of the cis-active element that mediates the effect of PMA, and whether the same or different transacting factor(s) mediates the effect of PMA and cAMP.

O 504 **THE HUMAN THYMIDINE KINASE PROMOTER: DELETION ANALYSIS AND SPECIFIC PROTEIN BINDING,** **Santosh S. Arcot, Erik K. Flemington and Prescott L. Deininger, Louisiana State University Medical Center, New Orleans, LA 70112.** **We have analysed the human thymidine kinase (tk) gene promoter by fusing 500bp of promoter sequences onto the heterologous CAT (Chloramphenicol Acetyl Transferase) gene. Deletions were carried out from the 5' end of the tk promoter and analysed for promoter strength by assaying for CAT activity after transfection into mouse L cells. This deletion analysis helped us to confirm the identity of several putative transcriptional elements. Analyses of the deletions suggests the importance of several possible "GC" elements located between -275 and -100 and a "CCAAT" element located at -40. Surprisingly, a deletion upstream at -281 decreases the promoter activity drastically indicating a possible repressor element. In addition, an evolutionary comparison identifies two highly conserved promoter elements: the -40 "CCAAT" element and a "TATA" element located at -21. Since the "CCAAT" elements have previously been implicated in the regulation of other genes, we have characterized the highly conserved -40 "CCAAT" element identified here in more detail using a protein binding analysis. From this study, we have determined that the predominant protein binding interaction with the human tk promoter occurs at the -40 "CCAAT" element.**

O 505 REGULATORY ELEMENTS INVOLVED IN THE TRANSCRIPTION OF THE MURINE C-MYC GENE. Claude Asselin and Kenneth B. Marcu, Department of Biochemistry, S.U.N.Y. at Stony Brook, Stony Brook, NY 11794-5215.

We have tested sequences in the first exon and the 5'-flanking region of the murine c-myc gene for their ability to form specific DNA-protein complexes with factors present in crude nuclear extracts prepared from Hela cells and other mammalian cell lines. Multiple binding sites were defined by gel retardation, binding competition with specific DNA fragments, dimethylsulfate(DMS) interference, DNAse I and DMS protection analysis. We have detected 4 protein binding sites 5' of the trnscription initiation site P_1, two of which bind the Spl transcription factor. Two other specific DNA-protein interactions have been found 5' of the major c-myc transcription initiation site, P_2. In order to assess the importance of these multiple DNA-protein binding sites for c-myc regulation, we have introduced deletions to inactivate one or many of these sites and we have synthesized them as oligonucleotides to assay their function independently in DNA transfection experiments. Data will be presented on these experiments.

O 506 THE TRANSITION TO ENHANCER INDEPENDENT TRANSCRIPTION OF Ig KAPPA GENES IS DEVELOPMENTALLY CONTROLLED AND CORRELATES WITH METHYLATION STATUS, Michael L. Atchison, Dawn E. Kelley and Robert P. Perry, Institute for Cancer Research, 7701 Burholme Ave., Philadelphia, PA 19111.
It has been proposed that immunoglobulin (Ig) enhancers are required to initiate Ig transcriptional activity, but became dispensable for the maintenance of transcription at certain stages of lymphoid development. This hypothesis derives from experiments in which enhancer activity was abolished, either by deletion of the enhancer DNA sequences or by lack of a critical trans-acting nuclear factor, and yet endogenous Ig transcription remained unaffected. We show here that modulation of Ig kappa (κ) enhancer activity by altering the levels of an enhancer binding nuclear factor, NF-κB, causes increased expression of endogenous κ genes in pre B cells but not in cells representative of the plasma cell stage. This developmental transition to enhancer independent transcription of κ genes correlates precisely with the methylation status of the Ig κ locus. Thus, at the pre B cell stage, κ enhancer activity and transcription are not followed by demethylation of the κ locus. However, at the plasma cell stage, the transcriptional activation of κ genes is coupled to hypomethylation of the κ locus and enhancer independence.

O 507 THE SV40 MAJOR LATE PROMOTER: A NOVEL TRIPARTITE STRUCTURE THAT INCLUDES INTRAGENIC SEQUENCES, Donald E. Ayer and William S. Dynan, University of Colorado, Boulder, CO 80309.
Unlike most genes transcribed by RNA polymerase II, the Simian Virus 40 late transcription unit does not have a TATA box. To determine what sequences are required for initiation at the major late mRNA cap site of SV40, clustered point mutations were constructed and tested for transcriptional activity in vitro and in vivo. Three promoter elements were defined. The first is centered 31 base pairs upstream of the mRNA cap site in a position normally reserved for a TATA box. The second is at the mRNA cap site. The third occupies a novel position centered 28 base pairs downstream of the mRNA cap site within a protein-coding sequence. The ability of RNA polymerase II to recognize this promoter suggests that there is greater variation in promoter architecture than has been previously believed. We are continuing to study the function of the SV40 late promoter using additional mutants and fractionation of the in vitro transcription activity.

O 508 A TRANSCRIPTION FACTOR OF SEA URCHIN EMBRYOS INTERACTS WITH THE PROMOTERS OF DEVELOPMENTALLY REGULATED H2B AND H2A GENES, Alcide Barberis, Iris Kemler, Giulio Superti-Furga and Meinrad Busslinger, Institute of Molecular Biology II, Hönggerberg, CH-8093 Zurich.
The sea urchin late H2B-2 and late H2A-2 histone genes are differentially expressed during embryogenesis. In the adult, these genes are expressed in a tissue-specific manner. Nuclear extracts of sea urchin embryos contain a novel factor which protects a 25 bp DNA segment from DNase I digestion on each promoter of two late H2B-2 and two late H2A-2 genes. This factor has been operationally termed LHTF (Late Histone Transcription Factor). LHTF binds to the -60 region of the late H2B-2 promoters between the octamer sequence and the TATA box, and to the -100 region of the late H2A-2 promoters. Competition experiments revealed an identical affinity of LHTF for all four binding sites, which exhibit surprisingly low sequence similarity. Deletion analysis of one of the late H2B-2 promoters showed that both the octamer element and the recognition sequence for LHTF are required for efficient in vitro transcription in the gastrula nuclear extract. Moreover, LHTF does not interact with the promoters of early and sperm histone genes and is absent from sea urchin testis nuclear extracts, thus suggesting its possible role in the developmental regulation of these late H2B-H2A gene pairs. Recent data indicate that LHTF, which has an apparent molecular weight of 70 kD, might even be responsible for the tissue-specific regulation of this particular class of late histone genes.

O 509 REGULATION OF GLOBIN GENE EXPRESSION BY STAGE-SPECIFIC TRANS-ACTING FACTORS, Margaret H. Baron and Tom Maniatis, Harvard University, Cambridge, MA 02138.
We have used a transient assay involving the preparation and analysis of interspecific heterokaryons (CELL 46:591-602) to show that: (1) erythroid cells contain stage-specific trans-acting molecules which function in the developmental regulation of globin genes; (2) these factors are active both in erythroid and in nonerythroid cells, whether primary or established; (3) globin genes in non-expressing differentiated cells are not irreversibly repressed; rather, the genome is plastic and can be rapidly reprogrammed in the presence of the appropriate trans-acting factors. Our observation that globin genes in non-expressing cells can be rapidly activated by diffusible factors implies that it should be possible to complement the presumptive deficiency in those cells by re-introducing the relevant factors, either in the form of RNA or DNA. We are developing a functional assay for the cloning of genes which encode such regulatory proteins. Hybrid fusion genes containing globin regulatory sequences and coding sequences for "reporter" genes (such as cell surface markers) are stably introduced into non-erythroid cells. These cell lines will serve as targets for microinjection, through glass capillaries, of erythroid cell mRNA, or for transfection with erythroid cell cDNA or total genomic DNA, with the goal of activating the hybrid gene in trans.

O 510 EVENTS DURING TRANSCRIPTION INITIATION OF ACANTHAMOEBA rRNA, E. Bateman, L.M. Hoffman, C.T. Iida, P. Kownin, W. Kubaska, Y. Qin, P.A. Risi, M.G. Zwick and M.R. Paule, Dept. of Biochemistry, Colorado State Univ., Fort Collins, CO 80523.
Transcription of the Acanthamoeba castellanii rRNA genes requires RNA polymerase I and a single transcription initiation factor (TIF). The interactions between these components and with the promoter have been studied using a combination of chemical and enzymatic footprinting probes and by various manipulations of the promoter DNA sequence. Transcription initiation requires TIF binding to part of the promoter which subsequently directs or allows polymerase binding. The complex formed between DNA, TIF and polymerase is apparently in a closed conformation. Following transcription initiation, polymerase translocates and unwinds at least 10 base pairs of DNA as judged by the sensitivity of a paused complex to diethylpyrocarbonate modification. Binding of RNA polymerase I to the TIF-promoter complex is independent of the DNA sequence around the transcription start site; however, transcription initiation requires a sequence at least resembling the wild type sequence. TIF thus precisely positions RNA polymerase by direct contact but such interactions are insufficient for transcription initiation in the absence of the correct DNA sequence. A consequence of contact between TIF and RNA polymerase I is that a negative regulatory mechanism may operate by preventing polymerase-TIF interactions.

O 511 ALTERATIONS IN THE EXPRESSION OF THE HEPATOCYTE SPECIFIC NUCLEAR FACTOR, HNF-1, ARE ASSOCIATED WITH EXTINCTION OF LIVER SPECIFIC GENE EXPRESSION. Susanne Baumhueter, Gilles Courtois and Gerald R. Crabtree, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.
The hepatocyte specific nuclear factor, HNF-1, binds to an essential cis-regulatory element present in promoters of several genes selectively expressed in hepatocytes. We adopted the hypothesis that HNF1 might be a positive trans-acting regulatory factor involved in establishing the liver phenotype during development and differentiation. We have analyzed HNF-1 and fibrinogen mRNA production in a well-differentiated hepatocyte cell line, Fao; a variant, FaofCC2, selected on the basis of a non-hepatic morphology, and a revertant, C2-Rev7, selected by growth in glucose-free media for the hepatocyte-specific gluconeogenic enzymes, phosphoenolpyruvatcarboxy kinase and pyruvate kinase. Using the gel retardation assay and UV cross-linking we detected the 90kDa protein HNF1 in nuclear extracts from Fao and the differentiated revertant, Rev 7 but not in C2. Instead, this dedifferentiated cell line expressed a variant nuclear protein (VNP) of only 70 kDa which bound to the same sequence as HNF1. The absence of HNF1 in C2 correlated with the absence or greatly reduced levels of fibrinogen gene mRNA. Surprisingly, VNP was also detected in intertypic hybrids showing extinction of liver specific traits and was present in nuclear extracts from many tissues of non-hepatic origin. In each case mRNA for the fibrinogen genes was not detectable. Although its role in the transcriptional regulation is not yet clear it is likely that the VNP is characteristic of an event occurring at a more fundamental level in the control of hepatocyte differentiation since it is found associated with such diverse events as dedifferentiation, extinction and in non-hepatic cell lineage.

O 512 OBP100, A 100 KD HELA CELL OCTAMER BINDING PROTEIN, DISPLAYS A REMARKABLY FLEXIBLE DNA BINDING SPECIFICITY, Thomas Baumruker, Richard Sturm, B. Robert Franza Jr. and Winship Herr, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, N.Y. 11724

Many eukaryotic upstream promoter and enhancer regions contain a functional octamer sequence ATGCAAAT, including small nuclear RNA histone H2B, and immunoglobulin promoters. An octamer related sequence, ATGCAAAG, is found in the SV40 enhancer region. We have purified and characterized the interaction between a 100 KD HeLa cell octamer binding protein (OBP100) and the SV40 and immunoglobulin heavy-chain (IgH) enhancers. A partially purified OBP100 containing fraction generates two complexes in a gel retardation assay with a fragment containing the SV40 enhancer but only one complex with an IgH enhancer fragment. By using dimethyl sulfate and diethyl pyrocarbonate modification interference assays, we have shown that the interactions with the SV40 enhancer are to two adjacent octamer-related sequences called Octa1 (ATGCAAAG) and Octa2 (ATGCATCT) whereas the interaction with the IgH enhancer is at the canonical octamer sequence ATGCAAAT. The base-specific interactions detected by the chemical interference assays display a remarkably different set of interactions with the Octa1 and Octa2 sites. Chemical modifications over a 10 bp sequence TATGCAAAGC affect Octa1 binding whereas Octa2 binding is affected by modifications spanning a 13 bp sequence ATGCATCTCAATT in which the octamer-like sequence is not centered. The protein responsible for the binding activitiy, OBP100, has been purified by heparin agarose chromatography, a DNA affinity precipitation procedure, and subsequent SDS gel electrophoresis. Gel purified OBP100 shows the same gel retardation activity with the SV40 and the IgH enhancers as the activity observed in the crude fractions. We are currently testing the full extent of the flexibility of OBP100 DNA binding specificity.

O 513 PROTEINS AND DNA SEQUENCES INVOLVED IN REGULATING THE HERPES SIMPLEX VIRUS EARLY GENE gD. P. Beard[1], D. Tedder[2] and L. Pizer[2]. [1]Swiss Institute for Experimental Cancer Research, Epalinges/Lausanne, Switzerland; [2]Dept. of Microbiology, Univ. of Colorado School of Medicine, Denver, CO.

The HSV immediate early protein ICP4 (α4) plays a major role in activating transcription of early and late genes. The way in which ICP4 acts, however, remains unclear. We have shown that purified ICP4 stimulates transcription of the gD gene in vitro. The gD gene has three ICP4-binding sites (I-III) in the promoter region. DNA fragments containing ICP4 site II or site III form specific complexes with ICP4 from infected cell extracts or a purified ICP4 preparation, in gel mobility shift assays. Proteins in the purified ICP4 preparation protect a region from -42 to -113 from nuclease digestion; deletion of the region from -84 to -129 greatly diminishes the strength of ICP4 binding.

Deletion of the strongest ICP4 binding site (II at -100) reduces the ability of ICP4 to stimulate transcription in vitro. The absence of site III (at +50) also results in less efficient activation by ICP4.

A cellular component from uninfected HeLa cells binds to a GC-rich region around -60, overlapping the footprint obtained with the ICP4 preparation. Deletion of nucleotides -56 to -67 abolishes binding. This deletion also stops gD transcription in vitro. However, the promoter with the deletion becomes active again when transcription is done in the presence of ICP4.

O 514 BINDING OF X.LAEVIS NUCLEAR FACTORS TO R-PROTEIN GENE PROMOTERS. E.Beccari and F.Carnevali, C.A.N.,Dept.Genetics and Molecular Biology,P.le A.Moro 5,ROMA-ITALY

We have undertaken a functional and structural characterization of the regions extending a few hundred bp upstream of the capsite of the L1 and L14 ribosomal protein genes of Xenopus laevis. L1 and L14 upstream regions and 5' deletion mutants were cloned into CAT vectors and expression of the CAT protein was tested by injecting the constructs into X.laevis oocyte nuclei. In the L14 promoter region, deletions from the 5' end progressively reduce CAT expression. 60% of the activity is retained with 60 bp, but only 2% with 30 bp upstream of the capsite. A more extended region is necessary for promoter activity in the L1 gene. A deletion mutant with 130 bp of 5' flanking sequences is expressed at low levels.

By the use of a gel-retardation assay, a specific binding between nuclear factors and DNA fragments spanning the promoter regions of L1 and L14 genes was evidenced. Specificity of the resulting complexes was demonstrated with competition assays. Protection by ExoIII digestion, performed in the presence and in the absence of crude nuclear extracts from X.laevis oocyte nuclei, identified short repeated sequences where the nuclease pauses. The repeated sequences of L1 are not homologous to the repeated sequences identified in L14.

O 515 STRUCTURE AND EXPRESSION OF THE PHO80 GENE OF SACCHAROMYCES CEREVISIAE, Lawrence W. Bergman, Stephen L. Madden, Caretha L. Creasy, Vickram Srinivas, William Fawcett, Ohio University, Athens, Ohio 45701.
In yeast, the repression of acid phosphatase under high phosphate growth conditions requires the trans-acting factor PHO80. We have determined the DNA sequence of the PHO80 gene and found that it encodes a protein of 293 amino acids. The expression of the PHO80 gene, as measured by Northern analysis and level of a PHO80-LacZ fusion protein is independent of the level of phosphate in the growth medium. Disruption of the PHO80 gene is a non-lethal event and causes a derepressed phenotype, with acid phosphatase levels which are 3-4 fold higher than the level found in derepressed wild type cells. Furthermore, over-expression of the PHO80 gene causes a reduction in the level of acid phosphatase produced under derepressed growth conditions. Finally, we have cloned, localized and sequenced a temperature-sensitive allel of PHO80 and found the phenotype to be due to T to C transition causing a substitution of a Ser for a Leu at amino acid 163 in the protein product.

O 516 **A RAT LIVER PROTEIN PROTEIN BINDS SPECIFICALLY TO CRUCIFORM DNA**
Marco E. Bianchi, EMBL, Meyerhofstrasse 1, D-6900 Heidelberg, West Germany.
Cruciform structures (now commonly called Holliday junctions) occur in DNA as a consequence of a) both general and site-specific recombination and b) the transition from interstrand to intrastrand base pairing in palindromic DNA sequences. We have constructed an artificial cruciform DNA by annealing in vitro four synthetic oligonucleotides of appropriate base complementarity: our artificial cruciform DNA is stable at moderate salt concentrations and has characteristic hydrodynamic properties. We have then incubated the labeled synthetic cruciforms with extracts from rat liver nuclei at 0°C and subjected them to electrophoresis in nondenaturing polyacrylamide gels. We have identified and partially purified a protein which retards the migration of the synthetic cruciforms, but not of control duplex molecules of identical sequence. Moreover, the activity binding to labeled cruciforms can be competed by cold cruciform molecules of different sequence, but not by linear duplex DNA. The rat liver cruciform-binding protein does not bind to single-stranded DNA with limited secondary structure (synthetic 50-mers), but binds weakly to single-stranded DNAs with extensive stem-and-loop structures, such as phage fd DNA. This protein has no associated endonucleolytic activity, and therefore it is unlikely to be the mammalian homolog of phage T4 or yeast Holliday-cleaving proteins.

O 517 **SEQUENCE-SPECIFIC DNA-BINDING OF V-MYB PROTEIN.** **Horst Biedenkapp and Karl-Heinz Klempnauer; ZMBH, University of Heidelberg, D-6900 Heidelberg, W.-Germany.**
The oncogene v-myb of Avian myeloblastosis virus encodes a nuclear DNA-binding protein predominantly associated with the chromatin.
In order to understand the role of myb protein-/DNA-interaction for myb-function it is interesting to analyze the DNA-binding activity of v-myb protein with respect to sequence-specificity. We have expressed the complete v-myb coding region in E.coli. The bacterially expressed v-myb protein, which is active in binding to DNA, was used to screen arbitrarily selected DNA molecules for the presence of myb-specific binding sites. Several in vitro DNA-binding assays have allowed us to identify a myb-specific binding site in bacteriophage Lambda DNA. Using bacterially expressed truncated v-myb proteins we could clearly demonstrate that the DNA-binding activity is correlated with the v-myb protein.
To identify possible v-myb specific target sites in the cellular genome we have analyzed binding to DNA fragments isolated by immunoprecipitation of chromatin fragments of cells expressing v-myb with myb-specific antibodies. Several of these fragments contain specific binding sites recognized by bacterially expressed v-myb protein. Comparison of the binding sites suggests that the v-myb protein recognizes a short sequence motif common to all sites.
The DNA-binding domain, located at the aminoterminus of the v-myb protein, does not show homology to 'helix-turn-helix' or 'zinc-finger' motifs found in other *sequence-specific* DNA-binding proteins. This may indicate that the v-myb protein represents a novel type of DNA-binding proteins.

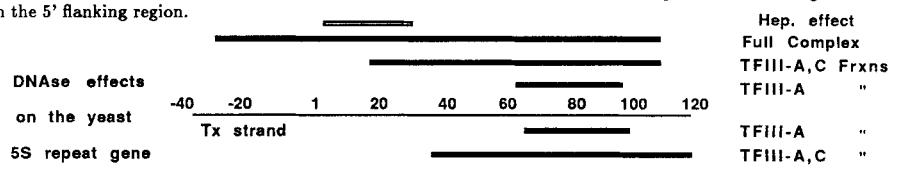
O 518 XENOPUS TRANSCRIPTION FACTOR IIIA FORMS A COMPLEX OF COVALENT CHARACTER WITH 5S DNA, Jeronimo Blanco and Joel M. Gottesfeld, Research Institute of Scripps Clinic, La Jolla, CA 92037.
The 5S gene-specific transcription factor TFIIIA forms an exceptionally stable complex with the internal promoter of the 5S RNA gene. Approximately 1 to 3% of the TFIIIA-DNA or deoxyoligonucleotide complexes are stable to harsh denaturation conditions and can be resolved by electrophoresis in the presence of SDS. These complexes are resistant to acidic conditions (0.1 N HCl, 55°C, 2h) suggesting that the interaction may be through a covalent bond. Complex formation does not result in DNA strand scission and studies of the chemical sensitivity of the complex suggest that the TFIIIA-DNA linkage may be through a phosphoramidate bond. Trypsin digestion of the complex correlates with the tryptic digest of free TFIIIA, showing that TFIIIA is, in fact, part of the complex. Complex formation is also observed with synthetic peptides corresponding to the metal-binding "fingers" of TFIIIA. The predominant site of TFIIIA-DNA interaction has been mapped to the 3' end of the 5S gene internal control region. These studies suggest that the TFIIIA-DNA complex may involve a transient covalent interaction of the metal binding loops or "fingers" with the phosphate backbone of DNA.

O 519 ROLE OF CHROMOSOMAL LOOP ATTACHMENT SEQUENCES ON IMMUNOGLOBULIN GENE EXPRESSION. Veronica C. Blasquez, Ming Xu, Robert E. Hammer, Steven C. Moses, Yasmin Mehrotra, Sharon L. Jones, and William T. Garrard, Departments of Biochemistry, Cell Biology and The Howard Hughes Medical Institute, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235
Sequences termed *matrix association regions* (MARs) appear to organize the eukaryotic genome into chromosomal loops inside the nucleus. However, the function(s) of these sequences is not yet understood. Previous work from this laboratory identified a MAR containing topoisomerase II sites just upstream of the enhancer in the mouse, rabbit, and human κ immunoglobulin genes. We thus hypothesized that MARs may work in conjunction with enhancers in establishing transcription. To test this we have stably introduced functionally rearranged κ gene constructs into plasmacytoma cells by co-transformation. Northern blot analysis reveals that wild-type transgenes exhibit a position effect, since only 10/17 clones are high expressors. Interestingly, constructs lacking the MAR exhibit a much more striking position effect, as only 1/13 clones is a high expressor. Furthermore, integration into sites permissive for expression of a linked selectable marker considerably reduces this effect, consistent with a role of the MAR in overriding position effects. To test this we are currently determining whether insertion of the MAR back into mutated constructs, 5' or 3' of the gene, can restore gene expression. As another approach, we have created transgenic mice with similar constructs and are currently investigating tissue-specificity and developmental timing of gene expression. Research supported by NIH and The Robert A. Welch Foundation.

O 520 Specific Interactions of a cAMP responsive Element from the hCGα Gene with Nuclear Factors from Choriocarcinoma Cells. J. Bokar, D. Kaetzel, and J. Nilson, Case Western Reserve University, Cleveland, OH 44106
We have recently shown that an 18 bp sequence directly repeated in the 5'-flanking region of the human glycoprotein hormone α-subunit gene is sufficient to confer cAMP responsiveness to a heterologous promoter, and is homologous to sequences in other cAMP responsive genes. In addition, a unique property of this cAMP responsive element (CRE) is it is a critical component of a basal level enhancer in choriocarcinoma cells, although the CRE alone is not sufficient for this activity. To further explore the mechanisms by which the CRE mediates these functions, we have begun to investigate protein-DNA interactions between a synthetic oligodeoxynucleotide corresponding to the CRE and nuclear proteins from BeWo cells using gel mobility shift assays. Based upon differences in gel mobility and affinity, at least two sequence specific protein-DNA interactions were detected. A series of mutant oligodeoxynucleotides was used to compete with the wild type 18bp CRE in gel mobility shift assays. We have shown that specific interaction with these nuclear factors depends heavily on the presence of nucleotides within the consensus sequence, but not on the nucleotides outside the consensus. We have shown that the CRE from the rat PEPCK gene binds similarly to these factors. Transfection experiments in BeWo cells revealed that mutant oligos with reduced binding affinity lost the ability to confer cAMP responsiveness to a heterologous promoter. Collectively, these data suggest that the protein-DNA interactions we are detecting are involved in cAMP regulated transcription.

O 521 V-*JUN* ENCODES A NUCLEAR PROTEIN WITH SEQUENCE SPECIFIC DNA BINDING ACTIVITY, Timothy J. Bos, Dirk Bohmann*, Hideaki Tsuchie, Robert Tjian* and Peter K. Vogt, Department of Microbiology, University of Southern California, School of Medicine, 2011 Zonal Ave - HMR 401, Los Angeles, CA 90033 and *Howard Hughes Medical Institute, Department of Biochemistry, University of California, Berkeley, CA 94720.
We have isolated a new oncogene, *jun*, from Avian Sarcoma Virus 17. *Jun* was found to share homology with the DNA binding domain of the yeast transcription factor, GCN4. The v-*jun* oncogene is expressed as a 65 kilodalton protein that contains retroviral *gag* sequences at its amino terminus fused to *jun* sequences that make up the carboxyl terminal two thirds of the molecule. This p65$^{gag\text{-}jun}$ product is found in the nucleus of chicken embryo fibroblasts transfected with DNA from the cloned genome of ASV17. p65$^{gag\text{-}jun}$ synthesized in bacteria binds to DNA with a sequence specificity similar to that of GCN4 and AP-1.

O 522 **INTERACTION OF YEAST TFIII-A AND OTHER FACTORS WITH THE 5S GENE**
Burkhard R. Braun, George A. Kassavetis, and E. Peter Geiduschek, Center for Molecular Genetics and the Department of Biology, M-034, University of California - San Diego, La Jolla, CA 92093.
We have characterized, by DNAse I footprinting, the 5S gene-specific transcription factor from *S. cerevisiae* analogous to *Xenopus* TFIII-A. The footprint of a crude TFIII-A fraction, as diagrammed below, corresponds only with the 3' two-thirds of the *Xenopus* TFIII-A footprint, which extends from bp. 45 to 97. Addition of partially purified TFIII-C to this complex extends the footprint as shown and also generates enhancements of DNAse I cleavage. TFIII-C stabilizes the DNA-protein complex against stripping by the polyanion, heparin, and against competition by subsequently added 5S RNA. When footprinted in a transcription system optimized for activity, protection is extended on the non-transcribed DNA strand to bp. -36. Heparin treatment, which strips RNA polymerase III from these complete transcription complexes, exposes a region near the transcription start site to DNAse I. Transcription to nucleotide 11 generates enhanced cleavage at bp. 34-36 from the transcription start. Transcription through the rest of the gene in the presence of heparin strips bound protein from the gene but not from the 5' flanking region.



O 523 **STRUCTURAL TRANSITIONS OCCURING IN VITRO UNDER MODERATE TORSIONAL STRESS IN A YEAST UPSTREAM ACTIVATING SEQUENCE CORRELATE WITH BINDING SITES FOR REGULATORY PROTEINS.** Karin D. Breunig and P. Kuger, Universität Düsseldorf, Düsseldorf, F.R.G.
The behaviour of regulatory sequences under torsional stress has been analysed in vitro. We used a small (380 bp) fragment with the Upstream Activating Sequence of the yeast β-galactosidase gene LAC4 (UAS_L). This fragment was ligated in vitro and probed with P1 nuclease after isolation of individual topoisomers. We saw an onset of specific structural transitions at moderate torsional stress. The positions of P1 hypersensitive sites (HSS) correlate with the binding sites for two regulatory proteins in a specific manner: the LAC9 binding site (1) is flanked by HSS but itself is P1 insensitive, the region of 50 bp containing the binding site for the second protein (2) is cleaved at 10 bp intervals, and the rest of the molecule is insensitive.
Binding studies with yeast proteins show that the affinity of LAC9 is not influenced by DNA topology whereas a second, as yet unidentified protein shows a preference for a template with a superhelical density of $\sigma \geq |0.05|$, where structural transitions occur.
As to whether changes in template topology affect regulation and may help to explain the position dependent variations in LAC4 expression on plasmids in vivo, is currently under investigation.

(1) Breunig, K.D. and P. Kuger, (1987) Mol. Cell. Biol., 7: in press
(2) Gödecke, A. and K.D. Breunig, in preparation

O 524 REGULATORY ELEMENTS FOR TRANSCRIPTIONAL STIMULATION BY PROGESTINS AND ANDROGENS OF THE MOUSE MAMMARY TUMOR VIRUS (MMTV) PROMOTER. Elena Buetti, Peter Gowland, Tea Meulia-Sossi, and Heidi Diggelmann, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland.

The DNA segment spanning 200 bp upstream of the MMTV promoter mediates the transcriptional regulation by certain steroid hormones (glucocorticoids, progestins, androgens, mineralocorticoids) through the interaction of the respective receptors with DNA. Using linker-insertion, deletion, or substitution mutants we had previously defined four regulatory elements required for maximal stimulation of transcription by glucocorticoid hormones (J. Mol. Biol. 190, 367-378 and 379-389, 1986). We have introduced some of these mutants into the T47D human mammary tumor cell line, which contains functional receptors for progestins and for androgens but not for glucocorticoids. The inducibility of the MMTV promoter was determined by S1 mapping of RNA of cells either stably or transiently transfected with mutant DNA. Stimulation by androgen was about 10 times lower than by progestins. With the latter, we observed that the promoter-proximal region (to -130 from the RNA start site) plays a more important role than the distal one. This is the reverse situation than with glucocorticoids, and it was confirmed by co-transfecting an active gene of the glucocorticoid receptor into the same cells along with mutant MMTV DNA. So, despite the described coincidence of the *in vitro* footprints of glucocorticoid and progesterone receptors, the contributions of proximal vs. distal regions are functionally distinct. Consistent with this result, a synthetic oligonucleotide of the distal site was unable to restore full progesterone stimulation when inserted into a proximal site mutant, while it did for glucocorticoid hormones.

O 525 REGULATION OF THE MUSCLE CREATINE KINASE GENE: TRANS-ACTING FACTORS, Jean. N. Buskin, Jane E. Johnson, and Stephen D. Hauschka, Department of Biochemistry, University of Washington, Seattle WA 98195

The mouse muscle creatine kinase (MCK) gene is expressed specifically in skeletal muscle and in heart. We are studying MCK induction in the mouse skeletal myoblast cell line MM14, in which the onset of differentiation and consequently of MCK expression is caused by deprivation of fibroblast growth factor (FGF).

A major effector of the mouse MCK gene expression is a transcriptional enhancer located 1100 nt upstream of the transcription start site. The enhancer is specific to differentiated myogenic cells and its activity is contained within a 110 nt fragment. Gel mobility shift assays have demonstrated the presence of binding factors, one of which binds within the 110 nt fragment and is present in nuclear extracts from differentiated myocytes, but not from myoblasts or lymphocytes. The specificity of binding has been investigated by using competitor DNAs from other regions of the MCK gene or from viral or cellular enhancers. DNAse I footprinting in combination with preparative mobility-shift gels revealed a protected site. Complementary DNA oligomers containing 21 bp of MCK enhancer sequences centered around the protected region are sufficient for myocyte factor binding. The function of these DNA sequences is under investigation, as is the requirement for other sequences within the enhancer fragment. Future investigations will attempt to understand the events between the withdrawal of FGF and the activation of the muscle-specific gene, MCK.

O 526 ORIENTATION-DEPENDENT FUNCTION OF AN 82 BASEPAIR CYC1 TERMINATOR FRAGMENT INSERTED WITHIN THE ACTIN INTRON OF YEAST, J. S. Butler, S. M. Baker, H. Ruohola, J. Hazen, R. Parker and T. Platt, University of Rochester Medical Center, Rochester, NY 14642

An integrative vector carrying the 5' end of the actin gene (including its intron) fused in frame to HIS4 sequences can confer the ability to grow on histidinol if HIS4C (encoding histidinol dehydrogenase) is sufficiently expressed. With an 82 bp region spanning the site of normal 3' end formation of Saccharomyces CYC1 mRNA cloned into the intron of this actin fusion, transformants cannot grow on histidinol. Cells transformed with the vector carrying the reverse orientation of this fragment can grow well, and Northern blots reveal normal levels of full length RNA. With the forward orientation, however, full length RNA is less than 40% of control levels, and a short polyadenylated RNA accounts for most of the transcription; its 3' end lies within the inserted fragment, corresponding to the normal CYC1 3' end. Suppressor mutations that permit growth on histidinol despite the inserted fragment have been isolated, and many are dominant or semi-dominant, suggesting cis-acting effects. We are also testing whether mutations in RPO22 (encoding an RNA polymerase II large subunit) can suppress our Hol$^-$ mutants. Analysis of the recessive mutations reveals a minimum of 3 complementation groups, indicating that defects in several different genes can restore higher levels of HIS4C expression. A parallel biochemical approach using an SP6 transcript with CYC1 3' sequences in a helicase assay is underway, to determine whether a rho-like transcription termination factor capable of unwinding an RNA-DNA duplex (as the bacterial protein can do) is detectable in extracts from yeast cells. This is expected to complement our genetic analysis, to illuminate the processes involved in the 3' end formation of mRNA in yeast.

O 527 **TRANSCRIPTIONAL ANALYSIS (IN VIVO AND IN VITRO) REVEALS DIFFERENTIAL REGULATION OF ß TUBULIN GENES DURING DROSOPHILA EMBRYOGENESIS,**
D. Buttgereit, A. Gasch, U. Hinz, D. Leiss, R. Renkawitz-Pohl, MPI f. Biochemie, Genzentrum, Munich, FRG

ß tubulin genes of Drosophila melanogaster are encoded by a small gene family, comprising at least four members. Two of them, the ß1 and the ß3 gene, are abundantly expressed during embryogenesis. In the early embryo, where no transcription occurs, the ß1 mRNA is supplied as a maternal message, transcribed in the nurse cells of the developing oocyte. Zygotic transcription seems to be restricted primaryly to the developing nerve system and later to the apodemes. In all stages the same transcription start site is used. In vitro transcription studies with nuclear extracts from embryos reflect the in vivo situtation so far, that no transcription from a ß1 template is observed in extracts containing embryos younger than 3 hours. It was shown by deletion analysis of the ß1 promoter, that 100 bp upstream of the cap-site are sufficient for a correct initiation of transcription in the cell-free system.
The ß3 tubulin message, in contrast, is not present as maternal mRNA. Transcription of the gene starts in the cephalic mesoderm after 4 hours of embryo development. Later, the mRNA accumulates also in the somatic and visceral mesoderm. By P-element mediated transformation, we demonstrated that separate elements regulate the expression in the cephalic, visceral and the somatic mesoderm.

O 528 TISSUE-SPECIFIC EXPRESSION OF THE TYROSINE HYDROXYLASE GENE,
F. Cambi, C.H. Harrington, E.J. Lewis and D.M. Chikaraishi,
Neurosciences Program, Tufts School of Medicine, Boston, MA.
Tyrosine Hydroxylase (TH) is the rate limiting enzyme in the synthesis of catecholamines and is expressed only in catecholaminergic neurons in both the central and peripheral nervous system and in the adrenal gland. TH is under a complex array of regulatory influences that act both at the transcriptional and post-transcriptional levels. To study the transcriptional regulation of TH, genomic DNA clones spanning 4.2 Kb of upstream region were isolated in this laboratory (C. Harrington et al. 1986). To explore the tissue-specific expression of the TH gene, 5′ upstream regions extending to -4200, -773 and -272 bp were cloned in front of the chloramphenicolacetyltransferase gene and were transfected into cultured cell lines. In all the experiments the cell line, PC8b, a rat pheochromocytoma that expresses TH endogenously, was used as a positive "expressing" line while lines of fibroblastic, hepatic, neuronal and neuroendocrine origin were used as the non-expressing lines. Elements that control tissue-specificity are located in the constructs containing 773 bp of TH 5′ upstream sequence information. This construct is expressed equally well in PC8b and in a neuroendocrine line (GH4), but at a very reduced level in the other cell lines. Furthermore the CAT construct containing 4.2 Kb is expressed in PC8b less efficiently than the construct containing only 773 bp suggesting the presence of a "repressor element". We are now in the process of defining the positive and negative elements by deletion analysis.

O 529 **Transcriptional and Post-transcriptional Regulation of the Human Transferrin Receptor: Two distinct genetic loci are responsible for two regulatory mechanisms.**
John L. Casey, David M. Koeller, Bruno DiJeso, Richard D. Klausner, and Joe B. Harford.
Cell Biology and Metabolism Branch, NICHD, NIH, Bethesda, MD 20892.

Transferrin receptors (TfR) provide cells with the essential nutrient iron through receptor-mediated endocytosis of diferric transferrin. It has been shown that intracellular iron levels regulate TfR mRNA levels and TfR biosynthesis. In desferrioxamine-treated (iron-starved) cells TfR mRNA levels are 20-30 times higher than in hemin-treated (iron loaded) cells. We have found that there are at least two genetic loci in the TfR gene which are required for iron regulation. One is in the 5' flanking region of the gene, the other is in the 3' end corresponding to the untranslated portion of the mRNA. We have analyzed the transcriptional activity of the promoter region of the TfR gene and found that there is a critical element between 86 and 70 base pairs (bp) upstream of the cap site that is identical in sequence to a portion of the polyoma virus enhancer. A fragment of the the TfR promoter region containing just 13 bp 3' of the cap site, when linked to the bacterial CAT gene, leads to iron-dependent accumulation and synthesis of CAT mRNA. A second iron-regulatory element is indicated by the observed regulation by iron of TfR mRNA levels in cells transfected with a heterologous construct containing the SV40 promoter and the TfR cDNA. The regulatory element in the 3' region of the gene has been localized to a 680 nucleotide (nt) region in the middle of the ~2500 nt 3' untranslated portion of the TfR mRNA. Comparisons of nuclear and cytoplasmic RNA from hemin- or desferrioxamine-treated mouse cells transfected with a human TfR minigene show that iron affects the level of TfR mRNA in the cytoplasm but not in the nucleus. We conclude that TfR mRNA levels are regulated by iron via 3-fold transcriptional and approximately 10-fold post-transcriptional effects.

O 530 SPECIFIC FACTORS BINDING TO UPSTREAM SEQUENCES OF THE HUMAN FOETAL $^{A}\gamma$ GLOBIN GENE. F. Catala*, M. Antoniou, E. deBoer, G. Habets, L. Wall and F. Grosveld, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K., *Institut Curie, Orsay, France.

Human $^{A}\gamma$ globin gene regulation has been studied by transfection into K562 cells. Individual promoter elements have been further analyzed by DNA retardation and footprinting assays. Retardation showed that K562 nuclear extracts contained at least three proteins, B1, B2 and B3 binding to an octamer at -182bp. B2 and B3 were not present in HeLa or B-lymphoid (J558L) nuclear extracts and thus appear to be erythroid specific. B1 could be separated from B2 and B3 through a heparin-agarose column with a high degree of purification. Footprinting assays with the same extracts showed that B1, or B2 and B3 gave different footprints of the octamer region. Footprints were also observed for the CCACCA box region and for an AGCAGCAGT motif. The latter was present only in K562 extracts and the corresponding erythroid factor was eluted in the same fraction as B1.

O 531 THE ENHANCER OF THE HUMAN PAPILLOMAVIRUS-16, Woon-Khiong Chan, Bernd Gloss, Hans-Ulrich Bernard, Institute of Molecular and Cell Biology, National University of Singapore.

The human papillomavirus-16 causes benign neoplasias in the genital region of men and women and is associated with up to 90% of all malignant genital carcinomas. The 7905 bp genome contains a 850 bp segment without open reading frames, which is supposed to be involved in regulation of transcription and replication. We have identified a 400 bp fragment cleaved out of this segment which functions as an enhancer in human cervical carcinoma cells but not in other human cell lines. This cell type specific enhancer contains at least 10 protein binding motifs as defined by DNA footprinting analysis. One of these motifs binds the glucocorticoid receptor and leads to glucocorticoid and progesterone responsiveness of HPV-16 gene expression. The enhancer also responds to induction by phorbolesters, most likely through binding of the AP1-protein. Three footprints coincide with the motif 5'-TTTGGCTT-3', found in the promoter of all keratin-genes. We are using synthetic oligonucleotides corresponding to these sequences to test whether cellular enhancer binding factors recognizing this motif may contribute to the cell-type specificity of the HPV-16 enhancer. The interaction of cellular DNA-binding factors and the HPV-16-E2-regulatory proteins (which do not bind the HPV-16 cell-type-specific enhancer) is under investigation.

Ref.: B. Gloss et al, EMBO J. 6, in press (Dec. issue)

O 532 A GENE ENCODING A PROTEIN WITH ZINC FINGERS IS ACTIVATED DURING G0/G1 TRANSITION IN CULTURED CELLS, Patrick Charnay, Phillipe Chavrier, Marino Zerial, Patrick Lemaire, José Almendral and Rodrigo Bravo, European Molecular Biology Laboratory, Meyerhofstraße 1, 6900 Heidelberg.

Zinc fingers are DNA-binding domains present in several eukaryotic regulatory proteins. We have identified a mouse gene, *Krox*-20, encoding a protein with zinc fingers and whose expression is activated during G0/G1 transition in cultured cells. The predicted protein is 445 amino-acids long, with an unmodified molecular weight of 48, 434d. It contains three zinc fingers which conform to the *Krüppel* finger consensus: Cys-X_2-Cys-X_3-Phe-X_5-Leu-X_2-His-X_3-His. A significant similarity is observed between *Krox*-20 fingers and those of the human transcription factor Sp1. An additional feature of the protein is its high content in proline residences (15.5%). These residues are concentrated within two regions flanking the zinc fingers and located respectively between positions 19 and 281 and 369 and 445. Serum stimulation of quiescent NIH 3T3 cells leads to rapid transient accumulation of *Krox*-20 mRNA, with kinetics similar to those of the *c-fos* nuclear oncogene. The induction does not require *de novo* protein synthesis and cycloheximide treatment leads to superinduction. The induction is due, at least in part, to transcriptional activation. *Krox*-20 is also activated after treatment of the cells with purified Epidermal Growth Factor (EGF) or Platelet Derived Growth Factor (PDGF). In the mouse, *Krox*-20 is expressed at low levels in thymus, spleen and testes. These tissues contain rapidly dividing cells and the expression of the *Krox-20* might thus be related to the proliferation of a subpopulation of cells. In conclusion, our observations suggest that *Krox*-20 encodes a transcription control factor, possibly involved in the modulation of cell proliferation.

O 533 Interaction of murine αA-crystallin gene cis-regulatory elements with lens nuclear factors. Ana B. Chepelinsky, Bernd Sommer, Eric Wawrousek and Joram Piatigorsky. Laboratory of Molecular and Developmental Biology. National Eye Institute. National Institutes of Health. Bethesda, Maryland 20892.

The 5' flanking sequence of the murine αA-crystallin gene contains several regulatory elements. A hybrid gene containing 111 base pairs of αA-crystallin 5' flanking sequence upstream of the bacterial gene chloramphenicol acetyltransferase (CAT) activates CAT gene expression in explanted chicken lens epithelia and in transgenic mice. In transgenic mice the CAT gene is expressed specifically in the lens. Those 111 bp of αA-crystallin 5' flanking sequence do not activate CAT gene transcription in cultured HeLa cells. However, they do promote CAT gene transcription in vitro in HeLa whole cell extract. Deletion mutants allowed us to define a core promoter element containing the TATA box (-34 to +46), functional in vitro in a HeLa whole cell extract. Two additional regulatory elements (-111 to -84 and -88 to -60) were functional in explanted lens epithelia. Synthetic double-stranded oligonucleotides corresponding to -111 to -84 and -83 to -55 bind two different lens nuclear factors identified by gel retardation and methylation interference. HeLa cell nuclear extracts also contain factors that interact with the αA-crystallin promoter. A series of point mutations are being used to map precisely the key nucleotides necessary for binding nuclear factors and for function of the αA-crystallin promoter.

O 534 NEGATIVE REGULATION OF THE HUMAN PAPILLOMAVIRUS TYPE 11 ENHANCER AND E6 PROMOTER BY HPV-11 E2-C PROTEIN. Michael T. Chin, Rei Hirochika, Hirohiko Hirochika, Thomas R. Broker and Louise T. Chow. Biochemistry, Univ. of Rochester, Rochester, NY 14642

The E2 open reading frame of papillomaviruses encodes at least two enhancer-regulating proteins. The full-length product is a trans-activator. The protein consisting mainly of the carboxyl-terminal 45% of human papillomavirus type 11 (HPV-11) E2 protein, called E2-C, has been expressed in monkey CV-1 cells. It corresponds closely to a protein inferred from the mRNA structure. E2-C represses E2-dependent HPV-11 enhancer activity in CV-1 cells, as measured by the chloramphenicol acetyltransferase (CAT) assay. An AUG^- mutation that prevents the synthesis of E2-C results in the loss of repression. E2-C was also made from an expression vector in E. coli. Crude lysates or E2-C purified by DNA affinity chromatography bind in vitro to a specific sequence, $ACCN_6GGT$, in filter-binding assays and have DNase I footprints identical to those of E2. This sequence has been shown to be necessary and sufficient for E2 binding and transactivation (Hirochika et al. 1988. Genes & Development, in press). Mutations in this sequence that prevent E2 from binding also preclude interaction with E2-C. The HPV-11 enhancer-E6 promoter has high E2-independent activity in certain human cervical carcinoma cell lines. HPV-11 E2 protein further stimulates this activity, whereas E2-C suppresses the E2-independent expression from this enhancer-promoter. Analysis of deletion mutants indicates that the sequences important for basal activity in cervical cells map outside the previously identified functional boundaries of the enhancer as defined in CV-1 cells (see abstract by Broker et al.). A model for the repression of E2 independent and dependent activities by E2-C protein will be presented.

O 535 TOPOISOMERASE I CLEAVAGE SITES IDENTIFY DNA BINDING PROTEIN DOMAINS. John Chiorini, Roger B. Cohen and Brian Safer, Section on Protein Biosynthesis, LMH, NHLBI, NIH, Bethesda, MD 20892

The topological state and accessibility of cis-acting regulatory sequences to specific DNA binding proteins are thought to modulate the activity of genes. Since several potential topoisomerase I binding sites are identified in the adenovirus 2 major late promoter, we analyzed the camptothecin-enhanced cleavage pattern of this gene produced by incubation of linear or supercoiled DNA with K562 nuclear extract. Prominent cleavages were located in regions flanking the upstream promoter sequence, TATA box and Ela-like enhancer sequence as well as adjacent to the newly identified downstream promoter sequence. Highly purified calf thymus topoisomerase I generated a similar pattern. An analogous correlation between topoisomerase I cleavages and cis-acting promoter elements was observed for the mouse β-globin and human eIF-2α genes. While the functional significance of this observation is not yet known, topoisomerase I mapping using unfractionated nuclear extracts and camptothecin appears to be useful in identifying potential cis-acting elements.

O 536 NUCLEAR FACTORS FROM EXPRESSING TISSUES INTERACT WITH A RAT ALPHA-2U GLOBULIN GENE INTRON, Henry A. Choy, Margaret McLaughlin and Philip Feigelson, Department of Biochemistry and Molecular Biophysics and the Institute of Cancer Research, Columbia University, New York, NY 10032.
Tissue-specific expression of alpha-2u globulins in the rat is characterized by developmentally and hormonally regulated activation of subsets of a large gene family. For example, expression of four of these genes in the liver is restricted to adult males. We are interested in defining the DNA elements and cellular factors which determine alpha-2u globulin expression. Sequence analysis of a hepatic-expressing member of the gene family revealed a potential regulatory region in the third intron containing both an uninterrupted run of 27 GT repeats and similarities to a viral enhancer core and the Sp1 transcription factor binding site. DNA fragments from this region were used in a DNase I footprinting assay to probe for tissue-specific DNA-binding factors in nuclear extracts. Strong sequence-specific interactions were detected between factors from all expressing tissues tested (liver, lachrymal, and salivary gland) and a 22 base site in intron 3 resembling the CCAT sequence in the 5'-flanking region of the gene. Weaker binding was seen at the sites resembling enhancer core; none was observed at the GT repeat region. Hepatic nuclei from prepubescent males and adult females, animals that do not express alpha-2u globulin in liver, contained similar footprinting activity. Non-expressing tissues from adult males (spleen, kidney, testis, brain) did not footprint. Thus, the tissue-specific interactions observed may act in conjunction with other hormonally regulated elements to produce tissue-specific expression.

O 537 GENE TRANSFER EXPERIMENTS DEFINE REGULATORY REGIONS FOR P450scc EXPRESSION Bon-chu Chung, Char-Chang Lai, Meng-Chun Hu, and Cheng-Hui Lin, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan
All steroid hormones are synthesized from a common precursor cholesterol which is converted to pregnenolone and isocaproic acid in a rate-limiting and hormonally regulated reaction catalyzed by a monooxygenase termed P450scc. We cloned the 5'-region of the P450scc gene including the first intron and exon, and about 5 kb of its 5'-flanking region. Messenger RNA start site was determined by primer extension and S1-nuclease mapping to be 62 nucleotides upstream of the ATG initiation codon. There is a TATAA and a CATT box approximately 30 and 70 bases upstream of the mRNA start site. Sequences at postions -200 and -75 are homologous to the cAMP responsive sites found in other cAMP regulated genes. In order to study regulation of the P450scc gene expression, we placed various parts of its 5'-flanking region upstream of the bacterial chloramphenicol acetyltransferase (CAT) gene and trasfected these plasmids into both steroid secreting and nonspecific cell lines. The expressed CAT activity represents promoter strength of input plasmids. About 230 bases of DNA at the 5'-flanking region of the P450scc gene is enough to direct CAT expression at a high level in steroid synthesizing cells but only weakly in nonspecific cells. We also found an inhibitory element situated around the -650 region of the P450scc gene.

O 538 BINDING OF PROTEINS TO THE CHICKEN BETA-GLOBIN PROMOTER REGION. S.P. Clark, C.D. Lewis, H. Gould* and G. Felsenfeld, NIH, Bethesda,MD. *Kings College, London, Eng.
The chicken beta-globin promoter region has several sites which are protected by protein during footprint analyses. Among these are the G-string (16 consecutive G's at -190), with a possible Sp1 site at its 3' end, a palindromic sequence (-155), the globin CACCC consensus (-135), an 11 base polypyrimidine stretch (-118) and a 15 base polypurine stretch (-52). Gel mobility-shift experiments show that most sites bind multiple proteins in a sequence-specific manner. Mutagenesis of the palindrome demonstrates that the central three nucleotides are not recognized, and only the proximal 6 of the 11 bases of each half-palindrome are required for binding. The general transcription factor Sp1 binds, but with low affinity, to the G-string, CACCC sequence and polypyrimidine stretch, and with high affinity to the polypurine stretch. Of these sites, only the G-string has the core Sp1 sequence GGGCGG. The predominant protein which interacts with the G-string, BGP1, binds with high affinity and is present only in erythrocytes. Despite the difference in sequence specificity and tissue distribution of Sp1 and BGP1, these proteins appear to be structurally related. Both are inhibited by a divalent cation chelator and have binding restored by Zn^{+2}. Furthermore, a monoclonal antibody which was selected for its ability to block binding of BGP1 to the G-string also recognizes Sp1, but with 10-20 fold lower affinity. Western blotting of a crude nuclear extract and silver staining after SDS-PAGE of material purified by affinity chromatography indicate that BGP1 has a molecular weight of 67 kD. The availability of pure BGP1 and an antibody which blocks its binding should enable us to determine the role of BGP1 in the transcriptional regulation of the globin gene family.

O 539 CHANGES IN HISTONE GENE DOSAGE ALTER TRANSCRIPTION IN YEAST, Chris D. Clark-Adams, David Norris, Mary Ann Osley, Jan S. Fassler and Fred Winston, Harvard Medical School, Boston MA 02115.

Chromatin structure is believed to be important for a number of cellular processes, including transcription. However, the role of nucleosomes in transcription is not well understood. If a wild type level of histones is required for normal transcription, a genetic screen for transcription mutants might identify mutations in histone genes. We have isolated many transcription mutants in the yeast Saccharomyces cerevisiae as suppressors of Ty and solo δ insertion mutations that inhibit normal transcription of adjacent genes. Mutations in SPT (Suppressor of Ty) genes suppress Ty and δ insertion mutations by restoring functional transcription to the adjacent genes. We have identified the yeast histone locus HTA1-HTB1, encoding histones H2A and H2B, as one such suppressor of solo δ insertion mutations. The HTA1-HTB1 locus causes suppression either when present on a high copy number plasmid or when mutant. These changes in HTA1-HTB1 alter transcription of genes adjacent to the δ insertions. Based on this result, we have examined the effects of increased and decreased histone gene dosage for all four yeast histone loci. From the types of histone gene dosage changes that cause suppression of insertion mutations, we conclude that altered stoichiometry of histone dimer sets can alter transcription in yeast. Experiments in progress will examine changes in chromatin structure caused by altering histone gene dosage.

O 540 THE TWO TRANSCRIPTION UNITS OF THE AUTONOMOUS PARVOVIRUS, MINUTE VIRUS OF MICE (MVM), ARE TRANSCRIBED IN A TEMPORAL ORDER WHICH MAY BE MEDIATED BY THE LARGE VIRAL NONSTRUCTURAL PROTEIN, Karen E. Clemens, Greg E. Tullis and David J. Pintel, University of Missouri-Columbia, Columbia, MO 65212.

The genome of the autonomous parvovirus, Minute Virus of Mice (MVM), is organized into two major coding regions which are expressed from two overlapping transcription units. MVM DNA replication and gene expression are entirely dependent upon one or more cellular function(s) expressed during the S phase of the cell cycle. Using a quantitative RNA hybridization/nuclease protection assay to probe infected RNA from highly synchronized cells, we have demonstrated that mRNAs generated from the MVM left hand end p4 promoter, which encode the viral nonstructural proteins, appear prior to mRNAs generated from the viral p39 promoter, which encode the viral capsid genes, providing direct evidence that there is a temporal order of expression between the two transcription units.

Preliminary experiments which examine the temporal expression of viral mRNA in cells infected by a recently constructed temperature sensitive mutant in the MVM NS-1 gene (Tullis, Labieniec-Pintel, Clemens, and Pintel, in preparation) has indicated that the regulation of the temporal expression of the p39 transcription unit is mediated by the NS-1 gene product which has recently been shown to stimulate the expression of the viral p39 promoter in transient co-transfection assays (Rhode, J. Virol. 55:886, 1985). Current experiments are designed to investigate the mechanism of this regulation.

O 541 IDENTIFICATION OF A DOWNSTREAM SEQUENCE AND BINDING PROTEIN THAT REGULATE ADENOVIRUS MAJOR LATE PROMOTER TRANSCRIPTION IN VITRO. Roger B. Cohen, John A. Thompson, and Brian Safer. Section on Protein Biosynthesis, LMH, NHLBI, NIH, Bethesda, MD, 20892.

We have used gel electrophoresis mobility shift and DNase I footprint assays to detect and purify a cellular nuclear protein from K562 cells which binds to a downstream promoter sequence (DPS) in the human adenovirus-2 major late promoter. By DNase I footprint and mutation analysis we have determined that this previously unidentified regulatory element extends from +146 to +174 relative to the cap site at +1. UV cross-linking studies using probes labelled uniquely in the DPS show that a 40,000 Dalton polypeptide (downstream transcription factor, or DTF) specifically binds the DPS. Sequence-specific DNA affinity chromatography of nuclear extracts has been used to extensively purify DTF. Mutations within the DPS which decrease protein binding by 80-90% also cause a 2.5-3-fold decrease in in vitro major late promoter expression. Alterations of the major late promoter in flanking regions of the DPS do not affect protein binding or transcription efficiency. A T→G transversion at +160 which increases protein binding impairs promoter activity. There is a 7/8 base similarity between sequences within the DPS and sequences within the upstream promoter sequence (UPS, -50 to -60) of the major late promoter. The proteins binding to the DPS and UPS are chromatographically distinct and have distinct molecular weights. The presence of the shared sequences, however, suggests the potential for competitive interactions between the factors binding to these two elements.

O 542 Developmental regulation of murine myelin proteolipid protein gene expression. Julia L. Cook and Prescott L. Deininger, Louisiana State University Medical Center, New Orleans, LA 70112. Proteolipid protein (PLP), the major protein component of CNS myelin sheath, is expressed in both a tissue-specific and a temporally regulated manner. The rat PLP gene is expressed exclusively in oligodendrocytes and maximum protein and mRNA levels have been demonstrated to coincide with myelination. These studies focus on the levels of regulation involved in achieving proper expression of PLP and subsequent normal neuronal myelination. Nuclear-runoff assays, employed to generate measurements of PLP transcription in the developing rat brain, indicate that transcription is predominantly responsible for the observed changes in PLP steady-state mRNA levels. Subsequent studies were conducted in order to identify putative regulatory regions within the promoter responsible for the regulatory phenomena observed. Various PLP 5'-flanking sequences were evaluated for promoter strength by testing the abilities of PLP promoter-CAT (chloramphenicol acetyltransferase) hybrid constructs to generate translatable CAT mRNA. 5'-flanking PLP sequences that possess distinct promoter activities are presently being testing for differential protein binding abilities in extracts derived from brain and non-brain cells.

O 543 **MULTIPLE CIS-ACTING ELEMENTS MEDIATE INDUCED EXPRESSION OF THE ALLANTOIN DEGRADATIVE GENES IN SACCHAROMYCES CEREVISIAE**, H-S. Yoo, & T.G. Cooper, University of Tennessee, Memphis, Tennessee 38163. Expression of the allantoin system genes of **S. cerevisiae** are induced by allophanate or its close analogue, oxalurate. We have shown that three distinct promoter elements mediate this induction process. The first element is an upstream activation sequence or **UAS**. When present in two or more copies, it supports constitutive B-galactosidase production in a **CYC1-LACZ** expression vector lacking the **CYC1 UAS**; there is no response to inducer. The second element is an upstream repression sequence or **URS**. When the **URS** is placed adjacent to the **DAL7 UAS**, it completely inhibits the latter's ability to support **LACZ** expression. The **URS** element is also capable of inhibiting operation of the heterologous **CYC1 UAS**. The third element we have designated **UIS** or upstream induction sequence. When this sequence is placed adjacent to the **UAS** and **URS** elements just described, the resulting construction (**UIS-UAS-URS**) supports normal oxalurate-mediated induction of **LACZ** expression. Neither single nor multiple copies of the **UIS** element possess **UAS** activity. These data are consistent with the hypothesis that induction results from **UIS**-mediated inhibition of **URS** function. Relief of **URS**-mediated inhibition of **UAS** function, in turn leaves the **UAS** free to activate gene transcription.

O 544 RECONSTITUTION, *IN VITRO*, OF TISSUE-SPECIFIC AND HORMONE-CONTROLLED TRANSCRIPTION OF THE XENOPUS LAEVIS B1 VITELLOGENIN GENE

Blaise Corthésy, Robert Hipskind, Irène Theulaz and Walter Wahli
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We have prepared nuclear extracts from Xenopus laevis liver that mimic the *in vivo* hormonally regulated expression of the B1 vitellogenin gene. This conclusion is based on the differiential induction of two templates either containing or lacking the estrogen-responsive element (ERE). In females and estrogen-stimulated male extracts, the hormone is necessary and sufficient to activate the ERE-containing B1 vitellogenin gene. In contrast, in untreated males extracts, the B1 promoter remains silent. Using female extracts and 5'-deletion mutants, we demonstrate that 344 base pairs (bp) of the 5'-flanking region of the gene are sufficient for estrogen-controlled, tissue-specific and efficient RNA polymerase II-dependent *in vitro* transcription. The hormonal effect is lost when the ERE is deleted and no transcription is dectected, even in presence of hormone. Interestingly, we observe that the promoter becomes constitutively active in deletions which only contain the 100-bp region upstream of the initiation site. Using DNAse I footprinting assays, we present evidence for the existence of a Xenopus liver factor that binds to a 29 bp region (-120 to -92) of the promoter. The putative role that this interaction plays in *in vitro* liver specific B1 vitellogenin gene expression is discussed.

O 545 PURIFICATION OF THE LIVER-SPECIFIC NUCLEAR FACTOR, HNF-1 AND CHARACTERIZATION OF ITS BINDING SITE, G. Courtois, S. Baumhauter, G. R. Crabtree, Stanford University Medical School, Stanford, CA 94305.
We have previously described a liver-specific nuclear factor, HNF-1, which interacts with regions essential for the activity of the α and β fibrinogen and α_1 antitrypsin promoters. Using a CAT assay and transient transfection of hepatic (HepG2) and nonhepatic cell lines (Hela and L cells) we find that the HNF-1 binding site is sufficient to confer liver-specific expression to a heterologous promoter. Moreover, we have purified HNF-1 to near homogeneity using an affinity chromotography procedure. The molecular weight of the isolated protein (90 kd) is in good agreement with the value determined using U.V. cross linking in which a radiolabeled bromo-substituted probe was incubated with a liver nuclear extract. Using purified HNF-1 we have detected its interaction with a functionally essential region of the albumin, and α fetoprotein promoters and with the promoter for the surface antigen of hepatitis B virus. In contrast, the protein does not interact with the SV-40 early control region, or the α globin promoter. A comparison of the different binding sites lead us to propose GTTAATNATTAAT as the consensus site for HNF-1. The specific interaction of HNF-1 with several different liver specific genes suggest that it could constitute an important factor in the establishment of the liver-phenotype.

Poster Session 4

O 546 THE HUMAN TRIMER UBIQUITIN GENE CONTAINS TWO PROMOTER REGIONS. Jack B. Cowland, Ove Wiborg*, Lars Theill* and Jens Vuust. Statens Seruminstitut, DK-2300 Copenhagen S, Denmark, and *Aarhus U iversity, DK-8000 Aarhus C, Denmark. Ubiquitin is a highly conserved, 76 amino acid protein that is involved in a number of important cellular functions, e.g. intracellular protein degradation and covalent modification of histones. Ubiquitin is encoded in the human genome as a multigene family. We and others have isolated and characterized a number of the human ubiquitin genes. One of these codes for a polyprotein with three ubiquitin molecules in tandem repeat. We have investigated the regulation of expression of this particular gene. Transient expression analyses (using the pSV2 CAT vector system) show the presence of two different promoter regions, one of which resides in a region corresponding to an intron of the pre-mRNA initiated from the other, distally located promoter region. Our investigations further indicate a cell-line specific utilization of the two promoters.

O 547 REGULATION OF THE HUMAN PAPILLOMAVIRUS E6-E7 ONCOGENE REGION PROMOTER; IMPLICATIONS FOR CERVICAL CARCINOGENESIS. T.P.Cripe[1], T.H.Haugen[1], M.Durst[2], L.Gissmann [2], A.Roman[3], and L.P.Turek[1], The University of Iowa, Iowa City, IA 52242, [2]DKFZ, D-6900 Heidelberg, F.R.G, and [3]Indiana University, Indianapolis, IN 46202.
The human papillomavirus (HPV)-16 E6-E7 gene region is preserved and expressed in cervical carcinoma cells, and facilitates the "immortalization" of human keratinocytes in culture. We have found that the transcriptional promoter of the E6-E7 transforming gene region (P_{97}) is active in transiently transfected cervical carcinoma cells when linked to the HSV-1 *tk* or bacterial *cat* genes. Sequences 5' to P_{97} contain a short enhancer element responding to cellular factor(s) in uninfected human foreskin keratinocytes and in cervical carcinoma cells, but not in human or animal fibroblasts. The enhancer activity correlates with the presence of cellular factors that specifically bind to the *cis* enhancer DNA. The E2 *trans*-activator products of HPV-16 or of the related bovine papillomavirus (BPV)-1 further elevate HPV-16-driven transcripts in cotransfections, and require the presence of E2-binding $ACC(N)_6GGT$ cores in *cis*. A "short E2" C-terminal repressor gene product (sE2) of HPV-16 or the BPV-1 sE2 repressor not only inhibits viral E2 *trans*-activation, but also suppresses enhancer response to keratinocytic factors. Suppression by the sE2 products is abolished by deletion of the *cis* E2-binding cores or in a non-binding sE2 mutant. We are currently characterizing the keratinocyte-dependent cellular factors, and testing the influence of the viral E2 proteins on cellular factor binding. The keratinocyte-dependent enhancer is likely to contribute to the epithelial cell tropism of HPV-16. The disruption of the HPV-16 E2 gene region observed in cervical carcinomas is likely to result in deregulated E6-E7 gene transcription in response to cellular factors, and may thus be one of the critical events in the malignant conversion of HPV-16-induced genital lesions.

O 548 MAPPING OF TRANSCRIPTIONAL REGULATORY ELEMENTS IN THE INTERLEUKIN-2 RECEPTOR ALPHA CHAIN PROMOTER, Sharon L. Cross, Julie B. Wolf, Nancy F. Halden, Charles H. Spencer and Warren J. Leonard, CBMB, NICHD, Bethesda, MD 20892.
The interleukin-2 receptor alpha chain (p55, Tac antigen, IL2Rα) gene is transcribed in normal peripheral blood T cells only after activation, but is constitutively transcribed in T cells transformed with human T cell lymphotropic virus (HTLV-I), the cause of adult T cell leukemia. Earlier work utilizing 5' deletions of the IL2Rα promoter linked to the bacterial chloramphenicol acetyltransferase reporter gene in transient transfection assays indicated that 5' flanking sequences regulated transcription in cell types that are inducible (Jurkat T cells) and constitutive (HTLV-I transformed T cell lines such as MT2) for IL2Rα expression. Furthermore, cotransfection of the HTLV-I tat-I ($p40^x$) gene with IL2Rα-CAT constructs demonstrated that this gene was capable of stimulating IL2Rα-CAT expression in Jurkat cells. The 5' promoter sequences required for this induction by tat-I were distinct from those required for phorbol ester stimulation. We have extended these observations by constructing a series of internal deletions in the promoter region. These constructs are being analyzed for their ability to direct CAT expression in various cell lines, as well as for their ability to be activated by tat-I or phorbol esters. Our studies have revealed that the IL2Rα promoter requires several separate regions for maximal activity, and that different promoter regions are utilized in HTLV-I transformed T cells than are needed in Jurkat cells. In conjunction with mapping the sequence elements required for promoter activity, we have identified areas of protein binding in the 580 basepair fragment used in these studies.

O 549 DOMAINS OF THE GLUCOCORTICOID RECEPTOR INVOLVED IN SPECIFIC AND NONSPECIFIC DEOXYRIBONUCLEIC ACID BINDING, HORMONE ACTIVATION, AND TRANSCRIPTIONAL ENHANCEMENT, Mark Danielsen, Jeffrey P. Northrop and Gordon M. Ringold*, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305 and *Syntex Research, 3401 Hillview Avenue, Palo Alto, CA 94304.

The glucocorticoid receptor (GR) consists of a core domain rich in the amino acids Cys, Lys, and Arg amino acids which can bind specific DNA sequences (glucocorticoid response elements) and activate transcription. We have functionally mapped this domain by a) deletion analysis, b) introducing point mutations, and c) constructing hybrid receptors that contain DNA binding domains composed partly of GR and partly of estrogen receptor sequences. The ability of these mutated receptors to bind specifically to a glucocorticoid response element and to activate transcription has been evaluated. We find that the activity of the centrally located DNA binding domain is modulated by functional regions of the N-terminal and C-terminal portions of the receptor. The N-terminal domain of the GR plays a role in decreasing nonspecific DNA binding and may therefore improve the ability of the protein to discriminate beween specific and nonspecific DNA binding sites. This activity maps to a small, highly acidic region of the N-terminal domain. The C-terminal domain of the receptor contains the glucocorticoid binding site and in addition represses the transcriptional activity of the GR in the absence of hormone. Hormone binding relieves the repression allowing transcription activation. The C-terminal domain contains a short sequence conserved among steroid receptors, deletion of which yields a receptor that activates transcription in the absence of hormone.

O 550 TRANSCRIPTION INITIATION IN BK VIRUS EARLY PROMOTER. Gokul C. Das, Tushar Chakraborty, and Sujoy DasGupta, Department of Molecular Biology, The University of Texas Health Center at Tyler, Tyler, Texas 75710.
BK virus, a human papovavirus, infects the majority of human population by the time of adolescence and is thought to be associated with a number of human diseases including cancer. Following the initial infection, the virus persists in a latent form and can be reactivated in immunosuppressed or immunodeficient individual. The intergenic enhancer-promoter region of the virus, consists of three 68 bp repeats with multiple copies of potential regulatory elements, in which an 18 bp sequence is deleted from the middle one. The present study is aimed at identifying the cis- and trans-acting elements of the viral early promoter. We show here that the transcriptional efficiency of the early promoter *in vitro* in a HeLa cell extract is reduced by about 80% when only the middle 50 bp copy of the repeats is deleted. No significant drop in promoter activity was observed with further deletion of another extra copy. Competition experiments with purified fragment containing all three repeats showed that the binding of transcription factor(s) from HeLa cells to this region of the promoter is important for transcription initiation *in vitro*. However, the transcription is not abolished when an SV40 promoter fragment (np 1-128) containing the TATA box and 6 copies of the CCGCCC box which binds a human transcription factor SP1, is used as competitor. These results suggest that the cellular factors involved in transcription initiation might be different in these two closely related viruses of the same family. *In vivo* characterization of viral promoter by chloramphenicol acetyl transferase (CAT) gene assay is underway.

O 551 CHARACTERIZATION OF AN INSULIN-INDUCIBLE GENE, CM Davis, N Chrapkiewicz, D Chu, and DK Granner, Vanderbilt University, Nashville, TN 37232.
We are studying a gene, p33, that is activated by several hormones, including insulin, in an effort to identify cis-acting insulin responsive transcription elements. In order to initiate these studies we have isolated several p33 genomic clones and have characterized the p33 gene. The gene is 14 kilobases (kb) long, and contains 4 exons. The first and second exons are separated by a 9 kb intron and the fourth exon contains 85% of the mature mRNA sequence. The primary transcript is processed into a 3.2 kb mature poly(A)$^+$ RNA which has a 270 nucleotide (nt) non-coding region followed by a 1377 nt open reading frame. This open reading frame is sufficient to code for a protein of the expected size. We have investigated the hormonal regulation of this gene in H4IIE cells, a permanent cell line derived from a rat hepatoma. Physiologic concentrations of insulin (5 nM) induce an 8-10 fold increase in mRNAP^{33} levels after 60 min, and dexamethasone (500 nM) increases mRNAP^{33} levels by 12-14 fold in 1 hour and by 20 fold at 3 hours. Nuclear run-on transcription assays demonstrate that these hormonal responses are due to changes in the rate of transcription of the p33 gene. The rate of transcription is enhanced in a specific and concentration dependent manner by insulin and dexamethasone. There is a 10 fold increase in transcription after 1 hour of insulin treatment (5 nM) and an 18 fold increase after 1 hour of dexamethasone treatment (500 nM). We are presently characterizing the 5'-flanking DNA of this gene in order to investigate the mechanism of the transcriptional regulation.

O 552 ANALYSIS OF A cDNA CLONE ENCODING THE TAT GENE OF VISNA VIRUS: TWO REGULATORY PROTEINS IDENTIFIED. Jennifer L. Davis and Janice E. Clements, Johns Hopkins University, Baltimore, MD. 21205. Visna virus is a lentivirus of sheep which causes a chronic, progressive disease involving the brain, lungs and lymph nodes. Restricted viral gene expression in vivo involves complex interactions between viral gene products, cell differentiation factors and host immunological factors. Prior studies in our laboratory have shown that the virus encodes trans-acting factor(s) which stimulate viral promoter driven gene expression. We report here the isolation of a cDNA clone (1436 nt long) that encodes at least one trans-acting protein. This clone corresponds to a 1.8kb mRNA which we had previously identified and characterized in Visna virus infected cells. Nucleotide sequence analysis of the cDNA clone shows that the transcript is quadruply spliced and, in one case, utilizes a non-consensus splice acceptor in the pol region of the genome. The message contains sequences derived from the 5' terminus of the genome, the 3' end of pol, the entire S open reading frame (which had previously been postulated to encode a regulatory function) and the 3' end of env. This multiply spliced structure contains two non-overlapping open reading frames (ORFs). The first ORF is derived entirely from the genomic S ORF and could encode a 94 amino acid polypeptide. The second ORF contains sequences from the 5' end of env joined to downstream sequences and could encode a 167 amino acid polypeptide. We have used this clone in a coupled in vitro transcription/translation system and shown that two distinct viral proteins are expressed (apparent molecular weight 18kD and 21kD, respectively) and correspond to the above ORFs. The regulatory effect of each protein and its contribution to the trans-activation of the viral promoter will be presented.

O 553 INDUCIBLE, AH RECEPTOR-DEPENDENT PROTEIN-DNA INTERACTIONS AT A DIOXIN-RESPONSIVE ENHANCER UPSTREAM OF THE CYTOCHROME P_1-450 GENE, Michael S. Denison, Joan M. Fisher and James P. Whitlock, Jr., Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305.
In mouse hepatoma cells, 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) activates transcription of the cytochrome $P_1$450 gene. The Ah receptor, together with cis-acting dioxin-responsive genomic elements, mediate the transcriptional response. We have identified a third cis-acting TCDD inducible enhancer within the 5' flanking region of the cytochrome $P_1$450 gene. This region, like the previously identified TCDD-inducible enhancers, requires TCDD:receptor complexes for its function. We have utilized gel retardation assays to study protein-DNA interactions at the TCDD-responsive enhancer. Nuclear extracts contain a protein that binds to the enhancer in a TCDD-inducible and sequence-specific fashion. The generation of the enhancer-binding protein occurs rapidly in response to TCDD, exhibits TCDD dose-dependence, and does not require ongoing protein synthesis. Nuclear extracts from a variant cell line defective in nuclear accumulation of TCDD:receptor complexes fail to form the specific DNA-protein complexes. Our results imply that the TCDD-inducible binding represents an interaction between the TCDD:receptor complex and the TCDD-responsive enhancer and demonstrates a relationship between in vitro protein-DNA binding and in vivo function.

O 554 HOMEODOMAIN CONTAINING PROTEINS CAN BIND COOPERATIVELY TO TANDEM REPEATS OF A SPECIFIC PALINDROMIC SEQUENCE. Claude Desplan, Jim Theis, Judy Kassis & Patrick O'Farrell, Dept. of Biochem, U.C.S.F., San Francisco CA, 94143.

In previous work, we have confirmed early suggestions that the homeodomain (HD) functions as a sequence specific DNA binding domain. Our recent experiments sugggest that the binding activity has several interesting properties: The engrailed (en) HD expressed in bacteria recognizes a specific set of sequences in the regulatory regions of the en and fushi tarazu (ftz) genes. These sequences share homology and are conserved in a distantly related species of Drosophila, D. virilis. The consensus sequence for the en protein, TCAATTAAATGPu, is nearly palindromic. The HD can also bind to synthetic versions of this consensus, or to a perfect palindrome, suggesting that the HD could bind as a dimer. Footprint analysis shows that the affinity of the protein for a tandem array of synthetic sequences is much higher than for a single copy, suggesting cooperative binding. The ftz protein has a class I HD, where one of the aminoacids in the putative recognition helix is different from en class II HD. Nonetheless, ftz and en HD's have closely related specificities; both bind to the consensus sequence but they have differing relative affinities to various sequences closely related to the consensus. This observation suggests that the various HD containing proteins could compete for binding to similar sequences. Furthermore, the closely related HD-containing proteins might interact with each other through heterodimerization and/or cooperativity, to recognize specific arrangments of sites in the regulatory regions of target genes.

O 555 IDENTIFICATION OF A DNA SEQUENCE THAT CONFERS NEGATIVE AUTOREGULATION OF IMMEDIATE EARLY GENE 3 FROM HERPES SIMPLEX VIRUS TYPE I. Joseph DiDonato & Mark Muller. Department of Molecular Genetics, Ohio State Univ. Columbus, OH 43210

We report fine mapping of sequences responsible for negative regulation of immediate early (IE) gene 3 of HSV-1 by its own gene product and we have correlated down regulation of the gene with its ability to form a specific protein-DNA complex in vitro. Previous work in this lab has shown that the IE gene 3 product, ICP4 forms a specific DNA-protein complex (the "A" complex) with sequences in the -10 to +3 region of the IE gene 3 (Muller, J. Virol. 61:858-865, 1987) suggesting that IE3 is autoregulated. We proposed that IE3 is negatively regulated and that the responder sequence from (-10 to +3) is a key site in down regulation. To test this, we disrupted the core sequence of the responder site by deleting 2 bp and then inserted the mutated sequence into the IE3 5' regulatory sequence (from -331 to +27). The wild type (pWT-GH) or deletion mutant (pD2-GH) constructions of the IE3 regulatory region were fused to a reporter gene (the human growth hormone or hGH gene) and then evaluated by transient expression assays. Expression of hGH in pWT-GH or pD2-GH was inducible to the same degree by the 65 K trans inducing factor ($65K_{tif}$). In contrast, co-transfection experiments revealed that ICP4 decreased expression of pWT-GH by a factor or 2 or more; however, in the deletion mutant (pD2-GH), ICP4 resulted in a less substantial decrease (about 10-20%) in expression of the reporter gene. The deletion had a clear effect on the ability of ICP4 to down regulate the reporter gene. We further demonstrated that the deletion mutant was defective in its ability to enter into a functional "A" complex. Finally, we have inserted the responder sequence (-17 to +27 of IE3) into a variety of other test promoters and have shown that this sequence unambiguously confers the ability of the promoter fragment to enter into a DNA-protein complex containing ICP4. The effects of this responder sequence on activity of these promoters in the presence and absence of ICP4 is currently under investigation. These findings are significant since this is the first demonstration of a negative regulatory control sequence in HSV-1 that has been directly correlated with a specific DNA-protein complex.

O 556 CYS- AND TRANS-ACTING ELEMENTS INVOLVED IN THYROID SPECIFIC EXPRESSION OF THE THYROGLOBULIN PROMOTER, Roberto Di Lauro, Donato Civitareale, Lina Ghibelli, Renata Lonigro and Alison Sinclair, European Molecular Biology Laboratory, 6900 Heidelberg, FRG.

A fragment of DNA, 170 base pairs long, containing the transcription start site of the rat thyroglobulin gene is able to function as a promoter only in a differentiated thyroid cell line (FRTL-5) which expresses the endogenous thyroglobulin gene. Protein binding studies have shown that a FRTL-5 specific nuclear protein recognizes at least two sites in this region, one centered at -70 and the other at -115. Two classes of mutants have been constructed. In the first class one of the two binding sites (the one closest to the transcription initiation site) has been replaced with the binding sites of several ubiquitous transcription factors, in the attempt to transform the promoter from tissue specific to constitutive. The second class aimed at inactivating the binding sites by replacing the recognition sequence with a random sequence. The analysis of these mutants by protein binding and in vivo expression may clarify the role of the FRTL-5 specific protein in the thyroid specific expression of the protein. In addition the first class of mutants could give information on the specificity of interaction, if any, of transcription factors.

O 557 **REGULATION OF MAMMALIAN ALCOHOL DEHYDROGENASE GENES.**
Howard J. Edenberg, Lucinda Carr, Ke Zhang, Mang Yu. Indiana University School of Medicine, Indianapolis, IN 46223.

The alcohol dehydrogenase (ADH) genes are expressed at high levels in adult mammalian liver. We have cloned both the human *ADH2*[3] gene and the closely related mouse *Adh-1* gene; there are several conserved sequence elements in the 5' regions. Initial studies fusing 5' regions of the mouse *Adh-1* gene to the reporter gene CAT reveal that gene expression is tissue-specific: strong in hepatoma cells and weak in CV-1 cells. The portion of the 5' region implicated in gene regulation by these CAT assays is bound by nuclear protein(s) from mouse liver extracts, as detected by gel mobility shift assays. The first intron of the mouse *Adh-1* gene contains a 288 bp alternating purine-pyrimidine sequence that has some characteristics of an enhancer; interestingly, a second mouse strain that is missing 101 bp of this region expresses only half as much ADH (protein or mRNA) in liver. Thus we have evidence for the location of sequences important to regulation of ADH gene expression, and for the existence of regulatory molecules that recognize these sequences.

O 558 ANTI-TOPOISOMERASE I IgG in NUCLEI OF CHIRONOMUS SALIVARY GLAND CELLS LEADS TO A BLOCK OF DNA TRANSCRIPTION, Endre Egyházi[1] and Egon Durban[2], [1]Dept. of Histology, Karolinska Institutet, Stockholm, Sweden, and [2]Baylor College of Medicine, Dept. of Pharmacology, Houston, Tx 77030, U.S.A.

Purified topoisomerase I anti-topoisomerase I IgG was microinjected into nuclei of Chironomus salivary gland cells and the effect on DNA transcription was investigated. Synthesis of nucleolar preribosomal 38 S RNA by RNA polymerase I and of chromosomal Balbiani ring RNA by RNA polymerase II was inhibited by about 80 %. The distribution of topoisomerase I within the polytene chromosome IV was observed by immunofluorescent staining with antitopoisomerase I IgG. The enzyme seemed preferentially associated with the highly active Balbiani rings. The size of the puffs appeared to be correlated with the intensity of staining. The inhibitory action of anti-topoisomerase I IgG could be reversed by the addition of exogenous topoisomerase I. The microinjection of topoisomerase I alone stimulated the DNA transcription by about 40 %. The pattern of inhibition of growing nascent Balbiani ring chains after microinjection of anti-topoisomerase I IgG indicates that the transcription process is interrupted at the level of chain elongation. The highly decondensed state of active Balbiani ring chromatin, however, remains unaffected after injection of topoisomerase I antibodies. These data are consistent with the interpretation that topoisomerase I is an essential component in the transcriptional process but not in the maintenance of the decondensed state of active chromatin.

O 559 TRANSFECTION AND EXPRESION OF RIBOSOMAL DNA IN TRYPANOSOMA BRUCEI,
Joslane Eid and Barbara Sollner-Webb,
The Johns Hopkins University School of Medicine, Baltimore MD 21205.

We have succeeded in introducing plasmid DNA into Trypanosoma brucei by electrophoration. Radiolabeled pBR322 is efficiently taken up (15%) upon giving a 1 kilovolt electric shock. When transfecting the DNA containing a T. brucei ribosomal region that includes the 5' end of the rRNA gene, transcription could be detected by dot blots and S1 nuclease protection assays under conditions specific for probe hybridization to RNA. Similar expression did not occur when the ribosomal region was deleted from the plasmid. This expression system was optimized for electric shock strength, concentration of transfecting DNA, etc. Trypanosomes exhibit many interesting and novel aspects of gene expresion that this technique should allow us to unravel. (PNAS 84:7812, 1987)

O 560 INHIBITION OF GC-BOX-DEPENDENT PROMOTER FUNCTION BY THE ANTI-CANCER DRUG CISPLATIN,
Gregory L. Evans and Jay D. Gralla,Department of Chemistry and Biochemistry and the Molecular Biology Institute,University of California,Los Angeles,CA. 90024.
The anti-cancer drug cisplatin covalently modifies GG dinucleotides preferentially in mammalian cell DNA. GG dinucleotides are prominent in GC-box DNA sequences,which form the core binding site for the human transcription factor Spl and which are important transcriptional control elements for many genes involved in growth control. We have determined the effect of cisplatin on the activity of a series of eukaryotic promoters in transient expression experiments in CV-1 monkey cells. All of the GC-box-dependent promoters(HIV,SV40 early,and human Harvey ras)we tested were 10-30 fold more sensitive to inhibition by cisplatin than most of the GC-box-independent promoters(adenovirus 2 major late,E3,and IVa2 promoters)we tested. Another GC-box-independent,very strong promoter,RSV, was as sensitive as the GC-box-dependent promoters. We are currently pursuing experiments to determine more directly the role of GC-box DNA sequences and promoter strength in these phenomena. Also,we are attempting to correlate the effects of cisplatin on individual promoters with its effects on bulk cellular transcription and DNA replication so as to evaluate the importance of promoter effects in cisplatin's mechanism of action as an anti-cancer drug.

O 561 TRANSCRIPTIONAL REGULATION OF THE ADULT DROSOPHILA ADH GENES,
Dean A. Falb and Tom Maniatis, Harvard University, Cambridge, MA 02138.
The promoter of the Drosophila mulleri Adh-2 gene and the distal promoter of the Drosophila melanogaster Adh gene are both activated in a tissue specific manner in the adult fly. We have localized tissue specific enhancer elements in the 5' flanking regions of each of these genes through P-element transformation experiments. Sequence comparisons between the D. mulleri and D. melanogaster enhancer elements reveal a 35/39 base pair similarity. We find that nuclear extracts prepared from adult nurse cells and from whole adult flies contain a factor that binds specifically to the common sequence element within these enhancers. Work is in progress to purify and characterize this factor with the objective of understanding its role in the tissue specific expression of Adh genes.

O 562 TRANSCRIPTIONAL CONTROL OF CELL CYCLE-REGULATED GENES,
Peggy J. Farnham, University of Wisconsin, Madison, WI 53706
Recent studies have suggested that altered transcription of growth-related genes can lead to neoplasia. We believe that understanding the mechanisms responsible for the normal cell cycle regulation of growth-related genes will be an important step in the identification of the molecular events associated with the loss of proliferation control in cancer cells. Towards this goal, my laboratory is studying three genes which are transcriptionally regulated in specific stages of the eukaryotic cell cycle: dihydrofolate reductase (DHFR), carbamyl phosphate synthetase-aspartate transcarbamoylase-dihydroorotase (CAD), and the c-myc proto-oncogene. We have developed in vitro transcription systems from both human and rodent cells which direct transcription from the normal in vivo initiation sites of these genes. We have prepared transcription extracts from cells in different stages of proliferation and differentiation in order to identify the DNA sequences and protein factors which are involved in transcriptional responses to changes in the growth state of the cell. A comparison of the different sequences and protein factors utilized by the DHFR, CAD, and c-myc genes will be presented.

O 563 SPECIFIC TRANS-ACTING FACTORS INTERACT WITH THE cAMP-RESPONSIVE AND TISSUE-SPECIFIC ENHANCERS OF THE GLYCOPROTEIN HORMONE α-SUBUNIT GENE.

Louis H. Ferland and Pamela L. Mellon, Regulatory Biology Laboratory, The Salk Institute, La Jolla, CA .

The single α-subunit of the glycoprotein hormones is expressed as a component of four hormones (LH, FSH, TSH and CG), each of which contains a distinct β-subunit, is expressed in a distinct cell type and is differently regulated by other hormones. Thus, the regulation of the α-subunit gene is quite complex. In earlier studies (Delegeane, Ferland and Mellon, MCB, 7:3994-4002, 1987), we identified regions of the 5'-flanking DNA which are involved in the tissue-specific and cAMP-regulated expression of this gene, as well as DNA-binding factors which recognize these sites. The cAMP-responsive element (CRE; -111 to -146) consists of a contiguous, direct, 18 bp repeat, with a core sequence found in the CREs of several other genes, while the tissue-specific element (TSE), located just upstream of the CRE, is a unique element whose activity requires the proximity of a functional CRE (see abstract by Delegeane and Mellon in this volume). In the present studies, we used 5' and 3' truncations of the DNA fragment of interest and mutations in the CRE to show that the factors that interact with these two elements are distinct and bind independently to their respective sites. We also report the observation, with extracts prepared from an expressing cell line (JEG-3), of 3 additional footprints farther upstream, one of which (-202 to -220) is not generated by extracts prepared from non-expressing cells (CV-1). In addition, a similar CRE in the oncogene *fos* (centered at -60) is protected by the same JEG-3 extracts. This footprint can be competed by the α-subunit or the somatostatin CREs, suggesting that the CREs from these 3 genes may interact with the same factor or with closely related ones.

O 564 EVIDENCE FOR DIFFUSION LIMITED KINETICS OF OPEN COMPLEX FORMATION BETWEEN σ^{32}-RNA POLYMERASE HOLOENZYME AND THE groE PROMOTER

Matthew A. Fisher (1), Deborah Cowing(2), Carol A. Gross(2), and M. Thomas Record,Jr.(1), (1)Departments of Chemistry and Biochemistry and (2)Department of Bacteriology, University of Wisconsin, Madison, WI. 53706

σ^{32}-RNA polymerase holoenzyme is essential for the recognition of promoters involved in the heat shock response in E. coli. We have investigated the kinetics of open complex formation and stability at the *groE* promoter with the nitrocellulose filter assay and quantitative DNase footprinting. At 37^0 C, 75 mM NaCl, 3 mM $MgCl_2$, 25mM Na Hepes, pH 7.5, we find that the value of k_a, the second order association rate constant for open complex formation, is approximately $(2\text{-}4)\times10^8$ M^{-1} sec^{-1}. This is within the range expected for a diffusion limited reaction between these two biopolymers. Consistent with this model is the weak temperature dependence of k_a and the insensitivity of k_a to salt concentration. The rate of association is the same for a 455bp DNA fragment and the linearized plasmid (4300 bp), which suggests that the reaction is controlled by three-dimensional diffusion. Both the filter-binding assay and DNase footprinting show that under similar buffer conditions (10 mM $MgCl_2$) the complexes have half lives greater than 45 minutes. Possible roles of the kinetics of open complex formation in the increase in transcription initiation at the *groE* promoter during the heat shock response will be discussed. Supported by NIH GM23467 and NIH GM36278

O 565 PURIFICATION AND CHARACTERIZATION OF HUMAN MITOCHONDRIAL TRANSCRIPTION FACTOR 1,

Robert P. Fisher and David A. Clayton, Stanford University, Stanford, CA 94305.

Mitochondrial transcription factor 1 (mtTF1) is a 25 kilodalton protein, purified from human KB cells, that binds to and activates both major promoters of human mtDNA. We have used in vitro transcriptional analyses, gel retardation assays and DNase I protection studies to define and characterize the mtTF1-promoter interaction. Using the gel retardation method, we have examined the dissociation kinetics of complexes formed by mtTF1 with a variety of wild-type and mutant promoter-containing DNA's, as well as with nonpromoter DNA. The results indicate that mtTF1 can activate transcription even through very weak or transient binding, albeit at reduced levels. Accurate initation, however, requires an additional promoter domain, not involved in mtTF1-binding, that encompasses the transcriptional start site. Methylation interference analysis of the strongest mtTF1-DNA interaction--with the light-strand promoter (LSP)--identifies contacts made by the protein in both the major and minor grooves of the DNA, over much of the length of the footprinted region. Only a subset of these contacts could be made at the heavy-strand promoter (HSP), which binds mtTF1 very weakly in vitro.

O 566 CIS AND TRANS ACTING FACTORS INVOLVED IN CENTROMERE FUNCTION IN YEAST, Jeff McGrew, Arlene Gaudet, Zhixiong Xiao and Molly Fitzgerald-Hayes, Department of Biochemistry and Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003.

Centromere DNA (CEN) from several yeast chromosomes has been sequenced and found to contain three conserved DNA elements (CDE I, CDE II and CDE III). We have made specific mutations in these conserved sequence elements and analyzed how these mutations affect the segregation of both plasmids and chromosomes in yeast. Single base pair mutations in a region of dyad symmetry, CDE III, can completely abolish centromere function during mitosis. Alterations in the other CDE sequences do not have as dramatic an effect on mitotic centromere function. Deletion mutations in the highly A+T rich CDE II cause premature chromosome disjunction at meiosis I, while mutations in CDE I appear to affect the meiotic segregation of plasmids, but not chromosomes.

Chromatin mapping and gel retardation studies have shown that specific proteins bind to CEN DNA. We have developed a genetic screen to identify genes in yeast which encode factors that interact with these sequences. Using this approach we have isolated four centromere related function (*crf1-4*) mutants that enhance the nondisjunction phenotype of chromosomes with mutant centromeres. One class of *crf* mutants has a nonspecific effect while a second class destabilizes chromosomes containing one type of CEN mutation significantly more than other types of centromere mutants. Conditionally lethal *crf* mutants will allow us to clone and characterize the gene products involved in kinetochore assembly and function.

O 567 TRANSCRIPTIONAL ACTIVATION IN YEAST BY THE DROSOPHILA *FUSHI TARAZU* POLYPEPTIDE, V. Danial Fitzpatrick and C. James Ingles, Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6.

Some of the polypeptides controlling embryonic development in Drosophila are DNA-binding transcriptional regulators. For some of these proteins such as *fushi tarazu* (*ftz*) the conserved homeobox domain appears to confer a sequence-specific binding activity on the *ftz* polypeptide; however, the actual mechanism of transcriptional regulation by *ftz* is unknown. To facilitate studies of the activities of the *ftz* protein we have expressed in yeast cells chimeras of *ftz* cDNA and the DNA binding domain of the yeast GAL4 gene. Using UAS_G-dependent ß-galactosidase expression as an assay of transcriptional activation, we have demonstrated that the GAL4/*ftz* chimeras, like GAL4 itself, activate transcription of the reporter gene. We have also observed that yeast cells transformed with the GAL4/*ftz* chimeric construct have a new phenotype; they agglutinate. Our results suggest that the *ftz* polypeptide can replace the transcriptional activation domains of GAL4. With *ftz* functioning in yeast cells, we now have a simplified system to delineate the mechanism of *ftz* action in transcription.

O 568 THE FATE OF THE VIRAL GENOME IN THE INTEGRATION PATHWAY OF POLYOMA VIRUS. David L. Hacker and Michele M. Fluck, Michigan State University, Microbiology Department, East Lansing, Mi. 48823-1101.

To determine the potential involvement of recombination in the generation of the concatameric species of the viral genome present in transformed cells, we have performed crosses with viral strains which differ in the presence of restriction endonuclease sites. Our results can be summarized as follows: Whereas no recombination can be detected in the replicating unintegrated pool of viral genomes detected during the first 3-5 days post infection, the viral genomes which become integrated in the transformed cells have undergone recombination at high frequency. These transformants with recombinant viral genomes have normal integration patterns. They often contain parental genomes in addition to the recombinant ones. In all the recombinant transformants examined multiple recombination events can be detected. An apparent gradient of recombination was discovered in the analysis of recombination events in 3 adjacent intervals. These results suggest that viral genomes in the process of integration interact with a highly recombinogenic machinery. In order to explore potential candidates for such a machinery, we have started to analyze the interaction of the viral genome with the host scaffold.

O 569 DNA-PROTEIN INTERACTIONS WITHIN A BIDIRECTIONAL PROMOTER, Marianne Frommer, Chiara Tyndall, Fujiko Watt and Peter Molloy, CSIRO Division of Molecular Biology, PO Box 184, North Ryde, Sydney 2113, Australia.

Associated with the 5' ends of a large number of vertebrate genes are segments of DNA known as CpG islands. These are regions, of around 1kb in length, which contain clustered CpG dinucleotides resulting from a very high G+C content and a lack of the characteristic CpG depletion of vertebrate genomes. CpG islands are found at the 5' ends of all widely-expressed genes and some tissue-specific genes (Gardiner-Garden and Frommer, 1987). In widely-expressed genes, the promoter region may lack a TATA box or any A+T-rich, TATA-like sequence. Furthermore, bidirectional transcription has been shown to occur from CpG islands associated with a few widely-expressed genes. One such CpG island from the mouse genome (HTF9) contains the TATA-less promoters for a pair of overlapping transcripts which are found in polyA $^+$RNA from all mouse tissues examined and which appear to code for housekeeping proteins (Lavia et al., 1987). We have used a DNaseI footprinting assay, with partially purified HeLa whole cell extracts (Samuels et al., 1982), to locate and characterise a number of strongly protected sites spanning the bidirectional promoter region of HTF9.

Gardiner-Garden, M. and Frommer, M. (1987). *J. Mol. Biol.* **196**, 261-282.
Lavia, P., Macleod, D. and Bird, A. (1987). *The EMBO Journal* **6**, 2773-2779.
Samuels, M., Fire, A. and Sharp, P.A. (1982). *J. Biol. Chem.* **257**, 14419-14427.

O 570 **PROTEIN FACTOR(S) WHICH REGULATE VIRUS-INDUCIBLE HUMAN β-INTERFERON GENE EXPRESSION** Takashi Fujita, Masaaki Miyamoto, Jun Sakakibara, Yoshiaki Sudo, Yoko Kimura and Tadatsugu Taniguchi, Inst. Mol Cell. Biol. Osaka Univ. Japan.
Human β-interferon (IFN-β) gene is transcriptionally activated by virus infection. In the absence of such stimulus, IFN-β gene expression is undetectable. We previously identified a DNA sequence which mediates the virally-induced signal (-117 to -39, from the cap site, **Cell, 41**, 484-496, 1985). Subsequent investigation revealed that the DNA sequence essentially consists of repetitive hexanucleotide units and that synthetic hexanucleotide repeats can function as virus inducible enhancers (**Cell, 49**, 357-367, 1987). In the present study, using gel retardation and DNAse I footprinting assays, we have identified nuclear protein(s) that specifically bind to the regulatory region of IFN-β gene. Point mutations which disrupt hexanucleotide units in natural 5' regulatory sequences reduce the viral inducibility as well as the protein binding capacity. By using various sequences and varying the number of haxanucleotide repeats, we have found that the inducibility of those repeats assayed in cells by DNA transfection, parallel with their binding ability to the regulatory protein(s). These results suggest that the protein(s) specifically mediate the function of the oligonucleotide repeats. Moreover we found evidence which suggest that the protein(s) function as negative and positive transcriptional factor in uninduced and induced cells, respectively.

O 571 **TWO TISSUE-SPECIFIC DNA-BINDING PROTEINS INTERACT WITH MOTIFS IN THE MOUSE β-GLOBIN GENE IVS2,** Deborah L. Galson and David E. Housman, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
Using an electrophoretic mobility shift assay, we have detected two novel sequence-specific DNA-binding proteins in nuclear extract from MEL cells that bind to motifs within the region of the tissue-specific DNase I hypersensitive site in the IVS2 of the mouse β-globin gene. The specific binding sites for these factors were delineated by both DNase I protection and methylation interference footprinting. Factor B1 binds specifically to two homologous sites, B1-A and B1-B, approximately 100-bp apart within the IVS2 on opposite strands. These two regions can interact with factor B1 independently. The proposed B1-binding site consensus is AAARRGGAARYG. Factor B2 binds to a site separated from the B1-A site by 5-bp. The proposed binding site for factor B2 is TCCTATCA. In addition, very close to site B1-B in β^M-globin is a close match (AGTTGCAT) to the octamer motif which is involved in the enhancer activity of a number of genes, including the immunoglobulin genes. We have established that this site can be bound by the ubiquitous factor NF-A1. Factor B1 is limited to cells of hematopoietic lineages and factor B2 is limited to cells of the erythroid lineage. The limited tissue distribution of the two novel factors, B1 and B2, and the location of the their binding sites, as well as that of factor NF-A1, suggest that the binding interactions of factors B1, B2, and NF-A1 may all have roles in the formation or maintenance of the tissue-specific DNaseI hypersensitive site in the mouse β-globin IVS2. Further characterization of these binding interactions and their significance in the control of β-globin gene expression is being pursued. We are also engaged in efforts to clone the genes for factors B1 and B2.

O 572 MOUSE B1 ALU SEQUENCE REQUIRED FOR ACTIVITY OF PROMOTION SENSITIVITY GENE,
Robert R. Garrity[1], John L. Seed[2] and Nancy H. Colburn[2], [1]BCDP, PRI, NCI-FCRF, Frederick, MD 21701-1013; [2]Cell Biology Section, LVC, NCI-FCRF, Frederick, MD 21701-1013.

Mouse JB6 promotion-sensitive variants (P^+) show a 100-fold greater sensitivity to promotion of neoplastic transformation by TPA than do their insensitive (P^-) counterparts. Two genes from P^+ cells have been identified that confer the promotion sensitive phenotype on P- recipient cells in DNA transfection assays. Sequence analysis of mouse pro-1 revealed elements for pol II transcription in one strand and pol III transcription in the complementary strand. Both transcription units share a B1 Alu domain. Northern analysis of cytoplasmic RNA from P^+ cells with single stranded RNA probes revealed low molecular weight RNAs hybridizing to probes recognizing only transcripts encoded by the pol III strand. These hybridization signals were not observed with Alu$^-$probes and appeared to represent a family of Alu-hybridizing transcripts. A unique pro-1 transcript may be masked within this signal. In addition, unique high molecular weight RNAs that hybridize to this same probe, and whose sizes are inconsistent with the 1 Kb pro-1 biologically active fragment, were absent in P^- clones and were expressed in P^+ and transformed variants. Bal 31 deletion analysis of pro-1 revealed dependence of its biological activity in transfection assays on the B1 Alu domain and its intragenic pol III promotors. These observations suggest the possibility that pro-1 may function as a mobile sequence controlled by pol III. A unique marker sequence has been inserted into the Alu domain of pro-1 in order to detect unique Alu transcripts from this gene.

O 573 TRANSCRIPTION OF THE MINUTE VIRUS OF MICE P4 PROMOTER IS DEPENDENT ON AN Sp-1 TYPE FACTOR IN HUMAN AND MURINE NUCLEAR EXTRACTS, Brian J. Gavin, Gyanendra Kumar, Jeong K. Ahn and David C. Ward, Yale University, New Haven, CT 06510

The genome of the prototype strain of Minute Virus of Mice (MVMp) contains two overlapping transcription units with promoters at map units 4 (P4) and 38 (P38). We have examined the transcriptional control of the P4 promoter in nuclear extracts of HeLa and mouse A9 cells, the normal host in culture. In vitro transcription of 5' deletion mutants in both extracts has shown that P4 promoter transcription is highly dependent on a 34 bp DraI fragment (-69 to -35), which contains homology to the consensus sequences for the Adeno E1A enhancer, the SV40 enhancer and the GC box recognition site for the Sp-1 transcription factor. Linker scanner and internal deletion mutants indicate that the important element within this region is the GC box motif. Specific protein binding to the 34 bp DraI fragment was demonstrated by competition gel mobility shift assays using homologous and heterologous fragments of MVM DNA. Furthermore, it was shown that competition with a heterologous GC box containing promoter (SV40) inhibited formation of the specific complex while a non-GC box containing promoter (Ad MLP) did not. Footprint analysis was used to directly demonstrate protein interaction with the GC box. Finally, UV-crosslinking of the 34 bp DraI fragment specifically labeled a protein of $\sim$ 95 kd in HeLa extract and 2 proteins of $\sim$ 98 and 130 kd in A9 extract. This is similar to the size reported for affinity purified HeLa Sp-1 (95 and 105 kd). These results suggest that the transcription of the MVM(p) P4 promoter in vitro is primarily dependent on the presence of a GC box motif and its interaction with an Sp-1 like protein.

O 574 A T CELL SPECIFIC ENHANCER IS LOCATED IN A DNaseI HYPERSENSITIVE AREA AT THE 3' END OF THE CD3-δ GENE. Katia Georgopoulos, Jannemieke Versteegen, Ethan Bier, Allan Maxam, and Cox Terhorst. Lab. of Mol. Immunology, Dana Farber Cancer Institute, Boston, MA 02115.

During intrathymic differentiation, the genes coding for the T cell receptor/CD3 complex are expressed in stages resulting in surface expression of competent receptors on the most mature T cells. The CD3-γ, δ, and ε proteins which are expressed intracellularly in the earliest detectable cells of T lineage form the core of the TCR/CD3 complex. In the present investigation aimed at understanding the tissue specific expression of the murine CD3-δ gene, a T cell specific DNaseI hypersensitive site was found 0.6kb downstream of the polyadenylation site of the gene. A 0.4 kb genomic fragment encompassing this hypersensitive site exhibited properties of a tissue-specific transcriptional enhancer which acts in a position- and orientation-independent manner. However, the activity of the promoter region was not found to be T cell restricted. Within the enhancer region, sequence motifs were found which are shared with the promoter of both the mouse and human CD3-δ gene. Sequence homologies were found between the T cell specific enhancer core element of the thymotropic viruses SL3 and RadLV and the CD3-δ enhancer region. Together these findings may identify the regulatory elements responsible for the exclusive expression of the CD3-δ gene in thymus derived lymphocytes. Given the fact that the CD3-γ gene, another member of the CD3 family, was mapped at 1 kb upstream of the CD3-δ gene and is transcribed from the opposite strand, it is possible that this 3' tissue-specific enhancer influences expression of both genes in a similar fashion. These findings suggest that gene expression of the members of the CD3 family may be governed by a common regulatory mechanism . The interaction and binding of cis-acting DNA elements present in the CD3-δ enhancer region and in the CD3-γ δ shared 5' region with tissue specific nuclear factors and furtermore their functional role in transcription is currently studied .

O 575 CIS-TRANS INTERACTIONS IN EPSTEIN-BARR VIRUS LATENT GENE TRANSCRIPTION, David Ghosh, Mary Hummel, and Elliott Kieff. Harvard University, Boston, MA 02115, and University of Chicago, Chicago, IL 60637.

By use of the in vivo CAT transient expression system, we have mapped multiple functional positive and negative cis-regulatory elements which modulate the activity of two latent gene promoters in the Epstein-Barr Virus genome. By use of the in vitro gel-retardation assay, a number of sequences in and around these regions have been identified which interact with sequence-specific DNA-binding proteins. One such sequence forms more than one DNA-protein complex, and in vivo competition experiments suggest that this sequence may be involved in both positive and negative types of control. This sequence may be involved in the differential expression of this particular latent gene promoter at different stages of the Epstein-Barr Virus life cycle. Other sequence-specific interactions occur at regions that probably function as constitutive cis- elements in EBV-positive B-lymphocytes.

O 576 DETAILED DNA CONTACT ANALYSIS OF A YEAST TRANSCRIPTIONAL ACTIVATOR, TYBF, Anurag Goel and Ronald E. Pearlman, York University, Toronto, Canada, M3J 1P3.

We have recently demonstrated that Ty-mediated activation of adjacent gene expression involves binding of a trans-acting factor to cis regulatory sequences in the 5' end of the yeast transposable elements Ty917(467) and Ty917(480). A single bp transition (G/C - T/A) at position +612 that drastically reduces adjacent gene expression in vivo also eliminates sequence-specific binding in vitro and in vivo (Goel, Feaver and Pearlman, submitted). The precise TyBF binding sequence has been determined by methylation interference analysis. We have further characterized this binding by "in situ" dimethyl sulphate protection experiments using native polyacrylamide gels. We have also determined the DNA backbone phosphate contacts made by TyBF using ethylation interference analysis. Taken together, our results indicate that unlike other eukaryotic transcriptional activators, TyBF binds rather extensively on both faces of the DNA helix. Further characterization and purification of TyBF are in progress.

O 577 Tn5 INSERTION MUTAGENESIS OF THE HSV TRANSCRIPTION ACTIVATING GENE PRODUCTS ICP0 AND ICP4: IDENTIFICATION OF DOMAINS INVOLVED IN INDEPENDENT AND SYNERGISTIC TRANS-ACTIVATION. William F. Goins, Peter C. Weber, M. Levine and J.C. Glorioso, University of Michigan Medical School, Ann Arbor, MI

Herpes simplex virus encodes five immediate early gene products. Among these, ICP is the essential major transactivator of viral gene expression during infection while ICP0 augments the activity of ICP4. In transient gene expression assays, ICP0 and ICP4, individually induce expression from herpesvirus promoters. In cotransfection assays ICP0 and ICP4 show 10-100 fold enhancement of promoter activity over the level observed with each immediate early gene alone. Panels of chain terminating Tn5 insertion mutants in each of these genes were tested independently and in combination in transient transfection assays for their ability to transactivate expression of the chloramphenicol acetyl transferase gene under control of either a typical delayed early or late virus gene promoter. The results confirmed that the carboxy termini of both gene products are required for transcription activation. Further, it was established that the amino terminus of ICP0 was associated with the synergistic effect on the transcription induction. The synergistic domain of ICP0 was localized to the second exon and lacked transcription inducing activity independent of ICP4. These mutant gene products are currently being tested for their role in virus reactivation from latent HSV infections of mouse ganglia.

O 578 MULTIPLE FORMS OF TFIIIA CONTROL THE DIFFERENTIAL EXPRESSION OF THE XENOPUS 5S RNA GENE FAMILIES. Jeronimo Blanco, Larry Millstein, Mona Razik and Joel Gottesfeld, Research Institute of Scripps Clinic, La Jolla, CA 92037.
We have previously shown that S-150 extracts prepared from mature oocytes or mid-blastula stage embryos of the frog Xenopus exhibit the differential transcription of the 5S RNA gene families observed in vivo in developing embryos. Inactive oocyte-type genes, as well as active somatic-type genes, are complexed with the 5S gene specific transcription factor TFIIIA in vitro. Oocyte-type gene transcription can be rescued in the S-150 by the addition of crude extracts from oocyte nuclei or immature oocytes. Protein fractionacton studies have revealed two forms of TFIIIA in the immature oocyte extract. The immature oocyte form of TFIIIA binds to the internal control regions of both oocyte and somatic 5S genes and stimulates transcription from both genes while TFIIIA derived from mature oocytes only acts as a positive transcription factor for the somatic-type genes. High level 5S gene transcription in the S-150 also requires a TFIIIC fraction from immature oocytes. These results suggest that the differential regulation of the 5S gene families observed in developing embryos may be related to postsynthetic modification states of TFIIIA.

O 579 TRANSMISSION OF BASAL AND REGULATORY SIGNALS IN A HUMAN hsp70 PROMOTER. John M. Greene and Robert E. Kingston. Department of Molecular Biology, Massachusetts General Hospital, Boston MA 02114 and Department of Genetics, Harvard Medical School. We have used a genetic approach to explore interactions between five promoter elements that are responsible for basal and heat regulated transcription of a human hsp70 gene. Four of these elements lie within 110 bases of the hsp70 transcription start site: a CCAATC box in inverted orientation at -67, a GC-rich element strongly resembling an SP1 binding site at -49, a TATA box at -28, and a heat shock element (HSE) at -106. Analysis of mutant promoters containing various combinations of these four elements demonstrates that they function independently in a multiplicative manner to stimulate transcription: the strength of any promoter containing more than one mutation can be accurately predicted by multiplying the effects of each individual mutation. Our data imply that these four elements all alter the same rate-limiting step of transcription, and show that proper levels of heat-induced transcripts from this promoter require not only the HSE, but the three proximal basal elements as well. A distal element, consisting of sequences between -1250 and -120, stimulates basal expression of this promoter five to ten-fold in human cell lines. We demonstrate that, in contrast to the HSE, this distal element functions only when the TATA element is intact. Thus, while the proximal elements can each directly and independently stimulate expression from this promoter, the distal element requires a TATA element to transmit its effect. We suggest that a general function of the mammalian TATA element is to mediate action of distal signals. Work is in progress to determine if function of the HSE can be made dependent upon a proximal element(s) by translocation to a distal location.

O 580 **IDENTIFICATION OF DNA SEQUENCES THAT MEDIATE TRANSCRIPTIONAL REPRESSION OF THE Mo-MuLV LTR IN EMBRYONAL CARCINOMA CELLS.** **Manuel Grez, Marion Ziegler and Wolfram Ostertag, Heinrich-Pette-Institut, Martinistr. 52, 2000 Hamburg 20, Federal Republic of Germany.**
Mouse preimplantation embryos and embryonal carcinoma cells are non-permissive for retrovirus transcription. Several lines of evidence suggest that the primary block in retroviral gene expression lies within the enhancer elements of the viral LTR. We have isolated and characterized retroviral mutants that are expressed in EC cells. Common feature to all of our mutants are alterations in or around the enhancer elements. Recombinants, constructed using the LTR of Mo-MuLV and one of our mutants (PCMV), were tested in a transient expression system. The exchange of a 150 bp fragment upstream from position -150 in the Mo-MuLV LTR with PCMV sequences restored expression of the Mo-MuLV LTR in EC cells. We have used this 150 bp fragment (Nhe I to Xba I) as a binding target for cellular factor(s) present in nuclear extracts obtained from PCC4 cells. We detected one factor that binds efficiently to the Mo-MuLV LTR but weakly to the corresponding sequences in PCMV. This factor is present only in undifferentiated cells and can be competed efficiently by Mo-MuLV sequences but not by PCMV sequences. Functional analysis suggests that the binding of this factor to the Mo-MuLV LTR represents the primary block of retroviral gene expression in EC cells.

O 581 STRUCTURE AND TRANSCRIPTIONAL REGULATION OF int-2; A GENE WHICH ENCODES A PROTEIN RELATED TO BASIC FIBROBLAST GROWTH FACTOR, Daniel Grinberg, Rosalind Smith, Jane Thurlow, Gordon Peters and Clive Dickson, Imperial Cancer Research Fund Laboratories, London, England.

Int-2 is a common proviral integration locus for the mouse mammary tumor virus in virally induced mammary tumors. Expression of this gene has been detected during early embryogenesis but as yet not found in adult mouse tissues, except when activated by the insertion of a provirus in adjacent DNA. From the sequence of cDNA's prepared from tumor RNA, an open reading of 27,000 daltons was predicted, which shows homology to the family of oncogene - growth factors typified by basic fibroblast growth factor. To examine the normal transcription of this gene we have used embryonal carcinoma cell lines which express four classes of int-2 RNA of 2.9, 2.7, 1.8 and 1.6 kb; similar in size to those identified in embryos and tumors. The structure of these transcripts has been established from the sequence of cDNA's and extensive RNA protection experiments. The results indicate two promoter regions initiating RNA at heterologous start sites. One promoter region (P2) is highly GC-rich and harbours several potential SP1 binding sites. In contrast the other promoter (P1) is further upstream and shows little resemblance to other known promoter regions. The RNA initiated from P1 continues to form a small exon (1a) which is spliced into the middle of the first exon initiated at P2. Additional complexity arises through usage of two distinct polyadenylation sites. Treatment of F9 cells with retinoic acid and dibutyryl cAMP appears to induce only RNA from the P1 promoter (2.7 and 1.6 kb classes), while similar treatment of PCC4 cells leads to an apparent rapid induction of all four RNA classes. We are currently examining the expression of int-2 transcripts in EC cells using nuclear run-off assays to establish the mechanism of the apparent induction, and using DNase protection and gel retardation assays to determine potential binding sites for transcription factors. To functionally identify important upstream control regions, we are employing transient transfection assays with portions of the int-2 promoter region linked to a marker gene.

O 582 TRANSCRIPTION FACTOR-DNA INTERACTIONS IN THE ENHANCER OF THE RETROVIRUS SL3-3, Anders Thornell, Bengt Hallberg, Pia Nilsson and Thomas Grundström, University of Umeå, Umeå, Sweden.

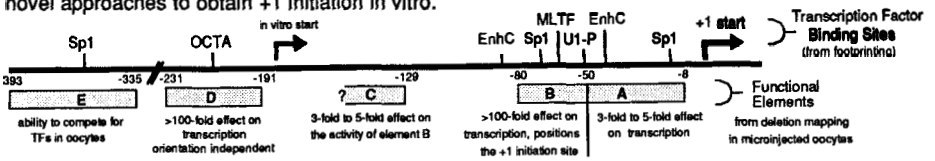
The mouse retrovirus SL3-3 has an enhancer preferentially active in T lymphocytes. We have identified DNA motifs essential *in vivo* for this differential enhancer activity in T lymphocytes and for the lower activity in other cell lines. The enhancer was found to contain multiple binding sites for SL3-3 enhancer factor 1 (SEF1). SEF1 proteins were found in much higher amounts in T lymphocytes and in lymphoid organs than in other differentiated cell lines and tissue types studied, and mutation of SEF1 binding sites affected transcription *in vivo* selectively in T lymphocytes. SEF1 was shown to have a structurally distinct DNA binding domain linked by a protease sensitive hinge region to another domain(s). The nucleotide sequence specificity of SEF1 binding, SEF1 purification data, and properties of SEF1 will be presented. Another identified DNA motif of the enhancer was found to bind nuclear factor 1 (NF-1). A mutant analysis of the nucleotide sequence specificity of NF1 binding to the SL3-3 enhancer sequence will be presented. Also the interactions of the glucocorticoid receptor and other proteins with the SL3-3 enhancer will be discussed.

O 583 IDENTIFICATION AND CHARACTERIZATION OF A SILENCER REGULATING CELL-TYPE SPECIFIC EXPRESSION OF THE RAT GROWTH HORMONE GENE (rGH). Sylvain L. Guérin and David D. Moore, Dept. Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

The cell-type specific expression of the rat growth hormone gene (rGH) is regulated, in part, by cell-type specific repression, which prevents transcription in all cells but those of the anterior lobe of the pituitary gland. We have mapped a negative regulatory sequence about 300 bp upstream from the transcription initiation site of the rGH gene, to a minimal 85 bp long fragment. This sequence, designated the rGH silencer, functions in an orientation-independent manner, but appears to be very sensitive to any modification of its position relative to the promoter region. Recognition of the silencer site by a trans-acting regulatory protein was suggested by in-vivo competition experiments and by the specific gel retardation of a silencer probe from -238 to -357 using crude nuclear extracts prepared from non-expressing L-cell fibroblasts. Site-specific contacts of the binding protein in the rGH silencer region were precisely located in a 15 bp segment from -303 to -318. Sequence comparison between this area of the silencer sequence and an area implicated in similar negative regulation of the human retinol binding protein gene shows a region of high similarity. Oligonucleotides containing this rGH silencer recognition sequence were shown to significantly reduce the level of CAT expression when inserted upstream of the minimal rGH promoter region in a CAT expression vector. The silencer binding factor is present in both pituitary and non-pituitary cells, suggesting either the presence of additional elements or the modification of the trans-acting silencer factor itself. In addition, transient transfections of plasmid derivatives in which the characterized rGH silencer sequence was deleted strongly suggest the presence of another silencer element located between positions -357 to -526. We believe that the mechanism of negative regulation of cell-type specific gene expression defined for rGH will represent an important, general aspect of tissue-specific regulation of gene expression.

O 584 Transcription and Footprinting of the Human U1 snRNA Gene. Samuel I. Gunderson, Mark W. Knuth, Thomas H. Steinberg, James T. Murphy 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 MLTF, Sp1, OCTA, enhancer core, AP-1, and CBF. Most of these sites correspond to functional domains in the U1 promoter that are required for efficient transcription in injected Xenopus oocytes. Footprints from partially purified HeLa nuclear extracts have been obtained for Sp1, MLTF, OCTA, enhancer core as well as for two U snRNA conserved sites. We are currently purifying these factors. To determine which if any of these factors are involved in U1 transcription we are developing an in vitro transcription system. However, to date, proper "+1" initiation in vitro does not exist for any of the snRNA genes (except for Pol III snRNAs and sea urchin U1 RNA). This lack of an in vitro system has hampered progress in understanding snRNA transcription. We are currently exploring novel approaches to obtain +1 initiation in vitro.



O 585 MINIMAL DNA SEQUENCE REQUIREMENTS FOR MULTIHORMONE REGULATION OF TRANSCRIPTION
John Ham, Paul Webb, Axel Thomson, Maurice Needham, Cathy Emmas and Malcolm Parker.
Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England.

Steroid Response Elements (SRE) are enhancers which are activated upon binding of steroid receptor. We have characterized the SRE in the LTR of Mouse Mammary Tumour Virus (MMTV) which responds to glucocorticoid, progestin and androgen but not oestrogen (Parker et al., J. Cell. Biochem., in press). Progesterone and glucocorticoid receptor bind to two regions of the MMTV SRE which together contain four imperfect inverted repeats of the sequence TGTTCT. We have synthesized oligonucleotides containing these sequences and inserted them upstream of the Herpes simplex TK promoter linked to the CAT gene. As little as 15 nucleotides is sufficient for progestin, glucocorticoid and androgen induction and the oligonucleotides confer a multihormone response in both orientations and at different distances from the TK mRNA cap site. TK CAT constructs containing single oligonucleotides for each of the four different TGTTCT palindromes in the MMTV LTR were compared. The results indicated that the relative hormone inductions mediated by each oligonucleotide are different suggesting that progesterone, glucocorticoid and androgen receptor have different affinities for each sequence.

TK promoter sequences from either position -112 to -53 or -112 to -39 in relation to the cap site were deleted from a construct containing a single oligonucleotide. In the resulting plasmids (pΔ-112 - 53 and pΔ-112 - 39) there are no TK upstream promoter elements and the centre of the TGTTCT palindrome is either 38 or 23 bases upstream of the TK TATA box. In the case of pΔ-112 - 39 the level of transcription in the absence of hormone was reduced but significant inductions by progestin, glucocorticoid and androgen were observed suggesting that a single receptor binding site and TATA box element contain sufficient sequence information for multihormone regulation of transcription. pΔ-112 - 53 showed much lower hormone inductions suggesting that the interaction between the response sequence and the TATA box may depend on the distance between the two elements and / or their relative position on the helix. The interaction is being studied further in vivo and in vitro.

O 586 THE MAPPING OF PHOSPHORYLATION SITES WITHIN SUBUNIT IIo OF MAMMALIAN RNA POLYMERASE II, Joyce R. Hamaguchi and Michael E. Dahmus, University of California, Davis, CA 95616.

The largest subunit of the transcriptionally active form of RNA polymerase II is extensively phosphorylated. **The objectives of these studies are to map the *in vivo* sites** of phosphorylation within subunit IIo and to relate these results to the specificity of phosphorylation catalyzed by purified protein kinases.

The *in vivo* phosphorylation of the HeLa cell RNA polymerase II is confined to the C-terminal **domain of subunit IIo. Subunit IIo is the highly phosphorylated form of subunit** IIa. ***In vitro*** **phosphorylation by casein kinases I and II also occurs predominantly within** the C-terminal domain. **Casein kinase I phosphorylates subunit IIa at multiple sites** whereas casein kinase II phosphorylates subunit IIa at only 1-2 sites.

Subunit IIo, phosphorylated *in vivo* or *in vitro* with casein kinase I, yield similar phosphopeptide maps upon digestion with clostripain. **These results suggest that** casein kinase I may be involved in the phosphorylation of RNA **polymerase II *in vivo*.**

Casein kinase II phosphorylates serine at position **1928.** This is supported by observations that: a) phosphopeptide maps produced by limited proteolytic digestion are consistent with phosphorylation at ser-1928, b) a synthetic peptide, identical in sequence to the C-terminal 11 residues of subunit IIa and containing ser-1928 is an active substrate for casein kinase II and c) ser-1928 is flanked by acidic residues and is similar in sequence to a synthetic peptide previously shown to be an effective substrate for casein kinase II.

O 587 TISSUE SPECIFIC EXPRESSION OF THE MAJOR HISTOCOMPATIBILITY COMPLEX Qa REGION CLASS I PROTEIN Q10 Diane E. Handy, Peter Burke+, Keiko Ozato+, and John E. Coligan. Biological Resources Branch, NIAID and +Lab of Developmental and Molecular Immunity, NICHD, NIH, Bethesda, MD 20892.

Mouse class I genes of the K or D region are expressed in all adult tissues except brain whereas genes from the Qa region have limited tissue expression. Q10 is a nonpolymorphic class I gene that maps to the Qa region and is reportedly expressed in fetal liver, yolk sac and adult liver. Sequences 5′ of the start of transcription of Q10 are homologous to class I regulatory sequences (CRE) that are important for the expression of classical (H-2) class I genes. Nuclear extracts from LH8 or L cells have three factors that interact with the CRE of H-2L^d: one binds to the direct repeat (region II), one to the small region of dyad symmetry (region I) and one to the larger region of dyad symmetry (region III) that includes the smaller inverted repeat. Only region II of the CRE from Q10 binds to a factor in nuclear extracts. Binding of this factor is inhibited by either L^d or Q10 derived oligonucleotides. Similar results were obtained for tissue nuclear extracts in that liver and other tissues contain a region II binding factor for both Q10 and L^d whereas additional factors bind only to the CRE of L^d. Sequence comparisons show that nucleotide differences in Q10 disrupt the region of dyad symmetry in the CRE and may account for the observed differences between Q10 and other class I genes. Thus, lack of expression of Q10 in cells other than the liver may be related to the inability to bind these additional factors, whereas expression of Q10 in the liver may involve DNA/protein interactions in regions other than the CRE.

O 588 THE INITIATION OF TRANSCRIPTION FROM SV40 MINICHROMOSOMES IN VITRO. Susan Batson, Catherine Heath, Mark Samuels, and Ulla Hansen. Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, 02115.

Experiments have been initiated to study the initiation of transcription from SV40 minichromosomes in vitro, in order to understand the role of chromatin in the regulation of gene expression. SV40 minichromosomes are isolated from CV-1 cells 48 hours post-infection, and purified by sucrose gradient sedimentation. The minichromosomes are transcribed in the presence of α-amanitin, to inhibit elongation of endogenous polymerases, and an α-amanitin resistant RNA polymerase II. All resulting transcripts are therefore newly initiated in vitro. With SV40 minichromosome as templates for transcription reactions in vitro, the pattern of transcripts generated is very similar to the pattern of mRNA observed in vivo at 48 hours post-infection in SV40 infected cells, but quite distinct from transcripts initiated on deproteinized SV40 DNA templates in vitro. This pattern of transcription from the minichromosomes is differentially stimulated by the addition of the SV40 specific transcription factors Sp1 or LSF. The early-early promoter is stimulated by the addition of Sp1, while the late and late-early promoters are stimulated by the addition of LSF. A low level of transcription however, appears to be unaffected by the addition of Sp1, and is thought to be due to the presence of endogenous transcription factors bound to the isolated minichromosomes. The population of minichromosomes capable of initiating transcription in vitro appears to be much larger than the population of minichromosomes transcribing in vivo, as determined by transcription of different minichromosome fractions across the sucrose gradient.

O 589 MODIFICATION OF CELLULAR SEQUENCE-SPECIFIC DNA BINDING ACTIVITIES BY ADENOVIRUS INFECTION, Stephen Hardy and Thomas Shenk, Department of Molecular Biology, Princeton University, Princeton, NJ 08544.

We are studying activities which bind to Adenovirus type 2 transcriptional control regions. One activity, which we will call alpha, binds to sites in control regions of the E1A and E2 genes. There are two sites in each control region. Nuclear extracts from normal cells yield two classes of alpha; these may be distinguished by the dissociation rate of their complexes with DNA. Each class of alpha is bound to DNA by the same factor. These activities are separable and stable. Infection of cells with adenovirus produces a modified version of alpha. The infection modified version recognizes the sites in E2 but not E1A as a substrate for cooperative binding. The evidence for this is that a unique complex is formed on E2 DNA containing two sites but not on a two site E1A DNA in a DNA band shift assay. The infection specific complex has a quadratic dependence upon protein concentration for binding to E2 DNA. The geometry of the sites in E2 is important, since inverting one of the sites will eliminate the infection specific complex. By comparing the normal arrangement of sites in E2 to a substrate with an inverted site we have developed an assay for this cooperative form. Infection with an E1A lacking virus at high multiplicity fails to produce the cooperative complex. Three cell types which express E1A proteins also fail to produce the modified complex. These data suggest a dependence on E1A expression which may not be direct. The involvement of other early genes and the time course of appearence of the infection specific alpha will be discussed.

O 590 MODULATION OF THE EXPRESSION OF A HUMAN HSP 70 /CAT CONSTRUCT USING A SYNTHETIC DOUBLE STRANDED REGULATORY ELEMENT AS A COMPETITOR, Annick Harel-Bellan, Douglas Ferris, Anna. T. Brini and William. L. Farrar. LMI, BRMP, NCI-FCRF, Frederick, MD 21701.
The 5' regulatory region of human HSP 70, a member of the heat shock protein family, contains a sequence highly homologous to the yeast Heat Shock Element (HSE), and necessary for heat shock induction of the gene. A synthetic double stranded 14 bp human HSP 70 HSE sequence was used to modulate the expression of a HSP 70 5' flanking region / CAT chimeric construct (pHBCAT) electroporated into a human lymphoid cell line, Jurkat (JK). The synthetic HSE, fulfilled two criteria: 1) it was shown in vitro to bind proteins from a JK cell nuclear extract in a sequence specific manner, as assessed by retardation assays; 2) when electroporated into the cells, significant amount of ^{32}P 5' end labeled synthetic HSE remained intact for up to 2 days, as assessed by gel electrophoresis, a portion of it showing an atypic migration behaviour, which interestingly seemed to be due to protein binding in vivo. In preliminary experiments, co-electroporation of the synthetic HSE surprisingly resulted in a 5-6 fold enhancement of the HSP 70/CAT hybrid construct basal level expression. The data indicate that an isolated regulatory sequence can capture DNA binding proteins in vivo and modulate transcription of an artificially introduced plasmid.

O 591 THE E2 *trans*-ACTIVATOR OF BPV-1 CONSISTS OF A C-TERMINAL DNA BINDING AND AN N-TERMINAL EFFECTOR DOMAIN THAT CAN FUNCTION INDEPENDENTLY. T.H.Haugen, T.P.Cripe, P.G.Schmid, and L.P.Turek, VAMC Iowa City, IA 52242.
The bovine papilloma virus (BPV)-1 E2 ORF encodes *trans*-acting proteins that bind to palindromic $ACC(N)_6GGT$ (*E2P*) cores upstream of the early viral promoter, P_{89}. A "short E2" repressor, corresponding to the C-terminal 62% of the E2 ORF, competitively inhibits transcriptional activation of the P_{89} promoter by the full-length E2 *trans*-activator gene product. In addition, molecular constructions expressing the full-length E2 ORF also *trans*-activate cotransfected heterologous promoters in transient assays, similar to the adenoviral E1A proteins. To map the E2 peptide domains needed for specific *trans*-activation of the *E2P cis* elements (E2 function A) and for *trans*-activation of unrelated promoters (function B), we have analyzed multiple mutants in the full-length BPV-1 E2 ORF. Premature termination (C-terminal deletion) E2 mutants did not *trans*-activate the P_{89} promoter (function A), and did not bind to *E2P* elements in gel shift experiments. In contrast, mutants expressing the N-terminal half of the E2 ORF still *trans*-activated heterologous promoters (function B). Both functions A and B were inactivated by N-terminal deletions, internal in-frame deletions, and most amino acid insertions in the N-terminal half of the E2 ORF. Mutants expressing intact C-terminal peptides as well as the "short E2" repressor construction inhibited P_{89} *trans*-activation by wild-type E2, and retained *E2P* binding. The full-length E2 *trans*-activator thus resembles the GCN4 protein of yeast: an N-terminal *trans*-activating effector domain which can also function independently, and a C-terminal DNA binding domain that can block binding of the full-length E2 *trans*-activator to the specific *cis* target cores, and is utilized by the C-terminal "short E2" repressor.

O 592 STUDIES ON TRANSCRIPTION INITIATION IN VITRO BY RNA POLYMERASE II, Diane Hawley, Mary Gilbert, Stefan Hermann, James LeBlanc, and Ann Seifried, University of Oregon, Eugene, OR 97403.
Our laboratory is investigating the mechanism of transcription initiation at the adenovirus major late promoter in a system reconstituted from partially fractionated HeLa nuclear extracts. We have separated the overall initiation reaction in vitro into several discrete functional steps by localizing precisely the steps at which known inhibitors of RNA polymerase II act. We are now using these inhibitors to define the DNA sequences and proteins required for these functional steps. Recently, we have developed another novel method for studying events occurring at initiation and during the initial phases of transcript elongation. We have constructed a series of DNA templates that allow transcription to proceed different defined distances in the absence of one ribonucleoside triphosphate. We are using these templates to investigate the processes of elongation, promoter clearance, and reinitiation of transcription in our in vitro system.

O 593 HUMAN PLACENTAL ALKALINE PHOSPHATASE (PAP) AS A REPORTER GENE FOR STUDIES OF TRANSCRIPTION REGULATION, Paula Henthorn, Paula Zervos, Harry Harris and Tom Kadesch, University of Pennsylvania, Philadelphia, PA 19104.

We describe the use of the human placental alkaline phosphatase (PAP) gene as a reporter gene for studies of transcription regulation in mammalian cells. The enzyme assay for PAP is quantitative, very rapid, and extremely inexpensive, using non-radioactive substrates and no specialized equipment or reagents. The enzyme's resistance to heat allows its activity to be easily distinguished from other endogenous alkaline phosphatase activities in mammalian cells. Moreover, the enzyme's highly restricted expression in mammalian tissues (it is primarily found in the placentas of higher primates only) is reflected by the virtual absence of activity in the mammalian tissue culture cell lines we have tested thus far. When a plasmid carrying the PAP gene under control of the SV40 early promoter (pSV2Apap) is transfected into a variety of cells, placental alkaline phosphatase activity can readily be detected using whole cell suspensions or cell extracts. Cotransfections of cells with pSV2Apap and a related plasmid carrying the bacterial CAT gene (pSV2Acat, encoding chloramphenicol acetyltransferase) indicate that expression of these two reporter genes is detected with roughly the same sensitivity. However, unlike the CAT gene, the PAP gene produces a relatively stable messenger RNA, allowing transcription start sites to be readily monitored.

O 594 **GROWTH-RELATED CHANGES IN POLY(I)-POLY(C) INDUCED TRANSCRIPTION OF THE HUMAN β_1-INTERFERON GENE,** Judith A. Hewitt and Paul O.P. Ts'o, Johns Hopkins University, Baltimore, MD 21205.

Poly(I)-poly(C) treatment of fibroblasts in culture results in a rapid induction of β_1-interferon (β_1-IFN) mRNA transcription. Several factors have been shown to interact with regulatory elements of the β_1-IFN gene before and after induction in a highly inducible cell line (Zinn & Maniatis, Cell, 45, 611-618, 1986). In our studies, HT1080 human fibrosarcoma cells were tested for IFN inducibility by biological assay of IFN activity, Northern blot hybridization, and mRNA *in situ* hybridization. Various parameters of cell culture were tested for their effects on inducibility, namely, seeding density, days in culture and medium replenishment. Very dense cultures ($>5\times10^5$ cells/cm^2) were found by all assays to be highly inducible (250 copies IFN mRNA per cell), while lower density cell cultures ($<10^5$ cells/cm^2) and cultures with shorter preinduction growth periods ($\leq$4 days) were not inducible (<5 copies IFN mRNA per cell). We have shown that IFN induction is not restricted to any stage of the cell cycle, nor is acquisition of inducibility affected by ploidy. This system, which provides uninducible and inducible physiological states in cell growth, enables us to study the differential expression and action of the factors responsible for IFN induction. (Supported by: DOE # DE-AC02-76-EV03280, NIEHS # ES03819, and NIH Training Grant # CA09110).

O 595 TRANSCRIPTIONAL REGULATION OF HEPATIC GLUCOKINASE BY THYROID HORMONES, Wolfgang Höppner and Hans J. Seitz, Institut f. Physiol. Chemie, Universität Hamburg, FRG.

Glucokinase (GK) (EC 2.7.1.2), rate limiting enzyme of glucose storage and utilization in the liver, is under multihormonal control: refeeding a carbohydrate rich diet glucokinase synthesis and activity is increased, while starvation or glucagon have a suppressive effect on both parameters. In the present study the effect of carbohydrate refeeding in the absence and presence of thyroid and glucocorticoid hormones on GK mRNA amount and rate of transcription was investigated using a specific cDNA.

Results: In intact rats carbohydrate refeeding resulted without delay in a more than 20fold increase in GK transcription and level of specific mRNA, followed by corresponding increases in GK synthesis and activity. In the absence of either thyroid or glucocorticoid hormones the response at the mRNA level is reduced by about 50%. Vice versa injection of thyroid hormones into hypothyroid rats restores GK mRNA to normal levels. This effect is the consequence of a parallel increase in thyroid hormone mediated rate of GK gene transcription.

To summarize, refeeding a carbohydrate rich diet rapidly increases GK gene transcription. This effect is enhanced by about factor 2 by thyroid hormones. The rate of GK transcription correlates with the cytosolic levels of GK mRNA. Thus alterations in GK gene expression predominantly reflect transcriptional events.

O 597 DNA-BOUND FOS AND MYC PROTEINS ACTIVATE TRANSCRIPTION IN YEAST,
Karen Lech, Kate Anderson, and Roger Brent, Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston,Mass. 02114

We have constructed genes encoding the DNA binding region of the bacterial LexA protein fused to the viral and cellular fos and myc oncogene products. The resultant LexA-Fos fusion proteins activated transcription in yeast. Transcription activation by these proteins was as strong as transcription activation by proteins native to yeast. The LexA-Myc fusion proteins also stimulated transcription, albeit much less strongly than the LexA-Fos fusion proteins. Transcription activation by all these fusion proteins showed an absolute dependence on binding to a LexA operator located upstream of the target genes. Transcription was not stimulated by native LexA or by proteins containing the DNA binding domain of LexA fused to bacteriophage 434 repressor or yeast MATα 2 protein. These results demonstrate that Fos and Myc proteins activate eukaryotic gene expression when they are bound to promoter DNA, and suggest that perhaps these proteins exert some of their effects because they stimulate transcription of cellular genes. We are currently using this transcriptional regulation phenotype in yeast to facilitate genetic analysis of the function of these proteins in higher organisms.

O 598 INDUCIBLE BINDING OF FACTORS TO INTERFERON CONSENSUS SEQUENCE OF MHC CLASS I GENES.
Yasuaki Shirayoshi, Peter Burke, *Ettore Appella and Keiko Ozato, NICHD and *NCI, NIH, Bethesda, MD, 20892.

The mouse H-2 L^d gene, like other MHC genes, is inducible by interferons (IFNs). The transcriptional regulation of the L^d gene by IFN-α/β partially depends on the 29-bp IFN consensus sequence (ICS) located in the promoter region. Nuclear extracts derived from T cell lymphoma cells and fibroblasts were assayed by gel retardation and methylation interference experiments for the presence of trans-acting proteins that bind to the ICS. One protein factor was detected in untreated cells. IFN treatment induced two other protein factors in less than one hour. Methylation interference and oligonucleotide competition experiments indicate that all three factors bind specifically to the core region of the ICS (AGTTTCACTTC). Specific mutations in the core region inhibit the binding of all three factors, and these mutations are able to abolish the IFN-response in vivo when introduced into CAT constructs. These results suggested that trans-acting proteins binding to the ICS are responsible for the IFN-inducibility of MHC class I genes. Purification of these factors is underway.

O 599 USE OF "TORSIONALLY TUNED PROBES" FOR QUANTITATION OF THE LEVEL OF UNRESTRAINED SUPERCOILING IN LIVING CELLS.
Richard R. Sinden, Tadeusz Kochel, Robert Brankamp, and Guoxing Zheng, University of Cincinnati, Cincinnati, OH 45267-0522.

One mechanism for regulating gene expression in prokaryotes is unquestionably through modulation of the level of negative supercoiling in DNA. Based on analogy with prokaryotes, and from several lines of evidence, it has been suggested that the expression of certain genes in eukaryotes is dependent on the introduction of unrestrained supercoils in DNA. While this laboratory has demonstrated unrestrained tension in prokaryotic DNA this topological state has yet to be unequivocally demonstrated in higher eukaryotes. Although it is clear that DNA in prokaryotic cells is supercoiled the actual level of unrestrained supercoils which represents the energy actually available for modulating gene expression is not yet well established; estimates range from σ<-0.025 to σ=-0.05. To approach this problem directly we have developed "torsionally tuned probes" to measure the level of unrestrained supercoiling *in vivo*. This approach, which is applicable in both prokaryotic and eukaryotic cells, is based on recent assays we have developed to quantitate the existence of both cruciforms and Z-DNA *in vivo* [Sinden, et al. (1983) Proc. Natl. Acad. Sci. USA 80, 1797; Sinden and Kochel (1987) Biochemistry 26, 1343]. We have constructed a series of palindromic DNA sequences (that can form cruciforms) and a series of Z-DNA forming sequences that undergo alternate secondary conformational transitions at different but precise levels of superhelical tension. Quantitation of Z-DNA *in vivo* has provided, to date, perhaps the most accurate estimate of the level of unrestrained torsional tension in *E. coli*; σ=-0.035 to -0.040. As expected, higher levels of negative supercoiling are observed in *E. coli* deficient in topoisomerase I. Application of these torsionally tuned probes in eukaryotic cells is in progress.

O 600 THE E.COLI LEXA REPRESSOR-OPERATOR SYSTEM WORKS IN MAMMALIAN CELLS, Gillian M. Smith, Karen A. Mileham, Susan E. Cooke, Sally J. Woolston, Helen K. George, Andrew D. Charles, William J. Brammar. Leicester University/ICI Joint Laboratory, Leicester, United Kingdom.

We have demonstrated the use of the *E.coli* LexA repressor-operator system to down-regulate gene expression in mouse cells. The LexA gene was placed downstream of the RSVLTR promoter with polyadenylation and splice signals from SV40. This expression unit was introduced into mouse L^{tk-} and C127 cells by calcium phosphate transfection and stable transfectants selected which expressed LexA protein. We have used the bacterial chloramphenicol acetyl transferase gene (CAT) as our reporter gene. Transcription from this gene was driven by the HSV *tk* promoter, into which we have introduced single or double inserts of synthetic LexA operator sequences in various positions throughout the promoter. Necessary 3' signals were from the HSV*tk* gene.

We have estimated repression by LexA by comparing the transient expression of CAT target constructs, after transfection into $LexA^+$ cells, with that in control cells not expressing LexA. We have observed up to 10-fold repression of CAT expression in $LexA^+$ cells.

O 601 PREPARATION AND PRELIMINARY X-RAY ANALYSIS OF CRYSTALS OF T7 RNA POLYMERASE, Rui Sousa, Eileen M. Lafer, and B.C. Wang, U. of Pittsburgh, Pittsburgh, PA 15260
After exhaustive screening of crystallization conditions of highly purified T7 RNA polymerase prepared in our laboratory from an overexpressing E. coli strain we have defined conditions which reproducibly give large single crystals of this enzyme. These crystals diffract to approximately 4 angstroms and contain 4 molecules per unit cell. The crystal is monoclinic with space group C_2 and cell dimensions A=125 Å, B=113 Å, C=139 Å, and α=98.5°. Determination of the structure of T7 RNA polymerase with these crystals appears feasible. Since no crystallographic studies of any DNA dependent RNA polymerases have been carried out to date, the structure of bacteriophage RNA polymerase would be of considerable interest to those interested in the field of protein-DNA interactions in general and transcription in particular.

O 602 EVIDENCE FOR A REPRESSOR CONTROLLING THE TRANSCRIPTION OF HLA CLASS I GENES IN SMALL CELLULAR LUNG CARCINOMA, Nico J. Stam, Frank van der Hoeven and Hidde L. Ploegh, The Netherlands Cancer Institute, Amsterdam, 1066 CX, The Netherlands.
Small Cellular Lung Carcinoma (SCLC) cells do not express HLA Class I genes, or do so in only minor amounts. Treatment of SCLC with gamma interferon results in induction of both Class I mRNA and protein. Measurements of rates of transcription in induced and uninduced SCLC suggest that the depletion of HLA Class I transcripts in uninduced cells is at the transcriptional level.
Transfection experiments with cloned HLA-A and -B genes into an HLA Class I negative SCLC resulted not only in the expression of the transfected genes, but in the expression of the endogenous HLA Class I genes as well. From these experiments we conclude the presence of a repressor molecule in SCLC, present in minor quantities, and responsible for the lack of transcription of HLA Class I genes.

O 603 TANDEM GENES ENCODING SIGMA FACTORS FOR CONSECUTIVE STEPS OF SPORULATION IN BACILLUS SUBTILIS, Patrick Stragier, Bruno Savelli and Céline Karmazyn-Campelli, Institut de Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France.

Sporulation in Bacilli is a simple model of differentiation induced by nutrient limitation. Two identical genomes issued from the last round of replication are segregated by an asymetrical septation in two adjacent and inequal compartments. The larger one engulfs the smaller one which becomes an intracellular organelle, the forespore. Different genes are coordinately expressed on the two chromosomes. The forespore progressively becomes endowed with multiple resistance properties and is released in the medium as a free spore.

As proposed by Losick and Pero, some of the spo genes are encoding new sigma factors that might play a crucial role by successively inducing the expression of new gene sets. Cloning and sequencing of the spoIIG region has revealed the presence of two adjacent genes encoding sigma factors controlling successive steps of development, spoIIGB (sigE) and spoIIIG (sigG). sigE codes for an inactive precursor of sigma-E (previously known as sigma-29) which is processed by the product of the first gene of the operon, spoIIGA, when the bacteria reach stage II. spoIIIG is an independent operon whose expression is switched on 2.5 h later than spoIIG. The respective role of sigma-E and sigma-G in the sporulation program will be discussed.

O 604 INFLUENCE OF A SPACER-tRNA ON TRANSCRIPTION, PAUSING AND PROCESSING OF THE RIBOSOMAL rrnB OPERON, Christof Szymkowiak and Rolf Wagner, Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Ihnestr. 73, D-1000 Berlin 33, West-Germany

In vitro transcription experiments with plasmids bearing the ribosomal rrnB operon with a truncated 16S RNA gene (a deletion of 1304 nucleotides) and several deletions in the spacer-tRNA gene show a remarkable difference in their transcription pattern. Two new RNase III cuts could be detected independent from the size of the deletion in the spacer-tRNA gene. Both cuts occur at the normal RNase III processing sites for the 16S RNA. There was no comparable RNase III cut without a deletion in the spacer-tRNA gene. These results were obtained by in vitro transcription assays using different FI-extracts from RNase III positiv and negativ strains and supplementation with purified RNase III. Interestingly the intensity of the RNase III cuts is strongly dependent on the superhelicity of the plasmid. Strong helicity of the plasmids favours the 3' processing site wereas weak helicity favours the 5' processing cut. From these results we infere that there is a defined order of processing in which processing of the spacer-tRNA direct the processing of 16S RNA.

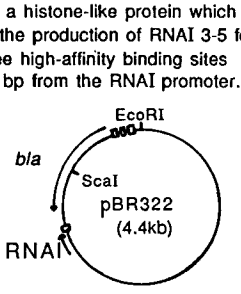
In vitro transcription of the whole ribosomal rrnB operon also show differences between a normal rrnB operon and operons with spacer-tRNA deletions. In the normal operon there is a termination site at position 300 of 16S RNA.

O 605 ENHANCER-LIKE EFFECTS OF PROTEIN-DNA INTERACTIONS IN A PROKARYOTIC, *in vitro*, TRANSCRIPTION SYSTEM

Bruce Teter, David J. Galas, Pierre Prentki,

Molecular Biology, University of Southern California, LosAngeles, CA 90089-1481.

Replication of pBR322 is controlled by antisense pairing of RNAI to the replication primer. The RNAI transcript is driven by a strong promoter which is stimulated by supercoiling. The major transcript is 106 nucleotides long and is terminated at a rho-independent terminator. We have been studying the effect of Integration Host Factor (IHF) on the transcription of RNAI *in vitro*. IHF is a histone-like protein which binds specifically and bends the DNA. Our experiments show that IHF enhances the production of RNAI 3-5 fold. The enhancement is dependent on supercoiling and on the presence of three high-affinity binding sites clustered between EcoRI and *bla* (figure). These clustered sites are >1200 bp from the RNAI promoter. A low-affinity site lies 10 bp downstream of the RNAI terminator. This level of enhancement is further increased when the EcoRI-ScaI fragment is tandemly repeated on pBR322. This "action at a distance" might be explained by several models (DNA looping, plectonemic wrapping, redistributed supercoil density). We are currently investigating the mechanism of the IHF-induced enhancement of RNAI production and will discuss our results in light of these models.



O 606 FOOTPRINTING OF TRANSCRIPTION INITIATION INTERMEDIATES AND ELONGATION COMPLEX IN lacUV5 WITH 1,10-PHENANTHROLINE-COPPER NUCLEASE. Theodore B. Thederahn and David S. Sigman, Molecular Biology Institute, UCLA , Los Angeles, CA. 90024. The 2:1 1,10-phenanthroline-copper complex is a nuclease that can be used to footprint DNA binding proteins including E. coli RNA polymerase. In addition to delineating the limits of the protein's binding site surrounding the promoter, the nuclease also produces a series of characteristic hypersensitive cleavage sites within the footprint that correspond to the region associated with the "transcription bubble" upon formation of the "open" complex. Upon addition of initiating nucleotides, these bands can be made to advance along the template strand in concert with transcription and thus act as direct probes of transcriptional processivity. By limiting the nucleotide complement, three initiation intermediates can be footprinted that correspond to mRNA production of four, nine and ten bases. The progression of the complex from the ninth to the tenth base induces a dramatic alteration in the interaction of the polymerase with the DNA. The number of observed hypersensitive bands decreases from a series of five to only two which are advanced by five bases along the template, even though there is only one nucleotide added. Since it previously has been observed that this is the step when the sigma factor is released, we believe that we have directly observed the transition from an initiation complex to an elongation complex. The utility of these processive footprints is demonstrated by examining the effects of the transcription initiation inhibitors daunomycin, actinomycin D, distamycin and proflavin on the initiation intermediates. We observe a direct correlation between the stability of the intermediate and its extent of transcription, reflected by decreasing sensitivity to the drugs, that culminates in an elongation complex that is insensitive to the effects of the drugs.

O 607 INTEGRATION HOST FACTOR (IHF) REPRESSES A CHLAMYDOMONAS CHLOROPLAST PROMOTER IN E. COLI, Robert J. Thompson and Gisela Mosig, Vanderbilt Univ., Nashville, TN 37235.
We are analyzing the control regions of a Chlamydomonas chloroplast promoter that we call P_A. Our previous results (Thompson and Mosig, Cell 48:281-287, 1987) have shown that apparent activity of this promoter is enhanced, both in the chloroplast and in E. coli, when cells are grown in the presence of sublethal doses of novobiocin, an inhibitor of E. coli DNA gyrase, of algal, and of pea chloroplast supercoiling type II topoisomerases. Our earlier results suggested that in situ activity of P_A is repressed by upstream silencers.

We show now that in E. coli, P_A is directly repressed by Integration Host Factor (IHF), suggesting that an analogous protein affects transcription within the chloroplast. In vivo, the himA 42 mutation affecting the α-subunit of E. coli IHF leads to an over-accumulation of P_A transcripts. This effect requires upstream chloroplast DNA sequences, indicating that it is not mediated at the level of RNA stability. In vitro, purified IHF, a generous gift from Howard Nash, protects a region overlapping P_A from DNAase and from methylation by DMS. An additional stronger IHF binding site is located ~100 bp upstream of P_A within one of the silencers. IHF retards formation of P_A-E. coli RNA polymerase open-complexes in vitro as determined by a gel-retardation assay. This inhibition occurs even if the upstream IHF site is deleted.

We interpret our results to mean that in E. coli, repression of P_A by IHF requires both binding of IHF to a site overlapping P_A and binding of one or more additional proteins, perhaps including IHF itself, to sequences upstream of P_A. We discuss possible mechanisms for this repression.

O 608 MULTIPLE ASYMMETRIC REPRESSOR BINDING SITES IN THE GENOME OF PHAGE P1, Mathias Velleman, Thorsten Heinzel, and Heinz Schuster, Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, Germany.

The P1 *c1* repressor gene was inserted into different expression vectors, the repressor protein was overproduced and purified to near homogeneity (1). By incubation of purified repressor with *Eco*RI-digested P1 DNA six repressor binding sites were identified in 5 of 26 *Eco*RI fragments. Binding sites were localized by the electrophoretic retardation of DNA fragment-repressor complexes and by DNase footprinting analysis. The repressor binding sites comprise a 17-bp-long consensus sequence (5') $_{\text{Pos.}}$ A_1T T G C_5T C T A A_{10}T A A A T $_{15}$T T (3') lacking symmetrical elements. Three of the six binding sites appear to be regulatory elements of known genes, whereas the function of the others is still unknown (2).

The repressor binding site or operator, Op72, of the P1 *ban* operon matches with the consensus sequence. Binding of RNA polymerase *in vitro* overlaps with this operator and is inhibited by P1 repressor as shown by electron microscopy (3). The mutant P1 *bac*, which renders *ban* expression constitutive, contains a single C/G → A/T bp exchange at position 5 of Op72. Additional *bac*-type mutants were isolated and the Op72 region sequenced. All but one of 27 mutants contain single bp exchanges in the highly conserved region (position 2 to 10) of the operator, and more repressor is required for the electrophoretic retardation of *bac*-type mutant DNA when compared to the corresponding wild-type fragment. A model of the interaction of P1 repressor and RNA polymerase with the operator Op72 is presented.

1. B. Dreiseikelmann, M. Velleman, and H. Schuster, *J. Biol. Chem.* **263**, (1988) in press
2. M. Velleman, B. Dreiseikelmann, and H. Schuster, *Proc. Natl. Acad. Sci.* (USA) **84**, 5570-5574 (1987)
3. R. Lurz, A. Heisig, M. Velleman, B. Dobrinski, and H. Schuster, *J. Biol. Chem.* **262**, (1987) in press

O 609 MULTIPLE PROTEIN-DNA INTERACTIONS WITHIN FUNCTIONAL ELEMENTS OF THE UROKINASE-TYPE PLASMINOGEN ACTIVATOR GENE. D. von der Ahe, D. Pearson and Y. Nagamine, Friedrich Miescher-Institut, P.O.Box 2543, CH-4002 Basel, Switzerland.

In cultured pig kidney epithelial cells, LLC-PK_1, urokinase-type plasminogen activator (uPA) mRNA is induced by a cAMP-elevating peptide hormone, calcitonin, and by tumor promoter phorbol esters (TPA). The induction does not require protein synthesis, suggesting that the activation of preexisting regulatory protein(s) is responsible for the enhanced uPA gene transcription. To understand the molecular mechanism of the transcriptional regulation by cAMP and TPA we have investigated the specific in vitro binding of nuclear proteins from LLC-PK_1 and several other cell lines to regulatory sequences of the uPA gene. We detected complex cis-acting elements recognized by multiple trans-acting regulatory proteins. These cis- and trans-acting elements are involved in controlling the basal level expression (TATA-, GC-, and CAAT-Boxes) and the regulation of the enhanced transcription by cAMP and TPA. Within 5000 bp upstream of the transcription start site we identified several cAMP and TPA-inducible regulatory sequences and their binding factors. In vitro competition experiments showed that there are cooperative effects among protein-DNA binding sites. We are currently analyzing the interplay between different cis elements and trans-acting proteins. Furthermore, we are purifying the regulatory proteins and characterize their biochemical activities.

O 610 PURIFIED HSF STIMULATES TRANSCRIPTION OF DROSOPHILA HSP 70 GENE IN XENOPUS OOCYTES, Barbara Walker, Susan Wilson, Igor Dawid, and Carl Wu, National Institutes of Health, Bethesda, Maryland 20895. Heat Shock Activator Protein, now commonly referred to as Heat Shock Factor (HSF), is a nuclear trans-acting factor which is induced to bind specifically to the regulatory sequences of heat shock promoters (HSEs) when cells are subjected to enviromental stress. We have purified HSF, a 110 kD protein, from Drosophila SL2 cells to greater than 95% homogeneity using a purification scheme which takes advantage of the protein's inducible DNA binding and its strong affinity for the heat shock concensus sequence CT_ GAA__CTT_AG. We have investigated the role of HSF in regulating the expression of Drosophila hsp 70 gene by in vivo assays using Xenopus oocytes. Oocytes were injected with either p122X14, which contains one complete copy of hsp 70, pHT 5'-186, a deletion mutant which contains 3 HSEs, or pHT5'-44, which contains only the TATA box. When oocytes injected with the complete gene are incubated at 19°C, the levels of specific hsp 70 transcription, assayed by primer extension, are just barely detectable. If the oocytes are heat shocked by incubating at 34°C, specific transcription is increased approximately 50 fold in oocytes injected with p122X14 or pHT5'-186, while the levels remain unchanged in oocytes injected with pHT5'-44, indicating that transcription is dependent both on an external stimulus and on the presence of the HSEs in the oocytes. When oocytes containing p122X14 are given a subsequent injection of purified HSF, specific hsp 70 transcription is stimulated approximately 30 fold in the absence of heat shock. Transcription in oocytes injected with either buffer or a control protein fraction remains at the very low basal level. (We thank Hugh Pelham for a gift of mutant hsp70 genes.)

O 611 SEQUENCES LINKED TO PROKARYOTIC PROMOTERS MODULATE THE EFFICIENCY OF DOWNSTREAM TERMINATION SITES. Alice Wieland and Michael Chamberlin, University of California, Berkeley, CA 94720

The efficiency of transcription termination at prokaryotic rho-independent terminators is widely believed to correlate with the ability to form an RNA stem-loop structure followed by a U-rich region in the transcript. However, we show that the efficiency of certain well-defined rho-independent terminators depends on the nature of the early transcribed region. Various changes in this region--as minor as a single base substitution--can reduce termination efficiency up to 20 fold at terminators as far as hundreds of nucleotides downstream. This effect is observed both in vivo and in in vitro reactions employing only purified E.coli RNA polymerase. Thus this effect cannot be attributed to antitermination factors separate from core RNA polymerase nor is it an artefact of in vitro transcription reaction conditions. Several lines of evidence indicate that the early transcribed sequences do not act by altering RNA secondary structure. Therefore, it appears that sequences the polymerase encounters during the transition from the initiation to the elongation phase of the transcription cycle influence its subsequent elongation properties, possibly by inducing modifications in polymerase analogous to those which the enzyme sustains at antitermination factor-dependent nut and qut sites. We are currently employing methods which allow the study of ternary transcription complexes paused at various positions throughout these transcription units to study the changes associated with the early elongation phase and to test models of how polymerase remembers the early transcribed region throughout elongation and how this memory affects termination efficiency.

O 612 TISSUE-SPECIFIC EXPRESSION OF THE RAT KALLIKREIN GENE FAMILY.
Debora R. Wines, James M. Brady, and Raymond J. MacDonald. Biochemistry Dept., University of Texas Southwestern Medical Center, Dallas, TX 75235

Glandular kallikreins are members of a highly related subfamily of the serine proteases and comprise a gene family of 12-20 members in the rat. Six unique, highly related rat kallikrein mRNAs with pairwise sequence identities ranging from 83-93% have been characterized. Two of these mRNAs encode the proteins true kallikrein and tonin, which function to process specific vasoactive peptides. The other four mRNAs appear to encode unique functional kallikrein-related enzymes. All six mRNAs are differentially expressed in a variety of tissues. Nine unique kallikrein-related genes (rGK1-9) have been isolated and characterized. True kallikrein and tonin are encoded by rGK1 and rGK2, respectively. The kallikrein-related P1 mRNA is encoded by rGK9. The genes rGK3 through 8 appear to be six new family members that have not been characterized at the mRNA level. The number, length, and boundaries of exons and introns in the rat kallikrein-related genes is conserved. These genes also appear to be tightly linked, as two genomic clones contain two genes separated by less than five thousand base pairs of DNA. Comparison of nucleotide sequences between kallikrein-related genes demonstrates striking sequence conservation in intron and 5′ flanking sequences as well as in coding regions. Despite high nucleotide sequence conservation in the proposed regulatory regions of these genes, they exhibit distinct patterns of expression. Functional studies of the DNA sequences necessary for the transcriptional regulation of the genes for true kallikrein and tonin are in progress. Comparisons between family members should identify discrete differences in gene regulatory sequences which result in unique patterns of tissue-specific gene expression.

O 613 PROTEIN-INDUCED DNA BENDING DURING TRANSCRIPTION INITIATION IN THE LAC OPERON
Sandra S. Zinkel and Donald M. Crothers, Yale University, New Haven, Ct. 06511

An isomeric set of DNA molecules has been constructed in which the phasing of a sequence-induced DNA bend relative to the regulatory region of the LAC operon has been varied over one helical turn. (Nature 328: 178-181 (1987).) The DNA bend enhances the sensitivity of our gel electrophoresis assay to the detection of protein-induced changes in the structure of the promoter DNA. We have used these molecules to study the changes in DNA structure induced by CAP and RNA polymerase that occur during transcription initiation. From the modulations in gel mobility of the various protein-DNA complexes due to phasing changes with respect to the sequence-induced bend, it appears that the DNA becomes progressively more bent as the initiation of transcription proceeds from open complex to a previously unresolved complex between open and initiated complexes. This new complex probably contains sigma factor and produces abortive transcripts of up to ten nucleotides in length. Upon incorporation of the eleventh nucleotide, the complex moves into the initiated form. At this point, the bend appears to return to the extent induced by CAP alone. Since the CAP-induced bend remains throughout transcription initiation, the bend energy does not seem to be used to open the promoter. Perhaps the bend serves a structural role in facilitating a protein-protein interaction between CAP and RNA polymerase.

O 700 NEGATIVE REGULATION OF PRIMARY B-CELL ACTIVATION AT THE IG RNA SPLICING/PROCESSING LEVEL, Una Chen, Basel Institute for Immunology, Basel, Switzerland.

Mouse primary B-cells can be activated to replicate for 1-2 cycles by anti-antigen receptor antibodies ($\alpha\mu$), but not to differentiate into Ig-secreting plasma cells. The differentiation event requires the help of both T-cells and A-cells or their lymphokines. B-cells can also be polyclonally activated both to proliferate and differentiate by bacterial lipopolysaccarides (LPS) via non-antigen receptor pathways. Activation of B- cells by means of LPS together with anti-receptor antibodies ($\alpha\mu$) leads to cell proliferation, yet the positive differentiation event to Ig secretion is very efficiently inhibited. Investigation of this anti-differentiation phenomenon at the molecular level has shown that there is a minor inhibition of Ig-transcription as measured by in vitro transcriptional run-on assay, transcriptional enhancer activity and gel retardation experiments. In situ hybridization experiments have demonstrated that every single B-cell is inhibited from μ-mRNA expression. The analysis of RNA expression at the cytoplasmic mRNA level has indicated that levels of secretory-μ-mRNA are affected first, and then those of membrane-μ-mRNA. Levels of K-chain and IgM J-chain mRNA are also subsequently decreased. The data suggest that the stage-specific processing factor(s) which are involved in the $\mu m \rightarrow \mu s$ switch is (are) the primary target(s) of anti-μ inhibition, and that there are trans-factors involving inter-chromosomal gene communication ($\mu \rightarrow K$ J chain gene). Analysis of RNA metabolism by H^3-uridine pulsing and DRB inhibition methods have shown that the level of inhibition is not at the RNA synthesis, degradation, transport, nor half-life level. Northern blot analysis of nuclear RNA precursors has strongly suggested that the negative regulation may be due to blockage of the RNA splicing/processing machinery, which slows down the RNA splicing rate and decreases the accumulation of RNA precursors. With the help of T-cell lymphokines, this negative effect could be partially reversed and multiple unprocessed RNA transcripts could be detected. These data imply that the regulatory event occurs during 5'-end capping, splicing and 3'- end cleavage/polyadenylation of the primary transcripts. This post transcriptional regulation affects exogenous as well as endogenous Ig gene expression. When a genomic plasmid is transfected and expressed in both sets of activated B-cells, the level of foreign Ig RNA detected in the LPS/un-treated B-cells is again less than that in the LPS treated B-cells, even though the 5'-end of the exogenous RNA is correctly initiated and capped as shown by S1 analysis. In summary, the interaction of anti-receptor antibodies with receptor delivers negative regulatory signals which affect the Ig RNA expression at the level of splicing/processing.

O 701 IDENTIFICATION OF NUCLEAR FACTORS THAT INTERACT WITH REGULATORY SEQUENCES WITHIN THE SOYBEAN HEAT SHOCK GENE *Gmhsp17.5E* PROMOTER. Eva Czarnecka, P. C. Fox and W. B. Gurley, Univ. of Florida, Gainesville, FL 32611. We have previously localized regulatory domains of the *Gmhsp17.5E* heat shock promoter of soybean by 5' and internal deletion analysis. Factors were found in crude nuclear extracts of soybean plumules that bind *in vitro* specifically to the *cis*-regulatory elements of *Gmhsp17.5E* promoter. Gel retardation assays and DNase I footprinting revealed a large number of interactions with the *Gmhsp17.5E* promoter proximal and distal domains consistent with observations that 179 nucleotides of the proximal domain contributed 50% of the promoter activity and the remaining 50% was due to the distal domain (-1175 to -180). The analysis was simplified by employing oligomer probes as well as by using partially fractionated extracts. We have identified seven to nine sites of DNA:protein interactions which correspond to regions previously shown by mutational analysis to be critical for activity. We have evidence for at least two discrete factors, heat shock transcription factor (HSTF) and AT binding factor (ATBF). The total binding activities for each of these factors increased in crude extracts prepared from heat shocked soybean plumules as compared to activity in control (28°C) extracts. Competition binding assays demonstrated that HSTF binding to the heat shock element consensus (HSE) was strongly dependent on the degree of homology to the *Drosophila* HSE and was distinct from the ATBF binding site.

O 702 LOCALIZATION 5' TO ZETA GLOBIN GENE OF BINDING SITES FOR TISSUE SPECIFIC AND DEVELOPMENTALLY STAGE SPECIFIC NUCLEAR PROTEINS PRESENT IN LEUKEMIA CELLS, C.Kane, Z.Howard, K.Purohit, and A. Deisseroth, M.D. Anderson Hospital, Houston, Texas, 77030.
The globin gene family exhibits differential expression of its members during the embryonic, fetal and adult stages of development. Gene transfer experiments in our own and other laboratories have suggested that nuclear transcriptional regulatory proteins specific for each stage of development govern the expression of these globin genes during development in man. In order to isolate and characterize such regulatory proteins, we first used nuclease hypersensitivity assays to identify binding sites for these proteins. We mapped two nuclease hypersensitivity sites specific for cells in which embryonic gene expression occurred 266 base pairs 5' to the start and 80 base pairs 3' to the start of exon I of the zeta globin gene. Three nuclear proteins bound to a 32 nucleotide oligomer in this region: one present in cells in which embryonic globin genes were expressed, one present in all erythroid cells tested, and one present predominantly in non-erythroid cells. We are currently attempting the isolation of these proteins by oligonucleotide affinity chromatography.

O 703 INTERACTION OF THE LexA REPRESSOR AND THE RecA PROTEIN WITH NATIVE AND CHEMICALLY MODIFIED DNA, Michèle Granger-Schnarr and Manfred Schnarr, Institut de Biologie Moléculaire et Cellulaire, CNRS, 15, rue Descartes, 67084 Strasbourg Cédex, France

The so-called "SOS system" in E.coli constitutes a regulatory network of about twenty unlinked genes which is under the control of two DNA-binding proteins: LexA, the common repressor of all these genes and the RecA protein that plays the role of an "inducer" in catalyzing the cleavage of LexA into two fragments. The affinity of LexA for its different operators varies over about two orders of magnitude from $3x10^7$ M^{-1} for the uvrA operator as determined in our laboratory (J.Mol.Biol. 1987,193,293-303) up to $5x10^9$ M^{-1} in the case of the umuDC operator.
The uvrA promoter/operator region allowed us to develop a new binding assay for copetitive transcription inhibitors like the LexA repressor. The assay is based on the measurement of abortive transcription kinetics with purified RNA polymerase in the presence and absence of LexA. This assay allowed us to determine DNA binding constants for the entire repressor and its more weakly binding N-terminal domain. We will further report results on the contacts between the LexA repressor and the backbone of the recA operator using both hydroxyl radical footprinting and ethylation interference mearurements.
The activated form of RecA is not only involved in the proteolytic cleavage of LexA, but also in SOS mutagenesis for a whole set of premutagenic lesions including AF- and AAF-adducts as well as apurinic sites (Mol.Gen.Genetics 1986,202,90-95). We will present in vitro results on the enhanced binding of RecA to AAF- and UV-modified double-stranded DNA.

O 704 CIS-ACTIVE ELEMENTS ASSOCIATED WITH THE cKI-RAS PROMOTER REGION, Eric K. Hoffman, Stephen P. Trusko and Donna L. George, University of Pennsylvania, Philadelphia, PA 19104.

From previous analysis of the 5' flanking region of the mouse cKi-ras proto-oncogene we identified a 700 base-pair (bp) fragment with promoter activity. This region is GC-rich (>82%) and contains a number of "GC" boxes, but lacks obvious CAAT- or TATA-box elements. To further define those structural features of the region which may play a role in controlling the expression of the cKi-ras gene, we constructed a series of 5'- and 3'-deletions of the 700 bp fragment. These DNA segments were placed upstream of the bacterial chloramphenicol acetyltransferase gene, the resulting constructs introduced into mouse NIH3T3 cells, and promoter activity examined by transient expression assays. We found that the step-wise deletion of DNA sequences resulted in a gradual loss of promoter activity consistent with the possibility that the promoter is comprised of multiple cis-active elements. We found no evidence for strong positive or negative control elements within this region. The experiments also identified a 160 bp fragment containing sequences important for promoter function. Electrophoretic gel mobility shift assays performed with the 160 bp fragment indicate that this segment does have the potential to bind nuclear protein(s). Sequences within this fragment are present within the promoter regions of several other "housekeeping" and "growth control" genes, and may define one or more elements important in regulating transcription among members of this class of growth-control genes.

O 705 PURIFICATION OF THE TRANSCRIPTION INITIATION FACTOR (TIF) FOR THE RIBOSOMAL RNA GENES IN THE EUKARYOTE ACANTHAMOEBA CASTELLANII. Laura M. Hoffman, Calvin T. Iida, and Marvin R. Paule, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523.

The components required for in vitro transcription of the ribosomal RNA genes from the eukaryote Acanthamoeba castellanii are being studied in our laboratory. The two required protein components are RNA Polymerase I (RNAP I), which provides the enzymatic activity for RNA synthesis, and Transcription Initiation Factor (TIF), which is responsible for directing RNAP I to the specific initiation site. RNAP I has previously been purified to homogeneity in our lab, while our recent efforts have been focussed on purification of TIF. The current purification scheme is a combination of conventional chromatography and more novel affinity chromatography. A side fraction from the phosphocellulose column in the RNAP I preparation is used as starting material. This material is purified through chromatography columns of DEAE-Cellulose and Green A Agarose, followed by a promoter DNA affinity column and a 5%-20% sucrose gradient centrifugation. SDS-PAGE of the purified material identifies a 150 kilodalton polypeptide. Ultraviolet light crosslinking experiments have also identified a DNA binding protein of 150 kd in transcriptionally active fractions. This data suggests that the 150 kd polypeptide is a major component of TIF, and may in fact be the only component of TIF.

O 706 COORDINATE REGULATION OF YEAST GLYCOLYTIC GENE EXPRESSION, Michael J. Holland, Janice P. Holland and Paul Brindle, Department of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616.

Efficient transcription of the enolase genes ENO1 and ENO2 as well as many other yeast glycolytic genes requires the product of the regulatory gene GCR1. Transcription of the enolase genes, for example, is reduced 50-fold in strains carrying a gcr1 deletion mutation. Complex upstream activation sites (UAS) which are required for gene expression have been mapped within the 5'-flanking sequences of ENO1 and ENO2. Interestingly, small deletion mutations within the UAS regions of each enolase gene have been identified which suppress the requirement for the GCR1 gene product (i.e. these mutant enolase genes are expressed at wild type levels in a strain carrying a gcr1 deletion mutation). This observation suggests that coordinate expression of the enolase genes is modulated by a repressor which binds to a site within the UAS regions of each gene. The GCR1 gene product, therefore, must interfere with repression. In order to identify the putative repressor, gel mobility shift assays were performed with a labeled DNA fragment containing the ENO2 UAS region and partially purified whole cell extracts prepared from a wild type strain and a gcr1 strain. Both extracts promote formation of discrete stable complexes with the DNA, however, the molecular weights of the complexes are distinctly different. DNase I footprinting and methylation interference assays show that the same protein/DNA interaction is responsible for both complexes suggesting that the GCR1-dependent difference in molecular weight of the complexes is due to additional protein/protein interactions. Most importantly, the DNA binding site for these complexes overlaps those sequences, which when deleted, render ENO2 transcription independent of GCR1. These observations suggest that the protein which binds specifically to ENO2 UAS sequences is the repressor predicted by the gcr1 suppressor mutations.

O 707 CIS AND TRANS ELEMENTS IN THE REGULATION OF PANCREATIC AMYLASE
Georgette Howard, Paul Keller, and Miriam Meisler Dept. of Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48109-0618

Interactions between nuclear trans-acting factors and specific fragments from the 5' flanking region of the Amy-2.2 gene are being analyzed. Previous work has demonstrated that the 5' flanking region between -208 and +19 is responsible for pancreas-specific expression as well as insulin regulation (Osborn et al, M.C.B. 7: 326 and unpublished observations). Two Amylase/ Elastase/CAT constructs were analyzed for expression in pancreatic cell lines AR42J and 266-6. Amylase fragments -208 to -82 and -172 to -110 were cloned upstream of the Elastase/ CAT hybrid gene pE1mCAT, which contains the pancreas-specific elastase region -205 to +22 (Hammer et al., M.C.B. 7:2956 and R. MacDonald, personal communication). The addition of the amylase fragments increased the expression of pE1mCAT by five-fold in the pancreatic cell lines. This enhancement was not observed in L cells. The amylase fragments thus appear to contain pancreas-specific enhancer activity. Band shift gels have been utilized to detect protein binding by the amylase enhancer. Nuclear extracts from pancreas of control and diabetic rats contain proteins which bind to these amylase fragments. These binding proteins are not present in nuclear extracts from spleen. Binding to the fragment -172 to -110 can be competed with the sequence -140 to -110, but not by the sequence -172 to -140. Further analysis and purification of the nuclear proteins by ion-exchange and affinity chromatography is in progress. The data suggests that the mouse amylase sequence -172 to -110 enhances transcription by the binding of pancreas-specific nuclear proteins.

O 708 TWO MECHANISMS OF STIMULATION OF SIMIAN VIRUS 40 LATE TRANSCRIPTION IN VITRO, BY BINDING CELLULAR FACTORS EITHER AT THE SV40 GC BOXES OR AT THE ENHANCERS.
Hui-Chuan Huang and Ulla Hansen, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115.

DNAase footprinting of the SV40 promoter region in the presence of various partially purified protein fractions that stimulate SV40 late gene transcription in vitro have revealed protection of several regions of the SV40 promoters, including the GC-box motifs in the 21 bp repeats and sequences within the 72 bp enhancers. By in vitro transcription assays, these partially purified fractions are devoid of Sp1, which stimulates transcription of SV40 early genes at the early-early promoter. The protected sites contained in the SV40 late stimulating preparations are: an AP1 binding site, an AP2 binding site, an octa protein binding site, and the GC-box motifs (boxes 1 through 3) which are different from the Sp1 high affinity binding sites. Three protein fractions from a Heparin Ultrogel column which all stimulate SV40 late transcription in vitro show different DNAase I footprinting patterns. One of the three protein fractions contains binding activity to the 21 bp repeat of the GC-rich region; this is LSF, which has been previously identified in our laboratory (Kim, et al, 1987, PNAS 84:6025). Two other transcription fractions contain proteins binding to the enhancer region but not to the GC boxes. Two independent mechanisms of regulating SV40 late gene transcription are hypothesized. One may involve stimulation of late gene transcription through binding to the GC boxes, and the other may involve stimulation through binding to the AP1 binding sites within the enhancer.

O 709 IDENTIFICATION OF NUCLEAR FACTORS THAT INTERACT WITH THE E1A-INDUCIBLE ADENOVIRUS E3 PROMOTER, Helen C. Hurst, Robert H. Jones and Nicholas C. Jones, Gene Regulation Laboratory, I.C.R.F., London, U.K.

We have identified four factors present in HeLa cell nuclei which bind to functionally important regions of the adenovirus E3 promoter. These four sites (BS1 to 4) are located between positions -7 to -33; -44 to -68; -81 to -103 and -154 to -183 relative to the E3 cap site. No qualitative or quantitative differences have been found in this binding pattern between nuclear extracts derived from uninfected or adenovirus infected HeLa cells. Competition experiments suggest that the factors binding to sites BS4 and BS3 are the previously identified nuclear proteins NF1 and AP1 respectively. The BS1 factor has not been well characterized, but preliminary mutational studies indicate that it may recognise the TATA box. The factor which binds to the BS2 site has been termed ATF and also interacts with other E1A inducible promoters and appears to be identical to the factor that binds to the cAMP responsive element of somatostatin. Interestingly, we have also found factors with identical DNA binding specificities to both AP1 and ATF in extracts prepared from fission yeast, Schitzosaccromyces pombe, though these extracts do not appear to contain NF1. Furthermore, E3 appears to be expressed well in S. pombe in the absence of E1A. We have now purified ATF from HeLa cells using an oligonucleotide affinity column to the BS2 sequence. The factor is a protein of 43 kd MW which can be eluted from a preparative acrylamide gel with some recovery of binding activity. Currently we are assaying this preparation for stimulating activity in in vitro transcription systems and whether E1A can alter the activity of ATF, perhaps via a post translational modification.

O 710 IDENTIFICATION OF CONTROL ELEMENTS IN THE PROMOTER OF A HIGHLY EXPRESSED MOUSE HISTONE GENE, Myra M. Hurt, Landie Liu, B. Jane Levine*, Arthur I. Skoultchi*, and William F. Marzluff, Florida State University, Tallahassee, Florida 32306 and Albert Einstein College of Medicine*,Bronx,NY 10461.

There are two clusters of mouse histone genes. One cluster, on chromosome 3, is expressed about 10 times more than the genes on chromosome 13. When an intact H3 gene is introduced into CHO cells, it shows accurately controlled expression both quantitatively and qualitatively. The transfected genes are present in low copy number and high levels of expression are obtained when the promoter region is intact. Expression of the transfected gene is regulated coordinately with DNA synthesis in cells synchronized by mitotic shake-off or treated with hydroxyurea. Bal 31 deletion analysis has revealed a 50 bp sequence 340bp distal to the mRNA start which is required for high expression. Removal of this sequence resulted in a 60% decrease in expression. Further deletions of a GC rich region (300 bp) containing several SP1-like elements down to 100 bp reduced expression to 15%. Cell cycle studies utilizing synchronized cells have also been used to identify the sequences required for coordinating transcription of this gene with DNA synthesis. These sequences are located in the 5' distal region (340bp) of the promoter. Removal of these regulatory sequences results in constitutive expression of the H3.2 gene. The adjacent H2a.2 gene contains similar control elements and may be controlled in a similar fashion. It is possible that the H3.2 control sequences involved in high expression and cell cycle regulation overlap.

O 711 A KINETIC ANALYSIS OF POLYOMAVIRUS TRANSCRIPTION.
Robin Hyde-DeRuyscher and Gordon Carmichael. Department of Microbiology, University of Connecticut Health Center, Farmington, CT 06032.

Polyoma virus transcription is controlled in a temporal fashion after productive infection of mouse cells. Transcripts from the early promoter are produced at both early and late times after viral infection, while transcripts from the late promoter are not detected before DNA replication. Late after infection late transcripts represent the great majority of viral-specific RNA in the cell. The mechanism by which the early-to-late switch is regulated is unknown. To further the understanding of the temporal control of the late promoter, we have carried out a kinetic analysis of polyoma virus transcription in mouse NIH3T3 cells. Nuclear run-on experiments have been performed using nuclei isolated from cells infected for 4, 8, 12, 18 or 24 hours. The resulting RNAs have then been hybridized to a number of early and late specific probes, which represent the entire polyoma genome. Preliminary results indicate that RNA polymerase molecules are unevenly distributed on the viral chromosome. In addition, this distribution changes dramatically as infection proceeds. The implications of these results will be discussed in terms of their relation to the temporal control of polyoma gene expression.

O 712 MULTIPLE NUCLEAR FACTORS ARE INVOLVED IN REGULATION OF THE MOUSE METALLOTHIONEIN-I GENE, Jean C. Imbert, Valeria Cizewski Culotta and Dean H. Hamer, Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland 20892.

The transcription of mammalian metallothionein genes is inducible by a wide variety of environmental and developmental signals. An analysis of Exonuclease III and DNAse I footprint experiments using the upstream control region of the mouse MT-I gene (extending from -200 to +68) and nuclear extracts from mouse L cells reveals that several nuclear factors bind to the region encompassing the 4 active metal responsive elements (MREs). Gel retardation assays performed using a set of synthetic oligonucleotides, corresponding to MREd and various substitution mutants, provide evidence that at least two factors interact with the isolated metal regulatory element. Moreover, transient transfection assays using various substitution mutants of MREd suggest that the multiple factor interactions observed *in vitro* are involved in metal regulated transcription of MT genes *in vivo*. Attempts to purify the essential factors and to elucidate the specific mechanism involved are in progress.

O 713 GENOMIC LOCALIZATION OF MOUSE LEUKEMIA (L1210) TOPOISOMERASE I-MEDIATED DNA BREAKS INDUCED BY CAMPTOTHECIN IN SV40 DNA. Christine Jaxel, Kurt W. Kohn, and Yves Pommier. Laboratory of Molecular Pharmacology, National Cancer Institute, Bethesda, MD 20892.

Camptothecin (CPT) is an antitumor drug, which is a specific inhibitor of mammalian topoisomerase I (topo I). Enzyme inhibition results in the formation of topo I-linked DNA strand breaks. The genomic and DNA sequence localization of topo I-mediated DNA breaks produced by CPT were determined by analyzing autoradiographies of neutral, alkaline 1% agarose or DNA sequencing gels of the reaction products of purified L1210 topo I with [^{32}P]-3'-end-labeled SV40 DNA (restriction fragment Ban I-Hpa II). Densitometer scanning of the autoradiographies of agarose gels were analyzed by computer with respect to the migration positions of [^{32}P]-labeled standards. The general conclusions of the analysis are that: 1) CPT-induced topo I-mediated DNA cleavage is predominantly single-stranded, since it is best detected by using DNA denaturing gels; 2) CPT-induced DNA cleavage is sequence selective, and most pronounced at a single site located near the origin of the early transcription region (T-antigens coding sequence); 3) CPT-induced DNA cleavage patterns were similar in supercoiled and linear DNAs; 4) The DNA sequence was determined at the major cleavage site (nucleotide position 4955); 5) Prominent cleavage sites also occur at a similar sequence in each of the two 72 bp-repeats in the major regulatory region of the SV40 genome.

O 714 REGULATORY CIS-ELEMENTS AND TRANS-ACTING FACTORS INVOLVED IN THE REGULATION OF NODULE SPECIFIC SOYBEAN GENES. Erik Ø. Jensen, Jens Stougaard, Jan-Elo Jørgensen, Carsten Poulsen, Niels Sandal, Frans de Bruijn, Jeff Schell and Kjeld A. Marcker. Department of Molecular Biology and Plantphysiology, DK-8000 Aarhus C, Denmark; Max Planck Institut für Züchtungsforschung, 5000 Köln 30, F.R.G.

Nodulins are a group of plant polypeptides specifically synthesized in the nitrogen fixing nodules on leguminous plants. Among the predominant nodulins are the leghemoglobins (Lb). Nodule specific expression of a soybean *lbc*$_3$ gene and a nodulin *N23* gene is mediated by DNA elements located within the 5' upstream sequences of the genes. This was established by analysing the expression of chimaeric genes in transgenic legumes. The positions of several positive regulatory elements in both the *lbc*$_3$ and *N23* upstream sequences were mapped by *Bal*31 deletion series. Furthermore a 37 bp. region containing sequences conserved in other nodulin genes was shown to be required for the nodule specific expression of the *lbc*$_3$ gene.

Nuclear extracts from soybean nodules, leaves and roots were used to investigate protein-DNA interactions in the *lbc*$_3$ upstream sequence. Two distinct sequences were identified, which strongly bind a nodule specific factor. The position of the two binding sites coincide with one of the positive elements. Competition experiments suggest that both elements bind the same nodule specific factor, although their A-T rich sequences differ substantially. Factors with the same binding properties have also been identified in nuclear extracts from nodules of two other legumes.

O 715 KINETICS OF ABORTIVE AND PRODUCTIVE RNA CHAIN ELONGATION WITH WHEAT-GERM RNA POLYMERASE II

Dominique Job and Claudette Job, C.B.M. du C.N.R.S., 31 chemin Joseph Aiguier 13402 MARSEILLE cedex 9 FRANCE

Kinetic results of abortive and productive RNA chain elongation with ribonucleoside triphosphates or various nucleotide derivatives as substrates show that the enzyme exhibits an apparent negative co-operative behaviour. The amplitude of the co-operativity is modified when the reactions are carried out in the presence of two alternating substrates or of a substrate and a substrate derivative behaving as an inhibitor. The results are discussed according to different minimal schemes: a two-sites model or an hysteretic model, with reference to the well-documented prokaryotic RNA polymerase. The influence of DNA sequence and conformation on the kinetic properties of the eukaryotic RNA polymerase has been studied using a variety of synthetic templates occuring in a right-handed or in a left-handed conformation. The general finding is that both the abortive and productive pathways are modified with the left-handed templates.

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O 716 UNUSUAL FEATURES OF THE PROMOTER AND POLYADENYLATION SIGNALS OF THE MOUSE THYMIDYLATE SYNTHASE (TS) GENE, Lee F. Johnson, Tiliang Deng, Christopher Harendza, James DeWille and Keith Jolliff, The Ohio State University, Columbus, Ohio 43210.
TS mRNA is unusual because it has multiple 5' termini and because the termination codon is followed immediately by the poly(A) tail. To study the sequences that are important for regulating transcription and polyadenylation, intronless minigenes were constructed that had the normal coding, 5' and 3' flanking regions of the TS gene. Normal minigenes, or minigenes altered in the 5' or 3' regions by deletion or site-directed mutagenesis, were transfected into ts⁻ hamster cells and the levels of expression determined. Full promoter activity was retained in minigenes that included only 60 nucleotides upstream of the longest mRNA molecules. This region contained potential binding sites for a variety of transcription factors including USF, NF1 and Sp1 (2 sites). Mobility shift and footprinting assays revealed that USF and Sp1 bound to their recognition sequences. Inactivation of the USF and/or the 5' Sp1 sites had only minor effects on promoter activity, whereas alteration of the potential NF1 site abolished expression. Unexpectedly, addition of introns 5 and 6 to the minigene stimulated expression by about 20-fold. Sequences that are important for polyadenylation were also studied. Alteration of the potential upstream signal AUUAAA (located in the coding region) to AUCAAA completely abolished polyadenylation at the normal site. However, alteration to AAUAAA had little effect. The location of downstream regulatory sequences is currently being determined.

O 717 ROLE OF THE CYSTEINE-ZINC FINGER IN DNA BINDING, M. Johnston, J. Dover, J. Kim* and C. Michels*, Dept. of Genetics, Washington University Medical School, St. Louis, MO 63110, and *Dept. of Biology, Queens College, Flushing, N.Y. 11367.
The 'cysteine-zinc DNA binding finger' is an evolutionarily-conserved DNA binding domain in many proteins. The distinguishing features of this motif are two pairs of cysteine (or histidine) residues that chelate a zinc ion and are separated by a stretch of amino acids that loops out to form the 'finger' that is thought to directly contact DNA. Evidence supporting the role of this sequence in binding to both DNA and zinc ions is provided by the effects of mutations that alter the 'finger' of the GAL4 protein, which binds to DNA and activates gene transcription in the yeast S. cerevisiae. These mutations abolish the ability of this protein to bind to DNA; some of them also reduce the affinity of the protein for zinc ions.
Because the several yeast proteins that contain this sequence motif have remarkably similar cysteine-zinc fingers but bind to different DNA sequences, the cysteine-zinc finger may not confer sequence-specific DNA binding capability. To test this hypothesis, we replaced the cysteine-zinc finger of GAL4 protein with the similar region from the yeast MAL63 protein. We find that a GAL4 protein with the cysteine-zinc finger of MAL63 protein retains GAL4-specific DNA binding activity, although this activity is reduced compared to normal GAL4 protein. This result is consistent with the idea that the cysteine-zinc finger is a general DNA binding domain.

O 719 TRANSCRIPTION ELONGATION AND TERMINATION BY PURIFIED EUKARYOTIC RNA POLYMERASES, Caroline M. Kane, David E. Adams, Dean W. Goddette, Tom Kerppola, and Daniel Reines, Department of Biochemistry, University of California, Berkeley, CA 94720.

We have previously reported that purified mammalian RNA polymerase II recognizes specific sequences as termination sites in vitro. The polymerase stops at these intrinsic termination sites and releases the nascent transcript. All sites share some common features. Several of these sites exist within eukaryotic gene sequences, and they may function to regulate gene expression in the cell. Using deletion analysis and site-directed mutagenesis, we are mapping the DNA sequences which signal the polymerase to terminate. These sequences define one of several probable classes of termination sites for RNA polymerase II. RNA polymerase II can recognize some termination sites without additional cellular factors; however, there are termination regions used efficiently by the cell which the purified polymerase does not recognize. These results are similar to those seen for the bacterial RNA polymerases which terminate through both factor-dependent and independent mechanisms. In addition, we are comparing the effect of the termination sites defined for RNA polymerase II on transcription by both RNA polymerases I and III in vitro.

O 720 REGULATION OF METALLOTHIONEIN GENE EXPRESSION BY 1α,25-DIHYDROXY-VITAMIN D_3 IN CULTURED CELLS AND IN MICE, Mika Karasawa, Junichi Hosoi, Hiroki Hashiba, Kiyoshi Nose and Toshio Kuroki, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, Japan.

Expression of metallothionein (MT) gene is known to be regulated by heavy metals, glucocorticoids, cytokines, phorbol ester tumor promoters and growth factors. We present here evidence that MT gene is also regulated by 1α,25-dihydroxyvitamin D_3 (1α,25(OH)$_2D_3$), a hormonally active form of vitamin D_3 (Karasawa et al. PNAS, 1987, in press). The exposure of FRSK cells, derived from fetal rat skin keratinocytes, to 1α,25(OH)$_2D_3$ at 0.5 ng/ml (1.2 nM) or more increased the level of MT mRNA time- and dose-dependently. Amount of MT protein, determined by RIA, was increased in paralled with the increase of MT mRNA. A receptor-mediated mechanism is indicated by the presence of a specific receptor for 1α,25(OH)$_2D_3$ (Kd, 39 pM; Nmax, 63 fmol/mg protein) and specific induction by 1α,25(OH)$_2D_3$ among vitamin D derivatives. Treatment with cycloheximide did not interfere with 1α,25(OH)$_2D_3$-induced MT mRNA, suggesting direct regulation by 1α,25(OH)$_2D_3$. Oral administration of 1α-hydroxyvitamin D_3, a synthetic precursor of 1α,25(OH)$_2D_3$, caused a marked increase in MT mRNA in the liver, kidney and skin. We are now investigating a cis-acting element by 1α,25(OH)$_2D_3$, if any, in the 5' promoter region which may regulate expression of MT gene by binding to the receptor/hormone complex.

O 721 IDENTIFICATION OF FACTORS THAT BIND TO HUMAN β-INTERFERON GENE REGULATORY SEQUENCES Andrew Keller and Tom Maniatis, Harvard University, Cambridge, MA 02138

Human β-interferon (β-IFN) gene expression is induced by virus or poly(I)-poly(C). This induction is due at least in part to an increase in the rate of transcription, and does not require protein synthesis. A 40 base-pair DNA sequence within the β-IFN promoter, termed the IRE, has been shown to be an inducible enhancer, and both positive and negative regulatory DNA sequences have been characterized within this element. We have identified three factors that bind specifically to two positive regulatory domains within the IRE. Two of these factors are present in nuclear extracts prepared from uninduced and induced cells; one is present only in extracts from induced cells. The appearance of the inducible binding activity is not prevented by inhibitors of protein synthesis, suggesting that this activity results from a post-translational modification of a pre-existing factor. Single base mutations that alter the expression of the β-IFN gene in vivo prevent the binding of these factors in vitro. Preliminary in vitro transcription experiments suggest that these factors are transcriptionally active.

Poster Session 5

O 722 FUNCTIONAL ANALYSIS OF THE CYCLOHEXIMIDE MEDIATED INHIBITION OF THE RAT ALPHA 1-ACID GLYCOPROTEIN GENE'S GLUCOCORTICOID REGULATORY ELEMENT, Elliott S. Klein*, Diego DiLorenzo and Gordon M. Ringold, *Department of Pharmacology, Stanford University, Stanford, CA 94305 and Syntex Research, 3401 Hillview Avenue, Palo Alto, CA 94304.

Alpha$_1$-acid glycoprotein (AGP), one of the "acute phase reactants", is induced by glucocorticoids in rat hepatoma cells at the level of gene transcription. Unlike many well characterized steroid responsive genes, this induction is diminished by inhibition of protein synthesis. We have previously shown that the 5'-flanking region of the AGP gene confers a hormonal responsiveness that is dependent upon ongoing protein synthesis.We now show that the glucocorticoid regulatory element (GRE) of the AGP gene located at position -121 to -107 bp., which has very high homology to the GRE consensus sequence ACAxxxTGTTCT , confers glucocorticoid responsiveness on a heterologous promoter. Such regulation is not diminished by concurrent inhibition of protein synthesis. Thus, the AGP glucocorticoid responsive element behaves as a classical GRE. However, inclusion of the AGP sequences from -120 to -42 bp. renders the hormonal induction sensitive to inhibition of protein synthesis. *In-vitro* DNAse-1 protection assays using nuclear extracts from HTC cells indicates the presence of several protected domains within the region -110 to -65 bp. of the AGP gene. Additional data to be presented supports the notion that a labile positive factor acting at a site near the GRE is required for efficient trancscription at the AGP promoter.

O 723 A TRANSCRIPTIONAL REGULATORY REGION IN THE 5' FLANKING SEQUENCE OF THE CHICKEN αA-CRYSTALLIN GENE. John F. Klement and Joram Piatigorsky. Laboratory of Molecular and Developmental Biology, National Eye Institute, NIH, Bethesda, MD 20892.

To delineate the chicken αA-crystallin 5' flanking region required for transcription, we fused various lengths of upstream DNA sequence, along with 72 bp of the first exon, to the bacterial chloramphenicol acetyltransferase (CAT) gene and transfected 14-day old chicken lens epithelial cells or fibroblasts. Our results indicated that 163 bp of αA-crystallin 5' DNA flanking sequence were required for efficient and tissue-specific expression of the αA-crystallin/CAT gene. The αA-crystallin 5' flanking region from -163 to -122 was protected from exonuclease digestion in the presence of lens nuclear extract from 14-day old chicken embryos. The 5' promoter deletion and DNA protection experiments suggest that the -163 to -122 region of the chicken αA-crystallin gene contains a binding site(s) for a transcription activating factor(s). Okazaki et al. (EMBO J. 4, 2589-2595, 1985) have reported that the chicken αA-crystallin 5' flanking region from -242 to -189 was required for the efficient expression of a chicken αA/δ-crystallin chimeric gene microinjected into mouse lens cells. Chepelinsky et al. (Mol. Cell Biol. 7, 1807-1814, 1987) demonstrated that 111 bp of 5' flanking sequence of the homologous mouse αA-crystallin gene was sufficient for the expression of an αA-crystallin/CAT gene in embryonic chicken lens epithelia. Together the data suggest that the regulation of the αA-crystallin gene in mice and chickens may involve species specific factors or different arrangements of common regulatory regions.

O 724 MECHANISM OF INHIBITION OF RNA POLYMERASE II-MEDIATED TRANSCRIPTION IN POLIOVIRUS-INFECTED CELLS. Steven Kliewer and Asim Dasgupta, University of California, Los Angeles, 90024.

Infection of mammalian cells with picornaviruses results in the inhibition of host-cell RNA synthesis. We are studying the mechanism of poliovirus-induced inhibition of RNA polymerase II transcription in vitro using extracts prepared from mock- and poliovirus-infected HeLa cells. By limiting transcription to one initiation event per template, we have shown that the lower activity of infected cell extracts is due to a decrease in the number of templates transcribed, suggesting a decrease in the number of active transcription factors in the infected extracts. In an effort to determine which transcription factors are deficient in infected extracts, we have fractionated mock- and poliovirus-infected extracts by chromatography on phosphocellulose into three fractions required for specific transcription from the adenovirus 2 major late promoter in a reconstituted system. Infected extracts have been shown to be deficient in an activity eluting in the 1.0 M KCl step fraction, the fraction containing the TATA box-binding factor. These results were corroborated by transcriptional "rescue" experiments, in which addition of the 1.0 M KCl step fraction prepared from uninfected cell extracts specifically restored transcription in extracts prepared from poliovirus-infected cells. Our results suggest that inactivation of the TATA box-binding factor is an early event in the inhibition of host-cell transcription by poliovirus.

O 725 IDENTIFICATION AND CHARACTERIZATION OF A CHICKEN α-GLOBIN ENHANCER, Joseph Knezetic and Gary Felsenfeld, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda,MD 20892.

The three chicken α-globin genes are linked together over an approximately 10kb stretch of DNA in the following orientation: 5'-π'- α^D- α^A-3'. The π'gene is transcribed in only the primitive cell types while the α^D and α^A genes are expressed in both primitive and definitive cell lineages. We have recently discovered an enhancer element in this chicken α -globin gene cluster. The enhancer maps to a DNaseI hypersensitive site approximately 750 bp 3' to the translation stop site of the α^A gene. This element stimulates expression from either the α^D or α^A globin gene promoters when assayed by transfection into primary chicken erythrocytes. No other enhancer activity was observed throughout the α-globin locus when these two promoters were used in this in vitro assay procedure. The enhancer is active in both primitive and definitive erythrocyte cell types. Moderate resolution DNaseI hypersensitivity studies and preliminary DNaseI footprinting studies show three regions of protein binding within the enhancer element, all containing a similar core DNA sequence.

O 726 MOLECULAR CLONING OF THE CHICKEN AVIDIN GENE FAMILY AND HORMONAL INDUCTION OF AVIDIN mRNA, Markku S. Kulomaa, Riitta A. Keinänen, Tarja A. Kunnas, Mika J. Wallén, Marja-Leena Laukkanen, Pekka M. Räisänen and Timo Joensuu, University of Tampere, 33101 Tampere, Finland.

Avidin is specifically induced by progesterone in the chicken oviduct and as a target of a steroid hormone its gene provides an interesting model system for studies of eukaryotic gene expression. Two recombinant clones were detected when a hen oviduct genomic library was screened using an avidin cDNA as a hybridization probe. The first clone, λgAV1, was digested with EcoRI or BamHI restriction enzyme, and the subfragments were subcloned into a plasmid (pBR322) and sequencing (M13) vector. Hybridization (Southern), restriction mapping and sequencing analysis of the genomic subclones indicated the presence of at least three avidin genes within the clone λgAV1. The genes were however unable to encode the correct peptide chain as compared to the known amino acid sequence of avidin. A dot matrix analysis (DIAGON) of one of the genes, an avidin related gene AVR1, suggested that it is split into four exons. A homology of 70-80% was observed when the amino acid sequence of preavidin was compared to that derived from the avidin related genes. Preliminary characterization of the other genomic clone, λgAV2, suggested at least partial overlap with λgAV1. By RNA hybridization (Northern, slot-blot) analysis, progesterone specific induction of the avidin mRNA was measurable in the oviduct for 2-4 hr and maximal for 16 hr after progesterone administration. The effect of estrogen prestimulation and other steroid hormones on the progesterone-dependent induction of the avidin mRNA is also under study.

O 727 5' FLANKING ELEMENTS REMINISCENT OF POL II GENES CONTROL THE EFFICIENCY OF THE POL III-TRANSCRIBED HUMAN U6 SMALL NUCLEAR GENE, Gary R. Kunkel and Thoru Pederson, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

U6 small nuclear RNA is transcribed by a different polymerase than the other abundant snRNAs (U1-U5), likely to be RNA polymerase III. The efficiency of U6 transcription is controlled by at least two elements in the 5' flanking region. Maximal in vitro transcription in a HeLa S100 extract requires templates containing the first 67 5' flanking nucleotides. Within this region is a sequence containing marked homology with the proximal control element utilized by U1 and U2 snRNA genes. Transient expression experiments with a transfected U6 maxigene in human 293 cells reveal another important control element between -245 and -149 relative to the U6 initiation site. An octamer motif (-222 to -215) is likely to constitute at least part of this distal transcription element. The octamer-containing human U2 gene enhancer ligated upstream of position -149 in the U6 gene can restore significant U6 RNA expression. U6 genes isolated from a variety of organisms contain a TATA sequence 25-30 bp upstream from the start site of transcription. Mutation of this sequence in the human gene (TATATA to TAGCTA) results in a decrease in U6 RNA transcription in vitro and in vivo, with no significant effect on the selection of the initiation site. We are now investigating the binding of nuclear factors to these putative control elements as well as the role of internal sequences in U6 transcription.

O 728 HIGH FREQUENCY OF THE HEAT-SHOCK CONSENSUS FOUND IN A REPETITIVE DNA FAMILY IN CAENORHABDITIS ELEGANS, Adriana La Volpe, Maria Ciaramella and Paolo Bazzicalupo International Institute of Genetics and Biophysics CNR, Naples Italy

We have identified a repetitive DNA family in C. elegans that is both interspersed in the genome and consists of individual elements that are internally tandemly repeated. Sequence analysis of some random subclones out of a 1 kb long element has revealed that the consensus of the repeated unit is the same as the consensus for the heat-shock promoter element (HSE), present in few copies upstream of the heat-shock genes of all eukaryotes. The entire element we analyzed seems made up of these HSE-like repeats whose length is 10 bp. The heat-shock consensus is 14 bp long but, as in front of some heat-shock genes, two HSEs can overlap by four base pairs. This is so for the whole length of our sequence, so about 100 HSEs can fit in the 1 kb long element. We failed to detect transcription, by Northern blotting, in the flanking sequences during normal growth and under heat-shock condition at this locus in C. elegans. Nevertheless this repeted sequence works as a heat-shock promoter in yeast. Infact we inserted a 244 bp subfragment of our element upstream of a deletion-substitution derivative of Saccharomyces cerevisiae HIS-3 gene deleted of its own UAS. The text vector alone does not transcribe HIS-3 seuq-nces. With our sequence cloned upstream the cding region we observe a high increase in the HIS-3 transcript after heat-induction but not atlower temperatures.

O 729 CONTROL OF HUMAN ζ GLOBIN GENE EXPRESSION, P.Lamb and N.J.Proudfoot, Sir William Dunn School of Pathology, South Parks Rd., Oxford OXI 3RE, England.
The human embryonic ζ-like globin gene is primarily expressed in primitive nucleated erythroblasts produced in the yolk sac. A switch in expression from ζ to the fetal/adult α globin genes is initiated at about 6 weeks of development. We are currently defining DNA sequences involved in the control of ζ globin gene expression. A cloned human ζ gene is expressed when introduced into cells with an early erythroid phenotype (K562), but not when introduced into Hela or COS cells. This specificity is also observed with constructs containing the cap site and 500 bp of 5' flanking sequence fused to the CAT gene. A series of 5' deletion mutants has been generated, none of which are active in Hela cells. In K562 cells deletion of sequences between -500 and -200 has no effect; however further deletion to -150 results in a 5-fold stimulation of transcription. The presence of possible negetive elements is now being investigated. Trans-acting factors that interact with the ζ promoter are also being studied. Two factors which bind to the CCAAT box region have been identified by gel retardation. Competition experiments show that one of these factors interacts with the promoter regions of several other human globin genes. However, the second factor appears to be ζ specific, and shows some cell-type specificity. Intriguingly, this factor also binds to a sequence upstream of the highly conserved ψζ gene promoter. This gene is transcriptionally inactive when introduced into K562 cells unless a deletion of the 5' flanking region is made which includes the ζ-specific factor binding site. The possibility that this binding site is required for repression of ψζ transcription is being investigated.

O 730 A LIVER SPECIFIC REGULATORY ELEMENT IN THE HUMAN APO CIII PROMOTER BINDS TO PROTEINS FROM BOTH HEPG2 AND HELA CELLS. Todd Leff, Karen Reue and Jan L. Breslow, Rockefeller University, New York, NY 10021.
Apolipoprotein CIII (apo CIII) is a structural component of triglyceride-rich and high density lipoprotein particles. The apo CIII gene is expressed primarily in the liver and to some degree in the intestine. In transfected tissue culture cells an apo CIII/CAT construction is expressed in HepG2 but not in HeLa cells. We have determined that this specificity is due, at least in part, to a cis-acting regulatory sequence located 80 nucleotides upstream from the start-site of apo CIII transcription. A small mutation in this sequence reduced transcription in HepG2 cells by about 8 fold, indicating that it is necessary for expression in hepatocytes. When this sequence element was inserted into the heterologous Adenovirus major late promoter it was found to stimulate transcription to a much greater extent in HepG2 than in HeLa cells, indicating that the element contributes to the cell type-specific expression of the apo CIII gene. Using a DNase I footprint analysis we have demonstrated that extracts from both HepG2 and HeLa cells contain a protein that binds to this liver specific sequence element. The binding proteins from both cell types give qualitatively identical footprints. When the gel mobility shift assay was performed with fragments containing the liver specific sequence element, shifted bands were observed in both cell types although the HepG2 protein appears to be larger then the HeLa protein. This may indicate that different proteins from these two cell types share the same binding specificity but have different transcriptional activities.

O 731 FORMATION OF THE 3' END OF snRNAs, Susan M. Lobo, Robert Lucito, Nouria Hernandez, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
The formation of the 3' ends of the human U1 and U2 snRNAs requires a 13 to 16 nucleotide long sequence, the 3' box, located downstream of the mature 3' end of the RNA. In addition, the reaction requires snRNA promoter elements; replacement of the polymerase II snRNA promoter by the promoter of an mRNA encoding gene results in the synthesis of RNAs that read through the 3' box. This surprising observation suggests that snRNA promoters might bind a termination factor; this factor could then be carried along the gene by the polymerase until the 3' box is encountered, or interact by protein-protein contact with a factor bound to the 3' box, resulting in looping out of the DNA separating the two binding sites.
To determine whether promoter sequences contain a binding site for a factor involved in 3' end formation, we have undertaken an extensive mutational analysis of the human U2 promoter, and have monitored the effects on initiation of transcription and on formation of the 3' end of the RNA. These experiments have delimited precisely the sequences required for initiation of transcription, and have shown that the human U2 promoter does not contain a binding site for a factor involved solely in termination. Indeed, deletions or substitutions of promoter sequences never affect 3' end formation without affecting initiation of transcription. However, 3' end formation can be inhibited by replacing the U2 enhancer with an artificial enhancer that presumably binds other trans-acting factors. Thus, a foreign transcriptional element is incompatible with U2 3' end formation. We are now in the process of characterizing the proteins that bind to the functional elements of the U2 promoter identified in the above experiments.

O 732 **RESTRICTION OF RETROVIRAL EXPRESSION IN MOUSE STEM CELLS,** Tatjana P. Loh and Richard W. Scott, E. I. du Pont de Nemours & Co., Inc., Central Research & Development, Experimental Station, Wilmington, DE 19898

Mouse embryonal carcinoma (EC) cells are non-permissive for retrovirus replication. Restriction of expression of Moloney murine leukemia virus (M-MuLV) in EC cells has been investigated using DNA transfection techniques. In addition to the inactivity of the M-MuLV enhancer sequences we have identified a region that spans the tRNA primer binding site that is responsible for restricting expression specifically in EC cells. Inhibition by the element ranges between 20 to 50 fold and is position dependent; relocation of the element outside of the transcription unit abolishes activity. The mechanism of action is unclear, however the half-lives of transcripts that do or do not contain the inhibitory sequences are virtually identical. Also, competition assays have demonstrated that repression can be relieved by co-transfection with a vast excess of the inhibitory element. An EC cell-specific footprint that maps to the tRNA primer binding site domain has also been identified. Currently, we are attempting to correlate specific binding in this region with inhibitory activity to further characterize the repression of retroviral expression in EC cells.

O 733 MURINE LEUKEMIA VIRUSES: TRANSCRIPTIONAL CONTROL AND ONCOGENESIS, Steen Lovmand, Allan Bækgaard, Hong Y. Dai, Henrik S. Olsen, Poul Jørgensen, Finn S. Pedersen and Niels O. Kjeldgaard, University of Aarhus, DK-8000 Aarhus C, Denmark.

Murine leukemia viruses represent a polymorphic group of retroviruses. Individual isolates differ in tissue tropism and in pathogenic properties. For some isolates these differences are determined by the nucleotide sequences of the transcriptional control elements in the long terminal repeat (LTR) of the proviral genome. The genome structure and biological properties of murine leukemia viruses associated with lymphoid and osteoid tumors have been analyzed. Genome structure analysis of a number of viruses has detected variation in the structure of the U_3 region in the LTR. These different U_3 structures can be grouped according to the number and relative order of a series of short DNA elements using the endogenous virus, Akv MuLV as a prototype. It is proposed that these DNA elements represent recognition sites for nuclear proteins involved in transcriptional regulation. To study the cis-function of these LTR elements we have developed a double marker selection strategy for easy generation of random LTR deletion and duplication mutants. Using deletion mutants, transient expression assays in murine fibroblasts indicate the use of different positive cis elements in the LTRs of variant viruses. In Akv MuLV we have identified a positive cis-element that differs in structure and relative LTR-location from elements described for Moloney MuLV and closely related viruses. Studies using nuclear extracts have identified specific protein binding to the Akv MuLV DNA element defined by mutational analysis.

O 734 **THE MECHANISM OF INITIATION BY RNA POLYMERASE II,** Donal S. Luse, George A. Jacob, Jonathan Neumann and Randy Bechard, Univ. of Cincinnati, Dept. of Biochemistry and Molecular Biology, Cincinnati, OH 45267-0522. We have been studying the process of transcription initiation by RNA polymerase II in vitro at the Ad 2 major late promoter. Our approach has been to assemble a preinitiation complex at Ad 2 ML by incubation of the DNA in nuclear extracts. The ability of such complexes to initiate is then assessed by synthesizing RNA in the presence of dinucleotide primers and/or a subset of the NTPs, in order to allow synthesis of only 1, 2 or about 10 phosphodiester bonds. We have shown with these approaches that at Ad 2 ML (a) a single bond is always made abortively (JBC 262, 289 and 14990), (b) once two bonds are made a partially stable ternary complex is obtained (JBC 262, 298) and (c) once about ten bonds are made a fully stable elongation complex is obtained (JBC 262, 298). We are now asking how these processes occur at other TATA box promoters (preliminary results with a strong β-globin promoter look like those at Ad ML) and how the deletion of the binding site for the stimulatory factor USF at Ad 2 ML will affect initiation there (preliminary results indicate that fewer active complexes are assembled without USF but no differences in the initiation process are seen).

O 735 Nuclear/Nucleolar Localization of the HIV tat Gene Product. Robert H. Lyons, David Brake, Hagai Rosenberg, Martin Rosenberg and Christine Debouck, Smith Kline & French Laboratories, King of Prussia PA.

The product of the HIV tat gene stimulates expression of the viral genome, probably by posttranscriptional as well as transcriptional mechanisms. The subcellular site to which the active protein localizes has been unknown. We have microinjected into mammalian cells an mRNA, produced in vitro, which codes for tat protein. At various times following RNA injection, the subcellular location of its protein product was determined by fixation and staining with an antibody specific for tat. Functional tat was produced after injection of RNA, as determined by the increased activity of a tat-responsive recorder gene and by immunofluorescent staining. Expression of tat was maximal two hours after injection, then decreased with a half-life of approximately 4 hours. Throughout this time, tat was found mainly in the nucleus, and was strikingly concentrated in the nucleolus, the site of ribosomal RNA synthesis and maturation. Some protein was additionally seen in the cytoplasm. We postulate that tat may influence nucleolar processes, and might thereby affect translation indirectly. The nucleolus contains three morphologically and functionally distinct types of compartment. We are currently using immunoelectron microscopy to determine the subnucleolar compartment to which tat localizes.

O 736 CIS-ACTING ELEMENT AND TRANS-ACTING FACTORS OF THE ADENOVIRUS 2 PEPTIDE IX GENE, Takashi Matsui, Univ. of Occup. and Environ. Health, Japan

DNA sequences required for maximal level of transcription of the adenovirus 2 peptide IX (pIX) gene have been identified using an in vivo transient expression system and an in vitro cell free transcription system. Deletion analysis of the upstream sequences revealed that in addition to TATA box, the sequences from - 70 to -40 are required for maximal and accurate initiation of transcription of pIX gene. Insertion analysis between two elements indicated that a distance between them is critical for the promoter function. Additionally, the upstream element when tandemly duplicated enhanced initiation of transcription. This transcriptional enhancement cleary depended on the distance between the elements. Therefore, function of the upstream sequences idenitfied within pIX gene seems to be equivalent to that of upstream elements known in several other genes. On the other hand, specific factors to the upstream sequences were identified in HeLa cell extracts, which form three complexes with different mobilities on a gel. DNase I footprinting and methylation interference footprinting showed that the sequences from -61 to -47 are similary covered with factor(s) in two of three complexes. In contrast, the third complex was formed by binding with the sequences from -47 to -54. Competitive binding with the upstream sequences of several other viral genes is also shown.

O 737 **FUNCTIONAL DOMAINS OF TRANSCRIPTIONAL REGULATORY PROTEINS ENCODED BY THE E2 ORF OF BPV-1**
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Lab of Tumour Virus Biology, NCI, NIH, Bethesda, MD 20892.

The E2 open reading frame of BPV-1 has been shown to encode both positive and negative acting transcriptional regulatory factors. The DNA binding properties of these factors were analysed to investigate the mechanism by which they might regulate gene expression. Polypeptides corresponding to the full-length E2 product and a shorter protein thought to represent the repressor function were synthesized in vitro by translation of T7 polymerase generated transcripts. Using rabbit antisera generated against synthetic peptides from the E2 open reading frame, it was possible to immunoprecipitate each of these products and show that each was capable of binding the same specific sequence located at several sites in the BPV-1 genome. This DNA binding property was mapped to a conserved carboxy-terminal domain of 101 amino acids by analysis of truncated polypeptides synthesized from the E2 open reading frame. This domain is shared by both the E2 transactivator and repressor.

Experiments are in progress to determine which regions of the E2 gene product are important for the transactivation function.

O 738 REGULATION OF RAT DYNORPHIN GENE EXPRESSION, Cynthia T. McMurray and James O. Douglass, Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Using Rnase protection analysis, we have mapped the cap site of the rat prodynorphin gene in rat striatum and testes. In both tissues the identical initiation site was used, located approximately 30 base pairs from a canonical TATAAA box. To conclusively identify the rat dynorphin promoter and to analyze its regulation, we have constructed a series of dynkat hybrids consisting of various lengths of sequence spanning 1.1 kb 5' to the cap site fused to the bacterial chloramphenicol acetyltransferase (CAT) gene. Each construct was transfected into mammalian cell lines and assayed for the ability of each dynorphin gene fragment to direct synthesis of the CAT gene product at the basal level and in response to cAMP. The protein factors presumably responsible for promoting basal and induced levels of CAT synthesis were functionally analyzed by measuring the amplitude, $\tau_{1/2}$, and kinetic rate constant for transcription directed by each dynkat construct. Additionally we have introduced dynkat constructs into primary rat leydig cell cultures, analyzed dynorphin promoter function, and compared these results with those obtained from the cell lines.

O 739 EXPRESSION OF A TRUNCATED MYB PROTEIN IN MYELOID CELL LINES CONTAINING RETROVIRAL INSERTIONS IN THE MYB LOCUS, Linda J. Miller, Yacob Weinstein, William Boyle* and James N. Ihle, NCI-Frederick Cancer Research Facility, Frederick, MD, and *Salk Institute for Biological Studies, San Diego, CA

The *myb* oncogene was initially identified as the transforming gene of the avian myeloblastosis virus (AMV), which specifically transforms myeloid cells both in vivo and in vitro. The myb proto-oncogene encodes a nuclear protein of 75 kd. In contrast, the transforming gene is a nuclear protein of 45 kd which is truncated at both the amino and carboxyl termini. Activation of the myb proto-oncogene has also been associated with murine myeloid leukemias. We have shown that retroviral insertions in the myb gene have occurred in two independently derived myeloid cell lines. The NFS-60 cell line is an IL-3 dependent myeloid cell line derived from a primary retrovirus induced tumor. The VFLJ2 cell line is a myeloid line derived from cultures of normal fetal liver cells infected with a retrovirus. In both cases, a retrovirus integrated into the middle of the myb gene. This insertion resulted in the overproduction of a truncated myb transcript. We have used myb specific antisera to characterize the protein products. These antisera immunoprecipitate a truncated myb protein of approximately 45 kd. Cell fractionation experiments indicate that the truncated protein is associated with the nuclear fraction. Our results suggest that the acquisition of transforming activity is associated with loss of a carboxyl region.

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O 740 REGULATION OF THE EARLY-LATE ($\beta\gamma$) GENES OF HERPES SIMPLEX VIRUS TYPE 1, Robert L. Millette, Jaya Lahiri, Patricia Perry, Diane Tomar, and Shin Chen. Department of Biology, Portland State University, Portland, OR 97207.

In early-late or $\beta\gamma$ genes of herpes simplex virus type 1 (HSV-1) code for essential structural proteins of the virus, including the major capsid protein, VP5 (ICP5). For maximum transcription of these genes, both immediate-early viral proteins and viral DNA replication is required. To characterize the trans- and cis-acting elements involved in this regulation, we have used i) in vitro stimulation of transcription, ii) mobility shift, and iii) DNA footprinting assays.

Using nuclear extracts, we have identified by mobility shift assay 2 major complexes involving the VP5 gene promoter. Both complexes appear to be formed by cellular proteins since the same pattern is obtained with uninfected and infected cell extracts. However, the complex of lower mobility (Complex A) was found to decrease at least 6-fold while the complex of higher mobility (Complex B) increased at least 10-fold by 8h after infection. Using DNAaseI protection footprinting of Complex A, we have found the same DNA sequence to be protected with both uninfected and infected cell nuclear extracts. The protected sequence, AGGGCCATCTTGAA, is located between -64 and -84 relative to the mRNA cap site. Although this sequence does not appear to be related to known eukaryotic control sequences, homologous sequences occur in certain other $\beta\gamma$ genes of HSV, in a number of eukaryotic cell and virus gene, and in certain retrovirus LTRs. The precise role of this sequence and that of Complex B in transcriptional regulation is being investigated using in vitro and in vivo transcription systems.

O 741 **STUDIES ON THE DIFFERENTIAL EXPRESSION OF THE BRAIN CREATINE KINASE GENE**, Mark T. Mitchell, Grace Hobson, Pamela Benfield, George Molloy*, and Mark L. Pearson, E. I. du Pont de Nemours & Co., Central Research & Development, Experimental Station, Wilmington, DE 19898 and *University of Delaware, Newark, DE 19713

The gene encoding rat brain creatine kinase (CKB) is highly expressed in brain but the steady state RNA is barely detectable in liver. Using nuclear extracts derived from rat brain and liver tissues, we are able to reconstitute the tissue specific expression of this gene *in vitro*. We have also examined the expression of CKB in extracts from HeLa and other cell lines. All of these extracts display distinct qualitative differences in their ability to recognize the multiple promoter-like elements of this gene. All of these extracts also display distinct qualitative differences in their factor binding patterns, as assayed by gel retardation and DNAaseI footprinting. Interestingly, a TATA like sequence which does not seem to function *in vivo*, does bind a sequence specific factor present in these extracts. We have begun to characterize more thoroughly the factors and sequences responsible for this differential expression of CKB.

O 742 **Promoter Structure Of The Human Cardiac α-Actin Gene**

Takeshi Miwa, Thomas Gustafson, Linda Boxer and Larry Kedes; Stanford University and Veterans Administration Medical Center, Palo Alto, CA 94304.

At least two upstream regions, proximal (-49 to -177) and distal (-263 to -443), of the human cardiac α-actin gene mediate high-level transcription in differentiated muscle cells. The proximal upstream sequence containing a core CC(A-T rich)$_6$GG sequence (CArG box), which is found in the promoter regions of several muscle associated genes, was sufficient for tissue-specific expression but it is not an enhancer element because its stimulation required correct orientation and position relative to the TATA box. We detected a binding activity to the CArG box in vivo and in vitro by trans-acting factors not only from muscle cells but also from non-muscle cells (HeLa and NIH3T3). Transcription in muscle cells can work with a single copy of the proximal CArG box as the upstream sequence. The trans-acting factors in non-muscle cells, however, can stimulate transcription but only in the presence of two tandem copies of it. Therefore, trans-acting factors from both cell types have distinguishable functional properties in transcriptional stimulation. The distal region acts as an enhancer-like element in muscle cells, but only when the CArG box is located in the promoter. Since we also detected factor-binding in this distal region, it may exert an effect through protein complexes with CArG binding factors.

O 743 STUDIES ON UBIQUITIN CARBOXY-EXTENSION PROTEINS, Brett P. Monia, David J. Ecker, Tauseef R. Butt and Stanley T. Crooke, Dept. Pharmacology, University of Pennsylvania School of Medicine, Phila., PA 19104 and Dept. Molecular Pharmacology, Smith, Kline and French Labs., King of Prussia, PA. 19406-0939.

Ubiquitin is a 76 amino acid protein believed to be essential for ATP-dependent, non-lysosomal intracellular protein degradation. It is found in eukaryotic cells either free or covalently joined to a variety of cytoplasmic and nuclear proteins. Ubiquitin, encoded in the genome as a multigene family, is synthesized either as polyubiquitin (3-9 tandem repeats of ubiquitin) or fused to sequences encoding a carboxy-terminus extension of 52-80 amino acids. These ubiquitin carboxy-extension proteins (CEPs) are strikingly similar to nucleic acid binding regulatory proteins in that they are highly basic (30% lys and arg) and contain amino acid sequence homology with the zinc-finger domains of transcription factors IIIA (Xenopus) and ADRI (Saccharomyces). We have initiated studies on the structure and function of CEPs in eukaryotes by expressing them in bacteria, yeast and mammalian cell lines. When expressed in eukaryotic cells, we have found that the ubiquitin-CEP fusion protein is processed to free ubiquitin and CEP. We have purified the intact fusion protein as well as CEP and are currently investigating their structure, nucleic acid binding properties and expression pattern in mammalian cell lines.

O 744 A FUNCTION FOR THE C-TERMINAL DOMAIN OF THE LARGEST SUBUNIT OF RNA POLYMERASE II, Matthew Moyle and C. James Ingles, Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6.

The *RPO21* polypeptide, the largest subunit of RNA polymerase II, contains an unusual eukaryotic-specific C-terminal domain. This domain, composed of the tandemly repeated heptapeptide sequence TyrSerProThrSerProSer appears to be present in the *RPO21* polypeptide of all eukaryotes. Previous studies have suggested that this polymerase II-specific domain is necessary for transcription in vitro from class II promoters. By making deletions in this domain in the yeast *RPO21* gene we established that a minimum of 9-11 of the 26 repeats is necessary for *RPO21* function in vivo (Allison et al Mol. Cell. Biol. In press). We have now shown that a synthetic peptide containing 5 repeats of the consensus heptapeptide sequence inhibits polymerase II transcription from both the adenovirus major late promoter and the mouse dihydrofolate reductase promoter in vitro. In contrast, this peptide has no effect on randomly initiated transcription of calf thymus DNA by polymerase II or accurately initiated transcription of VA1 DNA by RNA polymerase III in vitro. The kinetics of this inhibition of specifically initiated polymerase II transcription indicate that this polymerase II C-terminal peptide analog antagonizes the formation of an initiation-competent transcription complex. We have suggested previously that this C-terminal domain of RNA polymerase II could provide sites for interaction with transcription factors that are required for initiation from class II promoters. To identify such *RPO21* binding proteins, we have used photoaffinity crosslinking techniques with ^{125}I labelled derivatives of the heptapeptide repeats. In these experiments the heptapeptide analog was specifically crosslinked to a protein of M_r ~72 000 that is present in Hela cell nuclear extracts. This protein may be one of the general factors required for initiation of transcription by RNA polymerase II.

O 745 ANALYSIS OF OPERATOR SEQUENCE ELEMENTS THAT BIND MAT REGULATORY PROTEINS a1 AND α2, Sarbjit S. Ner, Jeanette A. Johnson and Michael Smith, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Cell type specificity in *S. cerevisiae* is governed by the transcriptional control exerted on unlinked genes by the products of the MAT locus. In the α cell type MATα1 switches on α-specific genes and MATα2 gene product represses a-specific genes. In the diploid cell type the expression of haploid specific genes is turned off by the combined actions of a1 and α2. Both a1 and α2 proteins share homology with the DNA binding helix-turn-helix sequences in prokaryotes and to the "homeo-box" sequences in eukaryotes. The operator sequences lying upstream of the target genes regulated by α2 (32 bp elements), and a1-α2 (18 bp elememt) have been identified, and are shown to be related to each other in that they both carry the motif ATGT....ACAT but differ in the spacer sequence that lies between the motif. We are currently addressing the question how α2 is able to interact with two related operator sequences.
We have chemically synthesised sequences that correspond to the BAR1 and MATα1 α2 and a1-α2 elements respectively, and have inserted these between the upstream activation sequence (UAS) and the TATA sequence of the CYC1 promoter fused to the β-galactosidase gene. In addition we have deleted the spacer sequence from the α2 element and have inserted into the a1-α2 element to make pseudo-MATα1 and pseudo-BAR1 operator elements. We show that the wild type operators confer cell type specificity and that the pseudo-BAR1 element acts as an activator. By the introduction of point substitutions into the BAR1 and pseudo-BAR1 elements we show which nucleotide residues are responsible for this effect.

O 746 DNA-PROTEIN INTERACTIONS WITHIN AN INTRON SERUM RESPONSIVE DOMAIN, Sun-Yu Ng, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan 11529, Republic of China

The human ß-actin gene first intron contains an evolutionarily conserved 30 base-pair sequence with a CArG motif. Similar motifs are found in other actin genes as well as in upstream promoter domains of the human ß-actin gene. This intron domain is inducible by serum and growth factors (FGF and PDGF). Since J.T. Elder *et al* (manuscript in preparation) have found DNase I hypersensitive sites in this region, I am examining DNA-protein interactions in this intron domain under various cellular growth conditions. Using an electrophoretic mobility shift assay, DNA-protein interactions in this region are sensitive to competition with the c-*fos* SRE sequence as well as an α-skeletal actin gene CArG sequence.

O 747 BINDING OF A HOST CELL FACTOR TO THE CONSERVED 21-NUCLEOTIDE REPEATS IN THE HTLV-I PROVIRAL LTR, Jennifer K. Nyborg and William S. Dynan, University of Colorado, Boulder.

Efficient expression of human T cell leukemia virus type I (HTLV-I) genes requires both host and viral proteins, and is dependent on DNA sequences in the proviral long terminal repeats (LTR's). Using DNase I footprinting with partially purified nuclear extracts from human cell lines, we have constructed a map of specific protein-DNA interactions over a 250-nucleotide region of the LTR upstream of the start site for viral RNA synthesis. We have identified a cellular factor, host expression factor-I (HEF-I), that binds to the imperfect 21-nucleotide repeats that have previously been shown to be necessary for efficient HTLV-I gene expression. HEF-I binding activity is present in preparations from both lymphoid and nonlymphoid cell lines. However, the boundaries of the protected regions vary with cell type. In addition to the HEF-I binding sites, several other regions of binding are detected, including a complex group of sites 40 to 90 nucleotides upstream of the RNA start site. We are continuing to purify HEF-I from lymphoid and non-lymphoid sources, and have begun *in vitro* transcription assays to study the functional role of HEF-I, as well as other cellular and viral factors, in HTLV-I gene expression.

O 748 SV40 ENHANCER ELEMENTS ARE COMPOSED OF INTERCHANGEABLE SUBUNITS Brian Ondek, Lisa Gloss and Winship Herr, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, N.Y. 11724

We have recently shown that three genetically defined SV40 enhancer elements, A, B, and C, each possess intrinsic enhancer activity. Using short synthetic DNA oligonucleotides, we showed that each of these elements can act autonomously as an enhancer when present as multiple tandem copies. Analysis of single and multiple B elements showed a single element is ineffective as an enhancer, whereas two or more elements are active. The SV40 enhancer is characterized by a complex pattern of short (8-10 bp) sequence motifs, including core ($GTGG^{A}/_{T}{}^{A}/_{T}{}^{A}/_{T}G$) and sph ($AAG^{T}/_{C}ATGCA$) motifs, many of which correlate with binding of trans-acting factors. Here we show that the genetically defined elements are structurally bipartite and consist of interchangeable sub-elements, which we refer to here as enhansons; these sub-elements or enhansons correlate with sequence motifs. New enhancer elements can be constructed by duplication of 9 bp long enhansons and by juxtaposition of two heterologous enhansons from separate wild-type elements. Isolated enhansons, however, are inactive. Analysis of spacing constraints between these sub-elements has been carried out using our most active synthetic enhancer element (coreA/coreA), six copies of which enhances transcription twice as well as the SV40 enhancer containing one 72 bp element, and have shown that while 1 and 2 bp insertions between the coreA repeat greatly decrease enhancer element function, 5 and 10 bp insertions inactivate the element. These strict spacing constraints contrast with that of two complete B elements where activity is readily apparent with 8-21 bp of inserted spacer DNA, weakly detectable with 40-70 bp and undetectable with >100 bp inserted. These studies have shown that the SV40 enhancer is organized into two distinct levels of cis-element organization: the enhancer element, which must be present in multiple copies for efficient activity, and the enhanson which when juxtaposed with one another forms a functional enhancer element.

O 749 IN VITRO ANALYSIS OF THE ACTION OF THE XENOPUS LAEVIS rRNA GENE ENHANCER, Louise Pape, Jolene Windle and Barbara Sollner-Webb, The Johns Hopkins University School of Medicine, Baltimore MD 21205.

The '60/81' bp repeats of the upstream spacer of the Xenopus laevis rRNA gene direct transcriptional enhancement by RNA polymerase I (Reeder, Cell 38:349, 1984). We recently demonstrated that there are two separable effects of the rDNA enhancer, stimulation of a promoter in cis and competition of a promoter in trans, and that both require the presence and function of the essential promoter domains at ∿-140 and ∿-30. Cis-stimulation, however, is sensitive to mutations in the 'enhancer cognate' promoter region whose sequence is highly homologous to the enhancer repeat. These data suggested that the rDNA enhancer acts by complexing with the polymerase I transcription factor(s) that binds to the ∿-140 and ∿-30 regions of the promoter (presumably the frog analogue of mouse factor D).

To test whether the enhancer functions by initially setting up a transcription complex or whether it is required for each round of transcription, we performed sequential addition assays. To this end, enhancerless and enhancer-bearing genes were either injected sequentially into Xenopus oocytes or were added sequentially to an in vitro transcription system that we have shown is sensitive to the effects of the enhancer. Once an rDNA promoter has preincubated with oocyte components and presumably assembled a transcription complex, it is rendered immune to the inhibitory effect of a subsequently added subcloned enhancer block. Additionally, the competitive ability of the subcloned enhancer block is increased by preincubating in the reaction, indicating that the enhancer can also stably bind required transcription factors.

O 750 A *Saccharomyces cerevisiae* **Protein Involved in Plasmid Maintenance Binds to and Affects the Transcription of Mating Type Specific Genes.** Steven Passmore, Randolph Elble, and Bik-Kwoon Tye. Section of Biochemistry, Molecular and Cell Biology. Cornell University, Ithaca, NY 14853.

The mcm1-1 mutation adversely affects the stability of minichromosomes in yeast. This mutation has a second phenotype: MATα mcm1-1 strains are sterile. The expression of both **a**- and α- specific genes are altered by this mutation. The steady state levels MFα1 and STE3 mRNAs in MATα mcm1-1 strains are dramatically reduced (< 5% of WT) . In addition , STE2 mRNA levels are slightly reduced (60% of WT) in MATa mcm1-1 strains. No derepression of STE2 mRNA was seen in MATα mcm1-1 strains. Both **a**-factor and BAR, however, are produced. We have overproduced and partially purified the MCM1 protein. We show, by both DNase protection and gel retardation assays, that the MCM1 protein binds the sequence 5'TTCCTAATTAGGAA (PAL). In addition, sequences related to PAL found within the MFα1 or STE2 operators are able to compete with PAL for binding. We will present evidence suggesting that the MCM1 protein and α1 interact.

O 751 EFFECTS OF A DNA HELIX-DESTABILIZING PROTEIN ON TRANSCRIPTION IN LIVING CELLS. E. Egyhazi,* M. Holst,* A. Pigon* and G. Patel.† (Dept. of Histology,* Karolinska Institute, Stockholm, Sweden and Dept. of Zoology†, Univ. of Georgia, Athens, Georgia, USA. Effects of microinjected rat liver DNA helix-destabilizing protein (HDP) and anti-HDP sera on the transcription of various RNAs in nuclei of Chironomus tenans salivary gland cells were investigated. The experimental protocol consisted of: (1) microinjection of pre-immune and immune sera into alternating nuclei of intact salivary glands; (2) radio-labelling of newly synthesized RNA; (3) separation of the nucleoli, Balbiani ring (BR) puffs and remaining chromosomal sections by microdissection; and (4) analyses of the radioactive RNA isolated from these nuclear components. Results showed that injected antisera had the greatest inhibitory effect on the RNA polymerase II-based transcription of BR puffs, 80%. Inhibition of RNA polymerase I-based transcription of nucleolar preribosomal RNA was about 70%, while effect on the hnRNA from the remaining chromosomes (I-IV) was about 40%. In all cases, the antiserum against the denatured sub-unit HDP was more inhibitory than that against the native HDP. In corollary experiments, microinjection of the HDP itself showed enhanced transcription of all RNAs but this effect was lesser than the inhibitory effect of antisera. Indirect localization by immunofluorescence showed immunoreactive-HDP to be heavily concentrated on BR 1 and BR 2. Western-blot analysis of C. tentans salivary gland nuclear proteins with anti-HDP (rat) sera showed cross-reactive protein bands with apparent M.W. ≅ 33,000, 42,000 and 65,000. These results suggest that a homologue of rat HDP and other C. tentans proteins, immunologically related to it, play an important role in transcription in vivo.

O 752 NEGATIVE REGULATION OF THE YEAST ENOLASE GENE *ENO1*, Alan E. Pepper and Michael J. Holland, Department of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616.

There are two yeast enolase genes, designated *ENO1* and *ENO2*. *ENO1* is constitutively expressed in cells grown on different carbon scources (glucose vs ethanol), whereas *ENO2* is induced twenty-fold in cells grown in a medium containing glucose. *Cis*-acting regulatory sites within the 5' flanking regions of these genes have been mapped by deletion analysis . *ENO1* and *ENO2* have functionally similar Upstream Activation Sequences (UASs) located ca. 500b.p. upstream from their respective translational initiation codons. *ENO1*, however, has an additional cis-acting site which acts to mask glucose induction. This Upstream Repression Sequence (URS) is 20-30b.p. in size, and is located ca. 210b.p. upstream from the *ENO1* initiation codon. *ENO1* genes containing small deletion mutations within the URS are induced in cells grown on glucose to levels similar to *ENO2*.

A genetic appoach has been deleloped to identify *trans*-acting factors which mediate URS function. To isolate mutations affecting repression of *ENO1*, a positive selection regime has been developed which selects for increased expression of the *ENO1* transcription unit in cells grown on glucose. A bacterial neomycin resistance gene, *APH(3')I* has been fused to *ENO1* 5' flanking sequences and integrated at the *ENO1* locus. Mutants, in *cis* or in *trans*, which relieve repression at the URS display resistance to high levels of the neomycin-related antibiotic G418. *Trans*-acting mutants are screened from the population of $G418^r$ mutants on the basis of their ability to synthesize high levels of ß-galactosidase from a second gene in the cell in which the *ENO1* 5' flanking sequences have been fused to the *E. coli LacZ* coding sequences. Utilizing this approach we hope to identify genes required for URS function.

O 753 THREE CHICKEN BETA-HATCHING GLOBIN GENE 5' TRANSACTING FACTORS, Neil D. Perkins, Robert H. Nicolas, Albrecht E. Sippel* and Graham G. Goodwin, Institute of Cancer Research : Royal Cancer Hospital, Chester Beatty Laboratories, Fulham Road, London, SW3 6JB, UK; *Zentrum fur Molekulare Biologie (ZMBH) der Universitat Heidelberg, Im Neuenheimer Feld 282, D-6900, Heidelberg, FRG.

The 5'-flanking DNAseI hypersensitive site of the chicken erythrocyte chromosomal β^H-globin gene has been shown to be present in fourteen day embryonic erythrocytes when the gene is transcriptionally active, but absent in adult chicken erythrocytes when the gene is inactive. Sequence specific nuclear DNA-binding proteins, which bind to this 5' DNAseI hypersensitive site, have been characterised by in vitro DNAseI footprinting, gel retardation and DMS methylation interference techniques. Three DNA-binding activities have, so far, been positively identified. One appears to be the ubiquitous DNA-binding protein, Nuclear Factor 1/'TGGCA' binding protein. Binding adjacent to this is an erythroid specific protein, designated H'. The third activity is a CCAAT binding protein, which is different from the CTF/NF-1 factor. These factors have been partially purified using DNA-affinity chromatography.

O 754 THE HIV-1 ENCODED *TRANS*-ACTIVATOR TAT FUNCTIONS AS A TRANSCRIPTIONAL ANTITERMINATOR. B. Matija Peterlin, Andrew F. Calman, Shaw-Yi Kao, Mark J. Selby, Howard Hughes Medical Institute, UCSF, SF, CA 94143

We investigated the mechanism of *trans*-activation by TAT which acts upon sequences in the HIV-1-LTR (TAR) to greatly increase the levels of viral replication and gene expression. Using a transient transfection assay into COS7 cells, where we cotransfected plasmids containg the HIV-1-LTR and non-functional or functional TAT, we found that increases in steady-state mRNA could account for the *trans*-activation by TAT. Next, we found that TAT affected the rates of transcriptional elongation through the HIV-1-LTR but not rates of initiation of transcription. In the absence of TAT, transcripts that initiated at the HIV-1 cap site terminated within the HIV-1-LTR. The predicted short transcripts (+55,+57, +59 nucleotides) were found in the cytoplasm of cells cotransfected with the non-functional TAT. In the presence of TAT, only full-length transcripts were found. Thus, TAT functions as a transcriptional antiterminator. We measured the stability of short and full-length HIV-1-LTR transcripts and the effects of cluster mutations in TAR upon *trans*-activation. In addition, we found no effect of TAT upon translation of mRNA containing the TAR sequence.

O 755 MOLECULAR INTERACTIONS BETWEEN CAMPTOTHECIN DERIVATIVES AND MAMMALIAN TOPOISOMERASE I. Yves Pommier, Christine Jaxel, Joseph M. Covey, Monroe E. Wall, and Kurt W. Kohn. National Cancer Institute, Bethesda, MD 20892.

Camptothecin (CPT) is a potent inhibitor of mammalian topoisomerase I (topo I). Topo I inhibition by CPT and derivatives was studied both by looking at purified Mouse leukemia (L1210) topo I-induced relaxation and cleavage of SV40 DNA, and by measuring DNA strand breaks in chinese hamster cells in culture by alkaline elution. Studies performed with purified topo I and CPT analogs substituted on the E ring showed that both the lactone and the 20-OH were necessary for activity, and that the 20-R stereoisomer was inactive. Analyses of CPT analogs modified on the A ring showed that substitutions at position 12 inactivated CPT, while similar 9-, 10-, or 11-substitutions did not. A good correlation was found between inhibition of DNA relaxation and induction of DNA cleavage, which suggests that topo I inhibition by CPT results from the stabilization of DNA cleavage intermediates. The genomic localization of drug-induced topo I-mediated DNA cleavage sites in SV40 DNA was similar for all derivatives. A consensus DNA sequence was found at the major cleavage sites. Alkaline elution measurements showed that CPT produced DNA single-strand breaks in DC3F cells and isolated nuclei. These breaks are all protein-associated, but it was necessary to use an appropriate SDS-containing lysis solution to demonstrate this point. The drug-induced protein-associated DNA breaks can form and reseal at 0^oC. Similar characteristics were found in cells as with purified topo I.

O 756 THE INTERACTION OF TWO TRANSCRIPTION FACTORS WITH DROSOPHILA RNA POLYMERASE II, David H. Price, Ann E. Sluder, and Arno L. Greenleaf, Department of Biochemistry, Duke University Medical Center, Durham NC 27710. Two transcription factors have been purified from Drosophila K_c cell nuclear extracts which affect the elongation properties of RNA polymerase II in vitro. Factor 5 (Price et al. (1987) J. Biol. Chem. 262, 3244-3255), which is also required for initiation of transcription, binds tightly to free RNA polymerase II and stimulates the rate of elongation of RNA polymerase II on a double-stranded DNA template. Another factor, SII, not absolutely required for initiation, eliminates the frequent pause sites that are encountered by RNA polymerase II. These studies utilize a dC-tailed double-stranded DNA template on which RNA polymerase II will initiate transcription without the need for accessory factors. The polymerase recognizes the single-strand/double-strand junction on this template and initiates transcription a precise distance upstream. One unexpected characteristic of the transcription of a dC-tailed template is that RNA polymerase II encounters a strong pause shortly after initiation. An RNase H activity has also been partially purified which causes the renaturation of the DNA template and displacement of the nascent RNA forming a transcription bubble similar to that seen in vivo. In the presence of RNase H, the two transcription factors stimulate the rate of elongation of RNA polymerase II to the in vivo rate. A model will be presented in which the transition from initiation to elongation is accompanied by a differential interaction of the two transcription factors with RNA polymerase II.

O 757 IDENTIFICATION OF THE GIBBON APE LEUKEMIA VIRUS ENHANCER BINDING PROTEIN. J. Quinn[1], M. Takimoto[1], N. Holbrook[2], and D. Levens[1]. 1. Lab. of Pathology, NCI. 2. Lab. of Molecular Genetics, NIA.
We have previously employed nuclease and gel retardation studies to localize a major determinant of enhancer function in the Gibbon Ape Leukemia Virus (GALV) to a 22 bp region. This region can function as an enhancer in transient expression assays and binds a nuclear protein. This 22bp segment contains a consensus binding sequence for the AP1 protein. To determine whether the GALV factor was the same as AP1, reciprocal competition studies with specific oligonucleotides and ion exchange chromatography were performed. Fractionation of a crude nuclear extract by Bio-rex 70 with gradient elution and assay with oligonucleotides for both the GALV region and a known AP1 site allowed us to seperate these proteins. Further analysis by gel retardation indicates that the GALV protein has much greater affinity than AP1 for the cognate GALV oligonucleotide and AP1 bound more tightly to its binding site than did the GALV factor; each of these oligonucleotides will cross compete when present in vast excess for both the GALV and AP1 proteins. Using a rapid affinity method for concentrating and enriching DNA binding proteins we can identify a protein which is specifically bound to the GALV enhancer region. This protein can be competed for by addition of oligonucleotides of the 22bp region containing enhancer function; no competition is observed with heterologous oligonucleotides. The GALV enhancer binding protein has a molecular weight of 40KD.

O 758 DNA BINDING PROPERTIES OF HUMAN GRANULOCYTE LACTOFERRIN-R.Ravazzolo, R.Barresi,V.Capra,C.Garré - Dept. Biology, I.S.M.I.,Univ.Genova,Italy.
Lactoferrin, a protein abundantly synthesized during the differentiation and maturation of the myeloid cells, was reported to play some role in the regulation of granulopoiesis. We have studied the DNA binding properties of this protein in granulocyte chromatin, looking for a possible regulatory role in transcription. Extraction of proteins from isolated nuclei with NaCl and subsequent electrophoretic analysis yielded, among other histonic and non histonic proteins, a very sharp band of 80 KD detectable in granulocytes and absent in lymphocytes and monocytes. The same band could be found after treatment of nuclei with DNase I or micrococcus nuclease or by incubation of nuclear suspensions with tRNA. Chromatography on single and double strand DNA cellulose columns confirmed the high affinity of this protein for DNA. Purification of the protein from nuclear salt extract by hydroxylapatite, heparin Sepharose and gel filtration chromatography, followed by immunochemical detection on western blot, allowed its identification as human lactoferrin. Work is in progress to verify if DNA binding is a true physiological event which can be related to the regulation of transcription and/or replication.

O 759 HUMAN PLASMINOGEN ACTIVATOR INHIBITOR-TYPE 1 (PAI-1) GENE: CHARACTERIZATION OF AN UNUSUAL PROMOTER REGION AND STUDY OF THE TRANSCRIPTIONAL REGULATION OF ITS EXPRESSION, Andrea Riccio, Leif R. Lund*, Peter A. Andreasen^, Keld Dano* and Francesco Blasi, Institute of Microbiology, University of Copenhagen, Oster Farimagsgade 2A, 1353 Copenhagen K., *Finsen Laboratory, Stranboulevarden 49, 2100 Copenhagen O, ^Institute of Biochemistry C, University of Copenhagen, Blegdamsvej 3C, 2200 Copenhagen, Denmark. Plasminogen activator inhibitor-type 1 is a Mr 54,000 specific inhibitor of urokinase-type (u-PA) and tissue-type (t-PA) plasminogen activators. PAI-1 production is influenced by several hormones and growth factors. In order to study the molecular mechanisms of this regulation we have cloned the human PAI-1 gene, identified its promoter region, and found that glucocorticoids, transforming growth factor-β and the phorbol ester PMA enhance PAI-1 transcription rate acting at a unique transcription initiation site. We have also found that 874 5' flanking nucleotides of the PAI-1 gene contain information enough to promote transcription and to respond to glucocorticoids when fused to the reporter CAT gene and transfected into human fibrosarcoma cells. Further analysis of the promoter region has revealed the presence of a moderately repetitive sequence, containing a TATA box, a GRE consensus, a Z-DNA forming sequence and two imperfect direct repeats at the extremities, few nucleotides 5' of the transcription initiation site. This finding raises the hypothesis that the human PAI-1 gene has been activated, by DNA insertion, during the evolution. Our efforts are now directed to analyze the role of the repetitive sequence in the regulation of PAI-1 gene by directed mutagenesis and to prove our hypothesis on gene activation by elucidating the PAI-1 promoter structure in species different from homo sapiens.

O 760 ANALYSIS OF THE PROMOTER-REGULATORY REGION OF THE RAT CYTOSOLIC P-ENOLPYRUVATE CARBOXYKINASE GENE, W.J. Roesler, G.R. Vandenbark and R.W. Hanson, Case Western Reserve University, Cleveland, OH 44106.

The transcription of the gene for P-enolpyruvate carboxykinase (GTP) (EC 4.1.1.32) (PEPCK) from the rat is stimulated by cAMP and glucocorticoids and inhibited by insulin. Putative hormone responsive elements have been identified within -550 bp of the start site of transcription by deletion analysis of stably transfected chimeric genes. When this region of the gene was analyzed by DNAase I footprinting using protein extracted from the nuclei of rat liver, six protected regions were noted. One of these included the sequence from -94 to -77 and corresponded to a putative cAMP regulatory element (CRE). An oligonucleotide which spanned this region of the promoter was analyzed for its ability to bind nuclear proteins using a gel retardation assay. Two major bands were shifted. A relative Kd of 4×10^{-10} M was calculated by Scatchard analysis. Mutations within the CRE core sequence of 5'-TTACGTCAG-3' drastically reduced the binding affinity of nuclear proteins as shown by gel shift competition, while mutations outside of this region had only a marginal effect on binding. A second region of the PEPCK promoter at -149 to -135, which contains a repeat (8 of 9 base match) of the core CRE between -94 and -77, also bound nuclear proteins. Competition analysis using the synthetic oligonucleotides in DNAase I footprinting indicated appropriate changes in digestion patterns of the two CRE's. Correlative functional studies and purification of the CRE binding protein from rat liver is currently in progress.

O 761 NUCLEAR PROTEINS WHICH BIND THE HUMAN FETAL GLOBIN GENE PROMOTER, Kirsten L. Rood, Deborah L. Gumucio, Todd A. Gray and Francis S. Collins, University of Michigan, Ann Arbor, MI 48109. The human fetal to adult hemoglobin switch is characterized by reduced γ-globin gene expression and increased activity of the adult β-globin gene. In the syndrome known as hereditary persistence of fetal hemoglobin (HPFH), this normal regulatory switch fails, resulting in elevated expression of the γ-globin gene in adult life. Point mutations have been identified in the promoter region of γ-globin genes cloned from some individuals with HPFH. These mutations appear to mark important cis-acting sequences since, after transfection into human erythroid (K562) cells, the mutant promoters direct over-expression of a linked reporter gene relative to the normal promoter. Using gel retardation and DNaseI footprinting assays, we are looking for trans-acting factors in K562 cells which may interact with these sequences. We have detected proteins which bind the duplicated CCAAT box motifs in the γ-globin promoter. The Greek HPFH mutation at -117 may increase the affinity of this interaction. A second protein binds in the vicinity of an octamer sequence, ATTTGCAT, found on the antisense strand. A synthetic double-stranded oligonucleotide (30-mer) containing the V_H immunoglobulin octamer sequence competes effectively for this binding activity. In addition, the binding of this protein is severely reduced by an HPFH mutation at -175. These findings suggest that this protein may represent a form of octamer binding protein with repressor activity.

O 762 PRODUCTION OF TRANSGENIC MICE EXPRESSING THE HUMAN K1 KERATIN GENE. D. Roop, C. Cheng, T. Mehrel, H. Nakazawa, D. Rosenthal, S. Yu, P. Steinert and S. Chung. NCI/NIH Bethesda, MD 20892

Two of the major differentiation products of mammalian epidermis are keratins K1 and K10. These proteins assemble into filaments (intermediate filaments) and together with microtubules and microfilaments form the cytoskeleton of epidermal cells. The genes encoding keratins K1 and K10 are expressed post-mitotically after cells have committed to terminal differentiation and migrated away from the basement membrane. Expression of the K1 gene preceeds that of the K10 gene. Attempts to identify sequences regulating the expression of these genes have been hampered by difficulties in inducing high levels of expression of these genes in primary epidermal cell cultures. As an alternative approach, we have produced transgenic mice containing the human K1 gene. The K1 gene is located within a 12Kb Eco RI fragment and flanked by 2 kb upstream and 3kb downstream. To discriminate between expression of the human K1 gene and the endogenous mouse K1 gene, an antibody was produced against a synthetic peptide corresponding to unique sequences at the C-terminal of the human protein. Immunoblot analysis indicates that the human K1 gene is expressed tissue specifically. In addition, developmental studies indicate that both the human and endogenous mouse K1 genes are induced at day 15 and this is coincident with the stratification of embryonic epidermis observed morphologically. Sequences responsible for tissue- and developmental-specific expression of the K1 gene are currently being determined by producing transgenic mice with deletions of the human K1 gene.

O 763 Purification and Characterization of RNA Polymerase I Transcription Factors, S. D. Smith, S.-B. Hong, H.-F. Yang-Yen, D. Lowe, and L. I. Rothblum, Baylor College of Medicine, Houston, TX 77030.

In vitro transcription by RNA polymerase I requires only one part of the ribosomal DNA promoter, referred to as the core promoter element (CPE), whereas in vivo transcription requires both the CPE and an upstream element (UPE). Transcription by RNA polymerase I is species specific, i.e. primate cells will not transcribe rodent ribosomal RNA genes. We have purified (25,000 fold) an activity from nuclear extracts of rat cells which is capable of reprogramming the heterologous transcription system. SDS-PAGE analysis of the purified material suggests that it is a 94 kd protein. Characterization of the activity indicates that it requires the CPE, and that it may form a stable complex.

A second activity (SF-1) that increases the efficiency of template utilization, and apparently requires two separate regions of the UPE has been partially purified. Sequences between -149 and -127 are required for SF-1 activity, whereas SF-1 forms a protein-DNA complex (detected by gel retardation assays) with nucleotides 3' of -126. Preliminary experiments indicate that these two aspects of SF-1 can be separated.

We have purified an HMG-like protein, p16. This protein binds both to the NTS of the rat ribosomal DNA repeat and to the ETS. p16 stimulates the transcrption of templates that contain either binding site.

O 764 A TRANSCRIPTIONALLY ENGAGED RNA POLYMERASE II MOLECULE IS PRESENT AT THE START OF UNINDUCED HEAT SHOCK GENES, Ann E. Rougvie and John T. Lis, Cornell University, Ithaca, NY 14853.

Within minutes of increasing the temperature of cells, a high density of RNA polymerase II molecules accumulates along the entire transcription unit of a heat shock gene. Surprisingly, even prior to the induction of heat shock, approximately one RNA polymerase II molecule is found on the promoter region of the major heat shock gene of Drosophila, hsp70 (Gilmour and Lis, 1986). This RNA polymerase molecule is detected *in vivo* by protein-DNA crosslinking of tissue culture cells and of intact Drosophila embryos, and also in isolated nuclei by sarkosyl-released run-on assays. The promoter-associated polymerase is transcriptionally engaged but apparently arrested (or pausing) after transcribing 10-30 bases and is unable to penetrate further into the hsp70 gene without heat shock induction. Crosslinking and nuclear run-on assays show RNA polymerase II is also associated with the transcription start of the uninduced hsp26 gene, suggesting a common step in the mechanism of transcription initiation and the subsequent activation of these genes.

DS Gilmour and JT Lis. 1986. RNA Polymerase II Interacts with the Promoter Region of the Noninduced hsp70 gene in Drosophila melanogaster cells. Mol. Cell. Biol.6, 3984-3989.

O 765 **PURIFICATION of TGGCA PROTEIN, a PROTEIN COMPONENT of a CELLULAR ENHANCER, R.A.W. Rupp, U. Borgmeyer and A.E. Sippel, ZMBH, INF282, 69 Heidelberg, FRG.**

The shortest, but still tissue-specificly active DNA-fragment of the distal (-6.1kb) chicken lysozyme gene enhancer contains a high-affinity binding-site (BS1b) for TGGCA protein, the chicken homologue of HeLa cell NF-I. **In vitro** methylation protection experiments revealed characteristics of TGGCA protein-binding to BS1b (i.e. 1 hypermethylated and 6 protected G(N7)-po- sitions). Genomic footprinting with lysozyme expressing (E26-transformed myeloblasts, MC29-transformed myelomonocytes) and non-expressing (AEV-transformed erythro-blasts) chicken cell lines prove **in vivo** binding of TGGCA protein in case of the active enhancer. We have isolated TGGCA protein from chicken liver. The final purification was achieved by preparative mobility shift electrophoresis (**PMSE**), using a synthetic DNA-binding site. Analyzing the components of the native protein-DNA complex on SDS-PAGE, we identified 6 protein species of slightly different molecular weight (36.8-29.8kd). From **in vitro** DMS-inter-ference experiments we conclude that all these protein species bind identi-cally to the TGGCA-binding site. Partial V8 protease digests indicate a high relationship in primary structure.

O 766 INTERACTION OF EBP20 WITH ENHANCERS OF AVIAN RETROVIRUSES, Tom Ryden and Karen Beemon, Johns Hopkins University, Baltimore, Md 21218.

We have established that the rat enhancer binding protein EBP20 (and an analogous protein from chicken), has high affinity for sequences between -225 and -188 within the LTR enhancer of Rous sarcoma virus (RSV) and sequences between 813 and 872 in the RSV *gag* enhancer. Binding was also observed within the LTRs of RAV-0 and Fujinami sarcoma virus. Inspection of other retroviral LTRs yielded an 18 bp identity to the RAV-0 binding site in the avian myeloblastosis virus LTR. Footprinting shows that it, as well as two other sites within the U3 region of the LTR, binds EBP20. From the avian virus binding sequences, as well as previously established footprints of other viral regulatory domains (Johnson et al., Genes and Dev. 1: 133; Graves et al., Cell 44: 565), we have derived a consensus sequence, $T^{T}/_{G}NNGPyAA^{T}/_{G}$. This is being used to make minimal mutations at the conserved nucleotides in order to directly assess the relationship between EBP20 binding and transcriptional activity.

O 767 TOPOISOMERASE I CLEAVAGE SITES IDENTIFY DNA BINDING PROTEIN DOMAINS. John Chiorini, Roger B. Cohen, John A. Thompson and Brian Safer, Section on Protein Biosynthesis, LMH, NHLBI, Bethesda, MD 20892

The topological state and accessibility of cis-acting regulatory sequences to specific DNA binding proteins are thought to modulate the activity of genes. Since several potential topoisomerase I binding sites are identified in the adenovirus 2 major late promoter, we analyzed the camptothecin-enhanced cleavage pattern of this gene produced by incubation of linear or supercoiled DNA with K562 nuclear extract. Prominent cleavages were located in regions flanking the upstream promoter sequence, TATA box and E1a-like enhancer sequence as well as adjacent to the newly identified downstream promoter sequence. Highly purified calf thymus topoisomerase I generated a similar pattern. An analogous correlation between toposisomerase I cleavages and cis-acting promoter elements was observed for the mouse β-globin and human eIF-2α genes. While the functional significance of this observation is not yet known, topoisomerae I mapping using unfractionated nuclear extracts and camptothecin appears to be useful in identifying potential cis-acting elements.

O 768 MULTIPLE BINDING SITES FOR GLUCOCORTICOID RECEPTOR WITHIN AMYLASE PROMOTERS, Linda Samuelson, Jan Carlstedt-Duke*, Jan-Ake Gustafsson*, and Miriam Meisler, University of Michigan, Ann Arbor, MI 48109, and *Karolinska Institute, Huddinge, Sweden. There are three different promoters for expression of the mouse amylase multigene family. The Amy-2 promoter is very active in the pancreas and expressed at a low level in the liver. The Amy-1 gene has two distinct promoters; the strong promoter Amy-1-S is active in the parotid gland, and the weak promoter Amy-1-L is active in the liver, parotid, and pancreas. All three amylase promoters are induced by glucocorticoids. To investigate the mechanism of this induction, we have studied the interaction of these promoters with purified glucocorticoid receptor. Glucocorticoid receptor protected several discrete regions of each promoter from DNAse digestion. Each binding site contains sequences with homology to the consensus TGTTCT. The footprints occur in several interesting regions. Within the region -374 to -158 of the Amy-1-L promoter there are two binding sites, separated by approximately 100 bp. One footprint is located within a potential stem loop structure (Mol. Cell. Biol. 6:969). There are five binding sites between -270 to +32 of the Amy-1-S promoter; one of these overlaps the TATA box. Within -208 to +115 of the Amy-2 promoter there are four binding sites. One overlaps a putative pancreatic enhancer, and another, centered at -8, occurs in a unique location between the TATA box and cap site. Site-directed mutagenesis has been used to change the sequence around position -8 from TGTTCT to TACTAT. This change eliminates the footprint over this region, but does not effect the other three footprints within the Amy-2 promoter.

O 769 CHARACTERIZATION OF THE POSITIVE AND NEGATIVE REGULATORY ELEMENTS THAT CONTROL THE OVALBUMIN GENE, Michel M. Sanders, Departments of Medicine and Biochemistry, University of Minnesota, Minneapolis, MN 55455.

Because four classes of steroid hormones induce the chicken egg white genes, the oviduct remains an ideal model system for examining the mechanism of action of steroid hormones. Our approach has been to test the functional significance of sequences in the 5'-flanking region of the ovalbumin gene by transferring recombinant genes into primary oviduct cell cultures that retain responsiveness to steroids. The promoter and 8 kb of 5'-flanking sequence from the ovalbumin gene was fused to the structural gene for chloramphenicol acetyltransferase (CAT). Expression of the transfected DNA is assessed by an assay based on the enzymatic activity of the CAT protein. Serial deletions revealed that sequences between -880 and -585 are essential for steroid-dependent expression of the ovalbumin promoter. A negative regulatory element (NRE) that represses the ovalbumin promoter was found between sequences -350 and -100. It is postulated that in the absence of steroids, the NRE represses expression of this gene and that steroids act either directly or indirectly through the steroid response element (SRE) to relieve this repression. To further define the SRE, deletional and insertional mutations were made in it and these indicate that sequences between -880 and -711 are not essential for steroid-dependent expression. This limits the SRE to the region -711 to -585. Heterologous genes containing the SRE, the NRE, or both regulatory elements fused to the thymidine kinase promoter did not confer their respective activities to this promoter. Thus, these regulatory elements may be conditional and may only regulate the homologous promoter. Experiments are in progress to test this hypothesis using other egg white promoters. In addition to steroids, the ovalbumin promoter requires the permissive effects of insulin for maximal expression. Transfection of serially deleted Ovalbumin/CAT fusion genes indicates that insulin is necessary for maximal expression of the promoter even when the SRE and NRE are both deleted, implying that the effect of insulin is on a general transcription factor(s) rather than on proteins that may interact with these elements. These results indicate that two promoter-specific regulatory elements control the ovalbumin promoter and that insulin does not exert its effects at either regulatory element.

O 770 TISSUE-SPECIFIC AND NON-SPECIFIC TRANSCRIPTION FACTORS BINDING TO THE RAT GROWTH HORMONE PROMOTER, F. Schaufele and T.L. Reudelhuber, University of California, San Francisco, CA 94143.

Rat growth hormone (rGH) gene expression is highly tissue-specific, being restricted to a subpopulation of cells within the anterior pituitary gland. Nuclear extracts prepared from rat pituitary tissue culture cell lines which either express the rGH gene (GC cells) or not (235-1 cells) were analysed for the presence of factors binding to rat growth hormone promoter sequences. The ability to bind of a factor, GHF, previously described as being localized to pituitary cell lines was important to in vitro as well as in vivo rGH transcription. GHF could not be detected in 235-1 cells confirming its tight association with rGH expression. Nuclear extracts from rat liver did not contain GHF binding activity but footprinted an overlapping activity (GAG) between -127 and -142 which was also present in GC extracts. Binding of GHF and GAG in GC extracts was mutually exclusive. A third factor binding between -218 and -237 (BBF) was present in GC, 235-1 and liver extracts but the gel retardations and footprints were slightly different between GC and 235-1 extracts; the liver footprint was identical to the GC footprint. Deletion of this footprinting sequence impaired expression from the growth hormone promoter transfected onto GC cells. Thus, it appears that optimal expression of the rat growth hormone gene involves promoter components displaying varying degrees of tissue-specificity.

O 771 A REGION WITHIN THE MVM CAPSID CODING ORF IS REQUIRED FOR ITS EFFICIENT EXPRESSION FROM A BPV SHUTTLE VECTOR, Robert Schoborg, Laura Labieniec-Pintel, Betty Glinsmann and David J. Pintel, University of Missouri-Columbia, Columbia, MO 65212.

MVM(p) and MVM(i) are two serologically similar variants of the autonomous parvovirus Minute Virus of Mice, which are reciprocally restricted for growth in mouse cells of dissimilar differentiated phenotype. MVM(p) replicates only in mouse fibroblast lines while MVM(i) replicates preferentially in T-lymphocyte lines (1). Hybrids between the two cell types are permissive for both viral strains suggesting that the restrictive host lacks a factor(s) required for growth of the restricted strain (1). The block to replication has been shown to be intracellular; the first stage of viral DNA synthesis proceeds normally but viral mRNA fails to accumulate (2). Genetically engineered recombinants have localized a viral determinant required for the growth of MVM in fibroblasts to a region within the capsid coding ORF (3).

In order to further characterize this differentiation - state sensitive viral element we have focused our analysis on the transcription of MVM(p) and MVM(i) recombinants in murine fibroblasts using Bovine papillomavirus (PBV) shuttle vectors. Our analysis has identified the same region defined by the recombinants described above to be required for efficient expression of the MVM capsid genes in fibroblasts. Current experiments are designed to determine whether the decrease in MVM mRNA seen in these recombinants is due to an inhibition of mRNA initiation and whether the viral element acts in cis or in trans. (1) Tattersall and Bratten, J. Virol. 46:944, 1983; (2) Spalholz and Tattersall, J. Virol. 46:937, 1983; (3) Tattersall and B. Hirt, IInd International Workshop on Parvoviruses, Oxford, 1987.

O 772 TRANSCRIPTIONAL CONTROL ELEMENTS IN THE RAT CORTICOTROPIN RELEASING HORMONE GENE. Audrey F. Seasholtz, Robert C. Thompson, and James Douglass, Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201

The key hypothalamic releasing factor involved in the mammalian stress response is corticotropin releasing hormone (CRH). CRH stimulates the synthesis and secretion of adrenocorticotropin from the anterior pituitary which in turn stimulates the synthesis and secretion of glucocorticoids from the adrenal cortex. The rat CRH gene has recently been isolated and sequenced (Thompson, R.C., Seasholtz, A.F. and Herbert, E. (1987) Mol. Endo. 1: 363). It exhibits a structural organization similar to that of the human gene, having two exons separated by an intervening sequence. The 335 bp of DNA immediately 5' to the putative mRNA cap sites for the human and rat CRH genes show 94% homology, suggesting that this region contains regulatory elements which have been conserved through evolution. In order to identify transcriptional control elements, different regions of the 5' flanking DNA from the rat CRH gene have been linked to the coding sequence of the E. coli chloramphenicol acetyltransferase gene. These fusion gene constructs have been transfected into a variety of cell lines including PC-12 (rat pheochromocytoma), AtT-20 (mouse anterior pituitary), and CV-1 (monkey kidney) cells. We have localized a cAMP responsive element between -238 and -182 bp relative to the CRH cap site. DNAse I footprinting and competition studies are being used to identify protein binding sites in the CRH 5' flanking DNA. We plan to determine whether the protein(s) involved in mediating cAMP induction of the rat CRH gene are specific for the CRH cAMP responsive element or recognize cAMP responsive elements from other genes. Additionally, we are attempting to localize glucocorticoid and tissue-specific transcriptional control elements in the rat CRH gene.

O 773 INTERACTION OF HUMAN TF III A WITH A HOMOLOGOUS GENE CODING FOR 5S rRNA Klaus H. Seifart, Rainer Waldschmidt, Lingru Wang, Dieter Jahn and Edgar Wingender, University of Marburg, D-3550 Marburg, F.R.G.

The gene coding for the major human 5S rRNA was completely synthesized chemically and cloned into pUC13. This approach was taken because attempts to isolate the human 5S gene have thusfar yielded pseudogenes or variant genes of unknown function. The synthetic gene was transcribed correctly and efficiently by RNA polymerase III either in a crude HeLa-cell extract or in a system reconstituted from purified transcription factors. Human TF III A was highly purified by a procedure to be presented. In contrast to TF III A from X. laevis oocytes, human TF III A does not footprint on the Xenopus 5S gene. Comparative footprint experiments will be presented showing an altered interaction of human TF III A with the synthetic human and X. laevis 5S gene respectively, reemphasizing the importance of homologous in vitro systems.

O 774 DNA-PROTEIN INTERACTIONS THAT REGULATE HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR GENE EXPRESSION. M.Frances Shannon, Jennifer R. Gamble, Jennifer Smith and Mathew A. Vadas, Division of Human Immunology, Institute of Medical and Veterinary Science, Box 14, Rundle Mall Post Office, Adelaide, South Australia.

The gene for human granulocyte/macrophage colony stimulating factor (GM-CSF) is expressed in a tissue specific as well as activation-dependent manner. The interaction of nuclear proteins with the promoter region of the GM-CSF gene that are likely to be responsible for this pattern of GM-CSF expression was investigated. We show that nuclear proteins interact with DNA fragments from the GM-CSF promoter in a cell specific manner. A region spanning two cytokine specific-sequences, cytokine-1 (5'GAGATTCCAC3') and cytokine-2 (5'TCAGGTA3') bound two nuclear proteins (nuclear factor (NF)-GMa and NF-GMb) from GM-CSF expressing cells in gel retardation assays. NF-GMb was inducible with phorbol myristate acetate and accompanied induction of GM-CSF message. NF-GMb was absent in cell lines not producing GM-CSF some of which had other distinct binding proteins. NF-GMa and NF-GMb eluted from a heparin sepharose column at 0.3 and 0.6M KCl respectively. We hypothesize that the sequences CK-1 and CK-2 bind specific proteins and are involved in regulating GM-CSF transcription.
The role of these sequences in directing cell-specific or inducible gene expression is being investigated using cell transfection assays. Results from deletion mutagenesis of the gene promoter and **in vivo** titration assays will be presented.

O 775 TRANSCRIPTIONAL REGULATION OF VSG GENE 118 OF TRYPANOSOMA BRUCEI, Cathy Shea, Mary Gwo-Shu Lee, Gloria Rudenko, Alan Morrison, and Lex H.T. Van der Ploeg, Columbia University, New York, NY 10032.

Antigenic variation in T. brucei results from the periodic activation of different variant cell surface glycoprotein (VSG) genes. One of the mechanisms by which a VSG gene can be activated is the duplicative transposition of a silent basic copy VSG gene to a transcriptionally active telomeric VSG gene expression site. We determined that the duplicative transposition of VSG gene 118 resulted in the activation of a cotransposed promoter with striking homology to the T. brucei rDNA promoter. Transcription at the expression site is insensitive to the drug alpha-amanitin, a characteristic of RNA polymerase I. Therefore, in contrast to transcription of other protein coding genes in T. brucei and other eukaryotes, transcription of the expression sites may occur by RNA pol I or an RNA pol I-like enzyme. Transcription of protein coding genes by RNA pol I may be made possible by the fact that in trypanosomes a 5' cap, essential for translation, is added to the mRNAs by trans-splicing of the pre-mRNA and the 140 nt pol II derived mini-exon precursor. The VSG pre-mRNA is polycistronic, containing two different VSG gene coding exons. The function of polycistronic pre-mRNAs in trypanosomes, the role of RNA pol I in VSG gene transcription and the importance of positional control for VSG gene transcription regulation is being studied.

O 776 CIS-TRANS REGULATION FOR LIVER-SPECIFIC EXPRESSION OF THE HUMAN ALPHA-1-ANTITRYPSIN GENE. Rong-Fong Shen, Yi Li, Sophia Y. Tsai and Savio L.C. Woo. Howard Hughes Medical Institute, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

The 5' flanking sequence of the human alpha-1-antitrypsin (AAT) gene contains multiple cis-regulatory elements that regulate its tissue-specific expression. These cis-regulatory elements include a distal enhancer and a proximal element located 348 bp upstream of the CAP site. Using band-shifting and foot-printing assays, we have shown that nuclear proteins of hepatic origin were able to specifically interact with the proximal element at the -52 to -78 region which contains a palindromic domain. To test whether the interaction between hepatic nuclear proteins and the cis-acting elements is essential for tissue-specific expression of the AAT gene, in vitro transcription assays were performed using nuclear extract prepared from rat liver or HeLa cells. It was observed that the AAT gene could be properly transcribed by the nuclear extract of rat liver but not by that of HeLa cells. However, by supplementing Hela nuclear extract with rat liver extract, proper transcription of the AAT gene was detectable by S_1 analysis, suggesting that positive hepatic nuclear factor(s) are responsible for AAT gene expression in the liver. Purification and biochemical characterization of the hepatic nuclear factor(s) will enhance our understanding of the regulatory mechanism for liver-specific gene expression.

O 777 DIFFERENTIAL EFFECT OF PROTEIN SYNTHESIS INHIBITION ON PROTO-ONCOGENE TRANSCRIPTION IN MOUSE LYMPHOCYTES, Maria C. Birchenall-Sparks and Fancis W. Ruscetti, Laboratory of Molecular Immunoregulation, BRMP, DCT, NCI, Frederick, MD 21701.
The mechanisms by which growth factors promote cellular proliferation are not clearly understood. We have found that treatment of lymphoid cells with growth promoting factors such as Interleukin 2 (IL 2) and Interleukin 3(IL 3) leads to transient expression of the cellular proto-oncogenes c-fos, c-myc and c-myb. In both IL 2 and IL 3 dependent cell lines c-fos transcription peaks 30 min after growth stimulation, whereas c-myc and c-myb expression is maximal 60 to 120 min after IL 2 treatment. The observation that c-fos gene expression is stimulated before the increase in transcription of c-myc and c-myb raises the question of whether activation of the synthesis of c-fos or other rapidly induced proteins migth be required for subsequent activation of genes such as c-myc and c-myb. We have used cycloheximide (CHX), a protein synthesis inhibitor, to investigate whether synthesis of new proteins plays a role in the rapid induction and subsequent repression of the transcription of these genes. CHX potentiates the transcription of c-fos when added before growth factor stimulation to CT6 (mouse cytotoxic T cells) as seen by nuclear run-off transcription assays. Under the same conditions, CHX inhibited the increase in transcription of c-myc and c-myb. The pretreatment with CHX caused a superinduction of the steady state levels of these proto-oncogenes. The results suggest: 1) in CT6 c-fos transcription is activated by a protein synthesis independent mechanism, while c-myc and c-myb stimulation requires new protein synthesis; 2) CHX affected the stability of these proto-oncogenes mRNAs, suggesting the involvement of CHX sensitive proteins in the degradation of the c-fos,c-myc and c-myb mRNAs.

O 778 CONTRIBUTION OF INDIVIDUAL NUCLEAR FACTORS TO THE TRANSCRIPTIONAL REGULATION OF THE MOLONEY MURINE LEUKEMIA VIRUS ENHANCER, Nancy A. Speck, Boris Renjifo, Erica Golemis, and Nancy Hopkins, Massachusetts Institute of Technology, Cambridge, MA 02139.
The Moloney murine leukemia virus (Mo-MuLV) contains a transcriptional enhancer located in the U3 region of the viral genome. The enhancer is organized as a 75 bp repeat, and contains binding sites for at least six distinct nuclear factors; LVa, LVb, LVc, and factors that bind to the NF-1, SV40 core, and glucocorticoid response elements (GRE) (Speck and Baltimore 1987 Mol. Cell. Biol. 7, 1101-1110). We have disrupted the binding sites for each of these factors by oligo-directed mutagenesis. Mutated enhancers have been cloned into the Mo-MuLV viral genome and transfected into NIH 3T3 cells to obtain viral stocks. We have analyzed the affect of each of these mutations on viral transcription in RAT-1 cells by S1 assays, and by comparing viral titers. Viral transcription can be stimulated 3 fold by the addition of dexamethasone to chronically infected RAT-1 cells. The response to dexamethasone was found to depend on sequences within the GRE, as expected. Several mutations (NF-1, core, and LVb) decreased the basal level of transcription by 3-5 fold, but did not affect the induction by dexamethasone.

O779 THE BPV E2 PROTEIN BLOCKS THE ACTIVITY OF A CELLULAR FACTOR REQUIRED FOR TRANCRIPTION, BY BINDING TO AN OVERLAPPING SITE.
A. Stenlund, R. Li, J. Knight and M. Botchan. Department of Molecular Biology, U C Berkeley, Berkeley CA 94740.
The BPV P1 promoter has an unusual structure with a major portion of its regulatory sequences located downstream of the cap site. A region that is absolutely required for transcription both in vitro and in vivo is located in a 28 nt sequence immediately downstream of the cap site. This sequence also contains the recognition sequence for a putative binding site for the viral transfactor E2. Co transfection of the P1 promoter with with an E2 expression construct leads to suppression of transcription from the P1 promoter. This is in contrast to several other BPV promoters which are activated by the E2 gene product. We will show by a variety of approaches, that this repression is caused by E2 binding to a site that overlaps with the binding site for a crucial cellular transcription factor, thereby preventing the cellular factor from exerting its function.

O 780 MULTIPLE FUNCTIONAL MOTIFS IN THE ENHANCERS OF CHICKEN U1 AND U4 SMALL NUCLEAR RNA GENES, William E. Stumph, Kenneth A. Roebuck, Kathleen J. McNamara and Zulkeflie Zamrod, San Diego State University, San Diego, CA 92182.

In vertebrates, transcription of small nuclear RNA genes by RNA polymerase II is stimulated by enhancer sequences located approximately 200 bp upstream of the transcription initiation site. In most cases, an octamer sequence (consensus ATGCAAAT) and a GC box (consensus [G/T]GGGCGGPuPuPy) are present in the snRNA enhancer region, and a number of expression studies have demonstrated that the octamer and GC box are important functional components of snRNA gene enhancers. Using a Xenopus oocyte expression assay, we have identified a third element essential for chicken U1 and U4B gene enhancer activity. Deletion of this "SPH motif" (SphI Postoctamer Homology) from either the U1 or U4B enhancer region results in the complete loss of enhancer activity in the oocyte system, even though these constructions retain both the octamer and GC box. In contrast, U1 and U4B templates which lack the octamer sequence but retain the SPH motif exhibit only a 2-fold reduction of enhancer activity. Moreover, in oocytes the SPH motif is required for the U1 and U4B genes to be efficient transcriptional competitors with intact snRNA genes. The correlation of the SPH motif with both enhancer activity and transcriptional competition suggests the existence of a cellular transcription factor which interacts with the SPH motif of snRNA gene enhancers.

O 781 THE CCAAT DISPLACEMENT PROTEIN : A POSSIBLE REPRESSOR OF γ-GLOBIN GENE TRANSCRIPTION, Giulio Superti-Furga and Meinrad Busslinger, Research Institute of Molecular Pathology Vienna, Austria and Genentech Inc., South San Francisco, California.

We have identified in nuclear extracts from several vertebrate cell lines (including Hela) a protein which is homologous to the CCAAT displacement protein (CDP) of sea urchins (Barberis et al., Cell 50, 347-359). The vertebrate CDP binds to the duplicated CCAAT region of the human Aγ-globin gene promoter but not to the equivalent regions of the ε-, β- or α1-globin genes. By themselves, neither of the two Aγ-globin CCAAT sequences is recognized by the CDP, while each of them can be bound by CCAAT-box binding factor(s) present in all extracts tested. The CDP can be clearly distinguished from the latter by several criteria : a) sequence requirements and competition behavior, b) molecular weight and size of protein-DNA complex, c) chromatographic behavior. Because the CDP competes favorably with the CCAAT-box binding factor, a putative transcriptional activator, for binding to overlapping sequences in *in vitro* binding reactions, we postulate that the CDP acts as a negative regulator of γ-globin gene transcription. The protein contacts three TGA-motifs, one of which was found to be mutated in individuals with the Greek type of hereditary persistence of fetal hemoglobin (HPFH). Point mutations in all three of these motifs affect the binding of the CDP *in vitro*. In stably transformed Hela cells these mutations lead to a derepression of Aγ-globin gene transcription.

O 782 SELF-SPLICING OF YEAST MITOCHONDRIAL GROUP I CONTAINING PRECURSOR RNAs, H.F. Tabak, G. Van der Horst, J. Smit, A.J. Winter and Y. Mul, Section for Molecular Biology,Laboratory of Biochemistry, University of Amsterdam, AMC - Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

A number of yeast mitochondrial group I containing precursor RNAs show self-splicing in vitro. Apart from the normal splicing products: intron+ 3'-exon, ligated exons and circularized intron, a number of abnormal products are also observed. We have previously shown that self-splicing of aI3 intron containing RNA transcribed from part of the cytochrome oxidase subunit I gene, results in formation of interlocked RNA circles. We now find that interlocked circle formation occurs also with large ribosomal precursor RNA containing large deletions in the intron internal reading frame. They consist of intron circles and circles containing 5'-exon sequences. Multiple forms have been identified using a two dimensional polyacrylamide gelelectrophoresis technique.

O 783 TRANSCRIPTION OF *XENOPUS* U snRNA GENES, Graham Tebb, Michael Kazmaier, Huw Parry, Nina Dathan and Iain W. Mattaj, EMBL, D-6900 Heidelberg, FRG.

We are studying the transcription of *Xenopus* U snRNA genes, especially U2, with the aims of defining which DNA sequences are involved in transcriptional control and of analysing the protein factors which interact with them. Of the four sites of factor-DNA interaction we observe within the U2 promoter, we have shown by microinjection of mutated genes that two are not required for efficient transcription of the gene in the *Xenopus* oocyte. The remaining two binding sites are the proximal and distal sequence elements (PSE and DSE): the PSE appears specific to U genes and is essential for transcription while the DSE is enhancer-like. The U2 DSE consists of adjacent binding sites for Spl and the "octamer binding protein", and both of these motifs contribute to DSE function although neither is essential. DSEs of other U snRNA genes also appear to be bipartite, and all contain an octamer motif. Among those we are studying is U6, which unlike the other U genes characterized thus far is transcribed by RNA polymerase III. We have shown that the U2 and U6 DSEs bind a common factor *in vitro* and *in vivo*, and that this factor binds to the octamer sequence. We have partially purified a protein able to bind to this sequence and are undertaking a similar purification to attempt to identify factors which can bind to the PSE.

O 784 ANALYSIS OF REGULATORY ELEMENTS FOR THE TISSUE SPECIFIC EXPRESSION OF THE MOUSE TRANSFERRIN GENE. Manfred Theisen, Rejean Idzerda, Wendy Ran, Richard R. Behringer*, Ralph L. Brinster* and G. Stanley McKnight, Department of Pharmacology Sj 30, University of Washington, Seattle WA 98195 and * Laboratory of Reproductive Physiology, University of Pennsylvania, Philadelphia PA 19104.

The iron binding protein transferrin is expressed in a tissue-specific and developmental manner in all vertebrates. In adult mice, transferrin mRNA is found at high levels in the liver and in the mammary gland of pregnant females. Using transgenic mice carrying 5' transferrin gene sequences linked to the coding region of the human growth hormone gene, we have shown that 581 base pairs (bp) of upstream sequences are sufficient to direct liver-specific expression of the transgene. By contrast, mammary gland-specific expression of the transgene requires sequences located upstream of the -581 region but within -3 kilobases.

Fusion constructs containing 581 bp of the transferrin promoter linked to the chloramphenicol acetyl-transferase (CAT) gene are highly active in both rat and mouse transferrin-expressing liver cell lines but not in a non-expressing rat liver cell line or in mouse fibroblasts. Deletion analyses of the transferrin promoter suggest that the liver specific regulatory element lies within 180 bp 5' of the transcription start site. *In vitro* DNA-protein binding experiments reveal a complex pattern of interactions between nuclear proteins derived from mouse liver extracts and this liver-specific element. The major protein-binding activity occurs within a region of high homology between the mouse, human and chicken transferrin genes.

O 785 FORMATION OF PROTEIN-DNA COMPLEXES BETWEEN HUMAN ESTROGEN RECEPTORS AND THE ESTROGEN RESPONSIVE ELEMENT

Irène Theulaz°, Robert Hipskind°, Béatrice ten Heggeler-Bordier°, Stephen Green*, Vijay Kumar*, Pierre Chambon* and Walter Wahli°

°Institut de Biologie animale, Université, Lausanne, Suisse, * INSERM U-184, Faculté de Médecine, Strasbourg, France

The human estrogen receptor (hER) is a trans-acting regulatory protein composed of putative DNA-binding and steroid binding domains and of two other regions influencing transcriptional acitivity, one adjacent to the DNA binding domain and the second mapping in the amino-terminus region of the protein.

We have prepared nuclear extracts from Xenopus oocytes which had been injected with a human estrogen receptor expression vector (HEO) and show here that these extracts are capable of forming complexes on the estrogen responsive element (ERE) of a Xenopus vitellogenin gene, as visualized by electron microscopy. Complex formation is inhibited by the estrogen antagonist tamoxifen, while the addition of estrogen reverses this inhibition. In order to assess the properties of the hER domains, we have analyzed several hER mutants. Mutants lacking the DNA binding domain are not able to induce complex formation, while those without the N terminal domain or the hormone binding domain (C terminal) do form complexes. The sensitivity to tamoxifen is lost when the hormone binding domain is removed.

The ability of the wild type and mutant hERs synthesized by the oocyte to trans-activate an estrogen-responsive chimeric CAT gene is compared to their capacity to induce protein-DNA complexes.

O 786 INTERACTIONS BETWEEN ACTIVE GENES AND NUCLEAR SUB-STRUCTURES UNDER IN VIVO CONDITIONS, Andrew Thorburn and John Knowland, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, UK

There is increasing evidence that DNA sequences which are to be transcribed are closely associated with a sub-nuclear structure called variously the "nuclear matrix", "nuclear scaffold" or "nuclear skeleton". A major problem with this work is that the procedures used to produce these structures involve the use of detergents and **hyper** or **hypo**tonic salt conditions. This has led to the suggestion that the results obtained have been artefactual.

We have tackled this problem by developing a method where we can study interactions between chromosomal genes and nuclear sub-structures without using detergents and under **isotonic** conditions. We find under these conditions that active genes are indeed associated with a large sub-nuclear structure. We are extending this work to look at the activation of Xenopus vitellogenin genes by estrogen and its receptor in relation to their association with sub-nuclear structures.

O 787 TISSUE-SPECIFIC EXPRESSION OF HEPATITIS B VIRAL GENES, Ling-Pai Ting, Hsiao-Kuey Chang and Shih-Min Cheng, National Yang-Ming Medical College, Taipei, Taiwan, Republic of China.

The infection of human hepatitis B virus has been associated with acute and chronic hepatitis, cirrhosis and hepatoma. When well-differentiated, poorly-differentiated human hepatoma cell lines and nonhepatic cells were transfected with HBV DNA, the HBV genes were preferentially expressed in well-differentiated human hepatoma cell lines. Using the heterologous CAT gene, we demonstrated that the flanking sequences of promoter in the presence of enhancer could exert the liver- and differentiation state-specificity. Comparing the transcriptional activity of promoter with the flanking sequence in the presence and absence of HBV enhancer, we demonstrated that the flanking regulatory sequences of SPI and SPII promoters of HBsAg gene and core promoter of HBcAg gene are responsible for the preferential expression of these genes in the well-differentiated hepatoma cells. The enhancer effect is common to many different cell types. Identification of crucial sequence for the specificity of well-differentiated hepatoma cells is on going.

O 788 MOLECULAR GENETIC ANALYSIS OF DNA METABOLISM DURING VACCINIA VIRUS INFECTION, Paula Traktman, Cornell University Medical College, New York, NY 10021.

Vaccinia virus is a large, complex DNA virus which grows lytically in the cytoplasm of infected cells. This cytoplasmic localization, coupled with the genetic autonomy of the virus from host cell functions, makes it an ideal system to analyze DNA metabolism. Biochemical and molecular genetic studies on transcription, DNA replication, recombination and DNA topology and condensation are highly feasible. We have been concentrating on purifying viral enzymes involved in DNA metabolism and in defining all of the viral genes which are essential for DNA replication. We have purified a type I topoisomerase encoded by the virus, and determined that it most closely resembles a eucaryotic enzyme. Biochemical and genetic studies are underway to determine the specificity of the topoisomerase-DNA interactions and its sensitivity to various pharmacological agents. Genetic and pharmacological studies are also underway to dissect the role of the topoisomerase in viral transcription and replication. We are also pursuing structure-function studies on the viral DNA polymerase, making use of temperature-sensitive and drug resistant mutants isolated after classical and directed mutagenesis. The structure and function of two additional viral gene products with an essential role in replication and an affinity for DNA are being investigated. Finally, the mode by which the genes for these early viral functions are regulated is being studied, as is the role which DNA replication plays in shifting viral gene expression to the late phase.

O 789 DNA METHYLATION AT SPECIFIC ^{me}CG SITES INHIBITS EARLY BPV-1 TRANSCRIPTION AND *trans*-ACTIVATION BY THE E2 FUNCTION. L.P.Turek, T.H.Haugen, T.P.Cripe and G.D.Ginder, Departments of Pathology and Internal Medicine, VAMC and The University of Iowa College of Medicine, Iowa City, IA 52242.
Bovine papilloma virus (BPV)-1 plasmids modified at C^{me}CGG sites *in vitro* induce neoplastic transformation in culture at a ~ 10-fold reduced frequency. We have found that methylation of BPV-1 DNA leads to a 4 to 8-fold reduced BPV-1 early gene mRNA levels in transient transfections. Transcription at the upstream early viral promoter, P_{89}, is modulated by viral E2 *trans*-acting gene products. The E2 proteins bind to palindromic ACC(N)$_6$GGT *cis* cores that are located 5' to the promoter and form an E2-responsive enhancer (*E2R*). Methylation of the P_{89} promoter or of the *E2R* element linked to the SV40 early promoter reduced their response to E2 *trans*-activation ~ 10 to 20-fold in transient transcription and enzymatic *cat* assays. This was due to one or more critical ^{me}CG sites in the P89 promoter or the *E2R cis* element itself, since the control SV40 early promoter-enhancer (which contains no C^{me}CGG sites) was not influenced by complete methylation of the rest of the equivalent *cat* gene construction. Using clones with smaller *E2R* fragments, we have identified a single CG site whose ^{me}CG modification reduced the E2 response > 25-fold. This site is a part of the P_{89}-proximal ACC(N)$_6$GGT E2-binding core. We are currently testing whether methylation abrogates the binding of the E2 *trans*-activator protein to this site or other E2-binding cores. Our results indicate that this E2-binding sequence is critical for P_{89} *trans*-activation, and provide a model for studies on the interaction between DNA binding *trans*-acting factors and modified *cis* elements in mammalian gene switching.

O 790 TRANSCRIPTIONAL ELEMENTS COMMON TO RIBOSOMAL RNA AND PROTEIN GENES OF NEUROSPORA Brett M. Tyler, Yuguang Shi and Karin Harrison, Research School of Biological Sciences, Australian National University, Canberra, ACT 2601, Australia

The synthesis of a balanced supply of components for ribosome assembly requires the co-ordination of three different nuclear RNA polymerases: RNA polymerase I for the large (40S) rRNA precursor, RNA polymerase II for the 70-80 ribosomal proteins, and RNA polymersae III for the 5S rRNA. In order to look for common transcriptional elements with the potential for co-regulating ribosomal genes, we have defined the sequences required for transcription of the 5S rRNA and the 40S rRNA genes.

We constructed comprehensive sets of deletions and point mutation clusters spanning the promoter regions of 5S and 40S rRNA genes and tested the effects of these mutations using homologous in vitro transcription assays. The results show that transcription of the 5S genes requires a TATA-box at -29, which fixes the startpoint of transcription, plus three internal elements including a novel element, the D box, at +19 to +30 (1).

Transcription of the 40S gene requires two broad domains located at -116 to -36 and -28 to +4, plus a sequence between -240 and -116 which provides a 2-3 fold stimulation. The downstream domain includes a TATA-box at -5 which is absolutely required for transcripton, while the upstream domain includes a sequence homologous to the 5S D-box located at -64. This D-box overlaps sequences absolutely required for transcription, raising the strong possibility that the D-box co-ordinates transcription of the 5S and 40S rRNA genes in Neurospora.

The one sequenced Neurospora ribosomal protein gene also contains a D-box in its promoter region at around -350. We are currently sequencing a second r-protein gene and are testing whether the r-protein D-box could be co-ordinating ribosomal protein and ribosomal RNA synthesis.

(1) Tyler, BM (1987) J. Mol. Biol. 196, 801-811

O 791 IMPLICATION OF TWO NUCLEAR PROTEINS IN THE ANTIGEN RECEPTOR DEPENDENT ACTIVATION OF THE IL-2 GENE AND THE HIV-LTR, J. P. Shaw, D. B. Durand, P. J. Utz, K. Ullman, B. Emmel and G. R. Crabtree, Stanford University, Stanford, CA 94305.
We have used the antigen receptor-dependent activation of the IL-2 gene to begin to define the nuclear events responsible for directing T-cell activation. Previous studies have shown that the IL-2 gene is controlled by a transcriptional enhancer which functions only in activated T cells. An analysis of internal deletions of this enhancer revealed three sequences essential for maximal activation of a linked reporter gene. When synthetic oligonucleotides of two of these sequences are concatanated and linked to an unrelated promoter, either sequence renders the promoter responsive to signals from the antigen receptor. As found with the IL-2 enhancer the ability of these oligonucleotides to activate a linked gene can be completely inhibited by 100 ng/ml cyclosporin A. These two oligonucleotides bind two different proteins, NFIL2A and NFIL2E. NFIL2A is constitutively expressed in all cells examined and binds to the SV40 enhancer. On the other hand NFIL2E is expressed only in activated T cells. The binding activity for NFIL2E appears by 20 minutes after T-cell activation and precedes IL-2 mRNA by 10 to 25 minutes. Inhibitors of protein synthesis inhibit the appearance of binding activity for NFIL2E and also inhibit IL-2 gene activation. Finally NFIL2E binds to essential regions of the promoters for other genes that are expressed during T-cell activation including the HIV LTR. For these reasons we believe that NFIL2E is involved in the early programming events in T-cell activation and propose the name NFAT-1 (Nuclear Factor of Activated T cells).

O 792 **ACTIVATION OF THE HUMAN IMMUNODEFICIENCY VIRUS LONG TERMINAL REPEAT BY AN SOS-LIKE RESPONSE IN HUMAN CELLS.** Kristoffer Valerie[1], Anne Delers[2], Claudine Bruck[2], Clothilde Thiriart[2], Hagai Rosenberg[1], Christine Debouck[1], & Martin Rosenberg[1]. [1]Smith Kline and French Laboratories, King of Prussia, Pa 19406, and [2]Smith Kline-RIT, B-1330 Rixensart, Belgium.

The *tat* gene product encoded by the human immunodeficiency virus (HIV) is a *trans*-activating factor responsible for enhancing viral gene expression directed by the HIV long terminal repeat (LTR). Recently, several laboratories have reported that certain viruses, and chemicals can also activate the HIV LTR in the absence of *tat* function. These studies have suggested that the HIV LTR may be responsive to certain cellular factors which are being affected by these various agents. A common denominator shared by these agents is their ability to cause stress responses in cells by affecting cellular processes such as DNA synthesis and cell cycle regulation. In an effort to determine whether stress responses might affect HIV gene expression, we examined the effects of the DNA damaging agents, ultraviolet light (UV) and mitomycin C, on HIV LTR-directed gene expression. We demonstrate that these agents enhance LTR-directed gene expression up to 150-fold in the absence of Tat protein, and that these levels match those obtained by *tat* activation alone. The increase in expression correlates with an accumulation of stable mRNA and is inhibited by α-amanitin, suggesting that activation requires transcription. Moreover, the increase in steady state mRNA is not inhibited by cycloheximide, indicating that protein synthesis is not required for activation. Most importantly, UV irradiation of human T-cells prior to viral infection significantly shortens the viral growth cycle. Apparently, UV exposure induces a cellular state which is highly conducive for viral replication and growth. We further demonstrate that even direct sunlight can activate LTR-directed gene expression. These results suggest that DNA damaging agents, as well as other agents which elicit SOS-like stress responses in mammalian cells, can activate HIV LTR-directed expression thereby enhancing viral growth. Supported in part by NIH grant AI24845.

O 793 A YEAST RIBOSOMAL DNA ENHANCER-BINDING FACTOR, Harm van Heerikhuizen, Jacobus Klootwijk, Jos Oliemans, Tanja Kulkens and Rudi J. Planta, Biochemisch Laboratorium, Vrije Universiteit, Amsterdam, The Netherlands.

In yeast the large ribosomal RNAs are transcribed by a specialized polymerase (RNA polymerase I) into a 37S precursor rRNA. The 37S transcription unit is repeated in yeast about 200 times in a head to tail orientation; the different transcription units being separated by spacer DNA: the so-called non transcribed spacer (NTS). This NTS of the yeast rDNA unit has been shown to contain an enhancer which measures about 170 bp and is located about 2 kb upstream of the site of initiation of the 37S precursor rRNA (Elion & Warner, 1986). Using the gel retardation assay we have identified a protein that binds, in a sequence-specific fashion, to a 50 bp fragment within the enhancer. This protein (EBP1) has been partially purified by means of heparin-agarose chromatography and protects a 20 bp region in the rDNA enhancer against DNaseI in an *in vitro* footprinting assay.

In vivo footprinting studies, using methylation of whole yeast cells with dimethylsulphate, are currently performed to detect whether the EBP1 binding site is also occupied *in vivo* and whether different metabolic conditions of the yeast cell exert any influence on the occupancy of the binding site.

We have deleted the EBP1 binding site *in vitro* from an artificial ribosomal minigene (ARES) and are now studying the effect of this deletion on the enhancement of rDNA transcription *in vivo* after transformation of the deleted minigene into yeast.

O 795 MATERNAL AND ZYGOTIC EXPRESSION OF SERENDIPITY DELTA, A DROSOPHILA FINGER PROTEIN GENE, François Payre & Alain Vincent, C.R.B.G.C., C.N.R.S., 118 rte de Narbonne, 31062 Toulouse, France.

The Drosophila serendipity (sry) locus encompasses 4 different genes clustered within 7.5 kb of DNA. Expression of each sry gene appears essentially autonomous although stage specific read-through transcription of some intergenic sequences results in the stable accumulation of polycistronic-like RNAs. The sry beta and delta genes code for closely related proteins containing respectively 6 and 7 TFIIIA-like DNA-binding fingers. The developmental pattern of synthesis and tissue distribution of the sry delta protein was studied using 1) antibodies raised against the protein 2) X-Gal/immunostaining of embryos, larvae and adult tissues from Drosophila lines transformed with a gene coding for a delta-lacZ chimaeric finger protein expressed under the control of the sry delta promoter. Maternal versus zygotic contribution to the early embryonic pattern will be discussed.

O 796 FACTORS AFFECTING TERMINATION OF TRANSCRIPTION FOR A HUMAN $tRNA_i^{Met3}$ GENE,

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We have isolated a third human $tRNA_i^{Met3}$ locus. Unlike two other loci, $tRNA_i^{Met3}$ exhibits three termination signals which appear to be rather inefficient. Deletion analysis has demonstrated that 5'-flanking sequences are required for optimal transcription rates in vitro. Deletion of sequences from -30 to -18 result in an 8-fold decrease in gene activity both in HeLa cell extracts and in Xenopus laevis oocytes. It appears that this decrease in transcription activity is due to the formation of less stable transcriptional complexes. We have shown that DEAE-cellulose fractionated HeLa cell extracts are deficient in transcript release compared to extracts which are not fractionated. Pure La antigen prepared in E. coli and missing 44 amino acids from the amino terminus prevents termination and release of transcripts from the transcription complex. Therefore, La antigen appears to function as an antitermination factor.

O 797 IN VITRO ANALYSIS OF PROTEIN-DNA INTERACTIONS AT THE 3' ENHANCER OF THE HUMAN BETA-GLOBIN GENE, Lee Wall, Ernie deBoer and Frank Grosveld,
National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

The human beta-globin gene has been shown to contain an enhancer in the 3' flanking region which in part confers the tissue and stage specific expression of this gene. In vitro analysis by DNaseI footprinting of a 260bp PstI DNA fragment, which has been shown to contain this enhancer activity, demonstrates four discrete regions of DNA which bind to factors present in nuclear extracts derived from both human and mouse erythroid cell lines, as well as from mouse fetal liver. Two of these regions appear to interact with erythroid specific factors as DNase protection, as well as the occurrence of hypersensitive sites, is not seen in extracts obtained from non-erythroid cell lines. The other two regions, one of which contains a 10 base pair sequence of dyad-symmetry, and the second of which contains a sequence homologous to the "octamer" motif found in several other enhancers, both show a pattern of DNaseI digestion which is specific to the cell type employed, suggesting that cell type specific nuclear factors may also bind in these regions. We are presently investigating which of these footprinted regions, or combination thereof, is responsible for the developmental specificity of this enhancer as well as characterizing the proteins which interact with these sequences.

O 798 INTERACTION OF ORIGIN BINDING PROTEINS AND TRANSCRIPTION FACTORS WITH MURINE ARS ELEMENTS, Michael Wegner, Sonja Schwender, Andra Klavinius, Wieslaw Siwka, Friedemann Müller and Friedrich Grummt, Institute of Biochemistry, University of Würzburg, D-8700 Würzburg, Germany.

Autonomously replicating sequences that allow the replication of plasmids in transfected mouse Ltk^- cells were isolated from chromosomal murine DNA. 11 so-called muARS elements were subcloned and sequenced. Two murine ARS binding factors (muABF1 and muABF2) have been detected and partially purified. muABF1 was shown to bind site-specifically to a subset of muARSs (muARS3, muARS4, muARS-NTS1 and muARS-NTS2). DNAaseI-footprint analyses revealed 47 bp long palindromic and extremely AT-rich recognition sites of muABF1 both in muARS-NTS1 and muARS-NTS2. muABF1 is a heat stable (10 min, 100°C) and acid resistant (1% trichloroacetic acid) protein which could be purified to apparent homogeneity by chromatography on heparin-Ultrogel and by SDS-PAGE elution and renaturation. Its native molecular weight is approximately 20 k. In mouse nuclear extracts muABF1 is physically associated with the cellular tumour antigen p53. muABF2 is heat labile and has a different recognition site in muARS-NTS1. The interaction of this factor with muARS-NTS1 is relatively weak since complexes need stabilization by glutaraldehyde fixation to be monitored in gel retardation experiments. Furthermore, recognition sites for the transcription/replication factor NFIII and the transcription initiation factors AP1 and AP3 were identified in various muARS elements.

O 799 ANALYSIS OF THE FUNCTION AND EXPRESSION OF THE MOUSE ESTROGEN RECEPTOR, Roger White, Jackie Lees, John Ham, Maurice Needham and Malcolm Parker, Imperial Cancer Research Fund, London, England, U.K.

Steroid hormone receptors are members of a family of proteins which are characterized by their ability to bind to specific DNA sequences and to enhance transcription from an adjacent promoter. cDNA and genomic clones corresponding to the mouse estrogen receptor (MOR) have been isolated and characterized (1). Nucleotide sequence analysis predicts that full length cDNA has the potential to code for a protein of 599 amino acids. Functional protein has been produced by the expression of cDNA in receptor deficient COS-1 cells, and analysed by co-transfection with a chimeric marker plasmid consisting of the estrogen response element from the Xenopus vitellogenin A2 gene linked to the thymidine kinase promoter and the chloramphenical acetyl transferase (CAT) gene. In this assay, CAT activity is stimulated by up to 80-fold in the presence of added receptor and estradiol at 10^{-7}M.

The MOR is coded for by a mRNA of approximately 6.5Kb. In the mammary gland, uterus and ovary, transcription is initiated at multiple sites which span a region of 62 nucleotides. There are 10 major starts in total, one of which is situated 31 nucleotides downstream from a TATA box-like motif. Preliminary transfection experiments in a number of cell lines, indicate that plasmids containing the promoter of the MOR linked to CAT result in a reduction in CAT activity relative to basal levels. Anaylsis of the sequence of the MOR gene promoter up to -1Kb reveals two regions homologous with "silencer" elements which have been described in the chicken lysozyme gene (2). Studies are now in progress using nuclease protection assays and deletion mutants of the MOR gene promoter to determine the position and role of DNA sequence elements in the expression of the estrogen receptor gene.

(1) White R, Lees JA, Needham M, Ham J and Parker M. Mol Endocrinology, Oct 1987
(2) Baniahmad A, Muller M, Steiner Ch and Renkawitz R 1987, EMBO J, 6 2297-2302.

O 800 EXPRESSION OF β-LACTOGLOBULIN FUSION CONSTRUCTS IN TRANSGENIC MICE AND CELLS. C. Bruce A. Whitelaw, Pam Brown, Maggie McClenaghan, Alan L. Archibald, J. Paul Simons and A. John Clark, Institute of Physiology and Genetics Research, Edinburgh, Scotland, U.K.

Our intention is to target expression of foreign genes to the mammary gland. Towards this aim we have cloned and characterised the entire sheep β-lactoglobulin (BLG) structural gene. BLG is the major whey protein in the milk of ruminants. In the sheep lactating mammary gland BLG transcripts comprise approximately 5% of polyA$^+$ RNA. Transgenic mice carrying the BLG gene specifically and abundantly express it in the mammary gland during lactation and large amounts of the foreign protein are secreted into the milk.

We have generated fusion constructs comprising DNA sequences encoding human plasma proteins and sequences derived from the sheep BLG gene. Expression of the fusion constructs was analysed by calcium phosphate mediated transient transfection into BHK cells. The SV40 enhancer was used to drive transcription since the BLG promoter does not function efficiently in these cells. The results obtained indicate the presence of mRNA which hybridises to probes derived from both human and sheep sequences, however evidence for aberrant splicing has also been found. To test these constructs for expression in the mammary gland, transgenic mice have been generated. Initial results from these mice indicate that some do express the fusion message in the mammary gland and secrete the human protein into the milk.

O 801 ICP4 IS A SEQUENCE-SPECIFIC DNA BINDING PROTEIN, Kent Wilcox and Patricia Kattar-Cooley, Medical College of Wisconsin, Milwaukee, WI 53226

The HSV immediate early polypeptide ICP4 (α4; IE175k) is a positive regulator of early viral gene transcription and a negative regulator of ICP4 gene transcription. Previous studies have shown that addition of specific target DNAs to protein extracts prepared from HSV-infected cells yield protein:DNA complexes that contain ICP4.

We have purified native ICP4 to apparent homogeneity by DNA affinity chromatography. The purified ICP4 consists of a mixture of oligomeric forms, including dimers and tetramers at a ratio of approximately 2:1. The DNA binding properties of purified ICP4 have been analyzed by gel mobility shift and filter binding assays. Both approaches demonstrate that ICP4 binds to DNA in the absence of any other cellular or viral proteins. Quantitative measurements of the affinities of purified ICP4 for sequences derived from the promoters of HSV immediate early genes encoding ICP0, 4 and 27 and the HSV early gene encoding thymidine kinase demonstrate that ICP4 is a sequence-specific DNA binding protein. ICP4 has a relatively high affinity for sequences in the ICP0 and ICP4 gene promoters. Binding of ICP4 to sequences derived from the thymidine kinase gene was relatively weak. There was no detectable binding of ICP4 to the ICP27 gene promoter. Preliminary results suggest that both the dimeric and tetrameric forms of ICP4 bind to DNA in a sequence-specific manner.

O 802 E1A TRANSACTIVATION OF THE HUMAN HSP 70 GENE. Gregg T. Williams and Richard I. Morimoto, Department of Biochemistry, Molecular Biology, and Cellular Biology, Northwestern University, Evanston, IL. 60208.

Human HSP70 gene expression is characterized by three diverse forms of regulation: 1) induction by a variety of forms of stress, 2) variation throughout the cell cycle, and 3) activation by the adenovirus E1A gene product. Studies in Hela cells using 5' deletion and linker-scanning mutants have determined that wild type basal mRNA levels require sequences to -74 containing functional CCAAT and TATA elements. Upstream sequences near -100 are required for heat shock induction. We have examined whether E1A activation is mediated through the previously identified promoter elements or through a distict mechanism. Co-transfection of an E1A containing plasmid with a series of 5' deletion mutants has demonstrated that removal of the CCAAT box at -68 reduces E1A activation 6-fold. Some residual activation remains, approximately 2-fold, which is lost when the TATA box is mutated in a -26 deletion mutant. Co-transfections using linker-scanning mutants confirm the requirement for a functional CCAAT box at -68. We propose that E1A trans-activation of the cellular HSP70 gene is mediated through the same genetic elements that control basal expression in the absence of E1A. We are currently attempting to determine the unique feature of the HSP70 promoter which distinguishes it from other promoters that contain similar elements and are not activated by E1A.

O 803 **A GENERAL METHOD FOR SELECTING MUTANTS IN THE RNA POLYMERASE III TRANSCRIPTION APPARATUS OF YEAST,** Ian Willis. Yale University, New Haven CT 06511.

Transcription of tRNA genes in yeast requires a minimum of 2 protein factors, TFIIIB and TFIIIC, in addition to RNA polymerase III. Both of these factors have been purified extensively from yeast cells (TFIIIB to apparent homogeneity) and characterized with respect to their physical and functional properties. In contrast to TFIIIB, the purification of TFIIIC is not yet complete. As a variety of factors may be responsible for the difficulties encountered in the biochemical purification of TFIIIC, we have considered genetic approaches to a molecular characterization of this factor. Using a number of transcriptionally defective tRNA genes, we have devised an assay/selection system that can monitor changes in transcriptional activity over 4 orders of magnitude. We are presently using this system in an effort to obtain yeast strains harboring mutant TFIIIC genes.

O 804 STEROID-DEPENDENT INTERACTION OF TRANSCRIPTION FACTORS WITH THE INDUCIBLE PROMOTER OF THE CHICKEN apo-VLDL II GENE IN VIVO. Jan Wijnholds, Sjaak Philipsen and Geert AB, Groningen University, Nijenborg 16, 9747 AG Groningen, Netherlands.

We have used in vivo methylation (genomic) footprinting to map interaction of transcription factors with the steroid-inducible promoter of the chicken apo-Very Low Density Lipoprotein II gene. In the region extending from -1250 to +200 protein/DNA contacts were detected in estrogen-induced liver cells that coincide with previously proposed estrogen-responsive enhancer elements (ERE's). The ERE's are a perfect and an imperfect copy of the palindromic sequence element, GGTCANNNTGACC. Other contact points are detected at a sequence element that resembles the binding box for the Coup-transcription factor which has been described for the ovalbumine promoter. The Coup-box is present as an imperfect repeat of the sequence GGGTCAAAGG. The above contacts were only observed in hormone-induced liver cells, and were absent in non-stimulated liver cells, erythrocytes and estrogen-stimulated oviduct tubular gland cells. Our results demonstrate a mechanism in which the regulation and transcription factors bind to the promoter region after hormone induction, rather than the activation of a pre-established transcription complex already bound on the inactive promoter.

O 805 ON THE STATE OF DNA IN SINGLE STRAND NUCLEASE SENSITIVE REGIONS.
G. Yagil, Department of Cell Biology, The Weizmann Institute of Science, Rehovoth, Israel.
A considerable number of eukaryotic genes have been found to contain regions sensitive to S1 and mung bean nucleases. As double stranded B form DNA is not attacked by these single stranded nucleases, the DNA is presumed to be in an alternate, non B, form in the sensitive regions. Several models have been proposed for this alternate form.

The sensitive regions have generally been found to be rich in polypurines in one or the other strand (Hentschel, Nature, **295**: 714, 1982; Evans et al., *Nucleic Acid Res.* **12**: 8043, 1984). We have therefore evaluated the frequency of polypurine runs in both translated and flanking regions of common genes. It is found that in SV40, for example, runs of six consecutive polypurines are more abundant by 75% than expected, while alternating purines-pyrimidines are found 20% less than expected. Most highly polypurine enriched sections are found in regions of regulatory significance; this includes the 21 base pair repeats and the T antigen binding sites, which have been found to be S1 sensitive (Evans *et al., loc. cit.*).

We have recently explored the possibility that DNA in the S1 sensitive regions can be in an entirely nonhelical form (EMBO J., **5**: 1917, 1986). Energy minimization of poly(G)•poly(C) leads to a structure which is locally stable, with an excess of energy which is only half that needed to melt DNA. The structure is characterized by a very high tilt, an unusual gauche torsion angle around the C5-O5 bond and a very exposed state of the bases. We have now extended our calculations to other polypurine-polypyrimidine structures. Poly(AG)•poly(CT) is found to be of particularly low excess energy (7.4 Kcal/base pair). As poly(AG) is also a most S1 sensitive sequence, the existence of sensitive regions in a nonhelical state ought thus be further explored.

O 806 DNA BINDING FACTORS FOR THE CpG-RICH ISLAND CONTAINING THE PROMOTER OF THE HUMAN X-LINKED PGK GENE. Thomas P. Yang, Judith Singer-Sam, Josephine Flores, and Arthur D. Riggs, Beckman Research Institute of the City of Hope, Duarte, CA 91010.
The gene coding for the glycolytic enzyme phosphoglycerate kinase (PGK-1) is X-linked in mammals and has a G+C-rich 5' region characteristic of several constitutive genes. Although PGK-1 is constitutively expressed, it is transcriptionally regulated in female cells by X-chromosome inactivation. To study the expression and regulation of the PGK-1 gene, we have analyzed the binding of trans-acting factors to the 5' region of the gene. We detect at least three distinct binding activities that interact in vitro with at least six different DNA sites. Two of these binding activities generate DNase I footprints at positions -360 and -130. We have examined the promoter specificity of the three binding activies in gel mobility-shift assays by competition with cloned promoter fragments of other genes. None of the activities interacts exclusively with X-linked promoters. However, one activity binds preferentially to G+C-rich promoters and another activity appears to bind to CAAT box sequences. Previous studies have demonstrated that all HpaII/MspI sites in this region are fully methylated in the inactive X chromosome and totally unmethylated on the active X. Competition studies using synthetic oligonucleotides containing 5-methylcytosine at all CpG sites in the -130 footprint region, which contains one HpaII site, show that DNA methylation does not significantly alter binding activity at this site. The function(s) of the DNA-protein interactions are being investigated by gene transfer and in vitro transcription experiments.

O 807 REGULATORY ELEMENTS OF YEAST ATP2, Patrice O. Yarbough and Michael G. Douglas, Department of Biochemistry, Southwestern Graduate School Dallas, TX 75235.
In an effort to elucidate the mechanism(s) of gene expression for yeast ATP2, which encodes the mitochondrial F_1 ATPase β-subunit precursor, a series of deletions within its 5' flanking region have been analyzed in order to identify cis-acting and trans-acting elements which may be involved in ATP2 expression. A 409 base pair deletion within the ATP2 5' flanking region completely abolishes ATP2 activity. Furthermore when this deletion is site-directed into the yeast chromosome at the ATP2 locus, the resulting yeast are unable to grow on glycerol. Wildtype sequences have been shown to complement this respiratory defective phenotype in cis. However these same sequences fail to restore growth on glycerol in trans. It has been shown by Northern analysis that the transcript levels of the deletion mutant are comparable to wildtype. Western blot analysis and pulse-labeling experiments demonstrate that the ATP2 protein levels are decreased 10-15-fold in the deletion mutant. The failure to grow on glycerol appears to be a direct consequence of the cell not having enough F_1 ATPase β-subunit to support growth on non-fermentable carbon sources.

It is believed that the transcript is not accessible to translation. The mechanism by which deletions in the 5' non-transcribed region of ATP2 can affect translation of the mature protein is of interest. The RNA secondary structure of the untranslated region of the ATP2 transcript is predicted to form an extensive and stable stem-loop structure (-38.9 kcal). The significance of such a structure is presently under investigation.

O 808 DIFFERENTIAL REGULATION OF ALA SYNTHASE GENES IN DBA/2 MOUSE. Ellora Young and Peter Dierks, Dept. of Biology, Gilmer Hall, Univ. of VA, Charlottesville, VA 22901.

Heme is required for many essential functions in mammalian organisms. It functions as the prosthetic group of cytochromes in electron transport, is involved in a wide variety of cytochrome P450 mediated biotransformation reactions, and functions as the oxygen carrier for hemoglobin in erythrocytes. The rate of heme biosynthesis in the cell appears to be controlled by the activity of the first enzyme in the pathway--aminolevulinate synthase (ALAS). In addition to a general feedback repression mechanism by heme, ALA synthase appears to be developmentally regulated in erythrocytes prior to globin induction. These observations, coupled with claims that different ALAS isozymes are expressed in hepatic and erythroid cells, have led to the suggestion that different genes are used to encode ALAS in these two tissues.

We have isolated two distinct ALA synthase cDNAs and their genes in DBA/2 mouse and can demonstrate that the expression of these genes *in vivo* is differentially regulated in erythroid and hepatic tissues. Expression of the α gene is induced during erythroid differentiation while the β gene is specifically derepressed in the liver in response to drugs that induce hepatic porphyria. We are currently utilizing a murine erythroleukemia cell line and a murine hepatoma line as model systems to decipher the many regulatory interactions between the physiological state of the cell and these two genes.

O 809 CHARACTERIZATION OF A TRANS-ACTING FACTOR BINDING SITE IN THE EMBRYONIC MYOSIN HEAVY CHAIN PROMOTER THAT ACTIVATES TRANSCRIPTION IN VIVO

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 positive and negative-regulatory elements are found throughout the promoter region up to -1413. By protein-DNA binding mobility shift assays, trans-acting factors which bind specifically to the MHC_{emb} promoter region have been detected in nuclear extracts prepared from mouse C2 myoblast, C2 myotubes, and HeLa cells. The exact binding site of one of these factors has also been identified by methylation interference foot-printing studies to be located between -93 and -84 with the sequence 5'-GTGTCAGTCA-3'.

The biological function of this protein-DNA interaction in promoter activity has been investigated by using a series of 5'-end deletion mutants in this region: pEMHC110, pEMHC102, pEMHC85, pEMHC80, and pEMHC74 with the 5'-end at -110, -102, -85, -80, and -74, respectively. These mutants were examined for the specific protein-DNA binding as well as promoter activity by transient expression studies in fused C2 myotubes. Mutants pEMHC85, pEMHC80, and pEMHC74 did not show protein-DNA binding activity and had 3 fold less promoter activity as compared with the promoter activity of pEMHC110 and pEMHC102, which retained the specific protein-DNA binding activity. However, this specific protein-DNA binding activity is not essential for MHC_{emb} promoter activity as indicated by the promoter activity of several internal deletion mutants: pEMHC1413d102, pEMHC1413d85, and pEMHC1413d80 which retain the upstream sequence from -1413 to -110, but contain the same sequence downstream from -110 as pEMHC102, pEMHC85, and pEMHC80, respectively. Mutants pEMHC1413d85 and pEMHC1413d80 showed 3 fold reduction in promoter activity as compared with pEMHC1413 and pEMHC1413d102. However, the deleted constructs still had significantly greater promoter activity than the basal promoter constructs as represented by pEMHC110. Since the nucleotide sequence of this binding site bears striking resemblance to AP-1 binding site, the possible induction of MHC_{emb} promoter by phorbol esters in C2 myotubes is being investigated.

O 810 REQUIREMENTS FOR MESENCHYMAL-EXPRESSION OF THE VIMENTIN GENE, Zendra E. Zehner, Christina M. Sax, Francis X. Farrell, Richard J. Garzon, Janet A. Cunningham, Denise M. Lafranchi, Virginia Commonwealth University, Richmond, VA 23298.

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, is growth regulated, is induced by exposure to the tumor promoter TPA, and is down-regulated during myogenesis. The 5'-sequence of the chicken vimentin gene reveals several elements in common with SV40 and other eukaryotic genes i.e., AP1, AP2, CAATT-, and five GC-boxes . We have begun an analysis of this region to determine which cis-acting elements are important for vimentin gene regulation. Transfected 5'-end: CAT fusion genes are expressed and regulated as the endogenous gene. These constructs express CAT in fibroblasts where 3% of the total protein is vimentin, are off in rat hepatoma cells which do not express vimentin, and are down-regulated during myogenesis where there is a switch to the muscle-specific IFP, desmin. The removal of three GC boxes and CAATT does little to affect overall gene activity. Therefore, we conclude that these are basal promoter elements and regulation of vimentin gene expression is due to upstream elements. In this regard, we have located a tissue-specific enhancer element (at -292 to -132) which is required for expression in fibroblasts. This same region controls the down-expression of vimentin during myogenesis. Surprisingly, there is a general silencer element (at -578 to -466) which represses gene expression in any cell-type. Experiments are in progress to determine how the general silencer and tissue-specific element serve to regulate vimentin gene expression.

O 811 EARLY EVENTS OF RNA POLYMERASE II TRANSCRIPTION INITIATION, Jimin Zhang, Kelley Logan and Steven Ackerman, University of Massachusetts at Boston, Biology Department, Harbor Campus, Boston, MA 02125.

We have investigated the earliest stages of assembly of the RNA polymerease II transcription complex. Transcription factor (TF) IIA interacts with the template first, and then TFIID interacts. TFIIA takes about 5 min. to fully interact, while TFIID interacts within 2 min. The IIA/DNA complex does not overcome the lag peiod, but IIA/IID/DNA does overcome the lag period. There are apparently interactions between IIA and IID, as a pre-incubation of these factors without DNA also overcomes the lag period. This DF complex binds to DNA slowly (40 min.) in contrast to the individual factors binding to DNA in an ordered assembly. Phosphocellulose fraction C, which contains several factors, binds to this pre-complex in a time dependent manner. All three (IIA, IID, fraction C) do interact with DNA at 0°C, but with slower kinetics. The binding to DNA at 0°C or 30°C is very rapid, as demonstrated by gel retardation. Thus, the slower kinetics is not at the DNA binding step. The ability of the complex to sequester a template is dependent on the stage of assembly. There is never complete sequestering.

O 812 **REGULATION OF 5S RNA SYNTHESIS IN ACANTHAMOEBA CASTELLANII,** **Michael G. Zwick and Marvin R. Paule, Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523.**

During encystment of the free-living protozoan Acanthamoeba castellanii, many genes are transcriptionally regulated. Pulse labelling studies show that by 10 hours into encystment, the large rRNA genes are shut off completely and 5S RNA synthesis is minimal. Evidence had been presented that regulation of large rRNA synthesis in this organism is due to a modification of RNA polymerase I. The 5S RNA gene is being cloned from an EMBL 3 genomic library in order to determine the mode of regulation of RNA polymerase III genes using an in vitro transription system as a model. Acanthamoeba S100 nuclear extracts are able to transribe Xenopus met-tRNA-I and yeast leu-tRNA-3 genes, although the corresponding 5S RNA genes are not transcribed. This may be due to lack of additional transcription factor(s) or transcription may be somewhat species specific. RNA polymerases I and III have been isolated from Acanthamoeba and share six common subunits. This, together with the fact that both these stable RNAs are down regulated at the same time during encystment, suggests that there may be a common regulatory scheme.