FUNDAMENTAL MECHANISMS OF TRANSCRIPTION

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March 28-April 3, 1992

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Keynote Address

R 001 YEAST RNA POLYMERASE II TRANSCRIPTION: STRUCTURE, MECHANISM, AND REGULATION, Daniel I. Chasman, Seth A. Darst, Aled M. Edwards, William J. Feaver, Peter M. Flanagan, Opher Gileadi, David Hinds, Raymond J. Kelleher III, Yang Li, Yahli Lorch, Gavin Meredith, Britt Park, Michael H. Sayre, Herbert Tschochner, and Roger D. Kornberg, Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305.

A fully defined RNA polymerase II transcription system from Saccharomyces cerevisiae comprises general initiation factors a, b, d (TFIID), e, and RNA polymerase II. Purification of the general factors to homogeneity and cloning of genes for their subunits will be described. Functional activities of three of the four general factors have so far been identified: CTD-kinase and DNA-dependent ATPase activities of factor b, TATA-binding by factor d, and TATA-to-start site distance determination by factor e. An initiation complex assembled from these components contains 19 different polypeptides, with a total mass of about one million Daltons. Structure determination of this complex has been pursued by X-ray crystallographic analysis of factor d and RNA polymerase II, and by electron crystallographic analysis of RNA polymerase II with associated factors. Aspects of RNA polymerase II structure, such as the locations of various subunits and of the CTD, are also being elucidated by electron crystallography. Additional factors required for transcriptional activation with both naked and nucleosomal templates will be described.

Structure and Physical Chemistry of DNA and Protein-DNA Interactions

R 002 MOLECULAR DISSECTION OF THE FOS/JUN LEUCINE ZIPPER INTERACTIONS, Peter S. Kim, Howard Hughes Medical Institute, Whitehead Institute for Biomedical Research, Department of Biology, M.I.T., Cambridge, MA 02142.

The leucine zipper motif was originally proposed by McKnight's group as a hypothetical dimerization motif in a new class of DNA binding proteins (1). A synthetic peptide corresponding to the leucine zipper of the yeast transcriptional activator, GCN4, folds as a parallel pair of α-helices: this led us to propose that leucine zippers are actually short coiled coils (2). Sequence requirements at the hydrophobic dimer interface have been probed using a genetic system in which the DNA binding domain of λ-repressor is used as a reporter for leucine zipper dimerization in a chimeric protein (3). Two-dimensional NMR studies (4) and recent X-ray crystallographic studies of this peptide (in collaboration with Prof. T. Alber's laboratory, University of Utah) confirm that the leucine zipper of GCN4 is a coiled coil (5). These studies also provide the first high resolution structure of a two-stranded parallel coiled coil (6).

The isolated leucine zipper regions from the nuclear oncogene products, Fos and Jun, are sufficient to mediate specific heterodimer formation (7). This provides a simple model system for studying the specificity of protein-protein interactions: two α-helices that prefer to interact with each other rather than with themselves. We find that destabilization of the Fos homodimer by acidic residues provides a thermodynamic driving force favoring heterodimers, and that eight amino acid residues from Fos and from Jun are sufficient to direct preferential heterodimer formation.

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R 003 DNA CONFORMATION DURING ACTIVE RNA TRANSCRIPTION, Xin Bi, Hai-Young Wu and Leroy F. Liu, Department of Biological Chemistry, Johns Hopkins University, School of Medicine, Baltimore, MD.

The effect of transcriptional elongation on DNA template conformation has been studied both *in vivo* and *in vitro*. In *E. coli* as well as *in vitro*, transcriptional elongation in the presence of *E. coli* DNA topoisomerase I resulted in positive supercoiling of the DNA template. In both *E. coli* and *Sacchromyces cerevisiae*, transcriptional elongation in DNA topoisomerase I null mutants resulted in hyper-negative supercoiling of the plasmid DNA template. These results are consistent with a model in which translocation of the RNA polymerase elongation complex on right-handed DNA produces positive supercoils in front of and negative supercoils behind the traversing RNA polymerase complex.

Several experiments have been designed to test the model. As predicted by the model, fusion of the positive and negative supercoils on a circular DNA can be retarded by a large anchor located in between. We have shown that looping of the circular DNA by the *lac* repressor tetrameric complex *in vivo* created such an anchor and fusion of positive and negative supercoils was indeed retarded within the looped domain. We have also tested whether transcription of a single transcription unit is sufficient to generate positive and negative supercoils. We showed that both *in vivo* and *in vitro* transcription of a single transcription unit was sufficient to generate supercoils efficiently. In addition, transcription from a single promoter was also shown to generate nuclease BAL31 sensitive sites. These experiments suggest that DNA conformation in the vicinity of the elongation complex is significantly altered.

To test whether transcriptional elongation affects template function *in vivo*, we have assayed for the recombination frequency between tandemly duplicated sequences in the presence and absence of transcription induction. We have created tandem duplications of the early and late part of the *tetA* gene and put the *tetA* gene under the control of the *lac* UV5 promoter. IPTG induction stimulated recombination between the tandemly duplicated regions about 10-fold. Surprisingly, the tandem duplication of the late part of the *tetA* gene was 1000 fold more efficient in recombination than the tandem duplication of the early part of the *tetA* gene. These results suggest that transcription stimulates recombination and a hot spot(s) for recombination is located in the late part of the *tetA* gene. We will present a model describing the effect of transcriptional elongation on DNA conformation and recombination.

R 004 STRUCTURE OF T7 RNA POLYMERASE, Bi-Cheng Wang[1,2], Rui Sousa[2], Yong Je Chung[1], John Rose[1] and Eileen M. Lafer[2], Departments of [1]Crystallography and [2]Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260

The three-dimensional image of T7 RNA polymerase has been visualized at 4 Å resolution by the multiple isomorphous replacement method. There are three molecules in the asymmetric unit. The overall dimensions of the molecules are 75 x 75 x 65 Å. The structure has a 50-60 Å long cleft which is about 15-25 Å wide and 25-40 Å deep, with a 40 Å long arm on one side of the cleft and a large structure on the opposite side. The cleft is capable of accommodating a double-helix. The 4 Å model of the molecule strongly indicates the existence of a small and a large domain, in agreement with biochemical studies, which predict a small N-terminal and a large C-terminal domain. The larger domain appears to be able to subdivide into two subdomains, also in agreement with suggestions from sequence analyses. The cleft is formed by the residues from these domains. The structure suggests that while *E. coli* RNA polymerase uses subunits to carry out its various functions, the phage RNA polymerases use subdomains to accomplish the comparable functions. We speculate that the subdomains of phage enzyme contain the essential functional structures that are located on the subunits of larger and more complex RNA polymerases, and in these latter enzymes, these essential structures converge to a cleft where DNA interacts with the enzyme. The electron density map has now been extended from 4 Å to 3.5 Å resolution. A tentative chain tracing has been made for all three independent molecules in the asymmetric unit. We have begun to align the chemical sequence and to assign the individual residues into the electron density map. A preliminary structural alignment with the Klenow fragment of DNA polymerase I has been made. The results of our analysis and its implications will be presented.

Initiation Mechanisms-I

R 005 INTERACTION OF RNA POLYMERASE WITH *E. coli* PROMOTERS IN THE PRESENCE AND IN THE ABSENCE OF THE CYCLIC AMP RECEPTOR PROTEIN, Henri Buc, Annie Kolb, Marc Lavigne, Malcolm Buckle, and Johannes Geiselmann, Institut Pasteur, Unité de Physicochimie des Macromolécules Biologiques (CNRS: URA 1149), F-75724 Paris Cedex 15

By binding upstream of the *lac*, *gal* and *malT* promoters, the cyclic AMP receptor protein (CAP) activates initiation of transcription at these three loci. The present evidence about the mechanism of activation will be reviewed : specific steps at which activation takes place, involvement of protein-protein interactions, and the role of DNA structure.

Particular emphasis will be placed on the role of the spacing between the start site of transcription and the center of the CAP binding site. The drastic effect of a deletion of 1 bp which affects spacing in a *gal* P1 promoter mutant will be detailed.

The structure of open complexes formed in the presence as well as in the absence of CAP *in vitro* and the origin of the positive interactions occuring in the ternary complex has been studied by crosslinking and by footprinting techniques. Implication of experiments using proteins altered by mutation and by deletion with impaired activation function will be discussed. (This part of the work is a cooperation with S. Busby's group at Birmingham-U.K. and with A. Ishihama's team at Mishima-Japan).

Technical improvements allowing to monitor distorsion of the DNA structure as well as the establishment of protein-nucleic acid contacts during open complex formation will be described.

R 006 SIGMA 54 TRANSCRIPTIONAL ACTIVATION, Jay D. Gralla, Department of Chemistry and Biochemistry and the Molecular Biology Institute, U.C.L.A., Los Angeles.

Comparative studies on the formation of open promoter complexes will be reported. The bacterial transcription factor sigma 54 confers on the bacterial transcription machinery the ability to be activated by compatible enhancer-like proteins. Domains within the protein that are essential for this include several DNA-binding domains, acidic and glutamine-rich activation domains, and a transcriptional masking domain. The current state of knowledge concerning the mechanism of activation will be discussed with particular attention to how the transcription start site is melted. The results will be compared to analogous studies of start site melting by mammalian RNA Polymerase II.

R 007 PARTIAL POLYPEPTIDES OF SIGMA-70 BIND SPECIFICALLY TO pTAC PROMOTER DNA, Carol A. Gross, Deborah A. Siegele, and Alicia J. Dombroski, Department of Bacteriology, University of Wisconsin, Madison, WI 53706

The σ^{70} transcription initiation factor of *E.coli* possesses two domains, referred to as Regions 2 and 4, that have been implicated genetically in recognition of the -10 and -35 consensus sequences of promoter DNA. Purified intact σ^{70} however has not been shown to interact specifically with promoters. We hypothesize that assembly of free σ^{70} with core RNA polymerase is necessary to induce a conformational change that reveals Sigma's DNA binding domains. We have examined the question of whether σ^{70}, in the absence of core RNA polymerase, is able to recognize and bind to promoter DNA by constructing a series of in-frame fusions between glutathione-S-transferase and various partial polypeptides of σ^{70}. This approach allowed us to separate the DNA binding domains from the remainder of σ^{70}. The purified fusions were characterized *in vitro*. A fusion carrying Region 4 binds preferentially to pTac and shows some specificity for the -35 consensus sequence. A fusion combining the putative DNA binding portions of region 2 with regions 3 and 4 demonstrates increased affinity, as well as specificity, for both the -10 and -35 consensus sequences. The greatest affinity and specificity occurs with a fusion carrying the putative core binding domain and Regions 2 through 4 (amino acids 360-613). Longer fusions become progressively less capable of binding DNA and discriminating promoter sequences from non-promoter sequences. These results therefore support the genetic evidence for σ^{70}'s participation in promoter recognition and support an allosteric model whereby DNA binding ability is only conferred when the amino-terminal segment of σ^{70}(amino acids 1-360) is deleted or assumes a conformation that unmasks the DNA binding domains.

Initiation Mechanisms-II

R 008 MECHANISM OF PROMOTER SELECTION BY MAMMALIAN RNA POLYMERASE II, Joan W. Conaway, Hiroaki Serizawa, Jeanene P. Hanley, John N. Bradsher, Karla P. Garrett, and Ronald C. Conaway, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

Productive binding of RNA polymerase II at the core region of TATA box-containing promoters is controlled by the action of the TATA factor and four additional transcription factors designated α (TFIIB), $\beta\gamma$ (RAP30/74), δ, and ϵ (TFIIE), which have each been purified to near homogeneity from rat liver. This process is accomplished in three distinguishable stages. In the first stage (Initial Complex formation), the core promoter is packaged with the TATA factor into a binary complex that serves as the recognition site for RNA polymerase II. In the second stage (Site Selection), transcription factors α and $\beta\gamma$ act in combination to promote selective binding of RNA polymerase II to the Initial Complex. Several lines of evidence argue that α and $\beta\gamma$ function at this stage by a mechanism analogous to that utilized by bacterial sigma factors. In the third stage, transcription factors δ and ϵ promote stable binding of the transcription apparatus to the Initiator element to form the complete preinitiation complex. Taken together, our findings argue that RNA polymerase II binds productively at the core promoter in at least two discrete steps, first "touching down" near the TATA element and finally extending its interaction downstream to encompass the capsite.

R 009 RNA POLYMERASE B (II) TRANSCRIPTION FACTORS : BTF2 PURIFICATION AND PROPERTIES, L. Fischer, C. Chalut, M. Kanno, N. Burton, V. Moncollin, and J.M. Egly, LGME/CNRS U/184 INSERM, Institut de Chimie Biologique, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg cédex.

It is now well established that the accurate and specific initiation of transcription of protein coding genes, involves the formation of a complex of proteins, which contains, in addition to RNA polymerase B (II), an, as yet poorly defined, number of general transcription factors. We will discuss the following points : (1) Comparison of the DNA binding and the transcription activities of both the endogeneous and the recombinant human TFIID. (2) Expression of both human and yeast TFIID ; we will point out the problems encoutered in obtaining of soluble TFIIDy having the same biological properties as the endogeneous factor. (3) We will show that TFIIB, which is different from BTF3, once bound to the stable preinitiation complex modifies the footprinting profile of recombinant yeast and human TFIID; the expression and purification of recombinant TFIIB will also be presented. (4) The purification and the interaction of the factor BTF2 will be described. This factor, which is a multi subunit protein according to our purification scheme, binds to RNA polymerase B in solution and to some of the other general transcription factors, whereas it does not bind to the adenovirus 2 major late upstream element factor (UEF/MLTF). This factor is different from TFIIE (according to Western blot analysis effected with D. Reinberg group, antibodies) and TFIIF (as shown by Western blot using RAP 30 antibodies.)

R 010 RAP30/74: MECHANISM OF ACTION OF A GENERAL TRANSCRIPTION FACTOR THAT INTERACTS WITH RNA POLYMERASE II,

Jack Greenblatt[1], Marie T. Killeen[1], Susan McCracken[1], Joyce Li[1], and Zachary F. Burton[2], [1]Banting and Best Department of Medical Research and Department of Molecular and Medical Genetics, University of Toronto, Toronto, Canada and [2]Department of Biochemistry and Agricultural Experiment Station, Michigan State University, E. Lansing, MI48824.

RAP30/74 (also known as TFIIF, βγ, or FC) is a heteromeric general initiation factor that binds to RNA polymerase II (1). It is required for initiation regardless of whether the promoter has a recognizable TATA box (1). Human cDNAs encoding both subunits, RAP30 and RAP74, have been cloned, sequenced, and expressed in *E. coli* (2,3). These cDNA's encode the active human initiation factors because RAP30 and RAP74 produced in *E. coli* can substitute for natural human RAP30/74 for initiation *in vitro* at the adenovirus major late promoter (Ad2MLP).

The small subunit RAP30 of RAP30/74 produced in *E. coli* binds to RNA polymerase II in the absence of RAP74(4). Both partially purified natural RAP30/74 (5) and recombinant RAP30 (4) prevent RNA polymerase II from binding non-specifically to DNA. Recombinant RAP30 also inhibits non-specific transcription by RNA polymerase II (4). However, unlike natural RAP30/74 (5), recombinant RAP30 cannot remove RNA polymerase II already bound non-specifically to DNA, implying RAP74 may be necessary for this activity. Part of RAP30 is weakly homologous to the segment of *E. coli* σ^{70} required for the association of σ^{70} with bacterial RNA polymerase (2). This relationship is likely to be functionally meaningful, firstly because RAP30 is the RNA polymerase-binding subunit of RAP30/74 (4,6), and secondly because human RAP30/74 binds to the core component of *E. coli* RNA polymerase and is displaced from RNA polymerase by σ^{70} (6). Natural RAP30/74 (or TFIIF) mediates the association of RNA polymerase II with a TATA box-containing promoter sequence containing the general factors TFIID and TFIIB, in the presence or absence of TFIIA (7,8). Both RAP30 and RAP74 are present in this pre-initiation complex containing RNA polymerase II, but recombinant RAP30 is sufficient for the recruitment of RNA polymerase II to a DB complex or DAB complex (7). Recombinant TFIID, TFIIB, and RAP30 are both necessary and sufficient for the specific binding of RNA polymerase II to the Ad2MLP, but other factors are subsequently required for initiation of transcription. The ability of RAP30 to recruit RNA polymerase II to a DB complex may be an indirect consequence of its ability to suppress non-specific binding of RNA polymerase II to DNA.

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R 011 INITIATION OF TRANSCRIPTION BY RNA POLYMERASE II. Danny Reinberg, Alejandro Merino, Ilho Ha, Leigh Zawel, Hua Lu, Lisa Weis, Jose Perez, Edio Maldonado, and Juan A. Inostroza. University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635.

Initiation of transcription from class II promoters, those transcribed by RNA polymerase II, requires seven protein factors (TFIIA,-IIB, IID, IIE, IIF, IIH, IIJ) in addition to RNA polymerase II. Of these seven factors only TFIID has a DNA binding activity with specificity for the TATA motif. Formation of a transcription competent complex on promoters that contain a TATA motif is initiated by the binding of TFIID. The other factors and RNA polymerase II enter into the preinitiation complex via protein:protein interactions. The structure of those general transcription factors (GTFs) which have been cloned, as well as the biochemical events that lead to initiation of basal levels of transcription (in the absence of activators) are currently under study.

The mechanisms of initiation of transcription on those promoters that lack a TATA motif are poorly understood. Toward this goal we have analyzed complex formation on different TATA-less promoters. Our findings indicate that transcription from TATA-less promoters requires the same factors utilized by promoters that contain the canonical TATA motif at -30. However, on TATA-less promoters the initiator (Inr) DNA element is recognized by specific DNA binding protein(s) (ITF). There appears to be a family of ITF factors. ITF, in addition to recognizing the Inr, interacts with the general transcription factors and/or RNA polymerase II. The interaction of ITF with the Inr and with components of the basal machinery results in a recognition site for the assembly of the general factors into a transcription competent complex.

Activation of basal transcription is a complex process. We have found that activation involves: i) the removal of factors that negatively affect basal transcription (DR-1 and DR-2). These factors interact with TFIID. ii) The direct interaction of the activator (acidic or proline rich) with one or more of the GTFs. iii) A protein fraction which contains components necessary for stimulation of basal levels of transcription. Dr-1 and Dr-2 have been extensively purified and a cDNA clones encoding Dr-1 have been isolated from HeLa cell. We have found that the interaction of Dr-1 with TFIID is regulated by phosphorylation.

R 012 MOLECULAR MECHANISMS OF TRANSCRIPTIONAL REGULATION IN YEAST

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The yeast GCN4 protein binds to upstream promoter sequences of 30-100 genes involved in amino acid biosynthesis and coordinately induces their transcription in response to amino acid starvation. The GCN4 DNA-binding domain is similar in sequence to the Jun oncoprotein, the oncogenic version of the vertebrate AP-1 transcription factor. Moreover, GCN4 and Jun bind the same DNA sequences (consensus ATGACTCAT), and jun efficiently activates transcription in yeast cells indicating a basic similarity in the molecular mechanism of eukaryotic transcriptional activation. However, unlike Jun or Fos, GCN4 can not transform rat embryo fibroblasts in combination with ras, even though it activates transcription to a comparable extent in these cells. This indicates that oncogenesis mediated by Jun and Fos does not simply reflect the ability to indiscriminately activate target genes containing AP-1 binding sites.

The GCN4 DNA-binding domain contains the bZIP structural motif which consists of a "leucine zipper" dimerization element and an adjacent basic region that undergoes a global folding transition upon specific interaction with DNA. GCN4 mutant proteins with altered DNA-binding specificities have provided an initial description of the protein-DNA contacts. In particular, mutations of asn235, ala238, and ser242 result in a GCN4 derivatives with altered sequence recognition at DNA residues ±4, ±3, and ±2 respectively, suggesting a tentative alignment of an α-helical surface of the protein along the target DNA. In addition to the DNA-binding domain, transcriptional activation by GCN4 requires a short acidic region in the center of the protein. The activation region is a repeated structure composed of small units that act additively which presumably interacts with other proteins of the transcriptional machinery. However, synergistic transcriptional activation on promoters with multiple binding sites does not depend on the number of acidic activation domains bound to promoter.

TFIID is the highly conserved, general transcription factor that binds TATA elements (consensus TATAAA) found in most eukaryotic promoter. A region of important for DNA binding has been identified by a yeast TFIID mutant that displays an altered specificity for TATA elements. In contrast to wild-type, the mutant protein permits transcription from promoters containing a mutated TATA element (TGTAAA) but can not support cell growth. Although eukaryotic TFIIDs have very similar amino acid sequences and biochemical properties, TFIID function is species-specific because human TFIID can not replace yeast TFIID *in vivo*. Surprisingly, this functional distinction reflects differences in the C-terminal core domains, not the divergent N-terminal region; indeed, the N-terminal region appears functionally unimportant for the essential function of yeast TFIID. An altered specificity version of human TFIID can support basal and GCN4-activated transcription in yeast cells from a TGTAAA-containing promoter, indicating that human TFIID can respond to acidic activator proteins together with the other components of the yeast transcription machinery. Finally, using temperature or proteolytically sensitive mutants of TFIID, it has been shown that TFIID is required for transcription from promoters lacking conventional TATA elements.

Initiation Mechanisms-III

R 013 MOLECULAR MECHANISMS MEDIATING GROWTH-DEPENDENT CONTROL OF RIBOSOMAL GENE TRANSCRIPTION, Ingrid Grummt, Anne Kuhn, Renate Voit, Andreas Schnapp, and Gisela Heilgenthal, German Cancer Research Center, 6900 Heidelberg, FRG.

We are studying the molecular mechanisms which regulate ribosomal RNA gene transcription according to the growth rate of the cells. Extracts prepared from exponentially growing mouse cells are active in rDNA transcription, whereas those of stationary cells are inactive. In an attempt to elucidate the mechanisms of this growth-dependent transcriptional regulation, we have purified the factors required for faithful initiation and studied their mode of action. We show that four positively acting transcription initiation factors (termed TIF-IA, TIF-IB, TIF-IC, and UBF) together with RNA polymerase I (pol I) to form a productive preinitiation complex at the core region (from -40 to -1) of the mouse rDNA promoter. Upstream sequences, the UCE (upstream control element, from -144 to -112) and the repetitive 140 bp elements in the spacer strongly increase the stability of the preinitiation complex and enhance the efficiency of transcription. Preincubation, template commitment, as well as order of addition protocols were used to analyze the various intermediate complexes formed during assembly of initiation complexes. As a first step, the species-specific factor TIF-IB binds to the rDNA promoter, a process that is facilitated by the upstream control element and factor UBF. Following binding of TIF-IB, pol I, TIF-IC and TIF-IA successively assemble in a well defined order. Interestingly, the transcriptional activities of two factors, TIF-IA and UBF, are affected by the physiological state of the cells. Transcriptionally inactive extracts which contain no TIF-IA activity can be functionally reconstituted by addition of purified TIF-IA, a regulatory protein that physicacally associates with pol I. In addition, changes in UBF activity are observed in response to cell growth. UBF purified from growing cells activates rDNA transcription in the presence of pol I and the other factors. However, UBF prepared from stationary cells is inactive, although it binds to its target DNA sequence with the same efficiency and specificity as UBF prepared from growing cells. We show that mUBF is phosphorylated in vitro by a protein kinase which by a number of criteria closely resembles casein kinase II (CKII). Peptide mapping and in vitro phosphorylation of recombinant UBF as well as functional analysis of several UBF mutants demonstrate that the hyperacidic carboxy-terminal domain of UBF is the major target for phosphorylation by CKII. Data will be presented showing that (i) UBF interacts specifically with pol I and (ii) that trans-activation by UBF is brought about by antagonizing the inhibitory action of (a) yet to be characterized DNA binding protein(s). This postulated negative-acting protein suppresses the assembly of preinitation complexes by competing for TIF-IB binding. Taken together, the results suggest that regulation of rDNA transcription initiation is the result of a complex interplay of positive- and negative-acting factors whose activity may be modulated by extracellular signals.

R 014 THE DNA-BINDING, INITIATION AND ELONGATION STEPS OF RNA POLYMERASE III-DIRECTED TRANSCRIPTION. George A. Kassavetis, Blaine Bartholomew[1], Burkhard Braun, Carolyne Bardeleben, and E. Peter Geiduschek, Dept. of Biology, U.C. San Diego, La Jolla; [1]present address: University of Southern Illinois, Carbondale.

The binding of RNA polymerase III (pol III) at the site of transcription initiation on tRNA and 5S rRNA genes requires the sequential assembly of transcription factors TFIIIA (5S-specific), TFIIIC and TFIIIB. On tRNA genes, TFIIIC acts first by binding to two variably-separated, intragenic promoter elements: *box A* and *box B*. The interaction of TFIIIC with the transcriptional start site-proximal *box A* promoter element determines the placement of TFIIIB upstream of the start site of transcription. Once bound to upstream DNA sequence, TFIIIB alone is capable of positioning pol III for accurate initiation. We have previously analyzed the disposition of the subunits of TFIIIC and TFIIIB along the *S. cerevisiae* SUP4 tRNA gene by a site-specific DNA photocrosslinking technique. We have now extended this analysis to 5S rRNA gene transcription complexes in which the sequential assembly of TFIIIC and TFIIIB is dependent on the initial interaction of TFIIIA to the *box C* intragenic promoter element. Although the *S. cerevisiae* 5S rRNA gene lacks both the *box A* and *box B* promoter elements, the disposition of the subunits of TFIIIC and TFIIIB is very similar in the space around the 5S rRNA and the SUP4 tRNA genes. The current DNA photo-crosslinking data on the disposition of the pol III subunits in binary and ternary transcription complexes will also be presented.

We have recently begun to examine the steps leading to productive RNA chain elongation. RNA polymerase III is capable of assembling onto a TFIII(B+C)-tDNA complex at 0 °C to form a stable "closed" promoter complex in which the DNA surrounding the transcriptional start retains its duplex form. Promoter opening is a temperature dependent and readily reversible process in which at least two regions appear to melt independently. When pol III initiates transcription under conditions that limit the nascent RNA chain to <6 nucleotides, it is in a distinguishable state from pol III in either the "open" promoter complex or the initiated complex containing a nascent 17 nucleotide RNA chain in terms of stablility and the properties of the transcription bubble. An analysis of RNA chain elongation and of the effect of DNA-bound TFIIIC on the ability of pol III to transcribe through the *box A* and *box B* promoter elements will also be presented.

R 015 MACROMOLECULAR INTERACTIONS DURING TRANSCRIPTION INITIATION BY RNA POLYMERASE I. Marvin R. Paule, Qin Yang, Christopher Terpening, Cathy Radebaugh, Han Li, Victor Duarte, Robin Jump, Philippe Georgel* and Kensal van Holde*. Department of Biochemistry, Colorado State University, Fort Collins, CO 80523, *Department of Biochemistry and Biophysics, Oregon Sate University, Corvallis, OR 97331

Initiation of ribosomal RNA transcription in *Acanthamoeba* involves the binding of a transcription factor, TIF-IB, to a promoter element extending from 17 bp to approximately 67 bp upstream of the transcription initiation site (*tis*) to form the committed complex. This complex persists through multiple rounds of transcription initiation. TIF-IB has been purified to near homogeneity by classical and DNA affinity chromatography and shown to consist of a 145 kD subunit which probably dimerizes to form the 289 kD native factor. While TIF fractions from after the second round of DNA affinity chromatography can produce the gel retardation and footprint characteristic of the committed complex, homogeneous (glycerol gradient) TIF-IB cannot do so in the absence of a second factor fraction with properties similar to vertebrate UBF. This latter protein factor from *Acanthamoeba* was identified by its ability to bind to a series of repeated sequences within the intergenic spacer, and which have characteristics of rRNA enhancer elements. Our results are compatible with the notion that rRNA enhancers maintain a high concentration of UBF in the nucleolus, and that UBF acts as an assembly factor for TIF-IB, probably by protein-protein interaction.

In vivo, it has been suggested that formation of the committed complex competes for the promoter with nucleosome assembly over the promoter. Certain DNA sequences, *Xenopus* 5S RNA genes, cloned downstream of the rRNA promoter preferentially position nucleosomes on the template such that a number of restriction enzyme sites are blocked. We find that the prior presence of TIF-IB and pol I on the template can alter this positioning of nucleosomes so that the restriction sites are accessible. Therefore, prior assembly of an active transcription complex does prevent the obstruction of the promoter by nucleosomes. Thus enhancers which localize assembly factor UBF in the viscinity of the promoter could prevent promoter obstruction by speeding committed complex formation.

The next step in initiation is the binding of RNA polymerase I to the committed complex. This is accomplished by protein-protein contacts with TIF-IB, and is DNA sequence-independent. DNA melting does not occur until after NTPs have been added, and we have been examining in detail the melting process by moving and stalling polymerase I on the template at various positions. The results suggest that initial melting occurs in an AT-rich region just upstream of the *tis*, following formation of the first phosphodiester bond(s), but not before. rRNA transcription is regulated by a modification of pol I which prevents it from binding to the promoter by interfering with polymerase-TIF interaction. Structural studies have identified an alteration of the 39 kD subunit, a subunit common to polymerases I and III and a homolog of the eubacterial α-subunit. The modification temporally correlates with transcriptional shutoff of the rRNA gene. Perhaps significantly, the same subunit is modified in RNA polymerase III during transcriptional shutoff of 5S RNA transcription.

Supported by grants GM22580, GM26059 and GM39901 to M.R.P. and GM22916 to K.v.H. from the U.S. Public Health Service.

Structure and Function of RNA Polymerases

R 016 MUTATIONAL ANALYSIS OF THE YEAST RNA POLYMERASE II C-TERMINAL DOMAIN, Jeffry L. Corden[1], Marilyn L. West[2], Hugh Y. Rienhoff[1], and Catharine E. Johnson[1], [1]Howard Hughes Medical Institute and Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, [2]Department of Botany, University of California, Davis.

The largest subunit of RNA polymerase II contains an unusual repetitive C-terminal domain (CTD) consisting of tandem repeats of the seven amino acid consensus sequence $Tyr_1Ser_2Pro_3Thr_4Ser_5Pro_6Ser_7$. The consensus heptapeptide is repeated from 17 to 52 times in different eucaryotes. Previous studies have shown that deletion of the CTD coding sequence is lethal indicating that the CTD plays an essential role in transcription in vivo. The nature of this essential role is unknown although a number of studies have suggested a role in initiation. We have developed several strategies to mutate the yeast CTD in order to study the nature of this essential function. These mutational studies have addressed the length requirement of the CTD and also its phosphorylation.

The CTD is known to be phosphorylated in vivo and we have previously identified a CTD kinase that can phosphorylate serines in positions two and five in vitro. To test the requirement for phosphorylation of these residues in vivo we have developed a cloning strategy to replace the natural CTD coding sequence with ligated oligonucleotides encoding heptapeptide repeats with amino acid substitutions. Changing all of the serines in either position two or five to alanine is lethal. Similarly changes to the constitutively charged amino acid glutamic acid are also lethal. These results are consistent with a requirement of cyclic phosphorylation of these residues. Other mutations that switch the order of serine and proline residues or substitute phenylalanine for tyrosine are also lethal.

We have also looked at the minimum number of heptapeptide repeats required for viability. CTDs containing as few as eight repeats are viable, although the growth rate of these cells is substantially reduced. Specific transcription by RNA polymerase IIB (lacking the CTD) has been demonstrated for several genes in vitro. We have examined transcription by RNA polymerase IIB in vivo using photoaffininty labeling. We detect no transcription by this polymerase in nuclei suggesting that the CTD is necessary for transcription initiation *in vivo*.

CTDs containing more than the wild-type number (26/27) of repeats have been constructed in yeast by cloning portions of the mouse CTD into the yeast gene. The longest of these CTDs confer a phenotype similar to that of the truncated CTD suggesting that there in an optimal length for the CTD.

R 017 THE ROLE OF CTD KINASE AND CTD PHOSPHATASE IN THE TRANSCRIPTION CYCLE OF RNA POLYMERASE II, Michael E. Dahmus, Jonathan D. Chesnut, John M. Payne and Joseph H. Stephens, Department of Biochemistry and Biophysics, University of California, Davis, CA 95616

The largest subunit of RNA polymerase II (RNAP II) contains at its C-terminus an unusual domain (CTD) consisting of tandem repeats of the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. The extensive phosphorylation of this domain is thought to play an important role in the initiation phase of transcription and may be of regulatory significance. The phosphorylated form of RNAP is designated IIO (contains subunit IIo) whereas the unphosphorylated form is designated IIA (contains subunit IIa). An analysis of the state of CTD phosphorylation as a function of the position of RNAP II in the transcription cycle has lead to the proposal that each round of transcription is associated with the reversible phosphorylation of the CTD. Considerable evidence supports the idea that RNAP IIA interacts with the promoter to form a stable preinitiation complex and that elongation is catalyzed by RNAP IIO. Consequently, phosphorylation of the CTD is thought to occur after the association of RNAP IIA with the promoter by a CTD kinase (CTDK) that is an integral component of the preinitiation complex. Completion of the cycle is presumed to be dependent on a CTD phosphatase (CTDP) that catalyzes the dephosphorylation of RNAP IIO thereby regenerating RNAP IIA.

An assay has been developed for CTDK and CTDP based on the differential mobility in SDS-PAGE of RNAP subunits IIo and IIa. ^{32}P-RNAP IIA was prepared by phosphorylation of the terminal serine of subunit IIa with casein kinase II. ^{32}P-RNAP IIO was prepared by phosphorylation of labeled RNAP IIA with partially purified CTD kinase in the presence of excess cold ATP. CTDKs catalyze a shift in the mobility of subunit IIa to a position of subunit IIo whereas CTDP catalyzes a shift in the mobility of subunit IIo to that of IIa. Two distinct CTDKs, designated CTDK1 and CTDK2, have been fractionated from a HeLa cell transcription extract by chromatography on Mono Q and Cibacron Blue Agarose. Both kinases catalyze the phosphorylation of serine and threonine, transferring approximately 100 mole phosphate per mole of subunit IIa. CTDK1 and 2 do not appear to be related to $p34^{cdc2/cdc28}$. CTDP, partially purified from HeLa cell transcription extracts, catalyzes dephosphorylation of the CTD thereby converting RNAP IIO to IIA. CTDP can utilize as substrate either purified RNAP IIO or RNAP IIO formed *in vitro* by phosphorylation of RNAP IIA with CTD kinase. Furthermore, CTDP is specific in that it does not catalyze the dephosphorylation of the serine residue phosphorylated by casein kinase II.

If indeed the dephosphorylation of RNAP IIO is essential before RNAP can initiate the next round of transcription, RNAP IIO should be inactive in *in vitro* transcription reactions that lack CTDP. In order to test this hypothesis, RNAP IIA and IIO were purified from calf thymus and their ability to transcribe the adenovirus 2 major late promoter (Ad2-MLP) was determined in the presence of a reconstituted transcription extract. In the absence of CTDP, the transcriptional activity of RNAP IIO is less than 20% that of RNAP IIA. The ability of ^{32}P-labeled RNAP IIA and IIO to form a stable preinitiation complex on the Ad2-MLP was also examined. These studies indicate that RNAP IIO cannot form a stable preinitiation complex under conditions that do result in stable complex formation with RNAP IIA. Consequently, the state of phosphorylation of the CTD can influence preinitiation complex formation.

R 018 IDENTIFICATION OF SIGNALS FOR PHOSPHORYLATION OF THE RNA POLYMERASE II CARBOXYL-TERMINAL DOMAIN, William S. Dynan[1], Scott R. Peterson[1], Arik Dvir[1], and Carl W. Anderson[2], [1]Dept. of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309, and [2]Biology Department, Brookhaven National Laboratory, Upton, N. Y. 11973

Previous studies have shown that a template-associated protein kinase phosphorylates the heptapeptide repeats of the carboxyl-terminal domain (CTD) of RNAP II. This phosphorylation reaction is closely associated with initiation of transcription. Although phosphorylation is dependent on a functional promoter sequence, the actual signal for phosphorylation is unknown. To identify this signal, we performed experiments using a recombinant protein substrate that contained the CTD of RNAP II fused to the DNA binding domain of a different protein. The presence of the DNA binding domain allowed us to localize the CTD substrate to the DNA independently of transcription complex formation.

We found that the fusion protein was multiply phosphorylated by a protein kinase that was detectible in crude nuclear extracts and enriched in fractions eluted from isolated transcription complexes. This enzyme was further purified and shown to closely resemble a previously known, DNA-dependent protein kinase (DNA-PK). CTD phosphorylation was dependent on the presence of the DNA binding domain in the fusion protein and on the presence in the reaction of a DNA fragment containing the cognate binding site. There was no absolute requirement for other transcription factors in the reaction. These results suggest that association of the CTD with DNA in itself may provide a signal for phosphorylation.

The design of the recombinant substrate allows us to colocalize the CTD on DNA fragments with various combinations of transcriptional activator proteins and general transcription factors. We are presently evaluating whether these other proteins provide additional signals that modulate the rate of CTD phosphorylation.

R 019 *IN VIVO* ANALYSIS OF THE C-TERMINAL REPEAT DOMAIN OF RNA POLYMERASE II LARGEST SUBUNIT, Jae Moon Lee, Steven Hardin, June Brickey, and Arno L. Greenleaf, Duke University Medical Center, Biochemistry Department, Durham, NC 27710.

The C-terminal repeat domain (CTD) of the largest subunit (IIa) of RNA polymerase II is essential *in vivo* but dispensable in some *in vitro* situations. This unusual domain, which contains the consensus repeat sequence YSPTSPS, is also subject to hyperphosphorylation on Ser and Thr residues. The hyperphosphorylation reduces the SDS gel mobility of the subunit, generating subunit IIo. Different experimental approaches are beginning to suggest specific functions for the CTD and modulations of those functions by phosphorylation, but more data are required to establish exactly what the physiological role(s) of the CTD might be and to determine what the significance of CTD hyperphosphorylation is in a physiological context. Toward these ends we are investigating the CTD and its phosphorylation *in vivo* in both *Drosophila* and yeast.

We have constructed a version of the *Drosophila* largest subunit gene that lacks the entire CTD and have used P element-mediated germline transformation to introduce this "IIt" subunit into the fly genome. Because the IIt subunit also carries a mutation causing amanitin-resistance, we can be sure that the transgene is expressed and the subunit is incorporated into catalytically active RNA polymerase II. Having shown that animals carrying only the truncated version of the subunit are inviable, we are in the process of attempting to determine how RNA polymerase II lacking the CTD is defective *in vivo*. We have also generated antibodies that are highly selective for either the unphosphorylated CTD or the hyperphosphorylated CTD, and we hope to employ these reagents to search for possible correlations between CTD phosphorylation state and RNA polymerase II activities.

We continue to investigate CTD phosphorylation in the yeast *Saccharomyces cerevisiae*. We previously showed that the *CTK1* gene, encoding the alpha subunit of the yeast CTD kinase we purified, is essential for normal growth (Lee and Greenleaf, 1991). That work also indicated that *ctk1* mutant cells which lack CTK1-containing CTD kinase were inviable at low temperatures (e.g. 13°C) and grew slower than wild type at 30 or 37°C. Analysis of RNA polymerase II in the *ctk1* mutants revealed that abnormally phosphorylated forms of the largest Pol II subunit were present. We concluded that the CTK1-containing CTD kinase normally plays a role in CTD phosphorylation *in vivo*. We further concluded that yeast cells contain at least one additional activity capable of phosphorylating the CTD. We are using genetic approaches to identify candidates for this additional activity. These approaches have already revealed that certain alleles of a second locus, *SLC1*, result in synthetic lethality at 37°C when present in *ctk1* mutant cells. We cloned the *SLC1* gene by *in vivo* complementation of the synthetic lethality and discovered that it is identical to a gene previously cloned by more than one group and referred to as either *SSD1* or *SRK1*. We are currently attempting to determine if and how the *SLC1* gene product might be involved in CTD phosphorylation.

Reference: Lee, J.M. and Greenleaf, A.L. (1991). "CTD kinase large subunit is encoded by *CTK1*, a gene required for normal growth of *Saccharomyces cerevisiae*." GENE EXPRESSION 1, 149-167.

Regulatory Mechanisms-I

R 020 PROKARYOTIC ENHANCERS AND ENHANCER-BINDING PROTEINS, D. S. Weiss[1], K. Klose[1], S. Porter[1], A. North[1], A. Wedel[1], and S. Kustu[1], [1]Departments of Plant Pathology and Molecular and Cell Biology, University of California, Berkeley.

A number of prokaryotic enhancer-binding proteins activate transcription by the alternative holoenzyme form of RNA polymerase, σ^{54}- holoenzyme, allowing this form of polymerase to transcribe in response to different physiological signals. The best-characterized of these enhancer-binding proteins is the NTRC protein (nitrogen regulatory protein C) of enteric bacteria. The function of NTRC has been studied intensively at the major promoter for the *glnA* gene, which encodes glutamine synthetase. NTRC stimulates the isomerization of closed complexes between σ^{54}-holoenzyme and the *glnA* promoter to open complexes in an ATP-dependent reaction. To do so NTRC binds to its enhancer-like sites, which are normally located between 100 and 150 bp upstream of the transcriptional startsite, and contacts the polymerase by means of DNA loop formation. One function of the enhancer is to tether NTRC in high local concentration near the promoter. To stimulate open complex formation NTRC must hydrolyze ATP. ATP hydrolysis depends upon phosphorylation of an aspartate residue located in a regulatory domain of NTRC that is distinct from the domain responsible for ATP hydrolysis and transcriptional activation. Phosphorylation of NTRC is increased under conditions of nitrogen limitation, conditions under which transcription of *glnA* is increased. It is our working hypothesis that ATP hydrolysis is not needed for specific contact between NTRC and polymerase per se, but rather functions to allow a change in configuration of polymerase once contact has occurred.

Regulatory Mechanisms-II

R 021 INTERACTIONS OF VIRAL TRANSCRIPTION FACTORS WITH THE TATA-BOX BINDING PROTEIN AND TFIID COMPLEX, Arnold J. Berk, Wes Lee, Cheng Kao, Xuan Liu, Eugene Bryant, Paul Lieberman, Qiang Zhou, Tom Boyer, and Naoko Kobayashi, Molecular Biology Institute, UCLA, Los Angeles, CA 90024-1570

The adenovirus E1A protein is a potent activator of transcription. We present several different approaches to demonstrate that the large E1A protein binds specifically and stably to the TATA-box binding protein (TBP). We demonstrate that the activation domain of E1A binds to TBP. It interacts with a 51 residue region from the concerved C-terminal domain of TBP that includes a repeat of basic residues between the homologous direct repeats of TBP. Analysis of TBP binding by E1A mutants indeicates that TBP binding is required, but not sufficient for E1A transactivation. Zta is a sequence specific DNA-binding transcription factor of the B-ZIP class which regulates induction of the lytic cycle in B lymphocytes latently infected with Ebstein-Barr virus. We show that the activation domain of Zta also interacts with the conserved domain of TBP. When Zta is bound to Zta-response-elements in the EBV BHLF1 promoter, it stabilizes TBP binding to a low affinity TATA-box. Nonetheless, Zta does not activate transcription in a standard in vitro transcription reaction with TBP. Zta does activate transcription in a reaction with TFIID complex prepared from HeLa cells. A characterization of the human TFIID complex will be presented.

R 022 IDENTIFICATION OF TRANSCRIPTIONAL INTERMEDIARY FACTORS MEDIATING THE ACTION OF SEVERAL TRANS-ACTIVATOR PROTEINS, Irwin Davidson, Sunita Chaudhary, Laszlo Tora, Christel Brou, Jun Wu, John White, Jung Joo Hwang, Lirim Shemshedini, Hinrich Gronemeyer, Jean Marc Egly, and Pierre Chambon, LGME/CNRS-U184/INSERM 11 Rue Humann 67085 Strasbourg France.

The transactivation functions of the activator proteins GAL4-VP16, Transcriptional enhancer factor 1 (TEF-1) and the estrogen and progesterone receptors are mediated by titratable transcriptional intermediary factors (TIFs) both *in vivo* and in cell free extracts *in vitro*. This hypothesis is supported by the observation that low concentrations of chimeric activator proteins containing the GAL4 DNA binding domain fused to the activation domains of VP16, TEF-1, the AB or EF regions of the estrogen receptor or the AB region of the progesterone receptor, activate transcription both *in vivo* and *in vitro* while at higher concentrations an inhibition of activation is observed. This inhibition of activation at high concentration is ascribed to the titration ("squelching") of a limiting TIF(s). In the case of GAL4-VP16, a fraction containing the TIF(s) is required to allow the VP16 activation domain to act early in the process of preinitiation complex formation to increase the number of functional preinitiation complexes which are formed in an *in vitro* transcription assay. The TIF containing fraction also allows the VP16 activation domain to interact with a component of the transcription apparatus as evidenced by the formation of a complex which is resistant to a monoclonal antibody directed against a peptide from the VP16 activation domain. In the case of TEF-1, the TIF(s) appears to be highly limiting *in vivo* as overexpression of cloned TEF-1 in cells containing endogenous TEF-1 does not lead to a superinduction of activity from reporter plasmids containing its cognate binding sites, but even at very low concentrations a squelching effect is observed. Moreover activation by TEF-1 shows cell type specificity both *in vivo* and *in vitro* suggesting that the TIF(s) also shows cell specificity. In addition the functional synergy of several domains within the TEF-1 protein is required for both transactivation and titration of the TIF(s). Fractions containing the TIFs for the above activators have been partially purified and assayed in reconstituted *in vitro* transcription systems and the results of this analysis will be discussed.

Mechanisms and Regulation of Transcription Elongation and Termination -I

R 023 FUNCTIONAL ANALYSIS OF TRANSCRIPT ELONGATION FACTOR SII FROM HUMAN AND YEAST CELLS, H. C. Chen, G. Cipres Casey, Z. Weaver, K. R. Christie, and C. M. Kane, Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, CA 94720.

The SII protein stimulates elongation by RNA polymerase II and promotes readthrough of some termination sites for the enzyme. Molecular genetics has been used to delete or alter sequences in the protein coding region for the human protein. Each of these altered proteins has been tested for its biochemical properties in vitro with calf thymus RNA polymerase II.

The structure of the human gene is also being examined with an eye toward determining whether or not a family of related sequences exists in humans, and other eukaryotes. This possibility has been suggested by Southern and Northern analysis of human DNA and RNA, and it has also been suggested by the isolation of a variety of related cDNAs from mouse tissues (1). In addition, disruption of the gene in Saccharomyces cerevisiae is not lethal, although growth is slowed (2). The yeast protein also can direct readthrough by yeast RNA polymerase II. Yet, as mentioned above, disruption of this gene is not lethal, although it does confer other phenotypes (3). While this suggests the existence of an SII functional homologue, such a protein has not been purified. Thus, we are using genetic complementation of SII disruption strains to identify other components of the transcription elongation machinery in yeast. Among such complementing genes we expect to find genes encoding functional homologues of SII itself as well as RNA polymerase II subunits. The progress of these genetic screens will be discussed.

(1) Kanai et al. (1991) J. Biochem. 109, 674.
(2) F. LaCroute, A. Ruet, and A. Guyonavarch, personal communication.
(3) Hubert et al. (1983) The EMBO J. 2, 2071.

R 024 **ROLE OF *E. COLI* RNA POLYMERASE SUBUNITS IN RNA CHAIN ELONGATION AND TERMINATION**, Robert Landick, Cathy L. Chan, Guo-Hua Feng, Clarissa Hebron, Donna N. Lee, Rodney Weilbaecher and Michelle Zimmerman, Department of Biology, Washington University, St. Louis, MO 63130

A large body of evidence supports the view that, in *E. coli*, altered interactions between RNA polymerase and the DNA and RNA chains in the transcription complex ultimately cause transcriptional pausing and termination. Changes in pausing or termination, in turn, influence the expression of many genes. At both pause and termination sites, changes in either an RNA stem-loop structure or downstream DNA sequences can affect the response of RNA polymerase. However, the way that these elements interact with RNA polymerase and the way that the interactions are altered when pausing or termination occurs remain unknown.

We have taken a combined biochemical and genetic approach to dissecting the interactions in the bacterial transcription complex. Isolation of termination-altering amino-acid substitutions in the ß subunit revealed that at least four regions of the polypeptide must somehow be involved in termination (1). Most of the regions exhibit strong similarity to the eukaryotic homolog of ß. Some changes in one conserved region (between residues 500 and 575), where most rifampicin-resistance amino-acid substitutions are located, appear to affect the elongation rate. Thus, the effects on termination for this class of substitutions may be explained by changes in the time polymerase resides at a terminator. Termination-altering amino-acid changes in two different regions, around codon 190 and between codons 800 and 850, increase termination efficiency at some terminators and decrease it at others. At least some termination-altering changes in the C-terminal region, between codons 1230 and 1342, also strongly affect assembly of the altered subunit into RNA polymerase.

Recently, we have begun a comparable study of termination-altering substitutions in the ß' subunit. Initial results are extremely interesting. As for ß, termination-altering substitutions in ß' are clustered in segments that correspond to regions conserved between prokaryotic and eukaryotic RNA polymerases. One cluster, between residues 1310 and 1360, is particularly striking. Nearly all are changes of histidine, serine, or threonine to hydrophobic amino acids, suggesting that hydrogen-bonding or chelating interactions in this region participate in elongation.

To better understand the structure of transcription complexes, we have studied the configuration of RNA and DNA chains in isolated paused transcription complexes. Probes for single-stranded DNA, $KMnO_4$ and DEPC, reveal an ~8 nt protected region on the template strand immediately preceding the site of NTP addition. RNase A, a probe for single-stranded RNA, is able to cleave the transcript within the region corresponding to these 8 nt without destroying the capacity of the complexes to resume elongation. The current status of these studies will be described.

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R 025 ELONGATION BY RNA POLYMERASE II: THE TRANSITION FROM INITIATION AND EFFECTS OF ELONGATION FACTORS
Donal S. Luse, Michael G. Izban, George A. Jacob and Joseph Kitzmiller, Dept. of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0524

Our laboratory has focused on two aspects of RNA polymerase II function: the nature of the transition between initiation and elongation (promoter clearance) and the structure and capabilities of the transcription complex once it arrives at the elongation state. We have shown that normal initiation by RNA polymerase II is accompanied by considerable abortive initiation. We have recently compared promoter clearance at the adenovirus major late (Ad ML) and mouse ß-globin promoters. Clearance at the globin promoter occurs rather abruptly 8 or 9 bases downstream of the initiation site, even in globin promoter variants with sequence changes at +8 or +9. Compared to the Ad ML promoter, the globin promoter supports much less productive RNA synthesis and loses proportionally more of its transcripts to the abortive pathway. Furthermore, we demonstrated with a nuclease protection assay that both promoters assemble preinitiation complex to the same extent. These results show that promoters can be limited at the clearance step. We have also extended our earlier observation that nucleosomal templates do not support efficient transcript elongation by RNA polymerase II by testing the effect of factors TFIIS, TFIIF and TFIIX. In our hands these factors stimulate the RNA polymerase II to physiological elongation rates, with minimal pausing, on pure DNA templates. However, the factors only slightly improve the much poorer elongation efficiencies on chromatin templates. Thus, new elongation factors and/or chromatin modifications essential for elongation remain to be discovered. Finally, in the course of testing elongation factors we had occasion to examine the effects of these factors on paused ternary complexes in the absence of NTPs. We found, to our considerable surprise, that the transcript is cleaved under these circumstances, apparently by the RNA polymerase itself (since the truncation is inhibited by α-amanitin). The truncated transcripts are retained in ternary complex since they are elongated when NTPs are added. The factor-dependent truncation process can proceed for over 25 bases; however, transcripts shortened to less than 9 bases cannot be elongated. The implications of these last observations for our understanding of the mechanism of transcript elongation will be discussed.

R 026 REGULATION OF *bgl* OPERON EXPRESSION BY PHOSPHORYLATION AND DEPHOSPHORYLATION OF AN RNA BINDING PROTEIN. Orna Amster-Choder, Maria Diaz-Torres and Andrew Wright, Tufts Medical School, Boston, MA 02111

The *bgl* operon in *E.coli* contains three genes *bgl*G, *bgl*F and *bgl*B required for catabolism of β-glucosides. Transcription from the *bgl* promoter is constitutive but in the absence of inducer it terminates at a rho-independent terminator, located upstream of the *bgl*G gene, yielding a 112 nucleotide transcript. A similar terminator is located in the intercistronic region between *bgl*G and *bgl*F. Thus *bgl* operon expression is regulated by transcription antitermination. We showed that the *bgl*G gene product is a positive regulator of transcription which functions by binding to an RNA sequence present in the two terminator structures, thereby preventing transcription termination (1). Using biochemical and genetic approaches we have now determined that the BglG protein binds to a 32 nucleotide bulged-hairpin structure formed in the nascent RNA, bulge nucleotides being crucial for recognition. We also showed that the ability of BglG to bind to its RNA target is regulated by the product of the *bgl*F gene, a phosphotransferase (PTS) transport protein present in the cytoplasmic membrane (2). In the absence of inducer, BglF transfers a phosphate group to a histidine residue on BglG, thus blocking its function as a transcriptional antiterminator. In the presence of β-glucoside substrate BglF removes the phosphate group from BglG-phosphate leading to activation of BglG. We have now shown that phosphorylation-dephosphorylation modulates a transition between monomeric and dimeric forms of BglG, the phosphorylated form being a monomer. Our results suggest a model in which RNA binding by BglG requires that it be present as a dimer in the cell.

1. Houman, Diaz-Torres and Wright (1990). Cell 62: 1153-1163.
2. Amster-Choder and Wright (1990). Science 249 : 540-542.

Regulatory Mechanisms in Transcription Elongation and Termination -II

R 027 THE RNA POLYMERASE II ELONGATION COMPLEX, Danny Reines, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.

The RNA polymerase II (pol II) elongation complex is the target of cellular regulatory influences, yet the composition and function of this specialized nucleoprotein complex is poorly understood. Transcription by pol II is efficiently arrested at a naturally occurring, intragenic site in a human histone gene that has been characterized previously. Using a monoclonal antibody against RNA, we have immunopurified active elongation complexes arrested at this and other sites. Complexes were formed *in vitro* from partially purified rat liver RNA polymerase II and general transcription factors. Immunoselection allowed us to separate complexes from unincorporated ribonucleotides. In the presence of elongation factor SII and a divalent metal cation, these washed elongation complexes displayed a heretofore unrecognized RNA hydrolysis activity. Recombinant mouse SII, translated *in vitro*, also activated the nuclease, providing evidence that the reaction requires this elongation factor. This SII-dependent RNA cleavage was not pyrophosphorolytic and was inhibited by α-amanitin. RNA must be in an elongation complex to serve as substrate. Shortened chains were capable of being re-extended when nucleotides were added. The simplest interpretation of these data is that, like many DNA polymerases, RNA polymerase II itself contains a nuclease function. Cleavage occurs near the 3'-end of the nascent RNA and extensive digestion can result in RNA chains shortened up to ≈100 nts. Elongation complexes stopped at various template locations, including the end of a linearized or "run-off" template, also underwent this reaction. Thus, this elongation complex-associated nuclease did not display sequence specificity and appeared to be a general property of SII-stimulated RNA polymerase II elongation complexes. A model will be presented suggesting how this novel, SII-activated enzymatic activity may participate in the stimulation of transcript elongation by RNA polymerase II. (Work in the author's laboratory was supported by grants from the National Institutes of Health and the American Cancer Society.)

Chromatin Structure and Transcriptional Regulation

R 028 THE INTERACTION OF CONSTITUTIVE AND TISSUE-SPECIFIC TRANSCRIPTION FACTORS WITH POSITIONED NUCLEOSOMES. Gordon Hager, Trevor Archer, Catharine Smith, Ken Carlson, Emery Bresnick, Sam John, and Philippe Lefebvre. Lab of Molecular Virology, National Cancer Institute, NIH, Bethesda MD 20892.

DNA sequences of the MMTV LTR are positioned in vivo on a phased array of six nucleosomes (A-F). Hormone activation of the MMTV promoter leads to modulation of nucleosome B structure in vivo, manifested as a region of DNA hypersensitive to nucleolytic reagents, and recruitment of transcription factors into an initiation complex. Analysis of nucleosome position using PCR amplification indicates that the nucleosomes are positioned at high resolution, and that the hypersensitivity is asymmetrically localized to the 3' side of nucleosome B and into the A-B linker region. NF1, a component of the MMTV initiation complex, is excluded from uninduced stable chromatin but binds constitutively to transiently introduced DNA. Hormone induction also leads to H1 depletion from the A-B region in stable chromatin, whereas the core histone complement of this region is unchanged. We find that a disomic structure composed of the A and B nucleosomes can be reconstituted in vitro, with the octamer cores accurately positioned, and that NF1 is excluded from this disomic structure[1]. The glucocorticoid receptor, in contrast, can bind to the disome. Thus, two potential mechanisms exist for the exclusion of NF1, either (1) exclusion by nucleosome positioning, or (2) exclusion by a higher-order, H1-dependent, chromatin structure. Modulation of the uninduced chromatin structure is necessary during transcription activation to permit binding of the initiation complex. These results indicate that a chromatin template containing specifically positioned nucleosomes is an active participant in transcriptional activation, and that modulation of this template structure is one feature of steroid hormone action.

Activation of cellular oncogenes by MMTV occurs by proviral insertion. We have identified a regulatory element in the viral regulatory sequences that is responsible for cell-specific viral transcription, and presumably also for tissue-specific oncogene activation[2]. This element, located at the 5' end of the LTR, acts as a cell-specific, positive enhancer region, and has been shown to contain binding sequences for at least two proteins, mp5 and mp4, both of which also appear to be limited in distribution to mammary cells. This element is also active in human mammary cells, and factors that bind to this region are uniquely present in human cell lines of mammary orgin. These observations suggest that MMTV protooncogene activation is mediated by a tissue-specific enhancer, which can constitutively activate a target promoter, but also function synergistically with the HRE of MMTV.

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2. Lefebvre, P., D.S. Berard, M.G. Cordingley, and G.L. Hager. J. Mol. and Cell Biol 11:2529-2537 (1991)

R 029 NUCLEOSOME POSITIONING AND CHROMATIN FUNCTION, S. Y. Roth, R. H. Morse, M. Shimizu, R. C. Parker and R. T. Simpson, Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892

Nucleosome positioning refers to the precise location of interactions of the histone octamer with a given DNA sequence. Stable positioning might influence the function of associated DNA sequences by limiting access for *trans*-acting factors. In the yeast, *S. cerevisiae*, α2 protein, produced in α mating type haploids and a/α diploids, together with MCM1, a non-cell type specific protein, represses transcription of a set of a cell type specific genes. The α2/MCM1 operator is located about 200 bp upstream of the structural genes. We have shown that repressor binding leads to a positioned nucleosome with a border 14 bp away from the α2 operator, both in minichromosomes and for two genomic a cell specific genes, *STE6* and *BAR1*. High resolution analysis demonstrates that the positioned nucleosomes are located with base pair precision in α cells. In contrast, in a cells, where the genes are expressed, the positioned nucleosome is absent and certain sites in the promoter abutting the operator are hypersensitive to nuclease digestion. Topological measurements are consistent with minichromosomes containing the α2 operator having one fewer nucleosome in a than in α cells.

The precision of location and occurrence in a variety of DNA sequence contexts suggested that positioning by α2 might involve interactions with protein domains in the core particle. Certain of the Grunstein histone H4 NH_2 terminal deletion or substitution mutants led to disruption of positioning of a nucleosome adjacent to the α2 operator, even in the presence of functional repressor. The domain of H4 defined by these mutations differs somewhat from that necessary for repression of the silent mating type loci. NH_2 terminal deletion mutants of H2B did not affect the positioned nucleosome.

The location of the positioned nucleosome next to the α2 operator places the TATA box in the center of the nucleosome, suggesting that physical occlusion of the *cis*-acting element to *trans*-acting factors might be the mechanism of repression of transcription. This hypothesis is supported by an experiment in which a series of deletions was constructed that moved another *cis*-acting DNA element, the ARS1 replication origin, successively further into a positioned nucleosome. Copy number, used as an indication of efficacy of ARS function, was drastically reduced when the origin was located within 50 bp of the pseudodyad of the nucleosome. A similar situation seems to hold for access of transcription factors to DNA within nucleosomes. Derepression of transcription of reporter genes linked to two different a cell specific promoters is in lockstep with the absence of a positioned nucleosome in yeast strains with mutated H4 NH_2 terminal regions. We suggest that chromatin structure, generated by interactions of the α2/MCM1 repressor complex with histones, is the mechanism of transcriptional repression of a cell specific genes in α cells.

In contrast to these results for replication origins and RNA polymerase II transcribed genes, when a positioning signal (either DNA sequence or the α2 operator) is engineered to locate a nucleosome such that the control elements of a tRNA gene are in the center of the predicted nucleosome, transcription is unimpaired and the positioned nucleosome is not present. Control experiments with mutated, inactive genes show that the positioning signals act independently of transcription. On the other hand, studies in progress show that transcription of a yeast 5S rRNA gene is blocked when the TFIIIB site is moved into the location of a predicted nucleosome; chromatin structural studies on these mutants are incomplete. An apparent competition exists between formation of positioned nucleosomes and interactions of other functional proteins with DNA in the cell, indicating that chromatin structure is an important consideration in studies of transcriptional regulation. The Stedmans were at least partially correct; chromatin is not a totally transparent package for DNA.

Late Abstract

DNA DISTORTION AND THE MECHANISM OF TRANSCRIPTIONAL ACTIVATION BY THE MerR PROTEIN, Tom O'Halloran,[1,2] Aseem Ansari,[2] Mark Chael[2], [1]Department of Chemistry and [2]Department of Biochemistry Molecular Biology and Cell Biology, Northwestern University, 2145 Sheridan Road, Evanston IL 60208-3113

Most mechanisms for positive control of transcription invoke stimulatory protein-protein interactions between regulatory factors and RNA polymerase. Based on results summarized below we propose that the MerR protein, an allosterically modulated transcriptional activator, communicates with the transcriptional apparatus through a protein-induced modulation of DNA structure. This distortion does not optimize the protein-DNA interface for sequence specific recognition: it apparently plays a direct role in lowering an activation barrier in the transcriptional initiation pathway.

MerR exerts both positive and negative control of plasmid-encoded prokaryotic mercury detoxification (*mer*) genes and it does not fall into one of the well documented classes of positive control factors such as upstream activators, alternate sigma factors, or two component signal-regulator systems. It binds tightly to a single *mer* operator in the presence and the absense of Hg(II). Allosteric modulation of the MerR/DNA complex by mercuric ion binding converts the repressor to an activator of transcription from *mer* TPAD promoter (P_T). The mer operator is located between the -10 and -35 regions of the P_T promoter. This spacer DNA between the -10 and -35 elements is 19 base pairs; two base pairs longer than the consensus *E.coli* promoter.

While the repressor and activator conformations of MerR bind to a site directly adjacent to both polymerase and the nascent transcription bubble, neither form shows detectable affinity for the holoenzyme. Evidence supporting a mechanistic role for protein induced DNA distortion is seen in the Hg(II) responsive hypersensitivity of MerR/DNA complex to the chemical nucleases Cu (5-phenyl-o-phenanthroline) and MPE. The degree of hypersensitivity, and thus of DNA distortion, parallels the extent of Hg(II) binding to MerR and correlates with and transcriptional activation at P_T in parallel transcription assays. To probe the nature of this distortion we have investigated the change in both the twisting and bending of the DNA helix induced by Hg-activated MerR. Both topoisomerase I and ligase assays on a plasmid bearing fifteen *mer* operators in tandem have been used to delineate MerR induced changes in helical twist of the DNA binding site in the presence and absence of effector. The topological data and nuclease results indicate that the activator form (Hg-MerR) stabilizes a localized kink in the promoter that is underwound by ca. 33 degrees relative to the Mer/DNA complex. Circular permutation assays indicate that MerR bends the DNA slightly, but that Hg-MerR bends the DNA to same degree. Thus signal-induced underwinding, but not bending, correlates with the activation event. The magnitude and direction of the Hg-MerR induced change in the local helical twist angle are consistent with a mechanism involving reorientation of conserved hexameric sequences which are otherwise suboptimally phased in this promoter.

These results lead us to propose a model in which underwinding of the spacer DNA is a critical step in MerR mediated transcriptional activation. Protein-induced distortion of DNA may be important not just for optimizing sequence specific recognition but also for lowering activation barriers in the transcriptional initiation pathways.

Structure and Physical Chemistry of DNA and Protein-DNA Interactions; Initiation Mechanisms-I

R 100 AN ATP-DEPENDENT INHIBITOR OF TFIID BINDING TO DNA, David T. Auble and Steven Hahn, Fred Hutchinson Cancer Research Center, Seattle, WA. 98104. We have identified an activity in yeast nuclear extracts which inhibits the binding of TFIID to DNA in an ATP-dependent manner. The effect is ATP-specific, rapid, requires ATP hydrolysis, and can be reversed with bacterial alkaline phosphatase. The inhibitory effect is not promoter-specific as TFIID-DNA complexes formed on the Adenovirus Major Late Promoter TATA sequence or the yeast *LEU2* TATA sequence are equally dissociated by the inhibitory activity. The inhibitory activity will dissociate complexes formed between E. coli-made yeast TFIID and DNA, and this assay has been used to identify fractions enriched in inhibitory activity over the course of purification. The inhibitor is specific for TFIID since three other protein-DNA complexes are unaffected by this activity. While inhibitory fractions contain kinase activity, TFIID itself does not appear to be phosphorylated and the mechanism of action of the inhibitor is not known. Interestingly, a ternary complex formed between TFIID, TFIIA and DNA is not dissociated by the inhibitor, suggesting that transcription complex assembly may be influenced by the interplay of TFIIA and the inhibitor.

R 101 STRUCTURAL POLYMORPHISM OF $d(GA{\cdot}CT)_n$ SEQUENCES: THE EFFECT OF ZINC IONS. F.Azorín, R.Beltrán, J.M.Casasnovas, J.Bernúes, A.Martínez-Balbás, A.Huertas and M.Ortiz Department of Molecular Biology. Centre de Investigación y Desarrollo, CSIC.Jorge Girona Salgado 18-26, 08034 Barcelona, Spain.

Homopurine-homopyrimidine $d(GA{\cdot}CT)_n$ sequences are structurally polymorphic. It has been shown that upon protonation they undergo transition to a triple-stranded conformation. Recently, we reported a zinc-induced structural transition of a d(GA.CT)n sequence[1,2]. This novel structure, called *H-DNA, is induced at neutral pH by moderate concentrations of zinc. Stabilisation of this altered conformation appears to be specific of nucleotide sequence and metal-ion. In this paper we will discuss the following aspects: (1) the determination of the conditions (degree of supercoiling, metal-ion concentration, ionic strength, pH, etc.) for the stabilisation of *H-DNA; (2) the determination of the conformation adopted by $d(GA{\cdot}CT)_n$ sequences in the presence of zinc. The modification patterns obtained with several chemical reagents (DMS, DEPC, OsO_4, MnO_4K, CAA, BAA) indicate that, at relatively low Zn/P ratios, the sequence adopts a Pur-Pur-Pyr triplex. Upon increasing the Zn/P ratio, the pyrimidine strand gets completely unpaired, while the purine strand remains associated by intrastrand pairing (GA-loop); (3) characterisation of the types of intra- and interstrand pairing of $d(GA)_n$ and $d(CT)_n$ sequences in the absence and presence of zinc, and (4) the possible role of this type of repeated sequences on DNA recombination[3].

1.- Bernúes et al., (1989) EMBO J. 8, 2087.
2.- Bernúes et al., (1990) Nucleic Acids Res. 18, 4067.
3.- Bernúes et al., (1991) Gene, in press.

R 102 Photoaffinity mapping of RNA polymerase III subunits assembled in preinitation and elongation complexes of the *S. cerevisiae* SUP4 tRNA gene. Blaine Bartholomew, George A. Kassavetis, David Durkovich, E. Peter Geiduschek. Depart. of Chem. and Biochem, SIUC, Carbondale, IL. 62901 and Department of Biology/Center for Molecular Genetics, UCSD, La Jolla, CA 92093.

Binding of transcription factors TFIIIC and TFIIIB to regions internal and upstream, respectively, of the SUP4 tRNA gene is essential for promoter-dependent transcription by RNA polymerase III (pol III). Binding of TFIIIC helps establish conditions whereby TFIIIB can bind to DNA upstream of the start site of transcription. TFIIIC can be selectively removed and is not required for promoter recognition by pol III. We report the use of DNA photoaffinity probes in helping to determine which pol III subunits are in close proximity to TFIIIB and to various locations in DNA in preinitiation and paused elongation transcription complexes.

The 128 and 37 kDa subunits of pol III are photoaffinity labeled from base pair -17 in the preinitiation complex. Photoaffinity labeling of these pol III subunits is dependent on specific binding of TFIIIC and TFIIIB. The 70 kDa component of TFIIIB is also photoaffinity labeled at this DNA site, which is well within the DNase I footprint region attributed to binding of TFIIIB. The largest subunit of pol III, the 160 kDa subunit, is photoaffinity labeled at positions in DNA more proximal to the start site of transcription. Dramatic changes in the photoaffinity labeling of pol III subunits are seen upon conversion of the preinitiation complex to a transcribing complex by the addition of ATP, CTP, and UTP. The omission of GTP causes the transcription complex to stall at nucleotide 17. The stalled transcripton complex was used to examine the location of pol III subunits in the transcribing complex. The 160 kDa pol III subunit is photoaffinity labeled at position +28/+29 on the 3' side of the transcription complex and the 37 kDa subunit and to a lesser extent the 128 kDa subunit are photoaffinty labeled at position -3/-2 still in close proximity to TFIIIB. The only pol III subunit that is significantly labeled from base pair +16, near the catalytic site, is the 128 kDa subunit.

R 103 DNA-BINDING SPECIFICITIES OF HELIX-LOOP-HELIX PROTEINS, T. Keith Blackwell and Harold Weintraub, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104. We are studying how MyoD, E2A, Myc-family, and other helix-loop-helix (HLH) proteins preferentially bind to different DNA sequences. To do so, we have developed a technique by which DNA sequences that can be specifically bound in vitro by a protein complex are isolated from synthesized templates that have regions of random sequence. These bound sites are amplified by the PCR, reiteratively re-bound and re-amplified, and finally can be sequenced directly as a pool, thus allowing rapid analysis. Our results indicate that MyoD and E2A homo- and heterooligomers preferentially bind a consensus CA -- TG motif, but at internal and surrounding positions select different sequence patterns that suggest half-site recognition. Myc-family proteins, and complexes of cMyc with the HLH protein max, also bind to CA -- TG sites, but with different specificities at internal positions such that these sites do not allow high-affinity binding by MyoD/E2A complexes, and vice-versa. The HLH protein twist binds to yet a different family of CA -- TG sites. We have begun to utilize mutagenesis of the MyoD DNA-binding region, along with the site-selection technique, to attempt to identify the amino acid residues that determine how these different proteins recognize their respective families of different but related preferred sites. For example, changing three MyoD residues to their cMyc counterparts allows this mutant protein to bind to sites that are preferentially recognized by cMyc, but not to a MyoD preferred site. We are now extending these results, and by a similar approach are also attempting to identify the residues that mediate interaction of these proteins with sequences that flank the CA -- TG consensus. From these experiments, we hope to derive some preliminary insights into the structure of HLH protein-DNA complexes.

R 104 MUTATIONAL ANALYSIS OF THE CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) GENE HLA-DRA SUGGESTS THAT THE DNA BINDING PROTEINS RFX AND X2BP REGULATE CLASS II GENES *IN VIVO*. Jeremy M. Boss, John H. Sloan, and Susan L. Hasegawa Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322.

Class II Major Histocompatibility Complex (MHC) molecules are heterodimers that function in the presentation of antigens during the immune response. Class II molecules are expressed predominantly on the surfaces of B cells, macrophages, activated T cells, and may be induced in other cell types by γ-interferon (γIFN). The genes that encode these molecules are regulated by a series of elements, W, X1, X2, and Y box, of which the X1 and X2 boxes are both necessary and sufficient for expression. The factors RFX and X2BP interact with the X1 and X2 boxes, respectively. Although these sequences are conserved, the base pairs that are important for transcriptional regulation have yet to be identified. Additionally, it is not known if the factors RFX and X2BP bind *in vivo*. To address these issue with regard to the MHC gene HLA-DRA, a series of point mutations spanning the conserved upstream sequences was created and analyzed for their effects on transcription in both B cells and γIFN treated fibroblasts. The effects of X1 and X2 box mutations on DNA/protein interactions were examined and compared to the transcriptional data. The results of these studies show that each of the conserved elements participate in maximal expression in B cells and that W, X1, and X2 boxes are important for γIFN induction in fibroblasts. Mutant templates designed to eliminate RFX and X2BP binding *in vivo* suggest that these factors may interact to promote transcription.

R 105 **X-ray Crystallographic and Biophysical Studies of Eukaryotic Transcription.** S.K. **Burley**, Laboratories of Molecular Biophysics, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

X-ray crystallographic and biophysical studies of the structure and mechanisms of action of eukaryotic transcription factors are underway in my laboratory. Current investigations include transcription factor *IID*, upstream stimulatory factor and hepatic nuclear factor 3. Recent results obtained from these systems will be presented.

R 106 **E4BP4, A LEUCINE ZIPPER FACTOR WITH TRANSCRIPTIONAL REPRESSOR ACTIVITY**

Ian G. Cowell, Ann Skinner and Helen C. Hurst, Gene Transcription Group, Imperial Cancer Research Fund, Hammersmith Hospital, Du Cane Road, London, W12 0HS, UK

We have cloned a novel leucine zipper factor, E4BP4, through its binding affinity for an ATF site in the adenovirus E4 promoter. The DNA binding specificity of E4BP4 was investigated using a binding-site selection approach, resulting in the definition of the consensus binding site (G/A)T(G/T)A(T/C)GTAA(C/T). In transient transfection assays E4BP4 is a transcriptional repressor. The repressing activity is dependent on the presence of E4BP4 binding sites in the target promoter and is relieved by coexpression of the adenovirus E1a product. Experiments are under way to map the repressing activity of E4BP4 and to elucidate its mechanism of repression and mode of interaction with E1a.

R 107 DISTINCT PROTEIN-DNA INTERACTIONS in vivo OF A VIRAL REGULATORY SEQUENCE IN NORMAL AND TRANSFORMED CELLS, Steffen Faisst, Manoussos Perros, Nathalie Spruyt, Jean Rommelaere, Oncologie Moléculaire, Institut Pasteur, BP 245, F-59019 Lille, France.

Transformation of rat cells by various oncogenes was previously shown to result in a striking stimulation of the expression of incoming minute-virus-of-mice (MVMp), a parvovirus endowed with oncosuppressive properties. Thus, the early promoter of MVMp (P4) was used as a model system to monitor changes induced by ras transformation in the interactions of a responsive regulatory region with proteins. Protein-DNA complexes formed in vivo were mapped and characterized by the UV footprinting method, and further confirmed in vitro by DNaseI footprinting and gel retardation assays. A number of proteins were found to associate with promoter P4 in vivo. Photoprotected elements in the P4 and upstream region were conserved between normal and ras-transformed cells. Yet, qualitative differences between both types of cells were consistently detected in their respective DNA photosensitivity patterns at most protected sites. These differences concerned either the whole or parts of the corresponding protected element and are interpreted in terms of multiple transformation-associated changes in protein-DNA complexes, including the induction of distinct DNA-binding activities and the modification of constitutive factors structure or interactions with other polypeptides. These changes were accompanied by a quantitative increase in the level of photosensitization of the TATA box, indicating a local unwinding of an active TATA box. The rate of TATA box-unwinding may be an indicator of the rate of transcripton initiation.

R 108 CHARACTERIZATION OF THE INTERACTION OF TRANSCRIPTION FACTORS USING A BACTERIAL REPRESSOR PROTEIN, Christiane Gatz, Claus Frohberg, Lisa Heins and Frank Roeder, Institut für Genbiologische Forschung Berlin GmbH, Ihnestr. 63, 1 Berlin 33, Germany

Transcription inititiation from a eucaryotic polymerase II promoter requires the functional interaction of regulatory transcriptional activators with at least one of the basal transcription factors binding in the vicinity of the TATA box. To characterize this type of interaction *in vivo*, we have inserted the bacterial Tet repressor-operator complex in nine different positions between an enhancer elementt (*as-1*) and the TATA-box of the cauliflower mosaic virus (CaMV) 35S RNA promoter. A direct contact between the transcriptional activator ASF-1, which binds to *as-1*, and the transcriptional machinery should be affected by a repressor protein bound between them, as the spacing of only 34 bp between *as-1* and the TATA box is too short to allow looping of the DNA around the repressor. In each construct, the distance of 34 bp was kept constant, while the position of the 19 bp *tet* operator relative to the TATA box differed by 2 bp. Thus, the position of the Tet repressor relative to the plant transcription factors was consecutively changed by 72°, which allowed us to investigate whether repression depended on the stereospecific alignment of the repressor with the transcription factors. Binding of the Tet repressor to the operator blocked transcription only when the operator was inserted less than 5 bp from the TATA-box. In all other promoter derivatives, no inhibitory effect of the repressor was observed, which suggests that ASF-1 does not directly interact with the general transcription machinery.

R 109 THE HMG DOMAIN OF LYMPHOID ENHANCER-BINDING FACTOR BENDS DNA AND FACILITATES ASSEMBLY OF FUNCTIONAL NUCLEOPROTEIN STRUCTURES, Klaus Giese, Jeffery Cox, and Rudolf Grosschedl, Department of Microbiology and Immunology, University of California and Howard Hughes Medical Institute, San Francisco, CA 94143.

Lymphoid enhancer-binding factor (LEF)-1 is a pre-B and T cell specific member of the HMG box family of DNA-binding proteins that recognizes a functionally important site in the T cell receptor α enhancer. We have previously shown that the HMG box represents a sequence-specific DNA binding domain. Recent studies indicated that DNA binding by LEF-1 induces a bend of approximately 130° in the DNA helix. Bending of DNA was found to be governed by the HMG domain of LEF-1 and influenced by nucleotides flanking the core-binding site. We have shown that multimerized LEF binding sites alone cannot augment basal promoter activity. Therefore, we examined whether LEF-induced DNA bends can facilitate communication between proteins bound at distant sites. Toward this goal, we replaced in the attP locus of bacteriophage λ one binding site for the bacterial integration host factor with one for LEF-1. LEF-1 was found to be capable of precisely aligning widely-separated integrase (Int) protein binding sites and stimulating Int-dependent recombination in vitro. These data suggest that LEF-1 can serve as an "architectural" element in the assembly of higher-order nucleoprotein structures.

R 110 THE E6 PROMOTER OF HUMAN PAPILLOMAVIRUS-16 IS REPRESSED BY COOPERATIVE BINDING OF 2 E2 PROTEIN DIMERS, DISPLACEMENT OF SP1 AND INTERFERENCE WITH THE TRANSCRIPTION INITIATION COMPLEX. Bernd Gloss, Shyh-Han Tan and Hans-Ulrich Bernard. Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511.

The E6 promoter of all genital human papillomaviruses has a characteristic arrangement of a Sp1 binding site, 2 palindromic binding sites of the viral E2 proteins, and the TATA box. The Sp1 binding site mediates as proximal promoter element activation of the E6 promoter by the remote epithelial specific enhancer. Both E2 binding sites are necessary to permit feedback repression of the viral gene expression, most likely through cooperative binding to the 2 flanking binding sites. One consequence of binding to these E2 sites is displacement of the Sp1 factor, which by itself could be a sufficient mechanism for repression. The other consequence of occupation of both E2 sites is interference with the function of the transcription initiation complex, a prerequisit for complete repression. Negative regulation may be the principal function of the HPV-16 E2 protein, since - in contrast to the BPV-1 E2 protein - it stimulates transcription only poorly, when binding in enhancer configuration relative to a promoter.

R 111 CHARACTERISATION OF IMMORTALISED PORCINE GASTRIC CHIEF CELLS BY STUDYING THE TRANSCRIPTIONAL CONTROL OF PEPSINOGEN. Christine J Grinham, Callum J Campbell, Allan Baxter and *Phillip Gallimore. Department of Biochemistry, Glaxo Group Research, Greenford, Middlesex, UB6 OHE, UK and *CRC Laboratories, Department of Cancer Studies, University of Birmingham, Medical School, Birmingham, B15 2TI, UK.

Pepsinogen A is synthesised in gastric chief cells. On activation in an acid environment it is converted to the aspartic proteinase pepsin. To our knowledge there are no known cell lines available which produce pepsinogen. Primary porcine chief cells have been isolated and maintained in culture for 4-8 weeks. The cells grow very slowly and they secrete pepsinogen for about 15 days after isolation, the peak response to secretory stimuli being at around 4 days. In order, therefore, to study the transcriptional control of pepsinogen a stable immortalised chief cell line is required which will synthesise pepsinogen in response to physiological stimuli.

Porcine chief cells have been immortalised by a replication defective hybrid human Adenovirus 5/SV40 origin$^-$ recombinant virus. Cells from the resulting clones have been characterised and assessed for indications of a differentiated phenotype by monitoring their ability to synthesise pepsinogen in response to secretory stimuli.

R 112 HOMEODOMAIN-DNA RECOGNITION: IS WHAT'S TRUE IN YEAST TRUE IN FLIES? Steve Hanes[1], Guy Riddihough[2] David Ish-Horowicz[2] & Roger Brent[1], [1]Dept. of Molecular Biology Massachusetts General Hospitial and Dept. of Genetics, Harvard Medical School, Boston, MA 02114, [2]Developmental Biology Unit, ICRF Dept. of Zoology, Oxford University, Oxford, UK OX1 3PS

We have been studying DNA binding by the Bicoid homeodomain protein using genetic assays in yeast (1, 2). We have examined, in detail, the contacts made by individual amino acids in homeodomain recognition α-helix with base-pairs in the binding site. The results led us to propose a general model of how the recognition helices of homeodomains are used to determine site-specificity (2). Here, we present tests of the essential features of our model carried out in the host organism, *Drosophila melanogaster*. The experiments take advantage of two types of mutant Bicoid protein: the first is defective in DNA binding, the second has a switched-specificity such that it no longer recognizes Bicoid binding sites, but instead recognizes sites bound by Antp-class proteins. As expected, both mutants fail to rescue Bicoid-deficient embryos. The mutant proteins may also interfere with wild-type Bicoid function to confer a dominant negative phenotype. Staining of blastoderm stage embryos shows that the switched specificity mutant, but not wild-type Bicoid, recognizes Bicoid binding sites that carry base substitutions that converted them into Antp-class binding sites. Thus, our results show the essential features of homeodomain-DNA recognition, worked out using genetic assays in yeast, hold true in the milieu of the *Drosophila* embryo. We do, however, observe a difference in the spacing requirement of adjacent binding sites, a result that may be informative with regard to cooperative protein-protein interactions necessary to bind DNA. The use of engineered proteins with novel DNA specificities may provide a useful way to dissect genetic regulatory hierarchies important in early development.

1. Hanes, S. D. and Brent, R. (1989). DNA Specificity of the Bicoid Activator Protein is Determined by Homeodomain Recognition Helix Residue 9. Cell *57*, 1275-1283.
2. Hanes, S. D. and Brent, R. (1991). A genetic model for interaction of the homeodomain recognition helix with DNA. Science *251*, 426-430)

R 113 *GENETIC SEQUENCES OF HORMONE RESPONSE ELEMENTS SHARE SIMILARITY WITH PREDICTED ALPHA HELICES WITHIN DNA BINDING DOMAINS OF STEROID RECEPTOR PROTEINS: A BASIS FOR SITE-SPECIFIC RECOGNITION,* Lester F. Harris, Michael R. Sullivan and David F. Hickok, Cancer Research Laboratory, Abbott-Northwestern Hospital, Minneapolis, MN 55407

We report conservation of genetic information between regulatory proteins' DNA recognition helices and the DNA sequences to which they bind. We also report findings of amino acid/nucleotide hydrogen bonding calculations using computer models derived from atomic coordinates of protein/DNA complexes. We present a hypothesis for a DNA site-specific recognition code where functional sites on amino acid side chains recognize genetically conserved sites of stereochemical complimentarity on their cognate codon or anticodon nucleotides within hormone response element major groove half-sites. Our observations support a deterministic origin of the genetic code and suggest that hormone response elements, operators and regulatory proteins' DNA binding alpha helices may be conserved remnants of a successful prebiotic process of molecular recognition and synthesis. Prior to the structural determination of steroid receptor proteins' DNA binding domains, we predicted the location, sequence and the alpha helical secondary structure of the DNA reading head, specifcally for the glucocorticoid receptor and by inference several other members of the steroid hormone receptor superfamily. These predictions were recently confirmed by NMR and X-ray crystallographic structural determinations of the DNA binding domains of the glucocorticoid and estrogen receptors. Our observation that the cDNA coding for the glucocorticoid receptor's DNA recognition helix contains a region of sequence similarity to the mouse mammary tumor virus 5' long terminal repeat glucocorticoid response element, suggests that this cDNA sequence may be a target site for glucocorticoid receptor binding and autoregulation.

R 114 FUNCTIONAL ANALYSIS OF THE COMPONENTS OF THE HUMAN TATA-BOX BINDING FACTOR COMPLEX (TFIID). Alexander Hoffmann, Ritsuko Takada, Masami Horikoshi, and Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

The human TATA-box binding factor (TFIID) plays a central role in the RNA polymerase II initiation complex, not only as its sole DNA binding component but also as a target for upstream activator proteins as functionally described with partially purified TFIID preparations.

More recent experiments have shown that the recombinant TATA-box binding factor (TFIIDτ) does not mediate upstream activator function, has a smaller footprint and lower mass than experiments with native TFIID fractions indicate. While immunoprecipitations of the TFIID complex from HeLa derived nuclear extract have identified TFIIDτ-associated factors, we have developed a precipitation system utilizing an affinity tag that is capable of isolating and eluting either the associated factors only or the whole TFIID complex in non-denaturing conditions permitting a functional analysis of the associated factors.

We will describe the method used here, which is of general interest and can find application in other protein-protein interaction studies. Furthermore, the results of functional assays with purified TFIID components will be presented.

R 115 THE EFFECT OF DNA TOPOLOGY ON ESTROGEN RECEPTOR BINDING, Sigrun H. Hofmann and Angelo C. Notides, Environmental Health Sciences Center, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642

Binding of the estrogen receptor to estrogen responsive elements (EREs) precedes the transcription of estrogen-regulated genes. In a novel approach to studying DNA binding of steroid hormone receptors, we used the technique of topoisomer gel retardation to investigate whether human estrogen receptor binds to altered geometries of EREs on supercoiled minicircles. The DNA-protein complexes were then treated with DNA modifying enzymes in order to probe for structural alterations. The estrogen receptor formed two sequence-specific complexes on a linear fragment containing two EREs (385II) and one complex on a fragment with one ERE (393I) with a greater than two-fold binding affinity for 385II consistent with the number of binding sites. Saturation binding studies with 385II showed that the high mobility complex B1 increased linearly with receptor concentration and became saturated. The lower mobility complex B2 was saturated at a two-fold higher receptor concentration over a range in which complex B1 decreased. This binding pattern supports a model in which B2 corresponds to receptor binding to both EREs by a non-cooperative addition of protein to a free site on B1 and suggests that a site-site interaction of DNA-bound receptors is not likely. Comparison of the relative mobilities of 385II and 393I protein-bound linear fragments which contain the ERE at different positions on the DNA suggest that receptor may induce a bending of the DNA. The receptor bound with highest affinity to linear molecules and covalently closed relaxed circles and with lower affinity to molecules containing one or three negative supercoils. No complexes were formed on the topoisomer with two negative supercoils. These findings show that small degrees of supercoiling cause distortions of optimal ERE binding sites that can limit or totally inhibit estrogen receptor binding. Topoisomerase I was used to probe complexes of receptor bound to supercoiled molecules for structural changes with the result that the receptor did not induce any global changes in topology and possessed no intrinsic topological activity.

R 116 DNA Binding Properties of Cloned TATA-Binding Protein from Potato Tubers. M.J.Holdsworth, C.S. Nall, [1]W. Schuch, M. Bevan. John Innes CPSR, Colney Lane, Norwich, Norfolk, U.K.[1] ICI International Seeds Business, plc, Jeallots Hill, Bracknell, Berks, U.K.

A full length cDNA clone encoding the TATA-Binding Protein (TBP), the DNA binding component of the general transcription factor TFIID, was cloned from potato tubers using an heterologous cDNA from *Arabidopsis thaliana* L.The DNA sequence of this cDNA showed that the predicted protein was highly homologous to cloned TBP from other species. Genomic southern analysis indicated that TBP is encoded in the potato genome as a low copy number sequence. The potato TBP cDNA clone encodes a functional protein that interacts in a sequence-specific way with the promoter region of a class-1 potato patatin gene. Functional analysis of carboxy-terminal truncated derivatives of potato TBP indicated that important components of DNA binding were located within the terminal 54 amino acids. Kinetic and thermodynamic properties of *in-vitro* synthesised potato TBP were investigated, and indicated strict salt and temperature preferences for maximum activity. In addition on and off-rate measurements indicated that both association and dissociation of TBP from DNA is slow. The specific and the non-specific equilibrium constants K_s and K_n were calculated as $5x10^9$ M^{-1} and $3.65x10^4$ M^{-1} respectively. These results indicate that the interaction of TFIID with the patatin promoter is highly specific. Cloned Potato TBP is being used to study the interactions between the general transcription machinery and plant 'activator' proteins *in-vitro*.

R 117 *GCR1* OF *Saccharomyces cerevisiae* ENCODES A DNA BINDING PROTEIN WHICH BINDS TO THE CTTCC SEQUENCE MOTIF. M.A. Huie, E.W. Scott and H.V. Baker. University of Florida, Department of Immunology and Medical Microbiology, Box 100266, JHMHC, Gainesville, FL 32610-0266, (904) 392-0680.

In *Saccharomyces cerevisiae* glycolysis enzymes constitute 30 - 60 percent of the soluble protein. *GCR1* gene function is required for high level glycolytic gene expression. In *gcr1* mutant strains the levels of most glycolytic enzymes are between 2 and 10% of wild-type. Binding sites for the global regulatory protein known as repressor activator protein 1 (RAP1)/general regulatory factor 1 (GRF1)/ translation upstream factor (TUF) are found in close proximity to one or more CTTCC sequence motifs in the controlling region of *GCR1*-dependent genes. *RAP1/GRF1/TUF*-binding sites are known to be essential elements of upstream activating sequences that control expression of many glycolytic genes. We recently demonstrated that *GCR1* encodes a DNA binding protein whose ability to bind DNA is dependent on the CTTCC sequence motif. In this report, we provide both *in vitro* and *in vivo* evidence to further define GCR1's binding site. Moreover, through the use of a series of MalE-GCR1 fusion proteins we were able to map the DNA binding domain of GCR1 to the caboxy-terminal 154 amino acids of the polypeptide. These findings in addition to the work of others suggests that the *GCR1* gene product and the *RAP1/GRF1/TUF* gene product act in concert to mediate high level glycolytic gene expression.

R 118 THE ACTIVATION DOMAIN OF GAL4 IS A β-SHEET STRUCTURE WHICH ALSO INTERACTS WITH A NEGATIVE REGULATOR, Stephen A. Johnston and Kerstin Leuther, Internal Medicine and Genetics and Development Program, University of Texas-Southwestern Medical Center, Dallas, Texas 75235-8573.

The C-terminus of GAL4 protein contains the archetypal "acid activation domain". Using "constrained mutagenesis" we have developed a physical model for the activation domain which involves a β-sheet-tightturn-β-sheet. We show that acidity is not essential for the activation function, but two acidic amino acids are essential for interaction with the negative regulatory protein, GAL80.

A peptide corresponding to the activation domain was purified from E. coli by a new protocol we developed. CD analysis of the peptide indicates that it contains a β-sheet region. Lower pH(~6) increases the β-sheet signal and makes the peptide more active in an in vitro assay for protein-protein interaction. We conclude that the activation/GAL80-interactive region of GAL4 is a β-sheet structure, not the acid amphipathic helix or acid blob commonly assumed. Other interesting biochemical features of this region will be discussed.

We also addressed the question of how GAL80 protein interacts with the activation region. The common view is that GAL80 blocks access to the activation domain under non-inducing conditions and then disassociates upon induction. Using a GAL80-VP16 fusion and mutations in GAL4 which fail to activate but interact with GAL80, we demonstrate that in vivo GAL80 stays associated with GAL4 upon induction.

R 119 PROTEIN-INDUCED DNA BENDING AND TWISTING BY CYCLIZATION KINETICS, Jason D. Kahn and Donald M. Crothers, Dept. of Chemistry, Yale University, New Haven, CT 06511

DNA bending and twisting by proteins have been demonstrated in many systems. Measurements of the bend angle have usually relied on comparative gel electrophoresis of protein-induced and sequence-directed bends, though the bend geometries may differ. We are using T4 ligase-mediated cyclization kinetics to determine the direction and magnitude of protein-induced DNA bends in solution. Cyclization kinetics has been applied to the determination of physical properties of DNA alone (Shore & Baldwin, 1983, *JMB* **170**, 957), and we have recently combined it with Monte Carlo simulation to determine the bend angle of A tract DNA sequences (Koo, Drak, Rice & Crothers, 1990, *Biochemistry* **29**, 4227).

We have constructed a set of short (150-160 bp) DNAs in which a CAP site is separated from a phased A tract multimer bend by a variable-length phasing adaptor. The total length of the molecule is independently varied. The cyclization probabilities or J factors of these molecules have been determined with and without bound CAP protein. The J factors measured to date range from 0.5 nM to 15 μM. CAP can increase J at least 150-fold when its site is "in phase" with the A tracts, and decrease J at least 5-fold when out of phase; this difference would indicate the presence of a bend and its direction for an unknown protein. The magnitude of the effect reflects the size of the bend angle. Enhancement by CAP is maximal for slightly overwound DNAs, and the optimum DNA length for cyclization of DNA with CAP bound is slightly longer than the optimum for DNA alone, suggesting protein-induced unwinding. Simulation methods for protein-induced bends have been developed, and calculations aimed at determining the bend angle, unwinding angle, and writhe are in progress. Results will be compared to those from the X-ray crystal structure (Schultz, Shields & Steitz, 1991, *Science* **253**, 1001).

The equilibrium constants for cyclization of DNA with and without CAP are connected to those for CAP binding to linear and circular DNA by a thermodynamic cycle. Gel shift competitions show that CAP binds 240-fold more tightly to circular than to linear DNA for a construct which cyclizes 150-fold better with CAP bound. This result suggests a general mechanism for cooperative or anticooperative protein-protein interaction without direct protein-protein contact, since the same thermodynamic linkage applies to DNA constrained by looping rather than cyclization.

R 120 DISSECTION OF RNA POLYMERASE I TRANSCRIPTIONAL ACTIVITIES MEDIATED BY PROTEIN BINDING SITES IN THE rDNA ENHANCER OF *SACCHAROMYCES CEREVISIAE*.

John J. Kang, Teresa J. Yokoi, and Michael J. Holland. Department of Biological Chemistry, School of Medicine, University of California, Davis, California 95616-8635, (916) 752-2928.

In *Saccharomyces cerevisiae*, a 190bp RNA polymerase I enhancer lies in the intergenic spacer between tandemly repeated 35S rRNA genes. A single copy of the rDNA enhancer stimulates transcription of a plasmid-bourne rDNA minigene about 20-fold *in vivo*. This enhancer element shows bipartite organization. The essential sequences for enhancement *in vivo* overlap those mediating spacer promoter activity *in vitro*. Presumably, this region binds an RNAP I-specific factor(s). The upstream portion of the rDNA enhancer includes 90bp of the rDNA enhancer sufficient to direct RNAP I-dependent termination *in vivo* and appears to modulate the activity of the RNAP I-specific region (Mestel, R et al. 1989. Mol Cell Biol 9:1243).

The modulator region contains three protein binding sites. A protein designated REB1 binds to Site1. ABF1 binds to Site2, and a third factor binds to sequences between the REB1 and ABF1 sites, designated Site3. To determine the transcriptional activities mediated by these factor binding sites *in vivo*, base substitution mutations were introduced in the rDNA enhancer which disrupt each of these sequence specific interactions *in vitro*.

Enhancer function was assayed *in vivo* using minigene *prib*1. Site1 and Site3 mutations, singly or in combination, caused moderate reduction of enhancer function comparable to that previously observed for corresponding deletion mutants. However, mutation of Site2 disrupted ABF1 binding *in vitro* and dramatically reduced enhancer function *in vivo*, implicating ABF1 in RNAP I-dependent enhancement.

Another minigene, *prib*2 was used to test RNAP I-dependent termination. In this case, the Site2 and Site3 mutations had no effect on termination. In contrast, mutation of Site1 abolished REB1 binding *in vitro* and RNA polymerase I-dependent termination *in vivo*. This result indicates a role for REB1 in termination of RNA polymerase I transcription *in vivo*.

Interestingly, the rDNA enhancer modulator region contains binding sites for two factors involved in transcription by RNA polymerase II, REB1 and ABF1, suggesting these factors may regulate transcription by a mechanism common to class I and class II genes.

R 121 RETINAL S-ANTIGEN: CHARACTERIZATION OF PROMOTER *IN VITRO* AND IN TRANSGENIC MICE.

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Photons are converted into visual signals (phototransduction) in the rod outer segments (ROS) of the retinal photoreceptor cells which contain highly specialized proteins. Renewal of the proteins are regulated under circadian rhythm. This rhythm in the mammalian species is considered to be regulated by melatonin, a hormone secreted by the pineal gland. The S-Antigen (S-Ag), an abundant soluble protein in both the photoreceptors and pinealocytes, modulates the visual transduction cascade. In order to study tissue specific expression of these proteins under circadian rhythm, we determined the detailed structure of the S-Ag genes in human, bovine, and mouse. These studies revealed that the upstream sequences of all 3 genes lack TATA, GC and CCAAT boxes, but the 5'-flanking sequences directed transcription from approximately 400 bp upstream of the initiation codon (ATG). Six fusion genes with CAT and the 5'-flanking segments of the mouse S-Ag, 50, 150, 240, 286, 400, and 1300 bp were constructed. Determination of the profile of CAT expression in transgenic animals established that the upstream 300 bp of S-Ag contains sufficient information for directing gene expression in the retina and pineal gland. Sites within these promoters that form specific complexes with nuclear proteins from the bovine retinal cells were subsequently identified by both footprinting and gel retardation assays. These data, together with the result of the *in vitro* transcription assays suggested that tissue specificity of S-Ag promoter is located within 1 to 286 bp of the transcriptional start site. Unexpectedly, a homologous element with the steroid/thyroid hormone-receptor binding was found in the promoter around -200 pb and the expression of S-Ag *in Vivo* was regulated by steroid hormone. In addition, hormonal and tissue specific elements and promoter activity was highly interdependent. Possible correlation between S-Ag expression and circadian rhythm will be discussed.

R 122 AMINO ACIDS NOT IN DIRECT CONTACT WITH DNA INFLUENCE THE BASE SPECIFICITY OF 434 REPRESSOR.,

Gerald B. Koudelka, Dept. of Biological Sciences, SUNY at Buffalo, Buffalo, N.Y. 14260.

Bacteriophage 434 repressor regulates the developmental decisions of the bacteriophage by binding to each of six binding sites on the phage chromosome with a distinct affinity. The most important decision depends on the ability of repressor to bind to O_R1 more tightly than to O_R3. The difference in repressor binding is due primarily an A->G change at position 4 in one half-site of O_R3. (O_R1: A-C-A-A-A-C-T-T-T-C-T-T-G-T; O_R3: A-C-A-G-T-T-T-T-T-C-T-T-G-T). The importance of position 4 is highlighted by the observation that 4A->N mutations in both half-sites of an operator decrease its repressor affinity >150-fold. Consistent with x-ray results showing that Gln-33 contacts O4 of the T at position 4 on the bottom strand in both half-sites of O_R1, changing Gln-33->Ala decreases the ability of repressor to distinguish between purines at position 4. This mutant protein however, fully retains the ability to discriminate between purines and pyrimidines, suggesting that other amino acids are also involved in recognizing bases at this position. To determine their identity, genes encoding doubly and triply repressors were constructed. The proteins were expressed, purified and the binding specificities of the mutant proteins determined *in vitro*. The types of substitutions made were guided by sequence homologies of 434 repressor to 434 Cro, a protein which naturally discriminates only between purines and pyrimidines at position 4. Mutating Thr-27->Lys or Glu-32->Gln, together with Gln-33->Ala, did not result in a repressor that had further lost specificity at position 4. Mutating both Glu-32 & Gln-33 to Ala caused repressor to lose specificity at both operator positions 3 & 4. Mutating both Thr-27->Lys & Glu-32->Gln together with Gln-33->Ala, did result in a repressor that had completely lost specificity at position 4 and retained specificity at position 3. This shows that at least three amino acids are involved in determining repressor's position 4 base specificity. X-ray data suggested an indirect role of Thr-27 at position 4 by hydrogen bonding to Ser-30, thereby holding this serine in van der Waals contact with bases at position 4. Contrary to this suggestion, a Ser-30->Gly, Glu-32->Gln, Gln-33->Ala did not have an additional loss of specificity. Model building suggests that the Lys-27 can make a new DNA-phosphate backbone contact at position 4. Apparently, this somehow contributes to loss of specificity. How the Glu-32->Gln can contribute to position 4 specificity is less clear, as neither amino acid is near the base or backbone at position 4. A role for an amino group in amino acid 32 is demonstrated by the observation that Lys-27, Asp-32, Ala-33 mutant repressor retained purine *vs.* pyrimidine specificity, but a Lys-27, Asp-32, Ala-33 protein did not. Model building suggests that $-NH_2$ of Gln or Asn-32 may hydrogen bond to the amino group of Gln-29. This would alter the position of the ß-carbon of Gln-29, allowing greater room for bulky purine bases on bottom strand at position 4.

R 123 STRUCTURAL STUDIES OF "ZINC-FINGER"/DNA INTERACTIONS: THE SP1 ZINC-DOMAINS WITH "GC-BOX" DNA SEQUENCES,

Richard W. Kriwacki[1], Steve C. Schultz[2], Bradley Bernstein[2], Priscilla L. Yang[1], Thomas A. Steitz[2], and John P. Caradonna[1], Department of Chemistry[1] and Department of Molecular Biophysics and Biochemistry[2], Yale University, New Haven, CT 06511.

The "zinc-finger" family of eukaryotic transcription factors has yielded a rich source of systems for the study of molecular recognition of DNA by proteins. In particular, the transcription factor Sp1 (1), known to be involved in the regulation of a large number of eukaryotic and viral genes and which possesses three Cys_2-His_2 "zinc-finger" motifs, binds with high affinity in a sequence-specific manner to a diverse set of DNA sequences. We are interested in understanding this particular DNA/protein recognition process and are pursuing these interests through the study of a model system composed of a peptide whose sequence contains the three Sp1 zinc-domains along with short length duplex DNAs. We will present the cloning, expression, and purification of a 92 amino-acid zinc-domain peptide as well as results illustrating the properties of this peptide binding to "GC-box" DNA fragments. Additionally, the results of structural studies involving the combined use of NMR spectroscopy and X-ray crystallography will be presented.

(1) J. T. Kadonaga, K. R. Carner, F. R. Masiarz, and R. Tjian, *Cell*, 51, 1079-1090 (1987).

R 124 USE OF A NEW TIGHTLY-REPRESSIBLE TEMPERATURE-INDUCIBLE SYSTEM FOR EXPRESSION OF FOREIGN GENES UNDER THE TRANSCRIPTIONAL CONTROL OF A HYBRID T7 PROMOTER FLANKED BY TWO OPERATORS, IN *ESCHERICHIA COLI* , Marina I. Lebedeva, Olga G. Shishkina, Nicolay Tsiba, Sergey V. Mashko, Institute of Genetics and Selection of Industrial Microorganisms, 113545, Moscow, USSR

A new system for expression of pro- and eukaryotic genes in *Escherichia coli* has been constructed. Heterologous transcription is achieved using modules based on genetic elements of phages T7 and lambda. Foreign genes are transcribed from the T7 late promoter ϕ10 by the phage T7 RNA polymerase (polT7), the gene encoding polT7 being expressed under the transcriptional control of the lambda phage promoter, p_R, regulated by a temperature-sensitive version of repressor CI. To achieve tightly-repressible regulation of transcription by polT7, its cognate chemically synthesized promoter ϕ10 has been flanked by two lambda operators - o_{R2} and o_{R3} in such a fashion that there are five integral DNA helix turns between the operators, so these sites can be recognized by two CI molecules in a cooperative manner (Hochschild & Ptashne: *Cell* 44 (1986) 681-687). The efficiency of the regulation of transcription in this system has been demonstrated in the use of the chloramphenicol acetyltransferase gene (*cat*) as a reporter.

R 125 THE PROTEIN-PROTEIN INTERACTIONS BETWEEN TFIID AND TFIIA AND MECHANISMS IMPLICATED IN THE STIMULATION OF TFIID BINDING TO DNA BY TFIIA. Dong Kun Lee, Jeff DeJong, Shigeru Hashimoto, Tae Kook Kim, Masami Horikoshi, and Robert G. Roeder. Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021. Analyses of contact points between yTFIID and DNA by various interference and protection assays, and by binding studies with deoxyuridine substituted oligonucleotides showed that TFIID interacts with the minor groove of the TATA element. Association of TFIIA with the TFIID-DNA complexes did not increase contact points between TFIID and DNA, instead decrease contact points only to the TATA element. Important domains of the TFIID for the interaction with TFIIA were mapped using yTFIID point-mutants. Association of TFIIA with yTFIID mutants which did not bind to DNA by themselves regenerated DNA binding activity of those mutants, suggesting structural changes of TFIID by TFIIA. Biochemical treatments were performed to regenerate DNA binding activity of those mutants without association of TFIIA, thereby to determine roles of TFIIA in the structural changes of TFIID. Kinetic studies of TFIIA/TFIID revealed how TFIIA stimulates TFIID binding to DNA.

R 126 THREE DIFFERENT POLYPEPTIDES ARE NECESSARY FOR DNA BINDING OF THE HETEROMERIC CCAAT BINDING FACTOR, CBF. Sankar N. Maity, Satrajit Sinha and Benoit de Crombrugghe, Department of Molecular Genetics, 1515 Holcombe Boulevard, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas 77030.

CCAAT binding factor (CBF) is a mammalian heteromeric transcription factor. Recently, full length cDNA clones for the CBF-A and CBF-B subunits have been isolated from both rat and mouse. Portions of the amino acid sequences of the two subunits show a remarkable sequence identity with segments of HAP3 and HAP2, respectively, two components of a yeast heteromeric CCAAT binding transcription factor. Recombinant CBF-B binds to DNA after complementation with a highly purified CBF-A fraction. Recombinant CBF-A protein is unable to bind to DNA after complementation with either the purified CBF-B or the recombinant CBF-B protein. However, when recombinant CBF-A, synthesized as a fusion protein with glutathione S-transferase, is denatured together with a highly purified CBF-A fraction in the presence of 6 M guanidine hydrochloride and subsequently renatured, the recombinant CBF-A binds to DNA after complementation with CBF-B. Using a Southwestern blot we demonstrated that a 40 kilodalton polypeptide, present in the purified CBF-A fraction, binds to DNA after complementation with both recombinant CBF-A and CBF-B. Our results indicate that this third polypeptide designated CBF-C, is tightly associated with CBF-A. Together with CBF-A and CBF-B, CBF-C is required for the DNA binding activity of CBF.

R 127 TOPOISOMER SELECTIVITY ASSAY FOR E. COLI RNA POLYMERASE, William McClure and Tin Tin Su, Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213.

The effect of DNA supercoiling on the association of *E. coli* RNA polymerase with the TAC promoter was investigated. The template used for the *in vitro* studies was a mini-circular DNA (1863bp) containing only the TAC promoter. The minicircles were formed *in vivo* using the phage λ *int-xis* recombination system. When limiting concentrations of RNA polymerase were added to defined mixtures of excess minicircles, competition occurred for the various supercoiled topoisomers. The bound and free fractions were separated in the first dimension of an agarose gel (gel retardation protocol). The enzyme was then removed from the DNA templates by soaking the gel in a solution of aurin tricarboxylic acid. The distribution of topoisomers in the bound and free fractions was then revealed in the second dimension of the gel using chloroquine to resolve the individual species. The ratio of bound/free for each topoisomer was used to calculate the change in promoter strength for each difference in supercoiling, as represented by the topoisomer species in the mixture. A simple graphical analysis showed that TAC promoter strength increased with increasing superhelical density to a maximum at about σ = -0.04.

R 128 **DIFFERENT THERMAL ENERGY REQUIREMENT FOR OPEN COMPLEX FORMATION BY ESCHERICHIA COLI RNA POLYMERASE AT TWO RELATED PROMOTERS.**

Steve Minchin, Steve Busby and Eileen Grimes

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We have studied the effect of temperature on transcription initiation *in vitro* at two related promoters $galP_{con}$ and *galP1*, which have the same nucleotide sequence around the -10 region and transcription start site, but differ in upstream sequences. One of the promoters, $galP_{con}$, carries the *Escherichia coli* promoter consensus sequence for the -35 region, 5'TTGACA 3', whilst *galP1* contains a block of "distortable" upstream sequences that allow promoter function in the absence of homology with the -35 region consensus. *E. coli* RNA polymerase can form complexes with both promoters at a range of temperatures. However, the thermal energy requirement for open complex formation differs: only *galP1* can form an open complex at low temperatures. The $galP_{con}$ promoter requires higher temperatures despite containing the same sequence as *galP1* in the region of strand separation. The thermal energy requirement for transcription from preformed open complexes is the same for both promoters. Temperature affects the half-time for open complex formation rather than the stability of pre-formed open complexes. We report the effect of mutations in *galP1* and $galP_{con}$.

R 129 A KI-GAL4 VARIANT DISPLAYS A DIFFERENT DNA-BINDING SPECIFICITY *IN VIVO* THAN *IN VITRO*.

Maria-Marek Nagiec, Malgorzata Czyz and Robert C. Dickson, Department of Biochemistry and L.P. Markey Cancer Center, University of Kentucky, Lexington KY 40536-0084

KI-GAL4 (aka LAC9) , a GAL4 homolog, regulates transcription of genes in the lactose-galactose regulon of *Kluyveromyces lactis*. By domain swap experiments it has been shown that the DNA-binding specificity of KI-GAL4 is determined by amino acids in a 14 residue region (residues 123 to 137) adjacent to the C6 Zn^{+2} finger. To identify which residue contacts a specific base in the DNA-binding site, UAS, we have developed a selection system using *Saccharomyces cerevisiae*. The system consists of (i) a *HIS3* reporter gene whose expression is driven by a symmetrical, consensus UAS (cUAS = CGGAATACTGTATTCCG) or by a mutant UAS (mUAS) carrying symmetrical base pair changes (ii) an activator plasmid carrying a library of *KI-GAL4* genes with mutations that produce all possible codons in a positions coding for amino acids that are thought to make base-specific contacts. From this selection system we have obtained a KI-GAL4 variant in which residue 133 is changed from Leu to Arg. This variant drives expression of *HIS3* only when the mUAS (-1T/+1A) and not the cUAS (-1C/+1G) is fused to *HIS3*. Thus, *in vivo* the variant is specific for the mUAS. In contrast to these results, a DNA mobility shift assay shows that the KI-GAL4 Leu→Arg variant protein has greater than 10-fold higher affinity for the cUAS than for the mUAS. We speculate that the conformation of the mUAS (-1T/+1A) is different *in vivo* than *in vitro* and that the variant KI-GAL4 protein only binds with high affinity to the conformation found *in vivo*. It is not known yet whether the -1/+1 base pairs are contacted directly by KI-GAL4 or whether they exert their influence on binding by an effect on the conformation of the UAS.

R 130 A GENOMIC ANALYSIS OF THE EF1(A), dbp, and YB1 CCAAT TRANSCRIPTION FACTOR GENE FAMILY REVEALS MULTIPLE PSUEDOGENE SEQUENCES,

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The ubiquity and multiplicity of the EF1(A), dbp, and YB1 gene family was initially examined by probing a vertebrate genomic "zoo" blot. Under high stringency conditions, a complex and markedly different hybridization pattern was revealed for all the vertebrate species examined when probed with either the entire or truncated rat EF1 (A) cDNA fragments. The least complex mammalian species assayed was bovinae (5 bands), while rat and mouse were the most complex (20 bands). A rat EF1(A) cDNA ORF probe was used to screen a EMBL3 rat and a EMBL3 bovine genomic library. Six rat genomic clones were obtained. Although each rat clone has a unique restriction and Southern blot recognition pattern, preliminary sequence data show high homology to the rat EF1(A) cDNA (over 90%). However, multiple detected translation deficits are prohibitive to production of intact novel EF1(A), dbp, and YB1 members. PCR assays yielded results indicative of no intronic sequences. Therefore, these rat clones are likely to be psuedogene sequences. In addition, four different bovine genomic and multiple bovine cDNA clones are currently being characterized. In conclusion, the multiplicity of homologous EF1(A), dbp, and YB1 sequences in vertebrate genomes includes both multiple psuedogene and functional gene copies.

R 131 CRYSTALLIZATION OF CAP WITH DNA FRAGMENTS GREATER THAN 40 BASE PAIRS IN LENGTH,

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The *E. coli* catabolite gene activator protein (CAP) activates transcription from a variety of promoters. The mechanism of transcriptional activation by CAP is unknown, but DNA bending induced by CAP likely plays an important role. The crystal structure of CAP bound to a 30 bp DNA fragment shows that the DNA is bent by an angle of roughly 90°(S. C. Schlutz *et al.*, *Science* **253**, 1001 (1991)). We have speculated that CAP might induce an even larger bend such that additional regions of positive potential further down on the CAP protein might be able to interact with the negatively charged backbone of a DNA fragment longer than 30 bp.

To crystallize CAP with DNA sequences >40 bp, we tried an approach based on the use of modular DNA. This was the method that lead to the crystallization of CAP with 30 bp DNA fragments(S. C. Schlutz *et al.*, *J. Mol. Biol.* **211**, 159 (1990)). Using modular DNA allows for rapid testing of a wide variety of lengths and termini for their ability to crystallize with CAP. This approach utilized a series of half sites, DNA duplexes which upon base pairing form whole symmetrical binding sites from 40 bp to 46 bp in length. CAP-DNA crystals were obtained for most of the DNA sequences. These crystals can grow quite large and diffract to 3.5Å. Surprisingly, several of the crystals obtained with different half sites appear to have the same unit cell dimensions and probably have the same space group ($P3_121$). This leads to the conclusion that in this crystal form the DNA does not stack end-to-end forming a pseudocontinuous helix as it does in most other protein-DNA complexes observed to date.

Data to 3.5Å was collected from a crystal of CAP bound to a blunt ended 46 bp DNA fragment. A model of CAP with the central 20 bp of DNA from the solved CAP-DNA crystal structure was used to obtain a molecular replacement solution. Attempts are currently being made to fit the rest of the DNA into the structure.

R 132 THE ACTIVATOR, NTRC BINDS COOPERATIVELY AT THE *glnA* ENHANCER

Susan C. Porter, Andrew B. Wedel, and Sydney Kustu; Department of Cell and Molecular Biology, U. C. Berkeley; Berkeley, California 94720

To activate transcription, the nitrogen regulatory protein NTRC binds to two primary sites upstream of the promoter for the *glnA* gene which encodes glutamine synthetase. These sites which function as a transcriptional enhancer are approximately three turns of the DNA helix apart and each has dyad symmetry. We have found that two NTRC dimers bind cooperatively to these two sites. By replacing each of the natural sites with a stronger site and using a gel mobility shift assay, we determined a cooperativity coefficient for binding of approximately 100. We were unable to completely disrupt cooperative binding by moving the sites an additional 1/2, 1, or 1 and 1/2 helical turns apart. The transcriptionally active form of NTRC is phosphorylated in its amino-terminal domain by the NTRB phosphotransferase. Phosphorylation increased the cooperativity of binding by approximately 20 fold but did not change the intrinsic binding affinity for an individual site. The isolated carboxyl terminal region of NTRC (90 amino acids) did not exhibit cooperative binding to two sites. This observation indicates that the protein interactions required for cooperative binding are localized outside the carboxyl terminus.

R 133 GENETIC AND STRUCTURAL ANALYSIS OF A HUMAN C-*MYC* P1 PROMOTER ELEMENT

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The -142 to -115 region of the human c-myc P1 promoter is essential for transcriptional regulation of the c-myc gene in vivo and in vitro (1,2), and is the site of interaction with at least one positively acting transcription initiation factor, known as PuF (2). Because of its hypersensitivity to DNaseI (site III_1) and S1, this region was designated as a nuclease-hypersensitive element, NHE (2 & refs within). As this NHE contains significant purine/pyrimidine asymmetry, it has also been selected as a high affinity site for triplex formation (3-5). Recently, we have undertaken a structural-functional analysis of NSE. To begin to elucidate the role of individual motifs and point mutations in protein binding and in vitro transcription of NHE, we have constructed deletion and site directed mutations of this region. Our data show that Mg^{++} ion concentration significantly alters the coseqeuences of certain mutations with respect to PuF binding and transcription, suggesting that transcription is regulated by DNA structural conformations of NHE. Data on probe-length dependence of protein binding in EMSAs can also be best explained by the presence of altered chromatin structure. We present evidence showing hyperreactivity of NHE to single-stranded DNA specific reagents, further implying the presence of unusual, although as yet undefined, DNA conformational states.

1. Hay et. al., 1987 Genes. Dev. 1:659; 2. Postel et. al., 1989 Mol. Cel. Biol. 9:5123; 3. Mirkin et. al., 1987 Nature 330:495; 4. Cooney et. al., 1988 Science 241:456; 5. Postel et. al., 1991 PNAS 88:8227

R 134 STUDY OF ACTIVE RNA POLYMERASE III TRANSCRIPTION COMPLEXES

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In vitro transcription of *Xenopus* 5S RNA genes has been investigated using both crude extracts and partially purified components. However, using these systems all of the transcription factors required and their interactions with the 5S RNA genes are not completely understood. We are using *Xenopus* oocyte extracts and a mutant 5S RNA gene that does not require UTP for transcription of the first 61 nucleotides to pause or stall RNA polymerase III in the middle of the gene within the TFIIIA binding region. At present we are trying to optimize a system that uses the nascent RNA chain attached to the transcription complex for the isolation of active versus inactive transcription complexes. The key step involves hybridization of a biotinylated RNA to the nascent chain of an active transcription complex that has RNA polymerase III stalled in the middle of the gene. Active transcription complexes can be selected for by binding the biotin groups of the hybrids to strepavidin paramagnetic beads. Inactive complexes can be washed away. The addition of all four NTPs to these transcription complexes extends the nascent chain to its full length. Single round transcription assays demonstrate that the active transcription complexes represent a small fraction of the input 5S RNA genes. Isolation of the active transcription complexes will permit a detailed analysis of the DNA-protein interactions during and after the passage of RNA polymerase III through the 5S RNA gene.

R 135 CONSERVATION OF GENETIC INFORMATION BETWEEN REGULATORY PROTEIN DNA - BINDING ALPHA HELICES AND THEIR COGNATE OPERATOR SITES: A SIMPLE CODE FOR SITE SPECIFIC RECOGNITION,

Lester F. Harris, Michael R. Sullivan, and David F. Hickok, Cancer Research Laboratory, Abbott Northwestern Hospital, Minneapolis, MN 55407

We present analyses of protein/DNA interactions based on information derived from genetic sequence comparisons, x-ray crystallography and point mutation studies. In addition, we report our findings of hydrogen bonding calculations using computer models derived from atomic coordinates of protein/DNA complexes. Research based on DNA operator site interactions with alpha helical amino acids of prokaryotic DNA regulatory proteins from bacteriophages and E. coli has described a specific, sensitive and complex regulatory event. However, a hypothesis describing the underlying mechanism of this recognition event has not been put forth; in fact, it has been said that there is no simple recognition code. We applied information derived from recently reported x-ray crystallography findings describing specific amino acid/nucleotide interactions of regulatory protein/DNA co-crystals. In addition, we applied information from point mutation studies of others using prokaryotic regulatory proteins and their operators. The results indicate that amino acids within alpha helices of DNA regulatory proteins specifically interact with their codon/anti-codon nucleotides within the major grooves of their cognate operator half-sites. Therefore, we present a hypothesis for a simple recognition code based on conservation of genetic information between regulatory proteins' alpha helices and their cognate operators.

R 136 SPECIFIC BINDING OF ARGININE TO TAR RNA, Jianshi Tao, Barbara Calnan, Bruce Tidor and Alan D. Frankel, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142

The HIV transactivator Tat interacts with TAR, an RNA stem loop located at the 5' end of the viral transcripts, and increases the transcription level. The Tat/TAR interaction is mediated by an arginine-rich region in Tat. Small peptides (nine amino acids) from Tat that contain the arginine-rich region bind specifically to a 3-nucleotide bulge in TAR. Surprisingly, this region of Tat can be replaced by nine arginines (R9) to give a fully active protein, whereas nine lysines (K9) cannot substitute. By starting with the K9 mutant and replacing the lysines with arginines, a single arginine was identified that is required for specific RNA binding and transactivation. Ethylation interference experiments suggest that this arginine contacts two adjacent phosphates at the junction of the double-stranded stem and bulge. Model building suggests that the arginine η nitrogens and the ε nitrogen can form specific networks of hydrogen bonds with adjacent pairs of phosphates, a structure termed the "arginine fork". To test the model, competition experiments were performed with the free amino acid arginine and related compounds. L-arginine blocks the specific peptide-TAR interaction, whereas L-lysine, and analogs of arginine that remove specific hydrogen bond donors, do not. Experiments using an L-arginine affinity column demonstrate that arginine and Tat peptides bind to the same site in TAR.

R 137 **HETEROLOGOUS COOPERATIVITY: THE cAMP-CRP/ CYTR NUCLEOPROTEIN COMPLEX IN E.coli.**
Poul Valentin-Hansen, Henrik Pedersen, Bjørn Holst, and Lotte Søgaard-Andersen, Department of Molecular Biology, Odense University, Denmark.
The Escherichia coli CytR regulon includes at least nine operons/genes coding for proteins involved either in uptake or catabolism of nucleosides and deoxynucleosides. A series of recent experiments have unambiguously shown that cAMP-CRP is an authentic component of the CytR repression apparatus and serves as an adaptor that allows the CytR repressor to associate with promoter-DNA. Using purified components we have now analyzed the interactions of the two regulators at all known CytR regulated promoters These analysis show that CytR is a sequence specific DNA binding protein that binds its targets with a relatively low affinity. In the presence of cAMP-CRP, however, DNA binding of CytR can be stimulated more than 1000 fold, and also, the presence of CytR enhances CRP DNA binding. This cooperative binding results in the formation of a nucleoprotein complex in which the regulators are located close to each other on the same side of the DNA helix. Moreover, the combined binding of the two regulators results, in some of the promoters, in a repositioning of the cAMP-CRP complex. Mutational analysis have established that amino acids 13, 17, 18, 108 and 110 of CRP are critical for its action as a co-repressor but not critical for transcriptional activation. In the structure of CRP these amino acids are located in two regions (loops) that are in very close proximity on the surface of the protein. We propose that these surface loops are involved in a direct protein-protein contact with the CytR repressor at the promoters.

R 138 HEAT STABILITY OF TRANSCRIPTION FACTOR SP1, Stephanie Vavra, Elaine Schenborn and Ken Lewis, Promega Corporation, Madison, WI 53711

It has been reported (Farhnam and Cornwell (1991), *Gene Expression* Vol. 1, Number 2, pp. 137-148) that the transcription factor SP1 as present in Hela nuclear extracts can be inactivated for DNA binding and transcriptional activation by heating at temperatures as low as 40° C. We find that this is not due to intrinsic heat lability of the protein. In fact, purified recombinant SP1 may be heated at temperatures as high as 90° C without any loss of specific DNA binding activity as measured by both a mobility shift assay and by footprinting. In addition, the heated protein retains the ability to activate transcription in *Drosophila* nuclear extracts. Purified SP1 is thus surprisingly heat stable. This finding indicates that the loss of SP1 binding activity in Hela nuclear extracts is due to some mechanism other than the thermal inactivation of the protein.

R 139 PROTEIN-PROTEIN INTERACTIONS DETERMINE THE DNA BINDING SPECIFICITY OF THE YEAST α2 REPRESSOR, A HOMEODOMAIN PROTEIN, Andrew K. Vershon and Alexander D. Johnson*, Waksman Institute, Rutgers University, Piscataway, NJ 08855, and *Department of Microbiology, University of California San Francisco, San Francisco, CA 94143

A simple example of combinatorial control of transcription in eukaryotes is the regulatory system that specifies cell type of the yeast *Saccharomyces cerevisiae*. This system involves the α2 and **a**1 proteins, members of the homeodomain super family, and the MCM1 protein, a protein that has strong identity to the DNA binding domain of the mammalian serum response factor, SRF. The α2 repressor works in combination with the MCM1 and **a**1 proteins to repress transcription of two different sets of genes. α2 and MCM1 combine to repress one set of target genes, while α2 and **a**1 work together to repress a second, different set of genes. The specificity of α2 repression is therefore dependent on which protein, MCM1 or **a**1, it interacts with. Our research investigates the molecular basis of this type of combinatorial control. In particular, how do the protein-protein interactions between regulatory proteins define which genes are regulated and how they are controlled?

Data will be presented that indicate: 1) Regions on either side of the α2 homeodomain are required for protein-protein interactions with MCM1 and **a**1, and that these contacts help determine the specificity of DNA binding. 2) α2 uses two different mechanisms to bind DNA. One mode is similar to that used by other homeodomain proteins. The second mode of binding is used only in conjunction with the **a**1 protein and it appears that α2 side chains are used differently to contact the DNA. 3) The protein-protein interactions between α2 and MCM1 are conserved between yeast and humans.

R 140 Dimerization of the Pituitary Specific Transcription Factor Pit-1 at Physiological Concentration is Inhibited by Phosphorylation. Jeffrey W. Voss* and Michael G. Rosenfeld**. *Eukaryotic Regulatory Biology Program and Center for Molecular Genetics School of Medicine, **Howard Hughes Medical Institute University of California, San Diego 9500 Gilman Drive La Jolla, California 92093-0648.

Pit-1 is a pituitary specific transcriptional regulator and a member of the POU domain class of transcriptional activators. Numerous reports have indicated that Pit-1 can activate growth hormone, prolactin and pit-1 gene expression in transient transfection experiments. Direct genetic evidence linking the expression of Pit-1 to the development of somatotroph and lactotroph cell lineages has been obtained through analysis of the Jackson and Snell dwarf mice. These strains are mutated at the pit-1 locus and fail to develop either growth hormone expressing somatotroph or prolactin expressing lactotroph cell lineages. The 1P element of the prolactin gene was shown to transfer Pit-1 responsiveness to chimeric reporter genes. Chemical crosslinking experiments have shown that Pit-1 binds to the 1P element of the prolactin gene cooperatively to form a dimeric complex. Heteromeric complexes consisting of Pit-1 and another POU domain protein, the widely expressed Oct-1 protein, were also found to form cooperatively on the 1P element, apparently, with synergistic transcriptional effects. The formation of multimeric complexes on the 1P element appears to be limited to Pit-1/Pit-1 homomeric and Pit-1/Oct-1 heteromeric complexes as determined from mobility shift experiments using other members of the POU class such as Brn-3. These studies also demonstrated, by immunoprecipitation and affinity chromatography, that these proteins possessed an intrinsic affinity for each other in the absence of DNA that appeared to be mediated by the POU-homeo domain. Recently, we have determined the physiological concentration of Pit-1 in two different pituitary derived cell lines and demonstrated, using chemical crosslinking techniques, that Pit-1 monomers can associate in the absence of DNA at these concentrations. Recently, it has been shown that Pit-1 is specifically phosphorylated *in vitro* at two consensus sites by protein kinase A. These sites also are found to be phosphorylated *in vivo*. Here, we show that the formation of dimeric Pit-1 molecules in the absence of DNA is dramatically inhibited by phosphorylation catalyzed by protein kinase A.

R 141 Purification of the mammary gland specific transcription factor (MGF) from lactating rats.

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The expression of the milk proteins is regulated both at the transcriptional and the post transcriptional level. It has been shown that insulin, glucocorticoid, and prolactin are necessary for the synthesis and accumulation of casein mRNA in rat and mouse mammary gland explants. We have chosen the ß-casein system as a model for the study of multihormonal action on gene expression. The analysis of the 5' flanking region of the murine ß-casein gene and the sequence comparison of the casein gene family revealed a highly conserved and unique sequence in their promotors, to which a predominant transacting factor bound. The amount of this factor increases during pregnancy and reaches maximum at birth. During the lactation, its level is kept constant, however, once pups are withdrawn no factor is detected. Our previous study revealed that this factor is indispensable for the responsiveness of the ß-casein gene promotor to the lactogenic hormones, glucocorticoid and prolactin. This factor is mammary gland specific. As a first step to elucidate the molecular mechanisms by which the expression of the ß-casein gene is regulated by these hormones, we purified this mammary gland specific factor (MGF). We describe here the purification of MGF from lactating rats by a combination of Bio-Rex 70 column, non specific DNA affinity column and sequence specific DNA affinity column chromatography. MGF was purified more than 2400 fold compared to the whole nuclear extract with 11 % yield of activity. Analysis of the purified fraction on SDS polyacrylamide gel electrophoresis revealed that it has been purified to near homogeneity, with apparent molecular weight of 89 kD. Renaturation has shown that this polypeptide is responsible for the binding to its cognate DNA sequence. Independent UV cross-linking experiments also supported the apparent molecular size. A homolog of MGF was also found in the mouse and bovine mammary gland, implying that this is crucial factor for casein genes expression.

R 142 AN UNUSUAL CELLULAR FACTOR STIMULATES Vmw65 DEPENDENT COMPLEX ASSEMBLY, Geoffrey Werstuck and John P. Capone, Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5

Vmw65 (Vp16, αTIF) is a major structural component of the herpes simplex virus (HSV) which, upon infection, transactivates viral immediate early (IE) gene transcription. Transactivation requires the assembly of a multi component complex which includes Vmw65 as well as the cellular factors Oct-1 and VCAF-1. This complex specifically recognizes cis-acting sequences found upstream of the IE genes. We have identified a Hela cellular factor, distinct from Oct-1 and VCAF-1, which enhances the stability of this complex in a dose dependent manner as observed by gel-retardation analysis. This factor, designated SF (stimulatory factor), is present in both the nuclear and cytosolic fractions of Hela cells. SF activity has been purified from Hela post-nuclear extracts using ultrafiltration, ion exchange and gel filtration chromatography and organic extraction. SF has a molecular weight of 1500-3000 Da and is resistant to heat inactivation as well as extensive protease, nuclease and phospholipase digestion. Treatment of SF with ß glucuronidase does not affect the ability of this factor to stimulate complex assembly but does change the mobility of the resulting complex on a native gel. These data suggest that SF is a component of the Vmw65 induced complex and may be composed at least partially of carbohydrates.

R 143 PROMOTER ACTIVITY AND REQUIREMENT FOR ENHANCERS IN EARLY MOUSE EMBRYOS IS DETERMINED BY CHROMATIN STRUCTURE

Maria Wiekowski, Miriam Miranda and Melvin L. DePamphilis Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110

When plasmids containing the luciferase gene under control of the HSV thymidine kinase promoter linked or unlinked to an embryo responsive enhancer are injected into early mouse embryos, high promoter activity is observed in the male pronucleus of 1-cell embryos, but ~6-fold less activity in the female pronucleus, the germinal vesicle of oocytes or nuclei of 2-cell embryos. The repression of promoter activity can be linked to chromatin structure, because treatment of embryos with butyrate stimulates promoter activity in all embryos to levels of "unrepressed" activity in the male pronucleus. Thus, the transcriptional capacity in all embryos is the same. The enhancer has no effect on promoter activity in oocytes and 1-cell embryos, presumably because its specific transcription factors are not present, but stimulates promoter activity in 2-cell embryos to levels like observed in the male pronucleus. Thus, the *in vivo* role of enhancers is to relieve repression on promoters applied by chromatin structure. At the 2-cell stage where embryonic gene expression is normally initiated, a repressive chromatin structure insures the expression of only specific genes. In contrast, at the 1-cell stage, DNA derived from the sperm is present in an "open" complex, whereas DNA derived from the oocyte remains in a more compacted structure. DNA injected into either male or female pronucleus is only expressed when 1-cell embryos are arrested at their S-phase, which allows initiation of embryonic gene expression, but prevents cell cleavage. In contrast, expression occurs even when 2-cell embryos undergo several mitoses after injection. Thus, chromatin is restructured during the first mitosis and association of Histone H1 with DNA might be involved in this process. Histone H1 coinjected with DNA represses activity in 2-cell embryos, but shows no effect in 1-cell embyros.

R 144 ANALYSIS OF THE ICP4 DNA BINDING DOMAIN. Kent Wilcox, Cheryl Bula, Jie Mi, and Chin-Lee Wu. Medical College of Wisconsin, Milwaukee, Wisconsin. 53226

ICP4 (IE175, Vmw175) is an immediate early HSV protein that contains a sequence-specific DNA binding domain within residues 262 to 490. To map the position of residues within this domain that contact DNA, we have conducted cross-linking experiments with a polypeptide that contains ICP4 residues 272 to 490 and oligonucleotides that contain photoreactive nucleotide derivatives. A portion of the ICP4 gene was expressed as a fusion protein in E. coli. Proteolytic cleavage of the fusion protein yielded peptide 272-490 (p272-490). This was purified to near homogeneity by DNA affinity chromatography. Based on sedimentation and chromatographic analyses, p272-490 is a homodimer. DNase footprinting results indicate that this peptide has the same DNA binding specificity as native ICP4. For the UV-crosslinking experiments, azido-nucleotides were incorporated into radiolabeled DNA targets by primer extension along an oligonucleotide template that contained a defined ICP4 binding site. Analysis by SDS-PAGE revealed that UV irradiation induced formation of stable cross-links between peptide 272-490 and these oligonucleotides. Chemical cleavage of the DNA-bound polypeptide revealed that cross-links were associated with a fragment extending from position 272 to 407. The position of these cross-links was confirmed by proteolytic cleavage.

R 145 **DNA CURVATURE AND TWISTING ESTIMATED BY THE TEMPERATURE DEPENDENCE OF THE ELECTROPHORETIC MOBILITY**, V.B. Zhurkin, L.S. Shlyakhtenko*, Yu.L. Lyubchenko*, R.E. Harrington* and E. Appella, National Cancer Institute, NIH, Bethesda, MD 20892, *Department of Biochemistry, University of Nevada-Reno, Reno, NV 89557

To analyze the temperature dependence of DNA curvature, a series of 21-base oligonucleotides containing An:Tn blocks was synthesized: A5-X5-A6-X5, where X5 are C5, G5, T5, CTCTC, GAGAG, TATAT and TCTCT. These 21-bp precursors were ligated and the electrophoretic mobility of their multimers in polyacrylamide gel was measured over the temperature range from 5° to 55°C. The temperature dependence of R-factor characterizing anomalous mobility of DNA is found to be extremely sequence dependent. For X5= GAGAG, G5 and C5, the R-factor is maximal at T= 5°C; for CTCTC and TCTCT max(R) corresponds to 15-20°C, and for TATAT and T5 the maximum is reached at 35° and 55°C respectively. Thus, our data refute the common belief that DNA curvature monotonically decreases with an increase in temperature. We interprete the non-monotonic R(T) dependence by a transition in the DNA shape from a right-handed superhelix to the planar loop (maximum R-factor), and then to a left-handed superhelix. In its turn, this macroscopic transition is a consequence of the decrease in the local DNA twisting upon heating. Basing on the above interpretation, we estimate the DNA twisting angle Ω in seven dimeric steps: Ω(AG)+ Ω(GA)< Ω(AC)+ Ω(CA)< Ω(AT)+ Ω(TA)< 2 Ω(AA). The electrophoresis data are compared with Monte Carlo simulations of DNA bending. The obtained results are discussed in relation to the regulation of gene expression by means of DNA looping.

Initiation Mechanisms-II & III

R 200 THE DNA BINDING DOMAIN OF C-JUN IS A TARGET FOR REPRESSION OF AP1 ACTIVITY BY E1A, P. Angel*, B. Hagmeyer+, T. Oehler*, I. Herr*, H. König*, A. van der Eb+ and P. Herrlich*, *Kernforschungs-zentrum Karlsruhe, Institut für Genetik, Postfach 3640, 7500 Karlsruhe, FRG; +Laboratory for Molecular Carcinogenesis, Sylvius Laboratories, Univ. of Leiden, P.O. Box 9503, 2300 RA Leiden, NL

The E1A protein of Adenovirus has been shown to repress transcription of various cellular genes including collagenase by interfering with the activity of the transcription factor AP1, the physiological regulators of collagenase expression. In order to define the mechanism of repression we analyzed whether the individual components of the AP1 complex, the cJun and cFos proteins, are affected by E1A. In transient transfection experiments in F9 teratocarcinoma cells, which do not express detectable amounts of endogenous cJun and cFos, both cJun homodimers and cFos-cJun heterodimers are incapable of activating transcription in the presence of E1A. To define the target for repression we used chimeric proteins composed of the DNA binding domain of cJun and the transactivation domain of the transcription factor GHF1. Transactivation of the collagenase promoter by the GHF1-cJun hybrid is completely inhibited by E1A while the activity of wild-type GHF1 is not affected showing that the DNA binding domain of cJun is the target for repression. In contrast, transactivation by a chimeric protein containing the transactivation domain of cJun and the DNA binding domain of GHF1 is not affected (F9 cells) or even enhanced (human fibroblasts). Because in a coprecipitation assay using a truncated form of Jun, E1A does not seem to interfere with dimer formation it is likely that the DNA binding activity of preformed Jun-Jun homodimers or Jun-Fos heterodimers is inhibited by an as yet unidentified mechanism. This interpretation is in agreement with our in vivo footprinting data on the collagenase promoter showing that in cells that constitutively express E1A, binding to the AP1 recognition sequence is completely abolished. E1A, however, does not inhibit all members of the AP1 family. For example, transcription of c-jun, that is regulated by two AP1 binding sites, is even induced by E1A.

R 201 AN IMMUNOLOGICAL DISSECTION OF THE β SUBUNIT OF *E.coli* DNA-DEPENDENT RNA POLYMERASE, William Ashraf and Robert E. Glass, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, England

We have generated and applied specific, characterised immunological probes for *E coli* RNA polymerase to facilitate structure-function studies on this important, complex and highly conserved enzyme. Defined regions of the *rpoB* gene coding for the extreme C-terminus of β were cloned into pUEX high-level expression vectors. This region of β has been implicated in σ^{70} and catalytic functions.

We have currently available three sequence-specific polyclonal antibody preparations directed against adjacent regions in the last 199 amino-acids of the extreme C-terminus. Data will be presented to show the epitope binding sites for these antibodies. These antibodies have been used to gain information relating to DNA-directed DNA transcription by assessing their effects on binary-complex formation (open-complexes) and transcriptional initiation (abortive initiation). Proteolysis using trypsin and V8 protease has also been employed to detect any conformational changes occurring during these initiation stages.

Finally, phylogenetic studies have been undertaken on RNA polymerase from a range of organisms making use of our antibodies against two adjacent conserved and non-conserved C-terminal regions.

R 202 CLONING AND CHARACTERIZATION OF cDNA FOR THE LARGE SUBUNIT OF THE GENERAL TRANSCRIPTION INITIATION FACTOR TFIIF

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RAP30/74, also known as TFIIF and FC, is a general transcription initiation factor that binds to mammalian RNA polymerase II. Apparently homogeneous TFIIF consists of 74KD and 30KD subunits that behave as a greater than 220KD complex on gel filtration. A cDNA clone for human RAP30 was cloned in 1989 by Greenblatt et al. The cDNA encoding RAP74 has been isolated using oligonucleotide probe based on peptide sequence data. The predicted molecular weight of this protein based on theoretical translation of full-length cDNA is 58KD, although E. coli expressed recombinant protein is apparently identical in size to the native HeLa protein on danaturing PAGE. Although the amino acid sequence of RAP74 has no obvious homology to other known proteins, it has regions that are remarkably rich in charged amino acids. Both E. coli expressed recombinant RAP74 and RAP30 were found to be required for reconstitution of in vitro transcription. We also investigated the association of RAP30 and RAP74 in vivo. The charged region of RAP74 was not required for this interaction.

R 203 ROLE OF AN ACTIVATOR IN THE ASSEMBLY OF THE POL. II TRANSCRIPTIONAL MACHINERY.

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Pol. II gene transcription requires general transcription factors which include the TATA-binding factor TFIID, TFIIA, TFIIB, TFIIE/F, and RNA polymerase itself. A reconstituted mammalian *in vitro* system, in which the TFIID fraction has been replaced by the cloned TATA-binding protein TBP, can support basal level transcription, yet cannot be stimulated above basal level by an activator. However, when TBP or cloned TFIIB are present in limiting concentrations, acidic activators help the assembly of the transcriptional machinery by overcoming the effect of an inhibitor (X) which coelutes with the TFIIE/F fraction. This effect of an activator depends on the presence of its activating domain and its binding site. Upon removal of the inhibitor X, basal level of transcription increases and the activator seems no longer able to help the assembly of the transcriptional machinery. Under these conditions, histone H1 can mimic the inhibitor X. However, inhibition by H1 can be relieved by an activator even in the absence of its activating domain, indicating that the inhibitor X is different from H1. Experiments are in progress to characterize the inhibitor X and to analyze the mechanism by which acidic activators counteract its action. Is relief of inhibition the only function that an activator has to perform to stimulate transcription? Results from our and other labs suggest that activators also have direct stimulatory effects on the transcriptional machinery by interacting with a positively acting target. Our results are consistent with a model in which this target is a component of the TFIID fraction.

R 204 ***ACANTHAMOEBA TFIID.*** *Erik Bateman, Feng Liu, Jie Min Wong and Diane Morgan. Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, Vermont 05495.*

We have cloned and expressed TFIID from the protozoan *Acanthamoeba castellanii*. TFIID cDNA encodes a protein with an approximate molecular weight of 28,500, whose C-terminal 180 amino acids show close homology to TFIID from humans and other organisms. The amino terminal domain is rich in proline, serine and glutamine, but is largely unique. An exception is the region just adjacent to the C-180 domain; several amino acids are conserved between plants and protozoans. Interestingly, a deletion of this region to the C-180 domain is less active than full length TFIID when assayed by footprinting or *in vitro* transcription. Other TFIID constructions are currently being prepared and analysed. We have also isolated and characterized the genomic copy of the Acanthamoeba TFIID gene.

Acanthamoeba TFIID is able to functionally replace human TFIID in HeLa nuclear extracts. An *in vitro* transcription system has been developed from Acanthamoeba, that accurately transcribes the adenovirus major late promoter, with initiation occuring at +1. Taken together, these results further underscore the conservation of the basic transcription apparatus throughout evolution.

R 205 PROMOTER STRUCTURE AND TRANSCRIPTION FACTORS OF THE HUMAN 7SK RNA GENE

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We have analyzed the sequence and factor requirements for transcription of the human 7S K RNA gene *in vitro* and *in vivo*. These results demonstrate that the two pol III promoters regulating 7S K and U6 RNA transcription reveal significant differences - though both promoters do look so much alike. In particular, the octamer-like sequence of the 7S K wild-type DSE (distal sequence element) definitely is not functional, either alone or in conjunction with the known essential element of the 7S K DSE, namely the CACCC-box. In contrast, like in the U6 DSE, an authentic octamer motif alone is able to confer to the 7S K DSE full functionality *in vivo*. In addition, other promoter elements known from the pol II transcription system are also able to replace the 7S K DSE functionally. Cooperativity between the 7S K "core" promoter (TATA-Box and proximal sequence element, PSE) reveals fairly low stringency, with respect to topological arrangement as well as to spacing. Finally, evidence is presented for differential factor requirements *in vitro* of 7S K and U6 RNA gene transcription. This was particularly surprising since the "core" promoters of both genes which are required and sufficient for *in vitro* transcription, contain identical TATA-boxes as well as PSE sequences.

R 206 GENETIC EVIDENCE THAT TFIIB AND RNA POLYMERASE II INTERACT TO AFFECT TRANSCRIPTION START SITE SELECTION IN YEAST,
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Mutations in the *SUA8* gene of *Saccharomyces cerevisiae* were uncovered as suppressors of an aberrant ATG translation initiation codon located at position -20 in the leader region of the *cyc1-5000* gene, which encodes iso-1-cytochrome *c*. Whereas *cyc1-5000 SUA8* strains contain <1% of the normal amount of iso-1-cytochrome *c*, *cyc1-5000 sua8* suppressor strains contain 25 - 35% of normal. Primer extension analysis of *cyc1* transcripts from *sua8* strains revealed that suppression is a consequence of altered transcription initiation: the normal initiation sites at -70, -62, -46 and -38 are all significantly diminished in favor of initiation at positions -28 and -14. Interestingly, mutations at the *sua7* locus suppress *cyc1-5000* by an identical mechanism; *SUA7* encodes a homologue of the general transcription factor TFIIB. Neither the *sua7* nor *sua8* suppressors are *cyc1*-gene specific since altered transcription start site selection was found at other yeast genes. The *SUA8* wild type gene was cloned by complementation of the temperature-sensitive phenotype conferred by the *sua8-1* allele. DNA sequencing revealed that *SUA8* is identical to *RPB1*, which encodes the largest subunit of RNA polymerase II. Mutations in *sua8* have also been uncovered as suppressors of the cold-sensitive defect associated with the *sua7-1* suppressor. These data provide genetic evidence that TFIIB and pol II interact to affect transcription start site selection in yeast.

R 207 GENETIC AND BIOCHEMICAL CHARACTERIZATION OF TFIID MUTANTS AND SUPPRESSORS
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Yeast genetic techniques were used to isolate mutant TFIID genes. Two classes of mutant TFIIDs have been identified. The first class comprises mutants which confer a dominant negative phenotype. These mutations all map within the repeats of TFIID. In vitro analysis of the mutant proteins indicate that they are defective for DNA binding, and probably cause growth reduction by titrating some essential factor into non-productive complexes.

A second class of TFIID mutants map within the basic region between the repeats. Mutations of the lysines in this region confer a temperature sensitive phenotype. In vitro analysis indicates that the mutant proteins are defective for interactions with TFIIA and TFIIB.

Several unlinked suppressors of TFIID mutations were isolated. These genes have been designated TDSs (for TFIID Suppressors). TDS1 suppresses the dominant negative TFIIDs, apparently by increasing levels of wild-type TFIID. The gene is non-essential, and encodes a 14 kDa protein. TDS2 encodes a 110 kDa protein which has an extremely acidic domain near the C-terminus. Overexpression of TDS2 suppresses several temperature sensitive alleles of TFIID. TDS4 is an essential gene which may encode a component of the RNA pol II initiation complex. It is an allele-specific suppressor of mutations within the basic region of TFIID.

R 208 IN VITRO STUDIES OF THE REGULATION OF DROSOPHILA rDNA TRANSCRIPTION.
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Regulation of rDNA transcription in Drosophila melanogaster was investigated by run-off in vitro transcription with nuclear extracts prepared from Schneider's line-2 cells treated with high serum and the tumor-promoting phorbol ester, TPA. The treatments stimulated the transcriptional activities so the regulatory mechanism may involve signalling by growth factors and activation of protein kinase C. To identify *cis*-acting element for regulation, the levels of transcriptional stimulation of various 5' and 3' deletion templates were compared. It was elucidated that sequences from +32 to +680 are not required for serum and TPA stimulation, regardless of the SRE element at +558 to +579. The sequences between -60 and -34 support a moderate increase (2- to 3-fold) in transcriptional activity while sequences between -60 and -150 fully augment the transcriptional stimulation (10-fold). In vitro, transcription from a spacer promoter showed a similar transcriptional control as that of gene promoter. It was also demonstrated that the stimulation is caused by an increased activity found after serum or TPA treatment. To identify the *trans*-acting factors, nuclear extract was fractionated with phosphocellulose (P-11) chromatography. In reconstitution experiments, only two essential activities eluted at 0.1 M and 0.6 M KCl are required for rDNA transcription. The two essential activities for rDNA transcription still reconstitute the transcriptional stimulation. In substitution experiments, the P-11 C fraction from treated cells is responsible for the stimulatory effect on transcriptional activity. The DNA-protein interaction for rDNA transcription was studied by gel shift assays and DNase I footprinting. In gel shift assays with a gene promoter-containing rDNA template, the treated extract produced more slow-moving complex and P-11 C fraction from treated cells has stronger DNA-binding activity. In DNase I footprinting experiments with nuclear extract and P-11 C fraction, two protection regions were observed: -75 to -92 and -122 to -135.

R 209 **THE ORGANIZATION AND FUNCTION OF NifA BINDING SITES.**
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The *nif* J and H promoters of *Klebsiella pneumoniae* are σ^{54}-dependent promoters that are positively activated by the NifA protein. This protein binds to Upstream Activator Sequences (UASs), located 60-200 bp upstream of the start of transcription(1). Bound NifA is presented to the RNA polymerase-σ^{54} complex ($E\sigma^{54}$) via DNA loop formation, mediated by the Integration Host Factor protein (IHF) bound between these two proteins. The *nif*J promoter is divergently transcribed from the *nif*H promoter, and sequence studies have revealed three potential NifA binding sites (UAS1, 2 and 3) and two potential RNA polymerase-σ^{54} binding sites (Downstream Promoter Elements, or DPEs), one located 422 bp into the coding region and the other overlapping UAS1 by 5bp. Thus the intergenic region between the *nif*J and *nif*H DPEs is very complex. Preliminary studies on *nif*J's two DPEs indicate that transcription is initiated at DPE1 but not DPE2. DPE1 but not DPE2 bound $E\sigma^{54}$ *in vitro*. *In vivo* dimethylsulphate protection experiments revealed NifA-dependent protection at UASs 2 and 3. Site-directed mutagenesis indicated both these sites to be involved in the positive control of the *nif*J promoter. The Integration Host Factor has a stimulatory effect. Simultaneous, divergent transcription of the *nif*J and *nif*H promoters is currently being carried out to assess the effect, on *nif*J transcription, of IHF and of NifA bound at the *nif*H UAS.

1. Cannon, W., Charlton, W. and Buck, M. (1991). Organisation and Function of Binding Sites for the Transcriptional Activator NifA in the *Klebsiella pneumoniae nif*E and *nif*U promoters. J. Mol. Biol. 220, 915-931.

R 210 OVER-EXPRESSION OF NFI REPRESSES STEROID HORMONE INDUCTION OF THE MMTV-LTR.

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Steroid hormone induction of the mouse mammary tumor virus long terminal repeat (MMTV-LTR) requires several site specific DNA binding proteins, including glucocorticoid receptor, OTF-1 and NFI/CTF. We are investigating the role of NFI in MMTV-LTR activation. The NFI family of proteins are encoded by 4 genes; NFI-A, NFI-B, NFI-C and NFI-X. NFI family members share amino acid homology at the N-terminal (DNA binding) domain, but diverge at their carboxyl termini which contains putative transactivation domains. To determine the role of the NFI activation domain in MMTV-LTR steroid induction, we have created an expression vector that over-expresses a truncated form of NFI consisting of the first 220 amino acids of NFI-C, (NFI-C.1-220). This protein contains the DNA binding domain, but lacks the previously characterized proline-rich activation domain. In Hela cells, cotransfection of NFI-C.1-220 with glucocorticoid receptor and a MMTV-LTR β-galactosidase, (β-gal) reporter gene construct leads to repression of β-gal expression. This finding suggests a requirement of the NFI-C carboxyl terminus for MMTV-LTR activation. However, surprisingly, repression is also observed when full length NFI-C is cotransfected with the MMTV-LTR β-gal construct. We are currently investigating if NFI-C.1-220 and full length NFI-C modulate repression through similar mechanisms. Preliminary titration data shows similar dose requirements of both NFI forms for MMTV-LTR repression.

R 211 COOPERATIVITY BETWEEN THE CRP/cAMP-COMPLEX AND THE CYT R REPRESSOR IN REGULATION OF THE *nup*G GENE IN *E. coli*.

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The expression of the *nup*G gene, encoding a nucleoside-transport system in *E.coli*, is controlled by at least three regulatory proteins; CRP/cAMP, CytR and DeoR [1].The *nup*G gene belongs to the CytR regulon, in which all the genes are repressed by CytR and activated by the CRP/cAMP complex. Almost all these genes have two upstream CRP-targets. In one of the systems (*deoP*$_2$) it has been shown, that CytR interacts with 2 CRP/cAMP complexes bound to the DNA if the distance between the CRP-targets is 53±1 bp but not 50 bp [2].

In Δcya strains the expression of the *nup*G gene is very low indicating activation by the CRP/cAMP complex. Furthermore repression of the gene by the CytR repressor is dependent on cAMP in the cell [1].

Our *in vitro* results indicate that CRP/cAMP complexes bind to the DNA at 3 targets near the promoter, and that CytR in a cooperative manner binds to two CRP/cAMP complexes. During this binding, the CRP/cAMP complex moves 2 bp resulting in a 52 bp distance between the 2 CRP/cAMP complexes in the CytR/DNA/CRP/cAMP complex. Detailed models are discussed.

1. Munch-Petersen, A. and Jensen, N. (1990) Eur. J. Biochem. **190**: 547-551
2. Søgaard-Andersen, L. *et al.* (1990) Mol Microbiol **4**: 1595-1601

R 212 LONG DISTANCE REGULATION OF THE *deo* OPERON EXPRESSION IN *E. coli*,

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The *deo* operon of *E.coli* which encodes genes that are involved in nucleoside catabolism is controled by at least three regulatory proteins. The regulatory region contains two promoters *deoP1* and *deoP2* placed 599 bp apart and upstream of the first structural gene *deoC*. Both promoters are regulated negatively by the deoR repressor which binds to O_1 and O_2 overlapping the -10 region in *deoP1* and *deoP2*, respectively. In addition, *deoP2* is regulated negatively by the cytR repressor and positively by the CRP/cAMP complex. We have found *in vivo* that both O_1 and O_2 are required in order to obtain an efficient deoR regulation of either promoter [1, 2]. Our current model is that the same deoR repressor molecule binds to both operators thereby forming a loop of the intervening DNA [1, 3]. This cooperative deoR binding occurs when the two operator sites are placed as far as 5000 bp apart [4], independent of the orientation of the operator sites, and also when one of the two operators are placed downstream of the gene that it regulates. Here we have studied the effect of changing the interoperator distance over short distances (<200 bp). The chelate model and the interplay between the long distance deoR regulation and the "short distance" cytR and CRP regulation is discussed.

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2. Dandanell, G. and Hammer, K. (1991) Mol Microbiol. 5(10): 2371-2376.
3. Hammer, K. and Dandanell, G. (1989) In: Nucleic Acids and Molecular Biology. F. Eckstein and D.M.J. Lilley (ed). Springer-Verlag. Berlin-Heidelberg.
4. Dandanell, G., Valentin-Hansen, P., Løve-Larsen, P.E. and Hammer, K. (1987) Nature. 325: 823-826.

R 213 REGULATION OF TRANSCRIPTION FROM THE β- AND γ-ACTIN PROMOTERS BY NF-Y AND p67^{SRF}.

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Efficient β-actin promoter activity in transfected HeLa cells requires only 114bp of 5' flanking sequences. Two of the regulatory elements within this region of the β-actin promoter, the CCAAT box at -91 and CCArGG box at -62, are specific in vitro binding sites for the transcription factors, nuclear factor Y (NF-Y) and serum response factor (p67^{SRF}), respectively. Point mutation in either element abolishes transcriptional activity of the native β-actin promoter or a heterologous promoter. These mutant templates fail to bind to or compete for interaction with their respective DNA binding protein. Therefore, we were interested in determining if the dual element requirement for transcriptional activity was due to protein-protein interactions between NF-Y bound at the CCAAT box and p67^{SRF} bound at the CCArGG box. Analysis of various insertion and deletion mutants revealed a requirement for a strict stereospecific alignment between the CCAAT and CCArGG boxes, but not between the CCArGG and TATA boxes.

The γ-actin promoter contains both a CCAAT box at -112 and a CCArGG box at -92. These regulatory elements are located a further 30bp upstream from the TATA box in the γ- as compared to β-actin promoter, however, the distances separating them are conserved between the vertebrate cytoskeletal actin genes. To determine whether NF-Y and p67^{SRF} play a role in regulating differential expression of the cytoskeletal actin genes, we analyzed the transcriptional and in vitro binding activities of the β- and γ-actin CCAAT and CCArGG boxes. We found that transcriptional activity in vivo correlated with the strength of nuclear factor binding in vitro: the most active construct (βγ) contained the stronger NF-Y and p67^{SRF} binding sites (β-actin CCAAT and γ-actin CCArGG boxes, respectively).

Using the βγ template as probe, we have detected a specific protein-DNA complex containing both NF-Y and p67^{SRF}. Studies are currently underway to delineate the composition of this higher order complex. We are also studying additional insertion and deletion constructs to understand the mechanism by which NF-Y and p67^{SRF} together regulate transcriptional activity of the β- and γ-actin promoters.

R 214 PARTIAL POLYPEPTIDES OF SIGMA-70 BIND SPECIFICALLY TO pTAC PROMOTER DNA, Alicia J.Dombroski and Carol A.Gross, Department of Bacteriology, University of Wisconsin, Madison, WI 53706

The Sigma-70 transcription initiation factor of *E.coli* possesses two regions, referred to as Regions 2 and 4, that have been implicated genetically in recognition of the -10 and -35 consensus sequences of promoter DNA. Purified intact Sigma-70 however has not been shown to interact specfically with promoters. We hypothesize that assembly of free Sigma-70 with core RNA polymerase is necessary to induce a conformational change that reveals Sigma's DNA binding domains. We have examined the question of whether Sigma-70, in the absence of core RNA polymerase, is able to recognize and bind to promoter DNA by constructing a series of in-frame fusions between glutathione-S-transferase and various partial polypeptides of Sigma-70. This approach allowed us to separate the DNA binding domains from the remainder of Sigma-70. The purified fusions were characterized *in vitro*. A fusion carrying Region 4 binds preferentially to pTac and shows some specificity for the -35 consensus sequence. A fusion combining the putative DNA binding portions of Region 2 and Regions 3 and 4 demonstrates increased affinity, and specificity, for both the -10 and -35 consensus sequences. The greatest affinity and specificity occurs with a fusion carrying the core binding domain and Regions 2 through 4 (amino acids 360-613). Longer fusions become progressively less capable of binding DNA and discriminating promoter sequences from non-promoter sequences. These results therefore support the genetic evidence for Sigma-70's participation in promoter recognition and support an allosteric model whereby DNA binding ability is only conferred when the amino-terminal segment of Sigma-70 (amino acids 1-360) is deleted or assumes a conformation that unmasks the DNA binding domains.

R 215 A NOVEL CIS-ACTING, ORIENTATION-DEPENDENT POSITIVE CONTROL SYSTEM WHICH CAN ACT AT LONG DISTANCES FROM ITS TARGET ACTIVATES PHEROMONE-INDUCIBLE CONJUGATION FUNCTIONS IN ENTEROCOCCUS FAECALIS. G.M. Dunny, and J.W. Chung, Univ. of Minnesota, St. Paul, MN 55108.

In Enterococcus (formerly Streptococcus) faecalis, the conjugal transfer of certain plasmids, such as the tetracycline resistance plasmid pCF10, is enhanced by peptide pheromones. A 12 kb region of pCF10 encodes two pheromone-inducible surface proteins, and several positive control genes required for expression of conjugation functions. The activation system functions when a negative control system encoded by another region of the plasmid is inactivated, either genetically, or by the addition of pheromone to donor cells carrying the plasmid. Previous studies indicated that several genes, including prg (Pheromone Responsive Gene) R, -S, and -T are required to activate expression of the prgB gene encoding the inducible surface protein Asc 10, which mediates attachment of donor cells to recipients. Northern blot and primer extension analysis indicate that prgB activation is at the level of transcription, and that the message initiates slightly upstream from the start codon. The distance (3-6 kb) between the regulatory genes and their target suggested that the activation mechanism might function in trans in any of these experiments. However, when the regulatory region and target genes were cloned in different sites of the same plasmid, separated by as much as 10-12 kb, activation of transcription from the prgB promoter was observed. The activation was dependent on the two regions being cloned in the same relative orientation in which they exist on wild-type pCF10. These studies also indicated that an additional upstream region encoding a gene called prgQ is also required for prgB activation. The model which best fits the currently available data is one in which one or more regulatory factors produced by the positive control region tracks along the DNA molecule in a specific direction to locate the target site.

R 216 A NUCLEAR 42-kDa PROTEIN FRACTION WITH PROTEIN KINASE AND PROMOTER-CONTAINING SINGLE-STRANDED DNA-BINDING ACTIVITIES, Endre Egyhazi and Jerker Stigare, Department of Medical Cell Biology, Karolinska Institutet, Stockholm, Sweden

A casein kinase II-like nuclear protein kinase has been identified and partially purified from an embryonal Chironomus cell line. By salt fractionation and affinity chromatography on casein-phosvitin-Sepharose a 42-kDa protein fraction with casein kinase activity was obtained. The kinase stimulates the phosphorylation of both casein and phosvitin with either ATP or GTP. The casein kinase activity of the 42-kDa protein is suppressed by heparin and 5,6-dichloro-1-ß-D-ribofuranosylbenzimidazole (DRB) but the inhibitory concentration of heparin is one order of magnitude higher than that required to inhibit CK II. In addition, the activity of our enzyme is markedly inhibited by treatment with spermine and spermidine, two polyamines which stimulate the activity of CK II. The results indicate that the nuclear 42-kDa polypeptide is a casein kinase the catalytic properties of which is similar but not identical with that of CK II. Analysis of the DNA-binding activity of the partially purified protein kinase by the Southwestern technique revealed that the 42-kDa protein preferentially binds single-stranded DNA. It binds singel-stranded promoter-containing restriction fragments including sequences from -204 to +74 from the ecdysterone controlled I-18C gene as well as sequences including the joint histone H2A/H2B promoters of Chironomus in a sequence selective matter. Previous and present results taken together suggest that the 42-kDa protein promotes the phosphorylation of some protein(s) involved in transcription and regulation of the protein coding genes.

R 217 A HYBRID SIGMA COMPLEMENTS A HYBRID PROMOTER. Richard Hayward, Ashok Kumar, Brenda Grimes and Mary Logan, Institute of Cell and Molecular Biology, University of Edinburgh, EH9 3JR, Scotland, UK.

Recent studies of the alternative sigma subunits associated with RNA polymerase from many different bacteria have established the existence of several homologous regions in the great majority of these proteins. Here we are concerned with the regions 2.4 and 4.2, which in the major sigma (σ^{70}) of *E.coli* are located respectively near the centre and the carboxy end of the protein, and apparently have respectively an α-helical and a helix-turn-helix structure. Elegant genetic studies of the *E.coli* σ^{70} and a *B.subtilis* σ have shown that single amino acid (aa) changes in region 2.4 can compensate for single basepair down-mutations in the -10 region of the corresponding promoter, and have revealed a similar relationship between region 4.2 and the -35 promoter segment. These results, and the inability of intact isolated sigma to bind specifically to DNA are compatible with a model in which regions 2.4 and 4.2 are independently allowed to interact directly, through the fitting of sigma subunits (of this majority class) into the core RNA polymerase, with the -10 and -35 promoter DNA sequences.

To test this model further we constructed a gene encoding a hybrid *E.coli* sigma, consisting of aa 1 to 529 of σ^{70} (including region 2.4) followed by the carboxy-terminal 82 aa of the heat-shock σ^{32} (including region 4.2), and fused it to *lacP* on a plasmid. On a compatible plasmid we placed a correspondingly hybrid promoter, with the -35 sequence of a heat-shock promoter suitably spaced from a "major" (TATAAT) -10 sequence, serving an otherwise transcriptionally isolated reporter gene. Studies of these and control constructions established that the hybrid sigma ($\sigma^{70\text{-}32}$) is necessary and sufficient to allow vigorous expression of the reporter gene from this hybrid promoter *in vivo*. These results indicate that sigma regions 2.4 and 4.2 function independently in promoter recognition, confirm that the carboxy-terminus of σ^{32} is sufficient to specify heat-shock -35 recognition, and suggest that $\sigma^{70\text{-}32}$ may be a conveniently stable and dispensible material for genetic study of sigma structure and function.

R 218 ANALYSIS OF THE SEQUENCES AND FACTORS REQUIRED FOR U-snRNA GENE EXPRESSION IN PLANTS, David J. Heard and Witold Filipowicz, Friedrich Miescher-Institut, Postfach 2543, CH-4002 Basel, Switzerland. All plant small nuclear RNAs (snRNAs) thus far described contain two highly conserved promoter elements which are necessary and sufficient for expression in dicot plants. These are a TATA-like sequence at -20 to -25 bases upstream of the transcription start site, and the highly conserved U-snRNA specific element (USE) positioned 3 helical turns upstream of the TATA box in pol III genes and 4 helical turns upstream in pol II transcribed genes. It is this spacing which determines promoter specificity (Waibel and Filipowicz, Nature 346, 199, 1990; Kiss *et al.* Cell 65, 517, 1991). To further show that internal sequences are not required for expression of these genes we have made several constructs in which the coding region of the *Arabidopsis* U6 gene is replaced by a fragment of the human β-globin gene, followed by a stretch of T residues as a pol III terminator. When analyzed in transfected protoplasts the β-globin constructs are highly expressed when the promoter has a pol III spacing but no transcripts are detectable with the pol II spacing. When further sequences containing a stem-loop and the 3' box of a pol II transcribed U-snRNA gene are included pol II transcripts are observed. The 3' box is a conserved sequence which has been shown to be required for accumulation of pol II transcribed snRNAs in plants (S. Connelly, unpublished results). It is not yet clear whether the 3' box is required for 3' end processing, or for termination of transcription.

We are also interested in determining the identity of transcription factors that interact with the USE and TATA elements in either the pol III or pol II spacing. Introduction of TATA box mutations into the pol II transcribed U2 snRNA gene or the pol III transcribed U6 gene have similar effects on transcription *in vivo*, whereas a completely different pattern of expression is seen when these same mutations are introduced into the CaMV 35S promoter linked to a leghemoglobin reporter gene. This suggests that the same TATA binding factor is interacting in a similar manner with the TATA box in both pol II and pol III U-snRNA gene transcription, but that this interaction is somehow different than that with mRNA gene promoters.

R 219 CATABOLITE REPRESSION AND XYLOSE INDUCTION OF *XYL* GENES IN *B. SUBTILIS* AND *B. MEGATERIUM*, Wolfgang Hillen, Dagmar Gärtner, Thomas Rygus and Christoph Hueck, Lehrstuhl für Mikrobiologie. Friedrich-Alexander Universität Erlangen-Nürnberg, Staudtstr. 5, W-8520 Erlangen. FRG. Tel. (09131) 858081.

The transcription of xylose utilization operons in *B. subtilis* and *B. megaterium* is about 150-fold induced by xylose and between 3- and 10-fold repressed by glucose. Footprinting experiments show a xylose relieved protection of a 25 bp palindromic sequence element by the Xyl repressor protein. Deletion of *xylR* encoding the Xyl repressor leads to xylose independent transcription but does not affect glucose repression. In gel shift experiments the repressors from both *Bacilli* are able to recognize and bind the respective heterologous *xyl* operator. Next to xylose only ribose led to about 4% induction *in vivo* and *in vitro*. The *cis* sites mediating glucose repression in both *Bacilli* are located in the translated portion of the respective *xylA* genes, about 130 bp downstream of the promoter. The efficiency of glucose repression is independent of translation of this element. Other sugars are able to repress, albeit with lower efficiencies. An oligonucleotide directed mutational analysis of the *cis* element identified important nucleotides in that sequence.

R 220 STRUCTURE-FUNCTION ANALYSIS OF GENERAL TRANSCRIPTION INITIATION FACTOR TFIIB, Koji Hisatake, Sohail Malik, Masami Horikoshi and Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021

General transcription initiation factor TFIIB plays a pivotal role in preinitiation complex formation by binding to TFIID/promoter complex and enabling the subsequent binding of RNA polymerase II/TFIIF. Like TFIID, TFIIB is also proposed to be a direct target of acidc activators such as VP16. We have recently purified human TFIIB and cloned its cDNA, which encodes a 316 amino acid protein with a calculated molecular weight of 34.7 kD. Analysis of amino acid sequence revealed several interesting motifs that include direct repeats, homology to the sigma subregion 2.1/2.2 and regular repeats of basic amino acids. Furthermore, the overall structural organization was similar to that of TFIID. Using human TFIIB as a probe, we have also cloned Xenopus TFIIB and found that it has 94% identity with human TFIIB. All the motifs are conserved between these species. The amino terminal region is relatively more divergent than the carboxy terminal region where all the motifs are located. These features are also reminiscent of the situation with TFIID.

We are currently trying to obtain TFIIB cDNA clones from other species to deduce the evolutionarily conserved domains. In addition, a series of deletion and point mutants are being constructed to analyze the functional importance of these motifs and conserved regions. The results of these structural and functional analyses will be presented.

R 221 THE EFFECT OF TATA BOX MUTATIONS ON TFIID BINDING, Barbara C. Hoopes, Stefan Hermann, James LeBlanc, D. Barry Starr and Diane K. Hawley, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The TATA box is the specific binding site for the general transcription factor TFIID. The DNA sequence of the TATA box is highly conserved and mutations in the TATA box alter the level of transcription both *in vivo* and *in vitro*. We are measuring the effect of TATA box mutations on both TFIID binding and transcription initiation *in vitro* to better understand the role of this conserved promoter element. We have used the combination of footprinting and gel mobility shift assays to measure the kinetics of association of yeast TFIID to derivatives of the Adenovirus Major Late (Ad ML) promoter, which contains the consensus TATA sequence, TATAAAA. We have also measured the stability of the resulting TFIID:TATA complexes. We have found that some mutations in the TATA box allow TFIID:TATA complex formation as assayed by gel mobility shift and have a negligible effect on association or complex stability. However, other mutations (CATAAAA, TATAAAC) do not appear to form TFIID:TATA complexes when assayed by gel mobility shift. However, we have shown using DNaseI footprinting that TFIID does associate with these sequences. We have analyzed the interaction of TFIID with the sequence TATAAAC in detail. The overall equilbrium for TFIID binding is reduced 100-fold for this sequence compared to the AdML TATA. A significant part of this effect is due to a large decrease in the stability of TFIID:TATAAAC complexes. While TFIID:AdML complexes have a half-life of over 60 minutes, TFIID:TATAAAC complexes have a half-life of only 3 minutes. The implications of these results, both in regard to the DNA binding of TFIID and the role of the TATA box in transcription initiation, will be discussed.

R 222 THE S300-II FACTOR IS A GENERAL TRANSCRIPTION FACTOR THAT INTERACTS WITH ENHANCER-BINDING FACTORS AND ACTIVATES TRANSCRIPTION FROM SEVERAL PROMOTERS, Nancy H. Ing, Sophia Y. Tsai, Ming-Jer Tsai, and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

The S300-II factor was discovered along with the chicken ovalbumin upstream promoter-transcription factor (COUP-TF) as being essential for transcription from the ovalbumin promoter *in vitro*. Our recent large scale purification of S300-II from HeLa nuclear extract has allowed us to further characterize and clone the factor. After four chromatographic steps, a 35,000 Mr band was electroeluted from SDS-polyacrylamide gels and found to contain the S300-II activity in G-free cassette *in vitro* transcription assays using the ovalbumin promoter, as well as adenoviral, SV40, synthetic progesterone receptor-dependent, and basal promoters. The S300-II band was cleaved with cyanogen bromide and internal peptide sequences were obtained. PCR primers were used to clone a cDNA fragment from HeLa poly A^+ RNA. The cDNA was then used as a probe to screen a lambda gt10 HeLa cDNA library. A nearly full length clone was obtained. Surprisingly, sequence analysis indicated that it is the general transcription factor TFIIB. Previous results demonstrating the stabilization of the complex between COUP-TF and its binding sequence by S300II/TFIIB suggested that COUP-TF may directly interact with S300II/TFIIB. Using a glutathione-S-transferase TFIIB fusion protein produced in *E. coli*, we can demonstrate specific interactions between TFIIB and COUP-TFI or human progesterone receptor, produced by an *in vitro* transcription/translation system. Therefore, in addition to its function in the assembly of general factors for a preinitiation complex, we believe that TFIIB plays a pivotal role in mediating induced transcription by interacting with enhancer factors.

R 223 TPA INDUCES SPECIFIC TRANSCRIPTION BY RNA POLYMERASE III IN *DROSOPHILA* SCHNEIDER CELLS, Deborah L. Johnson and Mitchell Garber, Departments of Molecular Pharmacology and Biochemistry., Schools of Pharmacy and Medicine, University of Southern California, Los Angeles, CA 90033. We have examined the ability of the tumor-promoting phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), to regulate RNA polymerase III gene expression in Drosophila. Using nuclear run-on assays, we detected a 3- to 5-fold increase in tRNA synthesis following TPA treatment of *Drosophila* Schneider S2 cells, whereas transcription from the actin 5C, *fos*-, and *jun*-related antigen promoters was unaffected. This response was rapid and transient, peaking at about a 45-minute exposure of the cells to TPA, and dissipating after 60 minutes. We have reproduced this stimulation *in vitro*. Extracts prepared from cells treated with TPA show an approximate 10-fold increase in specific transcription using a 5 S RNA and various tRNA gene templates. The nonspecific transcription by RNA polymerase III in these extracts, however, is essentially unchanged. With the availability of an *in vitro* system, we have characterized in more detail the mechanism of RNA polymerase III transcription activation. Stable complex formation assays and single round transcription conditions suggest that the induced and noninduced extracts contain the same number of transcription complexes, both active and inactive, however, the induced extract has a higher proportion of active to inactive complexes.

R 224 OVEREXPRESSION OF THE TRANSCRIPTION FACTOR AP-2 IS INDUCED BY *N-ras* ONCOGENE BUT AP-2 FAILS TO ACTIVATE TRANSCRIPTION, P. Kannan, Reinhard Buettner, Paul J. Chiao, Sun O. Yim and Michael A. Tainsky. The University of Texas M.D. Anderson Cancer Center, Houston,TX 77030

The teratocarcinoma-derived cell line, PA-1 has been established as a human cell model to understand the processes of multistage carcinogenesis and differentiation. Expression of AP-2 and its transactivation activity were induced by retinoic acid in PA-1 cells that do not contain an activated *N-ras*. The transactivation activity of AP-2 was measured by transient transfection of PA-1 cells with plasmids containing three synthetic AP-2 binding sites, a *tk* promoter and a reporter gene CAT. We explored the consequences of *N-ras* oncogene transformation on the expression and activity of AP-2. Expression of AP-2 was very low in non tumorigenic variants of PA-1 cells. The expression was enhanced more than 10-fold in *N-ras* or *v-myc* transformed PA-1 cells. Paradoxically, the transactivation of AP-2 was inhibited in these *N-ras* transformed PA-1 cells. Co-transfection of these cells with anti-sense *N-ras* restored AP-2 activity. We simulated the high level expression of AP-2 in *N-ras* transformed cells by cloning AP-2 cDNA adjacent to a SV40 early promoter and establishing stable transfectants in non *N-ras* transformed PA-1 cells. Results obtained from these overexpressors were very similar to *N-ras* transformed cells with high expression of AP-2 but paradoxical inhibition of its transactivation activity. Similar to *N-ras* transformed cells the AP-2 overexpressors also promoted anchorage independent growth. In summary, the reduced AP-2 activity correlates with carcinogenesis and the elevated AP-2 activity correlates with differentiation of PA-1 cells. In mobility shift assays the nuclear extracts of the *N-ras* transformed cells exhibited AP-2 specific DNA binding activity. These results indicate the presence of AP-2 protein in these *N-ras* transformed cells but AP-2 fails to initiate transcription suggesting that AP-2 may require an elevated level of a co-factor to activate transcription in PA-1 cells.

R 225 SRB2: A NOVEL RNA POLYMERASE II GENERAL TRANSCRIPTION FACTOR, Anthony J. Koleske and Richard A. Young, Whitehead Institute for Biomedical Research and Dept. of Biology, M.I.T., 9 Cambridge Ctr., Cambridge, MA 02142

The carboxy-terminal domain (CTD) of the largest subunit in RNA polymerase II in S. cerevisiae S288C consists of 27 repeats of a heptapeptide sequence (PTSPSYS). This domain plays an important role in specific initiation of transcription (L.A. Allison et al., PNAS 86: 2794-2798, 1989; C. Scafe et al., Nature 347: 494-494, 1990; S.-M. Liao et al. Genes and Development, in press, 1991). To better define the function of the CTD, we have isolated extragenic suppressors of CTD-truncation mutations. Genetic and biochemical studies reveal that one of the genes, *SRB2*, encodes a novel general transcription factor.

Genetic analysis of *SRB2* suggests that it encodes a protein that interacts functionally with the CTD. The *SRB2-1* allele was isolated in a screen for suppressors of the conditional lethality of certain CTD truncation mutations (Nonet and Young, Genetics 123: 715-724, 1989). *SRB2-1* suppresses the phenotypes of all conditional CTD-truncation alleles of *RPB1* but does not suppress the conditional phenotypes associated with other (non-CTD) RNA polymerase II mutations. Cells containing a complete deletion allele of *SRB2* (*srb2Δ1*) are viable, yet they exhibit the same phenotypes as severe CTD truncation mutations.

RNA polymerase II CTD truncations result in reduced responses to signals from certain upstream activating sequences (UASs) and *SRB2* mutations produce similar effects *in vivo*. Cells with wild-type RNA polymerase II that contain the deletion allele *srb2Δ1* are defective in their transcriptional response to the *GAL10*, *INO1*, and *HIS4* UASs. The suppressor allele, *SRB2-1*, suppresses the defect in the transcriptional response to the *INO1* and *GAL10* UASs of cells containing *RPB1* CTD truncations.

SRB2 is required for efficient promoter-dependent transcription *in vitro*. Nuclear extracts made from *srb2Δ1* cells were unable to generate a specific transcript *in vitro*. The addition of purified recombinant SRB2 restored the ability of the *srb2Δ1* extract to synthesize the specific transcript *in vitro*. The addition of a strong activator (GAL4- VP16) to the *srb2Δ1* extract permitted low levels of synthesis of the specific transcript. However, both purified recombinant SRB2 and GAL4-VP16 are necessary for restoration of transcription to normal activated levels in *srb2Δ1* extracts. Finally, we have demonstrated that SRB2 is required for commitment of the transcriptional apparatus to a template.

R 226 CIS-ACTING ELEMENTS OF THE CAD PROMOTER REQUIRED FOR ACCURATE AND EFFICIENT INITIATION OF TRANSCRIPTION, Richard Kollmar and Peggy J. Farnham, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

The Syrian hamster CAD gene encodes a trifunctional protein that catalyzes the first three steps in the *de novo* biosynthesis of pyrimidine nucleotides. Similar to many other eukaryotic housekeeping genes, it is growth-regulated at the level of transcription. The CAD promoter lacks a TATA box; transcription factor Sp1 binds to two GC boxes and stabilizes initiation complexes. To understand the mechanism of complex formation at the CAD promoter, we are investigating how RNA polymerase II is positioned accurately and how Sp1 interacts with other components of the initiation complex. We have defined a minimal functional promoter by deletion analysis and have created a series of substitution mutants throughout this region. We have now begun to determine the protein binding patterns and the transcriptional activities of these mutants *in vitro* and *in vivo* to define the *cis*-acting elements of the CAD promoter.

R 227 PRODUCTION OF ACTIVE HUMAN RAP30/74 IN BACTERIAL CELLS, Corwin F. Kostrub, Ann Finkelstein, Chun-hsiang Chang and Zachary F. Burton, Department of Biochemistry, Michigan State University, E. Lansing, MI 48824

RAP30/74 is a general initiation factor that binds to RNA polymerase II through its RAP30 subunit. RAP30/74 is the same as factor TFIIF, βγ and FC isolated by others. We and our collaborators previously reported the molecular cloning of cDNAs encoding the RAP30[1] and RAP74[2] subunits of this factor. We have subcloned these cDNAs into the expression vector pET11d (Novagen), which places a cloned gene under control of the bacteriophage T7 promoter. RAP30 and RAP74 were produced using *E. coli* strain BL21(DE3), which carries the gene for T7 RNA polymerase under control of P_{lac}. Approximately 300 mg RAP30 is obtained from a 1 liter culture. RAP30 accumulates in occlusion bodies and is solubilized using a guanidine-HCl renaturation procedure. Approximately 1 mg intact RAP74 is produced from a 1 liter culture. RAP74 is soluble when produced in *E. coli* and has been purified from extracts using phosphocellulose and Affi-gel blue chromatography. After induction, fragments of RAP74 as well as intact protein were detected in *E. coli* extracts. Co-expression of RAP30 and RAP74 cDNAs in *E. coli* did not lead to stabilization of RAP74 and, in fact, destabilized RAP30. Antibodies specific for the NH_2- and COOH-terminal domains of RAP74 have been generated and affinity-purified using T7 Gene 10-RAP74 fusion proteins, produced using expression vector pET3xa. Recombinant RAP30 and RAP74 support accurate initiation by RNA polymerase II *in vitro*. Some physical and functional properties of these recombinant proteins are reported.

References:
1. Sopta, M., Burton, Z.F. and Greenblatt, J. (1989). Nature (Lond.) 341, 410-414.
2. Finkelstein, A., Kostrub, C.F., Li, J., Chavez, D.P., Wang, B.Q., Fang, S.M., Greenblatt, J. and Burton, Z.F. (1992). Nature (Lond.) (*in press*).

R 228 PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF TWO DISTINCT FORMS OF TFIIIC, Robert Kovelman and Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021. We have previously shown that HeLa cell extracts contain at least two forms of TFIIIC, one of which supported higher levels of transcription. Changes in the relative levels of these two forms correlated with changes in transcriptional activity effected by the adenovirus E1A protein or by serum starvation, and preliminary studies with partially purified factors suggested phosphorylation as a potential difference between the two forms. To further characterize the two forms, we devised a novel purification procedure based upon both DNA-binding assays and transcriptional activity. TFIIIC transcriptional activity was selectively inactivated by heat treatment of nuclear extracts from HeLa cells, and this heat-treated nuclear extract was used to supply the other components required for transcription. Using a combination of conventional, high-pressure, and affinity chromatography, we found that the DNA-binding and transcriptional activities of TFIIIC co-purified with five polypeptides of estimated molecular weights 220kD, 110kD, 102kD, 90kD, and 63kD. Purification of the transcriptionally inactive form of TFIIIC revealed a similar polypeptide pattern, except for the apparent absence or modification of the 110kD subunit. We have also performed detailed analyses comparing the DNA-binding properties of the two forms and the apparent conversion of the active form to the inactive form by in vitro treatment with various phosphatases.

R 229 SIGMA^{70}-del4, LACKING REGION 4.2, IS SUFFICIENT TO ALLOW INITIATION AT AN "EXTENDED MINUS 10" PROMOTER. Ashok Kumar, Richard Malloch, David Smillie, Nobuyuki Fujita[1], Akira Ishihama[1], and Richard Hayward, Institute of Cell and Molecular Biology, University of Edinburgh, EH9 3JR, Scotland, U.K. and [1]National Institute of Genetics, Mishima, Shizuoka 411, Japan.

It is known that *E.coli* promoters having a good sequence agreement with the major -10 consensus (TATAAT), but lacking any significant resemblance to consensus near -35, allow quite strong transcriptional initiation *in vivo* if they have an additional motif, TGn, immediately upstream of -10. Presumably such "extended minus 10" promoters are recognised by RNA polymerase carrying the major sigma, σ^{70}.

Our previous studies of hybrid sigmas and hybrid promoters had suggested that the -10 and -35 recognition elements of sigma, regions 2.4 and 4.2, can function independently of one another. Thus if σ^{70} is indeed responsible for initiation at extended minus 10 promoters, it might be active in this respect even after removal of the -35 recognition region (4.2). To test this we synthesised an extended minus 10 promoter, and two derivatives having down mutations in the TG motif. When fused to a plasmid-borne reporter gene the first allowed strong expression of the reporter in *E.coli*, while the down mutants allowed only weak expression. We also fused the σ^{70} gene (*rpoD*) to a T7 late promoter on a plasmid, and effectively introduced a stop codon such that it encoded a polypeptide, σ^{70}-del4, retaining the first 529 aa of σ^{70}, but lacking the distal residues which include regions 4.1 and 4.2. σ^{70}-del4 was then over-produced, purified, and used in run-off transcription studies *in vitro* together with purified core RNA polymerase, normal σ^{70}, and DNA template fragments carrying the extended minus 10, down-mutant, or normal promoters.

The results show that σ^{70} indeed allows transcription from the extended minus 10 promoter, dependent on the integrity of the TGn motif. Moreover σ^{70}-del4, although detectably less efficient in binding to core enzyme than intact σ^{70}, and incompetent to serve a consensus -35/-10 promoter, is proficient in allowing initiation at an extended minus 10 promoter, provided that the TGn element is intact.

R 230 ANTAGONISTIC EFFECTS OF E1A 12S AND THE RETINOBLASTOMA PROTEIN ON HUMAN PROLIFERATING CELL NUCLEAR ANTIGEN PROMOTER ACTIVITY, Claude Labrie, Gilbert F. Morris and Michael B. Mathews, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724-2208.

The mechanism of cellular transformation by adenovirus is believed to involve the association of E1A oncogene products with a number of cellular proteins, resulting in the disruption of normal growth control. The E1A 12S product activates CAT expression from the human proliferating cell nuclear antigen (PCNA) promoter in transfected Hela cells. PCNA is a DNA replication factor whose expression increases during infection and subsequent transformation of rodent cells by adenovirus. The PCNA promoter sequences which mediate the E1A 12S effect reside within 87 base pairs of the transcription start site (Morris & Mathews, J. Biol. Chem. 265: 16116, 1990). These promoter sequences also contain elements similar to the retinoblastoma control element which mediates repression of c-fos by the retinoblastoma gene product (RB) in mouse 3T3 cells (Robbins et al., Nature 346: 668, 1990). Since E1A binds RB and RB is implicated in cell cycle regulation, we have investigated the possible effect of RB on the PCNA promoter. The first 87 base pairs of PCNA promoter sequence were fused to CAT (PCNA-87 CAT) at + 60 from the transcription start site. PCNA-87 CAT was cotransfected into Hela cells with combinations of CMV expression plasmids expressing either full-length RB, wild type E1A 12S, or E1A 12S pm928, which does not bind RB. RB represses basal PCNA-CAT expression in the absence of E1A but it fails to reverse the 14-fold stimulation of CAT activity induced by E1A 12S. On the other hand, transactivation by pm928 is greatly reduced by RB. Thus it seems that E1A 12S stimulates transcription from the PCNA promoter and, by a separate mechanism, also relieves the inhibitory effect of RB.

R 231 CIS-ACTING TRANSCRIPTIONAL ELEMENTS REQUIRED FOR THE EXPRESSION OF THE CIT1 GENE OF YEAST, Sobomabo Lawson, Keshav Singh and Alfred Lewin, Department of Immunology and Medical Microbiology, University of Florida College of Medicine, Gainesville, FL 32610

Citrate synthase is required for two essential cyclic metabolic pathways--the tricarboxylic acid cycle and the glyoxylate cycle. CIT1 is a nuclear gene encoding the mitochondrial form of citrate synthase, that form involved in the Krebs cycle. Its expression is regulated by the availability of oxygen and of glucose. In hypoxic cells and in cells grown in the presence of high levels of glucose, synthesis of citrate synthase and of CIT1 mRNA is repressed. Cells grown aerobically on non-fermentable carbon sources produce high levels of the enzyme. Using CIT1-lacZ fusions and nuclease protection assays, we have identified several regions in the 5' non-translated region of CIT1 that are required for high level expression of this gene. Mobility-shift assays confirm that they contain the binding sites for one or more proteins. Although one region contains a consensus binding site for the hap2/3/4 regulatory protein, deletion of this sequence does not impair regulated synthesis. Nor is functional hap2 required for expression of fusions containing the complete CIT1 promoter. Nevertheless, the highest levels of induction are observed in HAP+ cells, suggesting that this trans-acting protein may influence expression. CIT1 regulation is also unusual in that the effect of oxygen does not appear to be mediated by heme as an intracellular messenger. We are using methylation interference assays to define the 5' sequences involved in the modulating expression response to glucose and to oxygen.

R 232 PURIFICATION OF HIP1, A DHFR TRANSCRIPTION INITIATOR BINDING PROTEIN, Yue Li, Anna L. Means, Peggy J. Farnham, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

The sequence spanning the mouse dihydrofolate reductase (DHFR) transcription start site (from -16 to +11) has been demonstrated to have transcriptional initiator function. Purification of a protein (HIP1) from HeLa nuclear extract that binds to this initiator sequence enriched for a 180 kDa polypeptide. Southwestern blot analysis has indicated that this 180 kDa protein has the initiator sequence binding activity. Gel mobility shift analyses revealed that the sequence 5'TTCGCGCCA3' is critical for efficient interaction between HIP1 and the initiator sequence. A kinetic analysis of the association and dissociation rates of the HIP1 protein indicates that bound HIP1 protein can exchange rapidly with free protein. In addition, protein binding sites at or near the initiation sites of several other genes were tested for their ability to compete HIP1 binding. The SV40 late initiation site, but not the initiation sites of the AdMLP, REP, or TdT promoters, could compete HIP1 binding, suggesting that there are at least two different initiator binding proteins for different promoters. Preliminary in vitro transcription studies suggested that HIP1 protein can direct specific transcription driven by mouse DHFR promoter but not by AdMLP. These results raise the possibility that transcription of different genes may require different initiator proteins.

R 233 ANALYSIS OF THE STRUCTURE-FUNCTION RELATIONSHIP OF TRANSCRIPTION FACTOR TFIIB, Edio Maldonado, Ilho Ha, Y.-S. Lin, Jin Shang, M. Green and Danny Reinberg, Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635.

Human transcription factor IIB (TFIIB) is required for transcription from all class II promoters. The sequence of TFIIB contains a direct repeated motif of 76 amino acids at the C-terminus and a potential amphipathic α-helix motif from amino acid 185 to 202. Analysis of deletion mutants of TFIIB shows that both repeats are required to form a complex with TFIID and TFIIA on the TATA motif of the MLP promoter (DAB complex). This motif seems also to be involved in the formation of a complex with RNA polymerase II and TFIIF. Additionally, analysis of point mutations shows that the positively charged residues of the amphipatic helix are required for the interaction of TFIIB with the acidic activator VP16.

Because of the lack of a well-defined genetic system in human cells, we are attempting to identify the TFIIB homologue of S. cerevisiae. Using degenerate oligonucleotides derived from conserved sequences between the human and Drosophila TFIIB clones as a probe, we have isolated two clones from a lambda DASH yeast genomic library. These contain a 2.5 kb restriction fragment that hybridizes specifically with the probe. Currently, we are sequencing to determine if the putative clones contain the TFIIB homologue.

R 234 FUNCTIONAL AND STRUCTURAL ANALYSIS OF MAMMALIAN TFIID COMPLEXES : SUBUNIT COMPOSITION OF B-TFIID, Rachel E. Meyers[1], H. Th. Marc Timmers[2], Phillip Sharp[1,]1Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge. [2]Laboratory of Physiological Chemistry, University of Utrecht, The Netherlands

The basal transcription factor TFIID, an essential component of the RNA Polymerase II transcription reaction, interacts with the TATA element of promoters. The DNA binding component of human TFIID has been cloned and antisera specific for this 38 kD component show that this protein fractionates into two distinct complexes of 300kD (B-TFIID) and >700 kD (D-TFIID); the majority of the protein being present in the B-TFIID complex. Both complexes stimulate the basal transcription reaction with comparable efficiency. However, in contrast to the previously characterized D-TFIID, transcriptional initiation complexes formed with the B-TFIID complex do not respond to upstream factors containing acidic or glutamine-rich activation domains.

These functional and physical differences are likely to be caused by differences in the protein composition of the TFIID complexes. Using conventional and immunoaffinity chromatography, the B-TFIID complex was shown to consist of the 38kD protein and a 170kD protein of unknown identity. The 170 kD protein appears to be a unique component of the B-TFIID complex and is not contained within the larger D-TFIID complex. We are currently investigating the functions of the B-TFIID complex, which recent experiments suggest exhibits a DNA dependent ATPase activity.

R 235 ACTIVATION OF THE BASAL PROMOTER FOR THE PROLIFERATING CELL NUCLEAR ANTIGEN BY THE ADENOVIRUS E1A 12S PRODUCT IS DEPENDENT UPON PROXIMAL TRANSCRIPTIONAL ACTIVATORS, G.F. Morris and M.B. Mathews, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724. The gene for PCNA, a DNA replication factor, is growth-responsive. We have shown that the adenovirus E1A 12S product can transactivate a chimeric construct (PCNA-CAT) that expresses CAT from the PCNA promoter upon cotransfection into HeLa cells. Mutation of a sequence with homology to an ATF consensus sequence 50 nucleotides upstream of the transcriptional initiation site abrogates the response to E1A 12S. To examine the requirements for this transcription factor binding site in the response of PCNA-CAT to E1A 12S, we have replaced it, as well as sequences upstream, with 5 copies of the GAL 4 recognition motif. Since the PCNA basal promoter is not well-characterized and does not possess an AT-rich sequence in the region 20 to 30 nucleotides upstream of the transcription initiation site, the response of this new construct, G5PCNA-CAT, to upstream activators was examined. Like promoters that contain a correctly positioned TATA motif, G5PCNA-CAT can be activated by a variety of transcriptional activation domains (Sp1, E1A, and VP16) expressed as GAL4-fusion proteins. However, G5PCNA-CAT differs from TATA-containing promoters (such as G5E1BCAT) in its response to these chimeric activators when E1A 12S is co-expressed. In the absence of fusion proteins that recognize the GAL 4 binding sites, G5PCNA-CAT is unresponsive to E1A 12S. Co-expression of GAL4-Sp1, a fusion protein that can bind the GAL 4 sites and weakly activates transcription via the glutamine-rich transcriptional activation domain of Sp1, produces a positive response to E1A 12S by G5PCNA-CAT. We propose that bound GAL4-Sp1 can serve the same function in the response of G5PCNA-CAT to E1A 12S that the ATF motif serves in the wild-type PCNA-CAT promoter. Co-expression of E1A 12S with the two strong transcriptional activators, GAL4-VP16 or GAL4-E1A, reduces expression from G5PCNA-CAT. E1A 12S produces little or no effect upon transcriptional activation by these three transcriptional activators with G5E1BCAT. These experiments indicate that the PCNA basal promoter is configured in a manner that permits the response of some transcriptional activators to be modulated by E1A 12S, while a TATA-containing promoter remains unresponsive to the effects of E1A 12S.

R 236 SEQUENCE-SPECIFIC INITIATOR ELEMENTS FOCUS INITIATION OF TRANSCRIPTION TO DISTINCT SITES IN THE YEAST TRP4 PROMOTER, Hans-Ulrich Mösch and Gerhard H. Braus, Institute of Microbiology, Swiss Federal Institute of Technology (ETH), Schmelzbergstr. 7, CH-8092 Zürich, Switzerland

Transcription from the yeast *TRP4* promoter initiates at two major (i127, i76) and three GCN4 dependent (i31, i26, i12) initiator elements (Mösch et al., 1990). All of these elements contain not more than one deviation from the initiator consensus sequence PuPuPyPuPu, i.e. a pyrimidine nucleotide flanked by two purine nucleotides. A point mutation analysis of these elements in various combinations was performed by substituting the central pyrimidine nucleotide of the PuPuPyPuPu sequences for a purine nucleotide to result in PuPuPuPuPu sequences. These point mutations were tested in vivo and led to a quantitative loss of transcription initiating at these mutated sites. The sequence specificity was identical for basal and GCN4 mediated transcription demonstrating that they are functionally homologous. Multiple cryptic transcription start sites which function independently whether they are located on the coding or on the non-coding strand can replace the function of the mutated initiator elements. Therefore the overall rate of transcription initiation is not affected. These findings imply that the role of initiator elements is to focus the start point(s) of transcription at distinct sites located in the region between the site(s) of the assembly of the transcriptional complex and the start codon of translation.

Mösch, H.-U., Graf, R., Schmidheini, T. and Braus, G. (1990) *EMBO J.*, **9**, 2951-2957.

R 237 PURIFIED ACTIVATING TRANSCRIPTION FACTOR (ATF) STIMULATES RNA POLYMERASE-III MEDIATED TRANSCRIPTION OF THE HUMAN 7S L RNA GENE *IN VITRO*. Jürgen Müller, Sebastian Bredow and Bernd-Joachim Benecke, Department of Biochemistry, Faculty of Chemistry, Ruhr-University, D-463 Bochum 1, Germany.

The human 7S L RNA gene is transcribed by RNA polymerase III and codes for a 299 nt molecule which represents the RNA compound of the signal recognition particle (srp). In addition to one gene internal promoter element and a so far unidentified distal sequence element, an upstream ATF binding-site has been identified as being essential for efficient transcription of this gene *in vitro* and *in vivo*. The nuclear protein recognizing this ATF site was purified by ion-exchange and affinity chromatography. Competition experiments of south-western, gel mobility shift and DNase I footprint analyses revealed that binding of the protein to the 7S L promoter was highly specific and depended on the integrity of the ATF site present within this pol III promoter. The DNA/protein complex formed resembled that obtained with recombinant ATF-43 but differed from the corresponding complex between CREB (ATF-47) and the 7S L ATF binding-site (recombinant proteins were kindly provided by H.C.Hurst, London). Furthermore, our purified 7S L transcription factor appeared to be immunologically related to the ATF protein family. ATF-depleted extract fractions revealed only basal level transcription of the 7S L RNA gene *in vitro*, but showed significant activation upon addition of the affinity purified factor protein.

R 238 GENE REGULATION BY THE TRANSCRIPTION-FACTOR JUN/AP1 IS MEDIATED BY A SPECIFIC COACTIVATOR THAT DEPENDS ON THE PRESENCE OF ACIDIC AMINO ACIDS WITHIN THE TRANSACTIVATION DOMAIN OF JUN

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AP1 is a transcription factor consisting of the members of the Jun and Fos families. AP1 is able to induce the transcription of several target genes, e.g. the collagenase. To exhibit its activity, AP1 relies on components of the transcriptional machinery like TFIIA, TFIIB, TFIIE and the TFIID/RNA polymerase II complex. It has been suggested, that in the process of transactivation at least one additional protein is involved, that is thought to link up AP1 and the TFIID/RNA polymerase II complex to perform transactivation. This type of bridging factor is called mediator-protein or co-activator. Using in vivo squelching experiments, we could show, that different transcription factors utilize specific types of co-activators and that they can be classified by that property into distinct families. The specific co-activator of Jun requires the presence of acidic amino acids within its transactivation domain. The Herpes simplex virus protein VP16, Gal4 and JunB use the same co-activator to mediate transactivation. The transcription factor myb utilizes different types of co-activators one of which is also entitled by the transcription factors, which perform transactivation by acidic amino acids. GHFI, SRF and the estrogen receptor, in which no obvious acidic stretches can be found, perform transactivation through a different type of co-activator or by other mechanisms.

R 239 STRUCTURE AND FUNCTIONAL INTERACTIONS OF THE GENERAL TRANSCRIPTION FACTOR TFIIE.

Yoshiaki Ohkuma, Shigeru Hashimoto, Masami Horikoshi, and Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

The human general transcription factor TFIIE consists of two subunits of 57-kDa (TFIIE-α) and 34-kDa (TFIIE-β), and forms a heterotetramer with a molecular mass of approximately 180 kDa. TFIIE joins the preinitiation complex after RNA polymerase II and TFIIF. However, little is known about its actual function at this step. In order to understand the functional role of TFIIE in transcription initiation, we purified human TFIIE and cloned each of the two subunits. Both subunits are absolutely required for transcription activity. By sequence analyses of two subunits, several putative structural motifs and sequence similarities were found in each. TFIIE-α has putative leucine repeat, zinc finger, and helix-turn-helix motifs and sequence similarity to bacterial σ-factors. TFIIE-β has a putative basic region-helix-loop motif and sequence similarities to bacterial σ-factors as well. Interestingly, both subunits contain sequence similarity to a region of RAP30 (the small subunit of TFIIF) that has been reported to be homologous to a σ-factor subregion implicated in binding to bacterial RNA polymerase. Together with the observation that all of the other general transcription factors analysed so far (TFIIB, TFIIDτ, RAP30) also have sequence similarities to one or more σ-factor subregions, these similarites suggest an evolutionary segregation of function. And the putative structural motifs indicate the possibility of TFIIE interactions with the other transcription factors and with DNA, although TFIIE alone cannot bind to DNA by itself. The results of structure-function analyses of TFIIE-α and TFIIE-β focused on these motifs will be presented.

R 240 FUNCTIONAL REPLACEMENT OF A YEAST MITOCHONDRIAL DNA BINDING PROTEIN BY HUMAN MITOCHONDRIAL TRANSCRIPTION FACTOR 1, Melissa A.Parisi, Baoji Xu, and David A. Clayton, Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305-5427

Eucaryotic mitochondrial DNA (mtDNA) is transcribed by nuclear-encoded proteins believed to be distinct from the three polymerase complexes involved in nuclear transcription. Unlike pol II promoters, there are only two vertebrate mtDNA promoters, one for each strand of the closed circular DNA molecule. However, the mt promoter structure and organization as well as species specificity and production of polycistronic transcripts resemble polymerase I transcription. We have purified to homogeneity a 25-kilodalton protein from human mitochondrial extracts which specifically binds mitochondrial promoters and stimulates accurate transcription in vitro when added to a partially-purified mitochondrial RNA polymerase fraction. This protein, mitochondrial transcription factor 1 (mtTF1), has been isolated from both mouse and human mitochondria and has an inherent flexibility in primary DNA sequence requirement. The gene for human mtTF1 has been cloned, and the amino acid sequence shows homology to high mobility group (HMG) proteins, once thought to be ubiquitous nuclear DNA-binding proteins with undetermined roles in transcription. One member of this family is a nucleolar transcription factor, hUBF, which appears to bind DNA with at least one of its four HMG boxes and stimulate polymerase I transcription in a non-species-specific manner, thus underscoring the similarity to mt transcription. Homology also exists between mtTF1 and a protein isolated from yeast mitochondria which can, like human mtTF1, bend and introduce negative supercoils into DNA and footprint control regions of mtDNA. The amino acid sequence of the yeast gene, called *ABF2* (ARS Binding Factor 2), reveals two HMG boxes with similarity to human mtTF1. Disruption of the yeast *abf2* locus produces yeast cells which are unable to grow on nonfermentable substrates such as glycerol because of loss of mtDNA and hence mitochondrial function. Transformation of this strain by plasmid shuffle with a construct containing the human gene produces yeast cells containing properly localized human protein and, more importantly, partial rescue of the yeast respiratory-deficient phenotype. Although the role of ABF2 in yeast mt transcription is unclear, it appears that the human and yeast proteins share properties of mtDNA maintenance and, in addition, may have some elements in common with pol I transcription.

R 241 PROMOTER SPECIFICITY OF BASAL TRANSCRIPTION FACTORS, Jeffrey D. Parvin, Marc Timmers, and Phillip A. Sharp, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139

Regulation of expression of protein-encoding genes in eukaryotes is frequently mediated by sequence-specific transcription factors which control the activities of the basal factors and RNA polymerase II. The same set of basal factors have been considered to be essential for all class II promoters. Studies of the basal factor requirements for transcription from the immunoglobulin heavy chain gene (IgH) core promoter and the adenovirus major late gene core promoter (MLP) suggest that this paradigm is inaccurate. Basal transcription from the IgH promoter was reconstituted by TFIID, TFIIB, TFIIF, and polymerase, whereas basal transcription from the MLP required TFIIE in addition to the above factors. Two novel protein activities, referred to as "700 kDa" and "90 kDa," further stimulated the basal reaction from the MLP. Transcription from the IgH promoter was not inhibited by high levels of antibody specific for the large subunit of TFIIE, while transcription from the MLP was very sensitive to this antiserum. These data indicate that the basal factors are not in fact general, and, further, could explain how different core promoters could be stimulated to different degrees by enhancer-binding proteins.

R 242 THE YEAST *SUA7* GENE ENCODES A HOMOLOG OF THE HUMAN TRANSCRIPTION FACTOR TFIIB AND IS REQUIRED FOR NORMAL START SITE SELECTION IN VIVO, Inés Pinto, Dan E. Ware and Michael Hampsey, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, LA 71130

Mutations in the *Saccharomyces cerevisiae SUA7* gene were isolated as suppressors of an aberrant ATG translation initiation codon in the leader region of the *cyc1* gene. Molecular and genetics characterization of the cloned *SUA7* gene demonstrated that *SUA7* is an essential gene encoding a basic protein (calculated M_r of 38,142) with extensive sequence similarity (35% identity and 17% similarity) to the human transcription factor TFIIB. Sequence similarity extends across the entire length of the two proteins and includes a Zn(II)-finger motif near the N-termini. *SUA7* has been mapped to the right arm of chromosome XVI, linked to *cdc67* and *aro7*. Analysis of *cyc1* transcripts from *sua7* strains revealed that suppression is a consequence of diminished transcription initiation at the normal start sites in favor of initiation at downstream sites, including a major site between the aberrant and normal ATG start codons. A similar effect was found at the *ADH1* locus, establishing that this effect is not gene-specific. We conclude that *SUA7* encodes a TFIIB homologue that functions in transcription start site selection in yeast.

Supported by NIH Grant GM39484.

R 243 THE DIOXIN RECEPTOR: AN INDUCIBLE GENE REGULATORY PROTEIN WHICH IS MODULATED BY AN INHIBITORY PROTEIN AND PHOSPHORYLATION, Lorenz Poellinger, Anna Wilhelmsson, Ingemar Pongratz, Murray Whitelaw and Grant Mason; Department of Medical Nutrition, Karolinska Institute, Huddinge University Hospital F-60, Novum, S-141 86 Huddinge, Sweden

Signal transduction by dioxin is mediated by an intracellular receptor which is activated by dioxin to translocate from the cytoplasm to the nucleus and to interact with dioxin-responsive positive control elements near the regulated promoters. We have reconstituted under cell-free conditions dioxin-induced conversion of the receptor from a cryptic to a DNA-binding form. We show that the cryptic receptor form is associated with an inhibitory protein (hsp90), the release of which is necessary to unmask functional activities of the receptor protein. Hsp90 plays dual roles in the modulation of functional receptor activities: it is critical for a dioxin-responsive conformation in that it is required for formation of a stable ligand-receptor complex; and it inhibits the DNA-binding activity of the receptor. Moreover, receptor activity appears to be modulated by phosphorylation, since (i) dephosphorylation of the dioxin-activated receptor generates a non-DNA-binding form of receptor; and (ii) it is possible to reconstitute the DNA-binding activity of the receptor in vitro by protein kinase C- but not by protein kinase A-mediated phosphorylation. Finally, we have recently identified a labile nuclear factor which constitutively binds to sequences overlapping with the dioxin response element. This factor is distinct from the dioxin receptor which does not bind DNA in its non-activated state. We are presently investigating whether the receptor derepresses promoter activity by displacement of the constitutive factor or whether the constitutive factor actually complements receptor function in reconstituted cell-free transcription experiments.

R 244 ISOLATION AND CHARACTERIZATION OF TFIID TEMPERATURE SENSITIVE MUTANTS WHICH COMPROMISE TRANSACTIVATION IN VIVO, David Poon, Roberta A. Knittle, Kimberly A. Salbelko and P.A. Weil; Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232

TFIID has a known activity (DNA binding) as well as presumptive ones (protein-protein interactions with TFIIA, and/or TFIIB). TFIID has also been implicated as a direct or indirect target for transcriptional regulatory factors. We are currently working to identify the functional domains of TFIID that are necessary for these activities. We previously assayed yeast TFIID deletion mutants both in vitro for TATA-box DNA binding and basal level transcription activities and, in vivo, for their ability to complement a chromosomal null allele of the gene encoding this protein. In those studies, the nonconserved amino-terminus was found to be dispensable for both in vitro and in vivo function. Furthermore, any deletion within the conserved carboxy-terminal region of TFIID (aa residues 62-240) inactivated the protein.

To expand upon these structure-function analyses the gene encoding TFIID was subjected to site-directed mutagenesis within the conserved carboxy-terminal region of the molecule. Using this approach we have isolated temperature sensitive mutants of TFIID which are deficient in their ability to mediate transactivation by Gal4 in vivo. We are currently working to further characterize the molecular defects of these mutants and the results will be discussed.

R 245 Genetics of the yeast TATA-Binding Protein: Isolation of a High Copy Suppressor of a Trans-Dominant Mutation in TBP, Raymond Ganster, Weiqun Shen & Martin C. Schmidt, Dept. of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

The committing step for the assembly of the RNA polymerase II transcription complex at the proximal promoter is the binding to the TATA binding protein (TBP) to the TATA box element. We have devised a selection to isolate the genes for proteins which interact with TBP. Overexpression of a trans-dominant allele of TBP (SPT15-57) causes a severe inhibition of growth in some yeast strains. We have screened a high copy plasmid library of yeast genomic DNA for suppression of this growth defect. Two clones were isolated in this screen, and one clone, p6A, has been analyzed is some detail. p6A contains 7 kb of genomic DNA that maps to the right arm of chromosome XV, between whi2 and tmp1. Cells transformed with p6A overexpress two mRNA's of 3.5 and 2.0 kb. Overexpression of the 2 kb message is not by itself sufficient for suppression. Apparent toxicity in *E. coli* has so far prevented the construction of a subclone overexpressing only the 3.5 kb message. DNA sequence data indicates that the 3.5 kb message encodes a 116 kDa protein identical to the RAT1 protein identified by Drs. David Amberg and Charles Cole of Dartmouth Medical School. They isolated RAT1 in a screen of ts mutants for the mislocalization of mRNA. Current work is focused on the connection between the general transcription factor TBP and mRNA transport in yeast.

R 246 SPECIFIC INTERACTIONS BETWEEN TRANSCRIPTION INITIATION FACTORS AND RNA POLYMERASE I AT THE MOUSE rDNA PROMOTER, Andreas Schnapp, Gisela Heilgenthal and Ingrid Grummt, Institute of Cell and Tumor Biology, German Cancer research Center, W-6900 Heidelberg, Germany

We have used purified transcription factors and RNA polymerase I (pol I) to analyze the individual steps involved in the formation of of transcription initiation complexes at the mouse rRNA gene promoter in vitro. Complete assembly of transcription complexes requires pol I and at least four auxiliary factors, termed TIF-IA, TIF-IB, TIF-IC, and UBF. Preincubation, template commitment, and order of addition experiments were used to discriminate between various intermediate complexes generated during assembly of the initiation complex. As a first step, TIF-IB binds to the core promoter, a process that is facilitated by the upstream control element and by UBF. Binding of TIF-IB to the rDNA promoter results in the formation of a functional preinitiation complex which is stable for many rounds of transcription. Transcription competent initiation complexes are then built up by the stepwise association of pol I, TIF-IC and TIF-IA. Only the complete complex consisting of pol I and all four factors shows resistance to 0.045% of Sarkosyl and is competent to catalyze the formation of the first phosphodiester bond.

In order to study the interaction between pol I and the individual transcription factors we covalently attached purified pol I to an agarose matrix and chromatographed in vitro translated recombinant UBF on this affinity column. We show that UBF is bound to this column and can be eluted by high salt. mUBF binds also to yeast pol I but not to yeast pol II. Therefore, this interaction is specific for class I RNA polymerases and appears to involve a domain in pol I which is conserved in evolution.

R 247 DITHIOCARBAMATES AS POTENT INHIBITORS OF NF-κB ACTIVATION IN INTACT CELLS, Ralf Schreck and Patrick A. Baeuerle, Laboratory for Molecuar Biology, Gene Center, Am Klopferspitz 18a, D-8033 Martinsried, F.R.G.

Nuclear factor κB (NF-κB) is a multisubunit transcription factor that inducibly activates the expression of genes involved in inflammatory, immune and acute phase response. Recently, we have shown that micromolar concentrations of H_2O_2 induce the expression and replication of HIV-1 in latently infected Jurkat T cells. This effect is mediated by NF-κB that is rapidly activated by an H_2O_2 treatment from its inactive cytoplasmic form. The H_2O_2 induced activation of NF-κB was inhibited by several radical scavenging dithiocarbamates and metal chelating agents. One agent, Pyrolidine dithiocarbamate (PDTC), was further investigated in detail.

PDTC specifically and reversibly blocked in a dose-dependent manner the activation of NF-κB by phorbolester, tumor necrosis factor and interleukin-1 in all cell lines tested. Overdosage or chronic treatment desensitized the cells for the inhibiting effect of PDTC. The effect of PDTC on the inducibility of NF-κB binding was reflected in the drug's effect on the transactivation of various reporter plasmids in transient transfection assays. While κB-dependent gene induction was inhibited by μM-concentrations of PDTC, the basal activities of reporter constructs were virtually unaffected by the drug. Because PDTC did not affect the level of the cytoplasmic NF-κB/IκB complex and did not interfere with DNA binding of NF-κB, it most likely blocked a reaction required for release of IκB from NF-κB in intact cells. This reaction seemed to require metal ions and hydroxyl radicals. Superoxide radicals, which are rapidly eliminated in cell lines overexpressing Mn-dependent superoxide dismutase, seem not to be directly involved in NF-κB activation.

R 248 BROKEN FINGER MUTATIONS IN TRANSCRIPTION FACTOR IIIA, David R. Setzer, Sandra Menezes, and Samuel Del Rio, Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH 44106. We have analyzed structural and functional properties of a series of mutant forms of *Xenopus* Transcription Factor IIIA (TFIIIA) in which each mutant protein contains a single amino acid substitution in one of the putative Zn^{++}-binding histidine residues of the nine consecutive Zn^{++} finger motifs of the protein. Chromatographic properties of the mutant proteins and the results of partial proteolysis studies indicate that the mutation in each case results in structural disruption of the Zn^{++} finger domain containing the mutation with no apparent effect on adjacent fingers. These "broken finger" proteins have been used to analyze the role of each Zn^{++} finger in the function of TFIIIA. While all fingers contribute to the binding energy when TFIIIA interacts with the internal control region (ICR) of a 5S rRNA gene, fingers 3 and 4 are clearly more important than other fingers, resulting in 50- to 100-fold reductions in binding affinity when disrupted. Using footprinting analysis, we have localized the sites of interactions of individual fingers with the 5S rRNA gene ICR by identifying short sequences which are protected by the wild-type protein but which become accessible to the footprinting probe when mutant proteins are analyzed. These data suggest a model for the TFIIIA/5S ICR interaction that is at odds with some previous models. Surprisingly, the qualitative nature of the footprint obtained with a finger 4 mutant is indistinguishable from that of wild-type TFIIIA, despite a 50-fold reduction in binding affinity. Similarly, disruption of finger 6 results in a footprint very similar to that of the wild-type protein. It is possible that these fingers contribute to the binding energy not by direct interactions with the DNA helix, but by positioning fingers 1-3, 5, and 7-9 so as to permit optimal contacts between these fingers and the DNA substrate. Analysis of "broken finger" mutants by in vitro transcription analysis suggests that finger 4 contributes not only to the affinity of TFIIIA for 5S DNA in a binary complex, but also to the stability of the multi-protein transcription complex, and that a structurally intact finger 4 may therefore be necessary for interaction of TFIIIA with other components of the transcription machinery.

R 249 SEARCHING FOR FURTHER SIGMA GENES IN *E. coli*. David A Smillie, Akira Ishihama[1], Nobuyuki Fujita[1], Fiona M Townsley, Stephen Wedgwood, and Richard S Hayward, ICMB, University of Edinburgh, EH9 3JR, Scotland, UK and [1] National Institute of Genetics, Mishima, Shizuoka 411, Japan. Sequence analysis has shown homologies between sigma subunits from a wide variety of bacteria. Seven species of sigma and the genes encoding six of them have been found in *Escherichia coli*. We have designed short defined or mixed sequence oligonucleotide probes, and long probes based on codon choice, which correspond to conserved regions within sigma factors. We have used these in attempts to locate further sigma genes by probing the library of Kohara *et al.*, comprising physically mapped *E. coli* genome fragments in λ vectors. To date we have identified, besides *rpoD*, *rpoH*, and *rpoS*, five distinct loci which may represent hitherto unmapped sigma genes. We are currently fine mapping these loci, and sub-cloning relevant restriction fragments for DNA sequencing, database comparisons and gene disruption tests. As another approach to the identification of candidate sigma genes, the Mishima group earlier purified and NH_2-terminally sequenced four *E. coli* proteins which cross-react with antisera raised against a tetradecapeptide which is identical in σ^{70} and σ^{32}. None of these sigma cross-reacting proteins (SCRP's) was able to bind to core RNA polymerase in the tests applied, and two of them were identified from their sequences as thioredoxin reductase and ribosomal protein S2. Our two groups have collaborated to investigate the remaining SCRPs, 23 kD and 27 kD in size. Using oligonucleotide probes based on the NH_2-terminal protein sequences we have mapped, sub-cloned, and determined the DNA sequences of the corresponding genes. SCRP23 is evidently the *E. coli* homologue of the small subunit of alkylhydroperoxide reductase, previously sequenced in *S. typhimurium* by Ames and colleagues. The gene (*ahpC*) lies at 652 kb on the physical map of *E. coli* and the large subunit (*ahpF*) gene lies downstream, as in *S. typhimurium*.

SCRP27 is encoded at 3423 kb on the physical map, 250 bp downstream of *arcB*. The latter, sequenced by Iuchi *et al*, encodes a sensor-regulator protein for anaerobic repression of genes in the *arc* modulon. The protein sequence of SCRP27, which appears to be inessential (judged by gene disruption), offers no clear clues as to its function.

R 250 TRANS-ACTIVATION OF HOMOLOGOUS AND HETEROLOGOUS PROMOTERS BY THE HCMV UL112-113 NUCLEAR PHOSPHOPROTEINS, Marvin Sommer and Deborah Spector, Department of Biology, University of California-San Diego, La Jolla, CA 92093

Our laboratory is interested in studying the function of the 4 nuclear phosphoproteins of 84, 50, 43, and 34 kDa (HCMV UL112-113) encoded by a family of transcripts arising from the EcoRI fragments R and d of the human cytomegalovirus (HCMV) AD169 genome. Using transient transfection assays with 2 HCMV early promoters (2.2 kb and 2.7 kb early promoters) or the HIV LTR linked to the chloramphenicol acetyltransferase (CAT) reporter gene, we have shown that a construct capable of expressing all 4 nuclear phosphoproteins can activate expression from these promoters in both HeLa and U373 (glial) cells. In U373 cells the level of activation observed for the UL112-113 proteins is lower than that observed for the activation of the promoters by a genomic construct specifying the HCMV IE1 and IE2 proteins. However, in HeLa cells the 2.7 kb early promoter is induced to equivalent levels with either the UL112-113 proteins or the IE proteins. A 2-fold higher level of induction of the HIV LTR was observed for the UL112-113 proteins as compared to the IE proteins. The UL112-113 and the IE proteins do not appear to act synergistically since co-transfection of the UL112-113 proteins and the IE proteins with a reporter plasmid leads to a decrease in the level of activation observed for the IE proteins alone. Studies are currently in progress to determine if the regions in the HIV LTR required for the induction by the HCMV IE proteins are similar to those required by the UL112-113 proteins. In addition we are trying to determine the role of the individual UL112-113 proteins in viral gene expression and to identify the cellular and/or viral factors that may interact with these proteins.

R 251 TATA BINDING PROTEIN OF *TETRAHYMENA*, Laurie A. Stargell and Martin A. Gorovsky, Department of Biology, University of Rochester, Rochester, NY 14627.

Little is known about the process of transcription initiation by RNA polymerase II in ciliates. A mandatory factor for assembly of the basal transcription apparatus in other systems is TATA box binding protein (TBP), which binds to the TATA box typically located a relatively fixed distance upstream of the start site of transcription. Although several dozen *Tetrahymena* genes have been cloned and sequenced, canonical TATA boxes located a fixed distance from the start sites of transcription have not been identified. However, upstream regions of *Tetrahymena* genes are greater than 80% AT. Thus, ciliates may either have a TBP binding site which differs from other eukaryotes or employ a promoter recognition mechanism which does not involve TBP. The TBP genes from yeast, mammals, flies and plants have been sequenced (Hahn, *et al.*, Cell 58:1173-1181, Horikoshi *et al.*, Nature 341:299-303, Fikes, *et al.*, Nature 346:291-294, Kao, *et al.*, Science 248:1646-1650, Peterson *et al.*, Science 248: 1625-1630, Hoey, *et al.*, Cell 61:1179-1186, Gasch, *et al.*, Nature 346:390-394). Using sequence information from the conserved carboxy terminus, synthetic primers were constructed and PCR was used to amplify a fragment of the *Tetrahymena* homologue of TBP (tetTBP), which was cloned and sequenced. Using the fragment as a probe, we have found that tetTBP is encoded by a message of 1.3 kilobases, from a single copy gene. This gene has been cloned and is partially sequenced. The derived amino acid sequence for the carboxy terminal 180 amino acids is the most divergent described and is 70% identical with the yeast sequence; others show 80 to 93% identity within this region. In addition, tetTBP has a unique 20 amino acid carboxy terminal extension. Since *Tetrahymena* uses a non-standard genetic code in which TAA and TAG code for glutamine, expression in bacteria of the entire protein without modifications is not possible. To assist in purification of native TBP, polyclonal antibodies have been generated to a 54 amino acid domain within the conserved carboxy terminus fused with the trpE' gene. This antiserum recognizes a 36 Kd protein which is enriched in nuclear fractions. Preliminary results show cross-reactivity with yeast TBP. We are also using site-directed mutagenesis to change the gln/stop codons for over-expression of the whole protein. We hope to ascertain whether genes in *Tetrahymena* truly lack TATA boxes (and whether initiator sequences and factors are the exclusive means of promoter recognition; Smale and Baltimore, Cell 57:103-113) or perhaps, due to the divergence of tetTBP, the TATA box is unusual in sequence and/or position.

R 252 ACTIVATION OF RNA POLYMERASE II TRANSCRIPTION BY THE SPECIFIC DNA-BINDING PROTEIN LSF: INCREASED RATE OF BINDING OF TFIIB, Rebecca Sundseth and Ulla Hansen, Laboratory of Eukaryotic Transcription, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

The multiple overlapping transcriptional elements of the SV40 early and late promoters (enhancers, GC-boxes, etc.) do not include consensus TATA-boxes for either promoter. However, our invitro analysis demonstrates an absolute requirement of the SV40 promoters for the TATA-box binding factor TFIID, for both basal and activated RNA pol II transcription. While TFIID and other components of the initiation complex at an RNA pol II basal promoter have been well characterized, few mechanistic studies have focused on how upstream DNA-binding, transcriptional activators influence protein assembly at the initiation site. Our analysis of basal transcription on both the SV40 and Adenovirus major late promoters demonstrates that two slow steps in initiation of transcription are the assembly of the general transcription factors TFIID and TFIIB onto the template DNA. On the SV40 major late promoter, the rate of initiation complex formation is dramatically increased in the presence of the cellular transcriptional activator LSF. Direct analysis by band mobility shift assays demonstrates that LSF has no effect on the rate of binding, or the stability of TFIID on the promoter, predicting that LSF would not affect the template commitment step. Rather, kinetic analyses demonstrate that LSF reduces the lag in the rate of initiation complex formation attributable to the slow addition of TFIIB, and suggest that LSF increases the rate of association of TFIIB with the committed template. In addition, LSF increases the total number of transcription complexes in long term assays, which is also consistent with LSF increasing the rate of association of TFIIB, where TFIIB is not saturating. These results indicate a mechanism for the activation of the initiation of RNA polymerase II transcription by one upstream activating protein, LSF. This mechanism may also be applicable to other activators that function in cases where limiting concentrations of TFIIB in the cell dictate slow binding of TFIIB.

R 253 INDUCIBLE EXPRESSION OF NONSENSE SUPPRESSOR tRNA GENES *IN VIVO* USING THE *LAC* OPERATOR/REPRESSOR SYSTEM, Dan E. Syroid, Richard I. Tapping and John P. Capone, Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5

The *Escherichia coli lac* operator/repressor system has been used to regulate the expression of a human serine amber suppressor tRNA gene. The insertion of single operator sequences upstream of the gene had no effect on the amount of functional tRNA made *in vivo* as measured by suppression of a nonsense mutation in the *E. coli* chloramphenicol acetyltransferase gene. Inclusion of a plasmid expressing the *lac* repressor in these cotransfections resulted in nearly complete inhibition of suppression activity of the *lac* operator containing constructs but had no effect on wild type tRNA gene expression. The degree of inhibition was enhanced depending on the position of the operator upstream of the gene and through the use of a higher affinity operator site. Inhibition could be quantitatively relieved with the allosteric inducer isopropyl-ß-D-thiogalacto-pyranoside. Similarly, *in vitro* transcription of *lac* operator containing tRNA genes, using Hela cell nuclear extracts, was selectively repressed by the addition of *lac* repressor protein and this inhibition was partially reversible using isopropyl-ß-D-thiogalacto-pyranoside. These results demonstrate for the first time that the *lac* operator/repressor system can be adapted to control the expression of a eukaryotic gene transcribed by RNA polymerase III. This allosterically regulated suppressor tRNA gene has practical applications for the study of mammalian viral and cellular nonsense mutations.

Supported by the Medical Research Council of Canada.

R 254 TRANSCRIPTION FACTOR REQUIREMENT FOR MULTIPLE ROUNDS OF INITIATION BY HUMAN RNA POLYMERASE II, Marilyn Szentirmay and Michèle Sawadogo, Department of Molecular Genetics, U. T. M. D. Anderson Cancer Center, Houston, TX 77030. We have investigated conditons to observe multiple rounds of transcription initiation from the adenovirus major late promoter in an in vitro system derived from HeLa cell nuclear extract. Templates containing G-less cassettes provided a direct assay to discriminate between reinitiated transcripts and transcripts generated by a first round of transcription initiations. When reactions were reconstituted with the previously characterized class II factors TFIIA, TFIIB, TFIID, and TFIIE/F, transcription from the adenovirus major late promoter by human RNA polymerase II was essentially restricted to a single round of initiations. Reinitiations at previously transcribed major late templates required an additional activity, designated RTF (for Reinitiation Transcription Factor). The RTF activity was separated from all other known class II general transcription initiation factors. Partially purified human RTF was found to also promote transcription reinitiations at minimal promoters derived from the human c-myc, histone H4, and hsp70 genes, indicating that the same reinitiation factor may be utilized by many, if not all, genes. We will present evidence for the identity and mechanism of action of RTF.

R 255 CHARACTERIZATION OF cDNAS ENCODING RNA POLYMERASE II INITIATION FACTOR α (TFIIB) FROM RAT LIVER

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RNA polymerase II transcription factor α (TFIIB) from rat liver consists of a 35 kDa polypeptide required for accurate initiation by RNA polymerase II at the core regions of many promoters (Conaway, J. W. *et al.*, 1987, *J. Biol. Chem.* 262, 8293). Studies of promoter selection indicate that α functions in concert with rat liver transcription factor βγ [RAP30/74 (TFIIF)] to promote selective binding of RNA polymerase II to the Initial Complex (Conaway, R. C. *et al.*, 1991, *Proc. Natl. Acad. Sci. U.S.A.*, 88, 6205). A precise role of α in this process, however, has not yet been established. To expedite mechanistic studies of α's function in transcription initiation, we have isolated cDNA clones encoding this factor. Sequence analysis reveals that α is nearly identical to the human transcription factor TFIIB.

R 256 THE ROLE OF NATIVE AND RECOMBINANT TFIID IN BASAL TRANSCRIPTION FROM TATA AND NON-TATA CONTAINING PROMOTERS, Curtis M. Tyree and James T. Kadonaga, Dept. of Biology, University of California, San Diego, La Jolla, CA 92093.

Basal transcription factors are those required with RNA polymerase II for specific transcription from promoters. These general transcription factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIE, TFIIF, TFIIG, TFIIH) have been purified to varying degrees and several have been cloned. We have fractionated a *Drosophila* nuclear extract into four fractions containing TFIIB, TFIID, TFIIE/F, and RNA polymerase II activities. This reconstituted system transcribes a variety of promoters, including the *Drosophila jockey* mobile element which contains an unusual internal pol II promoter. We have replaced the TFIID fraction from embryos with recombinant *Drosophila* TFIID expressed in *E. coli.* in reconstituted *in vitro* transcription assays. We find a much different promoter specificity with the recombinant TFIID compared with the native TFIID fraction. The recombinant TFIID has high activity on the *Drosophila Adh* promoter, but little (<10%) or no activity on either the *Drosophila Krüppel* or *jockey* promoters. We have further fractionated TFIID from *Drosophila* embryos and determined that TFIID is probably not involved in transcription from the *jockey* promoter. We have fractionated a component that stimulates jockey transcription in the absence of TFIID polypeptide. In addition, in the absence of TFIID, we do not see a requirement for the TFIIE/F fraction for transcription of the *jockey* promoter. Experiments will be presented describing requirements for *in vitro* transcription from TATA-box-containing and non-TATA promoters.

R 257 THE INITIATOR ELEMENT DIRECTS THE DOWNSTREAM PROMOTER INTERACTION OF TFIID

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Native, human TFIID has been found to interact with DNA sequences downstream of the transcription initiation site (Sawadogo & Roeder, Cell 43:165, 1985), and this interaction has been suggested to play an essential role in the transcription stimulatory effect observed with transcription factors ATF and GAL4 (Horikoshi et al., Cell 54:665, 1988; *ibid.* 54:1033, 1988). We have investigated the DNA sequence requirements for this downstream promoter interaction on the human hsp70 promoter. Nucleotides between -3 and +5, a region previously identified as the initiator element (Smale et al., PNAS 87:4509, 1990), were found to be essential for this extended mode of binding. Correlations between the mode of TFIID-DNA binding and the levels of transcription, both basal and stimulated, will also be discussed.

R 258 BIOCHEMICAL ANALYSES OF mRNA TRANSCRIPTION IN *SACCHAROMYCES CEREVISIAE*

Paul A. Wade, Susan D. Shaffer, Mark R. Parthun, Michael Woontner and Judith A. Jaehning
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Previous analyses of a whole-cell transcription extract from *Saccharomyces cerevisiae* have demonstrated that the extract faithfully reproduces accurate initiation by RNA polymerase II *in vitro*.[1,2] In addition, the system responds to the presence of activator proteins including GAL4, GAL4-VP16, ACE1 and HSF in a physiologically relevant fashion.[2,3] Simple modifications to these procedures allow the production of an active transcription extract from hundreds of grams of cells suitable for preparative biochemistry. The extract can be fractionated to yield protein pools which lack transcriptional activity on their own but can be combined to reconstitute transcription.

We have utilized this preparative-scale extract, and transcription reconstitution assays, to purify the yeast counterpart of TFIIA to near homogeneity. Purification proceeds via successive chromatography on heparin-Sepharose, DEAE-Sepharose, Mono-Q, phenyl-Superose and Superose-6. TFIIA activity detected in transcription assays coelutes with polypeptides of 44 and 12 kDa. The native protein complex has an apparent molecular weight of 90 kDa based on gel filtration data. The purified complex interacts with purified recombinant TFIID and TATA box containing DNA in mobility shift assays. The composition and properties of the TFIIA purified by the reconstitution assay are indistinguishable from those isolated by TFIID affinity chromatography.[4]

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2. Woontner, M., P. A. Wade, J. Bonner, and J. A. Jaehning, 1991. Mol. Cell. Biol. **11**:4555-4560.
3. Parthun, M.R. and J.A. Jaehning, submitted for publication.
4. Ranish, J.A. and S. Hahn, 1991. J. Biol. Chem. **266**:19320-19327.

R 259 CASSETTE MUTAGENESIS OF REGION 2.3 OF *E.coli* σ^{70}

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We have used a cassette mutagenesis scheme to examine the informational content in a conserved region of the σ^{70} subunit of E. coli RNA polymerase. Region 2.3 of σ^{70} has been predicted by Helmann and Chamberlin to be involved in DNA melting around the startpoint of transcription due to its sequence similiarity with various eukaryotic ribonucleoproteins. These ribonucleoproteins contain two conserved RNA binding domains consisting of two highly conserved sequences. The first of these conserved sequences, termed RNP-1, is an octamer shown here aligned with part of the conserved region 2.3 of σ^{70}:

		Lys		Tyr Ala Tyr			Tyr
RNP-1 CS		Arg-	Gly-	Phe-	Gly-	Phe-	Val- X -Phe
σ^{70}		Arg-	Gly-	Tyr-	Lys-	Phe-	Ser- Thr- Tyr

The cassette mutagenesis technique consists of simultaneously randomizing a stretch of 3-7 amino acids by oligonuceotide cassette mutagenesis, screening for functional proteins and sequencing *rpoD* (σ^{70}) to determine the spectrum of allowed substitutions at each position.

Our results show most of the residues corresponding to the RNP-1 octamer can tolerate a wide variety of amino acid substitutions. Threonine is an exception and the only change at this position which yielded a functional protein was a serine substitution which retained only partial activity. These results suggest that if region 2.3 is involved in DNA melting, the mechanism is probably unlike single strand nucleic acid binding by ribonucleoproteins. Also, the conserved threonine residue in region 2.3 is important for protein structure or function.

R 260 PURIFICATION, CLONING, AND CHARACTERIZATION OF DROSOPHILA TFIIB, Sharon L. Wampler and James T. Kadonaga, Department of Biology, University of California, San Diego, La Jolla, CA 92093

Basal transcription of class II genes relies upon the formation of an initiation complex comprising RNA polymerase II and several auxiliary factors. To understand regulated transcription and gene activation, it is important to unravel the mechanisms involved in basal transcription. We have subdivided the components of the basic RNA polymerase II machinery into RNA polymerase II and three fractions containing transcription factors (TF) IIB, IID, and IIE/F. These fractions can be individually substituted with purified TFIIB, TFIID, and TFIIE recombinant proteins, respectively. More extensive purification of the TFIIB fraction yields a 34kD protein that correlates with transcriptional activity. In order to study Drosophila (d)TFIIB, we have isolated a cDNA clone encoding this initiation factor. The 1.5 kb cDNA encodes a 315 amino acid protein which is 80% identical to the human TFIIB sequence (Ha, I. et al. Nature 352, 689-695). In addition, we have isolated a genomic clone and will be analyzing the promoter region of dTFIIB. Recent results will be presented.

R 261 TRANSCRIPTIONAL ANALYSIS OF VACUOLAR MEMBRANE ATPASE GENES OF *NEUROSPORA CRASSA*

M.A. Wechser, E.J. Bowman, and B.J. Bowman, Department of Biology, University of California, Santa Cruz, CA 95064

The *vma* genes of *Neurospora crassa* encode subunits of the vacuolar membrane ATPase. This is a member of the family of V-type ATPases which are located on membranes of acidic compartments in eukaryotes or on the plasma membranes of archaebacteria. The V-ATPases are complex enzymes, composed of at least nine different polypeptides.

We are studying the expression of the *vma* genes, and wish to see how the stoichiometry of their products is established. To do this we are analyzing the transcription of these suspected housekeeping genes. The chromosomal locations of three *vma* genes have been determined. Two of them are on the same chromosome, but at least 20 kb apart. A third gene is on a different chromosome. The putative promoter regions of these genes have been isolated, sequenced, and their transcription initiation sites identified. The promoters for these genes do not appear to contain TATA sequences, but have a conserved octamer near the transcription initiation sites. Northern analysis indicates that the relative steady-state mRNA levels for the *vma* genes parallels the levels of their products in the enzyme. This suggests a transcriptional role in the stoichiometric expression of the unlinked genes. Promoter deletion analysis is being used to identify the cis-acting sequences which permit transcription of these genes. The *vma* promoters are also able to function in *Saccharomyces cerevisiae*, indicating that there is conservation of these promoters and the transcriptional machinery that recognizes them.

R 262 TWO PROTEIN-BINDING ELEMENTS INVOLVED IN TRANSCRIPTION FROM A TATA-LESS PROMOTER, Lisa Weis, Jose Perez, Kyoungsook Park, Michael Atchison, and Danny Reinberg, Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635.

Previous studies in our laboratory have shown that RNA polymerase II weakly recognizes the initiator element present in the adenovirus major late, IVa2 and TDT promoters. Together with TFIID, TFIIB, and TFIIF, RNA polymerase II forms a stable, transcriptionally competent complex on these initiators. There appear to be different families of Inr; consequently, we are extending our studies of how transcription competent complexes are formed on non-TATA promoters containing different initiator DNA elements (Inr). The promoter of the human DNA polymerase beta gene is relatively simple, containing one upstream element which is recognized by factors of the ATF family of proteins and three sequences matching Sp1 binding sites. Two additional transcriptional elements were identified through mutational analysis; one, located between -2 to +6, encompasses the initiator element, and the other is located downstream, from +26 to +31. Mutations in either region affect transcription *in vitro* and *in vivo*. the initiator element binds a protein, the initiator transcription factor (ITF), which appears to be identical to NF-E1, δ, and YY1. The downstream element is recognized by another DNA binding protein, LBP-1 in directing the formation of a transcription competent complex on the β-DNA polymerase promoter is currently under study.

R 263 DNA SUPERCOILING RESPONSE OF THE σ^{54}-DEPENDENT *nifL* PROMOTER. Simon K. Whitehall and Ray A. Dixon. AFRC-IPSR Nitrogen Fixation Laboratory, University of Sussex, Brighton BN1 9RQ, U.K.

Transcription from the σ^{54}-dependent *Klebsiella pneumoniae nifL* and *glnAp2* promoters is activated by the general nitrogen regulatory protein NTRC. Unlike the *glnAp2* promoter, transcription from *nifL in vitro* in a chloride-based buffer is supercoiling-dependent. The replacement of chloride with an acetate-based buffer decreases the stringency of the *nifL* supercoiling response but open complexes formed on linear *nifL* promoter DNA under these conditions are unstable and less extensive than those found on supercoiled DNA (form 1). We have introduced mutations in particular elements of the *nifL* promoter which increase its homology to *glnAp2*. At the wild-type *nifL* promoter, σ^{54}-RNA polymerase makes only limited contacts with the promoter in the absence of NTRC. However a G to T change at -26 (*nifL74*) allows the formation of a stable closed complex with σ^{54}-holoenzyme on both linear and form 1 templates in the absence of the activator. The combination of C to T mutations at -3 and -1 (*nifL18*) increases the A-T rich nature of the melted region and stabilises open complexes formed on linear DNA. Open complex formation as a function of superhelical density was assessed at each promoter. At the wild-type *nifL* promoter, open complex formation peaks at -0.067 and is not detectable at superhelical densities less than -0.032. Both the *nifL74* and *nifL18* mutations altered the supercoiling response, increasing the ability to form open complexes at low superhelical densities. The presence of *nifL74* and *nifL18* mutations in combination further altered the response of the promoter to DNA supercoiling. This suggests that the promoter as a whole, and not any one promoter element, mediates the transcriptional response to DNA supercoiling.

R 264 FUNCTIONAL BINDING OF THE TATA BOX BINDING COMPONENT OF TFIID TO THE -30 REGION OF TATA-LESS PROMOTERS, Steven Wiley, Richard Kraus, and Janet E. Mertz, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

Many viral and cellular promoters transcribed in higher eukaryotes by RNA polymerase II lack obvious AT-rich sequences, called TATA boxes, that bind the transcription factor TFIID. One such TATA-less promoter, the SV40 major late promoter (SV40-MLP), contains a genetically important sequence element 30 bp upstream of its transcription initiation site that has no obvious sequence similarity to a TATA box. We show here that the cloned human TATA box-binding protein functionally binds to this upstream sequence element, although with an affinity one-sixth of that to which it binds the adenovirus type 2 major late promoter's TATA box. Analysis of point mutations in the SV40-MLP's -30 element shows that the affinity of binding correlates with the efficiency of transcription from this promoter. Furthermore, this element has genetic properties identical to those of a TATA box: (i) it directs polymerase to initiate transcription approximately 30 bp downstream of its location; and (ii) inactivation of this element results in increased heterogeneity in the sites of transcription initiation. All TATA-less promoters tested were found to contain a sequence approximately 30 bp upstream of their major transcription initiation site to which the cloned TATA box binding component of TFIID binds. We conclude that many, if not all TATA-less promoters differ from TATA box-containing ones simply in the affinity of their -30 regions for binding of TFIID, with functional binding of TFIID being determined in part by other nearby sequence elements of the promoter. in the formation of a transcription initiation complex at nt 325. Whether binding of proteins that interact with the genetically important downstream and initiation site sequence elements (Mertz et al., these abstracts) stabilizes binding of TFIID to the -30 region element remains to be determined.

R 265 TRANSCRIPTIONAL ACTIVATION OF RNA POLYMERASE III, Ian Willis, Gerald Rameau and Alfredo López-De-León, Biochemistry Department, Albert Einstein College of Medicine, Bronx, NY 10461

Through a genetic selection our laboratory has isolated 3 dominant mutant genes in *Saccharomyces cerevisiae* that display increased transcription, *in vivo* and *in vitro*, by RNA polymerase III (pol III). The three genes have been designated *PCF1*, *PCF2* and *PCF4* for Polymerase C Factors 1, 2 and 4, respectively. *In vitro* studies have shown that whole cell extracts from all 3 mutant strains activate pol III transcription from 3 to 13-fold relative to wild type. None of the *PCF* mutations is allele specific; transcription is increased for all pol III genes tested. *PCF1-1* exerts its effects at two stages during the transcription process. The mutation increases the rate of pre-initiation complex formation as well as the number of these complexes that are transcriptionally active. Fractionation of *PCF1-1* extracts has shown that the mutant activity copurifies with the biochemically defined fraction TFIIIB. We propose that the *PCF1* gene encodes one of the two polypeptides believed to comprise the transcription factor IIIB. In contrast to *PCF1-1*, transcriptional activation in *PCF2-1* and *PCF4-1* extracts results simply from an increase in the number of transcriptionally active pre-initiation complexes. Preincubation of a gene with an excess amount of purified wild type TFIIIC blocks transcriptional activation by *PCF2-1* and *PCF4-1*. Other experiments have shown that the mutant activities in *PCF2-1* and *PCF4-1* extracts are present in limiting quantities and are required in stoichiometric amounts for transcription initiation. Present data suggests that the factors encoded by the *PCF2-1* and *PCF4-1* genes are subunits or regulators of the transcription factor IIIC.

R 266 VACCINIA VIRUS LATE TRANSCRIPTION FACTOR, Cynthia F. Wright[1], James G. Keck[2], and Bernard Moss[2]. [1]Dept. of Cellular Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306 and [2]Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20892. An in vitro transcription system has been developed from extracts of vaccinia virus-infected cells which can accurately transcribe vaccinia virus late gene templates. This system was fractionated by chromatography on phosphocellulose into three components, all of which were necessary for maximal late transcription. Using a transcription complementation assay, one component of the phosphocellulose flow-through was partially purified and designated VLTF-1. Antibodies raised against VLTF-1 immunoprecipitated the protein product of a vaccinia virus gene identified by genetic experiments as being necessary for late transcription in vivo and having the potential to code for a 30 kDa protein. Also, antibodies to the 30-kDa recombinant product of this gene reacted on Western blots with a protein of 30 kDa that cochromatographed and cosedimented with VLTF-1 activity. Taken together, these experiments identified the vaccinia virus gene encoding VLTF-1. This transcription factor has now been overexpressed using the vaccinia virus/T7 hybrid expression system and further purified by column chromatography and glycerol gradient sedimentation. Its role in vaccinia virus late transcription is currently being investigated.

R 267 PURIFICATION AND CHARACTERIZATION OF RNA POLYMERASE I TRANSCRIPTION INITIATION FACTORS FROM *Acanthamoeba castellanii*. Qin Yang, Catherine Radebaugh, and Marvin Paule. Department of Biochemistry, Colorado State University, Fort Collins, CO 80523

The specific initiation of rRNA transcription catalyzed by RNA polymerase I (pol I) in *Acanthamoeba castellanii* requires two protein factors designated TIF-IB and SBF. TIF-IB has been purified to near homogeneity by sequential anion exchange, cation exchange, and oligo-affinity chromatography followed by rate zonal centrifugation. The TIF-IB factor consists of a 145 kD subunit which is thought to dimerize to form the 289 kD native initiation factor.

SBF has been partially purified and completely separated from TIF-IB by standard chromatography methodology. The SBF protein binds to 140 bp repeat elements in the rRNA intergenic spacer and is thought to be a homolog of vertebrate UBF. Analogous to higher eukaryotic pol I systems, these spacer repeat elements have been shown to cause both *trans*-competition and *cis*-enhancement in an *in vitro* transcription system.

Using gel retardation and DNase I footprint analysis we have demonstrated that both TIF-IB and SBF are required to form the committed initiation complex. Purified TIF-IB requires the addition of SBF to bind to the core promoter element.

R 268 FUNCTIONAL DISSECTION OF A DROSOPHILA U1 snRNA GENE PROMOTER IN VITRO, Zulkeflie Zamrod and William E. Stumph, Department of Chemistry and Molecular Biology Institute, San Diego State University, San Diego, CA 92182.

Small nuclear RNAs of the U family (snRNA U1, U2, etc.) are involved in various types of RNA processing in eukaryotes. Transcription of snRNA genes is carried out by RNA polymerase II (with the exception of U6), but initiation elements specific to snRNA genes are involved. For example, the transcription initiation site of vertebrate snRNA genes is determined by a unique element, termed the PSE, located about 50 to 60 bp upstream of the cap site. Compared to vertebrates, there is much less known about the identity and function of promoter elements of invertebrate snRNA genes. Sequence comparisons of the 5'-flanking regions of several Drosophila snRNA genes (U1, U2, and U4) revealed the presence of two highly conserved sequence elements. The most proximal element is located at approximately positions -24 to -32; we will refer to it as Promoter Element Box 1 (PEB1). The second element, PEB2, is a 21 bp sequence stretching from about position -41 to -61. We have constructed deletions and scanning mutations in the 5'-flanking DNA of a Drosophila U1 gene and analysed the effects of the mutations on transcription in vitro. Deletion of sequences located 390 to 126 bp upstream of the cap site did not significantly affect Drosophila U1 transcription in vitro. When sequences were deleted to position -72, transcription was reduced approximately 2.5 fold. Scanning mutations within PEB1 and PEB2 reduced transcription in vitro approximately 10 fold and 20 fold respectively. Moreover, the conserved spacing between PEB1 and PEB2 was required for efficient promoter utilization. These studies provide evidence that, as for vertebrate snRNA genes, unique promoter elements are required for Drosophila snRNA gene transcription.

Structure and Function of RNA Polymerases; Regulatory Mechanisms

R 300 THE HUMAN HEAT SHOCK PROTEIN HSP70 INTERACTS WITH ITS OWN TRANSCRIPTIONAL REGULATOR, THE HEAT SHOCK TRANSCRIPTION FACTOR (HSF), Klara Abravaya, Shawn Murphy, Mike Myers, Kevin Sarge, and Richard Morimoto, Department of Biochemistry, Molecular and Cell Biology, Northwestern University, Evanston IL 60208

The rapid transcriptional induction of heat shock genes upon thermal stress is mediated by a heat shock transcription factor (HSF). HSF is present in non-heat shocked cells and is activated to bind to its target sequence, the heat shock element (HSE), upon heat shock. At moderate heat shock temperature (42C), the transcriptional induction of the human hsp70 gene is transient, peaking an hour after heat shock and then attenuating. We have previously demonstrated, by in vivo genomic footprinting, that transcriptional activation is mediated by the binding of HSF to HSE and the subsequent attenuation by the release of HSF from HSE. After an hour of heat shock, despite continued exposure of cells to the elevated temperature, HSF activity diminishes. An attractive hypothesis is that hsp70 itself, which accumulates during heat shock, may be involved in an autoregulatory loop by preventing the activation or contributing to the deactivation of HSF. To test this hypothesis we investigated whether hsp70 interacts with HSF. Using anti-hsp70 antibodies, hsp70/HSF complexes were detected at later times of heat shock, when hsp70 accumulates in the cell. Consistent with the current model of 70 kD stress protein function, hsp70 could be dissociated from the complex by ATP. We also demonstrated that in cells lacking the complex (early times of heat shock), the complex could be reconstituted by the addition of purified hsp70 protein. Deletion mutants of the hsp70 protein could not complex with HSF. Finally we have shown that addition of the hsp70 protein inhibits the activation of HSF in vitro.

R 301 **RNA POLYMERASE-ASSOCIATED TRANSCRIPTION SPECIFICITY FACTOR ENCODED BY VACCINIA VIRUS.**

Byung-Yoon Ahn and Bernard Moss. National Institute of Allergy and infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, U.S.A.

Vaccinia virus encodes a eukaryotic-like multisubunit DNA-dependent RNA polymerase and stage-specific transcription factors. The RNA polymerase packaged in the virion was found to contain a submolar polypeptide of 92 kDa in addition to the core subunits which consist of 2 larger and 8 smaller polypeptides. The RNA polymerase containing the 92 kDa polypeptide was separated from the core enzyme by column chromatography and tested for specific and non-specific transcription in vitro. Both forms of RNA polymerase transcribed random single-stranded DNA templates. However, only the form with the 92 kDa polypeptide exhibited specific transcription, in conjunction with the early transcription factor, upon a double-stranded DNA template containing a viral early promoter. The viral gene encoding the 92 kDa polypeptide was identified and its role in the specific transcription was confirmed by gene transfection experiments.

R 302 DISTINCT CONFORMATIONAL CHANGES INDUCED BY HORMONE AND ANTIHORMONE ARE THE PRIMARY STEP IN PROGESTERONE RECEPTOR ACTIVATION, George F. Allan, Sophia Y. Tsai, Bert W. O'Malley and Ming-Jer Tsai, Dept. of Cell Biology, Baylor College of Medicine, Houston TX 77030

Steroid hormone receptors are ligand-induced transcription factors which have potent effects on gene expression *in vivo*. Antihormones, typified by the abortifacient Ru38486, also bind to steroid receptors and thereby antagonize the positive transcriptional effects of steroid hormones. The molecular basis for the action of antihormones is not understood. The human progesterone receptor translated *in vitro* binds to its DNA response element in a ligand-dependent manner. Partial proteolytic analysis of [^{35}S]methionine-labeled receptor revealed the presence of a 30 kilodalton protease-resistant fragment following progesterone binding. A distinct 27 kilodalton fragment was produced following digestion of Ru38486-bound receptor. Immunoprecipitation with monoclonal antibodies directed against different regions of the progesterone receptor showed that both resistant fragments were derived from the carboxy-terminal ligand-binding domain. The antihormone-specific fragment differed from the hormone-specific fragment by truncation from the carboxy-terminus of the domain. The conformational change could be induced with the heat shock proteins bound, and was not induced by the removal of heat shock proteins by ligand-independent means. Thus hormone and antihormone induce the formation of a more compact, tightly folded ligand binding domain, and this structural change may trigger heat shock protein dissociation, dimerization and DNA binding. The antihormone-induced change leaves a region in the carboxy-terminal tail exposed to the solution which is hidden following hormone binding. This structural difference may be the mechanistic basis for the antagonistic activity of antihormones.

R 303 VACCINIA VIRUS ENCODED MULTISUBUNIT DNA-DEPENDENT RNA POLYMERASE.

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Vaccinia virus encodes a DNA-dependent RNA polymerase with an apparent molecular mass of about 500 kDa that structurally and functionally resembles eukaryotic RNA polymerase II. The purified RNA polymerase from either virions or infected cells is a multisubunit enzyme composed of two large and eight small subunits forming a core enzyme capable of non-specific transcription of single-stranded DNA templates. All the subunits of the enzyme are virus encoded, and the genes encoding these subunits have now been identified and characterized. Three of the vaccinia virus RNA polymerase subunits (RPO147, RPO132, and RPO7) show sequence homology to the corresponding eukaryotic subunits. The three subunits have the conserved zinc finger and metal-binding motifs found in their putative homologs and may perform similar or identical functions. Another vaccinia virus encoded subunit, RPO30 shows sequence homology to eukaryotic transcription elongation factor SII. RPO30 exists in two forms resulting from initiation at different methionine codons. Two forms of RPO19 also exist. All of the core subunit genes are expressed at early time after infection, and in addition some of the genes are also expressed at late times after infection.

R 304 **OPERATOR UNTWISTING BY Hg-MERR IS A CENTRAL STEP IN THE TRANSCRIPTIONAL ACTIVATION AT THE merTPAD PROMOTER**, Aseem Z. Ansari & Thomas V. O'Halloran, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, 2153 Sheridan Rd, Evanston, IL 60208. MerR binds the mer operator which is located between the -10 and the -35 regions of the RNA polymerase binding sites of the merTPAD (P_T) promoter. In this novel topological complex MerR represses transcription from the P_T promoter and in the presence of mercury it activates transcription while bound to the same site. DNA structure sensitive chemical nuclease reactivities suggested that the activator form of Mer (Hg-MerR) distorts the centre of the operator. Topological characterization of the protein induced distortion indicates that the activator form of MerR (Hg-MerR) untwists the operator by ~33° relative to repressor form of MerR. The -10 and -35 polymerase binding sites are spaced 19 bp apart in the P_T promoter as opposed to 17 ± 1 bp in consensus E.coli promoters. Thus untwisting the spacer, which is the mer operator, would realign the the phase of the polymerase binding sites such that they would resemble a promoter with an ~18 bp spacer. This would facilitate transcription from the P_T promoter. This novel mechanism of transcriptional activation is corroborated by the evidence that MerR mutants that activate transcription in the absence of mercury also distort the mer operator as detected by hyperreactive cleavage by Cu-phenyl phenanthroline.

R 305 FACTORS DETERMINING INITIATION OF TRANSCRIPTION FROM THE HUMAN β-GLOBIN GENE.
Michael Antoniou, Ernie deBoer, Eugenia Spanopoulou and Frank Grosveld, Laboratory of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA. UK.
DNA/protein binding studies in vitro revealed several factors interacting over the TATA box and transcriptional start regions of the human β-globin gene. These included TFIID, initiator protein, GATA-1, SP-1 and other previously uncharacterized proteins. In order to assess the role of these factors in initiation of transcription, β-globin genes were constructed containing point mutants in their promoter which specifically abolish binding of one or more proteins. These β-genes were then linked to the locus control region (LCR) of the β-globin locus to provide quantitative, gene copy dependent expression when stably transfected into murine erythroleukaemia (MEL) cells. Expression analysis in MEL cells revealed that TFIID was the only factor required at the TATA box. More interestingly, a novel factor binding over the cap site was also needed for full efficiency and correct initiation of transcription.

R 306 CHARACTERISATION OF THE ATPASE ACTIVITY OF A BACTERIAL TRANSCRIPTIONAL ACTIVATOR
Sara Austin & Ray Dixon, AFRC Nitrogen Fixation Laboratory, University of Sussex, Brighton BN1 9RQ, UK.

The nitrogen regulatory protein NTRC is a member of a family of proteins which activate transcription at promoters recognised by core RNA polymerase associated with an alternative sigma factor σ^{54} ($E\sigma^{54}$). The activity of NTRC is controlled by phosphorylation in response to changes in the levels of fixed nitrogen. Only phosphorylated NTRC will activate transcription. ATP is required for NTRC to catalyse isomerisation of closed complexes with $E\sigma^{54}$ and the promoter DNA to transcriptionally active open complexes. The presence of a putative nucleotide binding site in the conserved central domain of NTRC and other σ^{54}-dependent activator proteins suggests a mechanism in which ATP hydrolysis is required for the isomerisation of closed to open complexes by these proteins.

Using highly purified NTRC protein from Klebsiella pneumoniae we have characterised the ATPase activity of the wild-type protein and a mutant NTRC (S160F) which does not require phosphorylation for transcriptional activity. We have defined the conditions under which the proteins hydrolyse ATP and demonstrated that S160F NTRC has an intrinsic ATPase activity in the absence of phosphorylation while wild-type NTRC requires phosphorylation to hydrolyse ATP. We have investigated the effects of phosphorylation and other factors, in particular DNA and $E\sigma^{54}$, on the ability of both proteins to hydrolyse ATP.

R 307 TRANSCRIPTION OF HEAT SHOCK GENES IN *CLOSTRIDIUM ACETOBUTYLICUM*: NO EVIDENCE FOR THE INVOLVEMENT OF AN ALTERNATE SIGMA FACTOR,
Hubert Bahl, Franz Narberhaus, and Andreas Pich, Institut für Mikrobiologie, Georg-August-Universität Göttingen, W-3400 Göttingen, FRG
During growth, *Clostridium acetobutylicum*, a Gram-positive strictly anaerobic sporeformer, first produces acids and later switches to a solventogenic phase. This metabolic shift is connected with sporulation and the heat shock response. To elucidate the regulation of heat shock gene expression in this organism, we have determined promoter regions of heat shock genes and characterized the DNA-dependent RNA polymerase from heat shocked cells.
Two heat shock operons of *C. acetobutylicum* (*dnaK* and *groELS* loci) were cloned and sequenced. The transcription start sites were determined by primer extension analysis. The similarity of the deduced promoter regions to consensus sequences for vegetative sigma factors in Gram-positve bacteria and σ^{70} of *Escherichia coli* and the absence of additional start sites after heat shock suggest that an alternate sigma factor is not involved in the regulation of the heat shock response in *C. acetobutylicum*. However, an 11-bp inverted repeat between the transcription and translation start sites may play an important role in the expression of these genes. Almost identical sequences are present in front of heat shock genes of other bacteria. Interestingly, the RNA polymerase of heat shocked cells of *C. acetobutylicum* contained an additional protein, which influenced the activity of the holoenzyme *in vitro* but did not function as a sigma factor. It is possible that this protein interacts with the hairpin loop structure described above in order to regulate the expression of heat shock genes in *C. acetobutylicum*.

R 308 A PROTEIN-KINASE WHICH POTENTIALLY PHOSPHORYLATES THE C-TERMINAL DOMAIN (CTD) OF EUKARYOTIC RNA POLYMERASE II IS ACTIVATED DURING HEAT-SHOCK. Olivier **Bensaude**, Marie Françoise **Dubois**, Département de Biologie, Ecole Normale Supérieure, 46 rue d'Ulm, Paris 75230 Cedex.

The C-terminal domain (CTD) of the eukaryotic RNA polymerase II largest subunit consists of multiple repeats of the heptapeptide motif Tyr-Ser-Pro-Thr-Ser-Pro-Ser. In lysates from heat-shocked mammalian cells, we have found an increased activity of a protein-kinase, which phosphorylates a synthetic peptide (hepta-4) made of four repeats of this motif. This activity increases within a few minutes of stress even in the absence of protein synthesis. The mammalian $p34^{cdc2}$ kinase does not seem to be involved.

In order to establish that a CTD kinase was activated during heat-shock, the phosphorylation of a β-galactosidase/CTD fusion protein was examined. The fusion protein was detected by Western blot and its apparent molecular weight was increased upon phosphorylation. This fusion protein was found to be hyperphosphorylated during stress in parallel with the hepta-4 kinase activation. Thus, a CTD-kinase was indeed activated by stress within cells although RNA polymerase II could be dephosphorylated during the same period.

R 309 RETINOIC ACID DEPENDENT TRANSCRIPTION IN VIVO IS MEDIATED THROUGH TFIID CORE AND REQUIRE AN EC CELL SPECIFIC FACTOR, Anders Berkenstam, Maria del Mar Vivanco-Ruiz, Masami Horikoshi*, Domingo Barettino and Hendrik Stunnenberg, EMBL, 6900 Heidelberg, Germany. *Laboratory of Biochemistry and Molecular Biology, Rockefeller University, New York, NY 10021

The effect of retinoic acid (RA) on patterns of gene expression is believed to be mediated by three different retinoic acid receptor (RAR) subtypes RAR-α, -β and γ. RA induces the differentiation of certain cell lines in vitro, such as the P19 line of emryonal carcinoma (EC) cells. Expression of the RAR-β2 gene occurs as an early event in RA induced cell differentiation and RAR-β2 is the predominant isoform of RAR-β which is expressed in response to RA in EC cells. We show that RAR and recombinant TFIID cooperate to activate the RAR-β2 promoter in vivo when expressed in EC cells. Functional interaction by RAR and TFIID is strictly dependent on their respective binding sites within the RAR-β2 promoter and the presence of RA. The core domain of human TFIID is shown to be sufficient to mediate activation of transcription by RAR. Drosophila but not yeast TFIID can substitute for recombinant human TFIID in trans-activation. Additional factors appear to be required for the EC cell specific activation of the RAR-β2 gene; these may include TFIID associated polypeptides. We will present evidence that RAR, following activation by RA functionally interacts with TFIID via an EC cell specific factor.

R 310 ACTIVATION OF THE EPITHELIAL SPECIFICITY OF THE HUMAN PAPILLOMAVIRUS-16 ENHANCER BY UBIQUITOUS TRANSCRIPTION FACTORS. Hans-Ulrich Bernard, Doris Apt, Bernd Gloss, Merlina Isa and Terence Chong. Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511.

The enhancer of human papillomavirus-16 is active in all analyzed epithelial cell lines. These include some, which do not represent cells that are infected under natural conditions, like those derived from cutaneous epithelia, and cells which are of non-human origin. The enhancer is inactive in all other cell lines tested, in particular in human fibroblasts. Transcriptional specificity may thus contribute to the epitheliotropism, although not to the species specificity of HPV-16. Enhancer activation occurs through 7 binding sites for NFI, 3 for AP-1, 1 for oct-1/NFA, 1-3 for TEF-2, 1 for the glucocorticoid/progesteron receptors, and possibly through some not yet characterized factors. NFI, although binding in an ubiquitous manner, plays a central function in epithelial specific activation. This is suggested by the dramatic loss of function of individual NFI mutations as well as by the epithelial specificity of non-overlapping subclones of the enhancer, that seem to share no common cis-responsive element but NFI binding sites. Our research aims toward the identification of epithelial specific variations or co-factors of NFI, and for the specificity of cooperative interactions of NFI with heterologous transcription factors.

R 311 TAT TRANSACTIVATES HUMAN IMMUNODEFICIENCY TRANSCRIPTION IN VITRO BY STIMULATING INITIATION AND ELONGATION, Cindy A. Bohan[1], Fatah Kashanchi[1], Barbara Ensoli[2], Luigi Buonaguro[2], Kathy Boris-Lawrie[2] and John Brady[1,2], [1]Laboratory of Molecular Virology, NCI/NIH; [2]Laboratory of Tumor Cell Biology, NCI/NIH, Bethesda, Maryland 20892.

Human immunodeficiency virus type 1 (HIV-1) gene expression is regulated by the transactivating protein Tat. We demonstrate that purified Tat transactivates HIV-1 transcription (25-60 fold) *in vitro* in Hela whole-cell extracts and in a reconstituted transcription system containing partially purified general transcription factors and RNA polymerase II. Specificity of Tat transactivation *in vitro* is indicated by antibody inhibition experiments and cotranscription assays. Transcription is blocked at various functional steps during preinitiation and productive initiation complex formation on HIV-1 templates by sarkosyl. The resistance of Tat transactivated HIV-1 transcription to 0.015% sarkosyl in the preincubation mix and prior to productive transcription initiation suggests that Tat facilitates preinitiation complex assembly. Kinetic analysis of Tat transactivation suggests that Tat preinitiation complexes are efficiently converted from transcription initiation to elongation complexes. Our results demonstrate that Tat facilitates transcription preinitiation complex formation in the absence of RNA synthesis, supporting a novel function for Tat that is independent of TAR RNA, and increases the efficiency of elongation after productive initiation of HIV-1 transcription.

R 312 INSIGHTS INTO TRANSCRIPTIONAL REGULATION OF THE C-FOS PROTO-ONCOGENE: BINDING OF TWO DISTINCT TRANSCRIPTION FACTORS IS NECESSARY FOR MAXIMAL SERUM INDUCTION, Amy M. Boulden and Dr. Linda J. Sealy, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232

Transcription of the c-fos proto-oncogene is up-regulated by a wide variety of mitogenic stimuli including individual growth factors, hormones, cAMP, and serum. Serum induction of the c-fos gene is mediated through the serum response element (SRE) to which a sequence-specific transcription factor, the serum response factor (SRF), binds. The mechanism by which SRF effects mitogenic induction is unclear; *in vitro* binding activity, occupancy of the c-fos promoter *in vivo*, and post-translational modification of the protein remain unchanged after serum stimulation. Recently, we have identified an additional SRE-binding protein, SRE-BP, by gel mobility shift analysis. Site-directed mutagenesis and methylation interference have localized the binding site for this novel factor immediately 3' to nucleotides essential for SRF binding. Transient transfection analyses suggest a functional relevance for this binding activity *in vivo*; constructs which mutate the SRE-BP binding element display much lower serum induction than the wildtype element. Conversely, addition of an SRE-BP binding element to a minimal SRF-binding motif increases serum induction 400%, to a level equivalent to that mediated by the wildtype c-fos SRE. We propose, therefore, that maximal serum induction is critically dependent on binding of both SRF and SRE-BP, and are currently undertaking studies to define the interaction of these two proteins.

R 313 ISOLATION AND CHARACTERIZATION OF THE MAJOR VEGETATIVE RNA POLYMERASE OF *STREPTOMYCES COELICOLOR* A3(2); RENATURATION OF A SIGMA SUBUNIT USING GroEL, [1]Mark J. Buttner, [1]Kelly L. Brown and [2]Steven Wood, [1]John Innes Institute, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK and [2]Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, UK

The promoter region of the *dagA* gene is complex; it consists of four distinct promoters with different -10 and -35 regions. We report the isolation of a form of RNA polymerase that mediates transcription *in vitro* from the *dagA*p4 promoter. The core components of this RNA polymerase in association with a polypeptide of ca. 66-kDa direct transcription from the *dagA*p4 promoter; holoenzyme reconstitution experiments show that the 66-kDa polypeptide functions as a sigma factor to direct transcription from both the *dagA*p4 and *veg* promoters *in vitro*. Alignment of the DNA sequences of these two promoters shows they have bases in common at the -10 and -35 regions and these sequences are similar to those observed for the major RNA polymerases of other bacteria. N-terminal amino acid sequence analysis of the 66-kDa polypeptide revealed it to be the product of the *hrdB* gene. Previous experiments showed that the predicted amino acid sequence of the *hrdB* product is very similar to major sigma subunits of other bacteria and that disruption of the *hrdB* gene is lethal. These observations together lead to the conclusion that we have isolated the major RNA polymerase of *Streptomyces coelicolor* A3(2).

We have developed an improved protocol for the renaturation of sigma factors that have been isolated by preparative SDS-polyacrylamide gel electrophoresis. This method involves renaturing the polypeptide in the presence of the bacterial chaperonin GroEL. We expect this protocol to find general application for renaturing other polypeptides that have been subjected to SDS-polyacrylamide gel electrophoresis.

R 314 Two additional common subunits, ABC10α and ABC10β, are shared by the three forms of yeast RNA polymerase, Christophe Carles[1], Isabelle Treich[1], Françoise Bouet[2], Michel Riva[1], and André Sentenac[1], 1; Service de Biochimie et de Génétique Moléculaire, DBCM, 2; Laboratoire d'Ingénierie Chimique et de Marquage des Protéines, DIEP , Centre d'Etudes de Saclay, F 91191 Gif sur Yvette cedex, France

Yeast RNA polymerases are multisubunit enzymes that contain in common some small subunits. We showed that the smallest 10 kDa component of the three forms of enzyme (A10, B10, C10) is heterogeneous. In each case, it could be resolved into two distinct polypeptides (α and β) by reverse-phase chromatography. A10α, B10α, and C10α were indistinguishable on the basis of their electrophoretic and chromatographic behaviour, zinc binding ability and characteristic silver-staining. Furthermore, the tryptic digestion pattern of the three polypeptides is identical. Microsequencing of tryptic fragments demonstrated that ABC10α is a novel subunit, shared by the three forms of enzyme. Similarly, A10β, B10β, and C10β were indistinguishable. The aminoterminal sequence of A10β and C10β corresponded to that of B10β cloned by Woychik and Young (1990) *J. Biol. Chem.* **265**, 17816-17819. The gene encoding ABC10α, RPC10, was cloned and sequenced. RPC10 is unique, mapped on chromosome VIII, and is essential for cell viability. It codes for a very basic protein of 70 aminoacids which contains a zinc binding domain that could explain the zinc binding observed *in vitro*.

R 315 AFFINITY LABELING AND CHARACTERIZATION BY CLASS SPECIFIC ANTISERA OF SEVERAL *TRYPANOSOMA BRUCEI* RNA POLYMERASE SUBUNITS, Alger Chapman and Nina Agabian, Departments of Pediatrics and Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA 94143.

Trypanosomes exhibit several novel mechanisms of gene processing, including trans splicing, polycistronic transcription, and RNA editing. In addition, transcription of the major surface proteins of *T. brucei* is resistant to α-amanitin leading to the proposal that these protein coding genes are transcribed by RNA polymerase I. To further characterize transcription in *T. brucei*, we have developed reagents to study the subunit composition of trypanosomal RNA polymerases. The RNA polymerases were affinity labeled by UV crosslinking newly incorporated radioactive RNAs using the photoactivatible nucleotide 4-thio-UTP. The labeled polymerase subunits were then visualized on a denaturing protein gel. Antibodies were raised against fusion proteins derived from the cloned trypanosomal RNA polymerase major subunits. These antisera were used to verify by Western analysis the identity of the largest radiolabeled polymerase subunits. One of these antisera effectively immunoprecipitated RNA polymerase II from an ^{35}S-methionine labeled nuclear extract. Analysis of the precipitated subunits suggests that despite the unusual polycistronic transcription in trypanosomes, the *T. brucei* RNA polymerase II has a subunit structure similar to that seen in other eukaryotic cells. Studies are currently underway to identify which affinity labeled polymerase is crosslinked to the amanitin resistant surface protein genes.

R 316 THE EFFECT OF PHOSPHORYLATION OF RNA POLYMERASE II ON PREINITIATION COMPLEX FORMATION Jonathan D. Chesnut, and Michael E. Dahmus Department of Biochemistry and Biophysics University of California, Davis, CA. 95616

The C-terminal domain (CTD) of the largest subunit of mammalian RNA polymerase II (RNAP II) is comprised of 52 tandem repeats of the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. Two subforms of RNAP II are found *in vivo* that differ solely in the extent of phosphorylation within the CTD. RNAP IIA is unphosphorylated while RNAP IIO is hyperphosphorylated on serine and threonine. The role of this phosphorylation is unclear. Phosphorylation of RNAP IIA appears to occur at approximately the same time as the initiation of a transcript. Furthermore, the protein kinase that phosphorylates the CTD is stably associated with the preinitiation complex and may be an essential transcription factor.

In order to examine the differences in promoter association between RNAPs IIA and IIO, preinitiation complexes were formed in the absence of ATP with either ^{32}P-RNAP IIA or ^{32}P-RNAP IIO along with a reconstituted HeLa cell extract and the Adenovirus 2 major late promoter. Preinitiation complexes formed in these reactions were isolated by gel filtration and the state of phosphorylation of the complexed polymerase was examined. Reactions in which ^{32}P-RNAP IIA was the input enzyme yielded preinitiation complexes containing only ^{32}P-RNAP IIA while reactions formed with ^{32}P-RNAP IIO yielded no significant complex formation. In addition, reactions in which equimolar amounts of both ^{32}P-RNAP IIA and ^{32}P-RNAP IIO were added resulted in complexes that contained only ^{32}P-RNAP IIA. Fractions containing isolated complexes were assayed for complex stability and transcriptional activity by incubation with nucleoside triphosphates in the presence of a competing template. In all cases, complexes formed with labeled RNAP II were found to be stable and transcriptionally competent. These data support the idea that CTD phosphorylation may act to destabilize protein-protein or protein-DNA interactions that occur during preinitiation complex formation.

If phosphorylation of the CTD is, in fact, required for the transition from a preinitiation complex to an initiated complex, then extracts lacking CTD kinase should not be able to support transcription with RNAP IIA. Ongoing experiments designed to address the obligatory nature of CTD phosphorylation on transcription will also be presented.

R 317 RNA POLYMERASE II SUBUNIT 4 IS REQUIRED FOR GENERAL TRANSCRIPTION AND CELL VIABILITY DURING HEAT SHOCK AND IN THE STATIONARY PHASE, Mordechai Choder and Richard A. Young, Whitehead Institute for Biomedical Research, Cambridge MA 02142.

As yeast cells enter the stationary phase the transcription of most genes is repressed and their mRNA levels are consequently reduced, whereas transcription of a very small subset of genes is maintained comparable in all growth phases (Choder, Genes & Dev.[1991] *5*, 2315-2326). We found that among the latter type of genes, which are not repressed in the stationary phase, is RNA Polymerase II Subunit 4 (*RPB4*). *RPB4*, which is not essential for viability of optimally growing cells, is shown here to be required for normal entry into the stationary phase and for maintaining viability in post-log growth phases. So long as cells are in the log phase, deletion of *RPB4* has only a marginal effect (<x2) on transcription of most genes. Following the log phase, however, efficient transcription of most genes becomes dependent on *RPB4*. *RPB4* has been shown before to be present at less than one copy per holoenzyme molecule. We found that, following the log phase, protein levels of the polymerase subunits are decreased, whereas *RPB4* protein level remains unchanged, suggesting that the relative stoichiometry of *RPB4* with respect to other subunits is increased in post-log phases. In addition, *rpb4* mutant cells fail to respond normally to heat shock. Specifically, following a temperature shift from 26°C to 39°C, an otherwise nonlethal temperature, they rapidly loose viability and are almost completely unable to induce heat shock genes. Surprisingly, *RPB4* also was found to be essential for transcription of most, if not all, genes during heat shock.

Taken together, these results suggest that *RPB4* plays an important role in transcription under stress conditions.

R 318 EXPRESSION OF HUMAN RETINOIC ACID RECEPTOR ALPHA GENE SUPPRESSES TRANSCRIPTION OF *HUMAN* C-MYC *GENE IN XENOPUS OOCYTES*. Robin H. Chou[+*], Marcos R. Ortega[+], Marcella M. Flubacher[*] and Diane E. Mapstone[*]. Dept. of Anatomy[+] and Program of Molecular Biology[*], Hahnemann Univ. School of Medicine, Philadelphia, PA 19102

Induction of cell differentiation by morphogen retinoic acid has been known to be associated with down-regulation of c-myc protooncogenes. Since no DNA sequence in c-myc gene is homologous to any known responsive elements of retinoic acid receptors (RAR), we have investigated the modulatory effect of retinoic acid receptors on c-myc expression by employing Xenopus oocytes as an expression system. Cloned plasmids containing human c-myc (pHSR-1) or RAR-alpha (hRAR-alpha) genes can be expressed in the oocytes by microinjection respectively. Co-injection of hRAR-alpha and pHSR-1 resulted in suppression of c-myc expression. At 10:1 ratio of hRAR/pHSR-1 plasmid concentrations, a 67% decrease in both c-myc transcripts using either one of the two polyadenylation signals was detected when compared to transcripts in control samples which were co-injected with 1:1 ratio of hRAR/pHSR-1. This is determined by S1 nuclease protection assay using a 1.4 kbp ClaI/EcoRI DNA probe containing exon 3 and two poly(A) sequences. A 92% decrease was detected when a 100:1 ratio of plasmids was co-injected. To verify the level of RAR RNAs expressed in these oocytes, RNAs were isolated from oocytes after microinjection and RAR transcripts were detected by Northern hybridization using hRAR-alpha as probe. The levels of RAR transcript were proportionally increased in samples injected with 1:1, 10:1 or 100:1 ratios of hRAR/pHSR-1. These results directly demonstrate that RAR is capable of modulating c-myc expression in Xenopus oocytes, and provide a model to elucidate the yet unidentified RAR responsive element on c-myc gene.

R 319 A LINGUISTIC MODEL OF THE REGULATORY ARRAYS OF AN E.COLI DATA BASE. Collado-Vides J. Department of Biology Room 56-437. Massachusetts Institute of Technology, Cambridge, MA. 02139.

The formal justification for a linguistic theory of the regulation of gene expression which may provide a framework for the integration of large amounts of information has been obtained (1). The collection of E.coli promoters and their associated regulatory sites is the data base (2) for a Grammar of the sigma 70 system of transcription and regulation. This Grammar should generate all and only those arrays that are consistent with the principles of the sigma 70 system. The complete sequences of promoter, operator and activator binding sites have been identified as elements methodologically equivalent to phonemes. The identification of pertinent regulatory features, or distinctive features of these categories, supports a linguistic representation of the different arrays - a sort of phonological anaysis of the collection - where a "word" includes features of DNA sequences and properties of the binding proteins. The component of grammatical rules involves syntactic structures which identify sets of sites that occur as units, i.e. sites for the binding of the same protein. Two levels of representation are used in the component of rules. An initial derivation is specified by selection of "words" at proximal sites. Features associated with the proteins determine the subsequent derivation of remote sites by means of transformational rules.

A Grammar constitutes an explicit organized description of all the pertinent regulatory properties that combine in different arrays within a particular collection. The linguistic paradigm addresses the question of how to systematically organize sets of already deciphered information, in order to enhance our biological understanding and facilitate predictions. This novel paradigm shall be particularly useful within the Human Genome Project.

(1) Collado-Vides J. (1991) CABIOS 7:321-324.
(2) Collado-Vides J. et al.(1991) Microbiol.Rev. 55:371-394

R 320 MULTIPLE ACTIVATION AND REPRESSION DOMAINS CONTROL THE FUNCTION OF THE YEAST TRANSCRIPTIONAL ACTIVATOR ADR1,
William J. Cook, Daniel Chase, Deborah C. Audino and Clyde L. Denis, Department of Biochemistry and Molecular Biology, University of New Hampshire, Durham, NH 03824
The transcriptional activator ADR1 is required for the expression of the ADH2 gene from Saccharomyces cerevisiae. ADR1 activity is controlled by both translational and post-translational mechanisms. We have used a mutational and deletional analysis of ADR1, combined with a transcription activation assay system, to dissect the functional and regulatory domains in ADR1.
Within the N-terminal 642 amino acids of the 1323 amino acid ADR1 protein are three separate domains which activate transcription. Interspersed among these three activation domains are two regions which inhibit transcriptional activation by ADR1. The first of these repressor domains was previously identified by single amino acid mutations which allow partial release of ADR1 from the effects of glucose repression. Deletion of the second repressor domain coupled with a mutation in the first repressor domain does not result in augmented ADR1 activity. Based on these results, we propose a model in which the interactions between repressor domains modulate the function of adjacent activation domains.
Recent reports have identified expanded roles for the ADR1 activator in the control of genes involved in peroxisomal function, polyubiquination and other stress-related functions. Therefore, interactions between the different positive and negative domains in ADR1 may generate the flexibility required for ADR1 to carry out its numerous and diverse activation roles.

R 321 COUP-TRANSCRIPTION FACTOR DISPLAYS BOTH POSITIVE AND NEGATIVE TRANSCRIPTIONAL EFFECTS.
Austin J. Cooney, Sophia Y. Tsai, Bert W. O'Malley and Ming-Jer Tsai. Dept. of Cell Biology, Baylor College of Medicine, Houton, TX 77030.
COUP transcription factor (COUP-TF), an orphan member of the steroid/thyroid hormone receptor superfamily has been previously shown to activate transcription after treatment of cells with okadaic acid, 8Br-cAMP or dopamine. We wished to determine the optimum response element for COUP-TF activity. COUP-TF is related by its P box amino acid sequence to the ER/TR subfamily, which predicts that its response element should contain the GGTCA half site motif. In fact, the COUP element does contain this element as an imperfect direct repeat with a 2 bp spacing. We employed competition analysis to determine the relative binding affinity of COUP-TF for different orientations and spacings of the GGTCA core sequence. This analysis showed that COUP-TF displayed a promiscuous ability to bind to GGTCA repeats with different orientations and spacings. Thus not only is the GGTCA direct repeat with a 2 bp spacing a potential target element for COUP-TF, but so also are the palindrome with no spacing (TRE) and direct repeats with 5 bp and 7 bp spacings. Direct repeats of 3, 4 and 5 bp spacings have been shown previously to be selective response elements for the vitamin D, thyroid hormone and retinoic acid receptors respectively. We examined the effect of COUP-TF expression on transcription from these potential response elements and also its effects on the transcriptional response from these elements to their cognate receptors. In the absence of exogenous activators, COUP-TF represses basal transcription from these reporters. It also represses the transcriptional response to ligand activated vitamin D, thyroid hormone and retinoic acid receptors. Thus COUP-TF, like thyroid hormone receptor, has both positive and negative transcriptional effects.

R 322 A GENETIC AND IMMUNOLOGICAL ANALYSIS OF THE β SUBUNIT OF *E.coli* RNA POLYMERASE.
Karen D Cromie, Susan Trigwell and Robert E. Glass, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham. NG7 2UH. England.
We are applying complementary genetic and immunological techniques to the analysis of the structure and function of *E.coli* RNA polymerase. The β subunit of RNA polymerase, encoded by the *rpoB* gene, is involved in many steps in transcription, including substrate binding, DNA binding and σ^{70} function.
Based on comparative sequence analysis of β homologues in nature, the β subunit was divided into four potential domains, the junctions of which are in non-conserved regions. The domains have been expressed as β-galactosidase fusion proteins and used to produce polyclonal antibodies. The purified antibodies are being used to probe subunit conformation and assembly, and transcriptional initiation. The four domains have also been expressed from a regulatable promoter *in vivo* to investigate the concept of individual domain folding and to look for interaction with or sequestering of other RNA polymerase subunits.
The extreme C-terminus of the β subunit of *E.coli* RNA polymerase is implicated in σ^{70} function. A genetic system for the isolation and characterisation of altered forms of β with amino acid substitutions in a specific region of the C-terminus of the subunit has been developed. Mutants, screened *in vivo*, are being characterised *in vivo* and *in vitro* for various functions, including altered transcriptional activity of regulons dependent on σ factors other than σ^{70}. This work will complement our detailed analysis of this region of β using segment-specific antibodies to small, contiguous stretches encompassing the C-terminus.

R 323 PROMOTER SELECTIVE TRANSCRIPTIONAL REGULATION BY POU-HOMEO DOMAIN PROTEINS OCT-1 AND OCT-2,
Gokul Das, Masafumi Tanaka, and Winship Herr, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724 USA

Combinatorial interactions of multiple trans-acting factors with different DNA regulatory elements form the basis of transcriptional regulation. The octamer motif ATGCAAAT is a regulatory element for many genes transcribed by RNA polymerase II and RNA polymerase III. Octamer-binding proteins, Oct-1 and Oct-2, are closely related members of the POU family of homeodomain proteins. As a prototype to analyze the basis of promoter specificity of transcriptional activators, we are studying transcriptional activation of the U6 snRNA gene by Oct-1 and Oct-2.

The U6 snRNA gene has a promoter structure incorporating cis-acting elements of both snRNA and mRNA promoters and yet is transcribed by RNA polymerase III. The regulatory region includes a TATA sequence, an snRNA-specific element (PSE), and a distal octamer motif. To study transcriptional regulation by Oct-1 and Oct-2, it was necessary to overcome the ubiquitous endogenous activity of Oct-1. For this, the DNA binding specificity of Oct-1 and Oct-2 was reprogrammed without changing the gross structure of the protein by replacing the Oct-1 POU domain with the POU domain of the pituitary factor Pit-1, which displays a different DNA binding specificity. By substituting a multimerized Pit-1 binding site from the prolactin gene promoter for the octamer motif in the U6 snRNA gene promoter, we have assayed the activity of Oct-1 and Oct-2 for U6 gene activation in HeLa and 293 cells. In this type of assay, an snRNA gene transcribed by RNA polymerase II is preferentially activated by Oct-1 and an mRNA promoter is activated by Oct-2. These distinct Oct-1 and Oct-2 activities result from the use of different promoter-selective activation domains. Unexpectedly, RNA polymerase III transcription of the U6 gene is activated by both Oct-1 and Oct-2. This dual response may reflect that the U6 promoter contains elements common to mRNA (TATA box) and RNA polymerase II snRNA (PSE) promoters.

R 324 THE TRANSCRIPTIONAL ACTIVATOR CCR4 REQUIRES A LEUCINE-RICH REPEAT TO CONTACT THE TRANSCRIPTIONAL MACHINERY,
Michael P. Draper, Thomas Malvar, Rachel W. Biron, Arthur L. Kruckeberg and Clyde L. Denis, Department of Biochemistry and Molecular Biology, University of New Hampshire, Durham NH 03824
The yeast CCR4 protein is a general transcriptional activator that is required for full expression of nonfermentative genes, such as ADH2, and for spt10- and spt6- induced expression at the ADH2 and his4-912 delta loci. The SPT10 and SPT6 genes are general transcriptional factors which when mutated cause promiscuous transcription. Related SPT family members include TFIID and histones H2A and H2B. The CCR4 contains two regions that display sequence similarity to other proteins. The first region, the amino-terminal segment, is rich in glutamines, a characteristic of a number of other transcriptional factors. The second region contains a leucine-rich tandem repeat motif. Leucine-rich repeat motifs have been found to mediate protein-protein interactions in such proteins as yeast adenyl cyclase, ribonuclease inhibitor and lutenizing hormone receptor. This domain in CCR4 may bind to the SPT10 or SPT6 protein or another core component of the transcriptional machinery. Deletion of two of the five leucine-rich repeats in CCR4 was shown to produce a nonfunctional protein. Moreover, CCR4 fused to the LexA repressor protein was capable of converting LexA into a yeast transcriptional activator. The leucine-rich repeat motif was required for LexA-CCR4 transcriptional activation function. These results suggest that the leucine-rich repeat of CCR4 makes contact with an important part of the transcriptional machinery. In addition, CCR4 transcriptional activation function was shown to be carbon source regulated. In the LexA system CCR4 also showed synergistic activation in conjunction with the acidic activators GAL4 and ADR1 (required for ADH2 expression). Taken together these data suggest a model where CCR4, which is under carbon source control, contacts part of the transcriptional machinery and in doing so enhances the transcriptional response of acidic activators such as ADR1.

R 325 PHOSPHORYLATION STATE OF THE RNA POLYMERASE II LARGEST SUBUNIT DURING HEAT-SHOCK. Marie-Françoise Dubois and Olivier Bensaude, Département de Biologie, Ecole Normale Supérieure, 46 rue d'Ulm, Paris 75230.
The phosphorylation state of the largest subunit of RNA polymerase II was examined in various species of eukaryotic cells submitted to heat-shocks. At normal growth temperature, the enzyme was distributed between the non-phosphorylated IIa form and the highly phosphorylated IIo form. During heat-shock in human cells, we observed an hyperphosphorylation of the carboxy-terminal domain (CTD) which resulted in a shift from the IIo to the IIa form. In contrast, in mouse cells and in drosophila cells, non-lethal heat-shocks provoqued a shift from the IIo to the IIa form resulting from a dephosphorylation of the IIo form.
Since phosphorylation of the CTD is supposed to play a role in transcription, the *in vitro* transcription of nuclei from heat-shocked cells was examined. The transcription of heat-shock gene hsp 70 was strongly stimulated in nuclei from both murine and human cells. Meanwhile the transcription of house keeping genes, such as actin and glyceraldehyde-3-phosphate dehydrogenase, was repressed in nuclei isolated from heat-shocked murine cells but not in nuclei from heat-shocked human cells. This suggests that the phosphorylation of the CTD from the RNA polymerase II largest subunit might be associated with house-keeping genes transcription and might not be necessary for hsp 70 gene transcription.

R 326 SEQUENCE IDENTIFICATION OF TFIIIR: A CLASS III TRANSCRIPTION FACTOR COMPOSED OF RNA,
Heather M. Dunstan, Lisa S. Young, Pamela R. Witte, and Karen U. Sprague, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403
Efficient transcription from class III promoters is facilitated by a complex set of molecular interactions involving the template, pol III, and several auxiliary transcription factors. With the goal of understanding of the nature and function of these interactions, we are dissecting the class III transcription machinery of the silkworm. We recently demonstrated that *in vitro* transcription by silkworm RNA polymerase III requires a ribonucleic acid, called TFIIIR, in addition to the previously described class III factors (TFIIIA, TFIIIB, TFIIIC, and TFIIID).[1] Silkworm RNA has been fractionated in order to identify the species of RNA with TFIIIR activity. The pattern of TFIIIR activity within these RNA fractions suggests that TFIIIR activity resides in a single RNA species. We have purified TFIIIR RNA to apparent homogeneity, and have obtained a putative nucleotide sequence by rapid RNA sequencing, fingerprinting, and DNA sequencing of PCR-amplified cDNA. We are using copies of this RNA species synthesized *in vitro* by T7 RNA polymerase to rigorously test the transcriptional activity of the candidate sequence.
1. Young, L. S., *et. al.*, 1991. *Science* 252:542.

R 327 PHAGE MU VIRULENT MUTANTS CARRY DOMINANT REPRESSOR MUTATIONS WHICH TRIGGER PROTEOLYTIC DEGRADATION OF THE WILD-TYPE PROTEIN, Geuskens V., Desmet L., Mhammedi Alaoui A., Toussaint A., Laboratoire de Génétique, Unité de Transposition Bactérienne, Université Libre de Bruxelles, B-1640 Rhode-Saint-Genèse, Belgium.

Bacteriophage Mu repressor, a 197 amino-acid protein that binds to the tripartite 184 bp Mu operator region, is the major protein involved in the establishment and maintenance of the prophage state.

Virulent mutants of Mu (Mu*vir*) were selected for their ability to grow on a Mu lysogen. They are trans-dominant since they induce the resident prophage upon infection of a Muc + lysogen.

We found that Mu*vir* carry one of two types of frameshift mutations which provoke the synthesis of a truncated repressor with a few new residues at the C-terminus. Repressor stability is completely altered by these modifications, the half-life of *vir* repressor being five fold lower than that of the wild-type protein. This instability is in addition trans-dominant: the wild-type repressor present in a lysogen is degraded as soon as mutated *vir* repressor is produced in the same cell.

Phage and bacterial mutations which suppress the Vir phenotype have been isolated. They all restore stability to normal. This suggested that the *vir* repressor is very sensitive to or activates a host protease and triggers the wild-type protein towards the same proteolytic degradation, probably as a result of interactions between wild-type and *vir* repressor protomers. We have good evidence that it is the Clp protease which is involved in this process. The induction of the prophage in a Mu lysogen superinfected with Mu*vir* is thus most likely due to the degradation by Clp, triggered by the mutated *vir* repressor, of active repressor.

R 328 ANALYSIS OF A TISSUE-SPECIFIC ENHANCER: IDENTIFICATION OF AN ADIPOCYTE-SPECIFIC FACTOR, Reed A. Graves, Peter Tontonoz, Susan R. Ross*, Yuan-Di Halvorsen and Bruce M. Spiegelman, Dept. of Biological Chemistry and Molecular Pharmacology, Dana-Farber Cancer Inst. and Harvard Medical School, Boston, MA 02115 and *Dept. of Biological Chemistry, Univ. of Illinois at Chicago, Chicago, IL 60612

The adipocyte P2 (aP2) gene encodes a member of the fatty acid binding protein family whose expression is restricted to adipose tissue. We have been characterizing a 518 bp enhancer located at -5.4 to -4.9 kb of the aP2 gene. This enhancer stimulates tissue-specific and differentiation-dependent gene expression in transgenic mice and cultured cells, respectively. Using transient transfection of cultured adipocytes combined with DNA mobility shift binding assays, we have identified 5 cis-acting elements in the enhancer. In the context of the whole enhancer each of these sites is important for activity since mutation of any individual site reduces enhancer function by more than 75%. However, isolated fragments derived from the enhancer are capable of significant differentiation-dependent enhancing activity. One fragment of 122 bp has enhancing activity approximately equal to that of the whole enhancer despite the deletion of 2 cis-acting elements. We have characterized the 3 elements present in this fragment. One of them, Adipocyte Regulatory Element 2 (ARE2), binds a protein that can be detected in the nuclear extracts from several cell types. Multiple copies of the ARE2 element function as an enhancer in several cell lines. The other 2 elements present in the 122 bp fragment, ARE6 and ARE7, bind the same protein, called ARF6, as judged by competition studies in mobility shift assays. ARF6 binding activity is detected in adipocyte derived nuclear extracts but not in extracts from other cell types including preadipocytes. The enhancing activity of multimerized ARE6 or ARE7 binding sites is likewise adipocyte specific. Thus the specificity and potency of the 518 enhancer derives from the coordinate interaction of several components some of which are ubiquitous. We have identified a factor, ARF6, that appears to be specific for adipocytes and propose that ARF6 is the switch that triggers the activity of the enhancer and that in the absence of ARF6, the other elements, e.g. ARE2, are inactive.

R 329 SEQUENCE ANALYSIS OF THE SECOND LARGEST SUBUNIT OF RNA POLYMERASE II FROM *ARABIDOPSIS THALIANA*. Tom Guilfoyle, Rob Larkin, Craig Chandler. University of Missouri-Columbia, Department of Biochemistry, Columbia, Missouri 65211.

We have cloned a single copy gene and the corresponding cDNA which encode the second largest subunit of RNA polymerase II of *Arabidopsis thaliana*. *Arabidopsis RPB2* was isolated using a segment of *RPB2* that was amplified from *Arabidopsis* genomic DNA using PCR and degenerate oligonucleotides derived from amino acid sequences of the second largest subunits of eukaryotic class II enzymes and the β subunits of prokaryotic and chloroplast enzymes. The open reading frame predicts a 1188 amino acid polypeptide of 135 kDa that is greater than 60 % identical to the sequence derived from yeast and *Drosophila RPB2* and 40 % and 29 % identical to the sequences derived for the homologous subunits from yeast RNA polymerase III and I, respectively. The open reading frame is encoded by 6509 bp of genomic DNA and contains 24 introns. The 3' noncoding region may contain an intron, similar to the 3' noncoding regions of *Arabidopsis* and soybean *RPB1*. The first intron is distinct from the other introns in that it is approximately seven times longer and contains repetitive sequences. The promoter region also contains repetitive sequences as well as a homopyrimidine/homopurine motif that is similar to sequences that form triple-helical structures *in vitro* and to promoter sequences that bind nuclear factors.

R 330 YEAST TRANSCRIPTIONAL COACTIVATOR GAL11 FUNCTIONS IN MAMMALIAN CELLS, Hiroshi Handa, Atsushi Kubota, Hiroshi Sakurai* and Toshio Fukasawa*, Department of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 227, Japan, *Laboratory of Molecular Genetics, Keio University School of Medicine, Tokyo 160, JAPAN

The GAL4 protein, a DNA-binding transcriptional activator for the galactose-inducible genes in the yeast Saccharomyces cerevisiae, is now known to function 'universally'; when introduced into any of a wide variety of eukaryotic cells, it activates transcription of a gene bearing the GAL4-binding sequence (UAS_G) in its upstream region. GAL11, also known as SPT13, is required for maximizing transcription of some of the galactose-inducible genes in yeast. Genetic studies have suggested that GAL11 functions as 'coactivator' or 'mediator' for GAL4: GAL11, not binding to DNA as itself, mediates the activator function of GAL4 to stimulate the basal transcription machinery, presumably by protein-protein interactions. We report here that GAL11 exerted dual effects on GAL4-dependent trans-activation of a reporter gene bearing the GAL4-binding site (UAS_G) in mammalian cells depending on the amount of GAL11 expressed: The expression of the reporter gene was repressed when the amount of GAL11 was relatively small. As the amount of GAL11 increased, expression of the reporter gene was enhanced, and finally, reached 3 to 5 times the initial level with GAL4 alone. Such enhacement was observed only when UAS_G was located far from the TATA box. When UAS_G was placed close to the TATA box, the reporter expression reached the initial level, but did not exceed it. These results led us to hypothesize the existence of a mammalian homolog(s) of GAL11.

R 331 CELL GROWTH REGULATION OF THE MAMMALIAN TRANSCRIPTION FACTOR LSF, Ulla Hansen, Lucia Rameh, and Janet Volker, Laboratory of Eukaryotic Transcription, Dana-Farber Cancer Institute; Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.

The mammalian transcription factor LSF specifically binds to and stimulates transcription from the SV40 major late promoter (H.-C. Huang, et al, Genes Dev. 4:287-298, 1991). The DNA-binding activity of this cellular protein has now been examined as a function of the growth state of the mammalian cell. Tissue culture cells (U2OS) were fractionated on the basis of position within the cell cycle in an elutriation centrifuge, and the DNA-binding activity of LSF assayed by gel mobility shift analysis. A striking peak of activity was observed, with maximal activity corresponding to fractions containing decreasing numbers of cells in G1 and increasing numbers of cells in S. Human peripheral T cells were also examined for their LSF DNA-binding activity, both upon growth stimulation from the resting state and in stimulated, cycling cells. A dramatic (5 to 10-fold) stimulation of LSF DNA-binding activity was observed with stimulation of T cells to grow. In addition, cycling cells exhibited a peak of activity in late G1, as observed above. In all cases, proof that the increase in binding activity was LSF came from supershift experiments using LSF-specific antibodies. Thus, increases in DNA-binding activity of LSF are observed both in response of resting cells to growth stimuli (G0 to G1 transition), and in cycling cells in late G1 (or near the G1 to S phase transition). LSF is known to bind cellular promoters activated at both these stages in the cell cycle: the human *c-fos* and mouse thymidylate synthase promoters. The source of the changes of LSF DNA-binding activity were examined for the G0 to G1 transition. The electrophoretic mobility of immunoprecipitated LSF decreased within 3 hours of stimulation of A31 tissue culture cells (NiH3T3 derived cell line) with serum, due apparently to a phosphorylation event. In addition, dephosphorylation of LSF reduced its DNA-binding activity.

R 332 Factors required for expression of vaccinia virus intermediate genes. Nicholas Harris, Ricardo Rosales and Bernard Moss. Laboratory of Viral Diseases, National Institutes of Health, Bethesda, MD 20892.

Vaccinia virus gene expression is regulated in a sequential fashion. The proteins required for intermediate gene expression are early gene products and are present before viral DNA replication. Intermediate gene promoters, ligated to a G-less cassette, served as templates in an in vitro transcription assay. A cytoplasmic cell extract, made from HeLa cells infected with virus in the presence of an inhibitor of DNA synthesis, transcribed the DNA templates in vitro. Intermediate gene transcription required viral RNA polymerase and two virally encoded transcription factors. One of the factors eluted from DEAE cellulose at low salt (0.1 M NaCl) with capping enzyme activity (guanylate transferase assay). Virion extract, which contains capping enzyme, is competent to use an early gene promoter, but not an intermediate promoter, for transcription. The virion extract, however, could replace the low salt transcription factor in the reconstituted transcription reaction. Capping enzyme, purified to homogeneity from virion extract, was found to be the low salt transactivator of intermediate gene transcription. The viral extract was not able to replace the intermediate gene transcription factor which eluted at relatively high salt (0.25 M NaCl). This factor has been extensively purified and is required for transcription of intermediate genes. The intermediate transcription factors have early promoters whereas the late transcription factors have intermediate promoters, providing for a cascade mechanism of gene regulation.

R 333 FUNCTIONAL ANALYSIS OF THE DNA BINDING AND TRANSACTIVATION DOMAINS OF TRANSCRIPTIONAL ENHANCER FACTOR-1 (TEF-1), Jung-Joo Hwang, Irwin Davidson and Pierre Chambon, LGME du CNRS/U-184 INSERM, 11 rue Humann, 67085 STRASBOURG, FRANCE

Transcription factor TEF-1 binds specifically to two enhansons of degenerate nucleotide sequences, GT-IIC and Sph, in the SV40 enhancer. Activation of transcription by TEF-1 requires the action of a titratable, limiting transcriptional intermediary factor as evidenced by the ability of TEF-1 or GAL4-TEF-1 chimeras to "squelch" the endogenous HeLa cell TEF-1. We have defined at least three domains in the TEF-1 protein which are required for the transactivation and squelching effects. Interestingly a functional synergy between these domains is required for each of these functions as individually each domain is inactive. Two of these domains, an acidic and a proline rich domain, resemble in amino acid composition those previously identified in other transcription factors while the third critical domain appears to have no similarity to those as yet identified in other transcription factors. In addition we will also present the results from an analysis of the ability of DNA binding domain mutations to recognize the GT-IIC and Sph motifs.

R 334 TARGETS OF THE ACIDIC TRANSCRIPTIONAL ACTIVATION DOMAIN OF VP16. C. J. Ingles, H. Xiao, K. F. Stringer, M. Shales, R. Gupta, B. Coulombe, V. D. Fitzpatrick, R. Truant, and J. Greenblatt, Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada M5G 1L6.

The HSV-encoded polypeptide VP16 has a C-terminal activation domain rich in acidic amino acids. The synergistic nature of transactivation suggests that VP16 binds multiple targets. Columns of pA-VP16 agarose but not pA agarose, can deplete Hela nuclear extracts of their TFIID activity. The biological significance of this interaction was investigated using mutant forms of VP16. For changes at a critical phenylalanine residue at position 442 of VP16 there was a good correlation between transactivation activity in murine cells and the binding of VP16 to yeast TFIID.

TFIID may not be the only target of the acidic activator VP16. A small proportion of human TFIIB also bound pA-VP16 columns and human TFIIB produced in *E. coli* did bind to wild-type VP16 derivatives. This binding of TFIIB was reduced by mutations in VP16 that alter net negative charge, but have minimal effects on transactivation. To establish which VP16 interactions are important it will be necessary to find mutations in TFIIB and TFIID which affect VP16-activated transcription. The VP16 binding site in yeast TFIID maps to its evolutionarily conserved C-terminal domain. Point mutations in TFIID currently under study may be informative. TFIIB, TFIID, and three other polypeptides >100 kDa from Hela cell extracts that also bind to VP16 may all be involved in transcriptional activation.

R 335 DR-1, A TFIID BINDING PROTEIN AND INHIBITOR OF CLASS II GENE TRANSCRIPTION, Juan Inostroza and Danny Reinberg, Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635.

Regulation of gene expression is a complex process that can be achieved at multiple steps. The first step during formation of a transcription competent complex is the binding of TFIID to the TATA motif, providing a recognition site for the association of RNA polymerase II and auxiliary proteins. TFIID is also known to interact with activator proteins. A great deal of effort has been directed towards the isolation of proteins that activate transcription. On the other hand, an equally important means of regulating gene expression is repression. We have isolated from HeLa cells a 19 kDa protein termed Dr-1, that interacts with TFIID. Dr prevents the association of TFIID with RNA polymerase II and auxiliary proteins, and thereby inhibits transcription of class II genes. Using reverse genetics we have isolated cDNA clones encoding Dr-1. We have found that Dr-1 is a phosphoprotein and phosphorylation of Dr-1 is necessary for its interaction with TFIID. The protein kinase regulating Dr-1 activity is currently under study.

R 336 MOLECULAR COMMUNICATION BETWEEN RNA POLYMERASE AND TRANSCRIPTION FACTORS: MAPPING OF CONTACT SITES ON *E. COLI* RNA POLYMERASE, Akira Ishihama, Department of Molecular Genetics, National Institute of Genetics, Mishima, Shizuoka 411, JAPAN

The *E. coli* RNA polymerase holoenzyme is composed of core enzyme with the subunit structure of $\alpha_2\beta\beta'$, and one of the multiple species of σ subunit. The promoter selectivity of RNA polymerase is subject to modulation by either replacement of σ subunit, or interaction with one of various accessory transcription factors. Some factors bind to the RNA polymerse in the absence of DNA, but others interact with the RNA polymerase when both bind to the respective target sites on DNA.

In the course of mapping of functional sites on each subunit polypeptide of the RNA polymerase, we found that the C-terminal proximal one-third of α subunit is involved in molecular communication with transcription activator proteins such as CRP (cAMP receptor protein; an activator for catabolite sensitive genes) and OmpR (an activator for the membrane protein porin regulon), only when they bind to the target sites located upstream from the promoter -35 (*Cell* 65:1015-1022, 1991). Mutant RNA polymerases consisting of C-terminally truncated α subunits were, however, activated when transcription factors such as CRP and PhoB (an activator protein for the phosphate regulon) bound to the target sites located within the promoter region, indicating that besides the "contact site I" on the α subunit, there exists at least one more site ("contact site II") for communication with transcription factors (*Proc. Natl. Acad. Sci. USA* 88:8958-8962, 1991).

In order to classify transcription factors with respect to the contact site selection, more than 15 laboratories are being involved for mapping the contact site for different transcription factors using both wild-type and mutant RNA polymerases lacking the contact site I. I will try to summarize the results of this international collaboration.

R 337 **STUDIES ON PHO4 AND PHO80, ACTIVATOR AND REPRESSOR PROTEIN RESPECTIVELY, OF PHOSPHATE REGULATED GENES IN *SACCHAROMYCES CEREVISIAE*.** **P.S.Jayaraman and C.Goding**
MARIE CURIE RES.INST.OXTED,SURREY,RH8 OTL U.K.

The PHO4 protein is a positive activator of the phosphate regulated gene PHO5. It is a basic region-helix loop helix (b-hlh) DNA-binding protein and binds to the PHO5 UAS across two palindromic CACGTG/T motifs. PHO80 has been defined genetically as a negative regulator of PHO5 expression but the mechanism of repression is unknown.
Using a series of C-terminal PHO4 deletions fused to the GAL4 DNA-binding domain we have shown that a highly acidic 25 amino acid region of PHO4 between amino acids 74 and 99 is sufficient to activate the GAL UAS efficiently. In addition, fusion of N-terminal PHO4 deletions to the C-terminal DNA-binding domain of another yeast b-hlh CACGTG-binding protein, CPF-1, demonstrated that amino acids 83-265 of the PHO4 protein are sufficient both for activation of the PHO5 UAS and for repression by PHO80.
A possible mechanism of PHO5 repression is the inhibition of PHO4 activation by a direct protein-protein interaction between PHO4 and PHO80. We present data on the results of co-expressing PHO80 repressor protein fusions tagged with heterologous DNA binding domains together with a PHO4 protein tagged with the strong activation domain of the viral activator VP16.
The CPF1 DNA binding domain can substitute for the PHO4 DNA binding domain, however the PHO4 protein is unable to bind all CPF1 sites. Site directed mutagenesis has been used to study the molecular basis of this binding site discrimination.

R 338 ISOLATION AND CHARACTERIZATION OF THE RNA POLYMERASE OF METHANOCOCCUS VOLTAE. Janice W. Kansy and Jordan Konisky. Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Understanding the role of RNA polymerase from the function of each of its subunits to its interaction with various promoters is essential to understanding the regulation of gene expression. The RNA polymerases isolated thus far from the three domains, the Bacteria, the Archaea, and the Eucarya, are distinct in structure and function. To study the regulation of gene expression in the Archaea, RNA polymerase was isolated from Methanococcus voltae. The RNA polymerase consists of 9 subunits, 6 of which were serologically related to the RNA polymerase of Escherichia coli. The largest subunits, A, B', B'', and C, have been identified by Western blot analyses using antibodies to the subunits of the RNA polymerase of Methanobacterium thermoautotrophicum. The RNA polymerase of M. voltae was used in gel mobility shift and DNase I footprinting assays to identify the promoter region of the hisA gene. The results of these studies showed that the promoter for the hisA gene lies between 95 and 205 base pairs upstream of the hisA structural gene. A novel component of the RNA polymerase of M. voltae was recently identified: RNA. The RNA of the polymerase is heterogeneous in size with an average length of 140 nucleotides. Southern blot analysis indicates a discrete region of the M. voltae chromosome hybridizes to the RNA. Studies are in progress to determine the role of the RNA in transcription.

R 339 OVER-EXPRESSION AND PURIFICATION OF VACCINIA VIRUS LATE GENE TRANSCRIPTION FACTORS. James G. Keck and Bernard Moss. Laboratory of Viral Diseases, National Institute of Aliergy and Infectious Disease, National Institutes of Health, Bethesda, MD 20892

Vaccinia virus transcription occurs in stages, in which early gene transcription is required for DNA replication and the onset of intermediate and then late gene transcription. By using a transfection assay and a vaccinia virus gene library, three intermediate genes were shown to be required for late gene transactivation. These genes encode polypeptides of 17, 26 and 30 kd. Two of these polypeptides, the 17 and 26, contain zinc finger motifs. Mutations of the 26 kd polypeptide demonstrated that the in vitro ability to bind radioactive zinc correlated with the ability to transactivate late gene transcription in vivo. Over-expression of the three transcription factors in Hela cells was achieved using a recombinant vaccinia virus system that employes the bacteriophage T7 promoter and T7 RNA polymerase. This over-expression resulted in a greater than ten fold increase in the late gene transcription activity over that of a normal infected cell extract. By using column chromatography these transactivators have been separated and extensively purified. Each has been shown to be required, with the virally encoded polymerase, for the in vitro transcription under late promoter control.

R 340 STRUCTURAL STUDIES OF THE ACIDIC ACTIVATION DOMAIN OF THE GCN4 PROTEIN,

Thomas Kodadek and Michael Van Hoy, Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712

The yeast GCN4 protein is a transcriptional activation factor and posesses a prototypical acidic activation domain. This domain is thought to contact some factor or factors in the basal transcriptional machinery, thus aiding its assembly. Current thinking about the structure of acidic activation domains suggests that they are either unstructured "negative noodles" or amphipathic α-helices. However, these models have never been addressed biophysically. In order to do so, we expressed large quantities of a fusion protein in which the activation domain of GCN4 (residues 107-144) was attached to the C-terminus of ubiquitin. After specific proteolysis the activation domain was purified to homogeneity. Preliminary biophysical analysis of this polypeptide, including gel filtration, circular dichroism and NMR, show that the domain is not a random coil, but that it has very little helical nature. The results are most consistent with about half of the amino acids being part of β-sheets along with some turns. A detailed two-dimensional NMR study is in progress. The results of these experiments will be presented and the generality of our findings with regard to other acidic activation domains will be discussed.

R 341 *IN VIVO* SPECIFICITY OF INDIVIDUAL ELEMENTS OF THE ELASTASE I ENHANCER. Fred Kruse, Scott D. Rose, Robert E. Hammer, Galvin H. Swift and Raymond J. MacDonald. Univ. of Texas Southwestern Medical Center, Dallas, TX 75235. Mutational analysis of the 134 bp elastase I (EI) enhancer (-72 to -205), which directs pancreas-specific transcription, identified three important sequence elements for activity in acinar cells. To determine the role of each element in EI enhancer activity, we generated transgenic mice bearing multiple copies of each individual element fused to either the EI or HSV thymidine kinase (tk) promoter and a human growth hormone (hGH) reporter gene. The EI enhancer A element (-96 to -115), which is also present in the 5' flanking sequences of several other exocrine-specific genes (e.g., amylase, trypsin I & chymotrypsin B), directed low-level expression of the hGH reporter gene in a pattern quite similar to that observed for the endogenous EI gene, that is, in the pancreas, stomach, and duodenum. In the pancreas expression was limited to the acinar cells. The EI enhancer B element (-146 to -160) also acted as a pancreas-specific transcriptional control element. However, expression of the hGH transgene was restricted to the β cells in the islet of Langerhans. Because mutations of the B element affect transcription in acinar cells, it is required for appropriate acinar-cell expression as well. The presence of the EI enhancer B element in functionally important regulatory regions of other acinar-specific genes (e.g., amylase) and islet-specific genes (e.g., insulin, glucagon & somatostatin) suggests that B-like elements play a role in the expression of both exocrine and endocrine-specific pancreatic genes. The EI enhancer C element (-166 to -195) did not activate transcription by itself, but did augment the activity of the A and B elements when combined with either of these elements. The presence of a repressor element within a 300 bp region immediately upstream of the EI enhancer selectively silences expression of the EI gene in the endocrine pancreas. The three EI enhancer transcriptional elements and the upstream repressor act in concert to yield high-level, acinar cell-specific transcription.

R 342 MOLECULAR MECHANISMS OF TRANSCRIPTION ACTIVATION BY mUBF, A MOUSE NUCLEOLAR TRANSCRIPTION FACTOR

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We have fractionated mouse extracts into five components (i.e. pol I, TIF-IA, TIF-IB, TIF-IC and UBF) each of which is required to direct transcription from the mouse rDNA promoter. Using this reconstituted system, we have analyzed the function of UBF, a trans-activating factor that is highly conserved in eukaryotes. mUBF, a doublet of 97 and 94 kD proteins, interacts with both the rDNA promoter and the 140 bp repetitive elements in the 5'terminal spacer which enhance pol I transcription both in vivo and in vitro. Both the 140 bp elements and the core promoter act cooperatively and thus are functionally linked. We show that in the presence of the enhancer repeats and UBF the preinitiation complexes assembled at the core promoter are stabililized. This functional cooperativity between the enhancer repeats and UBF can also be visualized by electron microscopy. After incubation of rDNA templates with TIF-IB and UBF, we noted a substantial fraction of DNA molecules in which the enhancer and the promoter was joined by a DNA loop. Thus the synergism between the distant sequence elements appears to be mediated by UBF and TIF-IB.

Furthermore we present experimental evidence which suggest that UBF counteracts the inhibitory action of a DNA-binding protein which co-purifies with pol I. This yet to be characterized repressor of rDNA transcription competes with TIF-IB for binding to the promoter. The data suggest that in the absence of this inhibitory protein UBF is not required for rDNA transcription initiation.

R 343 EVIDENCE THAT AGGGA DOMAIN, IN ITS NATURAL CONTEXT, IS INVOLVED IN THE POSITIVE TRANSCRIPTIONAL REGULATION OF JC VIRUS EARLY PROMOTER, G. Kumar*, D.K. Srivastava, A.K. Sharma, W. Tefera and D. Patel, Department of Molecular Biology and Genetics, Center for Molecular Biology, Wayne State University, School of Medicine, Detroit, MI 48201

The human papovavirus JC (JCV), although prevalent in human population, causes a fatal disease called progressive multifocal leukoencephalopathy (PML) in immunocompromised individuals. The glial specificity of the expression of viral early genes has been demonstrated to be due to an interplay of both positive and negative regulatory factors . In previous studies a tandem repeat of "AGGGA" present between the TATA box and the NF1 protein binding site have been implicated to play a negative role in the regulation of the JCV early promoter.

In order to assess the role of the AGGGAAGGGA domain in the natural context of the JCV transcription, selected deletion and linker scanning mutants for the JCV (Mad 1) promoter were constructed. Analysis of the in-vivo and in-vitro transcriptional activity of these mutants reveal that mutation of the GGG region of only one AGGGA domain (p1.3E and p1.4E) resulted in the complete loss of transcription , whereby indicating that the AGGGA domain either interacts with a positive activator protein or it binds to a protein which helps the binding of other positive activator protein(s) at the neighboring cis elements.

UV crosslinking studies with duplex-3 , in which BrdU was selectively incorporated in TCCCT (AGGGA domain), were carried out to identify the proteins which interact with the AGGGA domain, in the presence of other proteins bound to the neighboring NF-1 and TATA domains. These studies reveal that while only 56-53kD protein binds to the AGGGA domain in the absence of other neighboring cis elements , another 80 kD protein(s) interact(s) with the same domain in duplex-3.

(Supported by NIH grant GM38228 to G.K.)

R 344 RNA- AND TEMPLATE- BINDING POLYPEPTIDES OF THE PEA CHLOROPLAST TRANSCRIPTION COMPLEX. Sujata Lakhani, Navin C. Khanna and K.K. Tewari. International Center for Genetic Engineering and Biotechnology, NII Campus, Shaheedjit Singh Marg, New Delhi 110 067, India.

The chloroplast (ct) RNA polymerase is a multisubunit complex but the function of each subunit is not understood. We have used photoaffinity labelling to study this problem. We developed a plastid run-on transcription system in which intact chloroplasts were lysed in the presence of α-^{32}P-ATP and under conditions optimized for *in vitro* transcription. Nascent transcripts synthesized from the endogenous organellar DNA (1-5 min incubation) were found to photocrosslink to a 48 kDa polypeptide after irradiation with UV for 15 min. The photoaffinity labelling of this polypeptide required Mn^{2+} ions which could not be replaced by Mg^{2+}. The Mn^{2+} appeared to stabilize the RNA-protein complex. This 48 kDa polypeptide was purified by 2-D gel electrophoresis and its N-terminal amino acid sequence obtained. In order to identify the template-binding polypeptide, the ct RNA polymerase was purified so that it could transcribe exogenously added DNA. Segments of DNA containing the promoters of 16S rRNA or *psbA* genes which encode two abundant ct RNAs, were amplified from ct DNA by the polymerase chain reaction and uniformly labelled with 5-bromo dUTP and α-^{32}P-dCTP. Exposure to UV light led to the crosslinkage of the template to a 150 kDa polypeptide. The photoaffinity labelling was dependent on the presence of ct promoters, and was not diminished by the addition of synthetic polynucleotides or non-plastid DNAs. This approach identified the 48 kDa and 150 kDa polypeptides as authentic components of the ct transcription complex.

R 345 THE RNA POLYMERASE II C-TERMINAL DOMAIN CONTRIBUTES TO THE RESPONSE TO MULTIPLE ACIDIC ACTIVATORS IN VITRO. Sha-Mei Liao[1], Ian C. A. Taylor[2], Robert E. Kingston[2], and Richard Young[1], [1]Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142; [2]Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA 02115.

The largest subunit of RNA polymerase II contains a unique carboxy terminal domain (CTD) that consists of repeats of the heptapeptide YSPTSPS. Here we report that progressive truncation of the yeast RNA polymerase II CTD causes progressive loss of trans-activator-dependent transcription in nuclear extracts but has little effect on elongation or termination. Specific transcription, which is reduced by up to 50-fold in these assays, can be restored in the defective nuclear extracts by adding purified wild-type RNA polymerase II. The defects in factor-dependent transcription are observed with templates that are assembled into nucleosomes as well as with templates that are not so assembled. Defects in factor-independent transcription are also observed, but these are not as profound as those observed in the presence of trans-activators. These results indicate that the RNA polymerase II CTD functions during transcription initiation and is required for normal levels of activated transcription in vitro.

R 346 THE TAIL OF THE LARGEST SUBUNIT OF RNA POLYMERASE II, Hua Lu and Danny Reinberg, Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635.

Two forms of RNA polymerase II that exist *in vivo*, phosphorylated (IIO) and non-phosphorylated (IIA), have been purified to apparent homogeneity from HeLa cells. A protein kinase that phosphorylates IIA polymerase has been identified and purified from HeLa cell nuclear extracts. This protein kinase shifts, independently of ATP, the complex formed with TFIID and TFIIB on the TATA motif (DB complex). this suggests that this protein kinase can bind to the DB DNA protein complex. We have previously reported that the association of RNA polymerase II with the preinitiation complex is mediated by the transcription factor IIF. We have now found that the protein kinase described here can also mediate the association of RNA polymerase II with the DB complex. Protein kinase-mediated association of RNA polymerase II with the preinitiation complex was dependent on transcription factors IIB and IID. In addition, this kinase, in the presence of ATP, extensively phosphorylated the carboxy terminal domain of the largest subunit (CTD) of RNA polymerase II. Extensive phosphorylation of the CTD requires the protein kinase and RNA polymerase II to be in association with the preinitiation complex. This was independent of whether or not the polymerase was delivered to the DB complex via TFIIF or via the protein kinase.

We have previously shown that both forms of RNA polymerase II could specifically associate with the preinitiation complex, but that the non-phosphorylated form of the enzyme preferentially associated with the transcription complex. We have now found that the protein kinase we have isolated from HeLa cells can, in an ATP dependent fashion, preclude the association of the phosphorylated form of RNA polymerase II with the preinitiation complex.

R 347 SITE-DIRECTED MUTAGENESIS OF BACTERIOPHAGE T7 DNA-DEPENDENT RNA POLYMERASE, Dmitry L.Lyakhov, Vladimir O.Rechinsky, Vera L.Tumitskaya, Boris K.Chernov, and Sergey N.Kochetkov, Institute of Molecular Biology, Acad. Sci. USSR, Moscow 117984, GSP-1, USSR

Bacteriophage T7 RNA polymerase (T7RNAP) is one of the simplest enzymes catalyzing RNA synthesis. Little is known about its functional groups and active site structure. Affinity labelling studies revealed two amino acid residues important for enzyme's activity, namely Lys-631 and Lys-172. The Lys-631 apparently belongs to the catalytic site of T7RNAP. The Lys-172 which is the site of limited proteolysis of T7RNAP in vivo and is likely to lie within an interdomain "stretch" is supposed to be involved in template binding. For further studies of the functional role of these residues the oligonucleotide-directed mutagenesis was carried out. The following changes have been made: Lys-631 to Gly, Leu, Arg; Lys-172 to Gly, Leu; deletion of residues 172-173 (Lys-Arg). Km values for 631-mutant enzymes were close to the one of the w.t. T7RNAP, while Vmax decreases in a row: Lys>Arg>> Gly, Leu. The kinetic parameters of 172-mutants do not differ significantly from those of the w.t. T7RNAP. For these proteins the change of the site and decrease of the limited proteolysis rate have been observed. It has been shown that deletion of residues 172-173 as well as mutation Lys-172 to Leu cause the changes in specificity of T7RNAP. These mutants can direct unspecific RNA synthesis on templates lacking the T7 promoter. Deletion (del.172-173) mutant is not able to synthesize extraneous RNA sequences in addition to the expected run-off transcripts. Both Leu-172 and deletion mutants reveal altered template specificity toward various DNA alternating and homopolymers and are able to utilize poly(rC) and poly(rU) as templates.

R 348 GLUCOCORTICOID REGULATION OF C-*MYC* PROMOTER UTILIZATION IN P1798 T-LYMPHOMA CELLS,

Tianlin Ma and E. Aubrey Thompson, Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch at Galveston, Galveston, Texas 77550

Glucocorticoids rapidly inhibit expression of c-*myc* mRNA in P1798 lymphoma cells. Statistically significant decreases can be observed within 5-10 minutes after addition of glucocorticoids. Although transcription of c-*myc* decreases within a few hours after dexamethasone is added to P1798 cell cultures, nuclear run-on transcription cannot be used to demonstrate that the very early changes in mRNA abundance reflect corresponding change in transcriptional activity. An RNase protection assay has been used to measure the abundance and the rates of turnover of the two major c-*myc* transcripts, arising from the P1 and P2 initiation sites. The relative rates of synthesis of the c-*myc* mRNAs(*i.e.* transcription) can be calculated from such data. The abundance of the P2 transcript exceeds that of P1 mRNA by 3-4 fold in mid-log phase cells. The turnover rates of the two c-*myc* mRNA are essentially identical, indicating that the P2 promoter is 3-4 times stronger than P1. The expression of the two mRNAs decreases at different rates in glucocorticoid-treated cells. A 50% decrease in the abundance of P1 mRNA occurs within 1hr after addition of dexamethasone. Expression of P2 mRNA is reduced by 50% within 4hr. However, the turnover rates of the two major c-*myc* transcripts do not change in glucocorticoid-treated cells. The T1/2 of P1 and P2 mRNAs are about 25-30min and not different from the turnover rates measured in control cells. The data indicate that glucocorticoid regulation of expression of c-*myc* in P1798 cells is almost entirely mediated through changes in transcription. Furthermore, glucocorticoids influence c-*myc* promoter utilization. The P1 promoter is inhibited to a greater extend, although utilization of the P2 promoter is reduced by >75%. As consequence, the P2 promoter is 16-20 times stronger than P1 in glucocorticoid-treated P1798 cells. Coincident results were obtained in *in vitro* transcription experiment. The P2 promoter of the c-*myc* was the major transcription initiation site in control cells and this initiation was decreased by dexamethasone to a level below that of detection. The initiation of P1 transcription was below the level of detection in both control and dexamethasone-treated cells.

R 349 AN IMMUNOLOGICAL DISSECTION OF THE β'SUBUNIT OF E.coli DNA-DEPENDENT RNA POLYMERASE, Stefanie A. Margarson and Robert E. Glass. Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham. NG7 2UH. England.

Our aim is to apply specific, characterised immunological probes against E.coli RNA polymerase as a means to facilitate structure-function studies on this important, complex and highly conserved enzyme. The β' subunit of RNA polymerase, encoded by the rpoC gene, has been implicated in DNA binding and in the catalytic activity of the enzyme. Using both existing and engineered restriction sites in rpoC, discrete domains spanning the entire gene were subcloned into the bacterial high level expression vectors (pUEX) to produce β-galactosidase fusion immunogens.

Epitope mapping of the purified antibodies against the various domains confirms the specificity of each antibody population. Immunoprecipitation of both core and holoenzyme with the domain-specific antibodies allows the demonstration of regions of the β' subunit which are accessible. Subunit assembly is studied by immunoprecipitation of size-separated fractions with the domain-specific antibodies, and subsequent immunostaining with anti-holoenzyme. Finally, immunological probing of early transcription complexes monitors conformational changes occuring during DNA binding and transcription initiation. These antibodies should also prove valuable for phylogenetic studies of RNA polymerase.

Presented here are the results from a range of experiments designed to investigate the interactions and conformational changes of RNA polymerase as determined by our sequence-specific antibodies.

R 350 CHARACTERIZATION OF T7 RNA POLYMERASE BY LINKER INSERTION MUTAGENESIS, W.T. McAllister, L. Gross, W-J Chen, H. Gartenstein, and M. Orlova, Department of Microbiology, SUNY-Health Science Center, Brooklyn, New York 11203

Thirty-five mutants in the T7 RNA polymerase were generated by linker-insertion mutagenesis and characterized with respect to the following properties: ability to synthesize functional messenger RNA in vivo; temperature sensitivity; ability to bind specifically to promoter-containing DNA fragments, or non-specifically to random DNA fragments; ability to carry out various stages of transcription initiation at a T7 promoter; promoter-independent catalytic activity on a variety of synthetic homopolymers; and the ability to recognize a T7 termination signal. A number of polymerase mutants with interesting biochemical properties were identified.

T7 RNA polymerase is a multifunctional enzyme that is involved not only in synthesis of late mRNA, but also in other processes such as DNA entry, replication, packaging and concatamer resolution. One mutant was identified which is transcriptionally active (able to synthesize functional mRNA in vivo) but which is unable to complement T7 RNAP phage particles. This mutant is presumably defective in its ability to participate in one (or more) of these functions.

R 351 MECHANISTIC STUDIES OF TRANSCRIPTIONAL ACTIVATION IN VIVO ROLE OF TFIIB IN GA 14 DERIVATIVES AND E1A TRANSCRIPTIONAL ACTIVATION, Alejandro Merino, Ilho Ha, Noburo Horikoshi, Roberto Weinmann, and Danny Reinberg, Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635.

It has been shown that some Gal4 derivatives bearing the VP16 activating domain or the synthetic amphipathic alpha helix activating region, can stimulate transcription by stabilizing the binding of TFIIB to the preinitiation complex formed by TFIIA, TFIID and the TATA motif. Studies *in vitro* have shown that these types of activators bind directly to TFIIB and stabilize its association with the preinitiation complex. This interaction requires certain amino acid sequences present in TFIIB. We have performed experiments to further examine the role of TFIIB in transcriptional activation *in vivo*. We have used an eukaryotic expression vector bearing the cDNA of the human transcription factor IIB (TFIIB) attached to an epitope tag. The first set of experiments performed in HeLa cells were attempted to overcome the squelching effect given by overexpression of the Gal4VP16 chimeric protein. We have found that the cotransfection of high amounts of Gal4VP16 expression vector with TFIIB expression vector resulted in a higher expression of a Gal4 chimeric construct driving the CAT gene. This was also shown in 293 cells that constitutively express the adenovirus E1a protein. These results suggest that the squelching caused by overexpression of Gal4VP16 is overcome by overexpressing TFIIB. Interestingly, the basal levels of transcription of a chimeric construct containing multiple Gal4 binding sites and directing expression of the CAT gene were affected differently in 293 and HeLa cells. Increasing amounts of the TFIIB expression vector lowers basal transcription in 293 cells while it enhances transcription of the same promoter in HeLa cells. We have extended this analysis and found that E1a proteins can interact with TFIIB. This interaction has been mapped within the TFIIB sequence by mutational analysis.

R 352 STRUCTURE/FUNCTION RELATIONSHIPS IN THE NOVEL RNA POLYMERASE SIGMA FACTOR σ^{54}.

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The RNA polymerase sigma factor σ^{54} is unlike other prokaryotic sigma factors in a number of respects. Its amino acid sequence shows no homology to the major sigma factor family which includes σ^{70} and σ^{32}, and transcription initiation by $E\sigma^{54}$ is totally dependent on an activator protein such as NTRC or NIFA. The structural gene for σ^{54} (rpoN) has now been sequenced from twelve different species. The predicted amino acid sequences show a high degree of conservation and conform to a domain structure which we proposed in 1987.

We have identified two very conserved features in the primary amino acid structure: a helix-turn-helix motif and a totally conserved stretch of 8 residues both near the C-terminus of the protein. Site-directed mutagenesis has been used to investigate the role of both regions in σ^{54} activity.

Mutations of conserved residues in both regions result in proteins which no longer allow binding of $E\sigma^{54}$ to the glnAp2 promoter, indicating that both regions function in DNA recognition. A series of mutant derivatives of glnAp2 has been used to investigate the predicted interaction between these regions of σ^{54} and the promoter.

R 353 CO-PURIFICATION OF FACTORS BINDING TO THE MAJOR LATE INITIATION SITE (IAP and IBP) AND DOWNSTREAM SEQUENCE ELEMENT (DAP) OF THE SV40 LATE PROMOTER, Janet E. Mertz, Steven Wiley, Richard Kraus, Elizabeth Murray, Fengrong Zuo, Nancy Zink, and Karla Loritz, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

The SV40 late promoter contains three sequence elements necessary for transcription initiation at nt 325, the major cap site. One maps approximately 30 bp upstream, one spans the major late initiation site, and one maps approximately 30 bp downstream of the major initiation site. We have purified factors from HeLa cell nuclear extracts by affinity chromatography with an oligonucleotide homologous the major late initiation site. The resulting fraction, consisting predominantly of three proteins with apparent electrophoretic mobilities of ~45-55 kd, contains two activities, IAP and IBP, that protect distinct parts of the initiation site sequence from DNase I cleavage in the absence of any upstream binding activity. Specifically labeled initiation site sequence oligonucleotides can be UV crosslinked to a protein of ~50 kd electrophoretic mobility. Levels of RNA from *in vitro* transcribed templates containing initiation site point mutations do not correlate well with DNase I protection of the mutants by IBP. This extensively purified fraction also contains an activity, DAP (Ayer and Dynan, MCB, July 1990, p. 3635-3645), that protects the SV40 major late downstream sequence element from DNase I cleavage. The purified protein does not protect the initiation site or the downstream sequence elements of the murine DHFR or TdT promoters. However, the Ad-ML promoter E-box sequence, which is known to bind a family of transcription factors including MLTF/USF and myc, can be protected from DNase I cleavage by the purified protein. This sequence can also efficiently compete binding of IAP and DAP to the SV40 late promoter. We conclude the following: i) Binding of IAP and DAP may play a role in initiation at nt 325; the role of IBP is not clear; and ii) IAP and DAP may interact by protein-protein association to give high sequence specificity and stable binding as an early step in the formation of a transcription initiation complex at nt 325. Whether binding of IAP and DAP stabilizes binding of TFIID to the -30 region element (Wiley et al., these abstracts) remains to be determined.

R 354 THE ROLE OF THE RNA POLYMERASE β' SUBUNIT IN SELECTIVITY OF TRANSCRIPTION,

Yoshikazu Nakamura and Koichi Ito, Institute of Medical Science, University of Tokyo, P.O. Takanawa, Tokyo 108, Japan

E. coli RNA polymerase is composed of four different subunits, 2α, β, β' and σ. Among them, the role of the β' subunit is poorly understood. We have isolated a mutation in the *rpoC* gene encoding the β' subunit which suppresses temperature-sensitive growth of the *nusA11* mutant. This mutation, *rpoC10*, is a glutamate-to-lysine change at position 402 (E402K) and this increased positive charge appears to compensate for the increased negative charge present in *nusA11* (G181D). This suggests a physical or functional interaction between the two regions of β' and NusA. *In vivo* transcription assays reveal that the *rpoC10* mutation enhances termination at ρ-dependent terminators and reduces termination at ρ-independent sites. Moreover, *rpoC10* does not affect λ *N*-mediated antitermination but inhibits the *Q*-mediated antitermination. This inhibition is eliminated by overproducing the Q protein. These results can be interpreted as indicating that the β' subunit of RNA polymerase is involved in the selective recognition of transcription terminators, and its glutamate-402 residue plays a crucial role in this recognition as well as in the interaction with the Q protein.

R 355 MUTATIONAL ANALYSIS OF A SUBUNIT COMMON TO NUCLEAR RNA POLYMERASES (RNAPs) IN SACCHAROMYCES CEREVISIAE, SHAHRZAD NOURAINI, JACQUES ARCHAMBAULT, JAMES.D. FRIESEN, Department of Molecular and Medical Genetics, University of Toronto, Canada, and Department of Genetics, Hospital for Sick Children, 555 university Ave. M5G-1X8.

We have used yeast genetics to identify functionally important regions of a subunit (RPO26) common to yeast nuclear RNAPs. Our genetic screen is based on the observation that a combination of various viable mutations in RPO26 results in synthetic lethality when combined with a temperature sensitive (ts) linker-insertion mutation (rpo21-4) in the gene encoding the yeast RNAPII largest subunit (RPO21). To isolate functionally defective alleles of RPO26, a copy of the gene carried on a single-copy plasmid, was mutagenized with hydroxylamine in vitro, and introduced into a yeast strain that contained rpo21-4, and the chromosomal RPO26 expressed from the repressible GAL10 promoter. Mutagenized plasmids that did not support growth when the expression of chromosomal RPO26 was repressed (in presence of glucose), were selected. Analysis of the phenotype associated with these plasmids in a RPO21 wild type background, revealed three phenotypic classes: null, conditional lethal, and neutral. The DNA sequence of the RPO26 open reading frame (ORF) was determined for half of the mutant plasmids. All sequenced plasmids contained mutations in the RPO26 ORF. All of the mutationally altered amino acids resided in the C-terminal two-thirds of the protein. Furthermore, a truncated allele of RPO26, encoding only the C-terminal two-thirds of the subunit, can support growth in combination with either the wild type RPO21 allele or the rpo21-4 mutant allele. These results suggest that amino acids essential for the function of RPO26 reside in the C-terminal two-thirds of the subunit.

R 356 DNA METHYLATION OF A B1 REPETITIVE SEQUENCE PROMOTER LOCATED IN THE FIRST INTRON OF THE MOUSE AFP GENE DOWN-REGULATES AFP PROMOTER ACTIVITY, Karin Opdecamp, Michèle Rivière, Pierre Drèze, Josiane Szpirer and Claude Szpirer, Département de Biologie Moléculaire, Université Libre de Bruxelles, Rue des Chevaux, 67. B-1640 Rhode-St-Genèse. Belgium.
Alpha-foetoprotein synthesis is a liver-specific function expressed in an oncofoetal manner. DNA methylation pattern studies of the mouse AFP gene have shown a good correlation between AFP expression and hypomethylation of a MspI site located in the first intron of the gene (site M0). A functional analysis, based on transfections of *in vitro* methylated constructions containing cloned AFP sequences linked to the reporter CAT gene has shown that i) a 140 bp fragment which contains the M0 site behaves as a cis-acting positive regulatory element stimulating the activity of the AFP promoter and ii) methylation at the M0 site suppresses this positive action. We showed by using band shift assays that methylation at the M0 site does not prevent the binding of nuclear factors to the 140 bp fragment and we assume that inhibition of AFP promoter activity by DNA modification is due to an indirect mechanism based on structural features. The 140 bp fragment corresponds to a B1 repetitive sequence which was shown by Tilghman and coworkers to be transcribed in antisense direction. The M0 site being located very close to the promoter of this B1 element, methylation at the M0 site might inhibit transcription of the B1 element which in turn might down-regulate AFP expression by the establishment of an inadequate conformation around the AFP promoter.

R 357 A YEAST MULTIFUNCTIONAL REGULATORY PROTEIN RAP1 BINDS TO A CONSERVED SEQUENCE IN ANIMAL VIRUS ENHANCERS. Alessandra Pollice†, John Pulitzer† Eric Gilson* and Susan Gasser* I.I.G.B †, via G. Marconi 10,. Naples, 80125, Italy and I.S.R.E.C.*, CH1066 Epalinges, Lausanne, Switzerland.
RAP1, is a scaffold associated multifunctional protein that plays a central role in the biology of **Saccharomyces cerevisiae.** Besides being involved in the transcriptional activation of many genes and in the silencing of others, RAP1 is important for chromosome maintenence. In dissecting the polyoma-virus enhancer to determine which sequences are essential for the ability (shown by us and others) to drive transcription in yeast, we found that a sequence corresponding to the binding site for yeast factor RAP1 is responsible for activation when linked to certain promoter contexts. We show that it is indeed RAP1 that binds to this site and is probably responsible for transcriptional activation in yeast. The RAP-binding sequence is conserved in SV40 and BPV enhancers as well as in the enhancer-like element in the yeast retrotransposon TY1. Enhancement of transcription in yeast by foreign promoter elements has been attributed to binding of yeast proteins that are homologues of higher eukaryote transcriptional activators. With this in mind we are currently studying proteins that bind to this enhancer site in mammalian cells.

R 358 TRANSCRIPTIONAL REGULATION OF THE PREPROTACHYKININ PROMOTER, S. Mendelson, C. Morrison, E. Clark, J. Morrow and J. Quinn. MRC Brain Metabolism Unit, Royal Edinburgh Hospital, Morningside Park, Edinburgh, EH10 5HF.

The rat preprotachykinin-A gene (PPT) is expressed in a very restricted population of cells, including a subset of dorsal root ganglia (*drg*) neurons, where its expression is regulated by nerve growth factor. No suitable cell line model is currently available which expresses from either the endogenous promoter or fragments of the PPT promoter linked to a reporter gene. We have initiated a functional dissection of the PPT promoter and a biochemical characterisation of the proteins which recognise the PPT promoter in various cells including *drg*. Several DNA elements have now been identified, some of which are recognised by neuronal-specific proteins. The characterisation of these potential regulatory elements provides a basis to elucidate the mechanism of PPT transcriptional regulation.

R 359 ACTIVATION OF CLASS II GENES, Alejandro Merino and Danny Reinberg, Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635.
The mechanism(s) by which different activators stimulate basal levels of transcription is not clear. Studies have demonstrated that basal transcription, as mediated by the TATA and/or Initiator (Inr) motifs, requires seven factors in addition to RNA polymerase II. Activators such as VP16 appear to directly contact some of the general transcription factors such as TFIIB and TFIID. Interestingly, while the interaction can be demonstrated *in vitro*, the addition of the activator to a reconstituted transcription system containing native human TFIID resulted in no activation. We were able to demonstrate that reconstitution of activation *in vitro* was dependent on three different protein fractions. Two of these protein fractions contain factors that repress basal levels of transcription (Dr-1 and Dr-2). These factors interact with TFIID and have been extensively purified. We found that the Dr-2-mediated repression can be overcome by TFIIA and/or an acidic activator. On the other hand, Dr-1 was found to displace the interaction between TFIID and TFIIA resulting in repression of transcription. The third fraction (ACF-1) appears to be necessary for activation. The role of ACF-1 in mediating activation of transcription is currently being analyzed.

R 360 Mapping the active site of yeast RNA polymerase B(II), Michel Riva[1], Christophe Carles[1], Isabelle Treich[1], E. F. Zaychikov[2], A.A. Mustaev[2],and André Sentenac[1], 1: Service de Biochimie et de Génétique Moléculaire, DBCM, Centre d'Etudes de Saclay, F91191 Gif sur Yvette cedex, France, 2: Limnological Institute of the Siberian Branch of the Academy of Sciences, Irkutsk, USSR

Yeast RNA polymerase B(II) was affinity labelled by using two different nucleotide derivatives (III and VIII) specifically reacting with free amino-group of lysyl residues. With both derivatives, the second largest subunit, i.e. B150, was found exclusively labelled. The affinity label was localized in the C-terminal part of the subunit by microsequencing a proteolytic fragment, then further mapped by a combination of extensive or single-hit chemical cleavage reactions and analysis of the peptide patterns. For derivative III, the label was mapped between Asn^{946} and Met^{999} within region H, one of the 9 conserved domains between B150 and the bacterial β subunit. Five lysyl residues at position 962, 965, 972, 979, and 987 could be the target of the affinity reagent. *In vitro* mutagenesis of these residues indicated that, at least, one of the two lysines 979 or 987 was labeled. Derivative VIII also labelled domain H together with the adjacent C-terminal conserved domain I. Labelling in domain I could be abolished by *in vitro* mutagenesis of the lysine 1102 indicating that this residue is the label target site. These results underscore the conservative evolution of eukaryotic and bacterial RNA polymerases.

R 361 COOPERATION BETWEEN THE ELEMENTS OF THE ELASTASE I ENHANCER IN TRANSGENIC MICE. Scott D. Rose, Fred Kruse, Galvin H. Swift, Robert E. Hammer, and Raymond J. MacDonald. University of Texas Southwestern Medical Center, Dallas, Tx 75235. The elastase I gene transcriptional enhancer (-205 to -72), which directs pancreas-specific transcription, has three functional elements: A, B, and C. A minimum of two elements are required for enhancer activity *in vivo*. Analysis of individual elements *in vivo* has shown that the A element directs acinar cell specific transcription, the B element directs islet β cell transcription, and the C element alone is inactive. To examine the cooperative interaction between the elements, human growth hormone reporter gene constructs containing triplicated pairwise combinations of the A, B, and C elements [$(CA)_3$, $(CB)_3$, $(AB)_3$] driving the elastase I promoter were introduced into the germline of mice. While the C element by itself is inactive, coupling it to either the A or B element increases both the average level of pancreatic expression (10 fold) and the probability that a given transgeic mouse will express the transgene, without altering the cell-type specificity of expression. Additionally, the $(AB)_3$ transgene displays additive cell-type specificity, as expression of the transgene is seen in both the exocrine acinar cells and the endocrine islet β cells, as well as a synergistic increase in expression. From these results we conclude that a pancreatic enhancer requires two element types, one that directs cell-specificity and one that augments enhancer activity. For the EI enhancer the A element is the acinar cell-type determinant, the B element has a dual role as either a general augmentor in the exocrine cells or a β cell-type determinant in islets, and the C element is a general augmentor. In the endogenous gene the β cell expression is silenced by a repressor domain immediately upstream of the enhancer.

R 362 ROLE OF HEAT SHOCK PROTEINS IN BACTERIOPHAGE MU LATE TRANSCRIPTION, Olivier Sand and Ariane Toussaint, Unité de transposition bactérienne, Laboratoire de Génétique, Université Libre de Bruxelles, 67 rue des chevaux, B1640 Rhode-St-Genèse, Belgium.

Transcription of bacteriophage Mu late functions is activated by the phage encoded C protein. The operon wich contains the C gene constitutes a middle transcript, between early and late transcripts. Late functions are responsible of host lysis, morphogenesis and DNA modifications. Some host heat shock proteins also play a role in Mu late transcription which is reduced in mutants of the heat shock gene *dnaK* at high temperature. Late transcription on Mu phages deleted from a part of their genome is also reduced in hosts mutated in the heat shock genes *dnaJ* and *grpE*. Heat shock proteins are known to interact with other proteins involved in most cellular events, to allow correct folding, subunit assembly, and nucleoprotein complex formation, to protect from stress, and to refold denatured proteins.

Our work aims at understanding where and how DnaK, DnaJ and GrpE, the products of the three heat shock genes mentionned earlier, act during Mu late function expression.

We found that DnaK, DnaJ and GrpE act beyond C synthesis and only at elevated temperature. C stability seems to be affected, at least in a *dnaK* strain. Experiments are in progress to define the reason and the meaning of C instability.

R 363 THE BIDIRECTIONAL REGULATORY MECHANISM OF DROSOPHILA ras2 / rop EXPRESSION, Orit Segev, Kurt Lightfoot, Lydia Maltby and Raqual Duarte, Department of Zoology, Witwatersrand University, Johannesburg, RSA

The Drosophila ras2 promoter region exhibits bidirectional activity as was demonstrated for the human c-Ha-ras1 , the mouse c-Ki-ras and the Drosophila ras1 promoters. Our studies indicate that the Drosophila ras2 promoter region shares with the human c-Ha-ras1 promoter a CACCC - box and an Ap-1 - like sequence which confers TPA inducibility. A specific transcription factor (factor B) binds the CACCC motif and can form a hetero-dimer with another specific DNA - binding protein (factor A). This large main complex was detected using embryo extract and S2 tissue culture cells, whereas a distinct protein factor which binds the ras2 promoter was observed in pupae and larvae stages. The binding sites (A+B) for these two protein factors were found to be essential for 100% expression of both genes flanking the promoter (ras2 and rop). Factor A was shown to interact very poorly with the full promoter region, while very high affinity was observed upon binding to single-stranded region A. By forming a complex with factor B, binding efficiency can be enhanced. Region A consists of three overlapping concensus sequences: a TATA-like element, a CArG-like motif (the core sequence of the Serum Response Element) and a DRE octamer which was shown to have a role in cell proliferation. Recent results show that alterations of DNA - protein binding specificities can be achieved by supplementing the growth media with different sera. We also suggest a role for species specific coactivators in ras2 transcriptional regulation.

R 364 VERTEBRATE U6 snRNA TRANSCRIPTION: THE CHARACTERIZATION OF A PSE BINDING PROTEIN
Kenneth A. Simmen, Jordi Bernués, Sam Gunderson, Joe Lewis and Iain W. Mattaj, The European Molecular Biology Laboratory, Heidelberg, D-6900, Germany.
The promoters of vertebrate U6 genes are essentially bipartite, comprising a proximal region and an upstream distal sequence element. In the proximal region lie two essential promoter elements,- a TATA motif at ~-25 and the proximal sequence element (PSE) at ~-60. We have previously shown that the TATA motif is an important determinant of the pol III specificity of U6 expression, and that recombinant TFIID is required for pol III transcription of human U6 in vitro. Given the role of TFIID in pol II transcription, TFIID bound at the TATA box must presumably act in conjunction with other factors to generate pol III specificity at the U6 promoter. In approaching which factors these may be, we have been examining a PSE binding activity (PBP, see Waldschmidt et al. 1990, *EMBO J.*, 10, 2595-2603) from a HeLa fraction known to be required for U6 transcription. In collaboration with R. Waldschmidt and K. Seifart we have established a correlation between the affinity of U6 PSEs for this factor and their transcriptional activity in HeLa cells and extracts. The transcriptional inactivity of the Xenopus U6 promoter in HeLa cells and extracts is attributable to the PSE, and its low affinity for human PBP. We have investigated the length and sequence requirements for binding of PBP to the human U6 promoter. PBP binding exhibits several unusual features. The DNAse I protection is large (30-40bp), and secondly, specific binding requires sequences outwith the PSE itself. The ability of PBP to interact with the U6 promoter and other U6 associated transcription factors is being studied by both in vitro transcription and binding studies.

R 365 Regulation of cJun activity by phosphorylation: implications for the mechanisms controlling the oncogenic activation of an transcription factor.
Tod Smeal*, Bernard Binetruy**, Daniel Mercola#, Michael Birrer^, and Michael Karin**. Departments of Pharmacology**, Biology* and Pathology#, Center for Molecular Genetics, University of California, San Diego, School of Medicine, La Jolla, Ca 92037 and Division of Cancer Prevention and Control^, National Cancer Institute Bethesda, MD 20814
In vivo, cJun is phosphorylated on at least five sites, three of which, reside within a single tryptic peptide flanking cJun's DNA binding domain and negatively regulate cJun's ability to bind DNA, Boyle et. al., Cell 64,573 (1991). The other two sites are in cJun's transcriptional activation domain and their phosphorylation is increased by activated ras, which also increases this domains transcriptional activity, Binetruy et. al., Nature 351, 122 (1991). cJun is hyperphosphorylated by a variety of transforming oncoproteins expressed transiently and stably. The n-terminal sites of human cJun map to serines 63 and 73. These sites have similair environments (L-T/A-S-P-D/E-V/L) and seem to be coordinately regulated. Upon replacement of the serines 63 and 73 by alanine, cJun's activation domain fails to respond to transforming oncoprotiens. Furthermore, these mutations block cJun's ability to cooperate with activated ras in the transformation rat embryo fibroblasts. Thus the dynamic regulation of cJun's DNA binding and transcriptional activities allows cJun to convert transient proliferative signals into changes in gene expression.

R 366 REGULATION OF THE ACTIVITY OF THE YEAST PGK UAS BY THE CO-ACTIVATOR GAL11.
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The transcriptional control exerted by the yeast PGK UAS has been shown to require the activity of both of the multifunctional nuclear factors RAP1 and ABF1 (Stanway et al., 1989; Chambers et al., 1989; Chambers et al., 1990). We have investigated the role of the co-activator GAL11 (Suzuki et al., 1988; Himmelfarb et al., 1991) in the process of transcriptional regulation by the PGK UAS and we have shown that it plays a significant positive role. Further analysis has revealed that this transcriptional effect is mediated through RAP1, not ABF1. Notably there appears to be a reduction in RAP1 DNA binding activity in a gal11 strain. These data suggest that GAL11 may influence a wide range of transactivator proteins, in addition to zinc finger containing activators such as GAL4 and PPR1.

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Stanway, C.A. et al. (1989) Nucl. Acids Res. 17, 9205-9218.
Suzuki, Y. et al. (1988) Mol. Cell Biol. 8, 4991-4999.

R 367 AN UPSTREAM NEGATIVE MODULATORY ELEMENT AFFECTS THE *IN VIVO* AND *IN VITRO* EXPRESSION OF A HUMAN SERINE TRANSFER RNA GENE,
Richard I. Tapping, Dan E. Syroid and John P. Capone, Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5
Due to isoacceptor transfer RNA redundancy it is difficult to demonstrate the *in vivo* activity of a particular gene. Presently, we are utilizing a system developed in our lab in which the phenotypic expression of a human serine amber suppressor tRNA gene can be quantitated *in vivo* by assaying the extent to which it is able to suppress an amber nonsense mutation in the *Escherichia coli* chloramphenicol acetyltransferase gene following cotransfection in mammalian cells. Through utilization of the above *in vivo* assay we tested the suppression activity of various extragenic deletion mutants of this gene. These assays revealed a negative element located immediately upstream of the gene whose deletion results in increased *in vivo* suppression. We have further characterized this element by performing *in vitro* transcription, competition and commitment studies with Hela cell nulcear extracts and have determined that this negative modulatory element is affecting the ability of this transfer RNA gene to sequester transcription components. This system thus provides a functional assay to study the regulation of mammalian tRNA gene expression *in vivo*.

Supported by the Medical Research Council of Canada.

R 368 EFFECTS OF CHROMATIN STRUCTURE AND PROTEIN MODIFICATION ON THE ACTIVITY OF *Acanthamoeba castellanii* RNA POLYMERASE I, Christopher Terpening, Philippe Georgel*, Borries Demeler*, Kensal van Holde*, and Marvin Paule, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523 and *Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331

The transcriptional activity of ribosomal RNA gene repeats in eukaryotes is thought to be affected by two different mechanisms. The first is a competition, presumptively with the chromatin proteins, for formation of protein complexes at the promoter. Sequences from the 5S RNA gene of *Xenopus laevis* have been demonstrated to position nucleosomes at defined locations *in vitro*. Multiple copies of these sequences were positioned downstream of the *Acanthamoeba castellanii* ribosomal RNA promoter. When nucleosomes were reconstituted on this closed circular plasmid in the absence of pol I and its species-specific transcription factor TIF-IB, aberrant positioning was noted. However, reconstitution with the prior presence of pol I and TIF-IB led to correct nucleosome positioning. Furthermore, pol I was able to transcribe the 5S gene following reconstitution as determined by primer extension analysis. The second mechanism involves modification of the polymerase itself, preventing its binding to the promoter. It has been suggested by others that ADP-ribosylation may activate pol I. This is intriguing in light of the fact that the *E. coli* RNA polymerase α subunit, a homolog of the 39 kDa subunit of *Acanthamoeba* pol I, is ADP-ribosylated during phage T4 infection. The 39 kDa subunit has been shown to be altered in its mobility in a SDS-polyacrylamide gel in concert with pol I inactivation during encystment. The transcriptionally inactive form shows increased mobility, which would be consistent with removal of an ADP-ribose moiety. However, treatment of the active polymerase with phosphodiesterase to remove the presumptive ADP-ribose moiety does not alter the activity of the polymerase in a specific *in vitro* transcription assay.

R 369 TWO PROTEINS THAT BIND TO THE HUMAN P53 AND HSV VP16 TRANSCRIPTIONAL ACTIVATION DOMAINS.

Ray Truant, C. James Ingles and Jack Greenblatt. Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6.

The mammalian tumor suppressor gene, p53, and the HSV VP16 virion protein gene both encode a transcriptional activation domain rich in acidic amino acids. Human p53 and VP16 were produced as fusion proteins in *E.coli* and used as ligands for affinity chromatography with HeLa cellular extracts. We identified two proteins, one >100 KDa and another 33 KDa, that bound specifically to wild type and not to mutant p53 derived from a tumor. Further affinity chromatography experiments with N and C-terminal truncations of p53 mapped the binding of the >100 KDa and 33 KDa proteins to the N-terminal 73 amino acid transcriptional activation domain of p53. The p53 mutant that we tested, (RH273), was not within this N-terminal domain, suggesting that tumorigenic p53 point mutations may change the conformation of p53 so that cellular proteins cannot bind to p53's activation domain. The >100 KDa and 33 KDa p53-binding proteins also bind to the activation domain of wild type VP16. The >100KDa protein was further purified to homogeneity and shown to bind directly to p53 and VP16. Experiments in progress should determine the exact identities of these acidic activator binding proteins as well as their roles in transcriptional activation and tumor suppression.

R 370 Stimulatory Mechanism, Characterization and Cloning of a Transcription Enhancer in Yeast.

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Previously our laboratory purified a trans-acting factor from *S. cerevisiae*, yIBF, which binds the internal enhancer sequence (IES2) of the Rous sarcoma virus (Karnitz et al., JBC 265:6131). *In vitro*, binding of yIBF to IES2 is absolutely dependent upon the phosphorylation state of the purified protein. *In vivo*, three repeats of IES2 inserted upstream of a *lacZ* reporter gene stimulate transcription as strongly as the GAL10 UAS. Stimulation of *lacZ* transcription by IES2 is independent of orientation and increases synergistically with IES2 concatamerization. yIBF binding to IES2 concatamers is non-cooperative by Hill Plot analysis. *In vivo* footprinting analysis of promoters containing one, two, or three IES2 repeats suggests that all IES2 sites are occupied. Therefore, synergistic stimulation of transcription for multiple binding sites most likely results not from increased occupancy, but rather an increased surface coverage which stimulates an increased frequency of transcription initiation.

We obtained a putative yIBF genomic clone by screening an expression library. Increased expression of this gene stimulates IES2 controlled *lacZ* reporter genes *in vivo*. Amino acid sequence analysis reveals an acidic peptide with a putative calcium binding EF-Hand motif.

R 371 ASP537, ASP812 ARE ESSENTIAL AND LYS631, HIS811 ARE CATALYTICALLY SIGNIFICANT IN T7 RNA POLYMERASE ACTIVITY, A-Young M. Woody, Patricia A. Osumi-Davis, Marcela de Aguilera, and Robert W. Woody, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523

To define catalytically essential residues of T7 RNA polymerase, we have generated five mutants of T7 RNA polymerase, D537N, K631M, Y639F, H811Q, and D812N, by site-directed mutagenesis and purified them to homogeneity. The choice of specific amino acids for mutagenesis was based upon photoaffinity labeling studies with 8-azidoATP and homology comparisons with the Klenow fragment and other DNA/RNA polymerases. Secondary structural analysis by CD indicates that the protein folding is intact in these mutants. The mutants D537N and D812N are totally inactive. The mutant K631M has 1% activity, confined to short oligonucleotide synthesis. The mutant H811Q has 25% activity for synthesis of both short and long oligonucleotides. The mutant Y639F retains full enzymatic activity although individual kinetic parameters are somewhat different. Kinetic parameters, $(k_{cat})_{app}$ and $(K_m)_{app}$ for the nucleotides, reveal that the mutation of Lys to Met has a much more drastic effect on $(k_{cat})_{app}$ than on $(K_m)_{app}$, indicative of the involvement of K631 primarily in phosphodiester bond formation. The mutation of His to Gln has effects on both $(k_{cat})_{app}$ and $(K_m)_{app}$, namely 3- to 5-fold reduction in $(k_{cat})_{app}$ and 2- to 3-fold increase in $(K_m)_{app}$, suggesting that His811 is involved in both nucleotide binding and phosphodiester bond formation. Mutations from Lys to Met and His to Gln have little effect on the $(K_m)_{app}$ for the template. We have shown that amino acids D537 and D812 are essential, that amino acids K631 and H811 play significant roles in catalysis, and that the active site of T7 RNA polymerase is composed of different regions of the polypeptide chain. Possible roles for these catalytically significant residues in the polymerase mechanism are discussed.

R 372 INTERACTION OF THE τ_1 TRANSACTIVATION DOMAIN OF THE HUMAN GLUCOCORTICOID RECEPTOR WITH THE TRANSCRIPTIONAL APPARATUS, Anthony P.H. Wright, Iain J. McEwan, Karin Dahlman-Wright, and Jan-Åke Gustafsson, Center for Biotechnology and Department of Medical Nutrition, Karolinska Institute, NOVUM, Huddinge University Hospital, Huddinge S-141 57, Sweden.

The glucocorticoid receptor (GR) is a member of a large family of ligand dependent sequence-specific transcription factors, that includes receptors for other steroid hormones, thyroid hormones, vitamin D_3 and retinoic acid. After the binding of it's cognate ligand and DNA response element the subsequent events whereby the GR effects the rate of target gene expresssion remain unclear. However, protein-protein interactions are likely to play a major part in this process. We have studied the role of protein-protein interactions in the mechanism of action of the τ_1 transactivation domain, using recombinant receptor proteins expressed in bacteria and yeast cells. Over expression of the τ_1 domain in yeast cells resulted in an inhibition of reporter gene activity (squelching) and severe impairment of cell growth. Kinetic studies revealed that the effect on cell growth occurred subsequently to the effect on gene expression. Analysis of different promoter constructs suggested that the τ_1 transactivation domain contacted directly a factor(s) required for the activity of a basal promoter. The implications of these studies for GR action together with results from *in vitro* studies will be discussed.

R 373 CYTOPLASMIC RETENTION OF THE PRECURSOR FOR THE P50 NF-κB SUBUNIT BY INTRAMOLECULAR MASKING OF A NUCLEAR LOCATION SIGNAL, Ulrike Zabel, Thomas Henkel, Karen van Zee, Judith M. Müller, Georg J. Arnold, Ellen Fanning and Patrick A. Baeuerle, Laboratory for Molecular Biology, Gene Center, Am Klopferspitz 18a, D-8033 Martinsried, F.R.G.

Nuclear factor κB (NF-κB) is an inducible transcription factor involved in activation of genes during inflammation and immune response. NF-κB binds as a heterodimer consisting of a 50 kD- and a 65 kD-subunit (p50, p65) to DNA. In most cell types, NF-κB exists as a non-DNA binding, cytoplasmic complex by association with the inhibitory subunit IκB.

The p50 subunit of NF-κB is synthesized as a non-DNA binding precursor protein of 110 kD (p110). p50 is contained in the N-terminal half of p110. We raised various anti-peptide antisera against p110 to investigate the subcellular distribution of overexpressed p50 and p110 by immunfluorescence and Western blot analysis. Transfected cells showed an exclusively nuclear location of p50, dependent on an intact nuclear location signal (NLS). In contrast to p50, p110 was located in the cytoplasm, although it contains the same NLS as p50. We could show that only p50, but not p110, is recognized by antibodies specific for the NLS sequence. Accessibility of the p110 NLS could be restored by denaturation or C-terminal truncation of p110. A p110 mutant lacking the C-terminal 191 amino acids was detected in the nucleus. This mutant contained all but one incomplete ankyrin repeat suggesting that the repeats are not required for cytoplasmic anchoring of the precursor.

These data led us to propose an intramolecular mechanism in which the C-terminal half of p110, which is linked to the p50 portion by a glycine-rich hinge, covers the NLS-motif in the p50 portion.

Although a week interaction between free C-terminus and p50 could be detected by immunocoprecipitation, we have no evidence that the C-terminal part of p110 alone can function as an IκB-like protein.

R 374 TFIIJ, THE EVENTS LEADING TO THE FORMATION OF AN OPEN COMPLEX AND THE MECHANISMS BY WHICH DEFINED ACTIVATORS STIMULATE TRANSCRIPTION, Leigh Zawel, Prussana Kumar, Jeanne Pierre Etchegaray, Ilho Ha and Danny Reinberg, Department of Biochemistry, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635.

Reconstitution of basal transcription requires seven general transcription factors (TFIID, IIA, IIB, IIE, IIF, IIH, IIJ) in addition to RNA polymerase II. Using recombinant TFIID, TFIIB, TFIIE and TFIIF and highly purified TFIIH, TFIIA and TFIIJ we have found that the requirement for TFIIA depends upon the source of TFIID. TFIIA, which has been purified to virtual homogeneity and consists of polypeptides of 34, 19 and 14 kDa, is not required and has no effect in reactions reconstituted with recombinant IID (TBP). When TBP is replaced with native TFIID, TFIIA stimulates transcription.

Our studies resulted in the discovery of a novel basal factor, TFIIJ, which is absolutely required when recombinant TBP is used to reconstitute transcription. When native TFIID is used in reconstituted transcription systems, the requirement for TFIIJ varies with the purity of the TFIID preparation used. Whereas a system containing crude TFIID exhibits no requirement for TFIIJ, transcription with more purified TFIID preparations is TFIIJ dependent, suggesting that TFIIJ interacts with either the core TBP or one of TBP-associated factors.

The mechanisms by which the GTF's and RNAPII assemble into a functional transcription initiation complex are unclear. Studies using gel mobility shift assays have indicated that preinitiation complex assembly proceeds in a highly ordered fashion. We have extended these studies using a template challenge format and have characterized the promoter sequences which contact preinitiation complex intermediates using DNase I and chemical footprinting. We are currently investigating the factor or factors necessary for the formation of the open complex. Subsequent to the formation of the open complex, we are also investigating (a) which factors remain at the promoter and which factors travel along the template with RNA polymerase II, and (b) the ability of defined activators to sequester components of the basal machinery at the promoter for reinitiation.

R 375 CHARACTERIZATION OF THE HEPATITIS B VIRUS X- AND NUCLEOCAPSID GENE TRANSCRIPTIONAL REGULATORY ELEMENTS, Pei Zhang, Anneke K. Raney and Alan McLachlan, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037

The regulatory DNA sequence elements that control the expression of the hepatitis B virus (HBV) X- and nucleocapsid genes in the differentiated human hepatoma cell lines, Huh7, Hep3B, PLC/PRF/5, and HepG2, the dedifferentiated human hepatoma cell line, HepG2.1, and the human cervical carcinoma cell line, HeLa S3, were analyzed using transient transfection assays. In this system, the hepatitis B virus enhancer I increases transcription from the X-gene promoter approximately five- to ten-fold in the differentiated hepatoma cell lines. In the dedifferentiated hepatoma cell line, HepG2.1, the enhancer I sequence does not appear to influence the level of transcription from the X-gene promoter. The enhancer I sequence increases transcription from the X-gene approximately two- to three-fold in HeLa S3 cells. In all of the cell lines, the X-gene minimal promoter element was within a 155 nucleotide sequence located between coordinates 1222 (-88) and 1376 (+67). The enhancer I sequence increased transcription from the nucleocapsid promoter approximately three- to ten-fold in the Huh7, Hep3B, PLC/PRF/5, and HeLa S3 cell lines, whereas it had little influence on the level of transcription from this promoter in HepG2 and HepG2.1 cells. The minimal nucleocapsid promoter element was within a 105 nucleotide sequence located between coordinates 1700 (-85) and 1804 (+20). This indicates that the levels of transcription from the X- and nucleocapsid gene promoters are determined in a cell-type specific manner, in part, by the hepatitis B virus enhancer I and the corresponding minimal promoter sequence. In the case of the dedifferentiated hepatoma cell line, HepG2.1, it appears that the enhancer I sequence does not influence the level of transcription from either the X- or nucleocapsid gene promoters. A model derived from these observations has been developed to account for the coordinate and cell-type specific regulation of HBV X- and nucleocapsid gene expression.

Regulatory Mechanisms in Transcription Elongation and Termination; Chromatin Structure and Transcriptional Regulation

R 400 TERMINATION OF TRANSCRIPTION BETWEEN HUMAN COMPLEMENT GENES C2 AND FACTOR B, Rebecca Ashfield, Helen Brown and Nick J. Proudfoot, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK.

The C2 and Factor B genes are separated by only 420bp of non-transcribed DNA. We postulate that termination signals exist in the intervening sequences of closely spaced genes to prevent transcriptional interference between them. We have defined a 120bp sequence 3' of the C2 poly(A) site which behaves as a termination signal when placed between two poly(A) signals, by favouring usage of the weaker, upstream signal. This sequence binds a protein, ME1a1, which is partially, but not wholly, responsible for this effect. We have evidence that the protein bends DNA, and this may explain the requirement for sequences outside the binding site. ME1a1 binds to the c-myc P2 promoter, where it appears to control promoter usage (K. Marcu and S. Bossone, personal communication). We have identified a second termination signal between closely spaced human genes g11 and C4, which also contains a binding site for ME1a1; we postulate that this protein may be widely used as a termination factor.

R 401 INTERACTIONS OF ELONGATING RNA POLYMERASE III WITH ITS TEMPLATE AND WITH TFIIIC, C. Bardeleben, H. Matsuzaki, G. A. Kassavetis, and E. P. Geiduschek, Department of Biology and Center for Molecular Genetics, University of California, San Diego, La Jolla, California 92093-0634

An *in vitro* transcription assay was used to analyze the kinetics of RNA chain elongation and the pausing pattern of RNA polymerase III (pol III) from *S. cerevisiae* on the SUP4 tRNA Tyr gene. Ternary complexes, arrested at a precise position, were formed by omission of one nucleotide and elongation was resumed synchronously by addition of the missing nucleotide . The pausing pattern was analyzed to determine which pauses are caused by sequence or by proteins bound to the gene. Addition of an A or U residue to a RNA chain with an UMP residue at its growing end is slow. No pauses were eliminated when ITP replaced GTP as substrate, suggesting that RNA secondary structure is not a major contributor to pausing.

The effect of bound protein on pausing was analyzed by examining the effect of treatments (Sarkosyl or high salt) that strip bound proteins. Only one pause, at bp54, 16 bp upstream of *box B*, was slightly affected. Using purified proteins and the ability of purified pol III to initiate on the ends of DNA molecules with a 3' overhang, this difference at bp54 was found to be a TFIIIC effect. Using the same initiation scheme, pol III and TFIIIC were found to interact in an orientation-dependent manner. Furthermore, it was determined that TFIIIC is released from its DNA-binding site during transcription. These results support the model that the DNA-binding protein TFIIIC is especially adapted to allow the rapid passage of pol III during RNA synthesis.

R 402 DNA CpG Methylation Inhibits Binding of NF-kB Proteins to the HIV LTR Cognate DNA Motifs Daniel P. Bednarik*, Colin Duckett+, Patricia, C. Guenthner, Song U. Kim, Kevin Griffis, Victor L. Perez, and Thomas M. Folks; Retrovirus Diseases Branch, Molecular Genetics Section, Centers for Disease Control, 1600 Clifton Road, Atlanta, Georgia 30333.

The regulation of cellular or viral gene expression is directly influenced by the pattern of methylated cytosine residues localized in the DNA of enhancer/promoter sequences. The mechanism of transcriptional silencing has been attributed to either an indirect model in which densely methylated DNA is recognized by proteins that may displace crucial transcription factors, or a direct model in which binding of a single transcription protein is prevented by the presence of a methyl CpG localized in a sensitive region of a DNA motif. In this study, we have determined that methylation of the core CpG located between the HIV LTR NF-kB repeated motifs can inhibit the binding of the NF-kB protein complex in crude nuclear extracts and specifically inhibit the binding of purified, recombinant p50 protein. We have employed the electrophoretic mobility shift assay (EMSA) and DNase I footprinting analysis to demonstrate that binding of the NF-kB proteins to their cognate motifs can be inhibited via the direct model proposed for methylation-mediated inhibition of DNA-protein interaction.

R 403 A CANDIDATE TRANSCRIPTION TERMINATION FACTOR BINDS THE C-MYC PROMOTER, David Bentley, and Sadia Roberts, ICRF, P.O. Box 123 Lincoln's Inn Fields, London , WC2A 3PX

Attenuation of transcription in the c-myc gene is an important control mechanism which is disrupted in Burkitt's lymphoma cells. This deregulation is linked to a switch from the P2 to the P1 promoter. P1 transcripts are not attenuated at the previously identified sites of premature termination near the end of exon 1 (Spencer et al. Genes and Dev.4, 75, 1990). We have found that P1 transcripts do not escape attenuation, but are in fact prematurely terminated at two sites T1A and T1B on either side of the TATA box of the P2 promoter in injected Xenopus oocytes and a Hela nuclear extract. Termination at T1A is disrupted by point mutations in the TATA box. A 28 b.p. oligonucleotide containing the TATA box is sufficient to cause accurate termination. We have identified a DNA-binding activity distinct from TFIID which binds this site with specificity that correlates with terminator function. The function of the T1 terminator in Burkitt's lymphoma cells will be discussed.

R 404 THE ROLE OF THE PHO85 GENE PRODUCT IN REPRESSION OF ACID PHOSPHATASE SYNTHESIS IN *SACCHAROMYCES CEREVISIAE* ,
Lawrence W. Bergman and David L. Johnson, Department of Microbiology and Immunology, Hahnemann University School of Medicine, Broad & Vine, Philadelphia, PA 19102.

The PHO85 gene product is required for the transcriptional repression of the yeast phosphate-repressible acid phosphatase (encoded by the PHO5 gene), under conditions of excess inorganic phosphate. The sequence of the coding region of PHO85 is highly homologous (approximately 52% identical) to the CDC28 gene of *Saccharomyces cerevisiae* , suggesting that PHO85 encodes a protein kinase. Mutation of a conserved Lys residue, involved in ATP binding in other protein kinases, causes constitutive synthesis of PHO5. This implicates the kinase activity of PHO85 in the repression of PHO5 transcription. Interestingly, mutation of a conserved Tyr residue in PHO85 also affects PHO5 expression, albeit to a lesser extent than the Lys mutation. To determine the domain of PHO85 responsible for the specificity of the PHO85 kinase, we have constructed a series of fusions between the CDC28 gene and the PHO85 gene. These fusions were then assayed for their ability to complement a *pho85* mutation and repress PHO5 expression. Finally, deletion of the PHO85 gene alters the cell's ability to utilize certain carbon sources suggesting the PHO85 kinase may have multiple substrates and function in both phosphate metabolism and carbon metabolism.

R 405 CONTROL OF THE EXPRESSION OF MOUSE RIBONUCLEOTIDE REDUCTASE R1 AND R2 SUBUNITS DURING THE CELL CYCLE,
Stefan Björklund and Lars Thelander, Department of Medical Biochemistry and Biophysics, Umeå University, S-901 87, Sweden.

Ribonucleotide reductase catalyzes the direct reduction of all four ribonucleotides to their corresponding deoxyribonucleotides. The activity of the holoenzyme is strictly coupled to the S phase of the cell cycle by de novo synthesis and breakdown of the R2 subunit. The R1 protein levels are constant throughout the cell cycle but only present in proliferating cells. We have previously reported that the mRNA's of both subunits are regulated in the cell cycle with low levels in G_0/G_1 and a synchronous increase just before S phase (Björklund et al. 1990 Biochemistry 29:5452-5458).

To study the regulation of the R1 and R2 subunits at the transcriptional level, we have cloned and sequenced the R1 and R2 promoter regions. Different regions of the promoters have been fused to the luciferase reporter gene and used to stably transform BALB/c cells. The R1 and R2 promoter regions have also been analyzed for binding of transcription factors using DNase footprinting and gel retardation. The R1 promoter contains two protected 23 mers that are identical except for one base pair. This single substitution disrupts a consensus sequence of 40 base pairs which also contains the consensus sequence for a CCAAT-box. The region binds recombinant Sp1 in its 3'-end which was unexpected since this DNA does not contain any Sp1 consensus sequence. We have also studied the regulation of the R1 and R2 genes at the level of elongation using in vitro nuclear run on assays and single stranded cDNA probes from different regions of the R1 and R2 genes. The results from these experiments will be presented.

R 406 THE PROMOTER REGION OF A YEAST TCA CYCLE GENE CONTAINS A COMPLEX ARRANGEMENT OF REGULATORY MOTIFS,
Susan B. Bowman, Geoff D. Kornfeld and Ian W. Dawes, School of Biochemistry and Molecular Genetics, University of New South Wales, Kensington, NSW 2033 Australia

The *LPD1* gene of *Saccharomyces cerevisiae* encoding lipoamide dehydrogenase has been cloned and sequenced.[1] The promoter region contains a number of potential regulatory motifs in a novel combination both upstream and downstream from the ATG start codon. Many of these motifs are consistent with the role of the protein in respiration, they include binding sites for the following proteins: GCN4, HAP2/HAP3/HAP4, CPF1, ABF1, RAP1.

The majority of these binding motifs are located in a cluster between -100 and -300 bp upstream of the start codon. This cluster contains a complex arrangement of binding motifs with several overlapping motifs. The function of these motifs has been investigated using gel retardation, site-directed mutagenesis and the use of *lacZ* fusion constructs.[2]

Additionally, preliminary data of the chromatin structure indicates hypersensitive sites are located 300 bp upstream and 300 bp downtream of the ATG start codon. Interestingly, the upstream hypersensitive site is located adjacent to the previously mentioned cluster of regulatory motifs. Data will be presented to show these hypersensitive sites and define the function of some of the regulatory motifs.

[1]Roy, D.J. and Dawes, I.W. (1987) Cloning and characterization of the gene encoding lipoamide dehydrogenase in *Saccharomyces cerevisiae*. J Gen Microbiol 133: 925-933.

[2]Bowman, S.B., Zaman, Z., Collinson, L.P., Brown, A.J.P., and Dawes, I.W. (1991) Positive regulation of the *LPD1* gene of *Saccharomyces cerevisiae* by the HAP2/HAP3/HAP4 acvtivation system. Mol Gen Genetics (in press).

R 407 TRANSCRIPTIONAL ACTIVATION OF THE S. cerevisiae ADHII GENE IN WILD TYPE AND TOPOISOMERASE I MUTANT STRAINS,
Micaela Caserta, Giorgio Camilloni and Ernesto Di Mauro, Dip. Genetica e Biologia Molecolare, Università di Roma "La Sapienza", Rome, Italy.

Regulation of RNA polymerase II transcription in Eukaryotes appears to be the result of a complex interplay between DNA and several different sets of proteins. These include nucleosomes, ubiquitous and specific factors, RNA polymerases and DNA topoisomerases. We have chosen the S. cerevisiae ADHII gene (1) as a model system for transcriptional regulation, because of the availability of naturally occurring promoter mutants (2). Primer extension analysis, using an oligo which recognizes both ADHI (consitutive) and ADHII (inducible) mRNAs on total yeast RNA, shows stronger transcriptional activation for the ADHII chromosomal gene in topoisomerase I mutant strains (3,4), compared to the isogenic wild type strains. We are currently analysing constructs to test whether the same effect can also be observed on episomal DNA. An analysis of the chromatin structure of the ADHII promoter both in glucose (repressing) and ethanol (inducing) conditions is in progress.

(1) Young, t. et al. (1982) Genetic engineering of microorganisms for chemicals. Ed. by A. Hollaender et al., Plenum Publishing Corporation.
(2) Williamson, V. M. et al. (1983) Mol. Cell. Biol. 3, 20-31.
(3) Goto, T. and Wang, J.C. (1985) PNAS 82, 7178-7182.
(4) Brill, S.J. and Sternglanz, R. (1988) Cell 54, 403-411.

R 408 A PERFECTLY PAIRED *his* LEADER A:B RNA SECONDARY STRUCTURE DISRUPTS INTERACTIONS THAT ENHANCE TRANSCRIPTIONAL PAUSING Cathy L. Chan and Robert Landick, Department of Biology, Washington University, St. Louis, MO 63130

During transcription, *E. coli* RNA polymerase pauses at discrete sites along the DNA. These pause sites have traditionally been divided into two classes, RNA hairpin-dependent and sequence-dependent sites. Both the *his* and *trp* biosynthetic operon leader regions contain RNA hairpin-dependent pause sites. We have found that RNA hairpin-dependent pause sites are multipartite and consist of (at least) a short RNA hairpin, a 3' proximal segment of transcript not involved in hairpin formation, and DNA sequences downstream from the pause site.

RNA polymerase pauses in the *his* leader just prior to addition of G103, following synthesis of the A:B hairpin from C65 to G100. This hairpin includes two mismatched regions. The results of base substitution studies suggest that only the upper portion of the *his* A:B structure forms at the pause site. Base substitutions in the lower region of the A:B stem-loop on the A side do not affect pausing. Substitutions in the upper region reduce pausing, which can be restored by compensatory substitutions in the B side. To test whether increasing the stability of the A:B secondary structure would strengthen the pause signal, we corrected the mismatched regions of the A:B stem (termed the perfect hairpin). The perfect hairpin reduced transcriptional pausing 3-fold. Similarly, we replaced the wild-type loop sequences with a tetra-loop sequence (UUCG), which is known to impart unusual stability to RNA structures. This change appeared to reduce pausing slightly.

Although increasing the Cl^- ion concentration slows the overall elongation rate, pausing in the *his* leader is *reduced* at increasing Cl^- ion concentrations, presumably due to masking of protein-nucleic acid interactions. In contrast, for the perfect hairpin elevated Cl^- ion concentrations have no effect on pausing. These studies suggest that ionic interactions between the wild-type pause hairpin and RNA polymerase may enhance pausing and that the perfect hairpin somehow prevents these interactions, perhaps by affecting the position of the stem or loop regions in the ternary complex.

R 409 OXYGEN TENSION REGULATION OF GLUTATHIONE PEROXIDASE GENE EXPRESSION IN CULTURED HUMAN CARDIOMYOCYTES. Douglas B. Cowan[1], R.D. Weisel[2], and D.A.G. Mickle[1,2]. Departments of Clinical Biochemistry[1] and Surgery[2], University of Toronto and Toronto General Hospital, Toronto, CANADA. M5G 2C4.

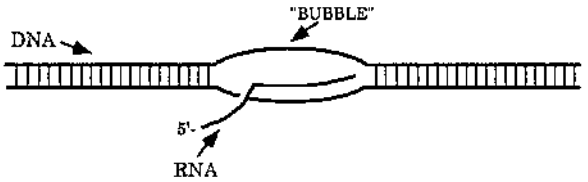
We have established an *in vitro* model with cultured ventricular cardiomyocytes from patients with tetralogy of Fallot to simulate the metabolic conditions of cardiovascular surgery for cyanotic heart disease. Previous work in our laboratory has shown that the antioxidant enzyme activity of glutathione peroxidase (GSH-Px) is regulated by oxygen tension, and that cardiomyocytes cultured at a low pO_2 (40 mm Hg) are more susceptible to oxygen mediated free-radical injury than cells cultured at a normal pO_2 (150 mm Hg). We propose that GSH-Px activities reflect enzyme concentrations and that O_2 tension acts to regulate the gene expression of GSH-Px. RNA isolated from cells cultured at the two oxygen tensions for 3, 7, 14, 21, and 28 days, was used for both Northern blot analysis and slot blot hybridization. GSH-Px mRNA levels were found to decrease with a drop in pO_2 and could be elevated with an increase in oxygen tension, essentially paralleling the results found for enzyme activities. These results were standardized against the housekeeping gene Hexosaminidase B (HexB) which displayed no difference in expression between pO_2's throughout the timecourse. Nuclear transcriptional run-off assays confirmed the role of O_2 tension in regulating nascent RNA synthesis from the GSH-Px gene. As a consequence of the work described herein, the form of GSH-Px expressed in the myocardium was clarified and both cDNA and genomic clones have been isolated. These findings have led us to hypothesize that within the vicinity of the GSH-Px gene, there lies a *cis*-acting element which binds an oxygen-responsive *trans*-acting factor subsequently modulating GSH-Px expression.

R 410 MOLECULAR ANALYSIS OF THE PHO81 GENE OF *SACCHAROMYCES CEREVISIAE* , Caretha L. Creasy and Lawrence W. Bergman, Department of Microbiology and Immunology, Hahnemann University School of Medicine, Broad & Vine, Philadelphia, PA 19102.

The PHO81 gene product is a positive regulatory factor required for the synthesis of repressible acid phosphatase in *Saccharomyces cerevisiae* (encoded by the PHO5 gene). Genetic analysis has suggested that PHO81 may be the signal acceptor molecule; however, the biochemical function of the PHO81 gene product is not known. Interestingly, the expression of the PHO81 gene is regulated by the level of inorganic phosphate and is controlled by the same trans-acting factors that regulate PHO5 expression. We have determined that overexpressing the wild type PHO81 gene, using the GAL1 promoter, results in constitutive synthesis of acid phosphatase. To elucidate the mechanism by which PHO81 functions, we have isolated and cloned dominant mutations in the PHO81 gene which confer constitutive synthesis of acid phosphatase. These mutations reside in the coding sequence of PHO81 and to date none have been isolated in the PHO81 promoter. We have demonstrated that overexpression of the negative regulatory factor, PHO80, partially blocks the constitutive acid phosphatase synthesis in a strain containing a dominant constitutive allele of PHO81. However, overexpression of the negative regulatory factor, PHO85, has no effect. This suggests that PHO81 may function by interacting with PHO80 or that these molecules compete for the same target.

R 411 RNA SYNTHESIS FROM A SYNTHETIC ELONGATION COMPLEX. Shirley S. Daube and Peter H. von Hippel. Institute of Molecular Biology, Univ. of Oregon, Eugene, OR 97403.

A nucleic acid structure was designed and constructed to resemble a transcription elongation complex. This structure is composed of 2 DNA oligomers partially complementary to each other, forming a "bubble" flanked by double - stranded regions, and an RNA oligomer which is complementary to 12 nucleotides within the bubble region.



Upon addition of either *E. coli* RNA polymerase or T7 RNA polymerase in trans to the bubble + RNA complex, the RNA is extended in an NTP dependent fashion. In addition, this synthesis is template dependent as shown by the formation of "stalled" complexes when only three NTP's are added. The fact that extension of the RNA is processive, is not inhibited by rifampicin, and is as efficient with both core and holo forms of *E. coli* RNA polymerase, suggests strongly that the system mimics the elongation mode of transcription.

This is a first demonstration of processive extension of an RNA primer with a defined sequence in a manner that circumvents initiation through a promoter.

R 412 THE HUMAN PAPILLOMAVIRUS TYPE 16 E6 PROTEIN BEHAVES LIKE A COACTIVATOR IN THE INDUCED TRANSCRIPTIONAL ACTIVATION OF THE THYMIDINE KINASE PROMOTER, Christian Desaintes, Sophie Hallez, Patrick Van Alphen, and Arsène Burny, Laboratoire de Chimie biologique, Université Libre de Bruxelles, 67 rue des chevaux, 1640 Rhode-St-Genèse, Belgium

The E6 protein of the human papillomavirus type 16 (HPV 16), along with E7, is responsible for the HPV-induced malignant transformation of the cervix. E6 has also been shown to promote in vitro ubiquitin-dependent degradation of the cellular p53 tumor suppressor protein after specific binding, and to activate transcription from a variety of viral promoters. The mechanism by which E6 activates transcription was investigated by co-transfecting into NIH 3T3 cells an E6 expression plasmid together with the reporter chloramphenicol acetyltransferase (CAT) gene linked to various truncated forms of the herpes simplex virus thymidine kinase (TK) promoter. Mutations or deletions that affected all upstream regulatory elements present in the TK promoter, such as the GC and CAAT boxes, reduced the level of E6-induced transcription. However, if compared to the basal level, these truncated promoters were still activated by E6. Site-directed mutations of the TATA sequence, although reducing the level of basal transcription, did not abolish the E6-mediated activation. Moreover, E6 could restore almost completely the full level of wild-type E6-induced transcription, as long as the upstream regulatory elements (GC/CAAT) were intact. This dual interaction of HPV 16 E6 is reminiscent of the activity of a coactivator.

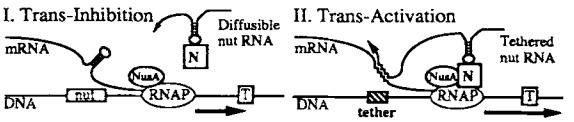
R 413 **Directing an Operon-Specific Antiterminator to the Target Polymerase by an Artificial Tethering Mechanism**
Joseph DeVito and Asis Das, Department of Microbiology, University of Connecticut Health Center, Farmington, CT 06030

The N protein of bacteriophage lambda functions as a transcriptional activator. It modifies RNA polymerase to a termination resistant form, permitting readthrough of multiple transcriptional terminators placed far downstream in the lambda genome, and allowing expression of genes required for both the lytic and lysogenic development of the phage. Antitermination in vitro requires at least one host factor, NusA, and a *cis*-acting site encoded by the promoter-proximal signal known as *nut*. The boxB component of the *nut* site RNA is known to bind the N protein. We propose that the *nut* RNA signal acts like DNA enhancers, capturing N protein and mediating a productive interaction between N and RNA polymerase.

In addition to bringing N in close proximity to the polymerase, *nut* RNA could work as an allosteric effector of N, binding N and converting it to a form capable of interacting with RNA polymerase. Alternatively, *nut* RNA may simply act as just an allosteric effector. Moreover, *nut* site might itself interact with RNA polymerase to induce the modified state necessary for readthrough of terminators.

We tested these hypotheses in vitro by adding freely diffusable *nut* RNA to a run-off transcription assay and measured its effect on N-dependent antitermination. We find that addition of *nut* RNA *in trans* does not allow antitermination in a *nut*-less transcription unit, suggesting *nut* site is not merely an allosteric effector. When added in excess, *nut* RNA inhibits N action on a *nut*-containing transcription unit. This inhibition is dependent upon the interaction of N with the *nut* site, and is overcome by replenishing N protein, but not NusA protein. Thus, we suggest that trans-inhibition of antitermination by diffusable *nut* RNA is mediated through a sequestering of N protein away from its site of action (see Figure I.)

More significantly, a diffusible *nut* RNA can activate N-dependent antitermination in a *nut*-less template when it is tethered to the nascent mRNA through an RNA:RNA hybrid (see Figure II.). We show that a tether sequence in the target mRNA can form a hybrid with the diffusable *nut* RNA, mediating N binding and antitermination. Detection of antitermination activity clearly demonstrates the role for *nut* site RNA as a tether for N. Experiments to further probe the function of *nut* site RNA are currently underway.



R 414 **IMPORTANCE OF THE PROMOTER ELEMENT SPACING FOR TRANSCRIPTION OF THE POL II AND POL III-SPECIFIC U-snRNA GENES OF PLANTS.**
D. Edoh, P. Vankan, T. Kiss, W. Filipowicz
Friedich Miescher Institut, 4002-Basel, Switzerland.

Plant small nuclear RNA (U-snRNA) gene promoters contain two conserved elements, the plant snRNA gene specific Upstream Element (USE) and the TATA box. These elements are necessary and sufficient for transcription by RNA polymerase II and III. It is a one helical DNA turn difference in the spacing between these two elements that determines the choice of the RNA polymerase. (Waibel & Filipowicz, Nature 346, 1990,; Kiss et al., Cell 65, 1991)

The distance between the USE and the TATA elements are highly conserved in all plant U-snRNA genes isolated to date. It corresponds to approximately four (pol II genes) or three (pol III genes) helical DNA turns, suggesting that the factors interacting with the USE and TATA elements have to interact at the same side of the helix. We are analyzing the effect of spacing mutations on the activity of the U-snRNA genes in transiently transfected and stably transformed plant cells. In the pol III-specific U6 gene the insertions and deletions of either odd or even numbers of half helical turns completely inactivate transcription in transfected protoplasts. In contrast, insertions of additional sequences (5, 10, 15 or 20bp) into the USE-TATA spacer of the pol II-specific U2 gene decreases transcription by only 50-70% and no correlation is observed between promoter activity and insertion of either an odd or an even number of half helical turns. Thus, in transfected cells, the requirement for the appropriate spacing between the USE and TATA elements are much more stringent for pol III genes than for pol II genes.

R 415 FUNCTIONAL ANALYSIS OF THE HUMAN β GLOBIN LOCUS CONTROL REGION 5' HYPERSENSITIVE SITE 2 (5'HS2). James Ellis, Dale Talbot, Niall Dillon, and Frank Grosveld. NIMR, The Ridgeway, Mill Hill, London, NW7 1AA, U.K.

5'HS2 confers position independent, high level expression on a linked β globin gene following stable introduction into Murine Erythroleukemia cells and transgenic mice. We previously showed that a tandem NFE-2 binding site in the 5' region of 5'HS2 is responsible for the enhanced level of transcription and that low level position independent expression is a characteristic of a subregion of 5'HS2 containing inverted GATA-1 sites and binding sites for the ubiquitous H and J factors. We have now generated point mutations in each of the latter 3 binding sites in order to identify the factor(s) responsible for position independent expression. We will present functional analysis of these mutated sites in MEL cells and transgenic mice.

R 416 THE MANY FACES OF *E. COLI* RNA POLYMERASE

Dorothy A. Erie, Omid Hajiseyedjavadi and Peter H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

We have been investigating the kinetics of misincorporation of nucleotides by *E. coli* RNA polymerase stalled at a specific site on a DNA template. These experiments were performed using a DNA template which contains the λP_R promoter and the transcript sequence shown below.

+1 +25
AUGUAGUAAGGAGGUUGUAUGGAACAAC—

As can be seen from the transcript sequence, stable stalled elongation complexes at position +24 can be made by performing transcription in the absence of CTP. Consequently, the rate of misincorporation at position +25, the first position in the transcript requiring CTP, can be measured. These experiments have revealed the existence of multiple conformations of ternary complexes stalled at position +24. We have deconvoluted the kinetic data in terms of complexes that misincorporate at +25 and arrest at +25, that misincorporate at +25 and extend to +27, and that do not misincorporate and do not chase when all four NTP's are added. This deconvolution reveals the existence of at least three enzyme conformations at +24. We have observed that the populations of the various conformations are affected by the history of the holoenzyme preparation as well as the history of the stalled ternary elongation complexes (that is, whether the stalled elongation complexes have been purified by spin columns or centricons, or not purified at all). Using changes in misincorporation kinetics as an indication of conformational changes, we are presently investigating environmental factors that determine the various conformational changes.

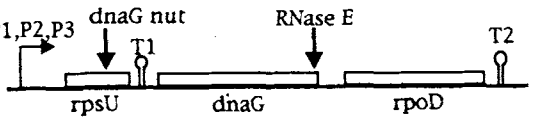
R 417 CLONING OF MSY-1, A MOUSE HOMOLOGUE OF THE Y-BOX TRANSCRIPTION FACTOR - FRGY-1

Mary Familari, David B. Sak, Alan P.Wolffe and Sherrie R.Tafuri. Laboratory of Molecular Embryology, NICHD. National Institutes of Health. Bethesda MD 20892.

MSY-1 was isolated from a mouse oocyte cDNA library. The full length sequence was found on two overlapping clones. The open reading frame codes for a 323 AA protein and shows greater than 95% AA sequence identity to FRGY-1 which was shown to activate transciption from the maternally expressed Xenopus hsp70 promoter (1). Moreover FRGY-1 interacts specfically with mRNA and is believed to function in translational regulation of maternal messages during oogenesis (2). We are testing the hypothesis that MSY-1 fulfills a comparable role in germ cell specific transcription and mRNA storage in the mouse. MSY-1 is expressed in low levels in the mouse kidney and muscle and is highly overexpressed in the testis. A developmental northern of mouse testis shows that MSY-1 expression begins at 15 days postpartum and continues through to adult. MSY-1 is a member of a multigene family in the mouse, some of which are psuedogenes. The transactivator function and testicular localisation of this protein is currently being examined.

(1) S. Tafuri and A Wolffe., PNAS 87 p9028, 1990
(2) S. Tafuri et al (submitted)

R 418 ANTITERMINATION OF CRYPTIC TERMINATORS IN THE rpsU-dnaG-rpoD OPERON, David S. Geller and G. Nigel Godson, Department of Biochemistry, NYU School of Medicine, New York, NY 10016



The genes for ribosomal protein S21, primase, and sigma-70 are all contained in the macromolecular synthesis operon but are discoordinately expressed.

A sequence upstream of T1, termed dnaG nut, possesses striking homology to the lambda nut antitermination site. In our test system, however, dnaG nut has no effect on T1. Surprisingly, though, we found that dnaG nut has substantial sequence-specific antitermination activity on a cryptic rho-dependent terminator. This suggests dnaG nut may act on terminator(s), presumably cryptic, other than T1. Since some dnaG amber mutants are partially polar on σ70 expression, these terminators must exist. We have mapped and cloned them and are testing the antitermination activity of dnaG nut on these sites.

Due to a weak ribosome binding site and a high content of rare codons, dnaG is poorly translated. In rRNA operons, it is thought that the rrn antiterminator site suppresses cryptic terminators revealed by the lack of translation. We suggest dnaG nut functions to overcome cryptic terminators in dnaG in a manner analogous to that of the rrn antiterminator. Prevention of premature transcription termination caused by the lack of translation would be crucial in this case, for expression of rpoD is dependent on full-length transcription of the operon.

R 419 3'-POLY(A) FORMATION BY VACCINIA VIRUS.

Paul D. Gershon and Bernard Moss, Laboratory of Viral Diseases, National institute of allergy and infectious diseases, National Institutes of Health, Bethesda, MD 20892, USA.

In the eukaryotic nucleus, the processes of mRNA 3'-end formation and 3'-poly(A) biogenesis are closely associated with transcriptional termination. Vaccinia virus transcripts are synthesized and polyadenylated within the cytoplasm of the infected cell and virus-induced RNA 3'-polyadenylation activity is associated exclusively with a heterodimer of two virus encoded polypeptides (VP55 and VP39). Although stable to chromatography and sedimentation, the heterodimer is readily dissociated by antibody to an N-terminal peptide of VP55. Catalytic activity is associated exclusively with VP55. VP39, in conjunction with VP55, stimulates 3'-poly(A) formation, and possesses poly(A) binding activity (CELL **66**, 1269-1278). Both VP55 and VP39 have now been individually overexpressed in eukaryotic cells and purified to homogeneity. We show that VP55 rapidly adds between 18 and 35 adenylates to an RNA primer in a processive manner, before polyadenylation decelerates and becomes non-processive. Studies with primers containing pre-formed poly(A) tails indicate that VP55 measures 3'-poly(A) length in contrast to counting added adenylate residues. VP39 confers processivity upon VP55 and increases the extension rate.

R 420 THE ISOLATION OF B3, A PROTEIN WHICH INTERACTS WITH A DEVELOPMENTALLY REGULATED ELEMENT IN THE *XENOPUS laevis* TFIIIA GENE, David Griffin and William Taylor, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232
We have recently isolated a cDNA clone coding for a protein, B3, which interacts with a developmentally regulated element in the TFIIIA gene of *Xenopus laevis*. We previously identified a region of the TFIIIA gene which contributes to the enhanced activity of the TFIIIA gene in stage III but not stage VI oocytes. A DNA fragment containing element 3 was subsequently used to screen a cDNA expression library from immature *Xenopus laevis* ovary, resulting in two positive clones totaling 2kb of sequence. Analysis of the sequence yields an open reading frame encoding a 285 a.a. protein. Co-injection into stage VI oocytes of an expression vector, pOEV, containing the B3 sequence with reporter constructs containing three repeats of element 3 with either a TFIIIA or Herpes-TK promoter sequence results in a 2-5 fold activation of transcription as measured by primer extension analysis. Bacterially expressed B3 protein has also been shown to bind element 3 by electrophoretic mobility shift analysis. This study has identified a protein which activates transcription from and specifically binds to element 3 from the Xenopus TFIIIA gene.

R 421 **Mutations in the heat shock activation sequence of yeast *HSP82* reveal a critical role for heat shock factor in establishing a nucleosome-free region over the TATA box.** *David S. Gross, Seewoo Lee, and Christopher C. Adams*, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, LA 71130.

Heat shock genes are poised for rapid transcriptional activation in response to environmental stress. A universal structural characteristic of such genes is the presence of a nuclease hypersensitive promoter region, free of canonical nucleosomes. We present evidence that *in situ* deletion or substitution of a 32 bp region encompassing the heat shock activation sequence, termed HSE1, in the *HSP82* gene of *Saccharomyces cerevisiae* virtually eliminates basal and induced levels of transcription (both are repressed 100- to 200-fold). Furthermore, such mutations abolish the nucleosome-free, DNase I hypersensitive zone over the promoter and lead to the *de novo* appearance of nucleosomes within the upstream regulatory region. One of these nucleosomes is centered over the TATA box and is rotationally positioned with respect to the underlying DNA sequence, as assayed by DNase I genomic footprinting. Strikingly, the TATA-associated genomic footprint is lost as well. These results suggest that constitutively bound heat shock factor (HSF) plays a critical role in excluding nucleosomes from the *HSP82* promoter. This notion is strengthened by the fact that mutagenesis of a poly(dT):(dA) element overlapping HSE1 has virtually no effect on the nucleoprotein architecture of the promoter, nor on its function. Moreover, a hierarchy of functions for HSF is suggested by the phenotype of other HSE1 mutants. For example, strains with double point transversions in HSE1 retain DNase I hypersensitivity over the promoter region, despite a preferential 50-fold reduction in basal transcription and the loss of a discernable HSF genomic footprint. Therefore, weak binding of HSF to DNA is sufficient to exclude nucleosomes from the *HSP82* upstream regulatory region, facilitate the binding of TFIID to the TATA element, and activate transcription during inducing conditions. Tight binding of HSF to the wild-type HSE1 sequence results, in addition, in high level transcription under basal conditions.

R 422 **Cloning, Isolation, and Characterization of the Human T-cell DNA-Cytosine 5-Methyltransferase Gene.** Patricia C. Guenthner, Song Kim, and Daniel P. Bednarik; Retrovirus Diseases Branch, Molecular Genetics Section, Centers for Disease Control, 1600 Clifton Road, Atlanta, Georgia 30333.

DNA methylation can affect the latency of HIV and HTLV by effectively silencing transcriptional expression. Previous studies have shown that a CpG island in the HIV LTR, when methylated by the human DNA-cytosine 5-methyltransferase (MeTase), inactivates HIV transcription *in cis*. Further characterization of the methyltransferase enzyme is therefore of obvious interest. A human T-cell cDNA library from the cell line Jurkatt has been screened, using a murine DNA methyltransferase cDNA clone as a probe, and a clone of approximately 0.7 Kb has been isolated (pMET2) and sequenced with 87% homology to an area in the 3' region of the murine cDNA. Based on the size of the mature protein (~172 Kdaltons), a gene of at least 5 Kb is expected. Southern blot analysis of human genomic DNA yielded single fragments of 4.2 Kb to over 12 Kb, using the pMET2 fragment as a probe. Northern blot analysis has been employed to analyze transcriptional expression, and to determine the size of the methyltransferase message. A human genomic library has been screened and four putative clones isolated and shown positive by PCR analysis, using primers to pMET2. Southern analysis on phage DNA digested with XbaI yields fragments large enough to include a full-length copy of the gene. Sub-cloning into an expression vector is underway in order to obtain sequence information, and to further characterize this gene.

R 423 DNA CpG METHYLATION INHIBITS TAT-MEDIATED TRANSCRIPTION OF THE HIV LTR IN A TIME-DEPENDENT MANNER. K.A. Gutekunst and D.P. Bednarik, Retrovirus Diseases Branch, Centers for Disease Control, Atlanta, GA 30333.
DNA methylation was shown to inhibit transcription from the HIV-1 LTR both in vitro and in vivo. Using the plasmid pU3RIIICAT, we studied the transient expression of CAT when the LTR was methylated. The enzymatic methylation of two CpG dinucleotide sites in the viral enhancer region resulted in suppression of transcription in vivo as assayed by the transient expression of CAT. This suppression can be overcome by the presence of the viral transactivator tat. Over increased time, the presence of tat had no effect in trans, suggesting that other methylation-dependent inhibitory factors may be involved. Using a CpG methylase to hypermethylate the DNA at all CpG residues, we have shown that transcription is blocked and that this block cannot be overcome by tat. In addition, we have demonstrated by in vitro transcription experiments, methylation of the LTR also resulted in a complete block of RNA polymerase II-dependent transcription. Our data suggests that methylation inhibits initiation of transcription from the HIV LTR. Understanding the role of methylation in suppression of transcription and the interaction of tat and other regulatory proteins with these methylated sequences will provide important information relating directly to latency of HIV.

R 424 ISOLATION AND CHARACTERIZATION OF STABLY ARRESTED RNA POLYMERASE II ELONGATION COMPLEXES, Diane K. Hawley, Daguang Wang, Debra Wiest, Angel Platas, and Melissa Holtz, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.
We are studying the mechanism of transcription arrest at a specific site downstream of the adenovirus major late promoter (MLP) in vitro. In the absence of the elongation factor SII, up to 80% of the elongation complexes from the MLP become blocked at this site and remain stably bound as ternary complexes with a lifetime of at least several hours. Most of these ternary complexes can resume elongation upon addition of SII. We have used several different approaches to isolate stably arrested ternary complexes. Purified RNA polymerase II molecules arrested at the site on a "dC-tailed-template" can be isolated either by gel filtration or by a polyacrylamide gel mobility shift assay. To isolate arrested ternary complexes in a promoter-dependent transcription system, we attached templates to agarose beads and showed that arrested complexes are stable to extensive washing, while other proteins are dissociated. Using these methods, we have observed that incubation of arrested complexes in the absence of nucleotides results in a progressive shortening of the associated RNA. This transcript shortening reflects removal of nucleotides from the 3' end of the RNA; the remaining transcript is still bound to functional ternary complexes and can be chased to longer transcripts. The process requires Mg++ and is prevented by α-amanitin. Similar behavior has recently been reported for E. coli RNA polymerase (Surratt et al. [1991] PNAS 88, 7983). Current experiments are addressing the mechanism of this event in the RNA polymerase II system.

R 425 CONSTRUCTION OF A POSITIVE FEEDBACK LOOP, Herman C., Dreze P., Thieffry D. and Thomas R., Department of Molecular Biology, Genetic Lab., 67 rue des Chevaux, 1640 Rhode-St-Genèse, Belgium.

Regulatory elements can interact with each other to form complex regulatory networks, in which one can distinguish two kinds of feedback loops, positive loops which generate multistationarity* (a choice between steady states) and negative loops which generate homeostasis*.
Our aim is to build a positive feedback loop that can be set on command in either of two stable positions by a *transient signal (epigenetic situation)*.
Using in vitro recombination, we have constructed a positive feedback loop with regulatory elements of phage lambda. The normal promoter of gene cII has been removed and replaced by the pE promoter that requires the cII product. This creates a vicious circle: either the cII product is present, in which case it will go on being synthetized, or it is absent and, since cII is necessary for transcription from promoter pE, it will continue not being synthetized and remain absent. This vicious circle can be disrupted by another copy of the *cII gene which is under the control of a* thermosensitive repressor.
Thus, we can transiently give the cII product to switch on the positive loop and study the conditions in which the loop remains on after the transient signal has disappeared.

* *Biological feedback* (1991), Thomas R. and D'Ari R., CRC Press.

R 426 REB1 IS REQUIRED FOR GCN4-INDEPENDENT *ILV1* BASAL LEVEL TRANSCRIPTION AND CAN BE FUNCTIONALLY REPLACED BY ABF1, Steen Holmberg and Jacques Remacle*, *Institute of Genetics, University of Copenhagen*, DK-1353 and * Dept. of Yeast Genetics, Carlsberg Laboratorium,DK-2500, Copenhagen, Denmark.
The *ILV1* gene of *S. cerevisiae* encodes the first committed step in isoleucine biosynthesis and is regulated by general control of amino acid biosynthesis. Deletion analysis of the *ILV1* promoter revealed a GC-rich element important for the basal level expression. This *cis*-acting element, called the *ILV1* basal element, is functional independently of whether GCN4 protein is present. The *ILV1* basal element has homology to the consensus REB1 binding sequence CGGGTARNNR. Gel retardation assays showed that REB1 binds specifically to this basal element. We show that REB1-binding sites normally situated in the *SIN3* promoter and in the 35S rRNA promoter, respectively, functionally can substitute for the *ILV1* REB1 site. Interestingly, an ABF1-binding site can also functionally replace the *ILV1*-binding site, suggesting that ABF1 and REB1 may have related functions within the cell.
Although the REB1-binding site is required for the *ILV1* basal level expression, the site behaves as a poor UAS when combined with the *CYC1* downstream promoter elements, suggesting that REB1 may need another promoter element to control *ILV1* basal level expression. A poly(dA) element situated between the *ILV1* REB1 site and the upper most transcription initiation site also displayed a poor UAS activity of its own. However, insertion of the REB1 site upstream of the poly(dA) element enhances synergistically the effect on transcription of this weak activator. This effect of REB1 is strongly distance dependent. In available data bases, we found many yeast promoters containing a REB1 site in front of a poly(dA) element. We suggest that such a combination may provide constitutive or high basal level expression to these genes.

R 427 TRANSCRIPTIONAL REGULATION OF THE PCNA GENE IN T-LYMPHOCYTES Danyang Huang, Ben Park, Pierette Appasamy, Michael B. Prystowsky, Department of Pathology, University of Pennsylvania, Philadelphia, PA 19104
The Proliferating Cell Nuclear Antigen(PCNA) is induced in the G1-S phase by Interleukin 2 (IL2) in T-lymphocytes and functions as an auxilary factor of DNA polymerase delta during DNA replication. IL2-stimulated PCNA expression in T lymphocytes is regulated by transcriptional and post-transcriptional mechanisms. Our studies are aimed at determining the cis elements which are required for IL2-stimulated PCNA expression and at defining the proteins in T cells which bind to these elements. For this purpose, a series of deletion constructs of the 5'-flanking region of the PCNA gene were made, and linked to the firefly luciferase coding sequence. In a transient expression assay, the luciferase activity was analyzed in the EL4 cell, a constitutively proliferating lymphocyte, and the L2 cell, a cloned T-cell that can be activated by IL2. Results of these experiments indicate that: (1) A 182 bp 5'-flanking region of PCNA gene is sufficient for full promoter activity in L2 cells, but not in EL4 cells, (2) the IL2-responsive element in the PCNA promoter was mapped to a 110-bp region (nucleotide -110 to +1 the transcription start site).
To define further this 110-bp region, we used a 37-bp oligonucleotide containing TGGCGTCATGACCTCG sequence (nt -52 to -36), a tandem repeat of a putative CRE (cAMP response element) site within this region as a probe in mobility shift assays to determine whether there are proteins that bind specifically to this region in cell extracts from both quiescent and stimulated L2 cells and splenocytes. The CRE-containing sequence interact specifically with proteins, which are induced by IL2 in L2 cells and by ConA in splenocytes.

R 428 *IN VITRO* TERMINATION OF TRANSCRIPTION IN YEAST. Linda Hyman and Claire Moore, Department of Microbiology and Molecular Biology, Tufts University School of Medicine, Boston, MA 02111.

The formation of the 3' end of eukaryotic mRNAs results from the cleavage of a pre-mRNA and subsequent poly(A) additon. Pre-mRNAs are rapidly processed *in vivo* and are not detectable due to their transient formation. Transcription may continue far downstream of the poly(A) site as shown for several mammalian and viral genes. However, it may be that in yeast, where genes are packed close together, transcription far downstream of the poly(A) site is not tolerated. In order to examine this possibility, we have developed an *in vitro* transcription termination assay for yeast.

Our earlier work demonstrated that sequences from the 3' untranslated region of the yeast *ADH2* gene (as well as other 3' end sequences) can direct 3' end formation of a fusion gene transcript *in vivo*. However, the downstream cleavage product is unstable and therefore intractable for analysis. We reasoned that we might circumvent this problem by studying transcription termination in an *in vitro* system, where the pre-mRNA intermediates might be more stable. Yeast nuclear extracts were prepared under conditions which accurately initiate transcription. We tested these extracts for the ability to terminate transcription at specific sites. Our results with the *ADH2* 3' sequence show that RNA synthesized *in vitro* is approximately the same length as the RNA made *in vivo*. This suggests that transcripts synthesized *in vitro* terminate at or near the same site as the transcrpts made *in vivo*. RNase protection analysis shows that the end of the RNA is approximately 80 nucleotides downstream of the major polyadenylation site. The RNA is not polyadenylated. Therefore, sequences from the 3' end of the *ADH2* gene can cause the termination of transcription *in vivo*, as well as *in vitro*. An important implication of this result is the uncoupling of mRNA cleavage/polyadenylation from transcription termination. We also tested the 3' end of the *GAL7* gene to establish the the generality of this result. The transcript synthesized *in vitro* was heterogeneous; there is a species which appears to end downstream of the poly(A) site and is $p(A)^-$. However, a significant amount of RNA is polyadenylated. Thus, depending on the sequence, transcripts made *in vitro* may accumulate terminated $p(A)^-$ precursors and/or accumulate transcripts which are cleaved and polyadenylated in the transcription extract. Further characterization of the *in vitro* system is underway. In addition we are engaged in a search for mutations in genes important for transcription termination.

R 429 SPECIFIC PROTEIN BINDING NEAR THE TRANSCRIPTIONAL ARREST SITE WITHIN INTRON ONE OF THE MURINE ADENOSINE DEAMINASE GENE, Jeffrey W. Innis, Shera F. Kash-Anderlik, Anne Jackson and Rodney E. Kellems, University of Michigan Department of Human Genetics, Ann Arbor, MI 48109 and Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030. Tissue-specific and developmental expression of the murine adenosine deaminase gene (ADA) is regulated by transcriptional arrest in the 5'end of the gene. A cell-free transcription system was developed to identify sequence requirements and factors involved in this process. Using this system, we identified two major sites of premature 3' end formation in the first 150 base pairs of the transcription unit. Specific protein binding to a DNA fragment encompassing these transcriptional arrest sites was detected in electrophoretic mobility shift assays. Competition studies have shown that the protein binds to the DNA near the transcriptional arrest site within intron one. Methylation interference analysis is being used to pinpoint the binding site in this region. Transcription assays with mutants of this binding site will be performed to establish whether binding is necessary for transcriptional arrest within this region.

R 430 3'- 5' CLEAVAGE OF NASCENT TRANSCRIPT IN RNA POLYMERASE II TERNARY COMPLEXES IS FACILITATED BY THE ELONGATION FACTOR SII, **Michael G. Izban and Donal S. Luse, Dept. of Mol. Genetics, Biochemistry and Microbiology, Univ. of Cincinnati, Col. of Medicine, Cincinnati, Ohio 45267-0524**

The process by which RNA polymerase II (RNAP) elongates RNA chains remains poorly understood. We have recently shown that physiological elongation rates can be achieved in vitro on naked DNA templates using highly purified ternary complexes supplemented with the elongation factor TFIIF; elongation factor SII was required to minimize pausing/termination at intrinsic pause sites. Further investigation of the effect of SII at intrinsic pause sites revealed that SII appears to facilitate transcript cleavage as judged by the shortening of transcription products generated by RNAP pausing at these sites. A similar effect was observed using a variety of artificially paused ternary complexes halted after the synthesis of 15 (15n complex), 20, 21, 23 and 35 nucleotide transcripts. Transcripts as short as 9 or 10 nucleotides were generated from 15n or 20n complexes in 5 minute incubations with SII. However, a fairly stable 31n complex was formed when 35n complex was incubated with SII for 5 minutes. Furthermore, 15n complexes generated from two different promoters showed different cleavage products. These data indicate that both the position at which the RNAP is halted and the sequence of the transcript affect transcript cleavage. In all cases transcripts cleaved to as short as 9-10 nucleotides remained in active ternary complex since they could be quantitatively chased. Moreover, we provide evidence that the catalytic site of RNAP moves backward when transcript cleavage occurs. Transcript cleavage requires a divalent cation, appears to proceed primarily in two nucleotide increments and is inhibitable by α-amanitin. Finally, SII appears to facilitate an activity inherent to the RNAP ternary complex since limited transcript cleavage occurs in the absence of SII.

R 431 *IN VITRO* AND *IN VIVO* COMPARISONS OF MOUSE α-A-CRYSTALLIN PROMOTER FOOTPRINTS REVEAL DIFFERENCES IN FACTOR BINDING BETWEEN LENS AND FIBROBLAST CELL LINES Marc Kantorow, Ales Cvekl, Christina M. Sax and Joram Piatigorsky, *LMDB, NEI, NIH, Bethesda, MD 20892.* The crystallins are a diverse group of soluble proteins that are primarily found in the transparent lens of the eye. Two α-crystallins, αA and αB, constitute a large proportion of crystallins in the mammalian lens and are present in all vertebrates. Both α-crystallins are preferentially expressed in the lens, as well as in lower concentrations in other tissues. Previous work showed that the mouse αA-crystallin -111 to +46 sequence is capable of directing lens preferred gene expression in transgenic mice. Here we have compared footprints of the mouse αA-crystallin promoter between an SV40 T-antigen transformed mouse lens cell line (αTN4-1) and a mouse fibroblast line (L929). Northern blot hybridization indicated that only the αTN4-1 cells express αA-crystallin. We performed *in vivo* footprinting by using ligation-mediated PCR to amplify genomic DNA isolated from DMS-treated whole cells or micrococcal nuclease treated-nuclei, *in vitro* footprinting by using DNAse I and partially purified nuclear extracts, and gel-shift assays with nuclear extracts and DNA sequences corresponding to footprinted regions. Four footprints were detected *in vivo* (-111 to -99, -72 to -55, -35-to -43, and -35 to +14) that exhibited significant differences between the two cell lines. The *in vitro* DNAse I footprints showed only three of the four *in vivo* footprints (-35 to -43 was missing). Although similar, the DNAse I footprints were not identical between preparations from the αTN4-1 and L929 nuclear extracts, indicating that different or modified DNA binding proteins are present in the two cell lines. Collectively, these results indicate that multiple proteins in both αA-crystallin expressing αTN4-1 cells and non-expressing fibroblasts bind to the αA-crystallin 5' flanking sequence in regions shown earlier to be functionally important for promoter activity, however, apparent modifications or interactions with other proteins affecting their precise binding characteristics *in vivo* may control their ability to promote transcription in lens cells.

R 432 SEQUENCE REQUIREMENTS FOR PREMATURE TRANSCRIPTION ARREST IN INTRON ONE OF THE MURINE ADENOSINE DEAMINASE GENE, Shera F. Kash-Anderlik^, Jeffrey W. Innis* and Rodney E. Kellems^, ^Inst. of Mol. Gen. and Dept. of Biochem., Baylor College of Medicine, Houston, TX 77030 and *Dept. of Hum. Gen., Univ. of Mich., Ann Arbor MI 48109. Premature transcription arrest plays a major role in the regulation of a number of genes, including murine adenosine deaminase (ADA). In a cell-free system, short transcripts with 3' ends at +96 to +100, +108 to +112, and +137 to +145 nucleotides (Sites I-III respectively) have been shown to accumulate. Sites I and II are in the first exon and transcripts accumulate by a sarkosyl-sensitive mechanism; site III is in the first intron and its generation is sarkosyl-resistant. To identify genetic requirements for site III transcript formation, the transcription properties of constructs containing mutant ADA gene fragments were examined in a cell-free system. By deletion analysis, the determinants for the site III transcript formation are located between +117 and +158. Additionally, we found that sequences upstream of +138 are not sufficient to generate site III transcripts. Transcript formation occurs independently of the orientation or sequence of the 5' untranslated region of the gene. In addition, site III transcript formation still occurs when the sequence determinants are moved 104 bp downstream from their natural position. To find out whether this region can function in a promoter-independent and/or orientation-independent manner, constructs which have this region (+123 to +158) in either orientation with respect to a heterologous promoter have been made and are currently being tested. In the future, further mutations will be constructed between +117 and +158 to identify critical residues for site III transcription arrest.

R 433 STABILITY OF *DROSOPHILA* RNA POLYMERASE II ELONGATION COMPLEXES *IN VITRO* Daniel D. Kephart, Nicholas F. Marshall, and David H. Price, Department of Biochemistry, University of Iowa, Iowa City, IA 52242
We show that nuclear extract from *Drosophila* K_c cells supports efficient elongation by RNA polymerase II initiated from the actin 5C promoter. The addition of 0.3% Sarkosyl, 1 mg/ml heparin or 250 mM KCl immediately after initiation has two effects. First, the elongation rate is reduced 80% - 90%, due to the inhibition of elongation factors. Second, there is an increase in the amount of long, run-off RNA suggesting that there is an early block to elongation that is relieved by the disruptive reagents. Consistent with the first effect, we find that the ability of factor 5 (TFIIF) to stimulate the elongation rate is inhibited by the disruptive agents when assayed in a defined system containing pure RNA polymerase II and a dC-tailed template. The disruptive agents also inhibit the ability of DmS-II to suppress transcriptional pausing, but only slightly reduce the ability of DmS-II to increase the elongation rate 2 fold. The pause sites encountered by RNA polymerase II after initiation at a promoter and subsequent treatment with the disruptive reagents are also recognized by pure polymerase transcribing a dC-tailed template. It has been suggested that 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) inhibits RNA polymerase II during elongation, but we find that the purine nucleoside analog has no effect on elongation complexes containing RNA over 500 nucleotides in length or on the action of factor 5 or DmS-II in the defined system.

R 434 BACTERIOPHAGE T4 INHIBITION OF TRANSCRIPT ELONGATION ON CYTOSINE-CONTAINING DNA TEMPLATES. Elizabeth Kutter, Rolf Drivdahl, Terry White, Joanna Wagner, William Canada, The Evergreen State College, Olympia, WA 98505 and Larry Snyder, Dept. of Microbiology, Michigan State Univ., E. Lansing, MI 48824
The bacteriophage T4 *alc* gene product is responsible for blocking transcript elongation on cytosine-containing DNA (Drivdahl and Kutter, J. Bact. 172:2716-2727, 1990) and, thus, shutting off host transcription. Various lines of in vivo and in vitro evidence, to be discussed, suggest a model whereby g*palc* interacts with the elongating polymerase and with stretches of the cytosine-containing DNA to slow elongation and facilitate termination. An Arg → His substitution at AA 368 of the polymerase ß subunit impedes this interaction, to a degree affected by the C/HMC ratio in the DNA.

We have cloned and expressed the *alc* gene product, taking advantage of the vector pET11a (a gift of F.W. Studier), which permits the cloning of very toxic genes. This vector contains a *lac* operator between the T7 promoter and the cloning site, as well as *lacI* encoded elsewhere, which act together to bestow tight control on expression of the cloned gene. High-level production of the protein is induced by infecting cells with λCE6 carrying the T7 polymerase gene. Most of the product is in inclusion bodies and migrates as a dimer on traditional SDS gels but as a monomer on SDS gels containing 6 M urea -- behavior similar to that of two other T4 RNA polymerase-binding proteins, gp55 and rpbA. We are currently purifying the *alc* gene product to study the details of its interactions with DNA and with RNA polymerase.

R 435 MEP-1, A MOUSE NUCLEAR FACTOR THAT BINDS TO THE REGULATORY ELEMENTS OF THE GENE ENCODING METALLOTHIONEIN-I. Simon Labbé and Carl Séguin. Centre de recherche en cancérologie de l'Université Laval, l'Hôtel-Dieu de Québec, Québec, Canada, G1R 2J6.
Metallothioneins (MTs) are small cystein-rich metal-binding proteins that serve notably to maintain intracellular levels of metals and to protect cells against heavy-metal toxicity. In higher eukaryotes, the synthesis of MTs is controlled at the transcriptional level by several stimuli including heavy metals, hormones, phorbol esters and interleukins. Heavy metal induction of MT gene transcription is dependent on the presence of specific nucleotide sequences, the metal-regulatory elements, or MREs. Protein blotting experiments revealed that a nuclear protein of 108 kDa, termed metal element protein-1 (MEP-1), binds to the mouse MRE elements with different affinities which are proportional to their relative transcriptional strength *in vivo*: MREd ≥ MREa = MREc > MREb > MREe > MREf. Similarly, human MRE4 and trout MREa bind to MEP-1. A protein similar in size to MEP-1 was also detected in HeLa-cell nuclear extracts. In footprinting analyses, synthetic oligodeoxynucleotides corresponding to the elements MREa, MREb, MREc, MREd and MREe of the mouse MT-I gene, as well as to the human MRE4 and trout MREa, all competed for the nuclear protein species binding to the mouse MREd region, MREe being the weakest competitor. In UV cross-linking experiments, the major protein species, complexed with MRE elements, had a molecular size corresponding to MEP-1. In the presence of EDTA, the formation of this complex was strongly inhibited, and Zn^{2+} restored the binding activity of the chelated extracts to 135% of the control value. We are currently purifying MEP-1 and have found that it is not glycosylated, since it eluted with the flow-through on a wheat germ Sepharose column. Moreover, it is retained by a zinc-chelating Sepharose column suggesting that histidine, cysteine and/or tryptophan residues are exposed on the protein surface. Supported by the M.R.C.

R 436 TAT PERMITS EFFICIENT TRANSCRIPTIONAL ELONGATION IN VITRO BY SUPPRESSING RNA POLYMERASE II PAUSING.
Michael F. Laspia, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
Tat, the transactivator protein encoded by HIV-1, acts in vivo to increase transcriptional initiation and to stabilize elongation. We examined the effect of purified bacterially-expressed Tat on HIV-1 transcription in a cell free system with HeLa cell nuclear extracts. Tat produced a 12 fold increase in transcription from the wild type HIV-1 promoter, but not from the adenovirus major late promoter or TAR mutant promoters. Tat acted through TAR RNA to elevate LTR-directed transcription since the addition of wild type, but not mutant, synthetic competitor TAR RNA inhibited transactivation. Transactivation in vitro was not due to increased transcriptional initiation since promoter proximal RNA levels and promoter *proximal transcription rates were not stimulated* by Tat. Rather, transactivation by Tat was due principally to increased efficiency of transcriptional elongation since increased transcription was accompanied by elevated promoter distal RNA levels and transcription rates. Kinetic analysis of HIV-1 transcription was consistent with the existence of two populations of initiating RNA polymerases: an efficiently elongating population of polymerases that was unresponsive to Tat and a population of polymerases whose efficiency of elongation was increased by Tat. Addition of Sarkosyl or high salt to transcription reactions, like Tat, increased the efficiency of HIV-1 transcriptional elongation to a high level suggesting that basal elongation is subject to repression. Repression of transcription elongation was due, in part, to pausing by elongation complexes at random locations downstream of the promoter. We postulate that low levels of HIV-1 transcription are due to repression of transcriptional elongation and that Tat acts though TAR RNA to overcome repression by increasing the efficiency of HIV-1 transcriptional elongation.

R 437 **The Mechanism of Antirepression of Histone H1 by Sequence-specific Transcription Factors on Chromatin. Paul J. Laybourn and James T. Kadonaga, Department of Biology, 0322, University of California, San Diego, CA 92111-0322**
Chromatin structure has an important role in the regulation of gene expression. The objective in these studies is to clarify the role of chromatin in the regulation of gene expression at the level of transcriptional initiation by RNA polymerase II. Chromatin was reconstituted from purified core histones and histone H1, which allowed the determination of the effect of core nucleosomes and histone H1 on transcription. Core nucleosomes located over the transcriptional start site block *transcription, but transcription can still occur from* chromatin templates that do not have a core nucleosome located directly over the start site [Laybourn and Kadonaga (1991) *Science* **254**, 238 - 245]. Histone H1 completely represses basal transcription from chromatin at a ratio of one H1 per nucleosome, but sequence-specific transcription factors Sp1 and GAL4-VP16 are able to counteract or antirepress the H1 mediated repression. Since the activated transcriptional level is the same on chromatin as it is on naked DNA the net result is a large increase in the fold activation. Using primer extension footprinting, we have shown that the DNA-binding domain of GAL4 (amino acids 1 through 94) binds chromatin at various histone H1 levels, but in transcription assays we found that the GAL4 DNA-binding domain alone is not able to antirepress histone H1. In addition, GAL4 DNA-binding sites are required by GAL4-VP16 to be able to counteract histone H1-mediated repression of transcription. Therefore, both the DNA-binding and the acidic activation domain are necessary for antirepression of histone H1 by GAL4-VP16.

R 438 **Unique Okadaic Acid Mediated Regulation of the Transin and c-jun Genes.**
Levy,J.P.[1],Karin,M.[2],Nicholson,R.[3],Milczarek,G.[1],Gensler,C.[1],Bowden,G.T[1]
1.Radiation Oncology, Tucson, Arizona
2.Pharmacology, La Jolla, California
3. University of New South Whales, Sydney, Australia
Okadaic Acid (OA) is a non phorbol ester tumor promoter in mouse skin and an inhibitor of protein phosphatases 1 and 2A. Okadaic acid treatment of mouse keratinocytes leads to a transriptional induction of the transin gene and a very strong, sustained induction of the c-jun gene(Molecular Carcinogenesis, 1991 in press). We have found that induction of the transin gene is dependent on AP-1. A CACCC box seven base pairs down stream of the AP-1 site in the transin promoter is bound by SP-1. Mutation of this site leads to a transcriptional upregulation. Gel shift analysis reveals that AP-1 and SP-1 compete for binding to their respective cis elements. Thus SP-1 appears to act as a repressor for the transin promoter. For the c-jun gene, we have mapped the OA mediated induced activity to the region between -132 and -1100 in the c-jun promoter. This region is upstream of that reported to be responsible for c-jun autoregulation
(Cell, Vol 55, 875-885, 1989) and for OA induction of the c-jun promoter (JBC, Vol 266, No. 15, 9363-9366, 1991). OA *mediated transcritional induction in this region can be* completely eliminated by expression of a dominant negative c-jun transcription factor mutant in the keratinocytes. Taken together we have demonstrated that OA mediates an induction of both the transin and c-jun genes through AP-1. The activity of AP-1 in the transin promoter is negatively modulated by SP-1. A unique, as yet undescribed, AP-1 activated region lies upstream of the -72 TRE in the c-jun promoter. These studies are aimed at understanding the interaction of AP-1 with other regulatory factors. (This work is supported in part by the USPHS grant CA-40584 awarded to G.T.B.)

R 439 OCCUPANCY OF FACTOR BINDING SITES AND THE EFFECT OF HISTONE HYPERACETYLATION IN SV40,
Leonard Lutter, Jyothi Eadara, Kenneth Hadlock, and LuAnn Judis, Molecular Biology Research Program, Henry Ford Hospital, 2799 West Grand Blvd., Detroit, MI 48202
We have used a restriction-enzyme-based functional footprinting approach to investigate the occupancy of the enhancer sites in the SV40 late transcription complex. We find that Sph I cuts the late transcription complex almost quantitatively, indicating that these sites are not occupied by factors. This is not necessarily unexpected, since the enhancer acts on early transcription, and we are currently developing a strategy whereby the occupancies of these sites can be assessed in both the early and late transcription complexes simultaneously.

We have also tested the model of Norton et al. [Cell 57 449 (1989)] which proposes that histone hyperacetylation induces gene activation by causing nucleosomes to release supercoils into the domain of the gene. A prediction of this model is that the level of constrained supercoils in the DNA of chromatin should decrease upon histone hyperacetylation. We have evaluated this prediction by determining the level of constrained supercoils present in SV40 and transfected plasmid minichromosomes in cells grown with or without butyrate, an agent which induces histone hyperacetylation. We found that in-vivo-assembled minichromosomes do not contain reduced supercoils when their histones are hyperacetylated. While hyperacetylation may indeed cause reduced wrapping of DNA on nucleosomes reconstituted in vitro to subsaturating levels, this effect is not seen in the native in vivo-assembled chromosome. Thus our results indicate that this proposal is unlikely to reflect the mechanism of gene activation in the cell.

R 440 CHARACTERIZATION OF A NOVEL *CIS*-ACTING ELEMENT IN THE HUMAN CARDIAC MYOSIN HEAVY CHAIN GENES, John D. Mably, Michael J. Sole and C.C. Liew The Centre for Cardiovascular Research, Departments of Clinical Biochemistry, Physiology and Medicine, University of Toronto, Toronto, CANADA.

The genes for the two human cardiac myosin heavy chain isoforms, α and ß, have been characterized and completely sequenced in our laboratory. The developmental and tissue-specific expression of these genes, however, is still not well understood. In this study, DNase I footprinting was used to define putative *cis*-acting regulatory sequences. Similar footprints have been detected at two positions in the α 5' upstream region (GAAAAATCT at -904 to -896 and GAAAATCT at -823 to -816). Sequence analysis of the 5' region of the ß gene has defined an element with the sequence AGATTTTTC, the reverse complement of the larger element found in the α gene. The defined sequence has an AT-rich core, as does the SRE/CArG motif found in the promoters of several muscle-associated genes. Because of this similarity in structure, we have designated this element the GArC motif (G,A-rich,C).

Oligomers were made to the GArC element and its flanking sequence and used in gel mobility shift assays to determine binding specificity. Binding was eliminated by the addition of unlabelled double-stranded oligomer as specific competitor. However, when non-specific competitor DNA was added (double-stranded oligomers of the *Xenopus* actin SRE and an AT-rich sequence from another region of the α gene were used) the binding to the labelled GArC probe was preserved. The molecular weight of the GArC-binding factor(s) was assessed using Southwestern blot analysis. Using the GArC oligomer as a probe, proteins at ≈67 and 55 kD were shown to bind this element. Thus, we describe a novel *cis*-element which is associated with a *trans*-acting factor(s). This may provide another mechanism of regulation to those previously reported for the cardiac myosin heavy chain genes.

R 441 INHIBITION OF HIV-1 TAT-DIRECTED TRANSACTIVATION BY α-AMANITIN AND DRB, S.K. Malcolm, T.E. Thais, S.R. Jaskunas, F. Victor, and W.A Spitzer. Virology Research, Lilly Research Laboratories, Indianapolis, IN 46285.

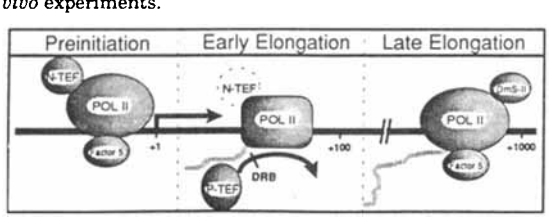
A major effect of transactivation by the HIV-1 tat protein is an enhancement in transcription elongation from the viral LTR promoter (1-5). α-Amanitin and DRB (5,6-dichloro-1-b-D-ribo-furanosylbenzimidazole) inhibit transcription elongation by RNA polymerase II (6-8). Therefore, the ability of these compounds to inhibit tat-dependent transactivation of a reporter gene was tested Other compounds that were tested included Ro5-3335, a reported inhibitor of transactivation (9), and phalloidin and phallacidin, which are structurally related to α-amanitin (10-11). In addition, the combined effect of Ro5-3335 and α-amanitin was examined.

The effect of these inhibitors on transactivation was examined in stable HeLa cell lines containing appropriate plasmids (12), or in transiently transfected HeLa cells. The reporter gene encoded tissue plasminogen activator for the stable cell lines or firefly luciferase for transient transfection experiments. Inhibition of the tat-dependent expression of the reporter gene transcribed from the HIV-1 LTR promoter was compared with inhibition of expression from the SV40 early promoter.

The results indicated that the tat/LTR-directed transcription was more sensitive to α-amanitin, DRB, and Ro5-3335 than transcription from the SV40 early promoter. Phalloidin and phallacidin had no selective inhibition on transactivation. The combined inhibition observed in the presence of both Ro5-3335 and α-amanitin was additive. These results suggest that the tat/LTR modified transcription complex of RNA polymerase II may have a greater affinity for α-amanitin and DRB than other transcription complexes, which may reflect the tat-induced modifications of the transcription complex resulting in enhanced transcription elongation.

R 442 CONTROL OF FORMATION OF TWO DISTINCT CLASSES OF RNA POLYMERASE II ELONGATION COMPLEXES, Nicholas F. Marshall and David H. Price, Department of Biochemistry, University of Iowa,Iowa City, IA 52242

We have examined the transition into elongation by RNA polymerase II initiated at a promoter and have identified two factors which are involved in controlling this process. One factor, which we call N-TEF (negative transcription elongation factor), causes all polymerase molecules which initiate to enter an abortive mode of transcription in which only short transcripts are made. The effect of N-TEF can be eliminated by the addition of 250 mM KCl or 1 mg/ml heparin soon after initiation. The second factor, P-TEF (positive transcription elongation factor), allows some polymerase molecules to enter a productive mode of transcription in which very long transcripts can be synthesized. P-TEF is inhibited by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) at concentrations which have no effect on the initiation of transcription. By using templates immobilized on paramagnetic particles we show that N-TEF, but not P-TEF, is tightly associated with preinitiation complexes. We show that isolated early elongation complexes normally yield only abortive elongation complexes unless P-TEF is added back to the reactions. A model is presented which describes the role of elongation factors in the formation and maintenance of elongation complexes. The model is consistent with the available *in vivo* data concerning control of elongation and is used to predict the outcome of other potential *in vitro* and *in vivo* experiments.



R 443 NusG: An *E. coli* Elongation Factor that Makes Termination by Rho Factor More Efficient and Antitermination by Bacteriophage λ N protein More Processive. Stephen Mason, Joyce Li, Jack Greenblatt. Department of Molecular and Medical Genetics and Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

Antitermination by the bacteriophage λ N protein involves the *E. coli* factors NusA, NusB, S10, and NusG. We have shown that transcription elongation complexes isolated from reactions containing purified RNA polymerase, NusA, NusB, S10, NusG, λ N protein, and a λ *nut* site-containing DNA template contain all five elongation factors. The assembly of this complex is highly cooperative and coordinated by the surface of elongating RNA polymerase. To determine the distance over which antitermination can occur we have used RNA-DNA hybridization to map λ transcripts made in our *in vitro* reactions. The presence of all five factors in the reaction allows for processive antitermination over distances as great as 8000 nucleotides. This efficient antitermination occurs from both the λ *pL* and *pR* promoters and depends on all five elongation factors. The great stability of N-modified transcription complexes containing NusA, NusB, S10, and NusG apparently allows processive antitermination over very long distances.

We have also found that NusG binds directly to termination factor Rho. NusG does not bind at high temperature to Rho factor with the *rho026* mutation, which prevents antitermination by N at high temperature. In addition, termination by Rho in the λ *pR* operon is greatly stimulated under certain reaction conditions by the presence of NusG. Therefore, NusG can aid both termination by Rho and antitermination by N.

R 444 TRANSCRIPTIONAL REGULATION OF *DROSOPHILA MELANOGASTER* F ELEMENTS, Gabriella Minchiotti, Cristina Contursi, Saverio Minucci and Pier Paolo Di Nocera, International Institute of Genetics and Biophysics, C.N.R., via Marconi 10, Naples; Dipartimento di Patologia e Biologia Cellulare e Molecolare, II Medical School, University of Naples, via S. Pansini 5, Naples.

D.melanogaster F elements are long, oligo-A terminated DNA sequences that belong to the superfamily of mobile DNA elements known as LINEs. The coding capacity and the structural organization suggest that F elements derive from the retrotranscription of RNA intermediates. Transient expression assays carried out in *Drosophila* Schneider II cells led to the identification of two oppositely oriented promoters in the 5' end of complete F family members: F_{in} and F_{out}. The F_{in} promoter transcribes from residue +6 toward the element body and probably controls the formation of the retrotransposition intermediates and gene products; the F_{out} promoter drives the synthesis of transcripts that initiate at multiple sites within the +92/+102 interval, on the opposite strand. DNA sequences important for the activity of the F_{in} promoter are included within the +1/+30 interval; regulatory *cis*-acting elements located within the +175/+218 interval influence the level of the accumulation of the F_{in} and F_{out} transcripts. In vivo, *out* transcripts were detected, by RNase protection assays, at different developmental stages; polyA$^+$ transcripts corresponding in length to full size F elements were identified by Northern analysis so far only in ovaries.

R 445 RHO-EK155: A NEARLY SILENT MUTATION IN E. COLI RHO FACTOR Keith W. Nehrke and Terry Platt, Department of Biochemistry, University of Rochester School of Medicine, Rochester, NY 14642 The overproduction vector p39AS contains a point mutation that results in the alteration of rho at amino acid 155 from Glu to Lys. Many of the major activities of rho and a large number of mutants have been analyzed using this variant, making it imperative to correct the error and characterize EK155 with respect to the bona-fide wild-type protein. Fortunately, the EK155 mutation is nearly silent: RNA binding, poly(C) ATPase and transcription termination activities in vitro are indistinguishable from wild-type. The only observable difference that true wild-type rho has is a two-fold higher ATPase and helicase activity using one particular RNA transcript, trp t'. The location of amino acid 155 in a "hinge" region of rho between the RNA and ATP binding domains suggests that Glu-155 may potentiate a rate-limiting conformational change that couples RNA binding to ATP hydrolysis.

R 446 A heterodimer of NusB and S10 binds to the *boxA* sequence RNA of the *rrnG* ribosomal RNA operon of *E. coli*. Justin Nodwell and Jack Greenblatt. Banting and Best Department of Medical Research and Department of Molecular and Medical Genetics, University of Toronto, 112 College Street, Toronto, Canada. M5G 1L6.

An element in the leader of the *rrnG* ribosomal RNA operon, which greatly resembles *boxA* of the phage λ *nut* sites, prevents downstream termination during transcription *in vivo* (Berg et al, 1989. *J. Mol. Biol.* 209. p345). NusA, NusB, S10 and NusG are *E. coli* transcription elongation factors which are involved in transcription antitermination by the phage λ N protein. We have used short RNA's encoding *rrnG boxA* for gel mobility shift experiments with purified NusA, NusB, S10, and NusG. None of the proteins shifts the mobility of *boxA* on its own, but NusB and S10 form a heterodimer which binds specifically to *boxA* RNA. The NusB-S10 shift is not affected by the presence of NusA and/or NusG. Binding of NusB-S10 to *boxA* is inhibited or abolished by antitermination defective point mutations in *boxA*. We suggest that the mechanism of transcription antitermination in the *E. coli* ribosomal RNA operons is closely related to that mediated by the N protein of bacteriophage λ, were antitermination factors form a ribonucleoprotein complex on *nut* site RNA that is carried along with the elongating polymerase (Nodwell and Greenblatt, 1991, *Genes & Dev*, 5, p2141).

R 447 MULTIPLE ELEMENTS FROM THE c-MOS ONCOGENE LOCUS INHIBIT mRNA ACCUMULATION IN A POSITION, ORIENTATION AND CELL-TYPE DEPENDENT MANNER, Steven K. Nordeen, Prakash Upadhya, Judith E. Spiegel, Department of Pathology, University of Colorado Health Sciences Center, Denver, CO 80262

The c-MOS oncogene is normally expressed just prior to meiosis in the maturing germ cells of both the testes and ovaries. The c-MOS protein, a serine-threonine kinase, is known to possess powerful fibroblast transforming activity even at extraordinarily low levels. In the rat, the c-MOS coding region is preceded by a region termed the rat inhibitory sequence (RIS) which is located 1.8 kb upstream of the reading frame. This sequence, originally defined by studies on the transforming activity of the c-MOS gene, abolished the ability of upstream viral promoters to direct transformation but had no activity on transformation when the promoters were placed between the inhibitor sequence and the c-MOS open reading frame. Thus the RIS may function as a fail-safe mechanism to prevent the inappropriate expression of c-MOS. In the present studies we show that the RIS sequence can function in a heterologous reporter gene context. Characterization of the originally described 821 bp element reveals that it is a complex element comprised of at least four functional domains. The individual domains of inhibitory activity have been localized to as little as 50 base pairs. The RIS inhibitory activity is very position dependent, functioning fully only when in the 5' end of a transcription unit and not at all in the 3' end. While the activity of the entire RIS is not dependent on its orientation, some of the domains are orientation-dependent. Also, the RIS functions in a cell-type dependent manner, active in mouse Ltk-fibroblasts and human T47D cells but inactive in HeLa cells. The definition and characterization of the inhibitory domains and their properties now permit mechanistic studies to distinguish whether RIS functions as a transcription terminator, an RNA destabilizing sequence or a combination of the two.

R 448 THE Aα MATING LOCUS OF SCHIZOPHYLLUM COMMUNE ENCODES TWO DISSIMILAR MULTIALLELIC HOMEODOMAIN PROTEINS. C.P. Novotny[1], H. Yang[2], C.A. Specht[2], G.P. Shen[1], M.S. Stankis[1], and R.C. Ullrich[1], Departments of Microbiology and Molecular Genetics[1] and Botany[2], University of Vermont, Burlington, Vt 05405. The Aα mating locus is one of four loci that regulate sexual development in the fungus Schizophyllum commune. We have determined the DNA sequence of Aα in three of the nine mating types. The locus consists of two multiallelic genes, AαY and AαZ. The Y polypeptides encoded by different alleles are 42% identical. The Z polypeptides exhibit 49-54% identity. The deduced Z and Y polypeptides have homeodomain motifs that may enable them to bind to DNA and thereby regulate the expression of developmental genes. Z has an acidic region that could be functionally analogous to the acid regions which activate transcription in Gal4 and GCN4 of yeast. Y as a serine rich region and a basic region which shows some identity to the lysine-rich region of H1 histones. Transformation experiments show that the A developmental pathway is activated when Z from one mating type interacts with Y from a different mating type. Northern blots reveal the presence of a third multiallelic gene (AαX) in the Aα locus. Expression of AαX is elevated 5-10 fold after mating which suggests that it is part of the Aα developmental cascade. The structure of AαX and the possibility that its expression is directly controlled by Z and Y is being investigated.

R 449 **Altered chromatin accessibility in the HPRT gene on integration of an enhancer-containing minigene.** Michael Pikaart and Bryant Villeponteau, Department of Biological Chemistry and the Institute of Gerontology, University of Michigan, Ann Arbor, MI 48109-2007.

Recent genetic data indicate that histones in chromatin function as generalized repressors of transcription. In vitro reconstitution experiments show that some enhancer-binding proteins can specifically counteract histone-mediated repression and increase local accessibility to transcription factors. Several in vivo studies further suggest that enhancers may have long-range effects on chromatin accessibility. We have inserted minigenes with or without the polyoma enhancer into the third exon of the hypoxanthine phosphoribosyltransferase (*HPRT*) gene by homologous recombination and assayed *HPRT* DNase I sensitivity to test for changes in chromatin accessibility. Relative rates of DNase I sensitivity are determined by a novel PCR assay that provides high-resolution maps of DNA accessibility for any sequenced locus in chromatin. In its native state, we find that the *HPRT* gene has low sensitivity to DNase I in human fibrosarcoma cells. Insertion of the polyoma enhancer and reporter gene into exon 3 confers altered *HPRT* DNase I sensitivity for 4 to 6 kb on either side of the enhancer. The changes in DNase I sensitivity peak near the enhancer and decline with distance from the enhancer. Deleting the enhancer from the minigene completely abolishes all effects of gene targeting on *HPRT* DNase I sensitivity. These experiments define the polyoma enhancer as a *cis*-acting mediator of chromatin accessibility. Data from other constructs containing glucocorticoid-inducible enhancer elements will also be presented.

R 450 IDENTIFICATION OF TWO CANDIDATES FOR SILENCER ELEMENTS ASSOCIATED WITH THE MURINE CD4 GENE, John F. Sands* and Janko Nikolic-Zugic#, *Department of Microbiology, Loma Linda University, Loma Linda, CA 92350 and #Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY 10021.

To determine the location of *cis*-acting DNA elements controlling the expression of the murine CD4 gene, we examined the chromatin of freshly isolated thymocyte and peripheral T cell subpopulations for DNase I hypersensitive (DH) sites associated with 10 kb of DNA surrounding the first exon of the CD4 gene. The presence or absence of the three T cell-specific DH sites in this region correlates with CD4 gene expression. Two of the DH sites are strongly present only when the CD4 gene is not expressed suggesting that these sites may participate in repression or silencing of the CD4 gene. One of these, called T7, is located about 300 bp 5' of the first exon, and it probably detects protein-DNA interactions near the CD4 promoter. The other site, T8, lies in the first intron about 2.5 kb 3' of T7. A third T cell-specific DH site, T9, located about 3.0 kb 3' of T7, does not vary with CD4 gene expression. T9 may detect a necessary control element that permits the regulation of CD4 during T cell development (perhaps to release the chromatin at the CD4 locus from an inactive transcriptional state). The DH sites were subcloned and analyzed for T cell-specific protein-DNA interactions using the electrophoretic mobility shift assay. Results of this assay demonstrate that all three DH sites exist as a consequence of T cell-specific protein-DNA interactions. The subcloned DNA restriction fragment corresponding to the T7 DH site binds a different set of proteins in $CD4^+$ cells as compared to $CD4^-$ cells. We found that all the T cell-specific protein-DNA interactions occurred in a DNA region of less than 250 bp. The restriction fragment that contains the intron DH sites binds two T cell-specific protein(s) present in both $CD8^+4^-$ and $CD8^-4^+$ T cells. In addition, we detect at least two $CD8^+$ specific protein(s) binding to this DNA region. Together, our data is consistent with the interpretation that these sites represent *cis*-acting control elements that repress CD4 gene expression during various stages of T cell development.

R 451 VACCINIA VIRUS mRNA (NUCLEOSIDE-2'-)-METHYLTRANSFERASE IS IDENTICAL WITH THE STIMULATORY SUBUNIT OF POLY(A) POLYMERASE. Barbara S. Schnierle, Paul D. Gershon and Bernard Moss. Laboratory of Viral Diseases, National Institutes of Health, Bethesda, MD 20892.

Vaccinia virus, a large DNA virus that replicates within the cytoplasm of infected cells, provides a unique system for studying transcription. The viral enzymes are capable of synthesizing mRNA with typical eukaryotic features. Processing of eukaryotic mRNA involves the formation of a 5' terminal $m^7G(5')pppN^m$ - cap structure and 3' polyadenylation. After methylation of the terminal guanosine, the second nucleotide is methylated by a specific nucleoside-2'-methyltransferase. The enzyme was purified from vaccinia virus cores as described before (Barbosa & Moss, JBC 253, 7692 (1978). The protein was transferred to a membrane, digested with trypsin and microsequenced. The peptide sequences matched the J3R open reading frame of vaccina virus, which encodes the stimulatory subunit (VP39) of poly(A) polymerase. Further studies demonstrated that the methyltransferase co-chromatographed with both free VP39 and the VP39-VP55 poly(A) polymerase heterodimer. VP39 overexpressed by recombinant DNA methods also had methyltransferase activity. From these data we conclude, that the stimulatory subunit of poly(A) polymerase is identical with the nucleoside-2'-methyltransferase.

R 452 GENOMIC FOOTPRINTING OF THE *TPI* PROMOTER OF *Saccharomyces cerevisiae* REVEALED FOUR PROTEIN BINDING SITES IN THE UAS REGION OF *TPI*. E.W. Scott and H.V. Baker. University of Florida, Department of Immunology and Medical Microbiology, College of Medicine, Box 100266, JHMHC, Gainesville, FL 32610-0266, (904) 392-0680.

In *Saccharomyces cerevisiae* the *TPI* gene product, triose-phosphate isomerase, makes up about two percent of the soluble protein. *TPI* gene expression is dependent upon the action of both the *GCR1* and *RAP1* gene products. Previously, we identified the site of an upstream activating sequence (UAS) to reside on a fragment that extended from -377 to -327. This fragment was shown to encode a RAP1-binding site. We have extended our analysis of the *TPI* controlling region. A series of deletions originating at -220 and extending towards the aforementioned RAP1-binding site suggested that the RAP1-binding site located between positions -339 and -349 was required for expression of the *TPI::lacZ* fusion. However, subcloning experiments with the UAS-less *TPI::lacZ* fusion indicated that a RAP1-binding site alone was not sufficient to drive expression of the fusion. Next, we carried out genomic footprinting experiments over the native *TPI* promoter in wild-type and *gcr1* mutant strains. We identified four sites of protection in the wild-type strain; whereas, only two sites were protected in the *gcr1* mutant strain. The proteins that bind these sites were identified. One site is bound by REB1, one site is bound by RAP1, and two sites are bound by GCR1. Each of the four sites were subjected to mutagenesis, and the effects of the mutations on expression of the *TPI::lacZ* gene fusion were assessed.

R 453 TRANSCRIPTION TERMINATION FACTOR RHO FUNCTIONS AS A DIMER WHICH RELEASES POLYNUCLEOTIDE COFACTOR DURING THE ATPase REACTION CYCLE Steven E. Seifried, Yan Wang, and Peter H. von Hippel. Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

Transcription termination factor rho from *E. coli* has been known to consist of six identical subunits. The assembled protein will translocate along the transcript in an ATPase dependent manner, interact with the RNAP complex, and bring about termination. We have used fluorescence, cryoelectron microscopy, HPLC, and other classical biophysical techniques to characterize the subunit association pathway and determine the symmetry of the intact rho hexamer. The D3 symmetry, a trimer of dimers, has observable functional manifestations.

Each protomer within a dimer can hydrolyze ATP, yet only one ATP binding site per dimer is filled at a time. Phosphohydrolysis requires the occupation of a polynucleotide binding site on each monomer. Affinity for these cofactor sites in the dimer is determined by the base composition of the polymer. Binding and ATPase assays with designed oligonucleotides, random copolymers, and homopolymers show that release of polymer from one of the two cofactor sites within the dimer is required for the continuation of the ATPase reaction cycle. The 2' hydroxyl of the ribose sugar increases the offrate of the polymer from an activated enzyme state. The RNA bind-release cycle, driven by ATPase, is coupled to the 3-D geometric elements to generate translocation of rho on nascent transcript.

R 454 MAPPING AND CHARACTERIZATION OF THE HIV-1 INDUCER OF SHORT TRANSCRIPTS (IST), Michael Sheldon, Ratneswaran Ratnasabapathy, Maureen Sullivan and Nouria Hernandez.

We have previously identified a novel transcription element in the HIV-1 LTR located between -5 and +82 relative to the start site of transcription. This element, which we have named IST (inducer of short transcripts), activates transcription from the HIV-1 promoter (and any other promoters we tested), but the resultant additional RNAs are all prematurely terminated around position +60. Because these short transcripts contain TAR, the Tat transactivation response element, they might be involved in regulating transactivation of viral gene expression.

We have now localized IST more precisely. Our results indicate that IST is most probably an RNA element contained within the lower half of a stable stem-and- loop structure that folds the first 60 nucleotides of HIV-1 mRNAs. IST is separable from TAR, which is contained within the upper half of the stem-and-loop and whose activity is completely dispensable for IST. The presence of both halves of the stem-and-loop is crucial for IST activity. Furthermore, maximal IST activity is dependent on the distance between the element and the promoter. As the element is moved farther downstream, short transcript formation decreases. The presence of a functional element in the lower half of the stem-and-loop structure is consistent with the high degree of sequence conservation of this region among different viral isolates.

R 455 **TWO DISTINCT NUCLEAR TRANSCRIPTION FACTORS RECOGNIZE LOOP AND BULGE RESIDUES OF THE HIV-1 TAR RNA HAIRPIN,**

Christian T. Sheline, Lawrence H. Milocco, and Katherine A. Jones, The Salk Institute, La Jolla, CA 92037

Transcriptional activation by the HIV-1 Tat protein requires specific residues in the hexanucleotide loop and trinucleotide bulge of the TAR RNA stem-loop structure found in the 5'-untranslated leader of all viral transcripts. Tat directly contacts residue U^{22} in the bulge, and is thought to act in concert with cellular factors bound to the loop. We find that Hela nuclear extracts contain two specific TAR RNA-binding proteins, designated TRP-1 and TRP-2, which compete for binding to the upper portion of the TAR hairpin. Analysis of point mutants in TAR RNA reveals that TRP-1 contacts residues in the loop which are important for trans-activation, whereas TRP-2 contacts the bulge, including the same residue (U^{22}) that is required for the Tat-TAR interaction. Glycerol gradient sedimentation and UV cross-linking experiments indicate that TRP-1 is a large heteromeric complex containing a 185 kDa RNA-binding protein, whereas TRP-2 activity derives from a family of 110-70 kDa proteins. Interestingly, both TRP-1 and TRP-2 promote TAR-dependent transcription *in vitro* in the presence of Tat, even though mixing experiments indicate that each of the three proteins must bind independently to TAR RNA. These findings suggest that the TAR element is recognized by two different nuclear RNA-binding proteins that affect transcriptional regulation by Tat.

R 456 ANALYSIS OF TWO CELLULAR FACTORS THAT BIND AT THE RNA START SITE OF THE HIV-1 PROMOTER. P.L. Sheridan, M. Schorpp, W.L. Brown and K.A. Jones, Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA. 92037.

Two factors, designated the HIV-1 Leader-binding (LBP-1) and Initiator (HIP) proteins, have been shown in DNAse I footprint experiments to bind to sequences centered around the viral RNA start site; from -17 to +35 and -25 to +2, respectively. This region has been demonstrated to be important for the transcriptional activity of the promoter and has also been implicated in the generation of attenuated RNAs. LBP-1 was purified to near homogeneity from HeLa cell nuclear extracts and found to migrate as a 63 kDa peptide in SDS-gels. Tryptic peptides of purified LBP-1 were subjected to automated protein sequence analysis and resulted in the identification of 8 unique peptides ranging from 5-18 amino acids in length. Two of these peptides suggest that LBP-1 may be post-translationally modified by phosphorylation. In addition, a third peptide is highly homologous to a 15 amino acid sequence of the *D. Melanogaster* transcription factor Elf-1. A cDNA for the initiator protein, HIP, was isolated following deoxynucleotide screening of a lambda gt11 HeLa cDNA expression library. Expression of the HIP cDNA in *E. Coli* as a trpE fusion gene produced a fusion protein that binds to the HIV-1 RNA start site in footprinting studies. The HIP cDNA contains a single continuous open reading frame that encodes 1008 amino acids (Mr: 115 kDa). The cDNA contains a number of recognizable protein motifs, most notably a novel Cys/His-element found in a unique group of nuclear regulatory factors. Interestingly, the HIP protein shows a high degree of homology to the SNF2 protein, a yeast transcription factor. Additional studies with purified protein and their cDNAs will determine what role these DNA-binding proteins play in HIV-1 transcription.

R 457 CELL-SPECIFIC AND UBIQUITOUS FACTORS ARE RESPONSIBLE FOR THE ENHANCER ACTIVITY OF THE RAT INSULIN II GENE, Sheau-Yann Shieh and Ming-Jer Tsai, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

Pancreatic β-cell-specific expression of the insulin gene is mediated, at least in part, by an enhancer element termed the rat insulin promoter element 3 (RIPE3) found within the rat insulin II gene between position -126 and -86. Here we identify three distinct factors interacting with RIPE3, namely 3a1, 3a2, and 3b1, which bind to the sequences between -100 to -90, -108 to -99, and -115 to -107, respectively. Factors 3a1 and 3b1 are β-cell specific, while 3a2 is ubiquitously distributed. The 3a1 site contains the consensus binding sequence (CANNTG) for a group of DNA-binding proteins called basic-helix-loop-helix (B-HLH) proteins. We showed in this study that the 3a1 binding activity contains E12/E47, a member of the B-HLH protein family, or an E12/E47-like protein. Sequence comparison of the 3a2 and 3b1 binding sites suggests that they are unique and may bind to novel transcription factors. Mutation analysis of each individual binding site in transient expression experiments indicates that all of the three binding sites contribute to the enhancer activity of the RIPE3 in β-cells. Mutation in any one of the three binding sites not only disrupts binding of the corresponding factor, but decreases RIPE3 enhancer activity by 4- to 7-fold. The results suggest that interactions between the 3a1, 3a2, and 3b1 factors are required for maximum enhancer activity of the RIPE3 in insulin-producing cells.

R 458 DIFFERENCE IN TOPOLOGY OF ACTIVE AND INACTIVE CHROMATIN. I.V.Smirnov, E.E.Minyat, S.G.Lezhnin, V.I.Popenko, V.L.Makarov. Engelhard Institute of Molecular Biology Acad.Sci.USSR, Moscow, USSR.

We decided to test the hypothesis that the loops of inactive chromatin of eucaryots are additionally folded into rosette-like structures, stabilized by some non-histone proteins, strongly associated with DNA. If this is the case, mild nuclease digestion of dehistonized nuclei would generate rosettes and small loops. We have developed a two dimensional electrophoretic approach, which enabled us to resolve DNA fragments according to their topology. In the case of transcriptionally inactive chicken erythrocytes nuclei, a slow moving fraction was detected at early stages of nuclease digestion. This fraction was analyzed by electron microscopy and rosette-like structures were indeed observed. When transcriptionally active fibroblast cells were used in the experiments, no such slow moving band was detected. Thus, our results strongly supports the existence of rosette-like level of inactive chromatin folding. The study of the "core" of this rosette-like structure is in progress.

R 459 THE BLOCK TO RNA POLYMERASE II TRANSCRIPTION ELONGATION IS RELATED TO OVERALL INITIATION LEVELS IN XENOPUS OOCYTE TRANSCRIPTION ASSAYS, Charlotte A. Spencer and M. Alison Kilvert, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7.

Transcription initiation and elongation both contribute to the regulation of steady-state c-myc RNA levels. We have used the Xenopus oocyte injection assay to study the premature block to transcription elongation that occurs in the first exon and intron of the human c-myc gene. Previous studies suggested that, after injection into Xenopus oocytes, transcription from the c-myc P1 promoter results in read-through transcription, whereas transcription from the stronger P2 promoter results in a combination of blocked and read-through transcription. We now demonstrate that this promoter-specific processivity results from the overall amount of RNA polymerase II transcription initiation occuring from either promoter. Experimental parameters that reduce the amount of transcription from P1 or P2, such as decreased template concentration or decreased incubation time, result in a reduction in the ratio of blocked to read-through c-myc transcripts. Conversely, when transcription levels are increased by co-injection of transcriptional activator protein, increased incubation time or addition of competitor promoters, the ratio of blocked to read-through transcripts increases. We are now investigating the hypothesis that, in the Xenopus oocyte transcription assay, an RNA polymerase processivity function is depleted above a threshold level of transcription initiation, and that this function can be reconstituted with fractionated and purified elongation factors.

R 460 TRANSCRIPTIONAL REGULATION, AUTOREGULATION AND COLD SHOCK INDUCTION OF hns EXPRESSION IN E. COLI, Roberto Spurio, Anna Brandi, Maurizio Falconi, Anna La Teana, N. Patrick Higgins*, Claudio O. Gualerzi & Cynthia L. Pon, Department of Biology, University of Camerino, 62032 Camerino (MC) Italy and *Dept. of Biochemistry, University of Alabama at Birmingham, USA.

Several DNA-binding proteins have been implicated in the organization of the prokaryotic nucleoid. Among these is H-NS (H1a), a neutral, heat-stable, dimeric protein containing 136 amino acids of known sequence localized in the nucleoid by immune electron microscopy. H-NS displays a fairly high affinity for double-stranded, curved DNA and its interaction with DNA causes a strong condensation of DNA both in vitro and in vivo. In E. coli, hns, the structural gene encoding H-NS, belongs to the cold shock regulon since its expression is enhanced approximately four-fold during the lag following the shift of the growth temperature from 37°C to 10°C. The "constitutive" and the cold shock regulation of hns expression have been studied by in vivo and in vitro experiments determining the levels of H-NS mRNA by Northern blot analysis and making use of hns-cat fusions in different genetic backgrounds, transcription in vitro, gel shift, footprinting and other related approaches. The stimulation of hns expression during cold shock depends on a transcriptional activator, the product of cspA, a protein homologous to eukaryotic CCAAT binding proteins, which binds specifically to a 110 bp DNA segment containing the promoter of hns. The level of hns expression at 37°C, on the other hand, depends on at least two regulatory loops, one involving the transcriptional autorepression by H-NS and, the second, a transcriptional activation/inhibition due to the Fis protein (Factor for Inversion Stimulation). Both proteins bind to specific sites within a curved region of DNA upstream of the hns promoter.

Acknowledgement. Supported in part by grants from the Italian MURST and National Research Council (CNR).

R 461 **Overexpression of yeast heat shock factor suppresses mutations within the *HSP82* heat shock gene promoter.** *Bruce Stentz and David S. Gross*, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, LA 71130.

The *HSP82* heat shock gene of *Saccharomyces cerevisiae* is normally expressed at a low basal level and is 10 - 20 fold inducible upon mild heat shock. Both basal and induced transcription of *HSP82* require binding of heat shock factor (HSF) to the principal heat shock element (HSE1) located upstream of the transcription start site. Two additional heat shock elements upstream of HSE1 (HSE2 and HSE3) have a weaker homology to the heat shock consensus sequence and are not detectably bound by HSF, as assayed by genomic footprinting (Gross *et al, J. Mol. Biol.* **216**:611-631 [1990]). We have found that point mutagenesis of conserved nucleotides within HSE1 reduces basal transcription 10 - 50 fold while only minimally affecting induced expression. In contrast, regional deletion or substitution of the entire HSE1 heat shock activation sequence essentially abolishes both basal and induced expression. We report here that overexpression of heat shock factor can suppress the phenotypes of the *cis*-acting *hsp82* mutants. Transient overexpression of HSF, achieved through use of an episomal *GAL1/HSF* fusion gene, leads to a dramatic increase in the basal transcription of single and multiple point HSE1 mutants of *hsp82*. We find that steady-state mRNA levels are elevated to levels nearly equal to that of wild-type; similar results are seen when HSF is constitutively overexpressed. Furthermore, under inducing conditions, a substantial (> 12 fold) increase in *HSP82* transcript levels is seen when HSF is constitutively overexpressed in an HSE1 regional substitution mutant termed Δ*HSE1::PET56*. These results suggest that one or both upstream heat shock elements, HSE2 and HSE3, may be functional in the presence of increased intracellular concentrations of HSF. We are currently conducting genetic and biochemical experiments to confirm this possibility.

R 462 TRANSCRIPTION ELONGATION AND TERMINATION IN THE VICINITY OF AN INTRINSIC TERMINATION SIGNAL. *Kevin S. Wilson and Peter H. von Hippel*, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

We are involved in studies on the kinetics of transcription elongation leading up to the tR2 intrinsic termination signal in phage lambda. On a DNA template linking the T7A1 promoter and the tR2 termination sequences, transcription complexes can be stalled immediately upstream of the tR2 termination signal. By sequencing the RNA transcribed from this template, we have determined the exact sites where termination occurs *in vitro* with purified *E. coli* RNA polymerase. The elongation of complexes stalled immediately upstream of the tR2 sites has enabled us to study in detail the kinetics of RNA chain elongation and the stability of complexes at individual positions as they approach the tR2 sites. The core protein ($\alpha_2\beta\beta'$) of RNA polymerase does not appear to pause for longer than 10 seconds at the tR2 sites before termination. This conclusion is derived from the kinetics of elongation through the tR2 termination sequence. The effects of NusA, sigma 70, and rho proteins on elongation and termination in the vicinity of the tR2 sites are now being explored.

R 463 TRANSFORMATION OF RAT-1 CELLS BY THE v-fos ONCOGENE INDUCES PREMATURE TERMINATION OF ALPHA 1 (I) PROCOLLAGEN GENE TRANSCRIPTION. , Helmut Zarbl, Caroline Hoemann, Monteserrat Corominas, Paul Bornstein† Division of Toxicology, Massachusetts Institute of Technology, Cambridge MA, 02139; †Departments of Biochemistry and Medicine, University of Washington, Seattle WA, 98195.

The FBJ *v-fos* oncogene encodes a nuclear protein capable of transactivating or trans-repressing promoters of specific genes. It is therefore likely that *v-fos* transforms cells by altering the cell's pattern of gene expression. We have used revertant cell lines (non-transformed variants) to identify the genes encoding the subunits of collagen (I), as targets of *v-fos* transformation-specific alterations in gene expression (Hoemann and Zarbl, Cell Growth & Diff.**1**:581, 1990). Results indicated that α_1(I) procollagen gene expression was suppressed at the transcriptional level, whereas the α_2(I) gene was suppressed at the post-transcriptional level. Unexpectedly, exogenous α_1(I) procollagen promotor-reporter gene constructs, including up to 3600 base pairs of 5' flanking regions and portions of exon 1 and intron 1, were several-fold more active in *v-fos* transformed cells than either Rat-1 cells or revertants. However, a human α_1(I) procollagen promoter construct, containing sequences which extended from -804 nucleotides upstream to +1440 bp in the first intron, was suppressed ten-fold in the *v-fos* transformed cells compared to Rat-1 cells or revertants. Mutation of a highly conserved inverted repeat at +89 to +110 bp in the first exon or removal of sequences from +292 to +1440 bp in the first intron failed to relieve the suppression of transcription in *v-fos* transformed cells. Transformation-related suppression was thus mediated by sequences between +115 bp in the first exon and +292 bp in the first intron. Analysis of runoff transcripts from *v-fos* transformed cells, using DNA probes derived from both the 5' and the 3' ends of the α_1(I) collagen gene, indicated that while an appreciable level of transcription was detectable from the 5' end of the α_1(I) collagen gene, a dramatic and graded decrease in the rate of transcription occurred towards the 3' end of the gene. Altogether, these studies suggest that suppression of α_1(I) procollagen gene expression in *v-fos* transformed cells occurs primarily by premature termination of transcription, which might be mediated by sequences contained in a 185 bp fragment spanning the intron 1/exon 1 boundary.

R 464 SPECIFICITY OF INTERACTION OF HISTONE H1 WITH SOME EUKARYOTIC DNA SEQUENCES, Jordanka Zlatanova and Julia Yaneva,* Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331 and *Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

The issue of whether histone H1 possesses some specificity of binding to certain nucleotide sequences in DNA is of fundamental importance to the suggested role of the linker histone in the regulation of transcription (reviewed in Zlatanova, TIBS 15, 1990, 273; Zlatanova & Yaneva, Mol. Biol. Rep. 15, 1991, 53). Despite some indications in the literature speaking in favor of sequence-specific binding of H1 to DNA, the problem is still unresolved. We report here our findings showing: (1) substantial preference of H1 for eukaryotic vs. prokaryotic sequences, and (2) selectivity of binding to some specific eukaryotic sequences. In a first series of experiments using the classical filter binding assay, we demonstrated that while pure bacterial plasmids were negligibly retained on filters by H1 binding, the same plasmids carrying eukaryotic inserts (the mouse α-globin gene) were substantially filter-bound (Yaneva et al., FEBS Lett. 263, 1990, 225). Moreover, when the insert was excised from the plasmid body and the mixture of the two fragments was incubated with H1, only the eukaryotic fragment was retained on the filter (ibid.). In a second study, the interaction of H1 with different regions of the mouse α-globin gene, subcloned in a bacterial plasmid, was investigated using several independent approaches (filter binding, precipitation, binding to paper-immobilized protein, agarose gel electrophoresis). The binding of some subclones, i.e. those encompassing the 5' end and the first half of the coding sequence is preferred over binding to other subclones. The selectivity is mainly expressed under conditions of non-cooperative binding of the histone to DNA. No correlation between the AT content of the distinct gene fragments and the relative affinity of their binding is observed. Clearly, other features of DNA structure are involved in the specific H1 binding. Results of the binding of H1 to the nuclear factor I recognition sequences are also reported.

Late Abstracts

mRNA PROCESSING AND NATURAL POLARITY IN THE HISTIDINE OPERON, Pietro Alifano, Flavia Rivellini, Anna Giulia Nappo, Claudia Piscitelli, Valeria Blasi, Massimo Picone and M. Stella Carlomagno, CEOS of CNR, University of Naples, S. Pansini 5, 80131 Napoli, Italy

The polycistronic primary transcript of the histidine operon of Salmonella typhimurium is subjected to mRNA processing leading to a stable distal mRNA species. The processing event requires a 5' cis element recognized by soluble factors and translation of the first cistron within the processed transcript.

As expected, the dependence on the translation apparatus results in modulation of the mRNA processing event in response to modified growth conditions. Starvation for folate, induced by drugs such as trimethoprim, leads to an increase in the amount of the processed species. The same effect is observed when the operon is derepressed by blocking the histidine biosynthetic pathway distally - but not proximally - to the step responsible for the production of ZMP (AICAR). High intracellular levels of this nucleotide by causing depletion of folate, of formilating sources, and consequently of fMet-tRNA interfere in the initiation step of protein synthesis that is involved in the stabilization of the processed transcript. Low levels of fMet-tRNA also cause enhancement of the "natural" transcription polarity in the histidine operon, due to the previously identified cryptic Rho-dependent intracistronic transcription terminators, and could result in an altered ratio between the proximal and distal gene products. The metabolic modulation of the processing event appears to represent a mechanism that compensates the transcriptional gradient and keeps the dosage of the gene products in balance.

REPRESSION OF E. COLI LACTOSE OPERON FROM DISTANT OPERATOR SITES: COOPERATION BETWEEN OPERATORS INDIVIDUALLY INEFFECTIVE, Michèle Amouyal[1] and Brigitte von Wilcken-Bergmann[2], [1]Unité de Physicochimie des macromolécules biologiques, Institut Pasteur, 25, Rue du Docteur Roux, 75724 Paris Cedex 15 (France), [2]Institut für Genetik, Weyertal 121, Cologne (Germany).

In vitro, lac repressor can bind to an operator site close to the start of transcription, lacO1, as well as to two distant sites of weaker affinity, lacO2 (+401bp) and lacO3 (-93bp). For long, the efficiency of the proximal site for repression has hidden the contribution of the distant sites and now raises the question of whether this site operates independently from the other ones or in concert with them.

In the synthetic system that we have designed, the synthesis of beta-galactosidase is controlled from new lac operator sites instead of the wild-type ones. The operator, whether proximal or distant, cannot induce repression. Two operators, one proximal, the other one distant, are required to repress the system (up to 35 fold), so that repression unequivocally results from the cooperation between the two sites.

In vitro, two distant lac operators can cooperate in protein binding through DNA loop formation. Our results support this mechanism in vivo.

Other aspects of this procaryotic "silencer" system will be presented.

CLONING OF ADAPTORS REQUIRED FOR TRANSCRIPTIONAL ACTIVATION USING GENETIC SELECTION IN S. CEREVISIAE, Shelley Berger, Benjamin Pina, Neal Silverman, Greg Marcus, Julie Agapite and Leonard Guarente, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139. Regulation of transcription requires interaction of upstream activator proteins with the basic machinery. Our recent research suggests a novel class of molecules, termed adaptors, bridges this interaction (Berger, et. al., 1990, Cell 61:1199). To clone adaptors we used genetic selection in S. cerevisiae. High level expression of GAL4-VP16 causes toxicity, which is alleviated by specific amino acid substitutions in the VP16 activation domain, in parallel with their reduction of activation. Thus, mutations in the adaptor might also prevent interaction with VP16, thereby relieving toxicity. Therefore, yeast expressing GAL4-VP16 were mutagenized and several strains were obtained which resisted toxicity, but did not reduce the amount of GAL4-VP16. Transcriptional effects caused by the mutations were consistant with a specific role in activation: transcription was selectively reduced in vivo from different promotors, as expected for mutations in an adaptor. A particularly strong effect was seen at promotors activated by GCN4. Similarly, activation by LEXA-GCN4, containing the DNA binding domain of LEXA and the activation domain of GCN4, was severely reduced in the mutant strains. Significantly, nuclear extracts prepared from the mutant strains supported a reduced level of activation by GAL4-VP16 and GCN4 compared to wild type extracts, but had no effect on basal level transcription. These results strongly suggest that the mutations disrupt the activity of an adaptor for acidic activators, such as VP16 and GCN4. In the absense of GAL4-VP16, the mutant strains grow poorly compared to wildtype, which allowed complementation using a wildtype genomic DNA library. Transformation of the mutant strains with the wild type genes complements the growth defect and, importantly, restores toxicity in the presence of GAL4-VP16. The two genes have been sequenced and do not correspond to any known genes.

SEARCHING FOR PEPTIDIC INHIBITORS OF THE FOS/JUN DIMERIZATION WITH PEPTIDE ON PHAGE LIBRARIES, Laurent Bracco, Marie-Noëlle Mary, Christine Pernelle, Christine Dureuil, François Clerc, Odile Boniface, Jean-Dominique Guitton and Bruno Tocque, CRVA, Rhône-Poulenc Rorer, 13 quai Jules Guesde, BP 14, 94403 Vitry-sur-Seine, France

Thanks to the increasing amount of data regarding the components and the mechanisms of the eukaryotic trancription machinery, it has become more feasible to specifically target transcription factors in order to affect gene expression. The AP-1 complex is probably one of the best studied case. Its components, the members of the Fos and Jun families can form homo and heterodimers via the association of domains referred as leucine zippers. We have chosen to target this specific interface in order to prevent the function of AP-1. We have already described the design of LexA/Jun hybrid repressors to probe the important contacts involved in leucine zipper interfaces (Schmidt-Dor, T. et al, Biochemistry (1991) 30, 9657-64). We now present the use of the peptide on phage technology to gain further information on these interactions and to discover potential peptidic inhibitors of the dimerization process. We show that an immobilized Jun leucine zipper synthetic peptide (but not a Fos analog) is able to selectively retain phages expressing the full length Fos leucine zipper at their surfaces but is inefficient regarding phages bearing a truncated or mutated Fos leucine zipper. The screening of a leucine zipper directed peptide library having a LX_6LX_6L format will also be discussed.

RNA POLYMERASE II STRUCTURE: Aled Edwards, Seth Darst, Gavin Meredith, Yang Li, Roger Kornberg. Department of Cell Biology, Stanford University, Stanford, CA 94305.

A mutant form of RNA polymerase II (pol IIΔ4/7), lacking two subunits, was purified from the Saccharomyces cerevisiae yeast strain *rpb-4*. This form of the enzyme formed well-ordered two-dimensional crystals that were analyzed by electron crystallography. The structure of this enzyme was determined to a resolution of 16Å and revealed a number of interesting features that include numerous grooves that might accommodate nucleic acid and a density that might represent the highly conserved C-terminal domain (CTD) of the largest subunit of pol II. A number of approaches are currently being used to better understand the pol II structure and our progress in these areas will be described. We will describe our progress in pol II X-ray crystallography, our efforts to localize the CTD using modified pol II, our efforts to localize specific subunits in the structure by antibody decoration and finally our progress in the formation and structural analysis of pol II/nucleic acid complexes.

X-RAY STRUCTURE OF A GCN4:DNA COMPLEX, Thomas E. Ellenberger*, Christopher Brandl*, Kevin Struhl* and Stephen C. Harrison*, Department of Biochemistry and Molecular Biology*, Harvard University, Cambridge, MA 02138 and the Department of Biological Chemistry and Molecular Pharmacology*, Harvard Medical School, Boston, MA 02115.

A crystal structure of the DNA-binding domain of the yeast transcription factor GCN4 complexed with DNA has been solved at 3.0 A resolution. The C-terminal 56 residues of GCN4, comprising the basic region and coiled-coil "leucine zipper", were expressed in *E. coli.* and crystals were obtained of this domain complexed with a consensus GCN4 (AP-1) DNA site. The GCN4 dimer binds in the major groove with the axis of the protein dimer nearly coincident with the pseudodyad of the AP-1 site. Overall, this DNA-binding domain has the appearance of an α-helical fork, straddling the major groove of the AP-1 site. The dimer interface of GCN4 is a parallel α-helical coiled-coil, essentially as described for an analogous peptide spanning the leucine heptad repeat [E.K. O'Shea, J. D. Klemm, P.S. Kim and T. Alber (1991) Science 254,539-544]. The amino terminal basic region of GCN4 forms an uninterrupted α-helix that is continuous with the α-helical dimerization region. The basic-region of each monomer diverges from the dimer axis in a smooth arc and tracks through the major groove over each of the AP-1 half sites, making extensive contacts with DNA bases and backbone phosphate oxygens. In this way, each GCN4 monomer contacts 4 to 5 basepairs without wrapping around the "back" side of the AP-1 site. The basic-region α-helix is essentially straight with no kink at the conserved asparagine (ASN235), the postulated "N-cap". The AP-1 DNA sequence is non-palindromic, and this asymmetry is mirrored by the bound protein. The GCN4 monomers in the complex are not precisely 2-fold symmetric about the dimer interface, and the central basepair of the AP-1 site is contacted differently by each monomer. The 20mer AP-1 DNA is B-form and essentially straight, with no pronounced kinks or bends. Further refinement of this structure and extension of resolution are underway.

CHEMILUMINESCENT DETECTION OF DIGOXIGENIN-LABELED NUCLEIC ACIDS: OPTIMIZATION OF THE DETECTION PROTOCOL

Hans-Joachim Höltke, Gregor Sagner, Waltraud Ankenbauer, Bruno Frey and Gudrun Schmitz, Boehringer Mannheim GmbH, Biochemical Research Center, Nonnenwald 2, D-8122 Penzberg

A fast and simple protocol for the chemiluminescent detection of digoxigenin (DIG) labeled nucleic acids with anti-digoxigenin antibody Fab-fragments coupled to alkaline phosphatase and 3-(4-methoxyspiro[1,2-dioxetane-3,2'-tricyclo-[3.3.1.1^{3,7}]decan]4-yl)phenyl phosphate (DPP) as substrate is described. The washing and blocking procedure was optimized to yield low background even on positively charged nylon membranes. The sensitivity of the system is equal or better than of radioactive methods. Exposure to X-ray or Polaroid film for up to 30 minutes is sufficient for the detection of 70 fg homologous DNA. Human single-copy genes are detected in Southern-blots of as low as 0.3 ug total placenta DNA. Blots can be reprobed multiple times very easily.
The advantages of the DIG-system, high sensitivity, absence of background and ease of reprobing, are illustrated by applications for single-copy gene detection in genomic blots of human DNA, Northern-hybridizations to rare mRNA, detection of E. coli genes on blots of genomic digests after pulse field gel electrophoresis as well as for nonradioactive DNA sequencing blots with DIG-labeled primers.

A subunit gene of an RNA polymerase II initiation factor and protein kinase

Opher Gileadi, William J. Feaver and Roger D. Kornberg. Department of Cell Biology, Stanford University Medical School, Stanford, CA 94305

Initiation by RNA polymerase II requires several additional protein factors, whose roles remain, for the most part, to be elucidated. One of the factors, discovered in HeLa cell extracts and termed TFIID, is known to bind the TATA element of a promoter. Another factor, which we have isolated from yeast and termed factor b, copurifies with a protein kinase activity directed towards the carboxy-terminal repeat domain (CTD) of RNA polymerase II. Phosphorylation of the CTD is believed to occur at every round of initiation and to be essential for the process. Purified factor b comprises polypeptides of 85, 75 and 50 kDa, and we report here the cloning of the gene encoding the 75 kDa component, termed TLB1. The predicted protein of 73 kDa is highly hydrophilic and shares no sequence homology with any other known gene. The TLB1 gene is essential for viability. Antibodies raised against the cloned gene product precipitate both transcription factor and CTD kinase, strengthening the notion that the two activities reside in the same molecule.

CHARACTERIZATION OF TERNARY COMPLEXES OF VACCINIA RNA POLYMERASE PAUSED AT PROMOTER-PROXIMAL SITES,

Jeremiah Hagler and Stewart Shuman, Molecular Biology Program, Sloan-Kettering Institute, New York, NY 10021

We have analyzed the structure of ternary complexes of vaccinia RNA polymerase, a virus encoded homolog of cellular RNA polymerase II. RNase footprinting of stably paused ternary transcription complexes suggests an RNA binding site within the RNA polymerase that includes 18 nt of RNA extending from the 3' growing point of the chain. The dimensions of the binding site do not change as the polymerase moves down the template. DNase I footprinting indicates that the polymerase complex covers about 39 bp on the template strand and 49 bp on the non-template strand. The leading edge of the transcription complex extends 16-17 bp downstream of the 3' end of the RNA growing point. Footprinting with o-phenanthroline/copper ion reveals a region of protection immediately surrounding the growing point of the RNA chain, extending 10 bp upstream and 4 bp downstream. We also show the transcription bubble to be at least 12 nt in length. The characteristics of these footprints do not change as the RNA polymerase complex translocates down the DNA template.

YEAST AND HUMAN TFIIDs ARE INTERCHANGEABLE FOR THE RESPONSE TO ACIDIC TRANSCRIPTIONAL ACTIVATORS *IN VITRO*

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Cloning and sequencing of TFIID genes from a variety of species have delineated a highly conserved C-terminal core domain and a divergent N-terminal region. Using a TFIID-dependent reconstituted yeast transcription system, we have found that human TFIID and yeast TFIID derivatives lacking part or all of the N-terminal region are fully functional in support of both basal transcription and the response to the acidic activator proteins GAL4-VP16 and GCN4. Conversely, yeast TFIID supports both basal and activated transcription in reactions reconstituted with human components. Yeast, human, and *Schizosaccharomyces pombe* TFIIDs are all similarly interchangeable in a recently developed TFIID-dependent transcription system from *Schizosaccharomyces pombe*. This functional interchangeability of TFIIDs across species argues strongly against species-specificity with regard to TFIID function in basal transcription and the response to acidic activator proteins. In addition, activated transcription with the conserved C-terminal domain of TFIID was dependent upon the previously identified mediator of transcriptional activation, thus eliminating the N-terminal region of TFIID as a potential target of interactions with acidic activation domains and mediator. These results further suggest that any intermediary factors between acidic activators and TFIID are conserved from yeast to man.

STRUCTURAL AND BIOCHEMICAL STUDIES OF THE CARBOXY-TERMINAL REPEAT DOMAIN OF YEAST RNA POLYMERASE II: Yang Li, Seth Darst, Aled Edwards, Roger Kornberg. Department of Cell Biology, Stanford University, Stanford, CA 94305.

A site for the protease Factor Xa (FXa) was engineered into the gene (RPB-1) for the largest subunit of yeast RNA polymerase II (pol II) between the region encoding the carboxy-terminal repeat domain (CTD) and the bulk of the subunit. The wild-type copy of the RPB-1 gene in yeast was replaced with the modified gene with little effect on cell viability. The modified enzyme (pol II-Xa) was expressed and purified from yeast and was fully active in assays of transcription *in vitro*. CTD-less polymerase (pol II-CTD) was generated by digesting pol II-Xa with immobilized factor Xa and was subsequently purified from uncleaved pol II-Xa and free CTD. The biochemical properties of the homogeneous tail-less enzyme will be described. The structure of this enzyme is being analyzed by electron crystallography.

ANALYSIS OF *cis*-ACTING DNA BINDING SITES FOR THE rel FAMILY OF TRANSCRIPTION FACTORS, Charles Kunsch, Maureen Maher, and Craig Rosen, Department of Gene Regulation, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

Nuclear factor κB (NF-κB) is an inducible transcription factor that binds to, and mediates transcription from, a *cis*-acting DNA element (κB motif) in the regulatory region of several viral and cellular genes. The two protein subunits of NF-κB (p50 and p65) are members of a related family of proteins each containing a well-conserved region of homology to the proto-oncogene c-*rel*. This region of homology contains the DNA binding and multimerization domains and these proteins are capable of binding to the κB element as both homodimeric and heterodimeric complexes. Although several κB-related sequences have been identified we have begun examining the preferred DNA binding sequences of several of the *rel*-related proteins. We have employed a binding-selection assay that consists of (i) binding of the purified *rel*-related proteins to a pool of random target DNA sequences, (ii) separation of bound vs. unbound DNA by gel-mobility shift assay and elution of the bound DNA, and (iii) amplification of the bound DNA by polymerase chain reaction (PCR) amplification. After several rounds of binding and PCR amplification, the DNA was cloned and individual clones were analyzed by DNA sequencing and also screened for their ability to bind differentially to homodimers of the various *rel*-related proteins. Results from DNA sequence analysis indicated that although some clones contained known κB motifs (GGGACTTTCC, and variations thereof), others contained novel sequences not previously described as potential κB binding motifs. These results indicate that the *rel* family of transcription factors can bind to a broader spectrum of DNA sequences than was previously believed. In addition, several clones were identified that were capable of binding to one or more of the *rel*-related proteins with relatively high affinity, but were either completely unable or severely reduced in their ability to bind to other *rel*-related proteins. These differential binding motifs will be tested in functional transient transfection assays to assess their ability to confer differential transcriptional activation in response to various *rel*-related proteins. DNA sequence analysis of these clones will provide important information about the precise nucleotide sequence requirements necessary for differential binding to individual *rel*-related protein members. These results suggest that differential binding of the *rel* family of transcriptional activators, as mediated by the *cis*-acting DNA binding elements, may provide another level of regulation to confer specific and selective induction of target genes.

ACTIVATION OF HTLV-I TRANSCRIPTION BY A GAL4-TAX$_1$ FUSION PROTEIN, Susan Marriott and Laurie Connor, Division of Molecular Virology, Baylor College of Medicine, Houston, TX 77030

Human T cell leukemia virus (HTLV-I) encodes several proteins which regulate viral gene expression at the level of transcription. One of these proteins, Tax$_1$, is a 40 kD positive transactivator of viral gene expression. Tax$_1$ is unique from many other transcriptional regulators in that it appears to be incapable of binding directly to DNA. However, Tax$_1$ can associate indirectly with DNA via a cellular transcription factor. This mechanism of indirect association with DNA is reminiscent of several other transcription factors including adenovirus E1A and herpesvirus VP16. To further investigate the activation of HTLV-I transcription by Tax$_1$ we have constructed a vector (TG14) which expresses a chimeric protein containing the DNA binding domain of the yeast transcription factor GAL4 (GAL4$_{1-147}$) fused to the complete Tax$_1$ coding sequence. Following transfection, Tax$_1$ and GAL4-Tax$_1$ protein expression levels were demonstrated by immunoprecipitation. HTLV-I LTR-CAT reporter plasmids were constructed in which specific Tax$_1$ responsive elements were replaced with GAL4 binding sites. Cotransfection of TG14 or Tax$_1$ with various LTR-CAT reporter constructs containing GAL4 binding sites demonstrated that GAL4 sequences were necessary for maximal activation of the promoter. Interestingly however, sequences surrounding the GAL4 binding sites were also important in determining the level of Tax$_1$ activation since the mere association of Tax$_1$ with a promoter which contained GAL4 binding sites, but lacked flanking LTR sequences, was not sufficient to allow maximal Tax$_1$ transactivation. LTR-CAT reporter constructs containing GAL4 binding sites flanked by two 21 base pair repeat elements demonstrated an 11-fold greater response to TG14 than those which lacked flanking 21 base pair elements. S1 nuclease protection analysis demonstrated that the activation of CAT expression induced by GAL4-Tax$_1$ occurred at the level of transcription. Taken together, these results suggest that Tax $_1$ activation of the HTLV-I LTR involves complex interactions with cellular proteins.

REGULATION OF HERPESVIRUS GENE EXPRESSION BY COMMON FACTOR-1 (CF-1, YY1, NF-E1, δ, UCRBP)

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The expression of leaky-late or βγ genes of herpes simplex virus (HSV-1) is characterized by having a partial requirement for viral DNA replication and absolute requirement for viral immediate-early (IE) proteins (ICPO, 4, and 27). We have recently reported that a cellular protein (the leaky-late binding factor or LBF) binds to sites (LBS) having the consensus sequence GCCATNT in promoters and downstream sites in a number of βγ genes of HSV-1. By using promoter deletion analysis we obtained evidence that this binding site is required for maximum transactivated expression of the major capsid protein (VP5) and glycoprotein D (gD) of HSV -1 in transient expression assays. By using oligonucleotides containing the binding sites for the LBF, CF-1, YY1, or NF- E1, in competition binding experiments, and proteolytic band clipping of complexes formed with VP5, δ, c-myc, and gD promoters, we have established that the LBF is the same as, or very similar to, a previously described cellular transcription factor, common factor-1 (CF-1, a.k.a. YY1, NF-E1, δ, UCRBP). This factor is unique in that it can act as either an activator or repressor of gene expression. In the case of certain βγ genes of HSV-1, it functions as an activator in the presence of viral IE regulatory proteins. We further show that sites containing homologs of LBS that occur in the major immediate-early promoter/enhancer of cytomegaloviruses and in simian retrovirus (SRV-1, SIV-1) LTRs bind this same cellular factor (LBF or CF-1). Evidence supporting the role of CF-1 in regulating genes of these viruses and in transactivation of certain genes by HSV-1 will be presented.

THE ROLE OF THE C-TERMINAL PHE-ALA-PHE-ALA RESIDUES IN THE MECHANISM OF T7 RNA POLYMERASE, Kasim A. Mookhtiar, John J. Dunn and Joseph E. Coleman, Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06511.

T7 RNA polymerase is a single polypeptide chain of 883 amino acids that carries out all the functions required for transcription from phage specific promoters (1). Structure- function studies from our laboratory have shown that the enzyme consists of two distinct domains, a 80 KD C-terminal domain that carries the promoter binding and the catalytic functions, and a 20 KD N-terminal domain that interacts with the growing RNA chain imparting a high degree of processivity to the enzyme (2). Recently, we have shown that the last two residues Phe^{882} -Ala^{883} play a crucial role in all phases of transcription (3). Removal of these residues, either genetically or proteolytically, to produce a "foot" mutant, results in a marked decrease in specific promoter affinity, catalysis of phosphodiester bond formation, and the processive nature of the enzyme. We have used site directed mutagenesis to evaulate the role of each of the last four residues and the terminal carboxyl group in transcription.

Deletion of the Ala^{883} has a very small effect on transcriptional activity. Extension of the C- terminus with Phe results in a phenotype that resembles the "foot" mutant, while addition of Phe-Ala to the native sequence results in slightly better binding and processivity than the latter enzyme. Substitution of Phe^{882} in the native protein with Ala results in an inactive protein, while the substitution of Tyr at this position is less severe. Suprisingly, any change at Ala^{881} results in a non-processive enzyme, since substitution of this residue with Gly, Thr or Leu leads to the "foot" mutant phenotype. These results show that 1) a carboxyl group is required in either position 882 or 883 , 2) Phe^{883} is crucial since substitution with even Tyr results significant loss in activity and 3) Ala^{881} may be involved in some steric interaction since subtitution with any larger or smaller side- chain is deleterious to enzyme activity.

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TBP-1, THE HIV TAT BINDING PROTEIN, BELONGS TO A NOVEL FAMILY OF TRANSCRIPTIONAL ACTIVATOR PROTEINS. Bella Ohana[1], Christopher Southgate[2], Michael Greene[2] and Craig Rosen[1], Roche Institute of Molecular Biology , Roche Research Center, 340 Kingsland Street, Nutley, NJ 07110[1], Program in Molecular Medicine, University of Massachusetts Medical Center, 373 Plantation Street, Worcester, MA 01605[2].

We previously described the identification of a cDNA that encodes a protein (TBP-1) that interacts with the HIV transcriptional trans-activator protein, Tat. Recent studies demonstrate that TBP-1 is one member of an evolutionarily conserved gene family. The observation that TBP-1 interacts with Tat, and is localized in the nucleus, suggested a potential involvement in transcriptional activation functions. To examine this possibility chimeric fusion proteins between the DNA binding domain of the yeast transcriptional activator protein GAL4 and TBP were constructed and their transcriptional activity was assessed by using a GAL4 upstream activation sequence-driven promoter-cloramphenicol acetyl transferase fusion. Our findings indicate that TBP-1 is a transcriptional activator and that activation may be both position and promoter dependent. Mutational analysis suggests that a well conserved motif, with homology to known ATPase domains, is important for the transcriptional activation function. The observation that both TBP-1 and the GAL4 TBP-1 fusion proteins can trans-activate an HIV LTR promoter synergistically with the Tat protein lends further support to the hypothesis that TBP may play a direct role in trans-activation mediated by the HIV Tat protein.

INSULIN-DEPENDENT TRANSCRIPTIONAL REGULATION OF TYROSINE AMINOTRANSFERASE (TAT) GENE EXPRESSION: OPPOSING EFFECTS ON BASAL EXPESSION ARE MEDIATED THROUGH A COMMON PROXIMAL *CIS*-ELEMENT. R. Peterson, D. Carmichael, and J. Koontz. Dept. of Biochemistry and Program in Cellular, Molecular and Developmental Biology. University of Tennessee. Knoxville, TN 37996.

Previous studies from our laboratory have shown that in the rat hepatoma cell line, KRC-7, insulin inhibits TAT gene expression through a selective decrease in TAT transcription (1). To further characterize the insulin response similar studies were conducted with the well-characterized hepatoma cell line, Fao, which is known to possess a differentiated phenotype. In agreement with a previous report (2), we find that insulin treatment results in an increased rate of TAT transcription followed by an increase in hybridizable $mRNA^{TAT}$. To further extend these results we have examined the effect of insulin on both dexamethasone- and cAMP-mediated induction of TAT gene expression in each cell line. Although insulin has opposing effects on basal expression in the two cell lines; insulin was found to inhibit, to an equal extent in each cell line, both dexamethasone- and cAMP-mediated induction. Transient transfectional analyses of 5'-flanking sequences, including a series of internal deletion mutants, cloned into the vector pBLCAT3 have allowed localization of the insulin-responsive region to the proximal promoter. DNase I footprinting of the TAT promoter (-351 to -2 bp) with nuclear extract from insulin-treated or untreated cells has revealed that several liver-enriched as well as ubiquitous factor binding sites are protected.

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SUPPRESSION OF NF-KB TRANSCRIPTIONAL ACTIVITY BY A *REL*-RELATED PROTEIN. Steven M. Ruben, John Klement, Maureen Maher, Chen-Hwa Chen and Craig A. Rosen, Department of Gene Regulation, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

NF-κB is a pleiotropic transcriptional activator involved in the inducible expression of a large number of genes including cytokines, cytokine receptors, major histocompatibility genes and several viral enhancer elements. The NF-κB complex is composed of a 50 kDa and 65 kDa protein subunit. Both of these subunits share a striking similarity to the family of proteins related to the *rel* proto-oncogene. This family of proteins contains a region of approximately 300 amino acids (*rel* homology domain) at the amino terminal end that contains residues important for both DNA binding and protein dimerization. The carboxy terminal region of these proteins diverges and is responsible for the transcriptional activity of *rel*-related proteins.

NF-κB is sequestered in the cytoplasm of uninduced cells through association with a repressor molecule, IκB. Following stimulation of cells, IκB is dissociated and NF-κB is translocated to the nucleus where is binds to the κB motif present in the regulatory region of numerous genes. We have isolated a cDNA encoding a novel 66 kDa *rel*-related protein, designated I-Rel, that suppresses NF-κB induced transcription. Unlike other *rel* family members, I-Rel does not interact with DNA. In addition, the carboxy terminal region of I-Rel contains no transcriptional activity on its own or when fused to the binding domain of the yeast GAL4 gene product However, it forms heterodimers with p50 and abolishes its DNA binding activity. Expression of I-Rel mRNA is induced by mitogenic stimulation and accumulates at a time following appearance of p50 transcripts. This suggests that I-Rel may be a component of a negative feedback pathway that modulates the expression of genes responsive to the NF-κB transcription factor complex.

Fractionation of the yeast RNA polymerase II transcription system and reconstitution of transcription with purified general factors

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A method has been developed for fractionating yeast whole cell extracts to obtain homogeneous RNA polymerase II (pol II) and three additional chromatographic fractions that lack pol II activity and are required, in addition to pol II and the general initiation factor TFIID, for accurate and efficient transcription *in vitro*. These fractions contain the activities that correspond to the previously reported yeast pol II initiation factors a, b, and e, and are necessary for the utilization of a variety of eukaryotic promoters by pol II. Factor a has been purified to homogeneity and consists of two polypeptide subunits with apparent molecular weights of 66 and 43 kilodaltons. Factor b has been extensively purified and is associated with three polypeptides of 85, 75, and 50 kDa. Homogeneous factor e consists of a single polypeptide of 40 kDa. Template utilization in reactions reconstituted with the purified factors is more efficient than in yeast nuclear and whole-cell extracts, with an RNA yield of 0.1-0.2 transcripts per template in 30 minutes.

PURIFICATION AND CHARACTERIZATION OF FACTOR E, A BASAL RNA POLYMERASE II TRANSCRIPTION FACTOR FROM *S.CEREVISIAE*

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Accurate initiation in a yeast *in vitro* transcription system requires four basal transcription factors (termed a,b,d and e) in addition to RNA polymerase II. Factor d can be replaced by the cloned yeast TFIID protein. The other three factors can be purified by fractionation of yeast whole cell extract. We used a reconstituted *in vitro* transcription system, which contained RNA Polymerase II and factors a,b and d in order to purify factor e. Factor e was purified to homogeneity in eight subsequent chromatographic steps. It consists of a single polypeptide of 40 kD. The transcriptional activity of this factor binds to an RNA Polymerase II affinity column and can be eluted with increasing salt concentrations. The activity coelutes with the 40kD polypeptide. Furthermore we show that factor e affects the position of the mRNA start site both in a *S.cerevisiae* and a *S. pombe* in vitro transcription system.

THE ANKYRIN REPEAT DOMAINS OF THE NF-kB PRECURSOR p105 AND THE PROTO-ONCOGENE BCL-3 ACT AS SPECIFIC INHIBITORS OF NF-kB DNA BINDING

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The inducible pleitropic transcription factor NF-kB is composed of two subunits, p50 and p65. The p50 subunit is encoded on the N-terminal half of a 105 kD open reading frame. To date, no function has been described for the C-terminal portion.
We show here that the C-terminal half of p105, when expressed as a separate molecule, binds to p50 or native NF-kB and can rapidly disrupt even preformed protein-DNA complexes. Deletion analysis of this precursor-derived inhibitor (pdI) activity indicated a domain containing ankyrin-like repeats as necessary for inhibition. The proto-oncogene bcl-3, which also contains ankyrin repeats, can equally inhibit DNA-binding of *rel*-like trancription factors. These observations identify bcl-3 as a novel inhibitor of NF-kB and suggest that the repeat domains in pdI and bcl-3 are mediating specific protein-protein interactions with the conserved *rel*-like domain in NF-kB. Both, bcl-3 and pdI have a different subdomain-specificity than the already described Ikb forms. We discuss the functional implications of our findings for NF-kB mediated signal transduction.

SPECIFIC INTERACTION BETWEEN THE NONPHOSPHORYLATED FORM OF THE HEPTAMER REPEAT PRESENT AT THE CARBOXYL END OF THE LARGEST SUBUNIT OF RNA POLYMERASE IIa AND TFIID

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Fractionation of a transcription competent HeLa extract on a column containing the heptamer repeat present in the caboxyl terminal domain of the largest subunit of RNA Polymerase II (YSPTSPS), resulted in the loss of its transcription activity. This extract could recover its ability to specifically transcribe different class II promoters upon addition of proteins that bind to the column, hTFIID, or cloned yTFIID. Fractionation of the extract on columns containing mutations of the heptamer did not affect its transcriptional activity. Fractionation of RNA polymerase II on columns containing hTFIID or yTFIID resulted in the specific retention of the nonphosphorylated form of RNA polymerase II. The phosphorylated form of the enzyme was unable to interact with TFIID. The specific interaction of RNA polymerase II with TFIID was mediated through the CTD.