BASIC ASPECTS OF TRANSCRIPTION

Organizers: Danny Reinberg and Jack Greenblatt

February 13 - 20, 1994; Keystone, Colorado

Plenary Sessions *Page*

Poster Sessions

Basic Aspects of Transcription

Basic Aspects of Initiation of Transcription by RNA Polymerase II

L 001 PROTEIN-PROTEIN INTERACTIONS THAT REGULATE TRANSCRIPTION BY RNA POLYMERASE II, Jack Greenblatt, Hua Xiao, Angela Pearson, Benoit Coulombe, Joyce Li, Jacques Archambault, J. Timothy Westwood, Ray Truant, Sherry Zhang, Zhigang He, Keith Stringer, Johnson Wong, Craig Dorrell, and C. James Ingles, Banting and Best Department of Medical Research and Department of Molecular and Medical Genetics, University of Toronto, Canada M5G 1L6.

The assembly of an active pre-initiation complex containing RNA polymerase II involves the general initiation factors TFIID, TFIIA, TFIIB, TFIIF (RAP30/74), TFIIE, and TFIIH. The formation of this complex involves multiple protein-protein and protein-DNA interactions. Interactions among RNA polymerase II, RAP30, TFIIB, and TBP, the TATA box-binding subunit of TFIID, probably explain why these four proteins are sufficient to form a stable complex at the adenovirus major late promoter (Ad2MLP) and are sufficient for transcriptional initiation at most promoters on supercoiled DNA templates. Site-specific protein-DNA photocrosslinking experiments with the Ad2MLP will be described that localize RAP30 just downstream of TBP at base pair -19 and localize the two largest subunits of TFIIA upstream of TBP around -40 and opposite TBP at the TATA box. TFIIH assembles into the pre-initiation complex after TFIIE and we will show that a C-terminal fragment of the large subunit of TFIIE contains a binding site for TFIIH.
Transcriptional activation probably involves multiple direct or indirect contacts between the activation domains of DNA-binding activator proteins and several general initiation factors. We have shown previously that the acidic activation domains of the herpes simplex virus protein VP16 and the human anti-oncogenic protein p53 directly bind TBP. The effects of mutations in TBP that reduce the binding to TBP of VP16 and p53 will be described. As well, experiments will be described showing that the retinoblastoma protein Rb, which represses transcriptional activation by the human cell cycle-regulated activator protein E2F-1, prevents the acidic activation domain of E2F-1 from binding TBP.
Two major cellular proteins that are directly bound by the activation domains of VP16 and p53 are methylene tetrahydrofolate dehydrogenase (MTHFD) and replication factor A (RFA). The significance of the MTHFD interaction, if any, is unknown and currently under investigation, while the interaction with RFA may explain why acidic activation domains can activate viral origins of DNA replication. We have also observed the VP16 and p53 efficiently bind the TFIIH activity in a HeLa cell extract. Like the TBP and RFA interactions, the interaction of TFIIH with VP16 is reduced by mutations that reduce the activity of the VP16 activation domain and does not require any other general initiation factor. Since RFA is a single-stranded DNA-binding protein and TFIIH has at least one DNA helicase subunit, we are presently investigating whether the VP16-TFIIH interaction is mediated by RFA.

L 002 THE YEAST RNA POLYMERASE II INITIATION COMPLEX, Roger Kornberg, Stefan Bjorklund, Brad Cairns, John Feaver, Young-Joon Kim, Lynn Henry, Yang Li, Yahli Lorch, and Jesper Svejstrup, Department of Cell Biology, Stanford University, School of Medicine, Stanford, CA 94305-5400.

Bacterially expressed yeast homologs of human/rat RNA polymerase II initiation factors TFIIB/α, TFIIE/ε, and TATA-binding protein (TBP), together with homogeneous yeast TFIIF/$\beta\gamma$, TFIIH/δ and polymerase, constitute a fully defined transcription system. Aspects of mechanism and regulation currently under study in this system include the following:

1) Dual roles of TFIIH in transcription and DNA repair (collaborative work with E.C. Friedberg and colleagues). Seven of eight polypeptides associated or interacting with TFIIH are required for nucleotide excision repair.
2) Functional interactions in transcription, revealed by interchange of factors between *S. cerevisiae* and *S. pombe* systems. Essential interaction of TFIIE with TFIIH may play a regulatory role, while that of TFIIB with polymerase determines the location of the transcription start site.
3) Additional factors important for basal transcription. One protein that stimulates basal transcription from many promoters has been identified, and the possible involvement of another will be described.
4) Mediator of transcription activation. A protein(s) that enables the stimulation of transcription by a variety of activator proteins has been resolved to near homogeneity.
5) Role of yeast TAFs in transcriptional activation (collaborative work with P.A. Weil and colleagues).

Role of RNA Polymerase II - CTD on Transcription

L 003 STRUCTURE AND FUNCTION OF THE CTD OF RNA POLYMERASE II, Jeffry L. Corden, Marilyn L. West, Anton Yuryev, Percy Cagas, Eytan Meisels, and Maha Gebara, Howard Hughes Medical Institute and Department of Molecular Biology and Genetics, Johns Hopkins Medical School, Baltimore, MD 21205.

A strategy was developed for mutation of each repeat within the yeast CTD. We have focused on mutations that alter serines in positions two and five ($Y_1S_2P_3T_4S_5P_6S_6$); corresponding to sites that can be phosphorylated by CTD kinases. Changing these residues to alanines is lethal, consistent with the hypothesis that phosphorylation of the CTD is essential for its function.

Lethal CTD substitution mutations have been used to isolate suppressors that allow growth of yeast cells harboring the mutant CTD. We have characterized one such suppressor gene named SCA1 (for suppressor of CTD alanine). This recessive suppressor encodes a novel protein of ~150kD. A `sca1` null mutation is able to suppress CTD mutations that alter serine residues in position two but not those in position five. We are currently studying these `sca1` null cells to determine the mechanism of suppression and the role of SCA1 in transcription regulation.

CTD kinases are able to discriminate between serines in position two and five. We have examined the substrate specificity of the CTD kinase activity associated with TFIIF and shown it to differ from that of $p34^{cdc2}$. The TFIIH-associated activity phosphorylates only one site per repeat while $p34^{cdc2}$ recognizes two sites. The different specificities of these kinases suggest that they have different roles in regulating RNA polymerase activity.

We have used two-dimensional NMR spectroscopy to analyze the structure of synthetic CTD peptides. The results of these experiments have led to a model of the unphosphorylated CTD consisting of a series of consecutive ß-turns. The proposed structure has two turns per repeat with the turns arranged in a right-handed helical structure. Serines in position two and five are differently situated in this structure which may explain their different accessibility to kinases and implies a different structural consequence upon their phosphorylation.

L 004 FUNCTIONAL CONSEQUENCES OF MODIFICATIONS WITHIN THE CTD OF MAMMALIAN RNA POLYMERASE II, Michael E. Dahmus, Mona E. Kang, Sang Soo Lee, Ross S. Chambers, Jonathan D. Chesnut, Grace K. Dahmus and Alan Lehman, Section of Molecular and Cellular Biology, University of California, Davis, CA 95616.

The C-terminus of the largest RNA polymerase (RNAP) II subunit in comprised of multiple heptapeptide repeats of the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. This C-terminal domain (CTD) is conserved in evolution and is essential for cell viability. A variety of experimental results have led to the idea that the interaction of RNAP II with the preinitiation complex is mediated, at least in part, by the interaction of the CTD with proteins assembled on the promoter. These interactions are in turn thought to be influenced by the extensive modifications that occur within the CTD. Modifications that have so far been demonstrated include the phosphorylation of serine and threonine, the phosphorylation of tyrosine and the addition of N-acetylglucosamine to serine and threonine. All of these modifications occur at multiple sites within the CTD of a given molecule of RNAP II.

The reversible phosphorylation of the CTD has been proposed to be integral to each cycle of transcription from the Adenovirus-2 major late promoter (Ad-2 MLP). However, since *in vitro* transcription from the Ad-2 MLP is not dependent on the CTD, this promoter is not the best paradigm for the study of CTD function. In an effort to investigate the role of the CTD and its phosphorylation, a RNAP II-dependent reconstituted transcription system specific for the DHFR promoter was established. In this reconstituted system, RNAP IIA, but not RNAP IIB which lacks the CTD, can transcribe from the DHFR promoter. Furthermore, RNAP IIB does not compete with RNAP IIA for preinitiation complex assembly. These results suggest that the CTD plays a critical role in the recruitment of RNAP II to the DHFR promoter.

The analysis of preinitiation complexes assembled on the DHFR promoter indicates that RNAP IIA readily assembles into functional preinitiation complexes in contrast to the inefficient assembly of RNAP IIO. However, transcript elongation is catalyzed by RNAP IIO as demonstrated by the photo-activated crosslinking of nascent DHFR transcripts to subunit IIo. These results indicate that transcription from the DHFR promoter involves the reversible phosphorylation of the CTD and support the idea that RNAPs IIA and IIO have essential but distinct functions.

The laboratories of Kadonaga and Sharp have recently reported that transcription from certain promoters in supercoiled templates requires RNA polymerase II and a minimal complement of transcription factors. Because transcription in such systems does not appear to be dependent on CTD phosphorylation, it was of interest to determine if the assembly of RNAP II into such complexes is influenced by the phosphorylation state of the CTD. Preliminary results indicate that RNAPs IIA and IIO are recruited with comparable efficiencies to preinitiation complexes formed on the Ad2-MLP in supercoiled templates in the presence of recombinant TBP, TFIIB and RAP 30. This is in contrast to studies utilizing linear templates and the complete complement of transcription factors in which phosphorylation of the CTD dramatically reduces the ability of RNAP II to assemble into preinitiation complexes.

L 005 RNA POLYMERASE II CTD PHOSPHORYLATION *IN VIVO*, John R. Weeks, Steven Hardin, Jianjun Shen, Jae Moon Lee, 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, which contains the consensus repeat sequence YSPTSPS, is subject to hyperphosphorylation primarily on Ser but also on Thr and Tyr residues. The hyperphosphorylation reduces the SDS gel mobility of the subunit, generating subunit IIo. We recently prepared goat antibodies selectively reactive toward the unphosphorylated CTD and rabbit antibodies selectively reactive toward the Ser/Thr hyperphosphorylated CTD. We are using these antibodies in double label immunofluorescent staining of polytene chromosomes to investigate the *in vivo* locus-specific distributions of Pol IIA and Pol IIO, with hypo- and hyperphosphorylated CTDs, respectively.

We find RNA polymerases IIA and IIO arrayed in markedly different, locus- and condition-specific patterns representing three classes of sites. One class contains only Pol IIO, another class contains only Pol IIA, and the third class contains a mixture of Pol IIA and IIO. To begin to understand these patterns, we have established correlations between the nature and activity of certain loci and the phosphorylation state of Pol II there. For example, major ecdysone-induced puffs stain exclusively for Pol IIO, indicating that hyperphosphorylated Pol II is the transcriptionally active form of the enzyme on these genes. In contrast, heat shock induced puffs stain strongly for both IIA and IIO, suggesting that heat shock genes are transcribed by a mixture of hypo- and hyperphosphorylated forms of Pol II. At the insertion sites of a transposon carrying a hybrid hsp70-*lacZ* transgene, we observe only Pol IIA prior to heat shock induction, consistent with the idea that Pol II arrested on the hsp70 gene is form IIA. After a 90 sec heat shock we detect heat shock factor (HSF) at the transposon insertion sites, and after a 5 min shock its spatial distribution on the induced transgene puffs is clearly resolved from that of Pol II.

We have also begun to monitor the co-localization of RNA polymerase IIA and IIO with other nuclear proteins involved in either pre-mRNA metabolism or transcriptional regulation. For example, using antibodies to hnRNP proteins and splicing components, we have discerned an apparent overall correlation between the presence and processing of nascent transcripts and the presence of Pol IIO.

References:

•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.

•Weeks, J. R., Hardin, S. E., Shen, J., Lee, J. M., and Greenleaf, A. L. 1993. Locus-Specific Variation in Phosphorylation State of RNA Polymerase II In Vivo: Correlations with Gene Activity and Transcript Processing. *Genes & Development* **in press.**

Mechanisms of Transcription Activation I

L 006 STRONG VIRAL ACTIVATORS STIMULATE TRANSCRIPTION THROUGH DIRECT INTERACTONS WITH TFIID AND TFIIA, Arnold J. Berk[1], Paul M. Lieberman[2], Thomas G. Boyer[1], Joseph V. Geisberg[3], Robert P. Ricciardi[3], Eugene Bryant[1], Ronald Koop[1], and Xuan Liu[1], [1] Molecular Biology Institute, U.C.L.A., Los Angeles, CA 90024-1570,[2]Roche Institute of Molecular Biology, Nutley NJ 07110-1199, [3]Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadephia, PA 19104

The adenovirus E1A activation domain and Epstein-Barr virus Zta protein were studied as model transcriptional activators. Activatation by E1A was found to require its ability to bind to TFIID. Conservative substitutions at all 49 residues in the E1A activation domain were assayed for their effects on binding to TBP *in vitro* and on actviation of an early adenovirus promoter in *in vivo*. Seven mutations which severely inhibited TBP binding were similarly defective in activation *in vivo*. Five mutations inhibited *in vivo* activation but did not affect TBP binding indicating that TBP binding is required for activation, and that a function(s) in addition to TBP binding is also required. Activation of *in vitro* transcription by a GAL4-E1A fusion requires the TAFs associated with TBP in the holo-TFIID multisubunit protein complex. The E1A activation domain was found to bind to holo-TFIID as well as it binds to isolated TBP. None of the detectable TAFs are displaced when E1A binds. Consequently, the surface of TBP which interacts with E1A is available for interaction in the holo-TFIID complex. High concetrations of GAL4-E1A squelche *in vitro* transcription, a process thought to be due to titration of the activation domain target. To identify the target we added additional general factors to a squelched reaction. Only holo-TFIID restored transcription to a squelched reaction, indicating that holo-TFIID is the target of the E1A activation domain which is titrated. Transcription complex assembly was studied in reactions using purified holo-TFIID and the Zta activator (a bZIP factor). Electrophoretic mobility shift assay (EMSA) in agarose gels and DNase I footprinting showed that Zta greatly stimulates the rate and extent of assembly of a stable holo TFIID-TFIIA complex by a process which requires both TAFs and the Zta activation domain.

L 007 **THE ROLE OF DISTINCT COFACTORS IN TRANSCRIPTIONAL ACTIVATION,** **Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021**

The TATA-binding subunit (TBP) of natural TFIID and a number of other general initiation factors sufice for basal transcription by RNA polymerase II from most core promoters. In constrast, the in vitro function of gene-specific DNA-binding activators requires, in addition: (i) one or more of the other TFIID subunits (TAFs), (ii) initiation factors (e.g., TFIIA) that may facilitate but are not essential for TBP-mediated basal transcription, (iii) one or more separable positive (PC1, PC2, PC3) and negative (NC1, PC3) components purified from the general cofactor USA, that may act individually or in concert and (iv) in some cases, activator-specific cofactors (e.g., the lymphoid specific Oct co-activator OCA-B) that show independent interactions with activators. Ongoing efforts have resulted in the further purification, cloning and characterization (structure and/or function) of these various cofactors and selected results relevant to activation mechanisms will be discussed.

L 008 ROLE OF HUMAN AND DROSOPHILA TBP ASSOCIATED FACTORS (TAFs) IN TRANSCRIPTION REGULATION, Verrijzer, C.P., Attardi, L.D., Chen, J.-l.,. Goodrich, J., Ruppert, S., Wang, E., Weinzierl, R., Yokomori, K., Tjian, R., Howard Hughes Medical Institute, Dept. of Molecular and Cell Biology, University of California, Berkeley.

TFIID is one of the general transcription factors that, in addition to RNA polymerase II, are required for transcription of eukaryotic protein-encoding genes. The initial step in the assembly of a preinitiation complex is binding of TFIID to the TATA-box. TFIID is a multisubunit protein complex composed of the TATA-box binding protein (TBP) and at least eight tightly associated polypeptides termed TAFs. Although TBP is sufficient to support basal transcription, activation of transcription by promoter-specific activators requires TAFs. We have cloned all the major Drosophila TAFs and most of the human homologues. Using recombinant proteins we have characterized multiple TAF-TAF and TAF-TBP interactions that mediate formation of the TFIID complex. Since at least some TAFs are predicted to function as coactivators, we investigated several activator-TAF interactions. Our results suggest that activators with distinct classes of activation domains, including Sp-1, VP-16 and NTF-1, target different TAFs and that TAFs mediate transcriptional activation by upstream factors in vitro. Surprisingly, the Drosophila dTAF$_{II}$150 shows a striking homology to the essential yeast gene TSM1 whose product, like dTAF$_{II}$150, interacts with TBP and other TAFs. Our results establish that there are also TAFs in yeast. Further studies on the function of TAFs in the regulation of transcription will be discussed.

L 009 **ACTIVATION/REPRESSION: E1A/P53,** Nobuo Horikoshi*, Anny Usheva*, Tom Shenk* and Roberto Weinmann*# Department of Molecular Biology*, Princeton University and Department of Molecular Drug Mechanisms, Division of Oncology & Drug Discovery, Bristol Myers Squibb, Princeton, NJ 08543

Direct interactions have been shown to occur between the adenovirus early E1A oncoprotein and the human TATA binding protein (TBP). These interactions can be demonstrated in mammalian cells and in vitro with recombinant TBP or native TBP complexes. We localized the regions of interaction between E1A and TBP to conserved domain 3 containing the Zn finger of E1A, and the basic sequences between aminoacids 214 and 270 of TBP. Point mutants within the helix 2 domain of TBP as well as in the Zn finger of E1A destroy this interaction as well as the transcriptional effects. This same helix 2 region of TBP as well as some adjacent sequences also are essential for the interaction with the cellular tumor suppressor p53 . When p53 is bound to TBP, it can be selectively displaced by purified E1A. In an in vitro transcriptional system reconstituted with purified components, p53 is able to repress transcription. Addition of purified E1A restitutes the transcriptional activity of the system. In transient assays, equivalent amounts of p53 and E1A plasmids are required to reproduce these effects. These data suggests that activation by conserved domain 3 of adenovirus E1A may be the result of displacement of p53, which acts as a repressor on promoters lacking p53 binding sites.

Mechanisms of Transcription Activation II

L 010 INTERACTIONS WITHIN EUKARYOTIC TRANSCRIPTION INITIATION COMPLEXES, Stephen Buratowski, Jacqueline DePaulo, and Paul Matsui, Whitehead Institute for Biomedical Research, Cambridge, MA 02142.

Eukaryotic transcription initiation complexes are assembled from multiple protein factors. It appears that a complicated set of protein-protein interactions are necessary for this assembly. A variety of genetic and biochemical techniques are being used to probe these interactions.

Yeast TFIIH (also known as factor b) consists of at least five subunits and appears to act both as a transcription factor and in DNA repair. The 73 kilodalton subunit (TFB1) and the 50 kilodalton subunit (SSL1) have been shown to interact by the "two-hybrid" system. The domains of the protein necessary for this interaction are being mapped by testing deletion mutants of these two proteins. Interestingly, mutants of TFB1 defective for interaction with SSL1 also cause hyper-sensitivity to ultraviolet light. We are also in the process of mapping interactions between these two proteins and other members of the TFIIH complex. Mutant strains defective in various transcription factors have been tested for sensitivity to ultraviolet light. So far, only mutations in TFIIH subunits lead to increased sensitivity. A model will be presented for a mechanism by which DNA repair and transcription can be coupled by TFIIH.

Transcription by RNA Polymerase III

L 011 RNA POLYMERASE II AND III TRANSCRIPTION OF THE HUMAN snRNA GENES, Nouria Hernandez, Cynthia L. Sadowski, R. William Henry, Renu Mital, and Susan Lobo, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

The human snRNA genes share very similar promoters, yet they are not all transcribed by the same RNA polymerase. Most snRNA genes, including the U1 and the U2 snRNA genes, are transcribed by RNA polymerase II, whereas the U6 snRNA gene is transcribed by RNA polymerase III. The basal RNA polymerase II snRNA promoters consist of a single element, the proximal sequence element or PSE, which is essential for transcription and localizes the start site. The basal U6 promoter also contains a PSE and, in addition, a TATA box similar to the TATA box found in many RNA polymerase II mRNA promoters.

We are characterizing the *trans*-acting factors involved in RNA polymerase II and III transcription of snRNA genes with the goal of understanding how RNA polymerase specificity is determined. We are also interested in comparing the factors required for transcription of the U6 snRNA gene with those involved in transcription of other RNA polymerase III genes such as the VAI gene. The U6 TATA box binds the TATA box binding factor TBP, which was first characterized as a factor involved in RNA polymerase II transcription from mRNA promoters. The U6 and U1 PSEs bind a large complex we refer to as the SNAP complex. Thus, SNAP appears to be both an RNA polymerase II and III transcription factor. TBP is also required for transcription of the VAI gene, as part of a complex we refer to as 0.38M-TFIIIB. We will describe our progress in characterizing these different factors.

L 012 MECHANISM AND REGULATION OF TRANSCRIPTION BY RNA POLYMERASE III, Robert J. White, Bernard Khoo and Stephen P. Jackson, Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR, U.K. and Department of Zoology, University of Cambridge.

We have shown that the TATA-binding protein, TBP, is a general transcription factor for RNA polymerase III despite the fact that most class III promoters lack TATA sequences. Through biochemical and immunological approaches, we have demonstrated that TBP functions as a component of the general RNA polymerase III factor TFIIIB. Another component of TFIIIB is the product of the BRF1/PCF4/TDS4 gene, which displays homology to the RNA polymerase II general factor TFIIB. To gain further insight into BRF1, we have isolated and sequenced its gene from a variety of divergent yeast species. In this way, we have identified several regions of the BRF1 protein that are particularly highly conserved and are thus likely to be important functionally. These regions are currently being tested for an ability to interact with TBP and other pol III general factors.

We are also interested in determining how pol III transcription can be regulated. In one approach, we are assessing the potential of known pol II activators and repressors to modulate transcription by RNA polymerase III in vitro. In another approach, we are investigating the molecular mechanisms by which the activities of pol III general factors can be regulated in response to physiological changes.

L 013 POL III TRANSCRIPTION COMPLEX ASSEMBLY AND RNA CHAIN ELONGATION, George A. Kassavetis, Claudio Joazeiro, Hajime Matsuzaki, Simon Whitehall, Carolyne Bardeleben, and E. Peter Geiduschek. Dept. of Biology, U.C. San Diego, La Jolla, CA 92093-0634.

The central transcription factor of the RNA polymerase (pol) III transcription apparatus, TFIIIB, serves as a beacon to direct pol III to the site on DNA at which it initiates RNA synthesis. Yeast TFIIIB is composed of three components that suffice for directing tRNA gene transcription: TBP, the TFIIB-related *BRF1* gene product (Brf) and a denaturing gel-isolated 90 kDa protein. TFIIIB is assembled upstream of tRNA genes without regard to DNA sequence by TFIIIC bound at the *box A* and *box B* intragenic promoter elements. This assembly is believed to involve the interaction of the Brf subunit of TFIIIB with the 120 kDa subunit of TFIIIC. The yeast U6 snRNA gene, *SNR6*, contains a TATA box at bp -30 and an extragenic *box B* element downstream of the gene that is suboptimally spaced relative to the start site-proximal *box A* element. It has been demonstrated by others that TFIIIC and *box B* are dispensable for U6 transcription with purified components, but are required *in vivo* and with some crude transcription systems *in vitro*. We have examined the assembly of TFIIIB on the *SNR6* gene and the properties of the TFIIIB-*SNR6* gene complex. Although TFIIIC is not required for TFIIIB-*SNR6* complex formation, the same three components of TFIIIB that are necessary for TFIIIC-dependent transcription of tRNA genes, are also required and sufficient for TATA box-directed *SNR6* transcription. The TFIIIB-*SNR6* gene complex displays properties that are indistinguishable from complexes formed on tRNA and 5S rRNA genes, such as the extent and location of its footprint, its stability and its resistance to heparin.

We have analyzed individual steps of RNA chain elongation by pol III on the SUP4 tRNA gene, in which the slow steps of transcription initiation were by-passed by forming 5' end-labelled, arrested and precisely positioned, ternary transcription complexes. RNA chain elongation with 1 mM NTPs at 20°C is uneven but relatively rapid, with a median elongation rate of ~20 nt/sec. At 0°C the elongation rate is reduced ~30-fold. Quantitative analysis of RNA chains after accurately timed periods of elongation generated kinetic constants for 58 sequential steps of nucleotide addition. These values were used in computer simulations of RNA chain growth. Elongation modelled as a simple sequence of pseudo-first order reactions yielded chain length distributions that remained relatively synchronous during elongation, while observed chain growth quickly became desynchronized. This discrepancy connotes a process of RNA chain elongation that is more complex. Computer simulations of more elaborate kinetic schemes have also been explored and suggest pol III may switch repeatedly between rapidly stepping and slowly stepping states during the elongation of a single RNA chain.

Hydrolytic cleavage at the 3' end of RNA chains has been documented to occur within ternary transcription complexes formed with pol II, vaccinia virus RNA polymerase and *E. coli* RNA polymerase. This nucleolytic activity can be either elongation factor-dependent (pol II: TFIIS; *E. coli* pol: Gre A and B) or an intrinsic property of the polymerase (vaccinia). Evidence will be presented demonstrating that ternary complexes formed with highly purified pol III contain a nucleolytic activity with properties similar to vaccinia virus RNA polymerase.

L 014 TBP/TAF COMPLEXES IN TRANSCRIPTION, Robert Coleman, Timothy Fisher, Amy Jackson, John Chicca, Andrew Taggart, Robert Carter, & Frank Pugh, Center for Gene Regulation, Department of Molecular & Cell Biology, Penn State University, University Park, PA, 16803.

Our research focuses on the role that the TATA binding protein (TBP) and associated factors (TAFs) play in transcription initiation of mammalian nuclear genes. We know that TBP and at least two TAFs, one tightly associated (TAF-172) and one loosely associated (TAF-L) comprise *TFIIIB and* are required for the expression of the pol III tRNA-type genes which contain downstream promoter elements. A distinct class of pol III transcribed genes, which includes the U6 snRNA gene, utilize only upstream elements. For U6, an essential PSE element and TATA box are required. Both of these elements are also found in Pol II transcribed genes. Does U6 involve a TBP/TAF complex akin to TFIIIB, TFIID, or does the U6 promoter involve a distinct TBP/TAF complex? Our findings indicate that multiple distinct TBP/TAF complexes can function at the U6 promoter. These TBP/TAF complexes are distinct from TFIIIB and at least one of them is distinct from TFIID. Our efforts are directed toward identifying the components of these TBP/TAF complexes and their role in polymerase selection and transcription initiation.

TBP is apparently required for transcription of all nuclear genes, even those which do not contain a TATA box. In the absence of a TATA box, how is TBP recruited to the promoter? Does TBP contact DNA when bound to a TATA-less promoter, or is it associated only indirectly via protein-protein interactions? Reports of TBP binding to alternative non-TATA sequences has lead us to investigate whether TBP has a high intrinsic affinity for DNA while having little sequence specificity. We have examined the association and dissociation kinetics as well as the temperature dependence of TBP binding to TATA and nonspecific DNA sequences. Interestingly we find that the properties of TBP binding to nonspecific DNA are very similar to its binding to TATA. These properties include rapid association at high TBP concentrations and slow dissociation rates. Association but not dissociation was found to be temperature sensitive. These findings argue that the binding of TBP to a TATA-less promoter is *no different* than the binding of TBP to a TATA containing promoter.

L 015 THE LIMITING STEPS IN RNA POLYMERASE III TRANSCRIPTION INITIATION, Ian M. Willis, Alfredo López-de-León, Gerald Rameau, Karen Puglia, Monett Librizzi and Indra Sethy, Department of Biochemistry, Albert Einstein College of Medicine, NY 10461.

A genetic approach to isolate mutations affecting transcription by RNA polymerase (pol) III has identified two novel genes, *PCF1* and *PCF4*. Dominant mutations in these genes suppress the negative effect a tRNA gene A block promoter mutation and increase pol III gene transcription *in vivo* and *in vitro*. Mutant and wild-type alleles of the two genes have been cloned and their products identified. Comparative studies of transcription using factors from wild-type and mutant cells have provided a biochemical understanding of the functions of the mutant gene products.

PCF4 encodes the 70 kD subunit of TFIIIB (TFIIIB $_{70}$) and is a TFIIB homolog. The genetic and biochemical properties of TFIIIB $_{70}$ indicate that it is a polymerase specificity factor. TFIIIB $_{70}$ is predicted to mediate interactions between the universally conserved factor, TBP and other pol III-specific factors and/or subunits of the polymerase. The *PCF4-1* mutation maps outside of the coding region and identifies a putative promoter element in the 5' flanking sequence of the gene. The suggestion that *PCF4-1* increases the amount of TFIIIB $_{70}$ has been proven by demonstrating that multiple copies of *PCF4* $^{+}$ suppress a tRNA gene promoter defect *in vivo* and increase transcription *in vitro*. These data indicate that TFIIIB $_{70}$ is stoichiometrically-limiting in yeast cells and in cell extracts and suggest that interactions involving the TFIIIB complex are thermodynamically limiting for transcription.

The *PCF1-1* mutation causes an amino acid substitution in a novel protein structural motif of the second largest subunit of TFIIIC (TFIIIC $_{131}$). The properties of the mutation indicate that it facilitates a rate-limiting step in transcription, specifically recruitment of TFIIIB into the preinitiation complex. This conclusion is consistent with other data suggesting that TFIIIC $_{131}$ and some component(s) of TFIIIB interact. Another property of the *PCF1-1* mutation which may be a consequence of the rate effect, is the presence of increased amounts of TFIIIB in mutant cell extracts. The biochemical basis of both effects of *PCF1-1* is being investigated.

Transcription by RNA Polymerase I

L 016 MOLECULAR MECHANISMS OF RNA POLYMERASE I TRANSCRIPTION INITIATION AND TERMINATION

Ingrid Grummt, Anne Kuhn, Udo Rudloff, Dirk Eberhard, Gisela Schnapp, and Raymond Evers,

German Cancer Research Center, Division of Molecular Biology of the Cell II, 69120 Heidelberg, Germany,

We are studying the molecular mechanisms which mediate specific initiation and termination of mammalian ribosomal gene transcription. Four positively acting transcription initiation factors (termed TIF-IA, TIF-IB, TIF-IC, and UBF) together with RNA polymerase I (Pol I) are required to form a productive preinitiation complex at the mouse rDNA promoter. Promoter selectivity is brought about by the species-specific factor TIF-IB/SL1. We have isolated TIF-IB from Ehrlich ascites and SL1 from HeLa cells and show that they are multiprotein complexes containing TBP and three TAFs. Data will be presented which compare the structure and the promoter specificity of the human and the mouse factor as well as the interaction of individual TAFs with TBP and rDNA, respectively. The results demonstrate that - similar to Pol II- and Pol III-specific TBP complexes - the conserved core of TBP is sufficient to nucleate the assembly of Pol I-specific TBP-TAF complexes. Binding of TIF-IB/SL1 to the promoter is stabilized by UBF, an HMG box-containing DNA binding protein. We have compared the transcriptional activity and DNA binding properties of UBF1&2 and show that UBF2, the splice variant lacking 37 amino acids of the second (of five) HMG boxes, fails to bind to rDNA and, therefore, is transcriptionally inactive. Thus, the individual HMG boxes are functionally diverse and HMG box2 plays an important role in binding of UBF to rDNA. However, both UBF isoforms bind efficiently to four-way junction DNA indicating that in addition to a certain sequence-specificity, the HMG boxes recognize defined DNA structures. Moreover, we show that both forms of UBF interact with a defined subunit of Pol I.

Termination of Pol I transcription is mediated by a transcription termination factor (TTF-I) which specificlly binds to a repeated 18 bp signal sequence downstream of the rDNA transcription unit. We have cloned the cDNA encoding the 130 kDa nuclear protein and present data describing its mode of action.

L 017 CHARACTERIZATION OF GENES RRN3, RRN6 AND RRN7 REQUIRED FOR rRNA TRANSCRIPTION IN SACCHAROMYCES CEREVISIAE, Masayasu Nomura[1], Daniel Keys[1], Yasuhisa Nogi[2], Loan Vu[1], Joan Steffan[1], Robert Yamamoto[1], and Jonathan Dodd[1], [1]Department of Biological Chemistry, University of California, Irvine, CA 92717-1700, [2]Department of Biochemistry, Saitama Medical School, Saitama 350-04, Japan.

Using a system in which the 35S rRNA gene is fused to the GAL7 promoter and transcribed by RNA polymerase II, we have isolated many mutants primarily defective in the transcription of 35S rRNA genes by RNA polymerase I (Pol I)[1]. These mutants were classified into seven new genes, (RRN3-7, RRN9, and RRN10) and into the two known RNA polymerase subunit genes, RPA190 (RRN1) and RPA135 (RRN2). A biochemical analysis of mutant cell extracts suggested that three of the new genes RRN3, RRN6, and RRN7, probably encode transcription factors required for in vitro rRNA synthesis by Pol I.

We have cloned these three genes and have determined their complete nucleotide sequences. From deduced amino acid sequences, RRN3 appears to encode a protein of 627 amino acids, RRN6 to encode a protein of 894 amino acids and RRN7 to encode a protein of 515 amino acids. Extracts prepared from these mutants fail to support specific transcription of rRNA by Pol I in vitro, and can be complemented by fractions from cellular extracts prepared from wild type strains or from strains containing epitope-tagged Rrn proteins. Using conventional biochemical fractionation combined with immuno-affinity purification, we have obtained evidence that Rrn6p and Rrn7p, and possibly Rrn3p, are associated with TATA binding protein (TBP) in crude extracts, forming a large protein complex. Properties of these three transcription factors as well as other new genes required for Pol I transcription will be presented.

[1] Nogi, Y., Vu, L. and Nomura, M. PNAS, 88, 7026 (1991).

L 018 EVENTS DURING RIBOSOMAL RNA TRANSCRIPTION INITIATION, Marvin R. Paule[1], Catherine A. Radebaugh[1], Han Li[1], Jennifer L. Matthews[1], Gary K. Geiss[1], Feng Liu[2], Jie-Min Wong[2], Erik Bateman[2], [1]Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80523, [2]Department of Microbiology and Molecular Genetics, and the Markey Center for Molecular Genetics, University of Vermont, Burlington, VT 05405

Despite the high degree of species specificity associated with ribosomal RNA transcription in eukaryotes, the fundamental mechanism of initiation appears almost identical across a wide range of phylogeny. *Acanthamoeba castellanii* is a small free-living amoeba phylogenetically close to yeast, and has the same protein requirements for accurate rRNA transcription as vertebrates. One of the *Acanthamoeba* proteins is functionally a homolog of vertebrate upstream binding factor, UBF. This protein acts as an assembly factor for the TBP-containing factor, TIF-IB. The latter is probably the fundamental initiation factor in analogy to TFIIIB of yeast. In *Acanthamoeba*, TBP is associated with TAFs with M_Rs of 145, 99, 96, (and 91) kDa, and has a native M_R of 290 kDa. We have compared the role of TBP in TFIID, TFIIIB and TIF-IB by examining inhibition of *in vitro* transcription by TATA-box specific oligonucleotides and anti-TBP antibodies. While transcription by polymerases II and III exhibit similar inhibition profiles by these reagents, results with the polymerase I system suggest that TBP is assembled in a distinct fashion in TIF-IB. To further investigate the assembly of these protein components in the initiation complex, we have initiated a detailed study of the structure of this complex. Electrophoretic mobility shift assays (EMSA), footprinting, and photo cross-linking have been used. EMSA-supershift assays with anti-TBP suggest that there are two molecules of TBP in each committed complex. Photo cross-linking to DNA with the sequence-region-specific reagent 5-[N-(*p*-azidobenzoyl)-3-aminoallyl]-dUMP has revealed that there are either two molecules of TIF-IB bound to the "core promoter" (-17 to -55) or the DNA is wrapped in a tight loop around the bound factor. In mouse and rat, TIF-IB alone appears capable of forming a committed complex, and we are investigating the presence of UBF in the *Acanthamoeba* complex. Polymerase I binds to the committed complex by protein:protein interactions, but the complex remains a closed promoter complex until after NTPs have been added. We have examined this requirement for nucleotides and find that βγ hydrolysis is not required, as it is for melting in polymerase II transcription. Furthermore, the formation of a stable melted bubble occurs in a stepwise fashion as the initial 4-nt long RNA is formed, based upon diethylpyrocarbonate modification. A steady-state sized melted bubble of between 12 and 16 base pairs is found in the stalled octamer complex; there is possibly a larger melted region after further elongation.

Supported by US Public Health Service Grant #GM22580 to MRP.

Regulation of Transcription Initiation in Bacterial Systems

L 019 **HIGH-SPECIFICITY SEMI-SYNTHETIC NUCLEASES: DESIGN, CONSTRUCTION, AND APPLICATION TO LARGE GENOMES**

R. H. Ebright, P.S. Pendergrast, and Y.W. Ebright, Department of Chemistry and Waksman Institute, Rutgers University, New Brunswick, NJ 08855

Synthetic or semi-synthetic site-specific nucleases can be constructed by covalently attaching a nucleolytic chelator-metal complex to a sequence-specific DNA binding molecule (*J. Amer. Chem. Soc.*, 104:6861, 1982; *Science* 238:645, 1987; *Science* 238:1129, 1987; *Science* 237:1197, 1987; *Proc. Natl. Acad. Sci. USA* 87:2882, 1990). However, previously reported synthetic or semi-synthetic nucleases have exhibited significant non-specific DNA cleavage activity (i.e., they have exhibited significant DNA cleavage activity at DNA sites other than the specific DNA recognition site).

In this report, we describe an approach to construct novel semi-synthetic nucleases that are able to cleave DNA when bound at the specific DNA recognition site, but that are not able to cleave DNA when bound at non-specific DNA sites. The approach is to incorporate a nucleolytic chelator-metal complex at an amino acid of a sequence-specific DNA binding protein that--due to differential DNA bending--is close to DNA in the specific protein-DNA complex, but is not close to DNA in the non-specific protein-DNA complex.

CAP sharply bends DNA in the specific CAP-DNA complex (*Nature* 308: 509, 1984; *EMBO J.* 3:2873, 1984; *Science* 253:1001, 1991). CAP does not sharply bend DNA in the non-specific CAP-DNA complex (*Biochem.* 18:255, 1979; *Nucleic Acids Res.* 7:1699, 1979). As a result, several amino acids of CAP are close to DNA in the specific CAP-DNA complex, but are not close to DNA in the non-specific CAP-DNA complex: i.e, amino acids 24 to 26 and amino acids 89 to 91. We have constructed a semi-synthetic nuclease by incorporation of 1,10-phenanthroline:copper at an amino acid of CAP that is close to DNA in the specific CAP-DNA complex, but is not close to DNA in the non-specific CAP-DNA complex: i.e., at amino acid 26 of CAP. The semi-synthetic nuclease is: [(acetyl-glycyl-5-amino-1,10-phenanthroline:copper)-Cys26;Ser178]CAP (*[(OP6)26]CAP*).

We have assayed the DNA-cleavage activity of [(OP6)26]CAP using 7.1 kb, 49 kb, and 4,700 kb DNA substrates having the DNA recognition site for CAP (i.e., recombinant bacteriophage M13 genomic DNA, recombinant bacteriophage lambda genomic DNA, and *Escherichia coli* genomic DNA). [(OP6)26]CAP cleaves each of the DNA substrates within the DNA recognition site(s) for CAP. DNA cleavage is highly efficient and highly specific.

[(OP6)26]CAP has applications in chromosome mapping, chromosome sequencing, and chromosome cloning. We are applying [(OP6)26]CAP to analysis of several prokaryotic and eukaryotic genomes.

L 020 THE N-TERMINUS OF SIGMA FACTOR REGULATES DNA BINDING, A.J. Dombroski, W.A. Walter and C.A. Gross, Department of Bacteriology, University of Wisconsin-Madison, Madison, WI

Prokaryotic transcription initiation factor σ is required for sequence-specific promoter recognition by RNA polymerase. Genetic studies have suggested that σ itself interacts with the template DNA at the -10 and -35 promoter consensus sequences. Direct binding of *E. coli* σ^{70} to DNA *in vitro*, in the absence of the core subunits ($\alpha_2\beta\beta'$) of RNA polymerase, however can only be observed for truncated σ polypeptides lacking all or part of the N-terminal domain. We have investigated the regulatory role of the N-terminus of *E. coli* σ^{70} in controlling DNA binding activity. Removal of the N-terminal 50 amino acids of σ^{70} restores DNA binding activity to the C-terminal DNA binding domains. Inhibition of binding by the N-terminal domain can also be observed *in trans*. The target site for the inhibitory action appears to be the DNA binding domain of conserved Region 4, which recognizes the -35 consensus sequence, but not conserved Region 2, which is responsible for for recognition of the -10 consensus sequence.

The alternative σ factors, or their derivatives, also bind to promoter DNA depending to varying degrees upon their N-terminal length and amino acid composition. *S. typhimurium* σ^F, which has no N-terminal extension, binds to DNA as a full length σ. The N-terminal extension of σ^K, a *B. subtilis* mother cell-specific σ involved in sporulation, modulates DNA binding ability, but does not completely inhibit binding. Additionally its N-terminal domain aids in stabilizing the polypeptide *in vivo*. The N-terminus of σ^{32}, the *E. coli* heat shock σ, on the other hand tightly controls DNA binding in a manner similar to σ^{70}. Interestingly, the N-terminal inhibitory domain of σ^{70} also effectively inhibits the DNA binding domain of σ^{32} suggesting that the mechanism of inhibition is similar for σ^{32} and σ^{70}. We propose that initiation of transcription is subject to regulation as a result of the composition and/or structure of the N-terminus of the σ subunit and that the σ family of proteins belongs to a larger class of intramolecularly regulated transcriptional effectors.

L 021 SINGLE-STRANDED DNA BINDING PROTEINS AS TRANSCRIPTIONAL ACTIVATORS, L. B. Rothman-Denes[2], X. Dai[1], M.Y. Choi[2], M. A.Glucksmann[2] and A. Miller[1]. Departments of [1]Biochemistry and Molecular Biology and [2]Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois, 60637.

Transcription of the 72kbp DNA linear genome of coliphage N4 is carried out by three different RNA polymerases: two phage-coded enzymes which transcribe early and middle phage genes and the E. coli σ^{70} RNA polymerase which transcribes the late phage genes. Single-stranded DNA binding proteins (SSB) are required for activation of early and late transcription.

Early transcription is carried out by a virion-associated, 320,000 kDa polypeptide (vRNAP) which requires supercoiled template and E. coli SSB for promoter utilization[1]. Results of genetic and biochemical experiments indicated that all determinants of promoter recognition are present in the template strand; both specific conserved bases and a hairpin structure on the template strand are required for promoter recognition. We have proposed that, on double-stranded templates, supercoiling-induced single-strandedness permits hairpin formation at the promoter. This structure is stabilized by E. coli SSB allowing productive vRNAP promoter recognition[2]. We will present results of experiments that corroborate this model and indicate that the sequences of the inverted repeats present at the N4 vRNAP promoter generate hairpins with unusually high stability. These results indicate that E. coli SSB activates vRNAP transcription by providing the correct DNA structure at the promoter.

E. coli RNAP recognition of N4 late promoters, which show poor homology to the σ^{70}-RNAP consensus sequence, requires the activity of the N4-coded SSB[3,4]. Extensive mutational analysis of N4 SSB indicates that the specific residues at the C-terminus are required for transcriptional activation but not for recombination or replication. These results suggest that N4SSB might activate transcription through RNAP-N4SSB interactions. The role, if any, of the N4 SSB single-stranded DNA binding activity in transcription activation remains to be determined.

1- *Markiewicz*, P. et al, Genes & Dev., 6: 2010-2019 (1992); 2- Glucksmann, M.A. et al. Cell, 70: 491-500 (1992); 3- Lindberg, G. et al. J. Biol. Chem, 264: 12700-12708 (1989); 4- Cho, N.Y et al. submitted.

Regulatory Mechanisms in Transcription Elongation and Termination in Bacteria

L 022 RNA POLYMERASE AS A MOLECULAR MACHINE: COUPLING BETWEEN CATALYTIC FUNCTION AND MOVEMENT, Alex Goldfarb, Mikhail Kashlev, and Evgeny Nudler, Public Helath Research Institute, New York, NY10016.

A directional structure-functional model of RNA polymerase is proposed which views the enzyme as a machine composed of at least three distinct elements: the DNA binding site located at the "front" of the molecule, the RNA binding site situated towards the "rear" and the active center between them. As RNA polymerase advances along the DNA template during elongation, the DNA and RNA binding sites are translocated synchronously reflecting their rigid connection. The active center, however, advances out of sync with the two other elements suggesting that it moves about within the RNA polymerase molecule. The advancement of the enzyme occurs by a two-step mechanism whereby the active center adds several nucleotides to the transcript within a "resting" elongation complex followed by a leap-like translocation of the DNA and RNA binding sites. The length of each synthesis/translocation cycle is strictly determined by DNA sequence. The model is based on DNA and RNA footprinting, affinity labeling of functional sites using crosslinkable substrate analogs and functional analysis of defined elongation complexes. It represents substantial detalization of the "inchworm" model of RNA polymerase movement first suggested by M. Chamberlin.

Regulatory Mechanisms in Transcription Elongation and Termination in Eukaryotic Systems

L 023 STRUCTURE-FUNCTION ANALYSIS OF TRANSCRIPT ELONGATION FACTOR TFIIS FROM HUMAN CELLS. Guadalupe Cipres-Palacin, Zoe Weaver, and Caroline M. Kane, Division of Biochemistry and Molecular Biology, University of California at Berkeley, Berkeley, CA 94720.

Transcript elongation by RNA polymerase II is often interrupted before the polymerase reaches the end of the transcription unit. This interruption occurs in a regulated manner and can lead to transcript release and termination, to pausing, or to the arrest of the elongation complex which begins transcription again only under the influence of regulatory protein factors. One such protein factor is TFIIS which promotes readthrough by RNA polymerase II of blocks to elongation within genes. This protein can interact with the polymerase during elongation and promote readthrough in part by inducing cleavage of the 3' end of the nascent transcript, a reaction also seen in elongation complexes containing other RNA polymerases in response to other factors (1). We have prepared a collection of site-directed mutations in the cDNA of human TFIIS. This collection has allowed us to characterize the quantitative properties of mutant TFIIS proteins in the transcript elongation process. For many of these mutants, there is direct correlation between a reduced rate of transcript cleavage in arrested ternary complexes and reduced readthrough promoted by TFIIS. However, there are also mutants in which the cleavage and readthrough promoting activities of TFIIS have been uncoupled. When such mutants are added to arrested complexes, transcript cleavage occurs quite efficiently while readthrough is dramatically reduced or eliminated.

In addition to the structure of the TFIIS protein, we have been studying the structure of the gene(s) encoding TFIIS in the human genome. Southern analyses have suggested that more than one gene for TFIIS related sequences is present in human DNA. We have identified at least two distinct clones related to the cDNA for the human protein, and sequence analysis of these clones coupled with a study of TFIIS expression in human cells is underway.

1. Kassavetis, G. A., and E. P. Geiduschek (1993) Science 259, 944-945.

L 024 TRANSCRIPT CLEAVAGE OVERCOMES ROADBLOCKS TO TRANSCRIPT ELONGATION BY RNA POLYMERASE II
Donal S. Luse, Michael G. Izban, Michael Rudd and Irakli Samkurashvili, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati, Cincinnati, OH 45267-0524

In the presence of factor SII, RNA polymerase II ternary complexes can cleave their nascent transcripts some distance from the 3' end, leaving the 5' portion of the RNA in active complex (1-3). We have shown (4) that polymerase II ternary complexes halted (or stalled) during elongation because of the absence of one of the NTPs generate primarily dinucleotides when incubated with SII. However, polymerases may pause even when NTPs are in excess, for instance in response to sequence signals. We refer to this sort of pause as an arrest. SII-mediated transcript cleavage leads to the release of 6-17 nt RNAs with arrested ternary complexes (5); relief of arrest by SII requires transcript cleavage (5,6). Pyrophosphorolysis can also cause cleavage from the 3' end of the transcript, leaving the ternary complex intact (3). In stalled complexes, PPi treatment causes the successive release of NTPs. We have recently made the surprising observation that incubation of arrested complexes with PPi leads to the production of the same set of fragments generated with SII-mediated cleavage (8-17 nt in the complexes we examined). However, these PPi-generated RNAs have 5'-triphosphate termini. Thus, transcript cleavage in the RNA polymerase II ternary complex, even at locations as far as 17 nt from the original 3' end, probably requires the direct participation of the polymerase active site; such cleavage can take place without the addition of SII. We have also asked whether transcriptional arrest occurs in response to other signals in addition to specific sequences in the template. Several groups had shown that when RNA polymerase II runs to the end of a linear template, some of the "run-off" transcripts are truncated upon incubation with SII (3,6). Using high resolution gels, we find that those polymerases which transcribe completely to the end of the template strand do not respond to SII. However, many polymerases pause about 5-6 bases prior to the end of the template; these polymerases are arrested, since they release 12-17 nt RNAs in response to SII. Thus, transcriptional arrest can be induced not only by particular sequence signals but possibly also by the loss of template contacts.

(1) Reines (1992), JBC **267**, 3795; **(2)** Izban and Luse (1992), Genes Dev. **6**, 1342; **(3)** Wang and Hawley (1993), PNAS **90**, 843; **(4)** Izban and Luse (1993), JBC **268**, 12864; **(5)** Izban and Luse (1993), JBC **268**, 12874; **(6)** Reines et al. (1992), JBC **267**, 15516.

L 025 **INCHING TOWARD A MECHANISM OF RNA POLYMERASE II ELONGATION,** David H. Price, Hongliang Guo, Daniel D. Kephart, David R. Chafin, Department of Biochemistry, University of Iowa, Iowa City IA, 52242.

In order to understand the action of factors which control the elongation phase of transcription by RNA polymerase II it is useful to first come to an understanding of the basic mechanism used by the enzyme to incorporate nucleotides into RNA chains. The traditional model of monotonic elongation by RNA polymerase II can not explain recent results obtained in several laboratories. We have examined the activity of RNA polymerase II under a variety of conditions using a defined transcription system comprised of *Drosophila* RNA polymerase II and a dC-tailed template. Both elongation and pyrophosphorolysis have been studied using DmS-II, individual subunits of TFIIF, ammonium and manganese ions, and α-amanitin as tools. We have found that there are kinetic barriers during pyrophosphorolysis that occur on average every two nucleotides. We hypothesize that these barriers occur because of the necessity of the elongation complex to undergo slow conformational changes. α-amanitin blocks this conformation change, but does not stop the limited addition or removal of nucleotides, suggesting that the active site is not directly inhibited, but rather that polymerase movement is inhibited. Results with DmS-II and TFIIF have indicated that there may be two conformational states of the polymerase, paused and elongation competent. Both DmS-II and TFIIF act on the paused state; DmS-II causing transcript cleavage and TFIIF facilitating the conversion of the paused state into the elongation competent state. Consistent with this we have found that TFIIF inhibits DmS-II mediated transcript cleavage. Surprisingly we have found that factor 5 (*Drosophila* TFIIF) or RAP30 can mediate low levels of transcript cleavage. Although transcript cleavage mediated by TFIIF is too slow to explain the ability of the factor to stimulate elongation, it does provide evidence for the mechanism of action of the factor. The kinetic barriers seen during pyrophosphorolysis and during extended cleavage mediated by DmS-II are similar suggesting that both processes share at least one step, but other evidence suggests that the processes do not follow the same pathway. Our results also strongly suggest that the active site of the polymerase and the cleavage site stimulated by DmS-II are not the same. A tentative model has been made that includes a slow discontinuous movement on average every two nucleotides during elongation and a paused to elongation competent conformational change that can occur in a sequence specific manner.

L 026 MECHANISM OF SII-DEPENDENT RNA SYNTHESIS, Danny Reines, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.

RNA polymerase II (Pol II) transcribes genes that can be as long as, or longer than, 10^6 base pairs. The enzyme also encounters obstacles to transcript elongation in the form of naturally-occurring arrest signals and DNA bound ligands. Stable arrested elongation complexes have been identified in vivo and in vitro. The molecular events involved in reading through these obstacles have been investigated in vitro by many laboratories. SII, a Pol II-binding protein with cryptic nucleic acid-binding activity, can stimulate Pol II to read through transcriptional impediments. SII exerts its function by activating a nascent RNA cleavage activity in arrested elongation complexes. RNA cleavage is an obligatory intermediate in the readthrough process. We have shown that SII functions in a general capacity to enable readthrough of at least 3 types of blockages to elongation: 1) a natural arrest site in a human gene coincident with a bend in the DNA helix, 2) a DNA major groove-binding protein, and 3) a DNA minor groove-binding drug. In all cases nascent RNA cleavage is observed. SII appears to target arrested Pol II and not the blockage per se. Accordingly, SII does not prevent the blockage but enhances the number of Pol II molecules that are able to bypass a blockage by enabling multiple attempts at transcript elongation via reiterative RNA cleavage and re-extension. Using an inhibitor of SII, sarkosyl, we provide evidence that multiple rounds of RNA extension and cleavage are required for readthrough by some enzyme molecules. Cleavage does not alter the probability of subsequent arrest but provides multiple attempts at elongation through a region of template that has a finite probability of stopping Pol II. In the case of a 50% blockage efficiency, six iterations of cleavage and elongation would, in theory, enable 99% of the enzyme molecules to read through this site. The Pol II elongation complex does not translocate upstream (backwards) after the nascent RNA is shortened by 7-9 nucleotides, the maximum distance required for the enzyme to escape from at least one arrest site. More extensive shortening of the RNA chain results in upstream movement of the complex. This is similar to the discontinuous movement observed for E. coli transcription complexes during RNA chain elongation.

We have also explored the effect of the DNA-binding drug distamycin on elongation by Pol II. We find that the structure of a template containing our arrest site is altered in response to distamycin. Distamycin can both block, as well as facilitate, elongation by Pol II. In the presence of drug, a natural pause site that impedes passage of Pol II can apparently attain a more readthrough-permissive form. We take this as independent evidence that template configuration is an important variable for elongation by Pol II. Interestingly, recent structural studies on *E. coli* RNA polymerase and HIV reverse transcriptase show that nucleic acid templates buried within these enzymes attain distorted configurations. A requirement for these conformations during elongation of nascent chains might explain why intrinsically bent DNA or drugs that alter DNA conformation influence elongation.

Chromatin and Regulation of Transcription

L 027 PLACEMENT OF THE DROSOPHILA MELANOGASTER EUCHROMATIC *HSP26* GENE INTO AN HP-1 HETEROCHROMATIC ENVIRONMENT, Lori L. Wallrath, Carolyn A. Craig, Lisa Rosman and Sarah C.R. Elgin, Biology Department, Washington University, St. Louis, Missouri.

When a euchromatic gene is placed in the vicinity of heterochromatin the gene frequently exhibits poistion effect variegation (PEV). PEV is the result of expression of a gene in some somatic cells and repression of that same gene in other cells. We wish to understand the mechanism by which genes packaged in heterochromatin are transcriptionally repressed. A P-element construct containing the *hsp26* gene as test gene and an *hsp70-white* gene as a reporter gene was transformed into *white* deficient *Drosophila melanogaster* embryos. Transformed lines with a single copy of the P-element inserted into euchromatin were obtained and are being used in a P-element mobilization scheme to recover inserts into heterochromatin by screening for PEV of *white* expression. Insertions into various heterochromatic regions of the genome (near centromeres, telomeres and along the fourth chromosome) were obtained. These are the same genomic regions that are associated with the highly conserved heterochromatin protein 1 (HP-1). It has previously been shown that mutations in the gene encoding HP-1 result in suppression of PEV. The affect of these mutations on the expression of the heterochromatic transgenes is being investigated. Heat shock induced expression and chromatin structure of the heterochromatic *hsp26* transgenes are being compared to that of a euchromatic transgene. The variegating transgenes are valuable for investigation of how higher order chromatin packaging influences gene expression and the role of chromosomal proteins such as HP-1.

L 028 FACTORS WHICH INTERACT WITH HISTONES TO REGULATE TRANSCRIPTION IN YEAST, Jeff Thompson, Andreas Hecht, Xiao-Jun Ma, Andrew Carmen, Steve Rundlett and Michael Grunstein. Molecular Biology Institute and the Department of Biology, University of California, Los Angeles, 90024.

One domain (residues 4-23) at the histone H4 N terminus is involved in the activation of regulated yeast genes. Another domain (residues 16-29) is responsible for repression of heterochromatin-like regions in yeast (telomeres, silent mating loci). Histone H3 also contains regions responsible for both the activation and repression of the GAL1 gene and for the repression of heterochromatin. One explanation we have pursued which may explain how histones have such varied functions involves the interaction of different regulatory factors with the histone N termini. We will present data showing the manner in which Sir3, a repressor of the silent mating loci and a suppressor of histone H4 mutations derepressing these regions, interacts in vitro with histone H4. In addition we will describe the results of both genetic and biochemical analyses for the interaction of other factors with the histone N termini.

L 029 ROLE OF CHROMATIN STRUCTURE IN THE REGULATION OF TRANSCRIPTION BY RNA POLYMERASE II, Rohinton T. Kamakaka, Michael J. Pazin, Elizabeth M. Blackwood, Michael Bulger, Leslie A. Kerrigan, Suman M. Paranjape, Catherine P. George, Thomas Burke, and James T. Kadonaga, Department of Biology, University of California, San Diego, La Jolla, CA 92093-0347.

In eukaryotes, synthesis of mRNA involves the basal RNA polymerase II transcriptional machinery, sequence-specific DNA binding factors that interact with cis-acting regulatory elements, and the template DNA, which is packaged into chromatin. We have been carrying out a biochemical analysis of the role of chromatin structure and DNA replication in the regulation of transcription by RNA polymerase II. The first portion of the talk will cover our recent studies of basal RNA polymerase II transcription in *Drosophila*. We have found, for instance, that accurate initiation of transcription from a subset of promoters can be achieved with a minimal set of purified factors that consist of RNA polymerase II, TBP (TATA box-binding protein), TFIIB, and the small subunit of TFIIF (RAP30). In addition, we have been investigating functional differences in the mechanism of transcription from different promoters. The remainder of the talk will describe the transcriptional properties of DNA templates that have been assembled into chromatin. The initial studies have led to the hypothesis that sequence-specific transcription factors can function both to counteract chromatin-mediated repression ("antirepression") and to facilitate the intrinsic transcription reaction ("true activation"). Transcription reactions performed with chromatin templates more accurately recreated in vitro effects that are observed in vivo. For example, the magnitude of transcriptional activation by either Gal4-VP16 or Sp1 that is observed in vivo was achieved in vitro with histone H1-containing chromatin templates, but not with naked DNA templates. In addition, long-distance (>1000bp) activation of transcription by Gal4-VP16 was observed in vitro with chromatin, but not naked DNA templates. We also employed a *Drosophila* S-190 chromatin assembly extract to replicate single-stranded DNA into double-stranded DNA and to assemble the newly synthesized DNA into chromatin in a coupled replication/assembly process. This reaction enabled us to examine the ability of Gal4-VP16 to potentiate the chromatin template either during or after DNA replication and chromatin assembly. The results of these studies suggest that DNA replication can function to disrupt chromatin structure and allow subsequent access of sequence-specific transcription factors to mediate gene activation.

L 030 HISTONE MODULATED TRANSCRIPTION: STRUCTURAL AND DEVELOPMENTAL ASPECTS, Alan P. Wolffe, Geneviève Almouzni, Philippe Bouvet, Stefan Dimitrov, Jeffrey J. Hayes, Nicoletta Landsberger, Dmitry Pruss, and Kiyoe Ura, Laboratory of Molecular Embryology, National Institute of Child Health and Human Development, NIH, Bethesda, MD. 20892.

A chromatin environment provides many advantages to the eukaryotic transcriptional machinery in regulating gene expression. Nucleosome formation can alternately either repress (1) or potentiate transcription (2). The three dimensional folding of DNA in chromatin has an important structural and regulatory role. We have characterized the influence of individual histones and their domains on nucleosome structure, nucleosome positioning and the capacity of nucleosomes to repress transcription (3, 4, 5). These in vitro experiments have been extended to chromatin function in vivo. Replication coupled chromatin assembly is required for the general repression of basal transcription in Xenopus oocyte nuclei (6). In spite of complete chromatin assembly, certain transcription factors (eg GAL4/VP16, HSF) activate transcription from the initially repressed state. These transcription factors have two functions: the relief of repression (chromatin disruption) and the activation of the transcription process (recruitment of the basal transcription machinery). We have examined the relative importance of these two functions during vertebrate development as the structural components of chromosomes are modified. These modifications include the replacement of linker histones eg B4, H1, H1° and alterations in histone acetylation (7). Some of the consequences of removal or overexpression of the linker histones for gene expression in the developing embryo have been determined.

1. Wolffe, A.P. and Dimitrov, S. (1993). Crit. Rev. Euk. Gene Exp. 3 167-191
2. Schild, C., Claret, F.X., Wahli, W. and Wolffe, A.P. (1993). EMBO J. 12 423-433
3. Lee, D.Y., Hayes, J.J., Pruss, D. and Wolffe, A.P. (1993). Cell 72 73-84
4. Hayes, J.J. and Wolffe, A.P. (1993). Proc. Natl. Acad. Sci. USA 90 6415-6419
5. Pruss, D. and Wolffe, A.P. (1993). Biochemistry 32 6810-6814
6. Almouzni, G. and Wolffe, A.P. (1993). Genes and Development (in press)
7. Dimitrov, S., Almouzni, G., Dasso, M. and Wolffe, A.P. (1993). Develop. Biol. (in press)

L 031 BINDING AND DISPLACEMENT OF NUCLEOSOMES BY REGULATORY TRANSCRIPTION FACTORS,

Baiyong Li, Hong Chen, Phillip Walter, Michelle Vettese-Dadey, Li -Jung Juan, Christopher C. Adams, Jacques Cote and Jerry L. Workman. Department of Molecular and Cell Biology and Center for Gene Regulation, The Pennsylvania State University, University Park, PA 16802.

To overcome repression of transcription from chromatin structures, regulatory and general transcription factors must replace nucleosomes which occupy crucial transcriptional control elements. To investigate mechanisms by which factors bind and displace nucleosomes, we have analyzed the binding of regulatory factors and histone displacement from nucleosome bearing factor binding sites. Inhibition of factor binding (i.e. GAL4 derivatives, Sp1, USF) to nucleosomal DNA is largely mediated through the core histone amino termini. The extent of inhibition from the amino termini was dependent on positions of binding sites within nucleosome cores. Thus, the amino termini are partly responsible for nucleosome translational position effects on factor binding. Inhibition of factor binding was extended to the outside of the nucleosome particle through the binding of histone H1 which inhibited the binding of some factors (i.e. USF) to a greater extent than others (i.e. GAL4). Inhibition of factor binding by the core histone amino termini was overcome through the cooperative, "facilitated", binding of multiple transcription factors to nucleosome cores (for example 5 GAL4-AH dimers). Facilitated binding of factors to nucleosomes also occurs between different factors. For example, the binding of a single GAL4-AH dimer to a nucleosome core increased the affinity of USF for an adjacent site by over two orders of magnitude. Thus, facilitated binding of factors to nucleosome cores appears to represent a general mechanism by which regulatory factors can enhance the affinity of adjacent factors to bring about complete occupancy, in chromatin, of regulatory regions with multiple factors.

Binding of transcription factors and nucleosome displacement can be stimulated by histone-binding proteins. We have found that the nucleosome assembly factor, nucleoplasmin, stimulates the binding of GAL4-AH, Sp1 and USF to nucleosome cores. Nucleoplasmin activity was not mimicked by the non-specific histone sinks, polyglutamate or RNA. Nucleoplasmin stimulated binding of multiple GAL4 derivatives was accompanied by depletion of the resulting GAL4/nucleosome complex of histones H2A/H2B. Thus, factor binding resulted in the initiation of nucleosome disassembly by nucleoplasmin.

DNA Topology and Gene Expression

L 032 ELECTRON MICROSCOPIC VISUALIZATION OF ACTIVE TRANSCRIPTION COMPLEXES, Jack D. Griffith[1], Yuh Hwa Wang[1], Tom Kerppola[2], Tom Curran[2], Lee Zawel[3], and Danny Reinberg[3]. [1]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, 27599-7295, [2]Roche Institute, [3]Rutgers University.

High resolution electron microscopy has been used to examine the structure of a series of complexes formed in vitro between DNA and specific transcriptional regulators and between the basal transcription factors isolated from yeast and human sources and classic TATA promoter elements.

The binding of full length or truncated variants of jun and fos to AP-1 sites on DNA has been examined in detail previously by gel electrophoretic methods where it was determined that a complex of fos and jun heterodimers interacts with the DNA to bend the DNA by approximately 94 degrees. Here we have used a direct EM approach to visualize a complex of full length fos and a truncated form of jun to an AP-1 site centered on a 350 bp DNA fragment. Preparation of the DNA for EM revealed protein complexes of at least 60 KDa bound to the AP-1 site. Binding was found to bend the DNA significantly; 41% of the complexes have a bending angle greater than 60 degrees, with an average angle of about 89 degrees. The very good agreement between EM and electrophoretic methods will aid in establishing the validity of both methods of measuring bending angles.

Using the same EM methods, a 390 bp DNA fragment containing the adenovirus major late promoter at its center was complexed with the yeast tbp protein. Complexes were visualized by EM whose sizes were consistent with either a monomer or dimer of tbp bound to the TATA element. At protein saturation, dimer binding predominated and the DNA was bent about the protein by approximately 90 degrees.

The binding of purified human basal transcription factors to the adenovirus major late promoter and to the HIV 5' LTR have been investigated by step-wise assembling the factors onto DNA in the presence or absence of ATP and preparing them for EM. Upon incubation of DNA with TFIIA+TFIID, an elongated complex bound to the TATA element and sitting off-center was observed. These complexes were similar in size and shape to complexes formed when TFIIB was also included. The DNA in these complexes did not appear to be bent by any consistent amount. When RNA polymerase II was included in the incubations, a much larger complex was observed over the TATA box. The most frequent appearance of this complex was that of a smaller protein complex bound directly to the DNA, and a larger spherical protein complex attached to the smaller one. We presume the larger complex to consist of RNA polymerase II. Inclusion of the additional factors required to form a fully competent transcription complex also yielded large complexes of this general appearance. Upon addition of nucleoside ribotriphosphates, movement of the RNA polymerase II from the complex could be detected by EM and frequently a complex consistent with its being TFIID+TFIIA was seen to remain over the TATA box. Finally, a frequent occurrence that was observed was a looping of the DNA induced by the TFIID+TFIIA+TFIIA and larger complexes --- in which one end of the loop was at the TATA box and the other end of the loop was either upstream or downstream.

L 033 TOPOLOGICAL COUPLING OF PROMOTERS THROUGH LOCAL DNA SUPERCOILING. David M.J. Lilley, Dongrong Chen and Richard Bowater Department of Biochemistry, The University, Dundee DD1 4HN, U.K.

leu-500 is a mutation in the -10 region of the *Salmonella typhimurium leu* operon that inactivates the promoter. However, the mutant promoter becomes active in a strain that has mutations in *topA*, the structural gene for DNA topoisomerase I. We have shown that the *leu-500* promoter can function on a plasmid when it is adjacent to the tetracycline resistance gene, *tetA*. The activation of the *leu-500* promoter requires that the *tetA* gene is transcribed, translated and exported, and that the host cell is *topA*. We propose that the A to G mutation in the -10 region of the *leu-500* promoter is compensated by local negative supercoiling arising from transcription of the *tetA* gene, which may reach elevated levels in a *topA* background, provided that diffusional dissipation is reduced due to anchoring of the TetA peptide in the membrane. This is a clear example of the modulation of the activity of a promoter by the activity of another promoter in *cis*, when they can be coupled through the topology of the template.

- The genetic control of DNA supercoiling in *Salmonella typhimurium*. S M H Richardson, C F Higgins & D M J Lilley *EMBO J.* **3**, 1745-1752 (1984).
- DNA supercoiling and the *leu-500* mutation of *Salmonella typhimurium*. S M H Richardson, C F Higgins & D M J Lilley *EMBO J.* **7**, 1863-1869 (1988).
- Superhelical torsion in cellular DNA responds directly to environmental and genetic factors. J A McClellan, P, Boublikova, E Palecek & D M J Lilley *Proc Natl Acad Sci USA* **87**, 8373-8377 (1990).
- Local DNA topology and gene expression: the case of the *leu-500* promoter. D M J Lilley & C F Higgins *Molec. Microbiol.* **5**, 779-783 (1991).
- Activity of a plasmid-borne *leu-500* promoter depends on the transcription and translation of an adjacent gene. D Chen, R Bowater, C J Dorman & D M J Lilley *Proc Natl Acad Sci USA* **89**, 8784-8788 (1992).
- Activation of the *leu-500* promoter: a topological domain generated by divergent transcription in a plasmid. D Chen, R Bowater & D M J Lilley *Biochemistry* In the press.

L 034 DNA CONFORMATIONAL DYNAMICS DURING RNA TRANSCRIPTION, Hai-young Wu[1], Marc Drolet[2], Yuri Panchenko[3] and Leroy F. Liu[3]; [1]Department of Pharmacology, Wayne State University, Detroit, MI 48201; [2]Department of Microbiology, University of Montreal, Canada; [3]Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Our recent studies have demonstrated that local DNA conformation can undergo significant variation during transcription elongation. Transcription elongation from a single T7 promoter is shown to affect DNA conformation both in *vivo* and *in vitro*. A plasmid which contains a single T7 promoter, was transcribed by T7 RNA polymerase *in vivo* in the presence of rifampicin. Under these conditions, inactivation of *E. coli* DNA gyrase resulted in rapid positive supercoiling of this plasmid DNA but not a compatible plasmid without the T7 promoter. *In vitro* transcription of a linear DNA template containing a single T7 promoter has also been studied in the presence of Bal31 nuclease. In the presence of all four ribonucleoside triphosphates, Bal 31 cleaves the linear DNA template at a major site within the transcribed region. This Bal 31-sensitive site is transiently generated during active transcription and is greatly enhanced in the presence of *E. coli* DNA topoisomerase I. In addition, Bal 31 is shown to cleave at the same site on a positively supercoiled, but not negatively supercoiled or relaxed, DNA template in the absence of transcription. 2D gel analysis has revealed that only highly positively supercoiled DNA topoisomers are Bal 31-sensitive. These results suggest that transcription elongation can generate a high degree of local positive supercoiling. Similar studies have also demonstrated R-loop formation on linear DNA template during transcription elongation. Based on these and other studies, we propose that due to the asynchronous movement of the RNA polymerase elongation complex, DNA conformation can undergo large fluctuation within the transcribed region in a sequence-specific manner. The large conformation fluctuation within the transcribed region is expected to have a major effect on DNA structure and function.

L 035 TARGETING OF SIR1 PROTEIN ESTABLISHES TRANSCRIPTIONAL SILENCING AT *HM* LOCI AND TELOMERES IN YEAST, Rolf Sternglanz[1], Cheng-ting Chien[1], Stephen Buck[2], and David Shore[2], [1]Department of Biochemistry and Cell Biology, SUNY, Stony Brook, NY 11794, [2]Department of Microbiology, College of Physicians & Surgeons of Columbia University, New York, NY 10032.

Previous studies suggest that the yeast SIR1 protein is involved in the establishment of transcriptional silencing at the *HM* mating-type loci. First, *sir1* mutations destabilize repression at the *HM* silent loci by greatly reducing the frequency of re-establishment of the repressed state. Second, overexpression of SIR1 can suppress many different mutants that are partially defective in silencing. We have now shown that a GAL4 DNA binding domain-SIR1 hybrid protein (G_{BD}-SIR1), when targeted to an *HMR* locus containing GAL4 binding sites (UAS_G), can establish silencing and bypass the requirement for the silencer element *HMR-E*. Silencing mediated by G_{BD}-SIR1 requires the trans-acting factors that normally participate in repression, namely SIR2, SIR3, SIR4 and histone H4. However, G_{BD} hybrids with SIR2, SIR3 , or SIR4 cannot establish silencing. Telomeric silencing, which does not require SIR1 and is normally unstable, is greatly improved by tethering G_{BD}-SIR1 to the telomere. These experimnets support a model in which native SIR1 protein is brought to the *HM* loci by proteins bound to the silencers where it acts to assure the efficient establishment of the silenced state. Telomeres appear to lack the ability to recruit SIR1 and that is why telomeric silencing is unstable. Normally the establishment of silencing requires passage through S-phase; we are investigating whether silencing mediated by targeted G_{BD}-SIR1 also requires DNA replication.

Structural Studies of Transcription Factors

L 036 X-RAY CRYSTALLOGRAPHIC STUDIES OF EUKARYOTIC TRANSCRIPTION FACTORS, **Stephen K. Burley,** Laboratories of Molecular Biophysics and Howard Hughes Medical Institute, The Rockefeller University, New York, New York 10021, USA.

My laboratory is working on the development of a detailed understanding of the physical principles governing molecular recognition in biological systems. We are examining model systems derived from the transcriptional control machinery responsible for regulating eukaryotic gene expression. Our approach is to use X-ray crystallography and other biophysical methods to determine and characterize the three-dimensional structures of biological macromolecules and their complexes with DNA or other ligands.

Our studies of protein-DNA recognition traverse the entire length of a typical eukaryotic gene promoter, including basal class II transcription factors, promoter proximal binding factors and distal enhancer binding factors. During the past year, we reported the crystal structures of TFIID TATA box-binding protein, Max homodimer complexed with the E-box sequence CACGTG, upstream stimulatory factor complexed with the same E-box, and hepatocyte nuclear factor-3γ complexed with its DNA binding site. Most recently, we solved the structure of the TATA box-binding protein complexed with the TATA element of the Adenovirus major late promoter.

L 037 THE ROLE OF DNA STRUCTURE AND HYDRATION IN TRANSCRIPTIONAL CONTROL, Zippora Shakked, Department of Structural Biology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Sequence–specific interactions between regulatory proteins and the base pairs of their DNA targets observed in high–resolution crystal structures are not sufficient to account for all the genetic and biochemical data. It has been therefore proposed that in addition to direct interactions, indirect structural effects and water-mediated interactions may be equally important in contributing to specific recognition. We have been investigating the crystal structures of various DNA targets in both their free and bound states in order to elucidate to what extent the intrinsic structure of DNA has a role in protein recognition and to what extent this structure is modulated by the protein. Our preliminary results indicate that the intrinsic structure and the inherent flexibility of certain regions of the DNA (e.g., bending at low energetic cost) as well as DNA hydration contribute to specificity in transcriptional control.

Basal Transcription Factors

L 100 MODIFICATIONS OF RNA POLYMERASE II PHOSPHORYLATION IN MAMMALIAN CELLS EXPOSED TO INHIBITORS OF TRANSCRIPTION, PHOSPHORYLATION AND DEPHOSPHORYLATION, Sylvain Bellier, Marie-Françoise Dubois, Van Trung Nguyen and Olivier Bensaude, Laboratoire de Génétique Moléculaire, Ecole Normale Supérieure, Paris, FRANCE.

Phosphorylation of the carboxy-terminal domain (CTD) of RNA polymerase II largest subunit is thought to play a role in transcription. Various CTD-kinases have been characterized, some are inhibited by 5,6-dichlorobenzimidazole riboside (DRB), others by the isoquinoline sulfonamides H-8 and H-7. We have found that DRB, H-8 and H-7 inhibited CTD phosphorylation in intact cells. Conversely, the phosphatase inhibitor, okadaic acid inhibited CTD dephosphorylation. DRB is a well-known transcription inhibitor. In this report, the effect of okadaic acid, H-7 and H-8 on transcription was established in intact cells.

The transcription inhibitor, actinomycin D, also affected the phosphorylation state of the CTD. From the actinomycin D concentration dependence of this effect, and the use of actinomycin D/DRB, actinomycin D/okadaic acid combinations, it is suggested that actinomycin D inhibit both the in vivo phosphorylation and dephosphorylation processes.

L 101 TRANSCRIPTION OF THE MINI-EXON GENES IN TRYPANOSOMATIDS; GENETIC AND BIOCHEMICAL ANALYSIS, Vivian Bellofatto and David Hartree, Department of Microbiology and Molecular Genetics, UMDNJ, Newark, NJ 07103

Members of the trypanosomatidae, a family of parasitic, unicellular organisms, produce mRNAs that are bipartite; all transcripts are the result of a trans-splicing reaction in which a short exon, called the mini-exon or spliced leader RNA, is joined to a pre-mRNA. We have begun to define the the molecular mechanisms responsible for mini-exon gene transcription. Using sequence substitution mutagenesis of tagged mini-exon genes and DNA-mediated transformation of the trypanosomatid *Leptomonas seymouri*, we have uncovered specific sequences essential for mini-exon gene expression. DNA foot printing experiments have identified specific DNA-protein interactions that occur near the transcription initiation site. Data concerning the partial purification of mini-exon-specific DNA binding proteins will be presented.

L 102 A CONSENSUS TATA BOX IS SUFFICIENT FOR TRANSCRIPTION BY RNAP IIB, Andrew B. Buermeyer, Lee A. Strasheim and Peggy J. Farnham, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

To understand the role of the conserved C-terminal domain (CTD) of RNAP II during transcription, we have studied transcription *in vitro* from the murine dihydrofolate reductase (*dhfr*) gene promoter, which requires RNAP II containing the CTD for activity, and the *rep-3b* gene promoter, which is active with RNAP II lacking the CTD (RNAP IIB) (1). Since the *rep-3b* gene promoter does not contain a consensus TATA box, its presence is not necessary for transcription by RNAP IIB. However, several groups have demonstrated a functional interaction between the CTD and TFIID. To test if CTD-dependence correlates with the strength of the TATA box, we determined whether mutations in the -30 region of the *dhfr* and *rep-3b* gene promoters affect activity with RNAP IIB. A mutant *dhfr* gene promoter containing a consensus TATA box was transcribed by RNAP IIB. Mutating a weak TATA box in the *rep-3b* gene promoter (TTAA to TTGC) resulted in reduced, CTD-dependent transcription. These data suggest that the presence of a consensus TATA box is sufficient for activity with RNAP IIB. We have confirmed this by demonstrating that synthetic constructs without a TATA box were not transcribed by RNAP IIB, whereas, contrary to our previously published findings (1), the TATA box-containing H2b gene promoter was transcribed by RNAP IIB. Further mutation of the *rep-3b* gene promoter identified an activating element in the start site region necessary for activity with RNAP IIB, suggesting that in promoters with a weak TATA box, certain activating elements can contribute to the ability to be transcribed by RNAP IIB. These data suggest that the CTD either may stabilize the preinitiation complex or contribute in recruitement of RNAP II or TFIID into the complex.

1. Buermeyer, A. B., et al. (1992) Mol. Cell. Biol. **12**:2250-2259.

L 103 TRANSCRIPTION FROM THE BACULOVIRUS POLYHEDRIN PROMOTER INVOLVES AN UNUSUAL DNA-BINDING PROTEIN, Sandeep Burma, Bipasha Mukherjee and Seyed E.Hasnain, Eukaryotic Gene Expression Laboratory, National Institute of Immunology, New Delhi 110067, INDIA

An unusual 30 kDa protein from insect (Spodoptera frugiperda) cells binding to the baculovirus polyhedrin gene promoter has been identified by gel retardation assay, affinity purified and characterised (Burma et al., J. Biol. Chem., 1993, In Press). This Polyhedrin Promoter Binding Protein (PPBP) appears to be an unusual DNA-binding protein with respect to its stability (binding was obtained at NaCl concentrations and temperatures ranging from 0.2 - 2 M and 0°C - 65°C, respectively), high binding affinity (binding even in the absence of non-specific DNA) with an apparent dissociation constant of ~$3.7X10^{-12}$ M and high specificity (binding was unaffected in the presence of a fifty thousand times excess of non-specific DNA). The size and recognition sequence of PPBP is comparable with that of TFIID. The 30 kDa mass of PPBP (as determined by UV-crosslinking and Southwestern analyses) is in the same range as that for TFIID. While TFIID binds to the TATA box sequence TATAAAA; PPBP needs related sequences - AATAAA and TAAGTATT, the latter is present at the transcription start point and is known to be essential for promoter activity. Thus, the apparent similarity of PPBP with TFIID suggests that PPBP might be a basal transcription unit which in association with virus specific factor(s) may regulate transcription from the polyhedrin promoter. The fact that phosphorylation is required for DNA-binding activity of PPBP further indicates that it might have a regulatory function.

L 104 TRANSCRIPTION FACTORS THAT BIND TO YEAST RNA POLYMERASE II, [1]Zachary F. Burton, [1]Wladyslaw Werel, [1]Richard C. Fentzke, [2]Paul A. Wade, [3]Nancy E. Thompson, [3]Richard R. Burgess, [2]Judith A. Jaehning, [1]Department of Biochemistry and Agricultural Experiment Station, Michigan State University, E. Lansing, MI 48824, [2]Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, CO 80262, [3]Biotechnology Center and McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706.

The largest subunit of eukaryotic RNA polymerase II has the consensus sequence YSPTSPS repeated multiple times at its carboxy-terminus. In the yeast *Saccharomyces cerevisiae* this sequence is repeated 26 times. This sequence is referred to as the carboxy-terminal domain or CTD. Using monoclonal antibodies that recognize the CTD that were immobilized on a Sepharose column, RNA polymerase II was isolated from an *S. cerevisiae* extract. Proteins bound to RNA polymerase II (RNA polymerase II-associating proteins [RAPs]) were dissociated from immobilized RNA polymerase II by elution with salt. As a control column, monoclonal antibodies of the same isotype directed against *E. coli* σ^{70} were immobilized on Sepharose. Comparison of the salt eluate of the anti-CTD column and the anti-σ^{70} column revealed a number of proteins that bound specifically to the anti-CTD column. Among these RAPs were identified yeast TFIIB and TFIIS (S-II), transcription factors that are known to bind to RNA polymerase II. RNA polymerase II and some additional RAPs were eluted from the anti-CTD column with 30 % ethylene glycol. As expected, no RNA polymerase II could be eluted from the anti-σ^{70} column with ethylene glycol. The quantities of RAPs obtained by this method are suitable for protein microsequencing methods. This approach should lead to the identification and isolation of novel transcription factors from yeast.

L 105 Interactions of yeast RNA polymerases with synthetic DNA templates, Christophe Carles, André Sentenac and Michel Riva, Service de Biochimie et de Génétique Moléculaire, DBCM, Centre d'Etudes de Saclay, F 91191 Gif sur Yvette cedex, France.

Characterization of the structure of the active site of RNA polymerases is important to understand the mechanisms of the transcription process. We have previously determined that the initiator nucleotide binding site is located in the second largest subunit of the three forms of yeast RNA polymerases. For the B150 subunit, this site has been mapped in conserved domains. In order to complete this study, we are investigating the interactions between DNA and RNA polymerases. We report the use of a variety of synthetic DNA probes in helping to determine which polymerase subunits contact the DNA template. Templates of the same length have been designed which contain a single stretch of BrdU at different positions from the stop site. The polymerase is incubated with a labeled DNA template in a non specific transcription assay in presence of three NTP. In these conditions, the enzyme is stalled at position +23. The arrested complexes are then irradiated at 256nm and submitted to SDS-PAGE.

In the case of the RNA polymerase II, the 150 and the 220 kDa subunits are photoaffinity labeled whatever the DNA labeled strand is transcribed or non transcribed. Evidences are shown which demonstrate that the CTD is not involved in the interaction of the B220 subunit with the DNA template. For both subunits, the interaction with the DNA template is related to the transcriptional activity of the enzyme.

Interactions of RNA polymerase I and III with DNA are currently investigated. Mapping of the labeled domains in the subunits sequence are under progress.

L 106 CTD PHOSPHATASE: A NOVEL PHOSPHATASE THAT DEPHOSPHORYLATES THE C-TERMINAL DOMAIN OF RNA POLYMERASE SUBUNIT IIo. R.S. Chambers & M.E. Dahmus. Section of Molecular & Cellular Biology, University of California, Davis, CA 95616.

The C-terminal domain (CTD) of the largest RNA polymerase II subunit contains multiple repeats of the consensus sequence YSPTSPS which can be extensively phosphorylated on serine, threonine and tyrosine. Phosphorylation of the CTD occurs during the transition from complex assembly to transcript elongation and may be involved in the release of RNA polymerase II from the pre-initiation complex. Presumably the phosphorylated form of RNA polymerase II is released from the template and must be dephosphorylated before it can enter a new pre-initiation complex. Consequently CTD phosphatase plays a critical role in the transcription cycle of RNA polymerase II.

A CTD phosphatase has been purified from a HeLa whole cell extract by Heparin sepharose, DE52, Mono S, Mono Q, phenyl Superose and Superose 12 chromatography. The phosphatase appears specific in that it removes phosphate from the CTD incorporated by CTD kinase but not by casein kinase II which phosphorylates a single serine five residues from the C-terminus. The phosphatase requires Mg ions for activity and appears to be insensitive to the phosphatase inhibitor okadaic acid. The latter two properties would suggest it is similar to the PP2C type phosphatases. The enzyme has a native molecular weight on gel filtration chromatography of 310 kDa but appears to be composed of a single subunit of 205 kDa by SDS PAGE. Several Lys-C peptides have been sequenced and experiments are in progress to clone the mammalian gene encoding the 205 kDa subunit.

L 107 CHARACTERIZATION OF THE TRANSCRIPTIONAL INITIATION SITES OF THE BRAIN-TYPE AND THE PERIPHERAL-TYPE HUMAN CANNABINOID RECEPTOR GENES, Gautam Chaudhuri[1] and Emmanuel S. Onaivi[2], [1]Division of Biomedical Sciences, and [2]Department of Pharmacology, Meharry Medical College, 1005 D. B. Todd, Jr. Boulevard, Nashville, TN 37208

The cannabinoid receptors are members of the G-protein linked superfamily of receptors. Computer selected primer pairs from their cDNA coding region sequences showed identical amplified DNA band sizes in both DNA-PCR and reverse-PCR, with human, rat and mouse templates, indicating intronlessness of the gene, in its coding region. There appears to be no difference in the size of the transcript in rat brain and testis or in the strains of mice tested. Thus, this receptor gene seems to be highly conserved and conform with the general intronless feature of most other G-protein associated receptor genes. Using antisense primers complimentary to the 5-end of the coding regions of the genes, we are doing primer extension analysis to find out the transcription initiation sites for these genes. The 5'-untranslated regions of these genes appear to have introns. We are characterizing these introns and the transcription initiation sites beyond these introns in the genomic clones containing the 5'-ends of these genes to understand the transcriptional initiation of these genes and also to locate promoters for these genes. Supported by NSF Research Center for Excellence in Cell and Molecular Biology Grant # HRD-9255157 and RCMI Grant #NIH 5 G12RR030208.

L 108 CHARACTERIZATION OF THE STRUCTURE OF HUMAN TFIIA, Jeff DeJong and Robert G. Roeder, The Rockefeller University, New York, NY

Human transcription factor IIA facilitates transcription initiation in vitro by interacting with the TATA-binding subunit of TFIID (TBP), and possibly by modulating activator-dependent enhancement of basal transcription. To understand mechanistically how TFIIA mediates these effects, it is necessary to understand the structure of the human factor, and how it may differ from other species. As purified from HeLa cell extracts, human TFIIA consists of three subunits of 35, 19, and 12kD. Using peptide-derived oligonucleotides a cDNA (hTFIIA/α) was isolated which encodes a 376 amino acid recombinant protein (p55) with homology to the yeast TFIIA gene TOA1. Surprisingly, peptides from both the 35 and 19kD subunits of TFIIA were located within the N- and C- terminal regions of hTFIIA/α, respectively. In addition, recombinant p55 could substitute for the two large subunits in a TBP-dependent bandshift assay using renatured natural p12. Thus, the p55 protein appears to be rapidly co- or post-translationally processed in vivo to generate the two large subunits observed in purified human TFIIA. In addition, a second cDNA (hTFIIA/β) was isolated which encodes a 12kd human TFIIA subunit with homology to the yeast TOA2 gene. Together the recombinant proteins generate a TBP-dependent bandshift indistinguishable from that seen using highly purified human TFIIA. Thus, human TFIIA is minimally encoded by two genes, hTFIIA/α and hTFIIA/β, which correspond to the TOA1 and TOA2 yeast TFIIA genes, respectively. Antibody depletion and TFIIA "add-back" experiments demonstrate that TFIIA is required for efficient transcription in nuclear extracts, a result which supports the contention that TFIIA plays a fundamental role in regulating transcription initiation.

L 109 MUTATIONAL ANALYSIS OF A ZINC-BINDING DOMAIN IN THE LARGEST SUBUNIT OF RNA POLYMERASE II FROM THE YEAST *SACCHAROMYCES CEREVISIAE*. Donaldson I.M. and J.D. Friesen. Dept. Genetics, Hospital for Sick Kids, 555 University Ave.Toronto, Ontario, Canada, M5G 1X8.

RNA polymerase is a zinc metalloprotein. The role of zinc in this enzyme remains largely unknown. Recently, a 72 amino acid domain of the largest subunit of yeast RNA polymerase II was shown to bind zinc in an *in vitro* assay (Treich, I., M. Riva, and A. Sentenac. 1991. J. Biol. Chem. **266**:21971-21976). Thirteen site directed mutations were generated in this zinc-binding domain (ZBD) of the largest subunit of RNA polymerase II (RPO21) from the yeast *Saccharomyces cereivisiae*. Among the nine potentially-zinc-coordinating amino acids chosen for mutagenesis, six were well-conserved among all three RNA polymerases from yeast and a variety of other eukaryotes. The effect of site-directed mutations within the ZBD of RPO21 was assayed by introducing mutant copies of the gene on a low-copy plasmid into a strain of yeast in which the normal, chromosomal copy had been deleted. Altering any one of the six conserved residues conferred either a lethal or conditional phenotype. Changes in any one of the less-well conserved amino acids had no effect under the conditions tested. Currently we are examining the effects of mutations within this domain on subunit stability and enzyme assembly.

The zinc-binding domain was overproduced in *E. coli* as a fusion to the maltose binding protein (MBP). The MBPZBD fusion protein was shown to bind zinc in an *in vitro* zinc-binding assay while MBP alone or fused to the α fragment of β-galactosidase did not bind zinc. Mutant forms of the ZBD fused to MBP had markedly reduced affinities for zinc compared to the WT form in this same assay. Currently we are using atomic absorption spectroscopy to examine the number of zinc ions bound by this fusion protein and its mutant forms.

L 110 MODIFICATIONS OF RNA POLYMERASE II CTD PHOSPHORYLATION DURING HEAT-SHOCK IN MAMMALIAN CELLS, Marie-Françoise Dubois, Sylvain Bellier and Olivier Bensaude, Laboratoire de Génétique Moléculaire, Ecole Normale Supérieure, Paris, France.

The phosphorylation of the C-terminal domain (CTD) of the largest subunit of eukaryotic RNA polymerase II has been investigated in human HeLa cells exposed to heat-shock. While in control cells, the phosphorylated subunit IIo and the dephosphorylated subunit IIa are found in similar amounts, during a 45°C heat-shock the phosphorylated subunit, IIo, accumulates and concomitantly the amount of the IIa subunit decreases. The same phenomenon is observed at lower temperature when cells are pretreated with a phophatase 1 and 2A inhibitor such as okadaic acid. This suggests that upon mild heat-shock conditions, the heat-shock induced CTD-phosphorylation process is operative although it can be counterbalanced by a CTD-dephosphorylation process.

Moreover, during heat-shock or exposure to sodium arsenite, a chemical inducer of the heat-shock response, the IIo form accumulates even in the presence of CTD-kinase inhibitors such as DRB, H-8 or H-7 and in the presence of actinomycin D. Thus, the heat-shock-induced CTD-phosphorylation process appears to be different from the pathway observed at normal growth temperature and might contribute to the regulation of transcription during heat-shock.

L 111 THE LIGHT-REGULATED *ARABIDOPSIS THALIANA* PLASTOCYANIN PROMOTER CONTAINS GT-1 BINDING SITES.

Ursula Fisscher, Peter Weisbeek and Sjef Smeekens. Department of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Plastocyanin (Pc) is part of the electron transport chain which provides the reducing equivalents for the dark reactions of photosynthesis. Pc is nuclear encoded by a single gene, its expression is positively regulated by light, transcription is light induced.

Transgenic plant studies revealed that the Pc promoter of *A.thaliana* contains a positively regulating element in the upstream region (-1580 to - 705 bp relative to the ATG translation start site) [1]. This region was used for *in vitro* protein binding studies. In gel mobility shift assays, two short sequences binding specifically to tobacco nuclear proteins were found. Nuclear protein isolates from light grown and dark adapted plants showed identical retardation patterns. This indicates that, if this promoter binding protein is involved in the light regulated transcription of Pc, a modification of the protein rather than *de novo* protein synthesis mediates the proces.

Sequence comparisons showed a modular resemblance with the pea ribulose-1,5-bisphosphate carboxylase small subunit 3A promoter. In this promoter two GT-1 binding sites flanking an A-T rich stretch were identified [2]. GT-1 binding can confer light regulated transcription on a heterologous promoter [3]. In the middle of the DNaseI protected region of the Pc promoter, a strikingly similar A-T rich stretch is found (AAAAGATAA). This box separates the two protein binding sites, suggesting a role for GT-1 in the light regulated transcription of the *A.thaliana* Pc gene. Competition experiments with mutant and wildtype synthetic oligo nucleotides strongly support this suggestion.

[1] Vorst *et al.* (1993). *The Plant Journal, in press.*
[2] Lam *et al.*(1990). *Science* 248, 471-474.
[3] Green *et al.* (1987). *EMBO J.* 6, 2543-2549.

L 112 ANALYSIS OF THE CHICKEN *GPAT/AIRC* BI-DIRECTIONAL PROMOTER SUGGESTS THE PRESENCE OF A BI-DIRECTIONAL Inr ELEMENT, Anthony Gavalas and Howard Zalkin, Department of Biochemistry, Purdue University, West Lafayette, IN 47907-1153

GPAT and *AIRC* encode the enzymes that catalyze steps 1 and 6 plus 7, respectively, of the purine biosynthesis pathway. Both the chicken and human genes are closely linked and divergently transcribed from a bi-directional promoter. The chicken promoter is TATA-less, GC rich, ~350 bp long and initiates bi-directional transcription at distinct sites ~230 bp apart. There is a symmetrical pattern of two pairs of Sp1 and CCAAT boxes located at optimal distance from the transcriptional start site of *AIRC*, and one pair of Sp1 and CCAAT boxes located at optimal distance from the transcriptional start site of *GPAT*.

The bi-directional promoter was scanned by deletion mutagenesis, and a bi-reporter vector was used to assay transcriptional activity in both directions in transfected HepG2 cells. The results show that the intergenic region is an integrated bi-directional promoter rather than a juxtaposition of two independent promoters. Removal of the two Sp1 or the two CCAAT boxes on the *AIRC* side reduced transcription in both directions by more than 60%. The removal of the *AIRC* side CCAAT box had a similar effect. Most strikingly, removal of ~50 bases surrounding the *AIRC* transcription start site severely impaired transcriptional activity in both directions. In contrast, deletion of DNA sequences downstream of each transcriptional start site reduced activity in a side-specific manner. Gel retardation assays using a HeLa nuclear extract detected a specific complex formed on the presumed *AIRC* initiator element which bears no identity with known Inr elements. On the *GPAT* side an octamer motif appears to be responsible for the formation of two specific complexes.

The data demonstrate that a novel Inr element plays a central role in coordinating coexpression of the divergently transcribed *AIRC* and *GPAT* genes from the intergenic bi-directional promoter. Experiments in progress will attempt to further characterize the protein binding sites around the transcriptional start sites.

L 113 ANALYSIS OF ARABIDOPSIS RNA POLYMERASE II PROMOTERS IN TRANSGENIC ARABIDOPSIS AND TOBACCO PLANTS, Tom J. Guilfoyle, Xiang Yang Shi, Tim Ulmasov, and Rob Larkin, Department of Biochemistry, University of Missouri, Columbia, MO 65211

We have cloned and sequenced genes encoding the three largest subunits as well as the fifth largest subunit of *Arabidopsis* RNA polymerase II. The three largest subunits are related to the β, β', and α subunits of *E. coli* RNAP and RPB1, RPB2, and RPB3 of yeast RNA polymerase II, and the fifth largest subunit is related to the RPB7 subunit in yeast RNA polymerase II. Each of these *Arabidopsis* genes is single copy with the exception of the third largest subunit which is present at two copies in the *Arabidopsis* genome. The homolog to the third largest subunit in RNA polymerases I and III is also present at two gene copies in *Arabidopsis*. Both copies of the genes related to the *E. coli* α subunit in RNA polymerase II as well as in RNA polymerase I and III are expressed during the plant life cycle. To study the expression of these genes during growth and development in plants, we have made translational fusions of the RNA polymerase II subunit promoters with the reporter gene that encodes β-glucuronidase (GUS). We have examined the expression of these reporter genes throughout the life cycle of *Arabidopsis* and tobacco. The genes show similar patterns of expression with the strongest expression in germinating cotyledons, root tips, lateral shoot meristems, pollen, and developing seeds. Within vegetative organs, the strongest expression occurs in vascular tissues. The plant hormone auxin, which acts as a mitogen when applied at high concentrations, induces the expression of the RNA polymerase II subunit genes in a variety of tissues. One of these genes, the fifth largest subunit, is also induced by reducing agents such as glutathione and dithiothreitol.

L 114 GENETIC ANALYSIS OF THE GENERAL TRANSCRIPTION APPARATUS IN *SACCHAROMYCES CEREVISIAE*, Michael Hampsey, Rhonda W. Berroteran, Zu-Wen Sun and Ines Pinto, Department of Biochemistry and Molecular Biology, LSU Medical Center, Shreveport, LA 71130

The *sua* genetic loci of *S. cerevisiae* were uncovered as suppressors of an aberrant ATG sequence (uATG) located in the leader region of the *cyc1* gene. The *sua7* and *sua8* suppressors compensate for the deleterious effect of the uATG by shifting transcription initiation downstream of normal, resulting in initiation between the uATG and normal ATG start codon. These suppressors confer multiple pleiotropic phenotypes, including cold-sensitivity, which suggests defects in assembly of a multisubunit complex. The *SUA7* and *SUA8* genes have been isolated and identified. *SUA7* encodes TFIIB (factor e) and *SUA8* is identical to *RPB1*, which encodes the largest subunit of RNA polymerase II. All *sua7* and *sua8* suppressor mutations encode single amino acid replacements at phylogenetically conserved or invariant positions. The *sua7* replacements occur in the most highly conserved region of TFIIB, adjacent to the Zn-finger motif. The *sua8* replacements are located primarily within or immediately preceding homology block D. Double *sua7 sua8* haploid mutants are inviable (synthetic lethality) and heterozygous *SUA7/sua7 SUA8/sua8* diploids display at least partial suppressor phenotypes even though the suppressor mutations are fully recessive (nonallelic noncomplementation). Taken together, these data define TFIIB and the largest pol II subunit as determinants of transcription start site selection *in vivo* and suggest that this function is conferred by interaction between these two proteins.

In an effort to define other components of the general transcriptional apparatus we are taking advantage of the conditional pleiotropic phenotypes conferred by the *sua7* and *sua8* mutations to isolate either extragenic or dosage-dependent suppressors of these defects. We have uncovered two different genes, designated *ssu71* and *ssu72* as suppressors of the cold-sensitivity phenotype associated with a TFIIB $E_{62}K$ replacement. Similarly, a single gene, *ssu81*, was uncovered as a suppressor of a pol II $N_{445}S$ replacement. All three genes have been cloned and are being characterized.

L 115 CAN TATA BINDING PROTEIN (TBP) BIND DNA FUNCTIONALLY IN DIFFERENT ORIENTATIONS? David J. Heard and Witold Filipowicz, Friedrich Miescher-Institut, Postfach 2543, CH-4002 Basel, Switzerland.

In plants the promoters of the pol II- and pol III-specific U-snRNA genes have identical *cis*-acting promoter elements which are both necessary and sufficient for transcription, the -30 TATA box and the highly conserved USE element. In pol III-transcribed genes the USE is located three helical turns upstream from the TATA box whereas in pol II genes the spacing is four helical turns. Remarkably, it is this single helical turn difference which determines RNA polymerase specificity (Waibel and Filipowicz, 1990 Nature 346, 199-202; Kiss *et al.*, 1991 Cell 65, 517-26). To learn how polymerase specificity is determined at these promoters it is important to determine whether these elements are bound by the same or different transcription factors in the two classes of genes. Interestingly, plants such as *Arabidopsis* and maize, unlike all other organisms described to date, have two genes coding for two different isoforms of TBP. Since no plant *in vitro* transcription systems are available we made use of mutants of TBP which bind specifically to TATA boxes with the mutation TATAAA to TGTAAA (Strubin and Struhl, 1992 Cell 68, 721-30) to study the interaction of TBP with these promoters *in vivo*. Using this system we were able to show that cotransfection of plasmids expressing altered specificity mutants of either of the *Arabidopsis* TBP isoforms, or for that matter human and yeast TBPs, rescue transcription of pol II and pol III U-snRNA genes carrying a TGTAAA mutation in plant protoplasts (Heard *et al.*, 1993 EMBO J. 12, 3519-28).

Surprisingly, unlike in yeast, the mutant *Arabidopsis* TBPs were able to rescue transcription of genes carrying TATA box mutations other than TGTAAA. This rescue is gene specific, as some mutations are rescued in one type of promoter but not in another. For example the mutation TCTAAA is rescued by mutant TBPs only in the context of the U-snRNA promoters (both pol III and pol II) but not in an mRNA promoter (CaMV 35S). In contrast another mutation, TATAGA, is not rescued in the pol III U-snRNA gene context but is in the pol II genes, both mRNA and U-snRNA (Heard *et al.*, ibid). We suggest two possible explanations for this gene specific rescue: 1) TBP bound into polymerase specific TBP/TAF complexes (ie. TFIID or TFIIIB) has different DNA binding properties *in vivo*. 2) TBP binds functionally in different orientations on different promoters. In this poster we will present arguments supporting the latter hypothesis and the results of experiments designed to test this possibility *in vivo*.

L 116 CHARACTERIZATION OF A PUTATIVE METAL REGULATORY ELEMENT-BINDING PROTEIN FROM *LEISHMANIA* NUCLEAR EXTRACT, Pamela L. Heard, Michael B. Seay and Gautam Chaudhuri, Division of Biomedical Sciences, Meharry Medical College, 1005 D. B. Todd, Jr. Boulevard, Nashville, TN 37208

The highly abundant surface glycoprotein (gp63) of the human pathogen *Leishmania*, implicated as essential at several aspects of its infective life cycle, is a zinc-proteinase. Zinc down regulates the transcription of this gene by a yet to be understood mechanism. We have found in the upstream region of the gp63 gene two canonical metal responsive elements (MRE) separated by a G-rich region, homologous to mammalian MT gene promoter. Using end-labeled 5'-GCCGTGCCCTGGACTCCCT-3'/3'-CGGCACGGGACCTGAGGG AG-5' as probe (contain the MRE-1 from gp63 gene upstream sequence), we have detected a protein in *Leishmania* nuclear extract that binds to this probe by EMSA. This binding is modulated by zinc ions dose-dependently. We are characterizing the λ-gt-11 clones screened from a *Leishmania* cDNA expression library with this probe to find out the probable amino acid sequence of this putative MRE-binding protein from *Leishmania*. Supported by NSF Research Center for Excellence in Cell and Molecular Biology Grant # HRD-9255157, NIH Grant # 3 S06 GM08037-21S1 and NIGMS Fellowship # 1 F31 GM15608-01A1 SRC-1.

L 117 MOLECULAR CLONING AND CHARACTERIZATION OF HUMAN TFIID SUBUNITS

Masami Horikoshi[1,2], Satoshi Hasegawa[1,2], Koji Hisatake[2], Ritsuko Takada[2], Tohru Yamamoto[1], Yoshihiro Nakatani[3] and Robert G. Roeder[2]
[1]Laboratory of Developmental Biology, Department of Cellular Biology, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo, JAPAN
[2]Laboratory of Biochemistry and Molecular Biology The Rockefeller University, New York, NY, USA
[3]Laboratory of Molecular Biology, NINDS, National Institutes of Health, Bethesda, MD, USA

Activity of transcription is vigorously regulated by thousands of regulatory factors which represent information indispensable for a living cell to respond properly to various signals. Action of regulatory factors are finally transmitted to one enzyme, RNA polymerase II, by certain mechanism. We speculated TFIID mediates the transmission of information represented by regulatory factors to RNA polymerase II, and proposed the model that TFIID is the target of transcriptional activators based on the results of experiments employing reconstituted system (Horikoshi et al. Cell, 54, 665 & 1043, 1988). We initiated molecular cloning of TFIID to evaluate the proposed model employing more defined system. We have isolated several cDNA clones encoding human TFIID subunits, and we are currently analyzing their characteristics. We will present results concerning functional and structural roles of human TFIID subunits to support models described above.

L 118 REGULATION OF STRINGENT MITOCHONDRIAL GENE EXPRESSION IN YEAST, Alan P. Hudson, Mahboob U. Rahman, Jabed Iqbal, Christina A. Nevel, and Catherine M. McEntee, Department Microbiology/Immunology, Medical College of Pennsylvania and D.V.A. Medical Center Research Service, Philadelphia, PA 19104

cAMP levels in yeast are controlled by RAS1 and RAS2; *ras1 RAS2* mutants double at wild-type rates in fermentative or respiratory medium, but respiring *RAS1 ras2* cells are known to grow poorly. Our Northern analyses showed that mitochondrial (mt) transcript levels are normal in *RAS1 ras2* cells during fermentative growth but only 10% of normal during respiratory growth. cAMP levels are known to be low in respiring *RAS1 ras2* cells, and nutritional manipulations can affect *RAS* transcription. Moreover, we had previously shown that transcription of mt DNA is curtailed in yeast during the stringent response elicited by either amino acid (aa) starvation or nutritional shiftdown. Our assays of cAMP levels in wild-type cells following shiftdown and aa deprivation showed that those levels fall 5-fold after downshift but are unaffected by aa starvation. Via *in vitro* transcription assays, we showed that stringent mt transcription after shiftdown is relieved by addition of cAMP to the assay, but that the similar curtailment after aa deprivation is not. Our data also indicated that *a bcy1* mutant, which lacks the regulatory subunit for cAMP-dependent protein kinase (cAPK), does not show the shiftdown-induced mt stringent response. These and other data suggest that mt gene expression in yeast is cAMP-sensitive, and that it may be *trans*-activated via cAPK activity. Using a filter-binding assay, we demonstrated a cAPK-mediated protein-mt DNA interaction and cloned a mt DNA fragment 5' to the 21S rRNA gene which is involved in that interaction. Sequencing of the fragment revealed a 36 bp GC-rich element, which computer analyses showed to be present in both orientations and at varying distances 5' to many promoter-containing yeast mt genes, but not in the flanking regions of promoterless mt genes. We are using nuclease protection to confirm that this putative *cis*-regulatory element is the site of interaction with one or more cAPK-phosphorylated proteins, and we are constructing mt deletion mutants to demonstrate that the element is involved in cAMP-mediated mt transcriptional control. (Supported by the D.V.A. Medical Research Service and the American Heart Association SE PA Affiliate.)

L 119 WHAT IS THE ROLE OF RPB9 IN TRANSCRIPTION?

Melissa W. Hull and Nancy A. Woychik, Roche Institute of Molecular Biology, 340 Kingsland St., Nutley, NJ 07110

Since RNA polymerase subunit sequence and composition are well conserved among eukaryotes, we are using the yeast *Saccharomyces cerevisiae* to investigate subunit structure and function. *S. cerevisiae* RNA polymerase II is composed 12 subunits, designated RPB1-RPB12, whose apparent sizes range from 220 kDa to 10 kDa. Recently, each of the 12 subuits genes has been cloned and sequenced. Although most of the subunit genes have also been shown to be essential for yeast cell viability, both RPB4 and RPB9 are only required for growth at high or low temperatures. We have taken both biochemical and genetic approaches to determine the role of RPB9 in yeast RNA polymerase II transcription. Comparison of the sequence of RPB9 to its homologues has aided us in identifying important regions of the protein. The amino acid sequence of RPB9 is well conserved with respect to its RNA polymerase II counterpart in *Drosophila melanogaster*. RPB9 is also similar to the *Saccharomyces cerevisiae* RNA polymerase I subunit RPA12. Each of these proteins is approximately the same size, and has two zinc fingers. Based on these sequence comparisons, we have constructed mutations in RPB9 in order to define the regions important for its function. We have found that the first zinc finger of RPB9 is required for function. We have also examined the RPB9 deletion strain for defects in *in vitro* transcription. As a genetic approach to the investigation of the role of RPB9 in transcription, we have isolated high copy suppressors of the temperature sensitive growth phenotype resulting from deletion of *RPB9* in yeast cells. We presently have identified and mapped several high copy suppressors. The high copy suppressor which we have investigated most extensively thus far has been identified as the single copy yeast protein kinase C gene *PKC1*. To determine why over-expression of *PKC1* suppresses the temperature sensitive growth defect of the *RPB9* deletion strain, we have constructed *PKC1* and *RPB9* deletions strains in which *PKC1* can be inserted on a high copy number plasmid. We have looked for differences in phosphorylation of RNA polymerase II *in vivo* in these strains, both with and without over-expression of PKC. We have also performed parallel experiments to examine the *in vitro* transcription activity of these same strains. We hope that further characterization of this and other suppressors may help to more clearly define the role of RPB9 in mRNA transcription.

L 120 STRUCTURE-FUNCTION ANALYSIS OF YEAST GENERAL TRANSCRIPTION FACTOR TFIIA, John J. Kang and Steven Hahn, Basic Sciences Division, F. Hutchinson Cancer Research Center, Seattle, WA 98104

The RNA polymerase II general transcription factor, TFIIA plays an important role in regulating initiation. In the yeast *Saccharomyces cerevisiae*, TFIIA consists of a heteromer of 32 kD and 13.5 kD subunits encoded by two essential genes, *TOA1* and *TOA2*, respectively. We have performed molecular genetic and biochemical experiments to elucidate structure-function relationships of TFIIA and thereby clarify its mechanism of function in transcription initiation.

By site-directed mutagenesis, we constructed deletion and amino acid substitution mutations in both subunits of TFIIA. We tested these mutations for their effects on yeast growth. Many internal deletions of *TOA1* showed no phenotype. In fact, deleting over half of *TOA1* resulted in a temperature-sensitive phenotype. The amino acid substitution mutations in *TOA1* which conferred a conditional lethal phenotype clustered in the carboxy terminal region. These mutant proteins appear defective in their ability to interact with TBP-TATA DNA complexes.

In contrast to the results obtained with the large subunit, most internal deletion mutations in *TOA2* resulted in death. We have performed preliminary biochemical analysis of three conditional lethal amino acid substitution mutations in *TOA2*. These mutant proteins form complexes with TATA-bound TBP normally. One of these mutants lacks the ability to block the action of the ATP-dependent inhibitor of TBP-TATA binding (ADI). Further biochemical analysis of TFIIA mutants will be presented.

L 121 **DIRECT RECOGNITION OF INITIATOR ELEMENTS BY A COMPONENT OF THE TFIID COMPLEX**, Jörg Kaufmann and Stephen Smale, Howard Hughes Medical Institute and Department of Microbiology and Immunology, U.C.L.A. School of Medicine, Los Angeles, California 90024

Accurate transcription initiation from mammalian protein-coding genes depends on recognition of core promoter elements. For TATA box containing promoters this recognition event is carried out by TFIID, a multiprotein complex containing TBP and several additional factors known as TAFs. However, numerous mammalian protein-coding genes lack a TATA box. The proteins that functionally recognize the core control elements of these TATA-less genes have not been identified. One class of TATA-lacking promoters are those that contain an Initiator (Inr) element.

An Inr can be defined as a basal control element that overlaps a transcription start site and is capable of determining the location of the start site in a promoter that lacks a TATA box. Furthermore, an Inr can enhance the strength of a promoter that contains a TATA box, if the Inr is located approximately 25 bp downstream of the TATA box. The Inr consensus sequence is PyPyANTPyPy. Although a few protein have been reported to bind to specific Inrs, the sequences required for binding of these proteins do not correlate with the requirements for Inr activity.

We have found that the TFIID complex specifically interacts with the Inr. Two lines of evidence are presented. First, during in vitro experiments, Inr activity is only observed in the presence of TFIID, but not in the presence of recombinant TBP. Second, DNase I footprinting experiments have revealed that TFIID contacts the DNA at the TATA box and also at the Inr. The interaction at the Inr correlates with the sequences required for Inr activity. Detection of the interaction was dependent either on a TATA box or on Sp1 bound to upstream sites. Furthermore, recombinant TFIIB specifically enhanced the contact between TFIID and the Inr, whereas TFIIA stabilized the TFIID-TATA interaction. These results demonstrate that both TATA and Inr elements are recognized by distinct components of the general transcription factor TFIID.

L 122 CO-CRYSTAL STRUCTURE OF *A. thaliana* TBP BOUND TO THE TATA-BOX OF THE ADENOVIRUS MAJOR LATE PROMOTER, Joseph L. Kim and Stephen K. Burley, Laboratory of Molecular Biophysics, The Rockefeller University, and Howard Hughes Medical Institute, New York, NY 10021

The TATA-box binding protein (TBP) is required for transcription by all three eukaryotic RNA polymerases. In transcription of class II nuclear genes, TBP comprises the DNA binding component of the general transcription factor TFIID. TBP binds specifically to the TATA element of class II promoters and thereby directs formation of a functional preinitiation complex with the other general transcription factors and RNA polymerase II. We have solved and refined the crystal structure of TBP from *Arabidopsis thaliana* complexed to a DNA oligonucleotide containing the TATA element of the Adenovirus major late promoter. This structure demonstrates a novel mode of specific protein-DNA interactions involving extensive van der Waals and hydrophobic contacts between the protein surface and DNA minor groove. Binding of TBP induces a sharp kink in the TATA element, resulting in a bend of approximately 80° in the DNA. Structural details and biological implications of this highly unusual protein-DNA complex will be presented. In addition, we have crystallized TBP with a series of mutant TATA-box containing oligonucleotides in order to arrive at a better understanding of the specificity determining elements in this protein-DNA complex.

L 123 THE MECHANISM OF TRANSCRIPTION INITIATION IS CONSERVED AMONG CLASS II PROMOTERS. Richard J. Kraus, Nancy M. Zink, Elizabeth E. Murray, Steven J. Wiley, and Karla J. Loritz and Janet E. Mertz. McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706 USA.

The SV40 major late promoter (SV40-MLP) contains three genetically important basal elements, mapping to the site of initiation and approximately 30 bp upstream and downstream of the +1 site (Ayer and Dynan M.C.B., 8: 2021, 1988.) We have shown that the upstream element, while not an obvious TATA box motif, functionally binds TFIID and cooperatively interacts with the initiator for proper start site selection (Wiley et al, P.N.A.S. 89: 5814, 1992). Recently, additional analysis of interactions at the -30 region of both strong and weak TATA promoters revealed that the combination of TBP and TFIIB binds each sequence approximately five-fold more efficiently than does TBP alone.

We also have attempted to purify positive-acting factors that bind the genetically important initiator element of the SV40-MLP. Instead, we identified an initiator binding protein, "IBP-s", that represses initiation from the SV40 late promoter when template copy number is low (Wiley et al., Genes & Dev., 1993, in press). Thus, the SV40-ML initiator may not be recognized by a novel protein that functions in the formation of initiation complexes. Rather, this promoter probably functions by a mechanism qualitatively similar to that of a TATA-box promoter.

We performed a saturation mutagenesis of the SV40-ML initiator and have defined the core bases of the initiator motif. They map from -2 to +3 relative to the start site, with the optimal sequence being 5'-TCAGT-3'. We found that transcriptional activity correlates with similarity to the optimal sequence and used our data to obtain a weight matrix that enables one to accurately predict the transcriptional activity of any given initiator sequence.

We conclude that only a single family of initiator elements exists and it is probably recognized by an initiation site complex consisting of RNA polymerase II and general transcription factors. Other initiator binding proteins simply recognize sequences overlapping the initiator and function as positive and negative regulators of transcription. We propose that the transcriptional start site is determined by the concurrent interactions of multiple trans-acting factors with each other and their binding sites on the promoter. Although the affinity of binding of any one of these factors may differ for various promoters, the mechanism of initiation complex formation is basically conserved for all class II promoters.

L 124 TRANSCRIPTIONAL CO-REGULATION OF A METALLOTHIONEIN-I LIKE GENE AND THE SURFACE METALLOPROTEINASE GENE IN *LEISHMANIA*, Clarence S. Lewis, Jr., Angelika K. Parl and Gautam Chaudhuri, Division of Biomedical Sciences, Meharry Medical College, 1005 D. B. Todd, Jr. Boulevard, Nashville, TN 37208

Metallothioneins are low molecular weight, cysteine- rich proteins that bind metal ions. These proteins have several potential physiological roles including their inducibility with metal ions, modulation of their transcription during cellular development, differentiation and proliferation, their capability to activate metalloenzymes and their action to alleviate the toxic effects of damaging free radicals. The highly abundant surface zinc-proteinase (gp63) of the human parasite *Leishmania* plays important role in the establishment and maintenance of infection in human macrophages. To understand the possible regulation of the activity of this parasite virulent factor by metallothionein, we have cloned a metallothionein-1 like gene from *L. mexicana amazonensis* cells using mouse MT-1 cDNA as probe. We are exploring the possibility of common cis and trans elements regulating both the surface zinc-proteinase gene and this metallothoinein gene by comparing the upstream sequences of these genes. We are also transfecting the cloned fragment of the metallothionein gene upstream sequence fragment to *Leishmania* cells transiently expressing CAT gene driven by the gp63 gene promoter to understand the linkage, if any, between the transcriptional regulation of these two proteins. Supported by NSF Research Center for Excellence in Cell and Molecular Biology Grant # HRD-9255157, NIH Grant # 3 S06 GM08037-21S1 and NIGMS Fellowship # 1 F31 GM16446-01 SRC-1.

L 125 ISOLATION OF A CDNA ENCODING THE LARGEST SUBUNIT OF TFIIA REVEALS FUNCTIONS IMPORTANT FOR ACTIVATED TRANSCRIPTION
Dongmin Ma[1],Hajime Watanabe[2],Fred Mermelstein[1],Xiaoqing Sun[1],Hiroshi Handa[2] and Danny Reinberg[1]. [1]Department of Biochemistry, Robert Wood Johnson Medical School,University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854-5635;[2]Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Midokiru, Yokohama227, Japan

Transcription factor TFIIA has been shown to interact with the TATA-binding protein and act early during preinitiation complex formation. The human factor is composed of three subunits (α,β,γ). A human cDNA clone encoding the largest subunit of TFIIA (α) was isolated. The recombinant α polypeptide, together with the β and γ subunits, was capable of reconstituting TFIIA activity. Studies using antibodies raised against recombinant α polypeptide demonstrate that TFIIA can be an integral component of the preinitiation complex. We demonstrate that TFIIA not only interacts with TBP but also associates with the TFIID complex. Functional assays establish that TFIIA has no role in basal transcription but plays an important role in activation of transcription. Interestingly, amino acid sequence analyses of the β subunit demonstrate these residues to be entirely contained within the carboxyl terminus of the cDNA clone encoding the α subunit, suggesting that protein processing or alternative splicing might be involved in the generation of the α and β subunits. Renaturation experiments with SDS gel-isolated α, β and γ subunits have shown that only α and γ subunits together could form a weak DA complex in the presence of TBP. Addition of the β subunit could dramatically stimulated the DA complex formation but did not change the migration of the complex. We are also engaged in the cloning of the γ subunit of TFIIA.

L 126 MUTATIONAL ANALYSIS OF ERCC3, INVOLVED IN DNA EXCISION REPAIR AND TRANSCRIPTION INITIATION: IDENTIFICATION OF DOMAINS ESSENTIAL FOR THE DNA REPAIR FUNCTION, Libin Ma, W. Vermeulen[#], A. Westbroek, A.G. Jochemsen, G. Weeda[#], D. Bootsma[#], J.H.J. Hoeijmakers[#], and A.J. van der Eb, Laboratory for Molecular Carcinogenesis, University of Leiden, 2300 RA Leiden, and [#]Department of Cell Biology and Genetics, Erasmus University Rotterdam, The Netherlands

The *ERCC3* gene specifically corrects the excision-repair defect of xeroderma pigmentosum (XP) group B and UV-sensitive CHO mutants of complementation group 3. Recently, ERCC3 has been identified as a component of the general transcription factor BTF2/TFIIH, which implies that this protein may participate in two cellular processes, transcription and nucleotide excision repair.

ERCC3 encodes a presumed DNA helicase containing a putative nuclear location signal, a potential "helix-turn-helix"-type DNA-binding motif, and seven conserved "helicase" domains with domain I corresponding to the Walker "A" site of the ATP-binding motif. Using site-directed mutagenesis, we have examined the functional significance of these domains in excision repair. A substitution of arginine for the invariant lysine residue in the ATPase motif ($Lys^{346}\rightarrow Arg$ mutant), six deletion mutations in the other "helicase" domains and a deletion in the potential DNA-binding motif fail to complement the excision-repair defect of rodent group 3 cells, implicating that the "helicase" domains as well as the potential DNA-binding motif are required for the proper functioning of ERCC3 in excision repair. Analysis of carboxy-terminal deletions suggests that the carboxy-terminal exon may comprise a distinct determinant for the DNA repair function. In addition, we show that an epitope-tagged version of ERCC3 retains the repair activity and accumulates in the nucleus. Deletion of the putative nuclear location signal does not impair the nuclear location nor the repair function, indicating that other sequences are also involved in translocation of ERCC3 to the nucleus. When expressed in normal cells, the $Lys^{346}\rightarrow Arg$ mutant completely abolished nucleotide excision repair and transcription, and induced a dramatic collapse of chromatin structure. The effect of other ERCC3 mutants on transcription remains to be determined.

L 127 EXPLORATION OF RNA POLYMERASE II STRUCTURE AND FUNCTION, Keith McKune and Nancy A. Woychik, Roche Institute of Molecular Biology, Nutley, NJ 07110

A major component of the eukaryotic transcription apparatus is the multisubunit enzyme RNA polymerase II. *Saccharomyces cerevisiae* RNA polymerase II has been extensively studied. The yeast enzyme comprises 12 known subunits, RPB1-RPB12, whose genes have been cloned, sequenced and characterized. At least nine of the twelve known RNA polymerase II subunits are related or identical to the subunits of RNA polymerases I and III, indicating that the three RNA polymerases have significant structural and functional similarities.

The RPB5, RPB6, RPB8, RPB10 and RPB12 (A10α) subunits are essential, shared components of RNA polymerases I, II and III. These subunits must carry functions common to the three RNA polymerases and may interact with one or more of the general transcription factors. We have isolated several conditional mutants of RPB5 and RPB6 and are assessing their impact on transcription *in vivo* and *in vitro*. Well defined single mutants of RPB5 and RPB6 are also being used to identify interacting proteins using second site suppressor analysis.

We have isolated the gene encoding the human RPB6 subunit and observed striking conservation in the carboxy half of the protein when compared to yeast RPB6. Over 80% of the residues are identical and over 90% are conserved in this 80 amino acid region. The same level of conservation is seen in this region of RPB6 from fission yeast, therefore identifying a critical functional domain. RPB6 also has a conserved acidic domain at its amino terminus. Complementation experiments revealed that the human RPB6 subunit is functional in *S. cerevisiae*. Southern analysis of digested genomic DNA from several higher eukaryotes also produced distinct bands, indicating a high level of conservation.

L 128 BACULOVIRUS MEDIATED CELL LINE DEPENDENT EXPRESSION OF GENES: REGULATION AT THE TRANSCRIPTIONAL LEVEL, Bipasha Mukherjee, Sandeep Burma and Seyed E. Hasnain, Eukaryotic Gene Expression Laboratory, National Institute of Immunology, New Delhi 110067, INDIA

Baculovirus mediated synthesis of two reporter proteins in five different lepidopteran cell lines derived from Spodoptera frugiperda (Sf21 & Sf9), Bombyx mori (BmN & Bm5) and Trichoplusia ni (TN368) was studied to understand the molecular basis of cell line dependent foreign gene expression. A recombinant baculovirus vAcßhCG-luc carrrying the genes encoding the ß subunit of human chorionic gonadotropin (ßhCG) and firefly luciferase (luc) under the transcriptional control of the Autographa californica nuclear polyhedrosis virus (AcNPV) polyhedrin gene promoters was used. TN368 expressed both ßhCG and luc to maximum levels, followed by BmN, Sf21 and Sf9. Bm5 did not show any evidence of synthesis of both the proteins. In-gel hybridisation analysis of RNA from the four expressing cell lines revealed a direct correlation of protein levels with levels of RNA implicating transcriptional control. Gel retardation assays carried out with nuclear proteins from the five cell lines and a 32 bp domain of the polyhedrin promoter revealed an interesting correlation between the levels of a Polyhedrin Promoter Binding Protein and expression levels. UV-crosslinking analyses comparing PPBP from Bm5 with that from the expressing cell lines indicated that additional factor(s) may mediate the interaction of PPBP with the basal transcription apparatus.

L 129 THE CONTINGENT REPLICATION ASSAY FOR PROTEIN INTERACTIONS: ANALYSIS OF THE MAMMALIAN TFIIF COMPLEX, Girish N Nallur, Haren A Vasavada and Sherman M Weissman, Department of Genetics, Yale University School of Medicine, New Haven, CT 06510.

Initiation of transcription by mammalian RNA polymerase II is a complicated process involving the ordered assembly of several basal transcription factors and the polymerase subunits on the template DNA. Many of these transcription factors are multisubunit complexes themselves. Cloning and characterization of these subunits would be a major step in understanding the process of transcription iniatiation *in vivo* , and be a tool for *in vitro* studies with purified components. We have used the Contingent Replication Assay (CRA) to analyze protein interactions in the TFIIF complex. Using human Rap30 as a target in the assay we recovered enriched levels of a cDNA encoding a portion of Rap74 from a normalized cDNA library from JY cells. The fold enrichment of the Rap74 cDNA in the output library suggests that the CRA is a valuable tool not only for the isolation of interacting cDNAs but also for the study of domain interactions *in vivo*. In addition, three other cDNAs were enriched. These cDNAs were isolated by a modified screening approach. Attempts are underway to characterize two of the enriched cDNAs whose sequences are not represented in the DNA data bases. Results pertaining to the CRA of Rap30 will be presented in addition to a detailed analysis of the CRA as a method to study protein interactions in mammalian cells.

L 130 CRYSTALLOGRAPHIC AND BIOPHYSICAL STUDIES OF TFIIB - A GENERAL TRANSCRIPTION INITIATION FACTOR, Dimitar B. Nikolov and Stephen K. Burley, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399

TFIIB is a general transcription initiation factor. It recognizes and binds to the TFIID-DNA promoter complex, containing the TATA-box binding protein (TBP), and facilitates the subsequent interaction with RNA polymerase II through the associated TFIIF. The binding of TFIIB can be the rate-limiting step in the preinitiation complex formation and consequently a target for transcriptional activation. The interaction of TFIIB with TBP and TBP-DNA complexes was studied by surface plasmon resonance, circular dichroism spectroscopy, photon correlation spectroscopy and analytical ultracentrifugation. Crystallographic investigations are underway. Recent advances will be discussed.

L 131 CHARACTERIZATION OF TRANSCRIPTION FACTOR(S) INVOLVED IN THE POSITIONING OF THE TRANSCRIPTIONAL START SITE, Ivan Olave, Edio Maldonado, and Danny Reinberg, Dept. of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, N.J. 08854.

RNA polymerase II dependent transcription requires a set of general transcription factors (GTFs) to accurate initiate transcription from an discrete start site. Different systems have been developed to characterize the specific roles of each of these factors in the transcription cycle.

We are interested in determining which of the basal factors is/are involved in the determination of the transcriptional start site. The position of the TATA motif in promoters from yeast *S. cerevisiae* and mammals is different. Although it would seem that the location of the TATA motif dictates the start site for transcription, previous studies suggest that specific protein-protein interactions are vital in governing accurate positioning of the polymerase at +1 in both systems. Among the observations that support this hypothesis are: i)TFIIB and SUA7, the *S. cerevisiae* homologue of human TFIIB, are not functionally interchangeable, though each can interact with its respective RNA polymerase II. ii) Our laboratory has recently shown that TFIIB can function as a molecular bridge to gap the DAB (DB) complex at the TATA motif with the polymerase, and the remaining GTFs, for accurate initiation of transcription, and iii) Hampsey and colleagues showed that mutations in SUA7 can alter the location of the start site.

In order to address the role of TFIIB or other factors in positioning the initiation site, we have chosen to study the yeast *Schizosaccharomyces pombe* because cellular processes are more phylogenetically conserved between *S. pombe* and mammalian systems than between *S. cerevisiae* and mammals.

We have developed a reconstituted transcription system *in vitro* from *S. pombe*. Three fractions, in addition to exogenous *pombe* TBP and polymerase, are absolutely required to initiate accurate transcription. Importantly, fraction C, which elutes approximately at 50kDa in gel filtration column, was shown to shift a DA complex to one resembling a DAB complex. Western analysis of fraction C revealed a 50kDa polypeptide reactive to SUA7 antibodies. Further studies will address whether this fraction can substitute for human TFIIB in a reconstitution assay and perhaps clarify the role of TFIIB-like proteins in positioning the initiation site.

L 132 BOTH RNA POLYMERASE III AND RNA POLYMERASE II RECOGNIZE THE HUMAN U6 PROMOTER IN VITRO, JungSun Park and Gary R. Kunkel, Department of Biochemistry and Biophysics, Texas A & M University, College Station, Texas 77843

U6 small nuclear RNA genes are transcribed by RNA polymerase III (polIII), unlike those encoding other snRNAs (U1-U5). The promoter of a vertebrate U6 gene is constituted of a proximal sequence element (PSE) and a TATA box, that in combination give rise to polIII-specific transcription in vivo and in vitro. Using a HeLa cell nuclear extract supplemented with TATA binding protein (TBP), we have detected both polIII and polII transcripts, as differentiated by their sensitivity to α-amanitin. The template for these reactions is the human U6 promoter ligated to a C-free cassette. The major polII-specific transcript is 2 nucleotides longer than polIII-specific U6 transcripts since synthesis bypasses the polIII terminator and stops at the first downstream C residue. The polII specific U6 transcription is independent of the PSE, but dependent on the presence of the TATA box. The polIII transcription from the U6 promoter is stimulated by proximal sequence binding protein (PBP). In contrast, the polII transcription is stimulated by TFIIB. A TBP-TFIIB (DB) complex is detected on the U6 promoter by a gel mobility shift assay. The kinetics of preinitiation complex formation of both transcripts has been investigated in the presence of Sarkosyl. Since TATA-dependent polII-specific transcription is detected from the U6 promoter, both polIII- and polII- preinitiation complexes can assemble on the U6 promoter. However, polII-specific transcription may be futile in vivo, since functional U6 RNAs are produced by polIII .

L 133 TRANSCRIPTIONAL INDUCTION AND CHARACTERIZATION OF *CYP6B1* GENE

H. Prapaipong[1], M.R. Berenbaum[2], and M.A. Schuler[1,3]
[1]Departments of Biochemistry, [2]Entomology, [3]Plant Biology, University of Illinois, Urbana IL 61801

Detoxification of hostplant defensive compounds by larval Lepidoptera is mediated partially by cytochrome P450 monooxygenses (P450s). In *Papilio polyxenes* (black swallowtail) larvae, expression of the CYP6B1 P450 polypeptide is induced in response to xanthotoxin, a linear furanocoumarin. Baculovirus expression of *CYP6B1* cDNAs in lepidopteran cell lines has demonstrated that two linear furanocoumarins, xanthotoxin and bergapten, are metabolized substantially by the *CYP6B1* alleles. To characterize the autoregulatory feature of this gene, a full-length *CYP6B1* genomic clone was isolated from a *P. polyxenes* genomic library using *CYP6B1* cDNA probes previously characterized. The open reading frame of the gene is interrupted by a single intron and potentially encodes a protein of 498 amino acids. Primer extension and ribonuclease protection analysis have identified the transcription initiation site at a point 28 nucleotides upstream from the AUG translation initiation codon. Putative TATA and CAAT boxes are located 30 and 129 nucleotides, respectively upstream from the transcription initiation site. Transfection of *CYP6B1* promoter-chloramphenicol acetyltransferase fusion constructs has domonstrated that a 800-nucleotide region 5' to the transcription initiation site retains basal and xanthotoxin inducible transcription elements. These data begin to elucidate the substrate-dependent mechanisms regulating transcription of this insect P450 gene. RNase protection analysis on RNA from larvae induced by linear and angular furanocoumarins has demonstrated that transcription of the *CYP6B1* gene is specifically induced *in vivo* in response to xanthotoxin and, to a lesser extent, by bergapten. Angular furanocoumarins, such as angelicin, which are not capable of being metabolized by the CYP6B1 gene product, do not induce transcription.

L 134 BINDING AFFINITY OF THE TRANSCRIPTION FACTOR FORK HEAD FROM DROSOPHILA TO VARIOUS DNA DUPLEXES, Karsten Rippe[1], Eckhard Kaufmann[2], Herbert Jäckle[3], and Peter von Hippel[1], Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403, USA[1], and Abteilung Biochemie, Universität Ulm, Oberer Eselsberg, Postfach 4066, D-89069 Ulm, Germany[2], and Abteilung Molekulare Entwicklungsbiologie, Max-Planck-Institut für biophysikalische Chemie, Am Faßberg, Postfach 2841, D-37018 Göttingen, Germany[3].

The *Drosophila* fork head protein is a region specific transcription factor that is involved in determining the development of internal organs like the gut, the salivary glands, and parts of the nervous system. The DNA-binding of fork head is mediated by a distinct domain of the protein which comprises ~110 amino acid residues. This domain is conserved in a number of other proteins from invertebrates to mammalians. We have over-expressed the ~110 amino acid DNA-binding domain and also the complete fork head protein in *E. coli* and studied the binding affinity to various DNA oligonucleotide duplexes by quantitative gel shift assays. The domain binds as a monomer with equilibrium dissociation constants ranging from $2 \cdot 10^{-8}$ M to $3 \cdot 10^{-9}$ M for the interaction with different specific binding sites. These results are compared to the binding of the complete fork head protein.

L 135 G-FREE TEMPLATE CONSTRUCTION FOR REGULATED *IN VITRO* TRANSCRIPTION ANALYSIS OF A FILAMENTOUS FUNGAL GENE, Yijun Ruan and David C. Straney, Department of Botany, University of Maryland, College Park, MD 20742

The *PDA*1 gene in the phytopathogenic fungus, *Nectria haematococca,* encodes pisatin demethylase, an enzyme which detoxifies pisatin, phytoalexin of pea. Expression of the *PDA*1 gene is induced by pisatin. In order to understand the transcriptional regulation of the *PDA* gene, we have established an *in vitro* transcription system for *N. haematococca* with a *PDA*1 promoter/G-free template. We have used a novel approach to add G-free sequence onto this fungal promoter by using PCR to generate downstream terminus of the promoter at designed site followed by terninal deoxynucleotidyl transferase addition of a G-free tail. Whole cell extracts from pisatin-induced mycelium of the fungus produce a transcript from the *PDA*1 promoter on a G-free template. The transcript is accurately initiated at an *in vivo* start site and its formation is dependent upon the *PDA*1 promoter's TATA box. The transcript is produced by RNA polymerase II based on its inhibition with α-amanitin and RNAPII-specific monoclonal antibody. Significantly, extract from uninduced mycelium does not produce this specific transcript, indicating that regulation is preserved in the extract. Both positive and negative elements have been identified by functional dissecting of the PDA1 promoter's pustream region.

L 136 DOWN REGULATION OF THE PROMOTER ACTIVITY OF AN UPSTREAM REGULATORY SEQUENCE OF *LEISHMANIA* SURFACE METALLOPROTEINASE GENE BY ZINC IONS, Michael B. Seay and Gautam Chaudhuri, Division of Biomedical Sciences, Meharry Medical College, 1005 D. B. Todd, Jr. Boulevard, Nashville, TN 37208

Zinc apparently plays important role in the human parasite, *Leishmania*. Its highly abundant surface glycoprotein (gp63), implicated as essential at several aspects of its infective life cycle, is a zinc-proteinase. While working with the gene of *L. mexicana amazonensis* gp63, we found indications of zinc ion regulation of its gene expression. A 450 bp upstream sequence (URS) of one of the surface metalloproteinase genes of *L. m. amazonensis* was amplified from specific chromosome by anchor PCR and was cycle-sequenced. This URS was found not to have a TATA element but to have medRNA splice site, GC box and two canonical metal responsive elements separated by a G-rich region, homologous (70-80%) to mammalian MT gene promoter. This URS was cloned in front of CAT reporter gene in pCATb and electroporated to *Leishmania* cells. This sequence orientation-dependently catalyze the expression of CAT gene in transfected *Leishmania* cells. The expression of CAT activity was dose-dependently down regulated by the presence of 1-10 μM zinc ions in the culture medium. We are exploring the mechanism of this regulation by quantitative RNA-PCR, nested deletion with exonuclease III and by oligonucleotide-directed mutagenesis studies. Supported by NSF Research Center for Excellence in Cell and Molecular Biology Grant # HRD-9255157, NIH Grant # 3 S06 GM08037-21S1 and NIH Training Grant # 5 T32 AI07281-09.

L 137 TWO TRANSCRIPTION FACTORS INVOLVED IN DNA REPAIR IN SACCHAROMYCES CEREVISIAE. Kevin S. Sweder and Philip .C. Hanawalt, Department of Biological Sciences, Stanford University, Stanford, California 94305

Recent studies in several different laboratories have implicated essential transcription factors in DNA repair, based upon UV sensitivity of the relevant mutants. However, UV sensitivity is not proof of a particular biochemical defect in nucleotide excision repair.

We examined yeast strains with mutations in the essential *SSL1* and *SSL2* (Suppressor of Stem Loop) genes for their ability to remove cyclobutane pyrimidine dimers (CPDs) from expressed genes, as well as from the genome overall. Both *SSL1* and *SSL2* genes had been identified by selecting for suppressor mutations that enabled cells to translate the *his4-316* gene, which contains a stem-loop mutation in the 5'-untranslated region of its mRNA (Gulyas and Donahue, *Cell* **69**(6):1031-1042). The product of *SSL1* is a component of the RNA pol II initiation complex factor b (BTF2/TFIIH) and the product of *SSL2* has been shown to interact with factor b (Feaver *et al.*; Buratowski and Zhou, *Cold Spring Harbor Abstract*, 1993).

Strains containing the supressor allele *SSL1-1* are temperature sensitive, exhibit a deficiency in polysome assembly at 37°C, and are UV sensitive (Yoon *et al.*, *Genes & Dev.*, **6**(12b):2463-2477). We determined the nature of the putative DNA repair defect in *SSL1-1* and *SSL1-3* mutants. We found that *SSL1* mutants are completely deficient in the preferential repair of the transcribed strand of the expressed *RPB2* gene and very deficient in the removal of CPDs from the genome overall.

Deletion of the carboxyl terminus of the wild-type *SSL2* gene results in the UV-sensitive strain, *SSL2-XP*. Since the *SSL2* protein has a high degree of sequence identity to the protein encoded by the human *ERCC3* gene [which complements xeroderma pigmentosum (group B)-Cockayne's syndrome (group C)], we investigated the basis for the UV-sensitivity in the *SSL2-XP* strain. The *SSL2-XP* strain, similar to *SSL1* mutants, was completely deficient in the preferential removal of CPDs from the transcribed strand of *RPB2* and very deficient in removal of CPDs from the entire genome. Thus, the *SSL2-XP* strain exhibits a phenotype very similar to that of human cells from patients possessing traits of both xeroderma pigmentosum (group B) and Cockayne's syndrome (group C). The similarities in UV sensitivity and repair defects between *SSL1* and *SSL2-XP* strains suggests an interaction *in vivo* between their respective gene products that is consistent with findings *in vitro* (Feaver *et al.*, Buratowski and Zhou).

L 138 SINGLE-STEP PURIFICATION OF HUMAN TRANSCRIPTION FACTOR TFIIB BY IMMUNOAFFINITY CHROMATOGRAPHY.
Nancy E. Thompson and Richard R. Burgess. McArdle Laboratory for Cancer Research, University of Wisconsin-Madison.

RNA polymerase II transcription factor IIB (TFIIB) was purified from a bacterial expression system by an immunoaffinity chromatography protocol. This protocol uses a monoclonal antibody (MAb) that is polyol-responsive. We have previously described the properties of this type of MAb (Thompson et al., J. Biol. Chem. 265:7069, 1990 and Thompson et al., Biochemistry 31:7003, 1992). The MAb (designated IIB8) is an IgG_{2a} MAb that inhibits transcription when added to HeLa cell nuclear extract and immunoprecipitates TFIIB from the nuclear extract. Using proteolytic cleavage of TFIIB, we believe that the epitope for this MAb lies between residues 52 and 105. The MAb was purified by Protein A and conjugated to cyanogen bromide-activated Sepharose. *Escherichia coli* strain BL21(DE3) containing the pLysS plasmid was transformed with hTFIIB contained in the pET11a vector. After induction with IPTG, the cells were harvested and lysed. The lysate was treated with 0.5% poly(ethyleneimine) and centrifuged. The supernatant was applied to the IIB8-Sepharose. After extensive washing, the TFIIB was eluted with Tris-EDTA buffer containing 0.75 M ammonium sulfate and 40% propylene glycol. The purified TFIIB was active in promoter-directed transcription when added to a IIB8-depleted nuclear extract and when used in the minimal IgH transcription system. We believe that this method will be very useful for the rapid isolation of TFIIB mutants and for the purification of large amounts of highly purified TFIIB for biochemical studies.

L 139 ROLES OF TFIIH AND TFIIE IN THE FORMATION OF THE OPEN PROMOTER COMPLEX, H.Th.Marc Timmers, P.C. van der Vliet and F.C.P. Holstege, Laboratory for Physiological Chemistry, Utrecht University, Vondellaan 24a, 3521 GG Utrecht, The Netherlands.

The ATP-dependent DNA helicase activity of TFIIH is presumed to be responsible for formation of the open promoter complex. We have obtained evidence that TFIIH is not essential for in vitro transcription of negatively supercoiled DNA templates. Efficient transcription from the adenovirus major-late (ML) promoter is observed with highly-purified transcription factor preparations of TBP, TFIIB, TFIIF, TFIIE and RNA polymerase II in the presence of AMP-PNP substituting ATP. The AMP-PNP analogue can be incorporated into RNA but does not support the DNA helicase activity of TFIIH, since it contains a non-hydrolyzable β–γ bond.
The basal factor TFIIE strongly stimulates transcription of ML-templates in the absence of TFIIH, but TFIIE has a weak effect on MMTV- and IgH transcription. These promoter-specific effects of TFIIE suggest that the function of this factor is not only to allow entry of TFIIH to the preinitiation complex. It may facilitate formation of the open promoter complex in an ATP-independent manner. We are currently examining the influence of these basal factors and of DNA topology on formation of the open complex as assayed by DNA-footprinting using chemical probes specific for single-stranded DNA.

L 140 A ROLE FOR THE N-TERMINAL REGION OF THE HUMAN TATA BINDING PROTEIN IN TRANSCRIPTION

Laszlo Tora, Alain Lescure#, Yves Lutz, Dirk Eberhard°, Xavier Jacq, Ingrid Grummt°, Alain Krol#, Irwin Davidson and Pierre Chambon
LGME du CNRS - U 184 de l'INSERM, Faculté de Médecine,11 rue Humann, 67085 Strasbourg, France; # UPR SMBMR, IBMC du CNRS 15, rue René Descartes, 67084 Strasbourg France. ° Institute of Cell and Tumor Biology, German Cancer Research Center,Heidelberg, Germany.

In eukaryotes, the TATA box-binding protein (TBP) is an integral component of the transcription initiation complexes of all three RNA polymerases. In this study the role of the N-terminal region of human TBP in transcription initiation from TATA-containing and TATA-less RNA polymerase (Pol) II and Pol III promoters, as well as on ribosomal RNA gene transcription directed by Pol I, has been examined using three monoclonal antibodies (MAbs). Each of these MAbs recognizes a distinct epitope located in the N-terminal domain of human TBP. MAb1C2, whose epitope comprises the beginning of the polyglutamine tract, selectively inhibits *in vitro* transcription from TATA-containing, but not from TATA-less promoters, irrespective of whether they are transcribed by Pol II or Pol III. Transcription by Pol I, on the other hand, was not affected by any of these MAbs. In contrast, no transcriptional inhibition from any of these promoters was observed with two other MAbs recognizing epitopes either upstream or downstream of the polyglutamine tract. Interestingly, selective inhibition of transcription from TATA-containing Pol II and III promoters was also observed using a synthetic peptide comprising the epitope of MAb1C2. Order of addition experiments indicated that MAb1C2 and its corresponding epitope peptide did not prevent binding of TBP to the TATA element, nor the formation of the TBP-TFIIA-TFIIB complex, but that they exerted their inhibitory effect on transcription by affecting a subsequent stage of preinitiation complex formation. These data indicate that a region within the N-terminal domain of human TBP may be required for specific protein-protein interactions neccessary for the formation of preinitiation complexes at TATA-containing but not TATA-less promoters.

L 141 BASAL TRANSCRIPTION FACTOR TFIIH CORRECTS THE DNA REPAIR DEFECT IN XP AND TTD, AND IDENTIFIES THESE DISORDERS AS LIKELY TRANSCRIPTION SYNDROMES

AJ van Vuuren, W Vermeulen, L Ma, G Weeda, E Appeldoorn, NGJ Jaspers, D Bootsma, JHJ Hoeijmakers, S Humbert*, L Schaeffer* and J-M Egly*. MGC Dept Cell Biol & Genet, Erasmus Univ. Rotterdam, The Netherlands; *Faculté de Médecine, Strasbourg, France.

Nucleotide excision repair (NER) removes a wide range of lesions, induced by UV-light or chemical agents, in a multi-enzyme reaction. NER defects are associated with heterogeneous disorders, xeroderma pigmentosum (XP), Cockayne's syndrome and PIBIDS (a photosensitive form of trichothiodystrophy). Besides hypersensitivity to UV and progressive neurological dysfunction, an increased risk of skin cancer in XP and developmental abnormalities in CS are clinical hallmarks. The *ERCC3* gene, encoding a protein with helicase activity, has been found to correct the NER defect in XP group B. Unexpectedly, the basal transcription factor BTF2/TFIIH contains a p89 subunit, which is identical to ERCC3.

In various purified BTF2 fractions, perfect parallels are observed between transcription, kinase and helicase activity and XP-B correcting repair activity. Besides NER correction in XP-B, also the repair defect in XP-D and TTD-A can be restored by the same BTF2 fractions. *ERCC2* is identified to correct the defect in XP-D. In normal extracts, all repair and ERCC3 activity are removed by immunodepletion with antibodies against the p62 subunit of BTF2, suggesting that p62 and perhaps the entire BTF2 complex are involved in NER. Expression of an ERCC3 cDNA carrying a mutation in the nucleotide binding domain in normal cells, completely blocks NER and RNA synthesis in a dominant fashion and induced a dramatic collapse of chromatin structure. Altogether, the BTF2 complex is associated with correction of the NER defect in XP-B, XP-D as well as in TTD-A and that it has a dual role in DNA repair and transcription, concluding that NER impairment is not a secondary effect of transcriptional inefficiency. Finally, the atypical clinical symptoms exhibited by the corresponding NER syndromes may be caused by a sublte defect in the transcription function of BTF2, affecting the expression of a specific subset of genes.

L 142 RECONSTITUTION OF THE RAP30/74 COMPLEX USING RECOMBINANT PROTEINS,

Bo Qing Wang, Lei Lei and Zachary F. Burton, Department of Biochemistry and Agricultural Experiment Station, Michigan State University, E. Lansing, MI 48824.

RAP30 and RAP74 are subunits of RAP30/74 (TFIIF; βγ), a general initiation and elongation factor for transcription by RNA polymerase II. cDNAs encoding human RAP30 and RAP74 have been expressed in *E. coli* to produce active proteins (1). RAP30 accumulated in inclusion bodies, was produced very efficiently and purified to near homogeneity. RAP74 was produced as a mixture of full length and NH_2-terminal fragments, which were the result of premature translation termination. Because bacterial ribosomes have difficulty translating the human sequence, portions of the RAP74 gene have been re-coded using codons found in highly expressed *E. coli* genes. Preliminary experiments indicate that this strategy will be successful to construct an improved RAP74 production system. The original human RAP74 cDNA was engineered to attach a COOH-terminal $(histidine)_6$ (H_6) sequence to the protein, and RAP74-H_6 was purified using Ni^{++}-affinity chromatography in 4 M urea. The RAP30/74 complex has been reconstituted by mixing recombinant RAP30 and RAP74 together in buffer containing 4 M urea and then dialyzing to remove urea. RAP30/74 complex prepared by this procedure has comparable transcriptional activity and solubility properties to human RAP30/74. This complex has an apparent native molecular mass of 280 kilodaltons, as measured by gel filtration, a value comparable to values reported in the literature for human TFIIF and rat βγ.

1. Bo Qing Wang, Corwin F. Kostrub, Ann Finkelstein and Zachary F. Burton (1993) Production of human RAP30 and RAP74 in bacterial cells, Protein Expression and Purification **4**, 207-214.

L 143 ANALYSIS OF TRANSCRIPTION INITIATION FROM THE TATA-LESS DNA POLYMERASE ß PROMOTER,

Lisa Weis and Danny Reinberg, Department of Biochemistry, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854

Previous studies in our laboratory and others have indicated that there are multiple pathways of transcription initiation by RNA polymerase II. Transcription initiation via a TATA motif has been well characterized. Alternatively, initiation has also been shown to be mediated through the Initiator element (Inr). Our work with the TATA-less DNA polymerase ß has contributed to what is known about Inr-mediated transcription. The promoter of the ß-pol 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. Mutational analysis has identified two additional cis elements. One, located between -2 and +6, encompasses the transcription start site and is homologous to the Inr element found in the adeno-associated virus p5 promoter. The second is located downstream, from +26 to +31. We have examined the effect these elements have on transcription in vivo and in vitro. We have also shown that the +2 to +6 element binds the transcription factor YY1 and the +26 to +31 element binds LBP-1, which also recognizes the transcription initiation site of the HIV-1 promoter. The way these proteins interact with the general transcription factors to form a transcription competent complex is currently under investigation.

L 144 PURIFICATION OF THE LATE TRANSCRIPTION SYSTEM OF VACCINIA VIRUS, Cynthia F. Wright and Asimina M. Coroneos, Department of Cellular Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306-6000

An extract made from HeLa cells infected with vaccinia virus and harvested late in infection can transcribe viral late genes. We have now resolved this system into four factors, each of which is absolutely necessary for late transcription to occur in vitro. Two of these factors, proteins of 30 and 17 kDa, are encoded by viral intermediate genes. These genes have been cloned into a baculovirus expression vector and the corresponding proteins purified to near homogeneity from infected insect cells. The proteins produced from this recombinant system are able to substitute for their counterparts purified from vaccinia virus-infected cells. The third factor has a sedimentation coefficient consistent with a protein of 30 to 38 kDa. Experimental results suggest that this is a previously unidentified factor encoded by a vaccinia virus early gene. The fourth component of this system is RNA polymerase. We have found that the RNA polymerase packaged in the virion can complement for the RNA polymerase fraction purified from infected cells. It is known that the packaged polymerase contains a submolar amount of an 85 kDa protein that is essential for early gene-specific transcription. Our experimental results suggest that the late transcription system uses a form of the RNA polymerase which is lacking this subunit.

L 145 INHIBITION OF RNA POLYMERASE II PREINITIATION COMPLEX ASSEMBLY BY HUMAN CYTOMEGALOVIRUS IE86 PROTEIN, Jun Wu[1], Osvaldo Flores*, Ray Jupp[1], Richard Stenberg[1], Jay A. Nelson[1] and Peter Ghazal[1]. [1]The Scripps Research Institute, La Jolla, CA 92037, USA. *Tularik, San Francisco, CA 94080.

The human cytomegalovirus major immediate-early gene encodes several protein isoforms which autoregulate their promoter (MIEP). One of these isoforms the IE86 protein, is a DNA-binding protein that represses the MIEP through its cognate recognition sequence (*crs*) located between the TATA box and the initiation site of transcription. Purified IE86 protein was shown to repress MIEP transcription *in vitro*, with nuclear extracts from a variety of cells. Core promoter constructs containing the *crs* were also specifically repressed by IE86 but not by a mutant IE86 protein indicating the general transcription machinery as the target for IE86 repression. Repression of the MIEP by IE86 was shown by two criteria to be dependent on the direct interaction of IE86 with the *crs*-element. Kinetic and template commitment experiments demonstrated that IE86 affects preinitiation complex formation but not the rate of reinitiation. Sarkosyl inhibition experiments further revealed that IE86 was unable to effect repression by either disassembling or preventing the elongation of a preexisting transcription complex. Further, the ability of IE86 to interact with the DNA-binding sub-unit of TFIID was shown not to be required for repression. In a reconstituted *in vitro* transcription system the core promoter of MIEP required the general transcription factors, TBP, TFIIB, TFIIE, and TFIIF/H, but not TFIIA. Wild-type but not a mutant protein of IE86 was shown to repress initiation in the reconstituted system in a *cis*-acting mediated pathway. The biological significance of these results and the precise mechanism by which IE86 represses transcription will be presented.

L 146 IDENTIFICATION OF SUPPRESSORS OF LETHAL SUBSTITUTIONS IN THE CTD OF YEAST RNA POLYMERASE II, Anton Yuryev and Jeffry L. Corden, Howard Hughes Medical Institute and Department of Molecular Biology and Genetics, Johns Hopkins Medical School, Baltimore, MD 21205

A series of lethal substitution mutations were constructed in the C-terminal domain (CTD) of yeast RNA-polymerase II (M.L. West and J. Corden in preparation). These mutations contain a Ser to Ala or Ser to Glu substitutions in every CTD repeat at positions 2 or 5 of the consensus sequence $Y_1S_2P_3T_4S_5P_6S_7$; positions that have previously been shown to be phosphorylated.

We devised a selection system for isolation of suppressors of these lethal CTD mutations in *S. cerevisiae* and isolated suppressors of the following mutations in the CTD: A2(18), CTD with 18 YAPTSPS repeats; A5(15), CTD with 15 YSPTAPS repeats; and E2(15), CTD with 15 YEPTSPS repeats. All of these suppressors are recessive and have a slow growth phenotype when growing with the lethal CTD mutation.

We have also devised a strategy to clone one of the A2(18) suppressor genes using complementation of its recessivity. Using this strategy we isolated a plasmid with a 10 Kb insert from a wild type yeast genomic library. This plasmid complements the A2(18) suppression. Upon deletion and sequencing analysis we have identified a novel gene named *SCA1* (suppressor of CTD-alanine). *SCA1* encodes a 150 KDa protein with no homology to any known protein in the database. The predicted amino acid sequence shows that the SCA1 gene product is highly charged and has a poly-Q stretch. We have determined that the *SCA1* gene is non-essential and located on the right arm of the chromosome IV.

We also have found that the deletion of the SCA1 gene has no detectable phenotype but suppresses the following CTD mutations: A2(18), E2(15) and WT7 (seven YSPTSPS repeats). It does not, however, suppress the A5(15), E5(18) or WT0 CTD mutations.

Our finding suggest that: 1) positions 2 and 5 of the CTD repeat have genetically distinct functions. 2) Substitution mutations in position 2 are genetically equivalent to the truncation of the CTD. Our current efforts are aimed at elucidating the molecular mechanisms of *SCA1* function.

L 147 ASSEMBLY OF RNA POLYMERASE II INITIATION COMPLEXES FOLLOWS A DISCRETE PATHWAY, Leigh Zawel, K. Prassana Kumar, Leung Kim, Alejandro Merino, Jack Griffith and Danny Reinberg, Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854

RNA Polymerase II requires the assistance of six protein factors to specifically initiate transcription: IID, IB, IIF, IIE, IIH and IIJ. Using highly purified and recombinant general transcription factors (GTF's), we have reconstituted RNA Polymerase II transcription *in vitro* to high efficiency. The pathway through which the GTF's and RNAPII assemble at a core promoter has been extensively characterized using a variety of approaches, including gel mobility shift assay, template competition and transcription from solid supports. Our studies indicate that the assembly of the initiation complex follows a rigid and precise pathway. We have gone on to examine, by chemical footprinting and electron microscopy, several of the intermediates formed during complex assembly. We find the intermediates DAB, DBPolF and the complete intiation complex are characterized by distinct protein-DNA contacts and gross physical structures. Furthermore, that the RNAPII appears to make a contact with the DNA downstream of the start site, at around +10. The use of immobilized DNA templates has allowed us to isolate complexes free of contaminants and to study the factor composition. In agreement with our complex assembly model, we find the complete complex contains TBP, IIB,RNAPII, IIF, IIE and IIH. The last factor thought to enter the intiation complex is TFIIJ. We find that synthesis of long transcripts, >400nt, is dependent on IIJ whereas synthesis of shorter transcipts is stimulated by TFIIJ. Further experiments indicate that TFIIJ is an elongation factor as it is (a) capable of associating with the initiation complex after formation of the first phosphodiester bond in the nascent RNA, (b) it increases the amount of full length RNA generated from synthetic DNA templates while concomittantly reducing the number of shorter, stalled RNA intermediates. TFIIJ has been purified to apparent homogeneity.

Activation I

L 200 The Hepatitis B Virus X Protein Targets the bZip Domain of CREB. Ourania M. Andrisani and John S. Williams. Dept. of Physiology and Pharmacology, School of Veterinary Medicine, Purdue University, W. Lafayette, IN 47907

The X protein of Hepatitis B virus, pX, is a promiscuous transactivator of viral and cellular genes. Although pX does not bind DNA, pX-responsive elements include the NF-κB, AP-1 and CRE sites. The transcription factors CREB and ATF interact directly with pX, altering their CRE-specific binding.

Here we examine the mechanism of the interactions between CREB and pX. We demonstrate that pX interacts with the bZip domain of CREB. This interaction increases the affinity of CREB for CRE by one order of magnitude. Methylation interference footprinting reveals differences between the CREB-DNA and CREB/pX-DNA complexes. However, the CREB/pX complex employs the same CREB determinants in CRE recognition. Transfection assays, using the CREB-dependent somatostatin promoter demonstrate a 8-fold transcriptional induction in the presence of pX, supporting the significance of these interactions *in vivo*. Thus the CREB/pX system is a unique example of auxiliary protein-protein interactions targeting the DNA binding domain of a cellular transcription factor.

L 201 THE *EVEN-SKIPPED* PROTEIN CONTAINS A REPRESSION DOMAIN AND CAN REPRESS TRANSCRIPTION IN A PURIFIED IN VITRO SYSTEM, Richard J. Austin and Mark D. Biggin, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520

The Drosophila developmental control gene *even-skipped* (*eve*) has previously been shown to repress transcription in vitro. To determine which regions of *eve* are important for repression, different portions of the *eve* protein have been fused to a heterologous DNA binding domain. These experiments have shown that CDGR, a protein containing the C-terminal half of *eve* (residues 140-376) fused to the glucocorticoid receptor DNA binding domain, can repress transcription in vitro from a basal promoter that contains glucocorticoid receptor binding sites.

To determine the mechanism by which the CDGR protein represses transcription, we have generated an in vitro transcription system containing purified fractions of the RNA Polymerase II transcription factors. Fractions of TFIIH, TFIIF, TFIID, and RNA Polymerase II (Pol II) were purified from Drosophila embryo extracts by column chromatography. Drosophila TFIIB and TBP and human TFIIE were obtained by expressing recombinant protein in E. coli. We have shown that the CDGR protein can repress transcription in our purified system using either TFIID fraction or TBP. We are now performing experiments to understand how the CDGR protein inhibits the action of the general transcription factors.

L 202 IN VITRO ANALYSIS OF THE CHICK BETA-GLOBIN LOCUS ASSEMBLED INTO SYNTHETIC NUCLEI: CHROMATIN RECONSTITUTION, DNA REPLICATION AND TRANSCRIPTION, Michelle Craig Barton and Beverly M. Emerson, Regulatory Biology Laboratory, The Salk Institute, 10010 N. Torrey Pines Road, La Jolla, CA 92037

The chick ß-globin gene locus is comprised of multiple genes that are expressed within erythroid cells at either embryonic or definitive stages of development. Changes in chromatin structure that are characteristic of the actively expressed gene are correlated with the interaction of multiple tissue-specific and developmental stage-specific proteins with the promoter and enhancer regulatory elements. Using in vitro chromatin assembly and transcription with staged erythroid extracts, we have previously determined the critical proteins required to activate expression of nucleosome reconstituted adult ß-globin genes. We found that the developmental regulation of ß-globin expression is achieved, in part, by the requirement of an erythroid protein, cGATA-1, and a stage-specific factor, NF-E4, rather than TBP, to activate chromatin through a specialized TATA box. We now extend these analyses of chromatin-assembled templates to encompass the regulation of the ß-globin gene within the entire globin locus. In addition, we can assemble the 35 kb locus template into synthetic nuclei by adding the membrane vesicular fraction of a Xenopus egg extract to DNA assembled into chromatin by the soluble fraction source of histone and nucleosome assembly factors. The formation of nuclear membrane structures is accompanied by a single round of semi-conservative DNA replication. By coupling chromatin assembly, double-stranded DNA replication and in vitro transcription in a step-wise manner, we have developed a system which allows the restructuring of chromatin in vitro. Introduction of activator proteins during DNA replication results in a developmental-like switch of expression from transcriptionally silent, closed chromatin to actively expressing open chromatin structures.

L 203 ADA2 FUNCTIONALLY INTERACTS WITH THE HERPES VIRUS VP16 TRANSCRIPTIONAL ACTIVATION DOMAIN AT BINDING SITES DISTINCT FROM THE ACTIVATION SUBDOMAINS, Shelley L. Berger, Paula Darpino, Reyes Candau, and Nickolai Barlev Wistar Institute, Philadelphia, PA 19104

Transcriptional activation requires communication of signals from *trans*-activator proteins to the general transcriptional machinery. This pathway involves contacts between *trans*-activators bound at upstream sites and general factors bound close to transcriptional start sites. A third class of factors, termed adaptors or coactivators may physically bridge interactions between upstream activators and the basal apparatus. We are characterizing two components (ADA2 and ADA3) of a potential adaptor complex required for activity of the herpes simplex virus *trans*-activatorVP16.

We have analyzed the acidic activation domain of VP16 as a fusion with the DNA binding domain from yeast GAL4, to identify a region required for transcriptional activation dependent on the adaptor. In vivo and in vitro analyses in S. cerevisiae indicated that there are two potent activation subdomains, but only one domain is strongly dependent on ADA2. First, we prepared a deletion series through VP16 and detected two activation domains that had strikingly different requirements for ADA2 in vivo. One domain within the amino-terminal region ($VP16_N$: amino acids 413-470) was completely dependent upon ADA2 for activity. A shorter version of $VP16_N$ (including amino acids 413-456) eliminated transcription in vivo, although this region contains a strong activation domain apparent in vitro (see below). In contrast, a second activation domain within the carboxyl-terminal region ($VP16_C$: amino acids 452-490) was nearly independent of ADA2 in vivo.

Second, all three regions $VP16_{FL}$ (413-490), $VP16_N$ (413-456) and $VP16_C$ (452-490) have potent activity in vitro. However, their activities are sharply dissimilar in an in vitro squelching assay of a heterologous *trans*-activator, where both $VP16_{FL}$ and $VP16_C$ selectively inhibited activated transcription by the heterologous *trans*activator, while $VP16_N$ did not. This suggested that $VP16_{FL}$ and $VP16_C$ both contain a binding site for a diffusable intermediary factor (i.e. the adaptor) required for activation by the heterologous *trans*-activator (and therefore presumably VP16 itself), whereas $VP16_N$ does not.

These data suggest a model where there are three functional domains within VP16: two activation domains at the N and C termini and a third domain to which the adaptor binds, located between amino acids 452 and 470. The N terminal domain is completely dependent on the adaptor for activity, while activation by the C terminal domain is relatively independent.

L 204 C-TERMINAL TRUNCATION OF RXRβ INHIBITS RETINOIC ACID INDUCED TRANSCRIPTION IN EMBRYONAL CARCINOMA CELLS

Jorge Blanco*, James Segars+, Jeffrey Medin*, Saverio Minucci* and Keiko Ozato*. *Laboratory of Molecular Growth Regulation and +Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda MD 20892.

Retinoid X receptors (RXRs) belong to the steroid/thyroid hormone receptor superfamily that mediate hormone dependent transcription. Members of this superfamily, retinoic acid (RA) receptor (RAR), thyroid hormone receptor, vitamin D receptor and peroxisome proliferator activated receptor have been shown to bind more efficiently to their respective response element and to regulate their transcription when they form heterodimers with RXRs than when they act alone. RXRs are also able to act as homodimers and upregulate CRBPII gene transcription through the RXR responsive element (RARE) when bound to its specific ligand, 9-cis RA (9cRA). In transient transfection analysis we found that deletion of 20 amino acids at the carboxyl terminus of the mouse RXRβ totally abolishes 9cRA activation of RXRE-Tk luc reporter in embryonal carcinoma cells. Similarly, this RXRβ deletion mutant blocked RA activation of βRARE-Tk luc reporter activity by about 90%. These data indicate that this mutant behaves as a powerful dominant negative receptor, and that the C-terminal amino acids play an important role in transcriptional activation function. Biochemical analysis indicates that this deletion mutant is capable of binding to 9cRA and to undergo a conformational change. These data suggest that ligand binding and transcriptional activation are dependent on separate domains. On the other hand, this domain is well conserved among all three isoforms of RXRs, and has the potential to fold in an α helix motif, with hydrophobic residues along one side of the interface and charged residues on the other. This structure is somewhat similar to acidic domains of several transcriptional activators, suggesting possible significance in the functionality of such domains.

L 205 BINDING OF NF-1 TO A NUCLEOSOMAL TARGET. Patrik Blomquist, Qiao Li and Örjan Wrange, Laboratory of Molecular Genetics, Department of Cell and Molecular Biology, Karolinska Institutet, S-171 77 Stockholm, Sweden

In the eukaryotic nucleus the transcription factors interact with DNA organised into chromatin rather than naked DNA. In recent years it has become evident that the local chromatin organisation can influence transcriptional activation. One of the most studied promoter is the Mouse Mammary Tumour Virus promoter, or the MMTV promoter. Part of the DNA in this promoter is organised into six phased nucleosomes. One of this nucleosomes contains binding sites for the Glucocorticoid receptor (GR) and Nuclear Factor 1 (NF-1). When GR binds to the DNA an alteration in this nucleosome is seen and binding of NF-1 can be detected. Experiments have suggested that one effect of GR in the MMTV activation is to change the nucleosomal structure so that NF-1 can bind. This does not exclude that GR also have more direct effects on MMTV activation.

Our investigation is focused on how the translational position of the nucleosome influence the binding of NF-1 to its target sequence. We address this question by reconstituting nucleosomes with an NF-1 binding site in a specific rotational and translational position. The position is determined by synthetic DNA bending sequences. The affinity of NF-1 for different positions is then revealed by gel-retardation assay. In this progress report we present the data obtained so far.

L 206 THE RETINOBLASTOMA PROTEIN HAS TRANSCRIPTIONAL REPRESSOR ACTIVITY THAT IS INDEPENDENT OF ITS INTERACTION WITH E2F, Kevin N.B. Chow, S.J. Weintraub, and D. Dean, Department of Internal Medicine, Washington University Medical Center, Saint Louis, Missouri 63110

The retinoblastoma protein (Rb) regulates progression through the cell cycle at least in part through its cell cycle–dependent interaction with the *trans*–activating protein E2F. Binding of Rb to E2F is controlled by the phosphorylation/dephosphorylation cycle of Rb that occurs with progression through the cell cycle. The dephosphorylated form of Rb binds to the transactivating domain of E2F and it had been thought that, when bound, Rb simply blocks *trans*–activation by E2F. However, we recently found that in the presence of Rb, E2F sites switch from transcriptional activators to silencers (Weintraub, et al., *Nature* 358, 259–261)—suggesting that interaction of Rb with E2F does not simply inactivate E2F, but results in the formation of an active transcriptional repressor complex.

To further understand the mechanism of transcriptional repression mediated by E2F sites in the presence of Rb, we have constructed vectors that express chimeric proteins in which either full length Rb or isolated regions of Rb are linked to the DNA binding domain of the yeast transcription factor Gal4, facilitating the tethering of Rb and specific regions of Rb to the promoter in an E2F independent fashion. Using these constructs we found that: 1. Rb has transcriptional repressor activity that is independent of its interaction with E2F. 2. Distinguishable domains of Rb are required for binding E2F and for repression.

L 207 A 5' ELEMENT OF THE CHICKEN β-GLOBIN DOMAIN SERVES AS TRANSCRIPTIONAL INSULATOR IN HUMAN ERYTHROID CELLS AND PROTECTS AGAINST POSITION EFFECT IN DROSOPHILA, Jay H. Chung, Mary Whitely and Gary Felsenfeld, Laboratory of Molecular Biology, National Institutes of Health, NIDDK, Bethesda, MD 20892

We have characterized an element near the 5' boundary of the chicken β-globin domain that insulates a reporter gene from the activating effects of a nearby β-globin locus control region(5'HS2) when assayed in the human erythroid cell line K562. We show that the insulation mechanism is directional, that it involves the alteration of chromatin structure over the promoter of the gene. The insulator has no significant stimulatory or inhibitory effects of its own. In transgenic Drosophila, the insulator protects the *white* minigene from position effects. The action of the insulator thus is not restricted to erythroid or mammalian cells, suggesting that such elements may serve an important and widely distributed function in the organization of chromatin structure. Furthermore, such insulating elements should be useful in protecting a gene of interest in gene therapy or transgenic mice.

L 208 **CHARACTERIZATION OF A NOVEL TRANSCRIPTIONAL REPRESSION DOMAIN.**

Ian G. Cowell* & Helen C. Hurst*, *Department of Biochemistry & Genetics, University of Newcastle Upon Tyne NE2 4HH. U.K. and *ICRF Oncology Group, Hammersmith Hospital, DuCane Road, London W12 OHS U.K.

We have previously shown that the bZIP protein E4BP4 is an active transcriptional repressor. Deletion studies showed that a discrete repressing activity resides in the C-terminal third of the protein. In this study we have mapped the repressing activity of E4BP4 to a small "domain" lying between residues 299-363. This 65 amino acid segment maintained repressing activity when transferred to the heterologous DNA binding domain of the yeast transcriptional activator GAL4. This segment of the E4BP4 polypeptide contains a high proportion of charged amino acids (26%) and a potential amphipathic α-helical sub-region. This structure does not resemble the repression domains that have been characterised so far from other active transcriptional repressors such as the *Drosophila* krüppel, engrailed or even-skipped proteins which are rich in alanine, glutamine or proline residues and which contain few charged amino acids. We therefore propose that E4BP4 represents a new class of transcriptional repressor. We are currently testing point mutants to determine key features of the E4BP4 repression domain. E4BP4 represses activated and basal transcription suggesting that it targets some component of the general transcription machinery rather than causing repression by inhibiting the transcriptional activation potential of one or more promoter-bound transcription factors. We will describe experiments to elucidate the mechanism of repression by E4BP4.

L 209 FUNCTIONAL SPECIFICITY OF PROMOTER SELECTIVE ACTIVATION DOMAINS. G. Das, C. Hinkley, M. Tanaka, and W. Herr, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

Transcriptional activators regulate transcription by communicating with the basal transcription machinery. We have previously shown that "promoter selective" activation domains within the POU homeodomain proteins Oct-1 and Oct-2 are able to preferentially activate the U2 snRNA or an mRNA promoter, respectively (Tanaka et al., 1992, Cell 68: 755-767). Here, we have tested whether other activation domains also display promoter-selective properties on a larger set of promoters. We tested promoters of three different types of genes: the c-fos gene, a typical mRNA-type gene transcribed by RNA polymerase II; the U2 snRNA gene also transcribed by RNA polymerase II; and the U6 snRNA gene, which is transcribed by RNA polymerase III. Though both the c-*fos* and U2 genes are transcribed by RNA polymerase II, their promoter sequences differ. The *fos* promoter contains a typical TATA box sequence whereas the U2 promoter has, instead of a TATA box, an snRNA gene-specific proximal sequence element called the PSE. The U6 gene has both an mRNA-type TATA box sequence and an snRNA-specific PSE element, which together paradoxically result in RNA polymerase III transcription.

The ability of various activation domains fused to the Gal-4 DNA-binding domain (Gal1-94) to transactivate these promoters containing Gal-4 binding sites was tested in mammalian cells in a transient expression assay. The response profile of the c-*fos* promoter to the various activators differed from that of the U2 and U6 snRNA promoters, which responded similarly, suggesting that the activation domains are distinguishing between the presence or absence of the snRNA-specific PSE element rather than the presence or absence of a TATA box or the type of RNA polymerase used for transcription. These studies also revealed three classes of promoter-selective activation domains: (i) those that activate both mRNA and snRNA promoters (e.g., Sp1 and Oct-2-derived glutamine-rich activation domains); (ii) those that activate an mRNA but not an snRNA promoter (e.g., the VP16 activation domain); and (iii) those that activate snRNA promoters but not an mRNA promoter (eg., an Oct-1-derived activation domain). Curiously, a point mutant of the Oct-2-derived activation domain was debilitated only for mRNA and not for snRNA promoter activation. These results emphasize the different promoter-selective activation domain requirements for stimulation of different promoters.

L 210 **THE ZINC FINGER REGION OF THE E1A ACTIVATION DOMAIN BINDS TO THE TATA BOX-BINDING PROTEIN,**

Joseph V. Geisberg[1], Wes S. Lee[2], Arnold J. Berk[2], and Robert P. Ricciardi[1]. [1]Department of Microbiology, The University of Pennsylvania School of Dental Medicine, Philadelphia, PA. 19104, and [2]Department of Microbiology, University of California at Los Angeles, Los Angeles, CA. 90024.

The Adenovirus 289R E1A is a potent transcriptional activator of many viral and cellular promoters. A direct interaction between the E1A activation domain and TBP has been previously demonstrated and is believed to play a key role in the mechanism of E1A-mediated transactivation. To identify the residues of the E1A activation domain that may be involved in contacting TBP, we co-immunoprecipitated bacterially purified human TBP with a series of *in vitro* translated E1A proteins containing single amino acid substitutions in each of the 46 amino acids of the E1A activating domain. In general, there was an excellent correlation between the E1A mutants' abilities to bind to TBP *in vitro* and to activate transcription *in vivo*, strongly arguing that the direct interaction between TBP and E1A is critical for E1A-mediated activation. Specifically, we found that TBP binds to a region of the E1A activating domain that contains a single Cys_4 zinc finger. The carboxyl region of the E1A activating domain, which was thought to bind a factor distinct from TBP, is dispensable for this interaction. A group of five E1A mutants provides strong evidence that while TBP binding to E1A is absolutely required for E1A transactivation, it alone is not sufficient. These results implicate the involvement of an additional factor in mediating activation by E1A. Evidence will be presented which may reveal the possible identity of this factor.

L 211 MOLECULAR INTERACTIONS BETWEEN THE GCN5 AND ADA2 CO- ACTIVATORS FOR FULL TRANSCRIPTIONAL ACTIVATION BY GCN4. Tassos Georgakopoulos and George Thireos, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, PO Box 1527, Heraklion 71110, Crete, Greece.

Two distinct transcriptional activators, the GCN4 and the HAP2/HAP3/HAP4 heterotrimer, require the function of the GCN5 protein to promote normal levels of transcription. This protein shares a similar region, the bromodomain, with other yeast, Drosophila and human proteins (1). We have demonstrated that this domain is necessary for the function of GCN5. Transcriptional activation by the GCN4 requires also the mediation of the ADA2 protein (2). The absence of both GCN5 and ADA2 products affects the transcriptional activation by the GCN4 to the same extend as the absence of each one independently. This suggest that GCN5 and ADA2 participate in the same pathway which mediates full transcriptional activation.

LEXA- GCN5 fusion proteins can direct transcriptional activation through a LEXA operator. This property does not depend on the bromodomain but requires both ADA2 and GCN4 proteins. These results suggest that GCN5 interacts with both GCN4 and ADA2. This possibility is presently being tested biochemically.

1. Georgakopoulos, T. and Thireos, G. (1992) *EMBO J.* 11, 4145 - 4152.
2. Berger, S. L. et al. (1992) *Cell* 70, 251 - 265.

L 212 PHOSPHORYLATION BY ERK REGULATES TERNARY COMPLEX ACTIVITY IN RESPONSE TO MITOGENIC SIGNALS. Hendrik Gille, O. Thomae, Melanie H. Cobb* and Peter E. Shaw, Max-Planck-Institut für Immunbiologie, Spemann Laboratories, Stübeweg 51, D-79108 Freiburg, Germany. *UT Southwestern Medical Center, Dallas, TX 7235-9041, USA

The *ets* protein p62^{TCF}/ELK-1 forms a ternary complex over the *c-fos* promoter whith a dimer of SRF and the serum response element, SRE. Binding of p62^{TCF} correlates with the responsiveness of the *c-fos* proto-oncogene to proliferative signals. p62^{TCF} and ELK-1 are substrates for the extracellular signal-regulated kinases ERK1 and ERK2, which are activated with rapid kinetics after stimulation of cells with mitogens. Phosphorylation of p62^{TCF} results in enhanced ternary complex formation *in vitro* and this change is paralled by an increase of TCF activity in nuclear extracts from growth factor stimulated cells. We have determined the ERK1 phosphorylation sites in ELK-1. High affinity ELK-1 binding *in vitro* and *c-fos* transcriptional activation in NIH3T3 cells depends on the phosphorylation status of a subset of these sites, which are also phosphorylated upon growth factor stimulation in intact cells.

L 213 MULTIPLE SEQUENCE-SPECIFIC INTERACTIONS CONTRIBUTE TO THE ASSOCIATION BETWEEN TFIID AND PROMOTERS IN DROSOPHILA, David S. Gilmour, Beverly A. Purnell, Peter A. Emanuel, Michael A. Sypes and Janet A. Weber, Department of Molecular and Cell Biology, Pennsylvania State University, University Park, PA 16802

The interaction of Drosophila TFIID with the hsp70 promoter depends on sequence-specific contacts in regions that include the TATA box, the transcription start and positions 18 and 28 nucleotides further downstream from the start. These interactions were detected by a "missing nucleoside" binding assay involving TFIID that had been immunopurified from Drosophila embryos. Mutations in these regions of the promoter also reduced the affinity of TFIID for the promoter. The preferred sequence at the transcription start was identified by binding TFIID to a pool of hsp70 promoters that contained random nucleotides in six positions surrounding the start. The preferred sequence was an excellent match to a previously identified Arthropod initiator element, and the two most highly preferred nucleotides in this region matched the most important residues in a mammalian initiator element. These results suggest that the initiator functions as a recognition element for TFIID in a wide spectrum of genes. Missing nucleoside analyses of the hsp26 and histone H3 promoter support this conclusion. The contacts that occur further downstream of the start site in other genes could also function as recognition elements for TFIID. Analysis of the TATA-less promoter for the Drosophila vermilion gene showed that nucleotides within the region from +19 to +36 were essential for TFIID-binding. While the contacts of TFIID downstream of the TATA element are detected in vitro, genomic footprinting analysis of a transformed hsp70 promoter only revealed evidence for contacts around the TATA element. Moreover, the region downstream of the transcription start was occupied by a paused RNA polymerase. In vitro crosslinking analysis of the TFIID/promoter complex has provided a possible explanation for how the interactions of TFIID and RNA polymerase II could be integrated in vivo. Subunits of TFIID crosslink to positions downstream of the transcription start while the TBP-subunit crosslinks at the TATA element. Elevating the salt concentration caused a preferential disruption of the downstream contacts on the promoter leaving the contacts in the TATA region mostly undisturbed. In vivo, the initiation complex could displace the downstream contacts of TFIID, leaving TFIID attached to the promoter by the TBP-subunit. The crosslinking analysis also identified a potential TFIID contact in vitro that occurred downstream of the region where RNA polymerase II pauses in vivo. This contact might participate in the mechanism that pauses RNA polymerase II on the hsp70 promoter.

L 214 IN VITRO REGULATION OF BASAL AND ACTIVATOR-DEPENDENT TRANSCRIPTION BY REPRESSORS, Andreas Goppelt, Gertraud Stelzer and Michael Meisterernst, Lab. for Molecular Biology, Genzentrum, LMU Munich, 82152 Martinsried, Germany

We have previously shown that negative cofactors (repressors) and positive cofactors in concert elevate activator-dependent transcription in vitro. In this study multiple defined repressors (histone H1, the NC1 component HMG2, NC2 and Topoisomerase I) were analyzed in a reconstituted class II gene transcription system. HMG2 and histone H1 both functioned cooperatively with a positive stimulatory activity (PC2) to enhance activator-dependent transcription. Histone H1 effects were mediated through DNA-binding domains of the GAL4 protein apparently involving an antirepression mechanism. In contrast, Topoisomerase I, which was cloned from a general cofactor fraction (USA), combined properties of both repressors such as histone H1 and stimulating cofactors such as PC2 since it repressed basal - and stimulated activated transcription. A distinct mechanism of activation by Topoisomerase I was further indicated by the observation that this bifunctional factor acted preferentially though the cryptic activation region located between aa 94 and 147 of GAL4.

Finally a previously defined repressor, NC2, was further purified and a possible relationship to the repressor Dr1 was investigated. With antibodies Dr1 was detected in both NC2 fractions and in TBP-NC2 promoter complexes, suggesting that Dr1 is a component of NC2. However, bacterially expressed Dr1 could not quantitatively replace NC2 in binding to TBP-promoter complexes and in basal repression. Experiments addressing the question whether NC2 function involves a modified Dr1, additional and/or alternative components and a functional analysis of NC2 in the context of an affinity purified TFIID complex will be presented.

L 215 A DYNAMIC INTERPLAY OF UPSTREAM FACTORS UNDERLIES THE NUCLEOSOME-FREE ENVIRONMENT OF THE YEAST HSP82 GENE PROMOTER, David S. Gross, Serena F. Simmons, and Tuba Diken, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, LA 71130

Heat shock genes are poised for instantaneous transcriptional activation in response to environmental stress. Facilitating this hair-trigger response is the presence of DNase I hypersensitive, nucleosome-free regions over the promoters of such genes. To identify biochemical determinants potentiating the *HSP82* heat shock promoter in *Saccharomyces cerevisiae*, we have introduced *in situ* mutations within sequences shown by genomic footprinting to be engaged in constitutive protein-DNA interactions *in vivo*. A critical role for heat shock factor (HSF) in displacing stably positioned nucleosomes over both UAS and TATA-initiation sites is indicated by two lines of evidence: [1] *In situ* deletion or substitution of the preferred HSF binding site, HSE1, leads to a dramatic transition in *HSP82* chromatin structure, as the 5' DNase I hypersensitive region is replaced by two stable, sequence-positioned nucleosomes. One of these is centered over the mutated heat shock element, while the other is rotationally positioned over the core promoter. [2] Fifteen- to 30-fold overexpression of yeast HSF results in a dramatic re-instatement of DNase I hypersensitivity and disruption of both promoter-associated nucleosomes within *hsp82*-ΔHSE1. Such suppression of the mutant phenotype is mediated through sequence disposed immediately upstream of HSE1 and containing two low affinity heat shock elements (Gross *et al*, *EMBO J.* 12: 3931-3945, 1993). That HSF may work in concert with distal factors in establishing the gene's potentiated state is indicated by phenotypes of *hsp82* alleles containing just a double point mutation in HSE1, just a deletion of far upstream sequences, or a combination of the two. While either mutation by itself confers no discernable alteration of chromatin structure, the double mutant appears to be more severely affected than the allele containing a 32 bp deletion in HSE1 (*hsp82*-ΔHSE1). Current work is focused on identifying these far upstream factors, as well as defining the *in vivo* role of the Swi1 and Swi2 proteins in establishing and maintaining the nucleosome-free environment over the *HSP82*$^{+}$ promoter.

L 216 THE DNA BINDING SITE SPECIFICITY OF BZIP TRANSCRIPTION FACTORS IS DETERMINED BY A COMBINATORIAL CODE THAT INVOLVES BOTH THE BASIC AND FORK REGIONS.
Naomi B. Haas, Peter F. Johnson* and John B.E. Burch. Fox Chase Cancer Center, Philadelphia, PA 19111 and *ABL-Basic Research Program, NCI-FCRDC, Frederick, MD 21702

Although crystal structures have been determined for GCN4-DNA complexes, it is still not clear how different bZIP transcription factors are able to recognize different binding sites since the amino acid residues that were shown to make base specific contacts in the GCN4-DNA complexes are present in the majority of bZIP transcription factors, including the drosophila giant protein and members of the related PAR family in vertebrates (DBP, VBP/TEF and HLF). Since the basic and fork regions of giant differ from the PAR bZIP factors in only five positions, we sought to determine which of these residues are critical for discriminating between VBP and giant binding sites. We found that the substitution of a single giant residue in the basic region of VBP was sufficient to allow recognition not only of giant sites but also CREB/ATF and C/EBP consensus binding sites. On the other hand, giant residues in the fork region of VBP did not allow recognition of giant, CREB/ATF or C/EBP sites. Quite unexpectedly, however, these fork region substitutions conferred the ability to discriminate between two VBP sites that differ only in the +5 and -5 positions of the palindrome. Whereas fork regions have previously been implicated as determinants for discriminating between overlapping and abutted versions of palindromic sequences, none of the fork region substitutions that we tested allowed recognition of overlapping palindromic sequences. We are currently using an optimal site selection protocol to address whether sequence differences in the fork region may also be involved in discriminating between target sequences that differ at positions other than +5 and -5.

L 217 **COMMON STRUCTURAL ELEMENTS IN THE VP16 ACTIVATION DOMAIN MEDIATE INTERACTION WITH TBP AND RPA,** John A. Hassell, Steven J. Triezenberg*, Bradford Brinton, and Paul Desjardins, Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario. *Department of Biochemistry, Michigan State University, East Lansing, Michigan. Analyses of polyomavirus DNA replication have revealed that transcription activators are involved directly in this process. Studies of GAL4 and GAL4-chimeras demonstrate that both the DNA binding and transcription activation domains are required for DNA replication. However, there is little evidence to support the notion that the very same domain mediates both processes because large regions bearing activation domains were used in these studies. To clarify this and to learn whether common or different structural elements in the activation domains are required for these activities, we analyzed the capacity of GAL4-VP16 chimeras and of transcriptionally-compromised derivatives thereof to activate transcription and replication of a common reporter plasmid in mouse cells. We observed that mutants impaired in their capacity to activate transcription were similarly affected in their capacity to augment DNA replication. Recently, we (He et al., Cell 73:1223-1232, 1993) and Li and Botchan (Cell 73: 1207-1221, 1993) discovered that the activation domain of VP16 binds to replication protein A (RPA), a cellular single-stranded DNA binding protein comprising three different subunits. The largest subunit binds directly to the activation domains of VP16, p53 and GAL4. In consequence we tested the capacity of GST-VP16 fusion proteins bearing the mutations for their capacity to bind to RPA, TBP and TFIIB, two components of the basal transcriptional machinery known to physically interact with VP16. Whereas all the mutants tested bound to TFIIB with the same affinity as wild type, each of the mutant derivatives bound to RPA and TBP with reduced affinity. There was a direct correspondence between the capacity of the mutants to activate transcription and DNA replication and their ability to bind to RPA and TBP. These results suggest that the same or closely-overlapping domains in VP16 mediate contact with TBP and RPA, and that these interactions are critical for the activation of transcription and DNA replication.

L 218 DETAILED MUTAGENESIS OF A REITERATED 18 AMINO ACID GLUTAMINE-RICH ACTIVATION DOMAIN, Craig S. Hinkley, Masafumi Tanaka, and Winship Herr, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

Transcription factors exhibit a remarkably modular structure consisting of both DNA-binding and transcriptional activation domains that can be separated functionally. Individual mutations within DNA-binding domains often have very dramatic effects on the ability of the protein to bind DNA. In contrast, transcriptional activation domains are relatively insensitive to individual mutations. We are using the ability to create activation domains by reiteration of short peptide sequences to study both the structure and function of a glutamine-rich activation domain derived from the POU-domain transcription factor Oct-2. One advantage of a reiterated activation domain is that the effects of individual mutations are amplified by the sequence reiteration. Previous work in our laboratory has shown that, when multimerized, an 18 amino acid subdomain of the Oct-2 N-terminal activation domain, called Q^{18}, can activate mRNA-type transcription in vivo and in vitro. Therefore, we examined the effects of individual substitution of each residue within the Q^{18} activation domain for an alanine residue.

The wild type and mutated segments were assayed as four tandemly reiterated copies fused to the Gal4 DNA-binding domain (Gal4-4XQ^{18}) after transient expression in HeLa cells. Wild type Gal4-4XQ^{18} activates transcription from a c-Fos promoter containing four Gal4 DNA binding sites approximately 30-fold to 100-fold greater than the Gal4 DNA-binding domain alone. Surprisingly, the results using the alanine-substituted mutants indicate that most of the amino acids in the Q^{18} sequence, including glutamine residues, are involved in transcriptional activation. A few of the alanine-substituted mutants, including a substituted phenylalanine residue, have a very dramatic effect on mRNA promoter activation. These results indicate that the overall intergrity of an activation domain can be critical for its function. Curiously, as shown by Das et al (see abstract for this meeting), alanine substitution of this phenylalanine residue enhances rather than suppresses the ability of the Q^{18} activation domain to activate an snRNA promoter.

L 219 A SPECIFIC "SYNERGY" DOMAIN WITHIN PIT-1 COORDINATES SYNERGISTIC TRANSACTIVATION OF THE PROLACTIN GENE WITH ESTROGEN RECEPTOR. Jeffrey M. Holloway, Daniel Szeto, and Michael G. Rosenfeld. Eukaryotic Regulatory Biology program, University of California, San Diego, HHMI, La Jolla, California 92093-0648.

Eukaryotic gene regulation involves complex organization of transcription factors to attenuate levels of gene transcripts. The pituitary specific transcription factor Pit-1 contains a N'-terminal transcriptional activation (tau) domain involved in activation of both the prolactin and growth hormone genes. In the case of the prolactin gene, full stimulation is dependent on an enhancer region located 1.6 kb downstream from the start of transcription that is also recognized by estrogen receptor, leading to synergistic activation of the gene. The mechanism by which ER and Pit-1 synergize was investigated using a number of methods. Our data indicate that synergism involves a specific "synergy" domain within Pit-1 that is necessay for synergism, but not basal transactivation. In addition, Pit-1 and estrogen receptor interact directly with each other, however this interaction does not appear to effect DNA binding affinities. Further studies show that interactions of ER with the core machinery factors TBP and TFIIb may contribute to the synergistic activation of the prolactin gene.

L 220 **ANALYSIS OF THE INTERACTION BETWEEN TBP AND THE TRANSCRIPTIONAL REPRESSOR Dr1.** J.A Inostroza, K.C. Yeung, F.H. Mermelstein C. Kannabiran and D. Reinberg. Dept. of Biochemistry, UMDNJ-Robert Wood Johnson Medical School 08854.

Initiation of transcription by RNA polymerase II is under the control of both positive and negative regulatory factors. We have identified and characterized a repressor factor termed Dr1. Dr1 interacts with the TATA-binding protein (TBP) within the TFIID complex. As a result, Dr1 represses transcription as measured*in vitro* using a reconstituted transcription system as well as *in vivo* by cotransfection of various promoters with Dr1. Repression can be overcome by overexpression of hTBP, but not TFIIB. To determine the domains of Dr1 important for its interaction with TBP, a series of deletion mutants representing N-and C-teminal truncations for both factors were generated and analyzed *in vitro* by affinity chromatography and *in vivo* by the yeast two-hybrid system. A 22-amino acid sequence within an amphipathic alpha helical region of Dr1 was shown to be required for its interaction with TBP. Moreover, a peptide encompassing this region fused to GST bound TBP specifically. The TBP binding sequence of Dr1 was essential for repression of transcription in both cotransfection assays and in the reconstituted system. Also, the C-terminal alpha helical region rich in glutamine and alanine residues was required for repression *in vitro*. Analysis of domains in TBP required for interaction with Dr1 include the first repeat and the basic region. Interestingly, TBP's basic region is also essential for its interaction with TFIIA and the activators VP16 and E1A. In conclusion, our data suggests that transcriptional repression by Dr1 is mediated through two mechanisms: Dr1 precludes the formation of a competent preinitiation transcription complex by interacting directly with TBP and secondly, through the C-terminal alpha helical region of Dr1.

L 221 INTERACTION BETWEEN THE HIV1 TAT TRANSACTIVATOR AND THE TATA-BOX BINDING PROTEIN, Pierre Jalinot, Philippe Veschambre and Pascale Simard, Laboratoire de Biologie Moléculaire et Cellulaire, CNRS UMR 49, Ecole Normale Supérieure de Lyon, 46 Allée d'Italie 69364, Lyon Cedex 07, France.

The Tat transactivator strongly activates transcription of the HIV1 provirus. This activity depends on binding of this small viral protein to the TAR RNA element and probably also to the Sp1 and NF-κB cellular transcription factors which associate with the upstream regulatory elements. In order to understand how Tat acts on the formation of the transcriptional initiation complex, we have investigated the activating properties of a fusion protein including the GAL4 DNA binding domain and the entire Tat coding sequence. Such a GAL4-Tat protein was very weakly, if at all, able to stimulate transcription, as determined by transient expression experiments using a reporter construct including four GAL4 binding sites upstream of the HIV1 TATA box. However, overexpression of TBP caused a very strong increase of the activity of the GAL4-Tat protein. Overexpression of the general transcription factor TFIIB had no effect. Analysis of different mutants indicated that deletion of the 49/57 basic domain does not impair and even increases this TBP-induced stimulatory effect. Mutations in the first three domains of Tat, between amino acids 1 and 46, led to a complete loss of this effect. In vitro biochemical experiments using purified proteins produced in bacteria showed that Tat can specifically interact with TBP. The stimulation of transcription by Tat is therefore likely to involve a direct contact between this transactivator and TBP. However, since the GAL4-Tat construct exhibited a strong self-squelching, which did not varied as a function of the TBP concentration, another event is likely to intervene in the process.

L 222 ROLE OF TFIIF IN SRF - ACTIVATED TRANSCRIPTION. Véronique Joliot, Hua Zhu, Mark Demma and Ron Prywes. Department of Biological Sciences, Columbia University, New York, New York 10027.

Transcription of the c-fos proto-oncogene is activated rapidly by growth factors in mammalian cells. The Serum Response Element (SRE) in the c-fos promoter can mediate this activation. Serum Response Factor (SRF), which binds specifically to the SRE, has been shown to be required for serum activation of the fos gene.

We have investigated how SRF activates transcription via the general transcription factors. We have found that the general transcription factor TFIIF has an important role in serum response factor (SRF)-activated transcription *in vitro*. A low amount of TFIIF was sufficient for basal transcription, while higher amounts were required for SRF, but not Sp1, activation. High TFIIF levels also increased activation by GAL4-VP16, while none of the other general transcription factors had these properties. TFIIF could also relieve squelching by SRF *in vitro*, suggesting that SRF may directly bind TFIIF. We found more direct evidence for SRF-TFIIF interaction by DNA binding assays where the RAP74 subunit of TFIIF bound DNA in conjunction with SRF, but not alone. RAP74 also bound DNA with GAL4-VP16, but not with Sp1 or the DNA binding domain of GAL4. More recently, we have found evidence for SRF-RAP74 interaction using protein blotting (farwestern) and yeast two-hybrid systems. These results suggest that the mechanism of transcriptional activation by SRF, and perhaps some other activators, involves their interaction with TFIIF. Results of the effects of deletion mutants in SRF and RAP74 on their interaction and transcriptional activation will be discussed.

L 223 DIRECT INTERACTION OF HUMAN TFIID WITH THE HIV-1 TRANSACTIVATOR TAT, Fatah Kashanchi, Graziella Piras, Michael F. Radonovich, Janet F. Duvall, Ali Fattaey* and John N. Brady, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 and *Massachusetts General Hospital Cancer Center, Building 149, 13th Street, Charlestown, MA 02129

The Tat gene of human immunodeficiency virus (HIV) plays a central role in the activation and life cycle of HIV. The mechansim by which Tat exerts its effects has been observed both at the level of transcriptional initiation and elongation. The transactivation response element of Tat, TAR RNA, is located between residues +19 to +44. Interestingly, even with the difference in nucleic acid specificities, Tat and DNA responsive acidic activators may act on a similar step in the transcription process. Here we present evidence that Tat is physically bound to the transcription initiation complex and interacts directly with the basal transcription factor TFIID. Incubation of Tat and HeLa nuclear extract with a biotin labeled DNA probe extending from -80 to +8 resulted in the specific association of Tat with the transcription initiation complex. Mutation of the TATA motif, but not the SP1 or Inr binding site, abolished Tat interaction with the DNA template. Affinity chromatography has been utilized to identify basal transcription factors which interact with Tat. Purified 86 amino acid Tat protein was coupled to an Affi-Gel-10 column matrix. The transcriptional activity of Hela extracts was depleted after chromatography on Tat affinity columns, which specifically retained the transcription factor TFIID. In contrast, TFIID failed to bind to a mutant Tat affinity column which contained a single amino acid substitution. TATA binding protein (TBP), expressed in and purified from *E. coli*, binds to Tat, suggesting that Tat interacts with the basic subunit of TFIID. Peptide competition and TBP binding analysis demonstrates that a specific domain of the Tat protein mediates the Tat-TFIID interaction. Our novel results suggests that Tat may transduce upstream or downstream regulatory signals by direct interaction with the basal transcripion factor TFIID.

L 224 BENT DNA IS A POTENT ACTIVATOR OF EUKARYOTIC TRANSCRIPTION, Jongsook Kim, Sherry Klooster, David Shapiro, Department of Biochemistry, University of Illinois, Urbana,IL, 61801

Binding of many transcription factors to their cognate sites induces DNA bending. To determine whether DNA bending itself could activate eukaryotic transcription, 2-8 copies of an A_6 tract which induces an intrinsic DNA bend were inserted into a synthetic minimal promoter containing only a TATA box. In CHO cells transfected with the intrinsic bending plasmids, transcription increased progressively with increasing DNA bending angles up to 80-fold over basal activity, and approached the activity of adenovirus major late promoter. Gel mobility shift assays revealed the existence of nuclear proteins which bind with high specificity to intrinsic DNA bending sequences. In both binding and competition studies using multimers of A_6 tracts and other related AT rich DNA fragments, there was an excellent correlation between the ability to bend DNA and to activate transcription and binding affinity in gel mobility shift assays. To study the mechanism of transactivation by intrinsic DNA bends in more detail, transcription was carried out in cell-free HeLa cell nuclear extracts. In the cell free system, the intrinsic DNA bending sequence activated transcription and the level of transcription was directly correlated with the degree of DNA bending, just as was observed in the whole cell experiments. The activation in the cell-free system could be effectively competed by the addition of a competitor containing DNA bending sequences. Only sequences which bent the DNA were effective competitors. Related sequences, which did not bend DNA were ineffective competitors. The results demonstrate that DNA bending can activate transcription from a minimal promoter both in whole cells and cell-free extracts and that activation is mediated by proteins that bind specifically to the bent DNA sequence.

L 225 INTERACTION BETWEEN HUMAN PROGESTERONE RECEPTOR AND THE 110 kDa TBP-ASSOCIATED FACTOR ($dTAF_{II}110$) FROM DROSOPHILA.

Christian Schwerk, Michael Klotzbücher, Verena Ulber and Ludger Klein-Hitpass, Institut für Zellbiologie (Tumorforschung), Universitäts-klinikum, D-45122 Essen, Germany.

The human progesterone receptor (hPR) is a member of the steroid receptor superfamily of ligand-inducible transcription factors that mediate the effects of their cognate hormones through binding to hormone response elements. Like other *trans*-activators, hPR consists of several autonomous functional domains, which are responsible for DNA-binding, *trans*-activation (activation function 1 and 2) and hormone binding. It is widely accepted that the mechanism of *trans*-activation involves direct or indirect protein-protein interactions between the activation functions of activators and basal transcription factors.

To study the role of the activation functions of hPR in *trans*-activation, we have expressed hPR isoforms A and B as well as a number of mutants lacking parts of the two activation functions as His-tagged fusion proteins in the baculovirus system. The analysis of the *trans*-activation properties of the purified proteins in cell-free transcription systems showed that the N-terminal activation function (AF1) is highly active. Sequences comprising the hormone binding domain also contribute to transcriptional activation, but this activation function cannot be modulated by hormone *in vitro*. In a protein-protein interaction assay we could demonstrate specific interactions between recombinant hPR and the 110 kDa subunit ($dTAF_{II}110$) of *Drosophila*, which did not depend on the presence of DNA. hPR mutants lacking the hormone binding domain bound $dTAF_{II}110$, suggesting the involvement of AF1 and/or the DNA-binding domain. In contrast to Sp1 (Hoey et al., Cell 72, 247-260, 1993), interaction with hPR required C-terminal sequences of $dTAF_{II}110$. Therefore, different *trans*-activators may interact with distinct domains of $dTAF_{II}110$.

L 226 EFFECT OF ACIDIC ACTIVATORS ON TBP-TATA BOX INTERACTIONS, Thomas Kodadek, Michael Van Hoy, David Fancy and Jean Stephens, Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712

The mechanism of action of "acidic" transcription activators is currently unknown. Since binding of transcription factor IID to the promoter is thought to be an early event in the assembly of the transcription complex, one possible model is that they promote the formation of, or stabilize, a TATA box-TFIID complex. This idea is consistent with the fact that many activators bind directly to TBP and mutations that weaken this interaction in vitro have in some cases been shown to correlate with a reduction in activation potential in vivo. To test this model, we have employed a simple in vitro system consisting of highly purified proteins to measure the rate of association and dissociation of a yeast TBP-promoter complex in the presence of various acidic activators. Results from these experiments are reported. We also present a detailed characterization of the complexes formed between TBP and acidic activation domains from GAL4 and VP16.

L 227 UPSTREAM STIMULATORY FACTOR USF REPRESSES TRANSCRIPTION FROM THE RAT PROTEASE NEXIN-1 PROMOTER

Patrick Küry, Henrik Ernø and Denis Monard, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland

The promoter of the rat PN-1 gene has been isolated and found to be highly GC-rich. It contains a TATA-like sequence (TGATAAA) and binding sites for several transcription factors, such as, Sp1, NGFI-A and -C, Krox-20, Wilms tumor factor (WT-1) and E-box binding proteins. Deletion analysis of the rat PN-1 promoter revealed the presence of a silencer element located between -383 and -239. Gel mobility-shift and missing contact analysis demonstrated that one major protein/DNA interaction occurs at a CACGTG site (E-box). The factor binding to this site is a heterodimer which is specifically recognized by antibodies to human upstream stimulatory factor (USF), but not by antibodies raised against other E-Box binding proteins thereby identifying it as the rat homologue of USF or a closely related transcription factor. This is the first time that USF has been found to be a negative regulator and therefore suggests that this factor may have a dual function in gene regulation. Furthermore, we could demonstrate that the binding of USF is specifically regulated after ibotenic acid or 6-hydroxydopamine induced lesions of the rat brain, suggesting that USF is involved in the upregulation of PN-1 in the astrocytes of the lesioned caudate putamen. These chemically induced lesions provide a model for neuronal loss in Parkinson's Disease.

L 228 THE IDENTIFICATION OF A POTENTIAL REPRESSOR PROTEIN, NF-ODC$_1$, IN ORNITHINE DECARBOXYLASE TRANSCRIPTIONAL REGULATION, G. Lynn Law, Run-Sheng Li, and David R. Morris, Department of Biochemistry, University of Washington, Seattle, WA 98195.

Polyamines are essential for proper cell growth and differentiation. The synthesis of these compounds is highly regulated during the normal cell cycle. The increased synthesis of these compounds has been associated with stimulated cell growth and tumor formation. Ornithine decarboxylase (ODC) catalyzes one of the major regulatory steps in polyamine synthesis and its activity directly correlates with the concentration of polyamines within cells. ODC is one of the most highly regulated enzymes known. Its activity can be regulated at several levels, including transcriptional, translational, and posttranslational. Increases in ODC activity result from cellular stimulation by a variety of mitogens and tumor promoting agents. Abnormally high levels of ODC activity have been found in many tumors including human breast, bladder, colon, and esophageal cancers.

Studies from our laboratory have shown that, in several cell lines, a major form of regulation is at the level of transcription and that a GC-rich region of the promoter plays a major role in the regulation of basal activity of this gene. Using methylation interference and DNase I protection assays, we have identified two overlapping binding sites in this GC-rich region. Gel mobility shift assays indicate that: 1) Sp1 or Sp1-like proteins bind to one of the two sites, and 2) that WT-1, a known tumor repressor that binds to GC-rich regions of DNA, does not appear to bind either site. We believe that a previously undescribed protein (NF-ODC$_1$) binds to the second site. Results of transfection experiments with promoters mutated in the NF-ODC$_1$ or Sp-1 binding site indicate that both sites are important for basal expression in several cell lines and suggest that NF-ODC$_1$ is a repressor of ODC transcription. We have partially purified this new protein using DNA affinity chromatography. The regulation of NF-ODC$_1$ activity may play an important role in the regulation of cell growth and deregulation of this protein may cause ODC overexpression, contributing to neoplastic growth.

L 229 TRANSCRIPTIONAL ACTIVATION OF HUMAN CDC2 GENE BY AN ONCOGENE, E1A 12S, Yuchi Li, Gang Niu and B. Robert Franza, Freeman Laboratory of Cancer Cell Biology, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724

We have cloned a 3.5 kb DNA fragment, 5' to the transcription initiation site of the human cdc2 gene. Deletion and site directed mutangensis studies revealed positive and negative regulatory regions and other regulatory sites in the cdc2 promoter. PMA/PHA and calcium ionophore treatments did not effect the transcription significantly. Cotransfection experiments showed that E1A 12S, an oncogene, activated transcription of the promoter. This activation is not affected by the presence of the negative regulatory regions and is mainly located in the region from - 170bp to -30bp of 5' to the initiation site. Site direct mutangenesis indicated that the activation of the cdc2 promoter involved E2F, SP1 and other sites.

L 230 STIMULATION OF A STABLE PREINITIATION COMPLEX INTERMEDIATE BY THE ZTA TRANSCRIPTIONAL ACTIVATOR

Paul Lieberman, The Roche Institute of Molecular Biology, Nutley, NJ 07110

Transcriptional activator proteins influence the rate of RNA polymerase II transcription, in part, by increasing the rate of formation of a preinitiation complex. The transcriptional activator protein Zta, encoded by Epstein-Barr virus, stimulates the formation of a preinitiation complex that is resistant to Sarkosyl and to ZRE oligonucleotide challenge. Components of the general transcription factors required for the formation of Zta mediated stable intermediates were determined by reconstitution of the preinitiation complex with partially purified general transcription factors. The formation of a Sarkosyl resistant preinitiation complex required the preincubation of the promoter DNA with Zta, holo-TFIID (TBP-TAFs), TFIIB, TFIIA and the coactivator USA. The formation of a ZRE resistant preinititation complex required the preincubation of promoter DNA with Zta, holo-TFIID, TFIIB and TFIIA. DNase I footprinting of preinitiation complex intermediates suggested that only Zta, hIID, and TFIIA make significant contacts with the promoter DNA. Agarose gel EMSA showed that USA may interact with holo-TFIID in a Zta dependent manner. Together, these results provide functional and physical evidence that the Zta transcriptional activator influences the formation of the TFIIA-TFIIB-hIID promoter complex, and changes the capacity of the preinitiation complex to interact with the coactivator USA, essential for high level transcription.

L 231 POSSIBLE ROLE FOR NEGATIVE TORSIONAL TENSION IN POISING THE *DHFR* GENE FOR ACTIVE TRANSCRIPTION, Mats Ljungman and Philip C. Hanawalt, Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA.

We set out to determine whether the DNA in the dihydrofolate reductase *(DHFR)* gene domain in Chinese hamster ovary B11 cells is under torsional tension and if so, whether this tension is due to transcriptional elongation or is introduced as part of an activation mechanism. Photoactivated psoralen was used as probe for torsional tension *in vivo* , producing interstrand cross-links preferentially in DNA sequences underwound by negative superhelical tension. The resulting cross-links were monitored in specific DNA sequences using the AFRHA/slotblot technique (Ljungman & Hanawalt *PNAS* 89, 6055-59, 1992). The topological status of six DNA regions within the *DHFR* gene domain was examined and negative torsional tension was found specifically and exclusively in the promoter. However, a domain much larger than the promoter must be topologically constrained since x-ray-induced nicks at an average spacing of more than 50 kb abolished the psoralen hypersensitivity in the promoter region. Furthermore, although the negative torsional tension in the promoter region was found to be dependent on serum stimulation, it was not significantly affected by drug-treatments that drastically reduced the rate of transcription. We suggest that unique features of the promoter region such as DNA sequence, methylation pattern and chromatin structure may predispose the DNA to unwind in the presence of negative superhelical tension. This unwinding would favor the assembly of the pre-initiation complex and/or aid in the formation of the open transcription complex leading to activated transcription.

L 232 IN VITRO ANALYSIS OF ACTIVATOR-DEPENDENT TRANSCRIPTION

Michael Meisterernst, Lab. for Molecular Biology, Genzentrum, LMU Munich, 82152 Martinsried, Germany

Our previous studies (Meisterernst et al., 1991. Cell 66, 981-993) indicated that activator-dependent transcription is strongly stimulated by a cofactor fraction (designated USA) in vitro. The USA activity was shown to consist of positive (PCs) and negative cofactors (NCs) that in concert efficiently enhanced induction of transcription by various activators. Here we have further investigated the issue of a cofactor requirement for different classes of activators in a highly purified class II gene transcription system. Four distinct positive cofactors were biochemically and functionally characterized. All four protein factors were isolated from the USA fraction, (to date with one exception) identified by protein sequencing and analyzed in basal and activator-dependent transcription in vitro. At the present characterized cofactor activities were apparently contained in a 15 kD peptide, a 100 kD protein (Topoisomerase I), a ca. 105 kD protein and in a 500 kD protein complex (native size) respectively. In this study we have compared the positive cofactors with respect to their specificity for distinct activators, we have investigated the general factor requirement for distinct cofactors, and have begun to analyze the mechanism of induction of activator-dependent transcription. Consistent with the observation that we found no apparent sequence homology between the PCs, this analysis revealed clear functional differences between the PCs with consequences for their putative role in vivo.

L 233 INVOLVEMENT OF TRANSCRIPTION FACTOR YY1 IN THE REGULATION OF HERPES SIMPLEX VIRUS (HSV-1) LEAKY-LATE GENES, Robert L. Millette and Lisa M. Mills, Department of Biology, Portland State University, and Department of Molecular Microbiology, Oregon Health Sciences University, Portland, OR 97207

We recently identified a cellular transcription factor, which we called the LBF, that binds to sites (LBS) in a number of leaky-late ($\beta\gamma$ or γ_1) genes of HSV-1. These include the major capsid protein (VP5) and glycoprotein D (gD) genes. By analyzing the effect of deletions (and, more recently, point mutations) in the VP5 promoter by gel shift analysis and transient expression assays, we established that the LBF is required for maximum transactivation of VP5 by HSV-1 superinfection or by cotransfected immediate-early (IE) genes (Chen et al, J. Virol. 66, 4304 [1992]). We have now confirmed by oligonucleotide competition, proteolytic 'band clipping', and inhibition by anti-YY1, that LBF is identical to transcription factor YY1.

To further elucidate the role of YY1 in HSV-1 gene regulation, we have constructed both deletion and point mutations in the gD YY1 site (bp -54 to -64) and in the adjacent and overlapping G-rich region ('G1', bp -61 to -71, previously shown by R. Everett to be critical for gD transactivation by the virus). Surprisingly, mutation of either site resulted in a severe inhibition of YY1 complex formation as well as gD promoter-driven CAT expression induced by HSV-1. The sequence of the G1 region (GGGGAGGGG) closely matches that of some known SP1 sites. By using SP1 oligonucleotide competition and anti-SP1 supershifts we have shown that an SP1-like factor binds to the G1 region. These results extend the known regulatory functions of YY1 and suggest that binding of both YY1 and SP1 to adjacent sites is required for transactivation of the gD promoter by HSV-1. How HSV-1 IE proteins such as ICP0 and 4 are involved in this transactivation is currently under investigation.

L 234 FOS-JUN HETERO-DIMERIAZTION AND BINDING TO DNA IS INHIBITED BY MAN 5

Bibhuti B. Mishra and Andrew V. Muchmore, Temple University Schol of Medicine, Philadelphia, PA 19140 and Metabolism Branch, NCI, NIH, Bethesda MD, 20892

$Man_5GlcNac_2asn$ (Man 5) is the core carbohydrate structure found on N-linked high mannose substituted glycoproteins. In the absence of protein, purified Man 5 exhibits a number of biologic effects including regulation of TNF and IL-1 production, activation of the hexose monosphate shunt, and inhibition of T cell proliferation. In a structurally specific fashion Man 5, but not a host of related mannose structures, inhibited fos-jun (AP-1) binding to DNA in a mobility shift assay. Man 5 also inhibited AP-3 and SP-1 binding to consensus DNA sequences but failed to affect either Oct 1 or Oct 2 binding in similiar assays. Based on cross linking studies as well as co-immunoprecipitation studies, it appears that Man 5 inhibits DNA binding by disrupting hetero-dimerization of fos and jun. These observations may help to explain the systemic immunologic abnormalities associated with circulating yeast mannan found in human chronic mucocutaneous candidiasis as well as the developmental defects seen with mannosidase deficiency. Furthermore, not only do these data offer a structurally unique approach towards regulating AP-1 and potentially other leucine zipper like transcription factors, but also they offer further evidence that specific glycosylation patterns are critical for regulated gene expression.

L 235 THE YEAST NUCLEAR ATPase, MOT1p, IS A REPRESSOR OF TRANSCRIPTION INITIATION

Chris G.F. Mueller*, Karin Hansen*, David Auble#, Steven Hahn# and Jeremy Thorner*. *Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720 and #Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

A mutation termed *mot1-1* was identified in *S. cerevisiae* because it causes elevated expression of β-galactosidase (β-gal) from reporter plasmids and temperature-sensitive growth (Davis, Kunisawa & Thorner, *Mol. Cell. Biol.* **12**: 1879-1892, 1992). The corresponding gene (*MOT1*) is an essential gene encoding a 1867-residue polypeptide that exhibits homology to known ATP-dependent DNA helicases, such as Rad3p, and is located in the nucleus. The conserved domain of Mot1p encompassing approximately the C-terminal third of the protein was overexpressed in *E.coli*, purified and showed readily detectable ATPase activity in vitro.

To understand how Mot1p affects gene expression, wild-type and *mot1-1* mutant cells were transformed with a series of β-gal reporter constructs. It was observed that, although the increase in β-gal seen in *mot1-1* cells was generally not promoter-specific, the elevation in expression was most evident with promoters lacking their cognate UAS elements. This suggests that defects in Mot1p lead primarily to enhancement of basal transcription. In support of this conclusion, nuclear extracts from *mot1-1* cells gave rise to a much greater number of transcripts from a *CYC1* promoter whose UAS have been replaced by a Gal4 binding sequence than did identically prepared extracts from normal cells in transcription assays in vitro.

A plausible role for Mot1p in controlling transcription initiation is based on the recent finding that an ATP-dependent inhibitor of the binding of TBP to DNA (termed "ADI"; Auble & Hahn, *Genes Dev.* 7: 844-856, 1993) appears to be identical to Mot1p. With the hydrolysis of ATP, ADI dissociates TBP from a TATA box unless the TBP-DNA complex is protected by association with TFIIA and/or TFIIB. Thus, when cells are deficient in Mot1p function, TBP (and the remainder of the transcriptional machinery) is recruited to the promoter more effectively thus raising basal expression. Various aspects of this model are currently under study.

L 236 THE THYROID HORMONE RECEPTOR INTERACTS DIRECTLY WITH TBP AND TFIIB, Anders M. Näär*, Thorsten Heinzel, Jeffrey M. Holloway and Michael G. Rosenfeld#. Eukaryotic Regulatory Biology Program. University of California, San Diego. School of Medicine. *Group in Molecular Pathology. #Howard Hughes Medical Institute. La Jolla, CA 92093-0648. USA.

Nuclear receptors for thyroid hormone and retinoids exert both positive and negative regulation of specific sets of genes by binding to DNA response elements exhibiting certain orientation and spacing of conserved core binding motifs. However, the precise molecular mechanisms whereby nuclear receptors influence the transcriptional activity of genes are not understood. As a first step to address this question we have investigated whether nuclear receptors may interact directly with components of the core transcriptional machinery. Several strong viral transcriptional activators have been shown to interact with the general transcription factors TBP and TFIIB, while other regulators such as Sp1 may require binding to TAFs for transcriptional activation. We have found that the receptors for thyroid hormone can bind directly to both TBP and TFIIB in a ligand-independent fashion. Furthermore, binding of thyroid hormone serves to alter the relative ratio of bound TBP and TFIIB, such that TFIIB binding is decreased while TBP binding is increased. Deletion analysis of the T_3Rs together with direct competition experiments indicate that TBP and TFIIB have overlapping binding sites on the T_3Rs. The thyroid hormone receptors harbor two "DB" interaction domains, one in the amino-terminal region, and one in the distal carboxy-terminus. Transfer of the carboxy-terminal ligand binding domain of the T_3R to the POU domain transcription factor Pit-1 inhibits the transactivation properties of Pit-1, while addition of hormone releases the inhibition. Together with our data indicating that TFIIB binding to the T_3Rs is decreased by hormone, these results suggest that TFIIB may be a target for T_3R transcriptional inhibition.

L 237 IDENTIFICATION OF CELLULAR PROTEINS THAT INTERACT WITH BOVINE PAPILLOMAVIRUS E2 TRANSCRIPTIONAL TRANSACTIVATOR IN VITRO, Nicole M. Rank and Paul F. Lambert. McArdle Laboratory for Cancer Research, University of Wisconsin, Madsion WI 53706

Bovine Papillomavirus type 1 (BPV-1) encodes a protein, the E2 transcriptional activator , E2TA, which regulates the transcription of multiple papillomaviral genes. Two components of the cellular basal transcription apparatus, TBP and TFIIB, have been previously shown to associate with other transcriptional activators. We demonstrate here that E2TA can likewise associate with TBP as well as with TFIIB. We found that Glutathione-S-Transferase E2TA (GST-E2) fusion protein binds to *in vitro* translated, radiolabeled TBP produced in a coupled transcription/translation system (TNT TBP) as well as to TBP present in HeLa Nuclear Extract (HNE). GST-E2 also binds *in vitro* translated, radiolabeled TFIIB (TNT TFIIB) and HNE endogenous TFIIB in parallel assays. Binding of TBP and GST-E2 requires no other intermediate factors as is shown by direct binding of GST-E2 to purified, bacterially expressed, recombinant TBP. The domains of the E2 transactivator required for interaction with TBP and TFIIB have been defined through the characterization of mutant GST-E2 proteins deleted in portions of E2TA coding region. Studies in progress are designed to address the potential biological significance of these interactions.

L 238 STAGE SPECIFIC ACTIVATOR PROTEIN: A TRANSCRIPTIONAL ACTIVATOR THAT RECOGNIZES SEQUENCE SPECIFIC SINGLE STRANDED DNA SEQUENCES, Lisa J. Rybacki, Jeffery DeFalco and Geoffrey Childs, Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461

In sea urchins there are distinct classes of histone gene families which are regulated temporally during the embryonic development of the organism. Previously, we demonstrated that a conserved region of DNA between positions -318 and -218 constitutes the embryonic enhancer of the *Strongylocentrotus purpuratus* histone H1β gene and is responsible for the transcriptional activation of the gene during the midblastula stage of embryogenesis. This region has been shown to consist of three binding sites (USE IV, site 2 and USE III) for a 43 kDa polypeptide which binds in its monomeric form or as part of a higher molecular weight complex (DeAngelo et al., 1993). This protein is referred to as Stage Specific Activator Protein or SSAP.

The SSAP gene has been cloned and sequenced. At the N-terminus of the protein are two consecutive RNP motifs linked to a glycine/glutamine rich region. Introduction of SSAP mRNA or an SSAP cDNA driven by a sea urchin promoter by microinjection into *Lytechinus pictus* zygotes results in the transactivation of a target gene containing the SSAP binding site. In addition, SSAP immunolocalizes to the nucleus where it is uniformly distributed at all developmental stages examined.

Both the authentic, purified SSAP protein as well as recombinant, bacterially produced SSAP (bSSAP) bind specifically to the USE IV enhancer element using mobility shift competition assays and also protect the USE IV core sequence by DNAse I footprinting analysis. While complexes formed by bSSAP and native SSAP are competed by the double stranded USE IV oligonucleotides; these complexes are also competed by the individual strands of the enhancer element. In addition, bSSAP exhibits the ability to bind to both strands individually, but not to mutant USE IV oligos.

Investigation of the structure of the DNA within the enhancer using DMS/S1 treatment as well as S1 treatment of supercoiled plasmid DNA strongly suggests that there is non B-form DNA within the SSAP binding sites.

We conclude that SSAP is a novel transcription factor which binds to single stranded DNA and activates the H1β gene at midblastula stage of embryonic development.

DeAngelo, D.J., DeFalco, J. and Childs, G. 1993. Purification and characterization of the stage-specific embryonic enhancer-binding protein SSAP-1. Mol. Cell. Biol. 13:1746-1758.

L 239 HYDROPHOBIC COILED-COIL DOMAINS REGULATE THE SUBCELLULAR LOCALIZATION OF HUMAN HSF2, Lynn A. Sheldon, Jeffrey S. Larson, and Robert E. Kingston, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

HSF2, one of the heat shock transcription factors in mammalian cells, is localized to the cytoplasm during normal growth and moves to the nucleus upon activation. Heat shock transcription factors in metazoans contain four hydrophobic heptad repeat sequences, three in the N-terminus and one in the C-terminus, that are predicted to form α-helical coiled-coil structures analogous to the leucine zipper. We show that mutations in either of two N-terminal zippers or in the C-terminal zipper disrupt normal localization of HSF2 and cause it to be constitutively nuclear. We further demonstrate that two sequences that immediately surround the N-terminal zipper domain are required for nuclear localization. These sequences fit the consensus for a bipartite nuclear localization signal (NLS). We suggest that interactions between the N and C-terminal zippers normally mask the NLS sequences of HSF2 and that these interactions are disrupted upon activation to expose the NLS sequences and allow transport of HSF2 to the nucleus. We conclude that zipper domains can regulate subcellular localization.

We have used the yeast two-hybrid system to test more directly whether the N-terminal and C-terminal zipper domains of HSF2 and other HSFs are able to interact with one another. The HSF2 N-terminal zipper domain was fused to the LexA DNA binding domain and the C-terminal zipper to the B42 activation domain. A reporter construct with LexA DNA binding sites and the lacZ gene was used to detect interactions. A strong interaction of the two zipper domains was seen as indicated by β-galactosidase activity. Further characterization of the potential interactions of zipper domains in other HSFs is now in progress.

L 240 A NOVEL DIMERIZATION REGION IS DELETED IN THE PRODUCT ENCODED BY AN ALTERNATIVELY SPLICED mRNA FOR THE CELL GROWTH REGULATED TRANSCRIPTION FACTOR LSF, Margaret Shirra[1,2], Quan Zhu[1,2], Janet Volker [1,2], Hui-Chuan Huang[1,2], David Pallas[1] and Ulla Hansen[1,2], [1]Dana Farber Cancer Institute and [2]Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA.

The transcription factor LSF has been previously purified as a 63 kDa protein that specifically binds to and stimulates transcription from the SV40 major late promoter in vitro (Genes Dev. 4:287-298). It has also been shown that LSF DNA-binding activity rapidly increases as cells progress from G0 to G1, apparently due to phosphorylation of LSF. We describe here the cloning of human cDNAs for two alternatively spliced forms of this transcription factor, LSF and LSF-ID. LSF is essentially identical to the transcription factor CP2 (Mol. Cell. Bio. 12:828-835), while LSF-ID is deleted for residues 189 and 239 of LSF. Structural analysis of the genomic DNA demonstrates that the shorter clone could result from either exon skipping and/or the novel mechanism of secondary splicing. Only the product of the longer cDNA binds DNA. The sequence of LSF is strongly similar to only one other protein in the database, Elf-1/NTF-1, a Drosophila transcription factor, but shows no recognizable DNA-binding or dimerization motifs. This similarity includes three regions of highest identity, two of which are absent in LSF-ID. Initial C-terminal and N-terminal deletion studies show that the region of the protein required for DNA binding is unusually large, greater than 238 amino acids. A consensus sequence of the known binding sites indicates that LSF binds to two weakly homologous direct repeats. Co-translation in wheat germ extracts of LSF and N-terminal truncation LSFΔ24 shows that LSF binds DNA as a dimer, using an epitope counting methodology to separate the similarly-sized complexes. However, LSF-ID does not affect the binding of LSF to DNA suggesting that these proteins do not interact. This implies that the dimerization domain may be coupled to the exon deleted in LSF-ID.

L 241 THE N-TERMINAL REGION OF TRβ2 CONTAINS STRONG TRANSACTIVATING DOMAINS ACTING VIA PALINDROMIC RESPONSE ELEMENTS

Maria Sjöberg and Björn Vennström, Lab. of Developmental Biology, Dept. of Cell and Molecular Biology, Karolinska Institute, S-171 77 Stockholm, Sweden

Vertebrates produce various forms of T3 receptors (TRs) from two related genes, α and β, which are differentially expressed in a tissue-specific manner. The cTRβ gene produces 2 mRNAs that encode receptors with distinct N-termini due to differential splicing. TRβ2 is the longer form having an extended N-terminal of 107 amino acids. We have shown previously that cTRβ2, as compared to cTRβ0, confers a stronger transactivation of a reporter gene containing a palindromic T3 response element, AGGTCATGACCT, upstream of the TK promoter of the CAT gene.

In contrast, a reporter gene containing a direct repeat element of AGGTCA spaced by four nucleotides (DR4), is equally well activated by the two receptors and the transactivation can be further enhanced by cotransfection of mRXRα. This suggests that the N-terminal region in TRβ2 confers the strong transactivation specifically through palindromic response elements.

This has been confirmed by molecular dissection of cTRβ2: the strong transactivation is due to transferable, ligand independent activating domains in the N-terminal region. A detailed analysis identified in one of the domains a 23 amino acid long sequence sufficient for strong transactivation. Replacement of Tyrosines in this domain for Methionines reduced transactivation by 80%. The amino acid sequence shows no similarities to previously described domains with transactivating functions.

Furthermore, the cTRβ2 transactivation through a palindromic response element is reduced by cotransfection with mRXRα, indicating that the strong transactivation is mediated by cTRβ2 homodimers. The data also suggest that transactivation through direct repeat elements is mediated by RXR/cTRβ heterodimers, in which case the N-terminal transactivating domain fails to exert its strong transactivating effect.

L 242 **TRANSCRIPTION ACTIVATION THROUGH AP1 BINDING SITES MEDIATED BY ADENOVIRUS E1A CONSERVED REGION 1.**

Kerstin Sollerbrant, Maria Bondesson, Mattias Mannervik, Göran Akusjärvi and Catharina Svensson. Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden.

The adenovirus E1A proteins harbor several transcription activating domains of which the major one maps to the E1A-289R unique domain designated CR3. We have identified a new transactivating domain, located within amino acids 28 to 90 of the E1A-243R, which mainly comprises of the conserved region 1 (CR1). When linked to the yeast Gal4 DNA binding domain, the Gal4/CR1 hybrid protein efficiently activated transcription of a CAT reporter containing Gal4 binding sites. On the other hand, transcription from a reporter driven by the SV40 enhancer was down-regulated by the Gal/CR1 fusion, indicating that amino acids 28 to 90 retains sufficient information to execute the previously described SV40 enhancer repressing function of the E1A-243R protein.

To search for the natural target for the CR1 activator, a panel of CAT reporters driven by individual transcription factors, were analysed for their response to Gal/CR1. Transcription driven by the ATF site from the adenovirus E3 promoter or the E2F site of the E2 promoter were both unaffected by the Gal/CR1 fusion protein. In contrast, two reporters containing dimerized AP1 factor binding sites, taken from the collagenase and adenovirus E3 promoters, respectively, were both efficiently activated by Gal/CR1. I general, transcription activation by Gal/CR1 was seen on several native promoters containing AP1 binding sites. This activation was much reduced in two cell lines lacking c-jun expression. In agreement with the possible requirement for c-jun, TPA induction or cotransfection with CMVc-jun , together with Gal/CR1, both resulted in a synergistic activation of the two AP1CAT reporters. Furthermore, "domain swap" experiments indicated that the activation domain, but not the DNA binding domain, of c-jun could serve as responding target for the Gal/CR1 activity.

It has previously been shown that the full length E1A-243R protein, through a CR1 dependent mechanism, represses the activity of AP1 by preventing its binding to the TRE element. Our current data indicate that sequences downstream of amino acid 90 in E1A-243R adversely affect the transactivation of AP1 driven transcription. This hypothesis is in agreement with our previous observation that E1A-dependent down regulation of metalloproteas expression (AP1-driven) in transformed rat cells, required a carboxy-terminal region of E1A.

L 243 IDENTIFICATION OF FACTORS REQUIRED FOR THE EFFICIENT TRANSCRIPTION OF A DROSOPHILA U1 snRNA GENE IN VITRO, William E. Stumph, Yuru Song, Yan Wang, and Zulkeflie Zamrod, Department of Chemistry and Molecular Biology Institute, San Diego State University, San Diego, CA 92182.

The basal promoter of a Drosophila U1 snRNA gene contains two elements essential for efficient in vitro initiation of transcription by RNA polymerase II. The first, termed PSEA, is located between positions -41 and -61 relative to the transcription start site. Because PSEA is crucial for promoter activity and is the dominant element for specifying the transcription start site, PSEA appears to be functionally equivalent to the Proximal Sequence Element (PSE) of vertebrate snRNA genes. Using a nuclear extract prepared from 0-12 hour Drosophila embryos, we have identified and partially purified a factor that binds sequence-specifically to the PSEA element. We term this the PSEA factor (PSEAF). A second cis-acting element, termed PSEB, is located at positions -25 to -32, and is required for an efficient level of transcription initiation. Mutation of PSEB, or alteration of the spacing between PSEA and PSEB, severely reduced transcriptional activity relative to the wild type promoter. Although PSEB is not closely related to the TATA box sequence, it can be converted to a canonical TATA sequence by making three nucleotide substitutions. These changes resulted in a 4 to 5 fold increase in promoter efficiency, but had no effect on the choice of start site nor on the RNA polymerase II specificity of the promoter. Moreover, antibody inhibition studies indicated that TBP is required for U1 transcription. A model consistent with these results is that TBP interacts with PSEB. However, binding of purified recombinant TBP to PSEB was not detectable by in vitro footprinting experiments. Recombinant TBP was also unable to complement TBP-depleted extracts for transcription of the wild type Drosophila U1 gene, suggesting that a TBP/TAF complex is necessary for the formation of the U1 gene pre-initiation complex. We propose that PSEAF, by binding to PSEA, recruits a TBP complex to the nearby PSEB element, which functions as a non-canonical (reduced-affinity) TATA box. This mechanism may assure that an snRNA-type (rather than an mRNA-type) transcription complex is formed on snRNA promoters.

L 244 TRANSCRIPTIONAL REGULATION OF THE HUMAN CFTR PROMOTER BY SP1 AND A NEGATIVE ELEMENT. Ting-Chung Suen and Lap-Chee Tsui. Department of Genetics, The Hospital for Sick Children and University of Toronto, Ontario, M5G 1X8, Canada.

Cystic fibrosis (CF) is a frequent autosomal recessive genetic disease in Caucasians caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Major advances have been made towards developing gene therapy protocol in animal models and patients. However, much less is known about how CFTR transcription is regulated. The proximal sequence of the CFTR promoter is G-C-rich and contains multiple consensus and potential Sp1 binding sites. In this report, we show by electrophoretic mobility shift assay (EMSA) that several protein-DNA complexes could be detected with a DNA fragment previously shown to have basal promoter activity (-214 to -96). The pattern of these shifted-bands and the result of competition assay with an Sp1 oligonucleotide suggest that Sp1 or its related proteins are involved. Methylation interference assay located the major binding site to a 10-bp sequence (GTGGGTGGAG) at -130, similar to the previously defined GT box. Functional stimulation was observed when an oligonucleotide containing this GT box was cloned upstream to a heterologous thymidine kinase promoter. Binding of purified Sp1 protein to the sequence was also detected by EMSA. Thus, Sp1 and/or other related transcription factors appeared to play a positive role in CFTR gene regulation. In addition, we have further localized the previously identified negative regulatory element to a 60-bp region (-335 to -277). We now show that this 60-bp DNA fragment could suppress the CFTR promoter even in the presence of multiple GC-boxes or the enhancer from SV40. Interestingly, cross-competition in EMSA was observed between the negative element and the basal promoter fragment (-214 to -96). Moreover, an EMSA complex was detected with a 30-bp fragment (-308 to -279) which contained the 3'-half of the negative element and the complex could be competed away by an oligonucleotide containing an Sp1 site. This result suggests an interaction between the putative protein factor binding to this region and Sp1. Thus, the regulation of the CFTR promoter is highly complex and further analysis of the regulatory elements is in progress.

L 245 **INDUCTION OF THE BRAIN CREATINE KINASE PROMOTER BY THE ESTROGEN RECEPTOR IN THE ABSENCE OF AN ERE MAPS TO A REGION THAT CONTAINS A BINDING SITE FOR MADS DOMAIN PROTEINS.** Drew A. Sukovich, Ranjan Mukherjee, and Pamela A. Benfield, Cardiovascular Molecular Biology, DuPont Merck Pharmaceutical Company, Wilmington DE 19880.

The estrogen receptor (ER) typically activates gene transcription by binding to estrogen responsive elements (ERE's). The brain creatine kinase gene (BCK) promoter is responsive to estrogen but contains no consensus ERE. To investigate the mechanism of estrogen induction, we introduced the estrogen receptor into HeLa, primary cardiomyocytes, and primary fibroblast cells with 195 bp of BCK promoter linked to a chloramphenicol acetyltransferase (CAT) reporter gene. A 10-fold and 5-fold stimulation of CAT activity was observed in HeLa and primary fibroblast cells, respectively. This stimulation is not sensitive to cyclohexamide and is blocked by estrogen antagonists. No stimulation of CAT activity was observed in the primary cardiomyocytes. This is in contrast to a CAT reporter construct containing the vitellogenin gene ERE linked to the TK promoter, which exhibited activation by estrogen in all three cell types. Deletion mutants of the ER demonstrate that the DNA binding and ligand binding domains of the ER are required for this induction although point mutations in the DNA binding domain which abolish DNA binding are still capable of estrogen induction. This suggests that ER binding to DNA is not required for estrogen induction. Analysis of 5' deletion and linker scan mutations indicates sequences between -45 and -75 including a TA-rich and CCAAT sequence to be crucial for stimulation of the BCK promoter by the ER. Furthermore, BCK promoter sequences (-37 to -195) confers estrogen inducibility when linked to the heterlogous β-globin promoter and mutations in the TA-rich sequence severly decreases the inducibility. We have previously shown that this TA-rich sequence binds a protein complex TARP (TA-rich binding protein). We propose a possible mechanism whereby ER stimulates transcription of the BCK gene by interacting with TARP.

L 246 IN VIVO ANALYSIS OF THE HUMAN TATA BINDING PROTEIN: SURFACES REQUIRED FOR ACTIVATED TRANSCRIPTION IN HUMAN CELLS. William P. Tansey and Winship Herr, Cold Spring Harbor Laboratory, 1 Bungtown Rd., PO Box 100, Cold Spring Harbor, NY 11724.

Activated transcription initiation results from the interaction of a DNA-bound activator protein with one or more of the general transcription factors (GTFs), assembled in a preinitiation complex around the TATA motif of RNA polymerase II-transcribed genes. One likely target for such interactions is the TATA binding protein (TBP), which is the first of the GTFs to enter the preinitiation complex, and has been shown to interact directly with a number of transcriptional activators in vitro.

We are interested in determining the role of TBP in activated transcription in human cells, by identifying the regions of TBP that are required to respond to various different classes of transcriptional activators in vivo. Adapting the altered specificity TBP assay (developed by Strubin and Struhl *Cell* ***68:721-730***) for use in HeLa cells, we have shown that transient coexpression of altered specificity human TBP can restore wild-type levels of activated transcription on a mutant promoter in which the canonical TATAAA motif had been changed to TGTAAA. We have used this assay to test the response of a number of mutant TBP molecules to multiple activators carrying the GAL4 DNA binding domain and activation domains belonging to the acidic, proline-rich, and glutamine-rich classes.

We have demonstrated that the conserved carboxyl-terminal domain of human TBP is sufficient to respond to all activator types tested. Yeast TBP, which is over 70 % identical in this region, responds similarly to most, but interestingly not all, of the activators used in this study. Additionally, we have found that double and triple point mutations in clustered regions on the surface of TBP have little effect on its response to most activators, with the exception of the acidic activation domain of VP16. Together, these results suggest that different regions of TBP may be involved in mediating the effects of different activators, and that, in general, multiple contacts across the surface of TBP are required for its function.

L 247 A DOMINANT MUTATION AFFECTS THE ACTIVATION POTENTIAL OF CERTAIN TRANSCRIPTION ACTIVATORS IN YEAST

Nektarios Tavernarakis and George Thireos.
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, PO Box 1527, Heraklion 711 10, Crete, Greece.

A genetic screening designed to isolate genes that communicate the effect of transcription activators to the basic transcription machinery was undertaken based on the observation that overproduction of the GCN4 protein has a deleterious effect on the growth of yeast cells. Generating genetic backgrounds where this effect would be alleviated should lead to the identification of molecules that participate in the transcriptional activation process at least by GCN4. Four unlinked mutations afr1,2,3,4, were obtained that upon GCN4 overproduction allowed for enhanced growth rate of yeast cells relatively to wild-type. Analysis of the properties of strains bearing the afr1 dominant mutation revealed complete immunity to toxic levels of GCN4 which was accompanied with reduction of the GCN4 activation function. In addition such strains exhibit impaired GAL4, HAP2/3/4, yAP1 and CAD1 activity *in vivo* while leaving a LexA-VP16 chimeric activator unaffected. We conclude that afr1 is a pleotropic mutation affecting specifically the activation potential of a number of transcription factors in yeast.

L 248 MODULATION OF YEAST HAP1 ACTIVITY BY COFACTOR PROTEIN(S), Bernard Turcotte, Department of Medicine, Royal Victoria Hospital, McGill University, Montréal, Canada H3A 1A1.

The expression of the yeast CYC1 and CYC7 genes is controlled by the HAP1 activator. A GAL4- like zinc finger (residues 1-148) specifies binding to the dissimilar sites UAS1 (of CYC1) and CYC7, and an acidic domain (residues 1307-1483) is essential for activation of transcription. To analyze how HAP1 binds to UAS1 and CYC7, saturation mutagenesis of the DNA binding domain was performed and mutants with altered activity were recovered. Class 1 mutants had a reduced activity at both UAS1 and CYC7, and class 2 mutants selectively eliminated activity at CYC7. Surprisingly, several mutants of both classes exhibited wild- type DNA-binding, indicating that they were specifically defective in activation. These positive control mutants alter residues that bracket the zinc finger. The phenotype of these mutants can be explained in a model involving cofactor proteins that bind UAS1 and CYC7 along with HAP1.

Suppressor strains showing increased activity for some positive control mutants have been isolated. Mutations are recessive and allele specific. These strains are being used to clone the gene encoding the putative cofactor protein.

L 249 **ANALYSIS OF GCR1, A TRANSCRIPTIONAL ACTIVATOR OF YEAST GLYCOLYTIC GENES**, Hiroshi Uemura, Masayuki Machida and Yoshifumi Jigami, Department of Molecular Biology, National Institute of Bioscience and Human-Technology, Tsukuba Research Center (MITI), Tsukuba, Ibaraki, 305, JAPAN

The Saccharomyces cerevisiae GCR1 and GCR2 genes affect expression of most of the glycolytic genes. Evidence for Gcr1/Gcr2 interaction has been presented earlier (1), and is now supported by the isolation of mutations in Gcr1 suppressing gcr2, as assessed by growth and enzyme assay. Four specific mutation sites were identified. Together with use of the two hybrid system of Fields and Song, they show that Gcr1 in its N-terminal half has a potential transcriptional activating function as well as elements for interaction with Gcr2, which perhaps acts normally as a coactivator. Complementation of various gcr1 mutant alleles and results with the two hybrid system also indicate that Gcr1 itself normally functions as, at least, dimer.

1. H. Uemura and Y. Jigami (1992) Mol. Cell. Biol. 12, 3834-3842.

L 250 CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF TRANSCRIPTION FACTOR IIB AND A TFIIB MONOCLONAL ANTIBODY, Stephanie Vavra, Mark Knuth, Nancy Thompson and Elaine Schenborn, Research and Development Eukaryotic Expression, Promega Corporation, Madison Wisconsin, 53511

The recombinant clone for eukaryotic transcription factor IIB (human TFIIB) has been over-expressed in *E.coli* BL21DE3 pLysS cells and purified. Gel shift analysis shows that the purified TFIIB protein is able to enhance the binding of TATA-binding protein (TBP) to a TFIID consensus oligo. Under suboptimal binding conditions of TBP to the DNA consensus site (no $MgCl_2$ in the binding reaction or in the gel system) TFIIB addition stabilizes the TBP/oligo complex and effectively increases TBP binding ability. A monoclonal antibody against TFIIB has also been generated. Depletion of TFIIB from Hela nuclear extract with the antibody coupled to *Staphylococcus aureus* cells results in an extract whose transcriptional activity (using HIV G-less and AdML promoter G-less templates) is several fold less than control extract. Adding purified TFIIB back to the depleted extract returns transcription levels to control values or higher. The TFIIB antibody has also been shown to supershift the TBP/TFIIB/DNA complex in gel shift reactions.

L 251 A CELL CYCLE REGULATED COMPLEX WITH E2F-LIKE PROPERTIES IN *SACCHAROMYCES CEREVISAE*, Sheela Vemu and Ronald R. Reichel, Department of Pharmacology and Molecular Biology, The Chicago Medical School, North Chicago, IL 60064

The mammalian transcription factor E2F has been implicated in the control of cell cycle progression. Because major aspects of cell cycle regulation are conserved between mammals and yeast, we sought to identify an E2F-like activity in the yeast, *Saccharomyces cerevisae*. Using gel retardation assays, we have detected an activity in *S.cerevisae* that recognizes the E2F binding site TTTCGCGC, which is present in several mammalian promoters. Purification of this activity, using ion exchange chromatography and glycerol gradient sedimentation, resulted in the identification of a complex of approximately 300kD containing at least five distinct proteins. UV cross-linking studies revealed the molecular weight of the DNA binding component to be 47kD. Additional DNA-binding studies showed that the 300kD complex also interacts with the cell cycle box-TTTTCGTG. In *S.cerevisae*, this sequence is known to interact with a protein complex consisting of SWI4 and SWI6 proteins. However, using mutant strains that are devoid of SWI4 or SWI6, we have demonstrated that our 300kD complex is clearly distinct from SWI4/6. Since the cell cycle box imparts cell cycle regulaion on a number of genes, we asked whether the 300kD complex fluctuates during the yeast cell cycle. Employing a temperature-sensitive mutant, we were able to show that the DNA-binding activity of 300kD complex oscillates during the cell cycle. Preliminary experiments suggest that protein phosphorylation plays an important role in the regulation process. Since yeast mutant strains that do not contain any functional SWI4/6 complex are still able to go through the cell cycle, it has been postulated that yeast cells contain activities that can replace SWI4/6. It is highly likely that our 300kD complex falls into this category. In summary, we have analyzed a yeast protein complex that shares several characteristics with mammalian E2F complexes and appears to be an important player in yeast cell cycle regulation.

L 252 THE S. cerevisiae 5S, ARS1-TRP1 AND TOP1 GENES AS SYSTEMS FOR THE STUDY OF PRINCIPLES THAT GOVERN CHROMATIN ORGANIZATION.

P. Venditti, M. Buttinelli, G. Camilloni, G. Costanzo, R. Negri, S. Venditti and E. Di Mauro. Dipartimento di Genetica e Biologia Molecolare, Università di Roma "La Sapienza", Italy.

Knowledge of the rules that control both the DNA accessibility, when wrapped around nucleosomes, and the localization-stability of histone octamers is essential for an understanding of chromatin functions. We analyzed the chromatin structure of three different S. cerevisiae gene systems: the 5S rRNA; the ARS1-TRP1 region; the DNA-Topoisomerase I. In order to study the nuclear structure in vivo, we developed a method to introduce active enzymatic probes into viable spheroplasts; this technique utilizes the antibiotic nystatin to create pores in the yeast cell membrane. A second technique has been developed to localize nucleosomal borders both in vivo and in vitro, using a no-background assay based on the purification and mapping of monomeric nucleosomal DNA. In all the three systems studied, high resolution nucleosome mapping showed multiple alternative positions always related to a unique rotational phase. This result suggests a major role for the DNA rotational signals in the DNA-histone octamer interaction. These signals are more evident in the 5S rRNA gene and weaker in the ARS1-TRP1, where we in addition observed a boundary effect by the transcription factor ABF I. In this context ABF I might act as a physical barrier to nucleosome invasion, thus limiting the number of possible nucleosome positions. These data were confirmed by the analysis of the chromatin arrangement in a plasmid carrying a mutagenized ABF I binding site. In conclusion: we suggest for the ABF I protein a role of fine tuner in the chromatin structure of the region examined.

L 253 RESOLUTION OF A COMPLEX FORM OF YEAST TBP,

Paul A. Wade, Michael Woontner and Judith A. Jaehning, University of Colorado Health Sciences Center, Department of Biochemistry, Biophysics, and Genetics, Campus Box B121, 4200 E. Ninth Ave., Denver, CO 80262

In vitro transcription by RNA polymerase II from *S. cerevisiae* differs from metazoan systems in several respects. For example, we have found that topoisomerase I is not a required transcription factor. In addition, the TATA-binding protein (TBP) from metazoans is found as part of a complex factor (TFIID) which is relatively stable to extraction and chromatography, while the equivalent protein from yeast (yTBP), is readily purified as a single polypeptide. We describe the resolution of a novel form of yTBP which resembles in some respects the TFIID complexes found in metazoans. A yeast transcription extract reconstituted using this complex yTBP fraction is dependent on TFIIA for activity, reminiscent of the metazoan TFIID complex and unlike systems that utilize a recombinant form of yTBP (rTBP). Repression and full levels of activation by the yeast Leu3p[1] activator require the use of the complex form of yTBP rather than rTBP. The protein/protein interactions required to maintain the TFIIA responsive form of yTBP are apparently disrupted by chromatography in ammonium sulfate but can be maintained with buffers containing potassium acetate. The response to regulatory factors associated with this complex form of yTBP does not appear to result from association with either Sug1p[2] or the products of the SRB2,4,5 genes[3] which have been postulated to interact with TBP.

1 Sze J-Y., Woontner M., Jaehning J.A. and Kohlhaw, G.B. (1992) Science 258:1143-1145.
2 Swaffield, J.C., Bromberg, J.F. and Johnston, S.A. (1992) Nature 357:698-700.
3 Thompson, C.M., Koleske, A.J., Chao, D.M. and Young, R.A. (1993) Cell 73:1361-1375.

L 254 IDENTIFICATION OF A DOMAIN IN ICP4 THAT FORMS A COMPLEX WITH THE TATA BINDING PROTEIN (TBP), Kent Wilcox, Jie Mi, James Taylor, and You-gang Lin, Department of Microbiology, Medical College of Wisconsin, Milwaukee, WI 53226

ICP4 is a large (1298 aa) immediate early HSV protein that activates transcription of delayed early and late viral genes. The absence of strong ICP4 binding sites in many promoters that are up-regulated by ICP4 suggests that ICP4 interactd with one or more protein components of the cellular transcription machinery. In particular, it has been proposed that ICP4 may function as a co-activator by binding to the TATA binding protein (TBP) component of TFIID.

To test this hypothesis, we radiolabeled full-length human TBP by in vitro translation and incubated it with native ICP4 . Using a co-immunoprecipitation procedure, we observed that TBP co-precipitated with native ICP4. When the procedure was repeated with four different immunologically-tagged portions of ICP4 produced from bacterial expression vectors, we observed a strong interaction with the carboxy-terminal domain of ICP4 and weak-to-non-detectable interactions with the other three portions of ICP4. Control immunoprecipitations with the E1a protein (a known TBP-binding protein) and unrelated antibodies as negative controls were performed to confirm the specificity of the TBP:ICP4 interaction. Co-immunoprecipitations performed with truncated forms of TBP revealed that two separate domains in the highly-conserved carboxy-terminal portion of TBP preferentially bound to ICP4. The control experiments with E1a revealed an additional site in TBP that interacts with E1a. We conclude that the carboxy-terminal domain of ICP4 has the potential to form a specific complex with TBP in vitro and propose that formation of this complex in vivo may contribute to the mechanism by which ICP4 activates transcription. The fact that the carboxy-terminal domain of ICP4 is highly conserved among alphaherpes virus immediate-early transcriptional activators lends support to this hypothesis. Site-specific mutants in the carboxy-terminal domain are being investigated to further map the location of the TBP-binding site.

L 255 TRANSCRIPTION ACTIVATION OF THE SV40 LATE PROMOTER IN VITRO IS REGULATED BY INTERACTION BETWEEN T ANTIGEN AND TEF1, Alan G. Wildeman, Lloyd Berger, Ellen Fanning*, Irwin Davidson[‡], and Donald Smith[†], Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, Canada, *Institute for Biochemistry, 23 Karlstrasse, Munich, FRG, [†]Department of Medical Microbiology, University of Edinburgh Medical School, Edinburgh, Scotland, and [‡]Faculté de Médecine, 11 rue Humann, Strasbourg, France.

Transcription of the SV40 genome shifts from the early to the late genes in response to the appearance of the early gene product T antigen and the onset of viral DNA replication. Although high levels of late transcription are seen only when DNA replication occurs, T antigen can activate the late promoter in the absence of replication. Transfection experiments have shown that sites within the enhancer to which the factor TEF1 binds are involved in mediating replication-independent activation. TEF1 is thought to be the same as the muscle-specific factor MCAT, and it shows extensive homology with the Drosphila protein *scalloped*.

Late transcription is initiated from multiple start sites in vivo. Using a Hela cell in vitro transcription extract, transcription of a subset of these can be activated by an NH_2 terminal fragment of T antigen produced in bacterial cells (Coulombe et al, J. Virol. 66:4591-4596). Further analysis has shown that activation does not require TAg binding sites on the DNA template, is repressed when TEF1 binds to a site (the GTIIC motif) on the late side of the enhancer, but requires that TEF1 binds to the Sph motifs situated near the early side within the 72 bp enhancer region. The DNA binding region of TEF1 is sufficient to bind to the GTIIC motif and repress late activation.

These results suggested that there might be a direct interaction between TAg and TEF1, thereby preventing TEF1 from binding to the GTIIC motif. Using bacterially produced proteins we find that TAg can bind to the DNA binding motif of TEF1, and that the region of TAg required for this interaction spans the DNA binding region of TAg. A minimal region of TAg that binds to TEF1 and TBP is not sufficient to activate transcription, suggesting that on the Sph motifs other as yet unidentified factors are required for activation in vitro.

L 256 ANALYSIS OF INTERACTIONS AMONG HUMAN TFIID SUBUNITS AND TRANSCRIPTION FACTORS

Tohru Yamamoto[1], Satoshi Hasegawa[1], Miho Yoshida[1], Yoshihiro Nakatani[2] and Masami Horikoshi[1]
[1]Laboratory of Developmental Biology, Department of Cellular Biology, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo, JAPAN
[2]Laboratory of Molecular Biology, NINDS, National Institutes of Health, Bethesda, MD, USA

Transcription is actively regulated to respond properly to various signals from the outside of a cell. These signals are transmitted into the inside of a cell and transduced to thousands of regulatory factors which represent information of signals. This action is finally transmitted to one enzyme, RNA polymerase II, by a certain mechanism as previously described (Horikoshi et al. Cell, 54, 665 & 1043, 1988). TFIID plays an important role in the transmission of information represented by regulatory factors to RNA polymerase II. We are presently analyzing interactions among regulatory factors, human TFIID subunits and transcription machinery to elucidate the molecular mechanism of the signal transmission in transcriptional regulation. Recent results will be shown and discussed.

Activation II

L 300 HUMAN NPY AND GAP-43 GENE EXPRESSION IN SH-SY5Y NEUROBLASTOMA CELLS, Göran Andersson,* Irja Johansson, ‡ Eva Örtoft, ‡ and Sven Påhlman ‡ *Department of Cell Research, Uppsala Genetic Center, Swedish University of Agricultural Sciences, Sweden. ‡ Department of Pathology, University Hospital, Uppsala, Sweden.

Treatment of the human SH-SY5Y neuroblastoma cells with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) leads to neuronal differentiation closely resembling that of sympathetic ganglion cells. A sustained induction of a single neuropeptide Y (NPY) mRNA species and two growth-associated protein, GAP-43 mRNA species was detected following phorbol ester treatment. The TPA-induced differentiation in these cells is dependent on activated protein kinase C (PKC) signal transduction events. Alterations in mRNA expression and activities of known transcription factors that are targets for PKC was analyzed. Induced activator protein-1 (AP-1) transcription activity was detected after TPA administration using transient transfection experiments with a chloramphenicol acetyltransferase (CAT)-reporter plasmid containing three AP-1 binding sites. Induced AP-1 DNA-binding activity consisting of cFos/cJun heterodimers to consensus AP-1 binding sites were detected using the electrophoretic mobility shift analyses (EMSA). Moreover, cooperative DNA-binding activities containing cJun and activator protein-2 (AP-2) were identified by EMSA to a region of the NPY promoter that contain AP-1-like as well as AP-2-like binding sites. AP-2 mRNA expression remained unaffected by TPA treatment suggesting post-transcriptional activation of AP-2 proteins in response to PKC. In contrast, expression of both c-fos and c-jun mRNAs was induced synchronously in a biphasic manner. The second peak of expression of these AP-1 mRNAs as well as the induced AP-1 and AP-2 DNA-binding activities, coincided with the onset of neuronal differentiation and with the expression of NPY and GAP-43 genes in SH-SY5Y cells. The GAP-43 expression was analyzed using RNase protection and DNase I footprinting analyses, respectively. Two clusters of putative transcription start sites were identified. Immediately 5' of these clusters, footprints were detected in extracts prepared from TPA treated cells. In these regions, binding sites for the Ets and HLH-families of transcription factors and one binding site for the transcription factor Olf-1 were identified. These results suggest that two GAP-43 promoters are activated in these cells following TPA treatment. Furthermore, several distinct regulatory pathways in SH-SY5Y cells, that are activated by TPA treatment, appear to regulate NPY and GAP-43 gene expression through the cooperative action of multiple transcription factors.

L 301 TRANSCRIPTIONAL AUTOREGULATION OF TWO YEAST BASIC HELIX-LOOP-HELIX ACTIVATOR PROTEINS, Brian P. Ashburner and John M. Lopes, Program in Molecular Biology and Department of Molecular and Cellular Biochemistry, Loyola University School of Medicine, Maywood, Il, 60153.

In the yeast Saccharomyces cerevisiae, the phospholipid biosynthetic genes are highly regulated at the transcriptional level in response to the phospholipid precursors, inositol and choline. Two proteins, the products of the INO2 and INO4 genes, are both required for activation of several structural genes involved in phospholipid biosynthesis. These proteins form a heteromeric complex which binds to a 10-bp element in the promoters of phospholipid biosynthetic genes to activate transcription. Curiously, we have found this 10-bp element in the promoters of both the INO2 and INO4 genes. In order to determine if INO2 or INO4 or both are regulated in response to inositol and choline, we have amplified the INO2 and INO4 promoter regions by PCR and inserted them upstream of the CAT reporter gene. These constructs were then transformed into wild type, ino2, and ino4 strains where they integrated in single copy at the GAL4 chromosomal locus and were subsequently assayed for CAT activity under repressing and derepressing conditions. We found that INO2-CAT was regulated approximately 10-fold in response to inositol and choline but that INO4-CAT is constitutively expressed. We have subsequently confirmed these results by quantitating native INO2 and INO4 transcripts by RT-PCR. INO2-CAT was not expressed in either the ino2 or ino4 strains while INO4-CAT was expressed at wild type levels in the ino2 strain but was not expressed in the ino4 strain. These results lead us to believe that INO2 requires both Ino2p and Ino4p for its expression while INO4 requires Ino4p and some other protein for its expression. These constructs were also tested in an opi1 strain. The product of the OPI1 gene, Opi1p, is a negative regulator of several phospholipid biosynthetic genes and opi1 mutant strains express these genes constitutively. INO2-CAT was expressed constitutvely at greater than wild type levels in the opi1 strain while INO4-CAT was expressed at wild type levels, thus INO2, but not INO4 appears to be sensitive to negative regulation by Opi1p. We are presently in the process of generating a series of INO2 promoter deletions to determine the cis-acting elements present in this promoter responsible for its regulation as well as its weak expression.

L 302 A NOVEL POSITIVE REGULATORY ELEMENT COOPERATES WITH OTHER UPSTREAM SEQUENCES TO MEDIATE DEVELOPMENTAL STAGE-SPECIFIC EXPRESSION OF THE HUMAN EMBRYONIC β-LIKE GLOBIN GENE (ε), Margaret H. Baron, William L. Trepicchio, and Michael A. Dyer, The Biological Laboratories, Harvard University, Cambridge, MA 02138.

The stage-specific regulation of embryonic globin genes in erythroid cells has been an experimentally elusive problem, in part due to the developmentally early timing of their expression. We have prepared a series of deletion mutations within the human embryonic β-globin 5'-flanking region and have analyzed their transcriptional behavior in several erythroid and non-erythroid cell lines. Comparison of the expression patterns of these mutated sequences revealed the presence of multiple positive and negative control elements within a 670 bp region upstream of the constitutive promoter. An embryonic erythroid-specific positive regulatory element (termed ε-PRE II) is of particular interest. Its developmental stage-specificity is observed only in the presence of other sequences, located further upstream, with which it interacts synergistically. Although neither region is active on its own in monomeric or multimerized form, when present together within the intact upstream control region, or when ligated in tandem, they activate a constitutive ε-globin or heterologous promoter in a tissue- and stage-specific manner. Both elements are evolutionarily conserved. A nuclear factor highly enriched in embryonic erythroid cells binds specifically to a novel six bp dyad repeat within ε-PRE II. Methylation interference and point mutagenesis studies have confirmed that residues within the dyad repeat are critical for both specific protein binding and transcriptional activity. Adult erythroid nuclei also contain a factor that binds to this region, but the complex formed migrates more rapidly during nondenaturing electrophoresis. Preliminary evidence suggests that this factor may be differentially modified in embryonic and adult erythroid cells.

L 303 A NOVEL DNA-BINDING PROTEIN CONTAINING A *FORKHEAD* DOMAIN IS INVOLVED IN MUSCLE-SPECIFIC TRANSCRIPTION, Rhonda S. Bassel-Duby, and R. Sanders Williams, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75235

A transcriptional control element, CCAC box, has been defined within a muscle-specific promoter region of the myoglobin gene. We used λgt11 expression cloning with oligonucleotides corresponding to the CCAC box to isolate a clone encoding a DNA-binding protein, termed CBF1. Protein expression of CBF1 is concurrent with muscle development and is elevated in muscle subjected to chronic contractile activity. Determination of the predicted amino acid sequence of CBF1 revealed it to be a member of a new family of DNA-binding proteins, defined by a 110 amino acid domain first identified in the *forkhead* gene of *Drosophila melanogaster*. *Forkhead* functions in the *Drosophila* embryo as a region-specific homeotic gene that promotes terminal, in contrast to segmental, development. Other members of the *forkhead* family, HNF-3α, β, and γ, also have been identified in vertebrates, where they also function as developmental regulators. Our current results provide the first suggestion that members of this family may be involved in control of gene expression in cardiac and skeletal muscle.

L 304 TRANSACTIVATION OF BOVINE HERPESVIRUS-4 (BHV-4) EARLY AND LATE PROMOTERS BY BHV-4 IMMEDIATE-EARLY 2 GENE PRODUCT. Bermudez, R. and van Santen, V. Department of Pathobiology, Auburn University, Auburn, AL 36849-5519.

BHV-4 encodes two immediate early genes: IE1 and IE2. IE1 shows predicted amino acid sequence similarity to IE110 of herpes simplex virus 1 in the amino-terminal regions. IE2 shares predicted amino acid sequence homology with Epstein-Barr virus and herpesvirus saimiri R transcriptional transactivators.

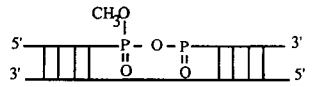
To study the mechanism by which BHV-4 IE gene products transactivate early (E) and late (L) viral genes, DNA segments 5' to the start of a 1.1-kb E RNA and a 1.7-kb L RNA were cloned into a CAT reporter gene vector and used as targets in transient expression co-transfection assays. The IE2 gene product transactivated both promoter-regulatory regions greater than 100-fold. The IE1 gene product did not transactivate either promoter-regulatory region and did not augment transactivation by IE2.

To map the IE2 response elements, deletions were made in each promoter-regulatory region and tested in co-transfection assays. IE2 response elements were found relatively close to the transcription start sites; in each case approximately 300 bp 5' to the start of transcription was sufficient for transactivation by IE2. To determine whether IE2 protein forms a complex with IE2 response elements, IE2 protein was generated by in vitro transcription and translation and used in gel shift assays. An IE2-specific complex was formed with the 300 bp fragments of each promoter which were sufficient for transactivation. These fragments were further subdivided and tested for IE2 complex formation. A 167-bp fragment of the E1.1 promoter and a 128-bp fragment of the L1.7 promoter formed complexes with IE2. These fragments are being tested for IE2-responsive enhancer properties in co-transfection assays.

L 305 INHIBITION OF SPECIFIC GENE EXPRESSION BY DOUBLE-STRANDED OLIGONUCLEOTIDES, Marta Blumenfeld, Svetlana A. Kuznetsova*, Catherine Clusel, Edgardo Ugarte, Zoe A. Shabarova* and Marc Vasseur. GENSET, 1, Passage Etienne Delaunay, 75011 Paris, France and *Department of Chemistry, Moscow State University, Moscow 119899, Russia.

We have previously demonstrated that double-stranded oligonucleotides carrying the recognition sites for DNA-binding proteins can specifically inhibit gene expression at nM concentrations (1). In order to improve the efficiency of the oligonucleotide competitors, we have designed modified oligonucleotides that are able to covalently crosslink to the targetted proteins when interacting with them.

The modification consists in an internucleotide trisubstituted pyrophosphate bond:



formed by template-induced chemical ligation of an oligonucleotide carrying an O-CH_3 substituted 3' P to another oligomer bearing a 5' P. The trisubstituted pyrophosphate can react in aqueous media with nucleophiles, *eg* amino groups present in the binding sites of DNA-binding proteins, thus forming a covalent phosphoamide bond (2).

We synthesized oligodeoxyribonucleotides containing the binding site for the liver-enriched transcription factor HNF-1 (Hepatocyte Nuclear Factor 1) and carrying single modifications at different positions on either strand. Radioactive HNF-1 duplexes were incubated with nuclear liver extracts and then analyzed by band shift assay (affinity study) and PAGE on SDS-denaturing gel (crosslinking efficiency). Our studies showed that HNF-1 was able to specifically bind and crosslink to the modified oligonucleotides. We observed a strict correlation between binding affinity and crosslinking efficiency of the different modified oligonucleotides. Our results indicate that oligonucleotides carrying trisubstituted pyrophosphate bonds can specifically crosslink DNA-binding proteins in crude preparations, thus constituting an attractive tool for both research and therapeutical applications.

1) Clusel *et al.* (1993). *Nucleic Acids Res.* **21**, 3405-3411.
2) Purmal *et al.* (1992). *Nucleic Acids Res.* **20**, 3713-3719.

L 306 THE CRE AND ATF ELEMENTS PLAY DIFFERING ROLES IN TAX TRANSACTIVATION OF THE HTLV-I LTR.

Laurie M. Connor and Susan J. Marroitt Baylor College of Medicine Houston, Texas 77030

The Tax protein of human T cell leukemia virus type I (HTLV-I) is a transactivator of viral gene expression. There are two Tax-responsive elements within the HTLV-I LTR (TRE-1 and TRE-2), and tandem TREs are necessary for full Tax transactivation. Tax does not bind directly to DNA but associates with the TREs via cellular transcription factors. One of the goals of this study was to determine whether both TREs are required for physical association of Tax with the LTR, or whether additional functions can be attributed to the TREs. We have demonstrated that a Gal4-Tax fusion protein can transactivate reporter constructs containing Gal4 binding sites. Interestingly, Gal4 binding sites were necessary but not sufficient for maximal activation by Gal4-Tax. LTR-CAT reporter constructs containing Gal4 binding sites flanked by none, one or two TRE-1 elements were activated by Gal4-Tax (7,48 or 72-fold, respectively). The TRE-1 elements contain binding sites for the cellular transcription factors CREB and ATF, thus we have separately tested the ability of CRE and ATF elements to replace TRE-1 elements adjacent to Gal4 binding sites. The results of these experiments demonstrated that a CRE element (35-fold), and to a much lesser extent , an ATF element (4-fold), was able to functionally replace the TRE-1 element in Gal4-Tax transactivation. Since we have previously demonstrated that Tax can associate with TRE-2 via a cellular transcription factor, we generated reporter constructs in which the Gal4 binding site was replaced by TRE-2. Reporter constructs containing TRE-2 flanked by a CRE element (22-fold), and to a much lesser extent an ATF element (8-fold), can influence the ability of Tax to function as a transactivator. This effect is not fully explained by the ability of CREB to physically direct Tax to the LTR. Thus, elucidating the mechanism of Tax transactivation of the HTLV-I LTR promises to provide new information regarding the differing roles that CREB and ATF play in Tax transactivation.

L 307 THE S. CEREVISIAE URS-1 BINDING PROTEIN, BUF, PARTICIPATES IN MULTIPLE DNA ASSOCIATED FUNCTIONS, T.G. Cooper, W.C. Smart, R. Luche, and R. Sumrada, Dept. of Microbiology and Immunology, Univ. Tennessee, Memphis, TN 38163

BUF protein is a global regulator in **S. cerevisiae** which negatively-controls expression of many unrelated genes by binding to **URS1** elements situated in their 5' regions. Among the genes regulated by BUF are those participating in carbon, nitrogen, and inositol metabolism, electron transport, meiosis, sporulation, and mating type switching. BUF protein consists of 37 and 73 kDa monomers that form a tight complex both in solution and when bound to DNA (PNAS 89:7412-7416).

At least one other protein, encoded by the **CAR80/UME6** gene, is also required for BUF-mediated transcriptional repression (NAR 8:1909-1915). Similar to mutation or deletion of the **URS1** site, disruption of **CAR80** results in loss of transcriptional repression. BUF-**URS1** complex formation does not require the **CAR80** product, since it occurs in a **car80** disruption strain. There is, however, a lower molecular weight DNA-protein complex formed in a wild type strain that disappears in the **car80** disruption mutant, raising the possibility that **CAR80** product also binds to DNA.

We used monoclonal antibodies generated against purified BUF proteins as probes to clone the **BUF1** and **BUF2** genes. We deduced the BUF proteins' amino acid sequences and find the larger one contains a zinc finger motif characteristic of DNA binding proteins. We demonstrate that pure BUF protein also binds to a DNA fragment containing sequences that mediate transcriptional activation. Mutations that destroy the ability of the DNA fragment to support transcriptional activation also destroy BUF binding. Finally, BUF protein is a required participant in DNA replication, because it is identical to the RFA/RPA replication protein. These data suggest that BUF/RFA/RPA protein is likely a new addition to the class of multifunctional proteins (including ABF1, MCM1, and RAP1) which participate in transcriptional activation and repression as well as in DNA replication. (Supported by NIH grant GM-35642).

L 308 MAREK'S DISEASE VIRUS VP16: A TRUNCATED HOMOLOG OF HSV VP16 EFFICIENTLY TRANSACTIVATES MDV AND HSV IMMEDIATE-EARLY PROMOTERS, Paul M. Coussens and Mekki Boussaha, Departments of Animal Science and Microbiology, Michigan State University, East Lansing, MI 48824

Herpes simplex virus encodes a potent transcriptional transactivator, VP16. Expressed late in infection and packaged into mature virus particles, VP16 activates Pol II-mediated transcription from a variety of viral immediate-early (IE) promoters. HSV VP16 has no intrinsic affinity for double-stranded DNA, but interacts with IE promoters through an Oct-1 binding sequence overlapping a larger sequence, TAATGARAT. Mutational analysis of HSV VP16 has identified the highly acidic carboxyl terminus as a potent transcription activating domain. However, recent characterization of VP16 homologues from different herpesviruses, including Marek's disease virus(MDV) and varicella-zoster virus(VZV), reveal molecules which lack an acidic carboxyl terminus yet are able to efficiently transactivate IE promoters. Sequence alignments between VP16 molecules of diverse herpesviruses reveal conserved motifs which may function as transcription activating domains. In this report, we demonstrate that MDV encodes a truncated form of HSV VP16 which is capable of transactivating Pol II-mediated transcription of IE promoters from both MDV and HSV. Multiple sequence alignments are used to examine conserved regions and suggest possible mechanisms of VP16 activity.

L 309 PEA3, AN ACTIVATOR OF TRANSCRIPTION AND DNA REPLICATION, INTERACTS WITH TBP AND RPA THROUGH COMMON DOMAINS, Paul Desjardins, Bradford Brinton, Ji-Hou Xin and John A. Hassell, Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario, Canada L8S 4K1

The *ets* gene family comprises numerous individual members that have been conserved among diverse species. mPEA3 is a relatively recently discovered member of this family of sequence-specific transcriptional regulators. The PEA3 DNA binding site was initially identified in the polyomavirus (PyV) enhancer where it is required for both transcription and DNA replication. Using PEA3 reporter and effector plasmids we have shown that mPEA3 is capable of enhancing transcription and PyV DNA replication. PEA3 is not unique in this regard: many different transcription activators are capable of activating PyV DNA replication. Recently we and others have shown that the transcription activation domains of several transcription factors bind to RPA, a single-stranded DNA binding protein composed of three different subunits. RPA is required for the initiation of PyV DNA replication as well as that of many other viruses. The transcription activation domains of several activators, GAL, p53 and VP16, bind directly to the large subunit of RPA. We have begun to map the functional domains of PEA3 required for transcription and DNA replication. Analyses of unidirectional amino- and carboxyl-terminal deletion mutants revealed that a 164 amino acid region that include the DNA binding ETS domain is sufficient for transcription activation. We have shown using GST-PEA3 fusion proteins and *in vitro* binding assays that this region of PEA3 harbours two independent TATA binding protein (TBP) binding sites. However, neither region alone is able to activate transcription after linkage to the DNA binding domain of GAL. RPA also binds to PEA3. Interestingly, the same two regions sufficient for TBP binding also mediate RPA binding. However, neither region is capable of independently binding to RPA, both regions together are needed to bind RPA. These results suggest that common features of the PEA3 activation domain mediates its interaction with TBP and RPA. We are currently defining the domain in the largest subunit of RPA and that in TBP required for PEA3 binding to learn whether they share sequence and structural similarities.

L 310 The transcriptional activator CCR4 is complexed via a leucine rich repeat domain with several other yeast proteins, one of which has homologs in mice and nematodes. ((M. Draper, C.Salvadore, H-Y. Liu, C. Denis)) Dept. of Biochemistry and Molecular Biology , Univ. of New Hampshire, Durham, NH 03824; 603/862-2438.

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 SPT genes are believed to be involved in the maintenance of chromatin structure. In an interaction trap screen using a LexA-CCR4 fusion protein as the bait we identified a protein from mouse which was capable of interacting with CCR4. We have called this protein m-CIP1 (mouse CCR4 interacting protein). Computer searches using the m-CIP1 protein sequence revealed the presence of m-CIP1 homologs in human, nematode and yeast. We have cloned the yeast homolog of m-CIP1 (y-CIP1) and have found that it also interacts with CCR4 as judged by the interaction trap method and co-immunoprecipitation experiments. Disruption of the y-CIP1 gene in yeast results in defects in transcription, temperature sensitivity on non-fermentative media and sporulation defects. These phenotypes are similar to those seen with disruptions of CCR4. Further the increased transcription seen in an spt10 or spt6 background is y-CIP1 dependent. Immunoprecipitation of a LexA-y-CIP1 fusion from whole cell yeast extracts have revealed that y-CIP1 not only associates with CCR4 in yeast but that it also is complexed with a 195 and a 205 kDa species. These 195 and 205 kDA proteins have previously been shown to co-immunoprecipitate with CCR4. We believe that CCR4 and its associated factors may make up an evolutionarily conserved complex which is involved in transcriptional regulation of a wide variety of genes.

L 311 TRANSCRIPTIONAL REGULATION OF GENES IN RESPONSE TO OXIDATIVE STRESS, Cheryl A. Edbauer-Nechamen, Sharon L. Salmon and Kelvin J.A. Davies, Department of Biochemistry and Molecular Biology, Albany Medical College, Albany, NY 12208

Cells are routinely exposed to a damaging milieu of oxidants. Complex systems, including antioxidants and DNA, lipid and protein repair systems have evolved to protect the cell from injury as a result of this exposure. Our group is interested in the control of gene expression after exposure to oxidative stress that will allow the cell to survive oxidative damage. We have established a model system in which HA-1 hamster fibroblasts show an adaptive response after exposure to oxidative stress. Cells that are pretreated with a low dose of hydrogen peroxide are able to survive a later lethal dose of hydrogen peroxide. The level of protection seen is up to 30-fold. This adaptive response is not observed when transcription is inhibited.

Of particular interest is the activity of repair systems that may be activated after exposure to hydrogen peroxide. These repair systems represent a "second line of defense" to repair damage caused by free radicals that escape the action of antioxidant enzymes. By northern blot analysis, we have observed a modulation of expression of several genes, including the tumor suppressor p53, the growth arrest and DNA damage-inducible genes GADD 45 and GADD 153 and the DNA repair enzyme AP endonuclease. A mechanism for regulation of transcription of these genes will be presented.

L 312 NEGATIVE REGULATORY ELEMENTS IN MAMMALIAN ALCOHOL DEHYDROGENASE GENES, Howard J. Edenberg[1,2], Mang Yu[1], Celeste J. Brown[1], Lu Zhang[1], Ronald E. Jerome[1], Zhonghua Lin[3], Lucinda G. Carr[3,4]. Depts. of [1]Biochemistry and Molecular Biology, [2]Medical and Molecular Genetics, [3]Pharmacology, and [4]Medicine, Indiana Univ. School of Medicine, Indianapolis IN 46260-5122.

Mammalian class I alcohol dehydrogenases (ADHs) catalyze the rate limiting step in ethanol metabolism. We are studying the regulation of mouse *Adh-1* and human *ADH1, ADH2* and *ADH3*.

In the mouse alcohol dehydrogenase gene *Adh-1*, the 5' region extending to -473 bp directed transcriptional activity of a linked reporter gene in H4IIE-C3 cells (a hepatoma cell line that expresses endogenous ADH activity). A longer construct, extending to -809 bp is 2-3-fold less active.

Gel retardation and DNaseI footprinting experiments localize a cis-acting element between -324 and -297 bp of *Adh-1* that reduces transcriptional activity to about half that seen in the construct extending to -473 bp; we call this element NRE-1. Mutation of 4 of the residues within NRE-1 abolish both the binding of nuclear proteins to this region and the negative effect upon gene expression.

A fragment located between -619 and -809 bp of *Adh-1* acts as a silencer element, reducing expression of a heterologous promoter/enhancer construct to about half that seen in the pCAT-Control construct alone, whether it is placed in front of the promoter or at the 3' end of the CAT gene, in either orientation.

The human *ADH1, ADH2* and *ADH3* genes all show a DNaseI footprint in the region from -100 to -146 bp that can be eliminated by competition with a consensus CTF/NF-I oligo. Despite great overall similarity in DNA sequence, they differ in the exact position at which this protein binds. This CTF/NF-I-related site reduces transcription two- to five-fold in the three different cell lines in which the promoter constructs have been tested.

L 313 MAPPING OF DOMAINS IN Sp1 NECESSARY FOR THE STIMULATION OF TRANSCRIPTION BY DIFFERENT ENHANCER ELEMENTS, Maud Forsberg, Anne-Christine Ström and Gunnar Westin, Department of Medical Genetics, Biomedical Center, Box 589, Uppsala University, S-751 23 Uppsala, Sweden

The transcription factor Sp1 regulates transcription of many cellular and viral genes by interacting synergistically with other site-specific transcription factors and with the basal transcriptional machinery. We have previously found that an enhancer which binds a single type of transcription factor (BPV E2) synergises with a minimal promoter containing two Sp1 binding sites. We are now mapping domains in Sp1 necessary for this E2-dependent stimulation. We also map Sp1 domains necessary for stimulation by the SV40 enhancer, by a glucocorticoid receptor-responsive enhancer and for the functional cooperation with the octamer element in the U2 snRNA gene promoter. The transcriptional activity of chimeric proteins, containing the DNA-binding domain of GAL4 fused to different parts of Sp1, have been measured from appropriate test genes in a transient expression assay. The results show that several regions of Sp1 can synergise with the different enhancer elements. We will also discuss results that suggest a particular role for the glutamine-rich activation domain A in E2-dependent stimulation of transcription.

L 314 CHARACTERIZATION OF NUCLEAR FACTORS INVOLVED IN 202 GENE INDUCTION BY IFN-α IN MURINE LEUKEMIA CELLS, Marisa Gariglio, Mirella Gaboli, Cristina Mana, Guo-Guang Ying and Santo Landolfo, Institute of Microbiology, Medical School, University of Torino, Italy.

The 5′ terminal flanking region of the IFN-inducible gene, 202, contains an interferon-stimulatable response element (ISRE), called GA box, that confers inducibility by IFN-α, but not by IFN-γ, on a reporter gene, such as the chloramphenicolacetyltransferase (CAT). Nuclear -extracts from L1210 murine leukemia cells, stimulated for various periods of time with IFN-α, were mixed with ^{32}P-labeled GA box and analyzed for the presence of retarded complexes in EMSA. In addition to a few constitutive retarded complexes, an inducible GA box-binding activity (GAbf-1) appeared after 5 min, peaked at about 2 hr, and was still abundant 12 hr after IFN-α treatment. In contrast, GAbf-1 was not appearent in the cytoplasmic fraction before 30 min, continued to increase up to 2 hr, but had disappeared within 12 hr. GAbf-1 activity was not observed in nuclear extracts treated with IFN-γ, and was not inhibited by pretreatment with the protein synthesis inhibitory cycloheximide. Photochemical cross-linking affinity demonstrated that nuclear extracts from IFN-α-treated cells give rise to three bands that migrate with a Mr of 43, 46 and 52 kDa. In contrast, nuclear extracts from untreated cells generated only two bands with Mr of 43 and 46 kDa, respectively. When the binding properties of GAbf-1 were compared with those of ISGF-3, the primary transcriptional activator for IFN-α-induced genes, a different pattern of retarded complexes was observed, indicating that GAbf-1 may be a novel transcription factor exploited by IFN-α to activate the 202 inducible gene.

L 315 FUNCTIONAL ANALYSES OF PROMOTER ELEMENTS RESPONSIBLE FOR METAL REGULATION OF THE TROUT METALLOTHIONEIN (MT)-B GENE, Lashitew Gedamu, Susan Samson, and Tapan Karchoudhury, Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada.

Metallothioneins (MTs) are conserved metal binding proteins which are regulated by metals at the transcriptional level. The rainbow trout (*Salmo gairdnerii*) MT-B gene promoter contains only two putative Metal Responsive Elements (MREs a and b) and lacks additional basal elements which complicate mammalian MT promoters. Because of this simplicity, the tMT-B promoter is particularly amenable to studies of MRE function using mutagenesis.

Native and mutant tMT-B promoters were assayed by transfection into rainbow trout hepatoma (RTH-149) cells. Inactivation of MREa resulted in reduction of metal fold induction from 19 to 7-fold. However, mutation of MREb nearly abolished basal and metal induced transcription. This confirms that the MREs display differential activity since MREb makes the largest contribution to metal fold induction. As well, MREs a and b interact co-operatively since the fold induction of the native promoter far exceeded the combined values for each MRE alone. Finally, mutations which increased the base pairing potential of the MREs also increased transcription activity.

The tMT-B promoter is also active when transfected into mouse L cells confirming the conservation of metal regulatory factors. The results generally parallel that of RTH-149 cells. However, MREa appears to make large contributions to basal activity since activity is drastically reduced by MREa mutation while metal induced levels are only slightly affected. This may indicate that additional constitutive factors are active in mouse L cells which bind MREa or an overlapping sequence. (Funded by MRC of Canada).

L 316 HUMAN HSF1 CONTAINS A POTENT REGULATED ACTIVATION DOMAIN, Marie Green, Elizabeth Newton, Thomas Schuetz, and Robert E. Kingston. Department of Molecular Biology. Massachusetts General Hospital. Boston, Massachusetts 02114

To characterize the transcriptional activation domains of the human heat shock factors, HSF1 and HSF2, we divided the carboxy two thirds of each factor into three domains, A, B and C, and fused these domains individually as well as in combinations to the DNA binding domain of bacterial LexA (for analysis in yeast) and the DNA binding domain of GAL4 (for analysis in mammalian cells). The amino terminal 200 amino acids of HSF compose the DNA binding and trimerization domains and were therefore deleted in the fusions constructs to avoid potential heterotrimerization with the endogenous factor. Transformations utilizing the LexA-HSF1 and HSF2 fusion proteins revealed that both HSF1 and HSF2 contain potent activation domains that function in *S. cerevisiae*. In HeLa cells however, we found that while domains B and C of HSF1 contain potent activation potential in the same range as VP16, domain A can function to repress this potential at control temperature when included in the same Gal4 fusion. When these cells are subjected to heat shock, the repression by the A domain is alleviated. In other words, the central domain of HSF1 is able to regulate the function of the C-terminal activation domains in a heat inducible manner. When transfections are performed in either Balb/c 3T3 cells or COS cells, the A domain still functions as a repressor however heat induction is not observed. Gel shift analysis and immunofluorescence studies indicate that the A domain does not affect the DNA binding or subcellular localization of the GAL4-HSF1 fusion proteins. In addition, the A domain can repress the artificial amphipathic helix activator, AH but not VP16. We have found, therefore, that HSF1 contains a very potent transcriptional activation domain(s) that is located in the C-terminal third of the protein and we have located a domain in the central region of HSF1 that can regulate the transcriptional activation domain in a heat inducible manner.

L 317 BIDIRECTIONAL TRANSCRIPTION OF THE HUMAN ET/PR264 LOCUS.

Cécile GUYON[1], Patricia CRISANTI[2], Johann SORET[1] and Bernard PERBAL[1,3]

[1]Laboratoire d'Oncologie Virale et Moléculaire, Institut Curie, 91405 Orsay ; [2]Centre de Biologie Cellulaire du CNRS, 94205 Ivry-sur-Seine ; [3]UFR de Biochimie, Université Paris 7 (D. Diderot)

We have reported that the expression of a class of thymic *c-myb* mRNA species requires the intermolecular recombination of ET (thymus specific) and *c-myb* coding sequences localized on different chromosomes both in chicken and human (1). We previously established that in chicken the ET locus is subjected to bidirectional transcription (2) and that antisense transcripts encode the general splicing factor PR264/SC35 (3).

In an attempt to establish whether bidirectional transcription also occurs in human cells, and whether both sense and antisense mRNA species are coexpressed in the same cell, we have performed RNAse mapping and *in situ* hybridization with ET or PR264-specific oligonucleotides and RNA probes.

The results presented indicated that ET and PR264 can be detected in various human cells (HL60, CCRF-CEM, HeLa) and that 10 % of the labelled HL60 cells expressed both ET and PR264.

We have recently shown that the expression of the human PR264 is modulated *in vitro* and *in vivo* by the *c-myb* proteins (4). To better understand the mechanisms controlling the bidirectional transcription of the ET/PR264 locus, we have undertaken a study of the ET transcription and promoter sequences, the results of which will also be presented.

The potential relationship between the bidirectional transcription of the ET/PR264 locus and hematopoietic differentiation will be discussed.

(1) Vellard et al. (1991) *Oncogene*, **6**, 505-514.
(2) Perbal and Vellard (1990) *C.R. Acad. Sci. Paris*, **311**, 467-472.
(3) Vellard et al. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 2511-2515.
(4) Sureau et al. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 11683-11687.

L 318 LESS SELECTIVE DNA BINDING OF v-Ets COMPARED TO c-Ets-1, Soonjung L. Hahn and Bohdan Wasylyk, LGME-CNRS and U184-INSERM, Institut de Chimie Biologique, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cedex, France

The oncogene *v-ets* and its cellular counterpart *c-ets-1* encode members of the Ets family of transcription factors. They specifically bind to DNA through the Ets domain, which is highly conserved among the members. Here we show that v-Ets has a less stringent DNA sequence requirement than c-Ets-1. v-Ets binds strongly to a broad spectrum of DNA sequence motifs, and therefore, weaker binding sites have a much greater affinity for v-Ets than c-Ets-1. The c-Ets-1 on-rate is higher with a strong DNA motif than with a weak motif. These results suggest that the strong binding motif is efficient in inducing a conformational change of c-Ets-1, facilitating its binding to DNA. On the other hand, v-Ets may not require such a process, presumably because the different C-terminal sequence results in a constitutively active conformation. This raises a possibility that v-Ets may transform cells by affecting the expression pattern of the genes carrying weak Ets binding sites. Our data have novel implications for the mechanisms of transformation by v-Ets.

L 319 **MOLECULAR CHARACTERIZATION OF THE YEAST TRANSCRIPTIONAL ACTIVATOR CHA4 .**

Steen Holmberg & P. Schjerling, Department of Genetics, Institute of Molecular Biology, University of Copenhagen, Denmark.

The ability of *Saccharomyces cerevisiae* to use serine or threonine as the sole nitrogen source depends on the *CHA1* gene, encoding the catabolic L-serine (L-threonine) dehydratase. *CHA1* is regulated by transcriptional induction by serine and threonine. We have identified two regulatory elements, $UAS1_{CHA}$ and $UAS2_{CHA}$, in the *CHA1* promoter which confer inducibility by serine and threonine on *Saccharomyces cerevisiae* genes. Serine-mediated induction through $UAS1_{CHA}$ and/or $UAS2_{CHA}$ depends on a functional *CHA4* gene. The *CHA4* gene has been cloned and sequenced. The CHA4 protein has a deduced length of 648 amino acids, including an N-proximal (residue 43 to residue 70) motif known as the $Zn(II)_2Cys_6$ binuclear cluster, suggesting that *CHA4* encodes a DNA binding protein. Furthermore, four acidic regions of 25 - 40 amino acids can be recognized. Gel retardation experiments employing an *E. coli* produced truncated version of CHA4 (residues 1 to 174) demonstrate that CHA4 *in vitro* specifically binds to $UAS1_{CHA}$ as well as to $UAS2_{CHA}$. Furthermore, the affinity of both $UASs_{CHA}$ to $CHA4_{1\text{-}174}$ can be abolished by a G to T mutation in the middle bases of two putative CEZ-elements (presumed binding sites for C_6 zinc finger proteins). The transcriptional activity *in vivo* of $UAS1_{CHA}$ and $UAS2_{CHA}$ and their mutated derivatives correlates with their ability to bind $CHA4_{1\text{-}174}$ *in vitro*.

An analysis of 23 published fungal C_6 zinc finger proteins indicates that many, but not all, contain coiled coil dimerization domains in the region following the zinc finger motif. Based on the presence of these coiled coils, we suggest that the C_6 zinc finger proteins can be divided into two groups, which use different ways of recognizing DNA.

L 320 SPECIFIC PROTEIN-PROTEIN INTERACTIONS OF TRANSCRIPTION FACTOR AP-2, Axel Imhof, Markus Moser, Armin Pscherer, Michael A. Tainsky* and Reinhard Büttner, Department of Pathology, University of Regensburg, 93053 Regensburg, Germany, * M.D. Anderson Cancer Center, Houston, Texas

Eukaryotic transcription factors are thought to mediate their transcriptional activation via specific protein-protein interactions. We have observed that overexpression of AP-2 in PA-1 teratocarcinoma cells leads to a strong inhibition of AP-2 transactivator function in CAT-assays. Furthermore the EMSA-pattern of nuclear extracts of PA-1 cells differs significantly as compared to the one that results from bacterially produced AP-2 protein. These two observations indicate that AP-2 may in vivo form a complex with other regulatory proteins. In order to characterize these AP-2 associated proteins we used two different approaches.

First we screened a 13.5 day old mouse embryo cDNA library with a full length AP-2 cDNA with low stringency in order to clone other AP-2 sequence-related genes which are important during mouse embryogenesis. The second approach was to purify AP-2 associated proteins with an affinity column using recombinant GST-AP-2 fusion protein as a matrix.

Resulting from these two approaches we cloned a new AP-2 related gene called AP2REL which differs from AP-2 mainly in its transactivation domain but is nearly identical in its DNA-binding and dimerization region. Bacterially expressed AP2REL protein has similar DNA-binding properties when compared to AP-2. We could also show that AP2REL heterodimerizes with AP-2 via binding of in vitro translated AP2REL to GST-AP-2 agarose. When using the affinity column to purify AP-2 associated proteins from ^{35}S-methionine labeled nuclear extracts, we demonstrated the specific interaction between AP-2 and a protein with an apparent molecular weight of 70 kDa.

L 321 **THE HUMAN CHORIONIC SOMATOMAMMOTROPIN GENE ENHANCER IS COMPOSED OF SV40-RELATED ENHANSONS AND REQUIRES PROMOTER ELEMENTS NEAR THE TRANSCRIPTION INITIATION SITE.** Shi-Wen Jiang and Norman L. Eberhardt, Endocrine Research Unit and Department of Biochemistry/Molecular Biology, Mayo Clinic/Foundation, Rochester, MN 55902

The human chorionic somatomammotropin (hCS) gene enhancer (CSEn) associated with the growth hormone (hGH) gene locus is involved in directing cell-specific expression of the hCS genes in placenta. We report a detailed structural analysis of this enhancer and its promoter requirements. CSEn stimulated transcription of the hCS, hGH, TK and RSV promoters, in human choriocarcinoma cell lines (BeWo and JEG-3) but not HeLa cells or rat somatolactotrophes (GC). Maximal enhancer activity was confined to a 242 bp DNA segment. Of several CSEn subfragments, only the En_57/242 subfragment retained significant activity (33.5% wild-type). The CSEn DNA sequence contained direct (DR) and inverted (IR) repeat motifs and sequences related to the SV40 enhansons, GT-IIC, GT-I, and Sph-I/Sph-II. DNAse I footprint analysis revealed that most of these sites were protected by nuclear proteins derived from BeWo, JEG-3, HeLa and GC cells. Site-specific block mutation of the GT-IIC-related and IR motifs virtually abolished enhancer activity, and mutation of all but the GT-I-related motif resulted in significant loss (30-60%) of activity. Promoter constructs containing deletions or site-specific mutations of the Sp1 and GHF-1 sites were examined for their ability to stimulate hCSp.LUC gene activity in BeWo cells. GHF-1 mutations had no significant effect on enhancer-stimulated promoter activity. There was a 74% reduction in the basal CS promoter activity upon mutation of the Sp1 site; however, the enhancer stimulated the activity above the original basal level (~4.8-fold stimulation). Thus Sp1 is required for maximal hCS promoter activity in placental cells and may contribute to overall enhancer efficiency. Examination of several proximal promoter mutants revealed that the TATA box and downstream sequences (nts -25/+1) were required for enhancer function. These data indicate that the CS enhancer is comprised of multiple SV40-related enhansons that interact cooperatively with factors that are localized to the hCS promoter near the transcription initiation site.

L 322 IN VITRO ANALYSIS OF MOUSE MAMMARY TUMOR VIRUS TRANSCRIPTION, Chang-Joong Kang, Brian E. Fee, Christopher M. Bral, and David O. Peterson, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843-2128

Transcription of the proviral DNA of the retrovirus mouse mammary tumor virus (MMTV) is induced by several classes of steroid hormones. Steroid responsiveness is a combination of repression of basal promoter activity by a negative regulatory element (NRE) in the absence of hormone and promoter activation by specific steroid receptor proteins bound to cognate hormone response elements (HREs) in the presence of hormone. Previous studies have identified four elements of the MMTV basal promoter that must in some way respond to these regulatory signals: a binding site for nuclear factor 1, a set of three overlapping octamer-related sequences, a TATA box, and an element near the transcription start site that is recognized by a protein we have termed initiation site binding protein. We have identified several binding sites for nuclear proteins within the MMTV NRE; mutation of these sites results in impaired NRE function in vivo, and it thus appears that these proteins are involved in mediating transcriptional repression. We have developed in vitro transcription assays based on fractionated extracts of cultured mammalian cells or bovine liver supplemented with purified transcription factors expressed in bacteria to define the mechanistic roles of specific proteins in basal, as well as regulated, MMTV promoter activity.

L 323 NFAT-SEQUENCE-SPECIFIC DNA-BINDING PROTEIN IS A NOVEL HETERODIMER OF 45-kDa AND 90-kDa SUBUNITS

Peter N. Kao and Blaise Corthesy, Pulmonary Medicine, Stanford University, Stanford, CA 94305-5487 and University of Lausanne, Institute of Animal Biology,CH-1015,Lausanne-Dorigny, Switzerland.

Expression of interleukin-2 (IL-2) by activated T-lymphocytes is regulated by the coordinated binding of transcription factors AP-1, Oct-1, NFkB and NFAT (Nuclear Factor of Activated T-cells) within the IL-2 enhancer. Using ion-exchange and DNA affinity chromatography we purified an NFAT-sequence-specific DNA-binding protein from nuclear extracts of activated Jurkat T-cells. The protein is a heterodimer of apparent molecular weights 45-kDa and 90-kDa; we obtained partial internal amino acid sequences and used these to clone the cDNAs encoding each subunit. The cDNAs predict proteins of novel structures with limited homologies to other proteins: NF45 is similar to prokaryotic transcription factor sigma-54, and NF90 contains a double-stranded RNA-binding motif identified in *Drosophila* staufen, human p68 kinase and human TAR-RNA binding protein. RNA encoding subunits 45.3 and 90.3 exists in nonstimulated Jurkat cells and in all other cell types examined. Antibodies demonstrated that both subunits are located in the nucleus of Jurkat T-cells. Clones NF45 and NF90 transiently expressed in Jurkat T-cells and recovered separately from endogenous protein through a polyhistidine fusion tail encode an NFAT-sequence-specific DNA-binding activity that is significantly enhanced following T-cell stimulation.

L 324 HEPATIC NUCLEAR FACTOR-4 REGULATION OF CYTOCHROME P450 2C GENES, Byron Kemper and David Chen, Department of Physiology & Biophysics, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

Hepatic cytochrome P450 (CYP) genes comprise the majority of the CYP gene superfamily. Previous studies demonstrated that an element, which is conserved in more than 20 CYP2A, CYP2C, and CYP2E genes and resembles the HNF-4 binding motif, mediated HepG2-specific transcriptional activation of two CYP2C genes. To determine whether HNF-4 functionally binds to this element, the tissue distribution, DNA binding characteristics, immunochemical reactivity of the protein that binds to the CYP genes was compared with HNF-4, as well as transcriptional activity of their recognition elements. DNase I footprint analyses and band shift assays indicated that the CYP binding activity, like HNF-4, was present in liver and kidney, but not brain and spleen. Both factors bound specifically to either the site in the CYP2C2 promoter or a known HNF-4 site in the human apolipoprotein CIII (apoCIII) promoter to form complexes with same electrophoretic mobilities which could be specifically "supershifted" by an antiserum to HNF-4. When the apoCIII HNF-4 sequence was substituted for that in the CYP2C promoter, comparable transcriptional activities were obtained and expression of HNF-4 in COS-1 cells transfected with CYP2C2 promoter-luciferase constructs activated transcription. Comparisons of promoters of closely related CYP2C genes demonstrated that HNF-4 was bound with significantly different affinities: 2-fold and 8-fold less for CYP2C1 and CYP2C3, respectively, compared to CYP2C2. These affinity differences correlated well with the transcriptional activities of minimal promoters or with CYP2C2 promoters containing substitutions of the CYP2C1 or CYP2C3 HNF-4 motifs. The HNF-4 element in the CYP2C3 promoter could be converted to a motif with binding affinity and hepatic cell specific transcriptional activity similar to that of CYP2C2 by a single nucleotide substitution. These results indicate that HNF-4 or a protein with similar properties is reponsible for the liver-specific expression of CYP2 genes and is an important determinant of basal activity in these genes.

L 325 NEURON-SPECIFIC EXPRESSION OF THE HUMAN DOPAMINE-β-HYDROXYLASE GENE REQUIRES BOTH THE cAMP RESPONSE ELEMENT AND A SILENCER REGION, Kwang-Soo Kim, Hiroshi Ishiguro and Tong H. Joh, Cornell University Medical College at the Burke Medical Research Institute, White Plains, NY 10605

Dopamine β-hydroxylase (DBH), the enzyme catalyzing the conversion of dopamine to norepinephrine, is specifically expressed in adrenergic and noradrenergic neurons in the central nervous system. DNase I hypersensitive sites are found in the 5' flanking region of the DBH gene in noradrenergic human neuroblastoma SK-N-BE(2)C cells, but not in HeLa cells. We report that the 4.3 kb upstream sequence of the human DBH gene confers cell type-specific expression as assessed by transient expression assay. Furthermore, deletion of the cAMP-response element abolishes >95% of the transcriptional activity by the DBH upstream promoter, thus implicating the CRE as an essential positive genetic element. In addition, co-transfection experiments and analyses of several mutant cell lines, which had been rendered deficient in cAMP-dependent protein kinase (PKA), indicate that PKA plays a crucial role in transcriptional regulation of the DBH gene. Second, deletion of a region between -490 and -263 bp results in 10-fold increase of reporter gene activity only in HeLa cell, indicating that this region contains a cell-specific silencer. A 13 bp fragment residing within that region shows 77% sequence identity with the neuron-specific silencer motif recently identified in three neuronal genes, i.e., SCG10, type II sodium channel and synapsin I genes. We propose that the interplay between the CRE and the neuron-specific silencer region plays an important role in the tissue-specific expression of the DBH gene in noradrenergic cells.

L 326 REGULATION OF THE BOVINE LEUKEMIA VIRUS BASAL TRANSCRIPTION, Kiss-Toth E., Unk I., Bachrati Cs. and Czibula A., Biological Research Center, Hungarian Academy of Sciences, Hungary Szeged, P.O. Box 521

Recent studies demonstrated, that the R-U5 region of the Bovine Leukemia Virus (BLV) Long Terminal Repeat (LTR) up-regulates the virus promoter, independently from the BLV trans-activator protein, p34 *tax*, encoded in the virus genome. Deletions were constructed to localize the important sequences responsible for this effect. The activity of the different constructs was determined in a transient expression system. Our results show that a 76 bp long sequence (Downstream Activator Sequence), present in the 3' end of the R region, is involved in the activation. The *in vivo* experiments show, that DAS can be divided into two independent but overlapping elements (DAS1,2). In these elements sequence comparison allows the identification of three conservative boxes. The gel-shift assay with DAS2, in good agreement with the *in vivo* results, demonstrates that only the full length element forms a low mobility DNA-protein complex.

L 327 SUPPRESSION OF PDGF-B/SIS EXPRESSION IN T24 BLADDER TUMOR X HUMAN FIBROBLAST HYBRIDS, Gregory C. Kujoth, Hua-Ming Jin and William E. Fahl. McArdle Laboratory for Cancer Research, Madison WI 53706

The PDGF-B/SIS gene is expressed in a variety of human tumor tissues and cell lines. Expression of PDGF-B /SIS could be regulated by suppression in non-expressing cells or by activation in expressing cells. In an attempt to distinguish between these possiblities, we have made somatic cell hybrids of diploid human fibroblasts, which are SIS mRNA negative, and T24 human bladder carcinoma cells, which express SIS at a high level. Of ~twenty hybrid clones examined, two clones exhibited SIS mRNA levels close to the T24 parent while the remainder were near the fibroblast parent or at an intermediate level. The SIS levels, averaged out over all of the hybrid clones, were suppressed to ~33% of the T24 parental level. The expression levels in the hybrids did not correlate with SIS gene copy number, thus ruling out the trivial explanation of loss of the gene resulting in the observed expression levels. Luciferase reporter constructs containing 4 kbp or 0.4 kbp of SIS promoter sequences were transfected into the parental cell lines to determine if transcriptional differences could account for the observed expression pattern. Both reporter constructs displayed significant activity in human fibroblasts, in stark contrast to the absence of detectable steady state SIS mRNA in these cells, but did exhibit the correct relative pattern of expression (higher in T24 than in fibroblasts). Thus, the activity of the exogenous SIS promoter may be due to one or more of the following scenarios: 1) the reporter constructs lack epigenetic alterations which normally keep the endogenous SIS gene turned off; 2) the reporter constructs do not contain all the sequences necessary for complete transcriptional regulation of the gene; or 3) additional regulation is occurring at a post-transcriptional level.

L 328 IDENTIFICATION AND CHARACTERIZATION OF AN ACTIVATING REGULATORY ELEMENT IN THE HUMAN SIS/PDGF-B PROMOTER, Yuxin Liang, Don Robinson, Hua-ming Jin and William E. Fahl, McArdle Laboratory For Cancer Research, University of Wisconsin-Madison, 1400 University Avenue, Madison, WI 53706

The SIS/PDGF-B gene, encoding the B polypeptide of platelet-derived growth factor, is transcriptionally activated (>50 fold) in human K562 erythroleukemia cells when they are induced to differentiate into megakaryocytic cells by treatment with TPA. A 250-bp PDGF-B gene promoter attached to a luciferase reporter gene reproduced this TPA-induced activation. Using systematic linker-scanning substitutions over the 250-bp region, a 20-bp sequence (5'-TCTCCACCCACCTCTCGCAC) at position -58 to -39 relative to the PDGF-B mRNA initiation site was identified to be essential for the TPA-induced activation. We named this the SIS proximal element (SPE, PNAS 90: 7563,1993). The SPE sequence was not found in the GenBank database of consensus sequences for transcription-factor binding sites. Gel mobility-shift assays using an SPE-oligonucleotide and K562 cell nuclear extracts showed three shifted complexes, one of which was formed only following TPA treatment of K562 cells. Present work involves saturation mutagenesis of the SPE to identify contact points for proteins which bind and activate through this element. A -58 to -56 TCT to GAG substitution showed no effect upon induction; mutant SPE elements showed differential binding abilities for proteins in the K562 cell nuclear extracts. High resolution mapping of the SPE will provide direction for efforts to purify and identify proteins important for PDGF-B gene activation.

L 329 Identification of proteins which are associated with the yeast transcriptional activator CCR4, a suppressor of mutations in genes thought to be involved in chromatin maintenance. (H-Y. Liu, M.draper, C. Denis) Dept. of Biochemistry and Molecular Biology, Univ. of New Hampshire, Durham, NH 03824; 603/862-2438.

The yeast CCR4 protein is required for the expression of a number of genes including the glucose repressible ADH2 gene. Mutations in CCR4 also suppresses mutations in SPT6 and SPT10, two genes believed to be required for the proper chromatin regulated maintenance of transcription. We show here that the transcriptional activation ability of CCR4, in contrast to many other activators, is glucose regulated. The leucine-rich repeat and the C-terminus of CCR4 were found to be absolutely required for CCR4 transcriptonal activity, although neither region was capable of activation on its own. Two activation domains were identified, one encompassing a glutamine-proline rich region similar to that found in eukaryotic transcriptional activators, but these regions required the leucine rich repeat and the C-terminus for proper function at the ADH2 locus. CCR4 was shown to form a complex with four other proteins, two of which , a 195 and a 185 kDa in size, were shown to bind specifically to the leucine rich repeat of CCR4. We propose that the leucine rich-repeat through its associated factors links CCR4 to its promoter context at the ADH2 locus where it is required for full glucose derepression and effects on chromatin structure.

L 330 CHARACTERIZATION OF THE MUSCLE SPECIFIC CORE PROMOTER OF THE RABBIT Na,K-ATPase $\alpha 2$ SUBUNIT GENE. Li-Mei Meng, Trine Kjærsig, and Peter L. Jørgensen. Biomembrane Research Centre, August Krogh Institute, Copenhagen University DK-2100, Denmark.

The Na,K-ATPase consists of one α and one β subunit. In mammals, there are at least three isoforms of the α subunit. The $\alpha 1$ subunit is expressed in most tissues, while the $\alpha 2$ and $\alpha 3$ subunits are expressed in brain, heart and muscle. To investigate the regulation of the expression in muscle, we cloned 5'-flanking sequences of rabbit $\alpha 2$ gene into the polylinker of pCAT-basic (Promega Corp. Madison). The constructs were transfected into myogenic cells (C2C12) and fibroblast cells (NIH3T3). These studies identified a myogenic cell specific basal promoter between nucleotides -165 to -43 from translation initiation. Just upstream of the TATA-like sequence, the promoter contained a well conserved 33 bp purine-rich sequence for which regulatory effects on transcription have been previosly studied (1). A TC-rich stretch of nucleotides surrounding the transcription start sites contained TCTCTG and TG(T/C)C motifs wich have been found in the initiator elements of various genes. Three copies of a GTTT(C/G) sequence, located just upstream of the 33-bp purine-rich sequence, activated the basal promoter activity by 4 fold. The GTTT(C/G) motif was well conserved in the $\alpha 2$ gene of human and rat. A DNA fragment (nucleotides -258 to -194) containing the consensus Sp1 binding site followed by a GGGGAGGAG sequence, activated the basal promoter activity by additional 2.5 fold. The GGGGAGGAG sequence has recently been shown to be a binding site for a nuclear factor, PAL (2). The Sp1-PAL motif were found in the promoter region of genes encoding neurofilaments. Work is in progress to identify the transcription factors involved in the regulation of the muscle specific core promoter.

1. Kennedy GC, Rutter WJ (1992) Proc. Natl. Acad. Sci. USA 89, 11498-11502.
2. Elder GA, Liang Z, Lee N, Friedrich Jr VL, Lazzarini RA (1992) Mol. Brain Res.15:85-98

L 331 BIOCHEMICAL AND GENETIC CHARACTERIZATION OF SBF, A FACTOR ESSENTIAL FOR ACTIVITY OF THE CHICKEN U1 AND U4 snRNA GENE TRANSCRIPTIONAL ENHANCERS, Jon H. Miyake, Cheung. H. Cheung, Daniel P. Szeto and William E. Stumph, Department of Biology, Department of Chemistry, and Molecular Biology Institute, San Diego State University, San Diego, CA 92182

The transcriptional enhancers of chicken U1 and U4 small nuclear RNA (snRNA) genes are composed of two functionally essential motifs: an octamer motif and an adjacent SPH motif. Using chicken embryo nuclear extract as a source material, we have identified a novel factor, termed SBF, that interacts sequence-specifically with the SPH motif. Southwestern analysis identifies SBF activity in a polypeptide with an apparent molecular weight of ~77 kD. Interestingly, SBF requires the presence of Mg^{2+} or Ca^{2+} at a concentration between 1-5 mM in order to efficiently bind to the SPH motif in vitro. Gel shift analysis has identified SBF-like activity to be present in the nuclear extracts of Hela cells and Xenopus laevis. A chicken embryonic expression library was screened with a double stranded oligonucleotide containing the SPH motif and a partial cDNA clone that is a candidate for SBF was isolated. This partial cDNA clone was subsequently used to screen another cDNA library from which overlapping clones were isolated. These clones contain a partial open reading frame that encodes a polypeptide composed of 727 amino acid residues that is not identical to any previously described protein. The amino acid sequence of this protein contains putative DNA binding and transcriptional activation domains. The ubiquitous transcription factor Oct-1 (which recognizes the octamer motif) and SBF function cooperatively to stimulate snRNA gene transcription in vivo. Extensive efforts, however, have revealed no evidence that Oct-1 and SBF bind cooperatively to DNA, and we are currently investigating alternative mechanisms that may be responsible for this functional synergism.

L 332 CHARACTERIZATION OF THE TRANSCRIPTIONAL ACTIVATION DOMAINS OF RelA (p65), P. A. Moore and C.A. Rosen, Department of Molecular Biology, Human Genome Sciences, Rockville, MD 20850.

NF-κB is a mammalian transcription factor induced by a variety of stimuli to regulate the expression of a wide range of genes encoding immunoreceptors, cytokines and viral proteins. In unstimulated cells, NF-κB is localized to the cytoplasm sequestered in a complex with IκB. When activated, it is present in the nucleus as a heterodimer composed of 50 kDa (NFKB-1) and 65 kDa (RelA) subunits. Both subunits exhibit strong homology at their N-terminal ends to the c-rel proto-oncogene and to other related family members. This rel-homology domain is required for DNA binding and dimerization. In contrast, NFKB-1, RelA and the other rel-family member genes differ significantly at their C-terminus. Previously it has been shown that the C-terminal region of RelA confers the strong transcriptional activity associated with NF-κB and contains three independent *trans*-activation domains (Moore et al., MCB 13:1666-1674). Work presented here dissects the C-terminal region of RelA further. Interestingly, a region was identified which appears to repress transcriptional activity suggesting that in addition to three transcriptional activation domains, RelA also contains a repressor region and can mediate its effect on transcription through interactions with more than one co-factor, perhaps in a cell type manner. To address this question we tested the ability of the activation domains to *trans*-activate in various cell lines. Each of the three activation domains were able to strongly *trans*-activate in HeLa (human cervical cell), Namalwa (human B-cell), Jurkat (human T-cell) in addition to COS cells (monkey kidney cell). In an effort to understand more fully the mechanism of RelA mediated activation we have conducted analyses of the RelA activation domains in *Saccharomyces cerevisiae*, an organism more amenable to genetic studies. As in mammalian cells, RelA functions as a transcriptional activator in yeast, with activation dependent on multimerization and inhibited by Ikb. In addition, the minimal activation domains of RelA functional in mammalian cells, are also functional in yeast demonstrating conservation of the transcriptional machinery required for NF-κB *trans*-activation. This has allowed us to begin dissecting the mechanism of RelA mediated *trans*-activation by taking advantage of the well defined genetic strategies available in yeast.

L 333 SELECTIVE INVOLVEMENT OF c-JUN IN AP-1-MEDIATED RESPONSE OF DETOXICATION ENZYMES TO HYPOXIA IN HUMAN COLON ADENOCARCINOMA CELLS. Peter J. O'Dwyer, Kang-Shen Yao, Fox Chase Cancer Center, Philadelphia, PA 19111.

Many solid tumors contain substantial fractions of hypoxic cells, which are relatively resistant to both radiation therapy and certain cytotoxic drugs. We have previously shown that exposure of HT29 cells to hypoxic conditions results in the overexpression of certain enzymes involved in the detoxication of xenobiotics, especially DT diaphorase and γ-glutamylcysteine synthetase, the rate limiting enzyme in glutathione synthesis. Nuclear run-on analysis reveals that the mechanism of this hypoxic effect on DT diaphorase is transcriptional induction. We have investigated the effects of hypoxia on elements in the promoter region of DT diaphorase. Electrophoretic mobility shift assays (EMSA) demonstrate the induction of a binding activity to the AP-1 response element of DT diaphorase. Supershift assays suggest that this binding is due to AP-1 proteins, and that Jun-Jun homodimers are induced to a greater degree than Fos-Jun heterodimers by hypoxia. Nuclear run-on assays indicate that selective induction of c-jun transcription occurs during the hypoxic exposure. These data suggest that the hypoxic response of detoxicating enzyme expression is mediated in part through AP-1, by a specific Jun-related mechanism.

The affinity of transcription factors for the AP-1 binding site depends upon the redox state of a cysteine residue located close to the DNA binding region of both Fos and Jun. Ref-1, a nuclear protein isolated by Xanthoudakis and Curran (EMBO J 99:653-665, 1992) maintains the reduced state, and promotes binding of AP-1. Following an 8 hour exposure to hypoxia, nuclear extracts from HT29 cells showed markedly increased Ref-1 protein content. During the hypoxic treatment, elevation of Ref-1 steady-state mRNA levels was evident as early as 2h, and levels increased throughout the hypoxic exposure. Nuclear run-on analysis showed this to result from induction of Ref-1 transcription. EMSA were used to further define the interaction of Ref-1 with specific AP-1 binding proteins under hypoxic conditions. We conclude that the response of HT29 cells to hypoxia is complex, and includes the induction of detoxicating enzyme expression in part through the induction and binding of transactivating factors to the AP-1 binding site.

L 334 MUTUALLY EXCLUSIVE INTERACTIONS OF NF-Y AND YY1 WITHIN A PROMOTER ELEMENT CRITICAL FOR THE CONSTITUTIVE ACTIVITY OF HERPES SIMPLEX VIRUS 1 IMMEDIATE EARLY 110K PROMOTER. **D. M. O'Rourke and P. O'Hare. Marie Curie Research Institute, Oxted, Surrey, UK.**

The regulation of IE110K promoter activity by cellular factors may be a critical determinant in the reactivation of viral latency.

We observed a high level of constitutive activity from the IE110K promoter in HeLa cells, comparable to that of the SV40 early promoter. Deletion of the upstream TAATGARAT elements, which are essential for the VP16 mediated immediate early specific regulation of this promoter, resulted in a minimal IE110K promoter which still retained strong constitutive activity. Subsequent further deletion of the minimal IE110K promoter identified an element essential for high level constitutive promoter activity. This element was refined to 20 bp located between -90 and -70 relative to the transcriptional start site at +1. Two nuclear proteins bound within the element, one protein has been identified as an NF-Y type CCAAT box binding protein, the other appears to be YY1, also known variously as UCRBP, ∂, NF-E1 and CF1. Mutational analysis of the promoter element and methylation interference data for YY1 and NF-Y suggest that the binding sites overlap, furthermore, *in vitro* binding studies indicate a mutually exclusive interaction of YY1 and NF-Y within the promoter element.

The mutual exclusion of CCAAT box binding proteins is involved in the developmental and tissue specific transcriptional regulation of a number of proteins, and YY1 has been shown to function as both a positive and negative regulator of transcription on a wide variety of promoters. We have therefore generated site directed mutations of the promoter element to determine the contribution of both proteins to promoter activity, and are currently screening latent and reactivated trigeminal ganglia extracts for relative levels and binding activities of YY1 and NF-Y. The mutually exclusive interactions of NF-Y and YY1 on the promoter element observed *in vitro* may modulate the activity of the IE110K promoter during latency and subsequent reactivation.

L 335 AP2 TRANSCRIPTION FACTOR IS INVOLVED IN THE TRANSCRIPTION OF THE LENS MIP GENE. **Chiaki Ohtaka-Maruyama, Xiaoyan Wang and Ana B. Chepelinsky.** Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20892, U.S.A.

MIP, the major intrinsic protein of the lens fiber membrane, is specifically expressed in the ocular lens fibers. MIP belongs to an ancient family of transmembrane channel proteins. MIP gene expression is temporal and spatially regulated during development. We are studying the *cis* regulatory elements responsible for the lens specificity and developmental regulation of the MIP gene.
We found that a DNA fragment containing 253 bp of 5' flanking sequence and 42 bp of exon one of the human MIP gene fused to the reporter gene chloramphenicol acetyltransferase (CAT) gene directs CAT gene expression to lens cells in transient assays.
Several motifs, known to bind transcription factors in other genes, like Sp1, AP2 and NF1 binding sites, are present in the 5' flanking sequence of the MIP gene. To elucidate the involvement of general factors in the expression of the MIP gene, we studied the *in vitro* transcription of the MIP gene using *Drosophila* nuclear extracts and purified transcription factors. We found that Sp1 and AP2 activate the *in vitro* transcription of the MIP gene. DNase I footprinting analysis and gel mobility shift assays showed that these factors bind to several domains located in the -253/+42 MIP sequence. Sp1 is non tissue-specific, whereas AP2 is expressed in a tissue-prefered manner. Mutations in the AP2 binding sites affect the binding to AP2. We are presently analyzing AP2 localization in the mouse lens. These studies will further our understanding of the role of general transcription factors on the tissue specific expression of the MIP gene.

L 336 THE HEPATOCYTE NUCLEAR FACTOR-3 (HNF-3) / FORKHEAD HOMOLOG (HFH) TRANSCRIPTION FACTORS EXHIBIT DIVERSE CELLULAR EXPRESSION PATTERNS AND BINDING SPECIFICITIES. David G. Overdier, Anna Porcella, Derek E. Clevidence, and Robert H. Costa. Department of Biochemistry (M/C 536), University of Illinois, College of Medicine, Chicago, IL 60612.

The HNF-3 transcription factors are three distinct proteins (α, β, γ) known to regulate the restricted expression of several genes important for liver function. The HNF-3α and -3β proteins are also important regulators in the lung and are expressed in early embryos (6.5 to 7.5 P.C.) suggesting that they play a more extensive role during development. The HNF-3 proteins share 95% homology in their DNA binding domain and possess amino acid conservation in their activation domains located at both amino and carboxy termini. The HNF-3 DNA binding domain also shares homology with the *Drosophila* intestinal differentiation factor, forkhead (fkh). Additional HNF-3/fkh-related proteins are known to be required for determination events during embryogenesis in *Drosophila* and *Xenopus*. The HNF-3/fkh proteins bind to DNA as monomers using a novel winged helix motif which consists of a modified helix turn helix motif. We have isolated an extensive family of tissue-specific rodent HFH genes.

Many of the HFH genes display tissue-restricted expression patterns, several of which are expressed in the lung. In situ hybridization studies reveal diverse cellular expression pattern of the HNF-3/fkh genes in the lung. HNF-3α and HFH-4 genes illustrate overlapping expression in the bronchial epithelial cells of the lung. In contrast, the HNF-3β gene is expressed in the smooth muscle layer surrounding bronchioles and arterioles while the HFH-1 is expressed throughout the lung at low levels. The HFH-8 is transcribed in the alveoli of the lung, but not surrounding the bronchioles or arterioles where HNF-3 and HFH-4 are expressed.

Using a site selection protocol we have identified high affinity DNA binding sites for the HFH-1 and HFH-2 proteins that are distinct from the HNF-3 DNA target sequence. Subtle nucleotide changes in the DNA recognition site are sufficient to distinguish between these different family members. Although these proteins bind to different DNA sequences the recognition helix constitutes the most conserved region of the protein. Analysis of chimeric proteins between the HNF-3 and HFH-1 DNA binding domains has defined a 20 amino acid sequence which is involved in DNA site specificity. Interestingly, these amino acid sequences are not involved in nucleotide contacts and contain the most divergent region of the HFH family. This sequence encompasses the helices which from a structure prediction may play an important role in positioning the recognition helix in the major groove of DNA. We propose that altered DNA binding specificity is mediated by the positioning of the conserved recognition helix in the major groove of DNA.

L 337 THE TRANSCRIPTIONAL ACTIVITY OF THE HUMAN INSULIN GENE IS DEPENDENT ON THE CT2 BOX WHICH INTERACTS WITH A STF1 LIKE PROTEIN, Helle V.Petersen, Leonard J., Serup P., Madsen O.D., Michelsen B., Hagedorn Research Institute, Niels Steensensvej 6, DK-2820 Gentofte, Denmark.
The human insulin enhancer contains three CTAATG sequences (CT boxes) which are thought to be involved in the tissue specific regulation of the human insulin gene. To investigate the importance of the CT2 box on the transcriptional activity of the human insulin gene we transiently transfected primary islet cells with constructs containing 337 bp of the human insulin enhancer with or without a mutated CT2 box linked to chloramphenicol acetyltransferase (CAT). Cat activity was found to be dependent on glucose concentration and an intact CT2 box. By electrophoretic mobility shift assays (EMSA) using oligonucleotides covering the CT boxes (CT1;-88 to -58, CT2;-231 to -200, CT3;-324 to -289) and nuclear extracts from insulin and non insulin producing cell lines we have identified a β-cell specific complex that has the highest relative binding affinity to the CT2 box. The protein binding to the CT boxes was by UV cross linking experiments found to be approximately 46 kDa. The transcription factor STF1 binds to a tissue specific element in the somatostatin gene containing the sequence CTAATG. Using in vitro translated STF1 in EMSA with the CT1 or CT2 box probes results in the appearance of a band of similar mobility as the β-cell specific complex. Furthermore, anti-STF1 antiserum blocks the binding of the β-cell specific complex. Together these results indicate that the CT2 box is required for transcriptional activity of the human insulin gene and that this dependence might be mediated by STF1.

L 338 CHARACTERIZATION OF TWO NUCLEAR FACTOR BINDING DOMAINS WITHIN A REGION OF THE APP PROMOTER THAT IS ESSENTIAL FOR ITS ACTIVITY. Wolfgang W. Quitschke, Department of Psychiatry and Behavioral Sciences, State University of New York at Stony Brook, Stony Brook, NY 11794-8101.

The major component of amyloid depositions in Alzheimer's disease is the amyloid beta protein, which is a truncated form of the larger amyloid precursor proteins (APP). The promoter of the APP gene was analyzed for its ability to direct cell type specific expression in different cell lines that showed variant levels of endogenous APP transcripts. The APP promoter and selected deletions were placed 5' to the reporter gene chloramphenicol acetyl transferase. Transient transfection assays showed that 96 base pairs 5' to the transcriptional start site are sufficient for full cell-type specific promoter activity.

Two nuclear factors that bind to this region in a sequence specific manner were identified by mobility shift electrophoresis, DNase foot printing, and methylation interference. One of the two factors, here designated as APBα, binds to a recognition sequence located between positions -30 and -58 upstream from the main transcriptional start site (+1). The other factor, APBβ, recognizes a sequence between positions -70 and -96.

The contribution of the two binding factors APBα and APBβ to the activity of the APP promoter were assessed by introducing block mutations into the respective recognition sequences, which were subsequently analyzed by transient transfection. The results suggested that factor APBβ contributes most of the transcriptional activity to the APP promoter. Mutations that were deleterious to the binding of factor APBβ *in vitro*, as determined by mobility shift assays, showed a marked reduction in promoter activity as compared to the unmodified wild type promoter. By comparison APBα only has a marginal role in transcriptional activity from the APP promoter. In quantitative terms, APBβ contributes approximately 70-90% and APBα 10-30% to the transcriptional activity from the APP promoter in all cell lines studied to date. In addition, both factors seem to function independently of each other.

L 339 THE TRANSCRIPTION FACTOR MTF-1 IS ESSENTIAL FOR BASAL AND HEAVY METAL - INDUCED METALLOTHIONEIN-I GENE EXPRESSION, Freddy Radtke, Rainer Heuchel, Oleg Georgiev, Gerlinde Stark#, Michel Aguet# & Walter Schaffner*,Institut für Molekularbiologie II, Universität Zürich, 8057 Zürich, Switzerland,#Institut für Molekularbiologie I, Universität Zürich, 8093 Zürich, Switzerland

About 36 years ago metallothionein (MT) was identified as a protein responsible for the natural accumulation of cadmium in equine kidney cortex. Over the past years the metallothionein genes have developed into one of the best studied systems for inducible gene regulation, a fact that is also reflected by the use of the MT-promoters for numerous transgenic studies. Although much knowledge about the cis-elements responsible for heavy metal induced MT-transcription has accumulated, the protein factor(s) involved remained elusive.

We have previously described and cloned a factor (MTF-1) that binds specifically to metal-responsive DNA sequence elements in the enhancer/promoter region of metallothionein genes. MTF-1 is a protein of 72.5 kDa that contains six zinc fingers and multiple domains for transcriptional activation. Here we report the disruption of both alleles of the MTF-1 gene in mouse embryonic stem cells by homologous recombination. The resulting null mutant cell line fails to produce detectable amounts of MTF-1. Moreover, due to the loss of MTF-1 the endogenous metallothionein-I gene is silent, showing that MTF-1 is required for both basal and metal-induced transcription. Accordingly, reporter genes containing either synthetic or natural metal-responsive promoters were not transcribed after transfection into the null mutant cells. Cotransfection of the MTF-1 expression vector rescued both basal and heavy metal-induced transcription. These results demonstrate that MTF-1 is essential for normal metallothionein gene regulation.

L 340 STRUCTURE-FUNCTION ANALYSIS OF THE TRANSCRIPTIONAL ACTIVATION DOMAIN OF THE LEU3 PROTEIN, Eumorphia Remboutsika and Gunter B. Kohlhaw, Department of Biochemistry, Purdue University, West Lafayette, IN 47907

The Leu3 protein (Leu3p) from the yeast *Saccharomyces cerevisiae* is unique among known eukaryotic regulators in that its function as a transcriptional activator depends entirely on a low molecular weight metabolite, α-isopropylmalate (α-IPM). In the absence of α-IPM, Leu3p is a repressor of basal level transcription. We have recently delimited the activation function of Leu3p to the 30 C-terminal residues of this 886-residue protein. A hybrid protein consisting of the DNA binding region of Gal4p and the 30 C-terminal residues of Leu3p caused strong expression of a reporter gene in mouse fibroblasts, providing evidence that this short region of Leu3p is both necessary and sufficient for transcriptional activation (J. Sze, E. Remboutsika, G.B. Kohlhaw, Mol. Cell. Biol. 13:5702-5709, 1993). Using the hybrid system, we now demonstrate by serial deletion that no more than 22 residues of Leu3p are required for activation. Deletion of the four or eight C-terminal residues from the 30-residue Leu3p sequence actually resulted in an increase in the efficiency of activation. Deletion of the C-terminal twelve residues caused total loss of activation. We believe that this abrupt change in function is consistent with the existence of a structural entity that was disrupted or prevented from forming in the 12-residue deletion peptide. Evidence for the presence of α-helical structure in the activation domain of Leu3p has come from circular dichroism analysis of a peptide containing the 28 C-terminal residues of Leu3p (experiments performed in collaboration with Dr. Stephen Johnston, Southwestern Medical Center, Dallas, TX). Work in progress is aimed at defining the minimum sequence required for transcriptional activation and at identifying the partner(s) that sequence interacts with.

Supported by PHS Grant GM15102 and a fellowship from the Purdue Research Foundation (E.R.).

L 341 **HUMAN T-CELL LEUKEMIA VIRUS TYPE-1 TAX TRANSACTIVATES THE PROLIFERATING CELL NUCLEAR ANTIGEN PROMOTER THROUGH A NOVEL *CIS* TAX-RESPONSIVE ELEMENT**

Steven Ressler, Gilbert F.Morris*, and Susan J.Marriott
Division of Molecular Virology, Baylor College of Medicine
One Baylor Plaza, Houston, TX 77030
*Department of Pathology, Tulane Medical Center, New Orleans, LA 70112-2699

The human T-cell leukemia virus type-1 viral transforming protein, Tax, is a potent transactivator of both viral and cellular gene expression. The ability of Tax to transform cells is believed to depend on its transactivation of cellular growth regulatory genes. Because expression of proliferating cell nuclear antigen (PCNA) is intimately linked to cell growth and DNA replication we were interested in studying whether Tax was able to transactivate the PCNA promoter. The PCNA promoter does not contain a TATA box, but instead has an initiator element that overlaps the start site of transcription; thus PCNA differs from other cellular genes known to be transactivated by Tax. We have demonstrated that the PCNA promoter can be transactivated by Tax through a novel responsive element located near the initiator. A series of PCNA promoter deletion constructs were tested for transactivation by Tax. From the largest (-1250 - +60) to the smallest (-46 - +60) promoter construct, the activation ranged from 3-12 fold, respectively. The smallest construct that was activated did not include the ATF binding site at -50 and mutations in the ATF element in the context of a larger promoter were still activated by Tax. Thus, we conclude that Tax transactivation of the PCNA promoter does not require the ATF element. Further, a DNA sequence, between -46 and -2, appears to be the primary responsive element for Tax on the PCNA promoter.

L 342 THE ACTIVATION DOMAIN OF CREB IS HIGHLY APERIODIC. Jane P. Richards[1,2], Hans Peter Bächinger[3,4], Richard H. Goodman[2], and Richard G. Brennan[3]. [1]Vollum Institute, [2]Department of Cell Biology and Anatomy, [3]Department of Biochemistry and Molecular Biology, and [4]Shriners' Hospital for Children, Oregon Health Sciences University, Portland, OR 97201.

We have carried out biophysical studies on the cAMP Responsive Element Binding protein (CREB) to elucidate the structural features of a transcription activation domain which contains both glutamine and serine/threonine-rich motifs. Circular Dichroism (CD) studies on CREB341 reveal a secondary structure content of 37% aperiodic, 34% β-turn, 9% β-strand and 20% α-helix. Similar studies on ACT265 (CREB activator domain residues 3-265) demonstrate that the activation domain alone contains 52% aperiodic, 29% β-turn, 16% β-strand and only 3% α-helix. The structure of ACT265 corresponds to that expected for the activation domain following the removal of the α-helical basic-leucine zipper (bZIP) domain. Aperiodic and β-strand structures have also been observed for peptides from the acidic transcription activation domains of the viral activator Vmw65 and the yeast proteins GCN4 and Gal4. These structures are therefore implicated as important activation motifs in glutamine and serine/threonine-rich as well as acidic transcription factors. In addition, we show that phosphorylation of CREB341 or ACT265 by the cAMP dependent protein kinase A (PKA) does not alter the CD spectra and therefore the secondary structure content of CREB. This suggests that the previously proposed structural changes, if present, are of a small and local nature undetectable by CD or involve a conformation change that does not significantly affect secondary structure content.

L 343 THE GENE RECRUITMENT OF AN ENZYME CRYSTALLIN: A LENS-SPECIFIC ALTERNATIVE PROMOTER IN ζ-CRYSTALLIN. Jill C. Richardson, Douglas C. Lee and Graeme J. Wistow, Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, USA.
Crystallins are the structural proteins of the eye lens and determine the optical properties of this tissue. ζ-Crystallin is a major taxon-specific crystallin in several mammals and is identical to the detoxifying enzyme NADPH:quinone oxidoreductase. This enzyme has been recruited to a second, structural role in lens by use of an alternative, highly tissue-preferred promoter. Analysis of the lens promoter of guinea pig ζ-crystallin shows strongly lens-preferred expression in cultured cells and in transgenic mice. DNAse footprinting assays with nuclear extracts from cultured lens cells reveal a major protected region, ZPE (zeta protected element), which is differently footprinted in fibroblast nuclear extract. Deletion studies show that the ZPE region is essential for promoter function. EMSA studies using the ZPE as a probe reveal two specific complexes. A region within the ZPE which contains C/EBP-like and octamer-like binding sites appears to be required for the formation of the lower complex. Competition studies indicate binding at these two sites is co-operative. The study of this gene is the first example of a tissue-preferred promoter in an enzyme crystallin gene and the first example of the use of alternative promoters in such a gene.

L 344 ACTIVATION OF CLASS II MHC GENES, James L. Riley and Jeremy M. Boss, Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322
The genes that encode the human class II Major Histocompatibility Complex products are expressed in B cells and are controlled in part by a series of upstream elements termed X1, X2 and Y. To probe the functional properties of the proteins that bind the X1, X2, and Y boxes, a series of reporter constructs that contain GAL4 DNA binding sites and the class II MHC regulatory sequences were made. These reporter constructs and GAL4-VP16 expression vector were co-transfected into wildtype B cells and two MHC class II transcriptional mutant B cell lines. The results of this study indicate that GAL4-VP16 mediated activity was synergistically enhanced by the factors that bind to the X2 and Y boxes but inhibited by proteins that bind the X1 box . Previous *in vitro* experiments with mutant cell line SJO have shown that it is missing the X1 binding factor, RFX. In agreement with these observations, the ability of the X1 box to interfere with GAL4-VP16 mediated activation was not seen SJO. Although defective in class II MHC gene transcription, the mutant cell line RJ2.2.5's X1 box binding factors were able to interfere with GAL4-VP16 activity. Additionally, the GAL4-VP16/X2Y box activity was also present to the same degree as it was in the wildtype B cell. Further investigation of the X1 blocking activity in both RJ2.2.5 and the wild-type cell line was done by placing two helical turns of DNA between the GAL4 site and the X1 box. In RJ2.2.5 the X1 blocking activity could be overcome by the additional space between the sites, suggesting a steric interference between the X1 factor and GAL4-VP16. In the wild-type cell line the X1 box blocking activity was still seen demonstrating that there is an additional higher order complex present in the wild-type cell line that is missing in the mutant RJ2.2.5.

L 345 CHARACTERIZATION OF A TRIPARTITE cAMP RESPONSE UNIT IN THE PHOSPHOENOL-PYRUVATE CARBOXYKINASE (GTP) GENE PROMOTER, William J. Roesler, Janet G. Graham, and Pam J. McFie, Department of Biochemistry, University of Saskatchewan, Saskatoon, SK, Canada S7N 0W0.
The gene encoding phosphoenolpyruvate carboxykinase (GTP) (PEPCK) is expressed to significant levels in both kidney and liver, but its activation by cAMP is much more robust in liver. Previous work out of our laboratory indicated that this stimulation is mediated by a "cAMP response unit', consisting of two inherently weak components; a typical CRE which binds CREB, and a second promoter region termed the LSR (liver-specific region) which contains multiple binding sites for liver-enriched factors. We demonstrate that over-expression of dominant repressors of CREB or C/EBP proteins significantly reduces the fold-activation of the PEPCK promoter by cAMP. Using gel mobility shift analysis, we show that preincubation of HepG2 nuclear extracts with either CREB or C/EBP antiserum prevents the formation of specific DNA-protein complexes using probes representing appropriate PEPCK promoter sequences. The C/EBP binding sites apparently possess some unique feature, in that other well-described C/EBP binding sites are unable to functionally replace those present in the PEPCK promoter. We also show that the LSR contains a *cis*-element in addition to the C/EBP binding sites which also participates in the cAMP responsiveness. This *cis*-element, which is an AP-1 binding site, plays an modulatory role, augmenting the weak synergism displayed between the CRE and the C/EBP binding sites. Thus, the cAMP response unit of the PEPCK promoter employs the activities of three distinct classes of transcription factors. This likely allows for a tissue-specific response to this signal, a mechanism whereby to fine-tune the extent of the response, and a mechanism whereby to integrate signals from separate signalling pathways into a coordinated response.

L 346 ANALYSIS OF THE BOX I AND II REGULATORY MOTIF OF THE MYELIN BASIC PROTEIN (Golli-mbp) PROMOTER. R.A. Saavedra[1, 2], K.S. Kimbro[1, 2], P. Rosenberg[1, 2], A. Lipson[2, 3] and C. Ljubetic[2]. [1]Harvard Medical School, [2]The Children's Hospital, Enders Pediatric Research Center, Room 1106, 300 Longwood Avenue, Boston, MA 02115 and [3]Dartmouth College, Hinman Box 2350, Hanover, NH 03755.

The Golli-mbp (gene expressed mainly in the oligodendrocyte lineage-myelin basic protein) complex contains two overlapping transcription units with two distinct promoters, of which the downstream (mbp) promoter may be more frequently used. A comparison of the downstream promoters from shark, the oldest living vertebrate with a mammal-like myelin, and mouse allowed the identification of two DNA sequences, the box I and II motif, that are identical between these two species. These DNA sequences are ten and eleven nucleotides long, respectively, separated from each other by five poorly conserved nucleotides, and located at -212 to -187 from the initiation of transcription site on the mouse MBP promoter. Box I contains sequences similar to simian virus 40 T-antigen and MyoD-binding sites. Box II contains sequences similar to a glucocorticoid receptor-binding site. We have synthesized a double-stranded DNA fragment that is 38 base pairs long and contains the sequences of the box I and II motif. We found that proteins within nuclear extracts from both mouse brain and rat cerebral cortex in culture bind specifically to this DNA fragment causing a retardation of its electrophoretic mobility. Three DNA-protein complexes (A, B and C) are seen with both adult mouse brain and rat cortical culture extracts. The expression of proteins responsible for the formation of these complexes appears to be developmentally regulated in mouse brain. Complexes A, B and C are also detected using nuclear extracts from brains of 14, 16 and 18-day old mouse embryos, however, the complexes formed with embryonic brain extracts migrate slightly faster than those formed with adult brain extracts. Moreover, complex B appears significantly more intense using brain extracts from 18-day old embryo as compared to the same complex formed with brain extracts from other ages. We are presently isolating these proteins from mouse brain.

L 347 FUNCTIONAL ANALYSES OF THE HUMAN METALLOTHIONEIN (MT)-IG GENE: *IN VITRO* AND *IN VIVO* STUDIES, Susan Samson, Nicholas Shworak, and Lashitew Gedamu, Department of Biological Sciences, University of Calgary, Calgary, Alberta, CANADA.

Metallothionein (MT) gene transcription is induced by metals. This is conferred by Metal Responsive Elements (MREs) in the promoter which are thought to interact with transcription factors activated by metals. We wish to discern the functional factor-promoter interactions which govern transcription of the human (h)MT-IG gene. Toward this goal, a mutant of the TATAA box (to TATCA) and six trinucleotide mutants of the proximal MREa region (18 b.p. in total) have been constructed to determine the contributions of both elements. We have employed transient transfection of human hepatoblastoma (Hep G2) cells complemented by rat liver nuclei *in vitro* transcription. To mimic metal induction *in vitro*, metals are added early in extract preparation such that the hMT-IG activity is increased 4-5 fold over regular extracts while activity of a control (AdML) remains the same.

The qualitative effects of each mutation are consistant *in vivo* and *in vitro*. The TATA box mutation decreases transcription substantially correlating with much reduced DNase I protection by human TATA Binding Protein. As well, mutations in the MREa core, TGC(A/G)CNC, are especially devastating. Contributions by the less conserved MRE flanking sequences are also apparent possibly because of trinucleotide rather than single point mutations. These results confirm that MRE binding factors are active *in vitro*. Also, there is a co-operative interaction of MREa with the TATA box since the activity of the native promoter is substantially higher than the combined contributions of each element when the other is inactivated by mutation. (Funded by MRC of Canada)

L 348 STRUCTURE AND PROMOTER STUDIES OF THE TROUT METALLOTHIONEIN (MT)-A GENE, Steven Schieman and Lashitew Gedamu. Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada.

Metallothionein (MT) genes are transcriptionally regulated in response to metal ions. There are, to date, two known MT genes in trout, tMT-A and tMT-B. The two genes are highly similar in their coding regions and structurally similar in their 5' regulatory regions. It is known from previous studies that tMT-A and tMT-B are regulated in a parallel manner but tMT-A is generally more highly induced in response to zinc and cadmium exposure. We have been studying the regulation of these two genes with the goal of explaining this observed differential regulation. The tMT-B promoter region has been studied intensively to reveal the roles of the cis-acting metal regulatory elements (MREs). We have recently cloned the tMT-A gene and similar studies have been undertaken on the promoter region. Our findings indicate the following; i) the physical structure of the 5' regulatory region of tMT-A is similar to that of tMT-B but differs in certain key areas such as MRE sequences and MRE location, ii) the MREs of tMT-A contribute to the metal inducibility of the gene in a fashion analogous to the contributions of tMT-B MREs. These studies now allow for a more quantitative analysis of the differential regulation of tMT-A and tMT-B. This work was supported by MRC Canada.

L 349 SITE-SPECIFIC BINDING OF THE HUMAN CYTOMEGALOVIRUS IE2 86 KDA PROTEIN TO AN EARLY GENE PROMOTER, Ruth Schwartz, Marvin H. Sommer and Deborah H. Spector, Department of Biology, University of California, San Diego, La Jolla, CA 92093-0116 USA

We have previously demonstrated that the human cytomegalovirus (HCMV) immediate early (IE) IE2 86 kDa protein is the major transactivator of the HCMV early promoter for the 2.2 kb class of RNAs (ORF UL 112-113). To define the mechanism of action of the IE2 86 kDa protein, we have further characterized the *cis* acting sequences in the promoter required for activation and the nature of the interaction of these sequences with the IE2 protein. We find that in transient expression assays, specific stimulation of this promoter by the IE2 86 kDa protein requires not only the TATAA box but also sequences located between nt -58 and -113 relative to the transcription start site. This is also a major regulatory region for this promoter during HCMV infection, although other upstream sites do contribute to full activation. Since site-specific DNA binding is critical for the function of numerous transcription factors, we have developed a highly sensitive assay to determine whether the IE2 86 kDa protein alone or in combination with other cellular factors can bind to this promoter. For these assays, we use a GST-IE2 86 kDa fusion protein, which is a fully functional transactivator for this promoter in *in vitro* transcription reactions. The IE2 fusion protein is incubated with the ^{32}P-labeled promoter either in the presence or absence of human nuclear extract, and specific binding is assessed by retention of the protein-DNA complex on glutathione agarose beads. DNase I footprint analysis has also been used to map the sequences involved in the binding. Our results indicate that the IE2 86 kDa protein can interact with two specific domains on this HCMV early promoter. One region, located between nt -146 and -120, binds strongly to the IE2 86 kDa protein in the absence of other cellular factors and shares some sequence homology with the previously identified *cis* repression signal (CRS) located near the cap site of the major HCMV IE gene. The other region, located between nt -113 and -86, binds weakly to the IE2 86kDa protein and has little homology to the strong binding region or to the CRS element. Binding to this region is enhanced two fold by the presence of U373MG nuclear extracts. With a set of deletion mutants for the IE2 86 kDa protein, we have shown that the DNA binding domain spans a large region in the carboxy terminal half of the protein. Mutants that do not bind the DNA fail to activate this early promoter.

L 350 ACTIVATION OF SOMATOSTATIN GENE EXPRESSION IN PANCREATIC ISLET CELLS: EVIDENCE FOR MODIFICATION OF A CCAAT-BOX BINDING FACTOR, Palle Serup, Frank G. Andersen and Ole D. Madsen, Hagedorn Research Institute, Gentofte, Denmark.

The somatostatin (SS) producing cell line Tu-6 is derived from an *in vivo* propagated pluripotent rat islet tumor cell culture; the MSL-G2 cells. In transient transfection assays SS-CAT fusion constructs exhibit nearly equal activities in Tu-6 and MSL-G2 cells despite a large difference in the ability of the cells to transcribe the endogenous SS gene. However, by using mutational analysis to define cis-acting elements in the two cell lines we have found a CCAAT-box-like sequence (TGATTGAT; -120 to -112) to be required for expression of SS-CAT in Tu-6 cells, but not MSL-G2 cells. Furthermore, nuclear extracts prepared from the two cell lines displayed different interaction to this sequence in DNase I footprinting assays. Using electrophoretic mobility shift assays (EMSA) we detected two sequence specific complexes. Formation of either of these complexes were abolished by mutation of the first three base pairs in either of the TGAT repeats. Interestingly, these complexes, although identical in sequence specificity between the two cell lines, consistently migrated faster when extracts from the SS-producing Tu-6 cells were compared with extracts from the pluripotent MSL-G2 cells, raising the possibility that modification of these factors leads to the differential sensitivity to mutation of the binding site observed between the cell lines, as well as the transcriptional activation of the endogenous SS gene that occurred during the derivation of the Tu-6 cells.

L 351 ACTIVATION OF INTERFERON-α GENE EXPRESSION IN SENDAI VIRUS-INFECTED NAMALWA CELLS, Nigel A. Sharp, Carolyn L. Dent, Sonya J. MacBride, and Dirk R. Gewert, Department of Cell Biology, Wellcome Foundation Ltd., Langley Court, Beckenham, Kent BR3 3BS, U.K.

Human α-interferons (α-IFNs) are produced by most cell types in response to virus infection. They are a family of closely related secreted proteins comprising at least 15 functional non-allelic genes with homology to the single copy IFN-β gene.

We have used several approaches to study the induction of specific IFN-α genes in the human lymphoblastoid cell line Namalwa following infection by Sendai virus. The induction of α -IFNs is transient, transcriptionally regulated and the subtypes are co-produced to different extents: the IFN-α1 promoter is strongly induced in response to virus infection whereas the IFN-α14 promoter is very weak. We have attempted to identify sequences in the promoters of these IFN-α subtypes that account for this differential induction, using either secreted alkaline phosphatase or cell surface antigen reporter gene systems. Fusions between the virus inducible elements of these 2 promoters have been made and indicate the lack of a positive transcriptional signal in the α14 promoter rather the presence of a constitutive repressor. This has been confirmed using a mutational analysis approach, aimed at identifying transcriptional control sequences within the virus inducible elements of the IFN-α promoters. In addition, dimerization of α1 promoter sequences results in a synergistic activation upon virus infection.

Bandshift analysis has also been used to look directly at proteins binding to the promoters; bands have been identified which are specific to IFN-α elements, and do not bind the IFN-β promoter.

L 352 A NOVEL METHOD FOR STUDYING PEPTIDE-DNA INTERACTIONS, Dušan Stanojević and Gregory L. Verdine, Department of Chemistry, Harvard University, Cambridge, MA 02138

The development of methodology for systematic manipulation of gene expression *in vivo* could find wide application in medicine and basic research. One of the possible approaches requires the generation of molecules that will specifically bind DNA sequences and influence the expression of nearby genes. Here we describe a new system for studying peptide-DNA interactions that might lead to a method for isolation of DNA-binding peptides having any desired sequence specificity. This system involves the formation of a reversible covalent bond between the modified DNA molecule and a peptide. Such a configuration allows the sequence-specific binding of even very short peptides.

L 353 TRANSCRIPTIONAL REGULATION OF LH RECEPTOR GENE EXPRESSION, C.H. Tsai-Morris, Y. Geng, L. Xie, E. Buczko, and M. Dufau, ERRB, NICHD, NIH, Bethesda, MD 20892

The functional importance of specific protein binding domains with the TATAless 173bp promoter on transcription in the LH receptor gene was studied with mutagenesis and gel retardation. Transcription was dependent on the presence of two SP1 elements within the promoter domain of both expressing (mLTC) and nonexpressing (CHO) cell types. Deletion of the 74bp domain downstream of the SP1 elements that contains two protein binding domains (C-box and M1) and the transcriptional start sites, gave a 50% reduction in transcriptional activity, although this domain by itself exhibited minimal promoter activity. Mutation of the M1 and C-box domains reveals a tissue specific influence by each domain on promoter activity, and gel retardation indicates the presence of multiple trans factors that bind to the C-box and M1 domains. The C-box binding factors either inhibit promoter activity or block inhibition through overlapping but not identical DNA binding domains. Competition and mutagenesis studies indicate that the C-box inhibitory factor binds to a consensus AP-2 element, and a neutral protein factor (complex) binds to an NF-1 element that overlaps the AP-2 element on the C-box . Trans factors on the M1 domain compete for C-box proteins, indicating protein interactions between the trans elements on the DNA domains. In addition, competition by an upstream regulatory element that is only inhibitory in nonexpressing CHO cells, for C-box factors, indicates that both the C-box inhibitory AP-2 protein and the C-box neutral NF-1 protein also compete for this upstream domain in a tissue specific manner. Competition between the inhibitory and neutral DNA binding factors within both upstream and promoter domains may be responsible for a mechanism that induces the on/off cycle of LHR gene expression in gonadal cells. These studies reveal a complex pattern of transcriptional activation and inhibition that may reflect target mechanisms for hormone regulation.

L 354 DNA METHYLATION MIGHT BE INVOLVED IN AP-1-MEDIATED REGULATION OF GENE EXPRESSION, Eugene Tulchinsky and Eugene Lukanidin, Danish Cancer Society, Division for Cancer Biology, Department of Molecular Cancer Biology, Strandboulevarden 49, DK-2100 Copenhagen, Denmark.

The transcriptional regulation of the mts1 gene was studied. This gene encodes a protein belonging to the S100 subfamily of small Ca^{++}-binding proteins and seems to be involved into the regulation of the motility of tumor cells (Ebralidze et al. Genes and Develop., 1989, 3, 1086). Promoter and enhancer sequences of the mts1 gene were characterized. Enhancer element which is located in the first intron of the gene has been studied in detail (Tulchinsky et al. Oncogene, 1993, 8, 79). Correlation between the level of DNA methylation and mts1 gene transcription was also observed (Tulchinsky et al., Proc. Natl. Acad. Sci. USA, 1992, 89, p. 914b). The aim of the present work was to identify some methyl-DNA binding proteins which could be involved in the regulation of the transcription of mts1 gene. Restriction fragments containing mts1 enhancer sequence were methylated in vitro and analyzed in motility shift assay. One of the observed complexes was strictly dependent on DNA methylation. The sequence involved in its formation corresponds to the AP-1 consensus with only one mismatch: TGACTCG. We have found, that this sequence in nonmethylated form can interact with the protein different from AP1. But when methylated it can form the complex with another protein which belongs to the AP1 family of transcription factors: AP1 consensus sequence acts as a specific competitor for this interaction. The work on the identification of this protein interacting with the sequence TGACT^{m}CG is in progress.

L 355 EXPRESSION-PATTERN OF HUMAN HEAT SHOCK TRANSCRIPTION FACTOR IN DIFFERENT CELLS

M. Victor and B.-J. Benecke
Lehrstuhl für Biochemie, Ruhr-Universität Bochum
44780 Bochum, Germany

By using synthetic oligonucleotides and PCR-Technology we have subcloned specific fragments of the human heat shock factors (HSF) HSF 1 and HSF 2, respectively, for analysing the pattern of expression in different cell lines.
HSF is synthezised constitutively and the factors known so far give response to different activators of the heat shock system. In this way human HSF 1 confers reactability to elevated temperatures and HSF 2 is activated by hemin during erythroid differentiation.
We have examined the expression of human HSF's in HeLa- and 293- cells, the latter of which are stably transfected with the adenoviral oncogenes E1A and E1B and constitutively express members of the heat shock protein family at a high level.
As a model system for human differentiation we chose the human teratocarcinoma cell line NT2, which can be induced to differentiate into a neuronlike cell type by using retinoic acid. Whereas in all cases the expression of HSF 2 was nearly below the level of detection, the expression of HSF 1 showed clear differences regarding the cell type.
Thus, it appears questionable that HSF 2 plays a major role in the heat shock response under standard experimental conditions.
The significance of two heat shock transcription factors being differently expressed in various cell types will be discussed.

L 356 IN VITRO TRANSCRIPTION OF THE HUMAN IL-2 RECEPTOR ALPHA GENE, Wang Yongjun, Qi Zuhe, Shen Yufei, Department of Biochemistry and Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, 5 Dong Dan San Tiao, Beijing 100006.

It has been clear for many years that in order to study the mechanisms of transcription as well as to identify the factors and nucleotide sequences that control and regulate gene expression, cell free systems that accurately and specifically transcribe exogenously added gene are required. Given the critical role played by IL-2R alpha gene expression in the control of the high affinity receptor display and T- cell proliferation, we have attempted to identify trans- acting factors and cis-regulatory sequences involved in transcriptional activation of their receptor gene. This analysis was begun by preparing two plasmids containing 5' deletion mutants of the IL- 2R promoter linked to its cDNA sequences, from -350 and -480 to 937, respectively.

A homologous in vitro transcription system was developed in which the human IL-2R alpha gene was faithfully transcribed. Results indicated that the +1 initiation site could be intiated accurately, and proper template concentration was needed for obtaining optimal transcription. Specific RNA synthesis was evident by 15 min, which was the earliest time point examined. Transcription reaction was maintained at least 2 hr at 30°C, but no transcript was observed when incubated at 37°C for 1 hr. Nuclear extracts, from B3D5 cells in which the IL-2R alpha peptides are constitutively expressed, augmented the in vitro transcription of the human IL-2R alpha gene.

L 357 ASYMMETRIC bHLH-DNA INTERACTIONS: SIMILARITY BETWEEN THE VERTEBRATE DIOXIN AND *DROSOPHILA* CNS MIDLINE TRANSCRIPTIONAL RESPONSE ELEMENTS, Keith A. Wharton, Jr., Robert G. Franks, Yumi Kasai, and *Stephen T. Crews, Department of Biology and Molecular Biology Institute, UCLA, Los Angeles, CA 90024

The *Drosophila single-minded* (*sim*) gene encodes a basic-helix-loop-helix (bHLH) transcription factor which acts as a master lineage regulator of the embryonic CNS midline. Using transgenic flies, we define a seven base-pair consensus midline response element (MRE) which: 1) is necessary for CNS midline gene expression in three putative target genes; 2) is sufficient for CNS midline gene expression when multimerized; and 3) resembles an asymmetric E-box. These results, coupled with the similarity between Sim and two bHLH subunits of the vertebrate dioxin receptor and its cognate binding site, the xenobiotic response element (XRE), suggest a model whereby Sim and an as yet uncharacterized partner protein bind to the asymmetric MREs present in midline enhancers. As many bHLH proteins are well conserved between vertebrates and flies, the *Drosophila single-minded* mutation may shed light on human midline genetic defects, collectively termed holoprosencephalies.

*Current address: Department of Biochemistry, UNC Chapel Hill, Chapel Hill, NC

L 358 ANALYSIS OF HTLV-I TRANSCRIPTION IN VITRO. Graziella Piras, Fatah Kashanchi, Michael Radonovich, Janet Duvall, John N. Brady. Laboratory of Molecular Virology, NCI, NIH, Bethesda, MD 20892.

Transcription of HTLV-I is regulated by cellular factors that interact with cis-acting sequences located in the 5' LTR and by the viral encoded trans-acting protein Tax_1. The HTLV-I promoter was studied by using an in vitro transcription system to analyse basal and Tax_1 transactivation at the molecular level. The HTLV-I promoter contains all the structural features of a typical RNA polymerase II promoter. HTLV-I transcription however, was not inhibited by the presence of α-amanitin at concentrations which inhibited a typical polymerase II promoter (6 μg/ml, AdML). HTLV-I transcription was only inhibited when a higher concentration of α-amanitin was used (60 μg/ml), in the range of a typical polymerase III promoter (VA1) . To rule out RNA polymerase III activity, we showed that HTLV-I transcription is resistant to tagetitoxin, a polymerase III specific inhibitor, and is not affected by antibodies against TFIIIC. Further in vitro reconstitution experiments with purified and recombinant basal transcription factors indicated that RNA polymerase II and polymerase II basal factors are indeed involved in the transcription of this promoter. These observations suggest that a distinct and specific initiation/elongation complex may be involved in HTLV-I transcription.

Elongation

L 400 RNA POLYMERASE II MUTANTS CAUSE A UBX-LIKE PHENOTYPE BY AN EFFECT OCCURRING PRIOR TO THE ELONGATION STEP IN TRANSCRIPTION, Lillian P. Burke and Mark A. Mortin, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

We have been using mutations in Drosophila RNA polymerase II to study the functional domains of this multimeric enzyme. Flies carrying *RpII215*^{4}, a mutation in the largest subunit of RNA polymerase II, are resistant to α-amanitin, a toxin which specifically inhibits the elongation step of transcription. Homozygous *RpII215*^{4} (*RpII215*^{4}/*RpII215*^{4}) or hemizygous (*RpII215*^{4} /-) flies have a normal phenotype, while *RpII215*^{4} /+ flies have an enlarged haltere, a mutant phenotype also caused by haplo-insufficiency of the transcription factor Ultrabithorax (*Ubx*$^-$/+). This genetic interaction suggests that the mutant and the wild-type proteins interfere with each other leading to the mutant phenotype. In order to study the mechanism of this effect, we have taken advantage of the α-amanitin resistance of RpII215^4 polymerase. *RpII215*^{4} flies were crossed with wild-type flies and the resulting progeny were raised on food containing 25 μg/ ml of α-amanitin, a concentration that kills all wild-type flies. This was done in order to determine if elongation by the wild-type polymerase was required for expression of the Ubx phenotype. **Results:** Adult *RpII215*^{4}/+ flies showed the Ubx-like phenotype in both the presence and the absence of α-amanitin. *RpII215*^{4} /+;*Ubx*$^-$/+ flies show an enhanced Ubx-effect and this enhancement is also present when these flies are raised on α-amanitin. Homozygous and hemizygous flies continued to show a normal phenotype. Measurements of α-amanitin in the developing larvae by HPLC show that the larvae have concentrations of α-amanitin known to inhibit transcription by wild-type RNA polymerase II. The presence of α-amanitin in the tissue that forms the haltere has also been demonstrated by detecting the presence of FITC-labeled α-amanitin. **Conclusion:** This suggests that the Ubx-like phenotype seen in *RpII215*^{4}/+ flies results from an effect of the wild-type polymerase occurring prior to the elongation step in transcription.

L 401 EVIDENCE FOR TWO CONFORMATIONS OF *DROSOPHILA* RNA POLYMERASE II DURING ELONGATION, David Chafin, Hongliang Guo and David H. Price, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.

During elongation on dC-tailed templates RNA polymerase II encounters many pause sites in the absence of elongation factors. RNA polymerase can also be induced to stall by limiting one of the nucleotides needed for efficient elongation. The addition of pyrophosphate or DmS-II causes the removal of nucleotides from the 3' end of the nascent transcript. Pyrophosphate drives the reverse reaction liberating NTP's while DmS-II catalyzes the removal of primarily dinucleotides. We have examined the kinetics of nucleotide removal from the 3' end of the nascent transcripts by pyrophosphate and DmS-II and have found that the two reagents caused the polymerase to retreat with different kinetics suggesting the existence of two conformational states of elongation complexes, paused and elongation competent. Paused polymerases are acted upon quickly by DmS-II and slowly by pyrophosphate. On stalled polymerase the reagents have the reverse preference.

Like the forward reaction, polymerase encounters kinetic barriers in the reverse direction. Polymerases retreating due to the action of DmS-II or pyrophosphorolysis encounter a kinetic barrier on average after every two nucleotides are removed. This data suggests that there is a step in common to both processes. The evidence indicates that the polymerase must move discontinuously covering a two nucleotide increment. Both pyrophosphorolysis and DmS-II mediated transcript shortening require the discontinuous movement during extended reactions.

L 402 RNA POLYMERASE INTERACTS WITH A NASCENT TRANSCRIPT RNA HAIRPIN AT THE *HIS* LEADER PAUSE SITE. Cathleen L. Chan and Robert Landick, Dept. of Biology, Washington University, St. Louis, MO 63130

To understand how RNA polymerase recognizes regulatory signals during transcript elongation, we studied its behavior at the pause site in the *his* operon leader region. Dissection of the pause signal by base substitution reveals it is multipartite and consists of four elements: (i) a 5-bp stem-loop RNA secondary structure (the pause hairpin) that forms 11 nt upstream of the 3'-end of the transcript, (ii) a 3'-proximal region of transcript or DNA template, (iii) the 3'-terminal nucleotide of the transcript, and (iv) the DNA sequence downstream of the pause site (Chan and Landick, *J. Mol. Biol.* 233:23-42). Regardless of the usual configuration of the 3'-proximal region of the transcript, formation of a pause RNA hairpin appears not to alter directly the configuration of the last 11 nt of the transcript. Rather, our studies suggest the phosphate backbone of the pause hairpin interacts ionically with a positively charged hairpin-binding pocket on RNA polymerase.

The existence of a hairpin-binding pocket is supported by several observations. First, a survey of the effects of neutral salts on transcript elongation revealed that anions can alter elongation in distinct ways, one of which appears to be disordering a hairpin-binding site on RNA polymerase. The effect of elevated concentrations of neutral salts on elongation is complex: at most sites anions inhibit elongation in the order HPO_4^{2-} > acetate = glutamate > SO_4^{2-} > ClO_4^- > Br^- ≥ Cl^-. This deviates from the general order of Hofmeister-series effects on protein structure (ClO_4^- > Br^- > Cl^- > acetate > SO_4^{2-}). Disruption of protein structure with concomitant loss of enzymatic activity can explain the effect of chaotropic anions such as ClO_4^- on elongation and corresponds to salt effects observed for other enzymes. However, the even more inhibitory effects of stabilizing anions such as SO_4^{2-}, which ordinarily favor protein-protein interaction, are unusual. Perhaps these anions restrict the conformational flexibility of RNA polymerase, thus inhibiting translocation on the DNA template. At the pause site, however, chaotropic anions such as Cl^- and ClO_4^- actually accelerate elongation, whereas SO_4^{2-} and acetate inhibit it; these effects follow the Hofmeister series. Mutations that eliminate the pause hairpin or that significantly alter its structure also eliminate the elongation-promoting effect of Cl^-, suggesting that chaotropic salts may disrupt pause hairpin-RNA polymerase interactions that ordinarily slow elongation by disordering the structure of a hairpin-binding pocket.

L 403 TAR-TAT RECOGNITION IN BOVINE IMMUNODEFICIENCY VIRUS - AN INTERESTING SYSTEM TO STUDY TRANSCRIPTIONAL REGULATION, Lily Chen and Alan D. Frankel, Department of Biochemistry and Biophysics, and Gladstone Institute of Virology and Immunology, University of California, San Francisco, PO Box 419100, San Francisco, CA 94141-9100

There is considerable evidence that RNA structure plays an important role in regulating gene expression. Extensive biochemical and NMR spectroscopic studies on the RNA recognition domain (amino acids 49-57) of the HIV transcriptional activator, Tat, have been completed. The arginine-rich RNA binding domain is unusually flexible both in amino acid sequence and in structure, with a single arginine providing the only sequence-specific RNA contact. A possible candidate RNA binding domain, which is also rich in arginines, is found in the BIV Tat protein. The transactivation domains of BIV Tat and HIV Tat have similar sequences and may use similar mechanisms to transactivate their viral long terminal repeats. A 17 amino acid arginine-rich peptide from the BIV Tat protein (amino acids 65-81, SGPRPRGTRGKGRRIRR) has been identified that binds specifically to an RNA hairpin at the 5' end of its mRNAs and recognizes unusual structural features of the RNA. Binding of the BIV peptide to BIV TAR is considerably tighter and more specific than binding of HIV Tat to HIV TAR. Mutagenesis, RNase mapping, and chemical interference experiments indicate that bulge and stem regions of BIV TAR are recognized simultaneously by the BIV peptide and that the RNA adopts an unexpected structure. In particular, two GC base pairs in the upper stem, near a single nucleotide bulge, are highly accessible to RNAse digestion. In vivo experiments confirmed the high specificity of the interaction and the identity of the RNA determinants. Transactivation experiments using HIV-BIV hybrid proteins in human and mouse cells have shown that, unlike HIV Tat, BIV Tat does not require additional cellular RNA loop-binding proteins for transactivation. Peptide mutagenesis, RNA binding experiments and immunoprecipitation experiments have identified amino acids in BIV Tat important for TAR binding. Unlike HIV TAR-Tat and RRE-Rev interactions, no conformational changes in BIV TAR have been detected by by circular dichroism upon peptide binding, however the thermal stability of TAR increases upon specific binding. The BIV system provides an interesting system to characterize the TAR-Tat interaction and to compare the mechanisms of BIV and HIV transactivation.

L 404 PURIFIED YEAST RNA POLYMERASE II READS THROUGH INTRINSIC BLOCKS TO ELONGATION IN RESPONSE TO THE YEAST TFIIS ANALOGUE, P37, **Karen R. Christie**[1], Donald E. Awrey[2], Aled M. Edwards[2], and Caroline M. Kane[1], [1]Division of Biochemistry and Molecular Biology; University of California, Berkeley, CA 94720; [2]Departments of Pathology and Biochemistry; McMaster University; 1200 Main St. W, Hamilton Ontario CANADA L8N 3Z5

The yeast *Saccharomyces cerevisiae* has a TFIIS-related transcription elongation factor, originally called P37. Like the mammalian and *Drosophila* TFIIS factors, this protein stimulates its cognate RNA polymerase II to read-through intrinsic blocks to elongation and binds directly to the polymerase. To elucidate functional features of this protein:protein interaction, we tested the ability of several forms of RNA polymerase II to respond to either full-length or an amino-terminal truncation of TFIIS. The variants of the polymerase differed in the structure of the carboxyl-terminal domain of the largest subunit or lacked two of the smaller subunits. The two forms of TFIIS were purified after overexpression in *E. coli*. No differences in ability to recognize intrinsic blocks to elongation or to read-through them in response to either form of TFIIS were detected among these structural variants.

Further, ternary complexes containing each variant form of RNA polymerase cleave the 3' end of the nascent transcripts in response to TFIIS, a reaction previously reported for mammalian and *Drosophila* TFIIS and likely to be important in TFIIS function. Thus the carboxyl-terminal domain of the largest subunit and subunits four and seven of the polymerase, while required *in vivo*, are not required *in vitro* for recognition of intrinsic blocks to elongation, read-through in response to TFIIS, or TFIIS-stimulated cleavage of the nascent transcript.

L 405 IDENTIFICATION AND CHARACTERISATION OF THE *xap* OPERON OF *E. COLI*, Gert Dandanell, Claus Poulsen and Corina Seeger, Department of Biological Chemistry, Institute of Molecular Biology, University of Copenhagen, Denmark.

In 1980, a second purine nucleoside phosphorylase was found in *E. coli*, named xanthosine phosphorylase (XapA). The product of the *xapA* gene is not present in measurable amounts in the cell, but when cells are grown in the presence of xanthosine, the expression is induced more than 600 fold (1,2). The isolation of regulatory mutants (*xapR*) that cotransduce 90% with *xapA*, indicated that the *xapA* gene expression is activated by the XapR protein in the presence of xanthosine. The *xapA* and *xapR* genes have been mapped to the 52' region on the *E. coli* chromosome (1). DNA sequencing of the 51' region revealed an open reading frame ORF294, which shows high homology to a family of transcriptional activators called the LysR family (3). ORF294 was suggested to encode XapR.

We have isolated and sequenced a 7.7 kb *Hind*III fragment from the Kohara library, that contains the xanthosine phosphorylase activity. DNA sequencing of this region revealed ORF294 (*xapR*) and three other open reading frames upstream of *xapR*. One of these is highly homologous to the human purine phosphorylase (60% identical) and has been identified as *xapA*, while another ORF (*xapB*) encodes a membrane protein that could be a transport protein. We have identified these gene products in minicells, and characterised the transcriptional start sites and potential XapR binding sites. Although XapR is a member of the LysR family, the proposed structure of the *xapABR* region appears to be arranged differently from other members of the lysR family.

1. Buxton, R.S.,Hammer-Jespersen, K.H., and Valentin-Hansen, P. (1980) *Molec. gen. Genet.* **179:** 331-340.
2. Hammer-Jespersen, K.,Buxton, R.S., and Hansen, T.D.H. (1980) *Molec. gen. Genet.* **179:** 341-348.
3. Brun, Y.V.,Breton, R.,Lanouette, P., and Lapointe, J. (1990) *J. Mol. Biol.* **214:** 825-843.

L 406 SII INDUCES CLEAVAGE OF NASCENT TRANSCRIPTS ARRESTED BY A CYCLOBUTANE PYRIMIDINE DIMER ON THE TEMPLATE DNA STRAND, Brian A. Donahue, Daniel Reines* and Philip C. Hanawalt, Department of Biological Sciences, Stanford University, Stanford, CA 94305 and *Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322

Transcription coupled repair has been characterized as the preferential repair of DNA lesions on the transcribed strand of an expressed gene compared to the nontranscribed strand and to unexpressed genomic domains. It has been proposed that the RNA polymerase elongation complex, stalled at a site of a lesion, serves as a signal to the DNA repair machinery. Such a signal should facilitate removal of the transcription-blocking lesion and allow transcription to resume. However, the stalled polymerase may obstruct access of repair enzymes to the damaged site. Also the DNA strands presumably must reanneal before excision-repair can begin.

The SII elongation factor of mammalian cells has been shown to facilitate the transcription of RNA polymerase II through a variety of pause sites in several genes. An essential feature of SII mediated bypass of these pause sites is the cleavage of the nascent transcripts before the polymerase can elongate. RNA cleavage is thought to enable the DNA to assume a structure that will allow the RNA polymerase to proceed past the site. SII mediated transcript cleavage may play a role in transcription coupled repair by altering the structure of the stalled polymerase complex so that the repair complex can excise the damage and restore the structural integrity of the DNA template.

In order to study the role of SII mediated transcript cleavage in transcription coupled repair we have constructed a DNA template in which a cyclobutane pyrimidine dimer (CPD) is located downstream of the major late promoter of adenovirus at a specific site on the transcribed strand of all templates. Our results have shown that the specific CPD is a strong block to RNA polymerase II. The arrested polymerase-RNA-DNA complex is very stable and blocks the CPD from recognition by photolyase, a prokaryotic DNA repair enzyme. Addition of the purified SII protein induces nascent transcript cleavage. The shortened RNAs can be elongated, at least up to the site of the CPD; thus the polymerase remains competent throughout this process. A model for the role of transcript cleavage in transcription coupled repair will be presented.

L 407 A PUTATIVE CELLULAR ANTITERMINATOR OF TRANSCRIPTION ELONGATION THAT ACTS BY BINDING THE DOWNSTREAM ELEMENT OF THE SV40 MAJOR LATE PROMOTER. Theresa L. Eisenbraun, Fengrong Zuo, and Janet Mertz, University of Wisconsin, Madison, WI 53706.

The SV40 major late promoter (MLP) contains a genetically important element located approximately 30 bp downstream from its transcription start site (Ayer and Dynan, Mol Cell Biol. 8:2021, 1988). Mutational analysis of this element indicated that it extends approximately from nt +17 to nt +36. Interestingly, double-stranded oligonucleotides containing the wild-type downstream sequence, but not ones containing mutated versions of it, compete for a factor(s) necessary for efficient transcription from the SV40 MLP *in vitro*. Thus, the downstream element is recognized by a factor(s) (DAP) at the DNA level. However, order-of-addition experiments indicate that the effect of the downstream element is at the level of transcription elongation. In these experiments, sarkosyl was added to an *in vitro* transcription reaction to a concentration at which transcription initiation was inhibited, but not elongation. Addition of double-stranded wild-type and mutant oligonucleotides to an *in vitro* transcription reaction after addition of NTPs and sarkosyl resulted in the inhibition of transcription. On the basis of these results, we hypothesize that DAP binds to the downstream element of the SV40 MLP at the DNA level and functions as an antiterminator of transcription elongation. A precedent for an interaction of this type is the phage lambda Q protein which binds to DNA but acts as an antiterminator of transcription elongation. DAP represents the first report of the existence of a comparable activity in higher eukaryotes.

Ayer and Dynan (Mol. and Cell. Biol. 10:3635, 1990) attempted with only marginal success to purify DAP from HeLa cells on the basis of its DNA binding ability. We are now attempting to do so using also as an assay its ability to stimulate transcription when added back to an *in vitro* transcription reaction.

L 408 INTERACTIONS BETWEEN RNA POLYMERASE AND TRANSCRIPT AFFECT GreA- AND GreB-MEDIATED REVERSE TRANSLOCATION, Guohua Feng, Daguang Wang and Robert Landick, Department of Biology and Molecular Biophysics, Washington University, St. Louis, MO 63130, U.S.A.

Proteins that stimulate cleavage of the nascent transcript in ternary transcription complexes have been found in both prokaryotes and eukaryotes. In *E. coli*, two ~17 kDa transcript-cleavage factors, GreA and GreB, have been identified. Although the biological functions of these proteins are not defined, they may play roles in preventing transcription arrest or increasing transcription fidelity. In order to understand the mechanism of transcript cleavage, we studied the effect of interactions between RNA polymerase and transcript on reverse translocation. Reverse translocation occurs when successive rounds of transcript cleavage allow RNA polymerase to back-up on the DNA template. We found that: (1) RNA polymerase could undergo reverse translocation when 2- to 3-nt fragments were successively cleaved from the RNA; (2) reverse translocation was blocked in the promoter-proximal region when the transcript was shortened to approximately 8 nt; (3) GreA and GreB could induce cleavage of abortive transcripts as short as 4 nt prior to release and stimulate productive initiation at a weak promoter; (4) with promoter-distal transcription complexes, both GreA and GreB could induce long range backing-up on a G-less template, but were blocked from processive reverse translocation when the transcript contained secondary structure; and (5) the *rpoB8* mutation, which confers rifampin resistance and is known to slow transcript elongation, accelerated reverse translocation. This suggests mutations in the Rif region of the β-subunit may affect the interactions between polymerase and transcript, thus changing its susceptibility to cleavage and elongation in opposite ways. To explain these data, we consider a model in which RNA polymerase contains three distinct transcript-binding sites (an ~8-nt site I proximal to the 3' end, a distal transcript-exit site II, and a hairpin-binding site III between them). GreA and GreB induce cleavage of the transcript only in site I. In the promoter-proximal region, reverse translocation is blocked simply because the site II can not be left empty. In the promoter-distal region, transcript may be fed into site II and then site I continuously, resulting in extensive backing-up of the polymerase. However, this continuous reverse translocation may be blocked by an RNA secondary structure binding to site III, such as the hairpin structure in *his* or *trp* paused complexes, or by structures outside the complex that prevent the transcript from entering site II.

L 409 MUTATIONAL ANALYSIS AT POSITION 882 IN T7 RNA POLYMERASE, Lisa P. Gardner and Joseph E. Coleman, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520-8114

Bacteriophages T3, T7 and SP6 all code for single-subunit RNAP's which have conserved C-termini of (Tyr,Phe)-Ala-Phe-Ala-COOH. Deletion of Phe^{882}-Ala^{883} in Bacteriophage T7 RNAP reduces polymerization activity to 5% relative to WT. We have probed the role of Phe^{882} by determining the effects on transcription *in vitro* of Tyr (FAYA), Trp (FAWA), and Ala (FAAA) substituted at position 882. A deletion mutant, ΔAla^{883} (FAF), is characterized as well to analyze the effect of the precise location of the COO- moiety.

The rate of formation of the initial phosphodiester bond can be measured using a template which codes for GGACU and limiting the substate to GTP. WT T7 RNAP forms GG products at 15 min^{-1}. Rates measured for FAF, FAWA, FAYA and FAAA are 1.8, 0.9, 0.4 and 0.3 min^{-1} respectively. Thus, all mutations severely affect the rate of initiation. The K_m of the initiating GTP for the WT enzyme is 0.9 mM. The K_m's for FAWA, FAYA and FAF are 1.2, 3.1 and 3.4 mM, respectively. In contrast, the K_m for FAAA is 15 mM. Clearly, the aromatic side chain at position 882 is one of the factors involved in binding the initiating nucleotide. The ratio of rates of formation of GGACU (4 NTP substrates) to GG (GTP as only substrate) reveals what effect the side chain at position 882 has on the rate of formation of bonds subsequent to the GG bond (elongation). If all bonds are formed at the same rate, this ratio would equal 0.25. The ratio calculated for FAWA and WT is 1.4, i.e. initiation is still rate controlling, and the rate of subsequent bond formation is much faster. The ratios for FAYA and FAAA are 0.23 and 0.1, suggesting that the rate of subsequent bond formation has decreased and is slower than initiation. We examined the effects on processivity of the enzyme after the polymerase has escaped the abortive phase by using a template which encodes a 20 base message. The ratio of the sum of full-length run-off transcripts to the sum of all products (full-length and those resulting from premature dissociation of the complex) indicates the processivity of the enzyme on a scale of 0 to 1. The processivity of WT is 0.7, whereas FAF and FAAA both measure 0.5, suggesting a slightly destabilized ternary complex. In contrast, FAWA shows a significantly destabilized ternary complex with a processivity of 0.1.

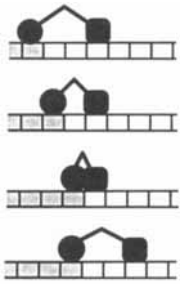
L 410 NASCENT RNA CLEAVAGE BY POLYMERASE II DOES NOT REQUIRE UPSTREAM TRANSLOCATION OF THE ELONGATION COMPLEX ON DNA, Weigang Gu, Wade Powell, John Mote, Jr., and Daniel Reines, Graduate Program in Biochemistry and Molecular Biology, Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322

Obstacles incurred by RNA polymerase II during primary transcript synthesis have been identified in vivo and in vitro. Transcription past these impediments requires SII, an RNA polymerase II-binding protein. SII also activates a nuclease activity in arrested elongation complexes and this nascent RNA shortening precedes transcriptional readthrough of many impediments. We show that no more than 7-9 nucleotides are removed before RNA polymerase II can resume RNA extension. Using E. coli exonuclease III as a probe, we mapped the boundaries of the elongation complex on DNA. We demonstrate that upstream movement of RNA polymerase II is not required for limited RNA shortening and reactivation of an arrested complex. Further cleavage of RNA does result in backward movement of the enzyme. We suggest that SII-mediated cleavage of RNA induces a structural rearrangement of the elongation complex distinct from upstream translocation on DNA that may reposition the 3' end of the nascent RNA.

L 411 **EVIDENCE FOR DISCONTINUOUS MOVEMENT DURING ELONGATION BY RNA POLYMERASE II**

Hongliang Guo and David H. Price, Department of Biochemistry, University of Iowa, Iowa City, IA 52242

The traditional model for transcription elongation implies that the polymerase moves monotonically along the DNA template. We have examined the basic mechanism of RNA polymerase II elongation in part by characterizing the reverse reaction, pyrophosphorolysis. The properties of *Drosophila* RNA polymerase II elongation complexes formed on a dC-tailed template were examined. During pyrophosphorolysis the polymerase encounters kinetic barriers every two nucleotides. α -amanitin does not completely inhibit nucleotide addition or removal catalyzed by the polymerase, but appears to interfere with the movement of the enzyme. As expected the factors that affect elongation have similar effects during the reverse reaction, pyrophosphorolysis. Similar results have been observed with calf thymus RNA polymerase II. Our results suggest that in addition to the translocation that must occur after every nucleotide addition there is another polymerase movement that occurs on average every two nucleotides. Support for the existence of this later discontinuous movement comes from a variety of experiments and has far reaching implications for the mechanism of elongation and the control of the elongation phase of transcription. The diagram at the right shows two nucleotide additions followed by one discontinuous movement. Open boxes, DNA; shaded boxes, RNA; round black shape, active site of polymerase; black arm and rounded square, rest of polymerase.



L 412 DIRECT AND INDIRECT EFFECTS OF MUTATIONS IN λ P_{RM} ON OPEN COMPLEX FORMATION AT P_R, Gary N. Gussin, Raymond S-C. Fong, and Scott T. Woody, Genetics Ph.D. Program and Department of Biological Sciences, University of Iowa, Iowa City, IA, 52242.

The transcription startsites of the phage λ promoters P_{RM} and P_R are separated by 82 bp. Even though RNA polymerase (RNAP) can bind simultaneously to the two promoters, mutating P_R increases the rate of open complex formation at P_{RM}. This results in a four-fold increase in k_f, (the rate constant for isomerization of closed to open complexes) and a three-fold decrease in K_B (the equilibrium constant for formation of closed complexes)[1]. In effect, RNAP bound at P_R acts as a "repressor" of open complex formation at P_{RM}, but the "repressor" acts at step subsequent to RNAP binding to P_{RM}[2,3].

Recent experiments indicate that interference between the two promoters is reciprocal. That is, mutations in P_{RM} can affect the rate of open complex formation at P_R. This phenomenon can be detected only when P_R is made less active by mutation so that the rates of open complex formation at P_{RM} (wild-type) and P_R (mutant) are comparable. These experiments were complicated by several additional observations: (i) When P_R is mutated, a weak promoter (P_α) that overlaps both P_{RM} and P_R and is potentially capable of directing transcription of cI, is activated. (ii) Mutations in P_α and P_{RM}, which are changes at -29 and -50 with respect to P_R, unexpectedly affect P_R promoter strength. We suggest that a 12-bp A:T-rich sequence flanking -50 is a determinant of the strength of P_R, and that this sequence functions in the same way that a similar upstream sequence functions during open complex formation at the *rrnB P1* promoter[4].

[1]Fong, R. S-C., Woody, S. T. & Gussin, G. N. (1993). *J. Mol. Biol.* 232: 792-804.
[2]Hershberger, P. A. & deHaseth, P. L. (1991). *J. Mol. Biol.* 222, 479-494.
[3]Woody, S. T., Fong, R. S-C. & Gussin, G. N. (1993). *J. Mol. Biol.* 229, 37-51.
[4]Rao, L., Ross, W., Appleman, J. A., Gaal, T., Leirmo, S., Schlax, P.J., Record, M. T. & Gourse, R. L. (1994). *J. Mol. Biol.* (in press).

L 413 CHROMOSOMAL PROTEIN HMG-14 INCREASES THE RATE OF TRANSCRIPTIONAL ELONGATION BY RNA POLYMERASE II ON CHROMATIN TEMPLATES, **Ulla Hansen**, Sylvie Rimsky, Susan C. Batson, Michael Bustin† and Han-Fei Ding, Dana-Farber Cancer Institute, and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, and †NCI/NIH, Bethesda, MD 20892

HMG-14 is a nonhistone chromosomal protein that may be involved in the generation or maintenance of the structure of active chromatin. To assess the transcriptional role of HMG-14, in vivo-assembled chromatin containing elevated levels of HMG-14 were obtained either by isolating Simian virus 40 (SV40) minichromosomes from a stable CV-1 cell line overexpressing human HMG-14 mRNA, or by adding recombinant human HMG-14 to purified minichromosomes. We show that elevated levels of HMG-14 enhanced transcription in vitro by RNA polymerase II specifically on chromatin templates, but not on DNA templates. In particular, HMG-14 increased the rate of elongation on the nucleosomal templates. These findings establish that the association of HMG-14 with nucleosomes directly facilitates transcription and may affect the transcriptional potential of the chromatin.

L 414 LIGHT-INDUCED RELEASE OF A SPECIALISED SIGMA FACTOR FROM THE MEMBRANE DURING LIGHT ACTIVATION OF GENE EXPRESSION IN *Myxococcus xanthus*, David A. Hodgson, Simon J. McGowan, Hazel C. Gorham, Dawn C. Miller, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, England

When cells of the Gram-negative bacterium *Myxococcus xanthus* are exposed to light, they turn orange due to the production of carotenoid pigments. These carotenoids protect the cells against the otherwise lethal effects of light. There are a number of genetically unlinked genes (*car* genes) that encode the enzymes responsible for synthesis of the carotenoids. These structural genes are under the control of three regulatory genes (*carQ, carR* and *carS*), which are arranged in an operon and translationally coupled. We have extensive genetic evidence that *carQ* and *carS* are positive regulators of the structural genes and *carQ* positively regulates the promoter of the *carQRS* operon. The *carR* gene product negatively regulates the promoter of the *carQRS* operon and is an inner membrane protein. We have further genetic evidence that CarQ is sequestered to the membrane by CarR in the dark and that light causes the release of CarQ to induced *carQRS* promoter activity and, subsequently, the rest of the *car* regulon.

Recent amino acid residue sequence analysis of CarQ (Mike Lonetto, personal communication) reveals that it is a member of a family of small (*c.* M_r 20,000) RNA polymerase sigma factors which includes the *Pseudomonas aeruginosa algU* gene product (involved in control of alginate production) and a *Streptomyces coelicolor* factor required for expression of an agarase gene promoter (Mark Buttner, personal communication). This sigma factor family is unusual in that they all contain virtually no region 3s and little or no region 1. We have evidence that CarQ is a sequence specific DNA binding protein

L 415 EXONUCLEOLYTIC CLEAVAGE OF TRANSCRIPTS IN RNA POLYMERASE II TERNARY ELONGATION COMPLEXES BY SII-STIMULATED HYDROLYSIS OR PYROPHOSPHOROLYSIS. Melissa S. Holtz and Diane K. Hawley, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

Exonucleolytic cleavage of RNA transcripts *in vitro* is observed when isolated RNA polymerase II elongation complexes are incubated with SII or pyrophosphate in the absence of nucleotides. Using transcripts stalled at the arrest site located in the Adenovirus major late transcription unit, we have shown that sequence specific pausing occurs as the transcripts are shortened. The sites where pausing occurs are dependent both on the sequence of the pause site and on the surrounding sequence context and are independent of the distance from the site where cleavage began. Most strong pauses are observed with both SII and pyrophosphate, suggesting that these pauses are independent of the mechanism of cleavage and may reflect shared processes such as translocation of the polymerase complex. Identification of transient pause sites during both elongation and exonucleolytic cleavage of transcripts may help to elucidate structural aspects of the ternary transcription complex.

When transcription complexes are arrested, hydrolysis of transcripts stimulated by SII initiates rapidly. At short times of incubation (5') all of the arrested transcripts are cleaved. In contrast, pyrophosphorolysis initiates slowly with some transcripts remaining at the arrest site after a long incubation (45'). This difference is seen at concentrations of SII and pyrophosphate where the apparent rate of cleavage, once initiated, is similar. This suggests that in an arrested complex the 3' end of the transcript may be interacting with a site on the polymerase where it is more accessible to hydrolysis than to pyrophosphorolysis.

L 416 ACTIVATION OF THE MIDDLE AND LATE PROMOTERS OF BACTERIOPHAGE MU BY THE RELATED ACTIVATORS MOR AND C. M.M. Howe, M. Kahmeyer, I. Artsimovitch, and Z. Zhao, Department of Microbiology and Immunology, University of Tennessee, Memphis, TN 38163

Transcription during lytic development of bacteriophage Mu is regulated by a cascade of transcriptional activators. The early gene product Mor is required to turn on the middle promoter P_m, and the middle gene product C is required to turn on the late promoters P_{lys}, P_I, P_P, and P_{mom}. Both proteins exert their effects by binding to their cognate promoters and activating transcription by *E. coli* RNA polymerase holoenzyme. Middle transcription also requires DNA replication and is blocked by mutations in the sites or genes involved in replicative transposition. Experiments with replicating and non-replicating heat-induced Mu prophages suggest that the replication requirement reflects a need for a particular template DNA conformation and not differing levels of Mor protein.

In this study we have used a promoter-*lacZ* fusion system to define the sequences involved in regulation of middle and late transcription. 5' and 3' deletion mapping has been used to define the promoter sequences required for transcription and to assess the role of additional proteins with potential binding sites in the promoter region. DNAseI footprinting with the activator proteins and additional regulatory proteins has been used to define their target binding sites. Point mutations have been isolated in P_m and P_{lys} by Mod-PCR (mutagenic oligo-nucleotide-directed PCR) to define the specific bases required for promoter activity.

Previous comparison of their amino acid sequences revealed that Mor and C are related proteins sharing 40% identical amino acids and 55% chemically similar amino acids. Both proteins possess an acidic N-terminus and basic C-terminus, and C possesses a recognizable helix-turn-helix DNA binding motif. The properties of hybrid proteins with the N-terminus from one gene and the C-terminus from the other gene will be described.

L 417 PURIFICATION AND CHARACTERIZATION OF THE δ PROTEIN FROM THE BACTERIOPHAGE P4.

Bryan Julien, Kaye Reiter, and Richard Calendar.
Department of Molecular and Cell Biology, 401 Barker Hall, University of California at Berkeley, Berkeley, CA. 94720.

The P4 δ gene encodes a transcriptional activator of the 2 late promoters of P4 as well as the 4 late promoters of the helper phage P2. Analysis of these promoters reveals little similarity in the -35 region to σ^{70} activated promoters, which is typical of positively controlled promoters. Positioned at -55, in all of these late promoters, is a partial dyad sequence that has been shown to be necessary for transcriptional activation in the Sid promoter of P4 and the F promoter of P2. It is proposed that δ binds to this partial dyad and activates transcription.

To understand the biochemistry of activation, we purified δ. We have fused the δ gene to *malE*, the maltose binding protein gene. Two different overproduction plasmids were constructed. In pBJ60, the δ gene was cloned into the pMalC-2 vector with its own Shine-Delgarno and therefore produces two proteins, a δ-maltose binding protein fusion and unfused δ. In the other vector, pBJ62, δ was cloned without its own Shine-Delgarno and as a consequence, only produces the δ-maltose binding protein fusion. When δ is purified from cells carrying pBJ60, using an amylose column, both the δ-maltose binding protein fusion and unfused δ are purified. This indicates that the δ protein dimerizes in solution. In addition, this purified mixture has transcriptional activity, as measured by an *in vitro* transcription and translation system (S30). When we purify the fusion protein alone, from cells carrying pBJ62, there is no transcriptional activity. Therefore, we conclude that the active protein in the mixture from pBJ60 is the unfused δ.

Filter binding and gel retardation experiments, with this purified mixture, show specific binding to a DNA fragment that contains the -10 to -90 region of the Sid promoter of P4 and to fragments that contain the other promoters activated by δ.

L 418 DIFFERENT INTERACTIONS OF PHAGE T7 AND SP6 RNA POLYMERASES WITH THEIR PROMOTERS,

Changwon Kang, Sang Soo Lee, Sung Su Kim, and Yeongjin Hong, Dept of Life Science, KAIST, Taejon 305-701, Korea

To reveal the molecular basis of specific interaction between phage RNA polymerases and promoters, 4 bp at positions −12, −10, −9 and −8 of the T7 promoter were substituted individually and multiply by SP6 promoter-specific bp, and 3 bp at −10, −9 and −8 of the SP6 promoter were replaced by T7-specific bp. Promoter activities of 28 sequences were measured *in vitro* with T7 and SP6 polymerases separately. Any T7 and SP6 variants that contain either or both changes at −9 and −8 show greatly reduced activity. Interestingly, the double substitution at −9 and −8 yields significant SP6 promoter activities and virtually no T7 promoter activity. Furthermore, the SP6 promoter variants with both T7-specific −9C and −8T show T7 promoter activities. All our results indicate that the two base pairs at −9 and −8 of both the T7 and SP6 promoters are the primary (if not the only) determinants of specificity, and that the hierarchy of importance of positions for promoter activity is −8, −9 > > −10 > −12.

The roles of the conserved TATA sequence from −4 to −1 of T7 and SP6 promoters were also investigated with promoter variants carrying all the possible multiple and single mutations at this region having A:T or T:A bp. Supercoiled template activities of T7 mutant promoters are not reduced much *in vitro* compared with the wild type at 37 and 25°C. However linear template activity varied substantially. All mutants were less active than the wild type and generally those needing more free energy for melting are less active. Substitutions at −4 and −3 make promoter substantially weaker than those at −2 and −1. In contrast to the results of T7, activities of SP6 promoter variants under supercoiled conditions are diverse. Alteration at −4 and −3 reduces promoter activity more seriously than those of −2 and −1. Linear template activities of all the SP6 mutant promoters are less than 20%. Thus, TATA sequence of T7 promoter is mostly involved in melting of DNA duplex, and that of SP6 promoter in both melting and specific binding with polymerase.

L 419 ARREST OF PURIFIED RNA POLYMERASES IIA AND IIO AT THE C-MYC T_{II} SITE. Richard G. Keene, Lucille London, Timothy I. Meier, Anja Mueller, and Robert Landick, Deptartment of Biology, Washington University, St. Louis, MO. 63130.

Current evidence suggests that RNA polymerase II (pol II) transcription complexes become modified in vivo either at the promoter or early in elongation to alter the efficiency of transcript elongation and the biochemical properties of elongation complexes (Roberts, S., and D.L. Bentley. (1992) EMBO J. 11, 1085-1093). Modifications which may influence elongation could include phosphorylation of the C-terminal domain (CTD) of the largest subunit of pol II during conversion of pol IIA to pol IIO or stable incorporation of factors that either promote transcript elongation, such as S-II and TFIIF (RAP 30/74), or incorporation of negative factors which inhibit elongation. We have examined the effects of CTD phosphorylation on elongation and on association of elongation factors by comparing the behavior of mammalian pol IIA and pol IIO at intrinsic c-*myc* pause, arrest and termination sites.

We used an immobilized Sma I-linkered oligo(dC)-tailed c-*myc* template to initiate transcription very near the authentic c-*myc* P2 promoter start site to produce primarily displaced transcripts. Pure pol IIA from calf thymus or pure pol IIO from HeLa cells were compared on this template. We found that: (i) pol IIO arrests at c-*myc* T_{II} with an almost two-fold lower efficiency than pol IIA (15 ± 5% vs. 27 ± 1%, respectively); (ii) washed HeLa nuclear extract elongation complexes initiated at a promoter and washed during elongation with 1% sarkosyl arrest with very low efficiency at T_{II} (< 8%); (iii) TFIIF remains associated with either pure pol IIA or pol IIO and increases elongation rates even after multiple washes in transcription buffers with ionic strengths ranging from 50 mM to 170 mM. Association studies at higher ionic strengths should reveal if TFIIF preferentially associates with pol IIA or pol IIO. Differences in T_{II} site arrest efficiency between pol IIO and pol IIA could occur because CTD phosphorylation alters intrinsic pol II:nucleic acid interactions at the T_{II} site. Alternatively, differences in arrest efficiency may occur because pol IIO displaces transcripts from the DNA template better than pol IIA. Transcription of the Sma I-linkered oligo(dC)-tailed template may still produce some transcription complexes which contain persistent RNA-DNA hybrids. We have found that transcription complexes containing persistent RNA-DNA hybrids arrest more efficiently at T_{II} than transcription complexes containing displaced transcripts.

L 420 FUNCTIONAL ANALYSIS OF THE LARGE SUBUNIT OF *DROSOPHILA* FACTOR 5 (TFIIF)

Daniel D. Kephart, Hongliang Guo, Bo Qing Wang[1], Zachary Burton[1] and David H. Price, Department of Biochemistry, University of Iowa, Iowa City, IA 52242, [1]Department of Biochemistry and Agricultural Experiment Station, Michigan State University, E. Lansing, MI 48824

Factor 5 is a *Drosophila* RNA polymerase II initiation factor which also affects the elongation phase of transcription. The large subunit of factor 5 (F5a) and several truncations were expressed in *E. coli*. A chimeric fly/human factor composed of recombinant F5a and RAP30, the small subunit of human TFIIF, functioned during initiation in either the *Drosophila* or human system. The chimeric factor also stimulated elongation by purified *Drosophila* RNA polymerase II. The N-terminal half of F5a was necessary and sufficient to stimulate elongation in the presence of RAP30.

We were surprised to find that RAP30 mediated transcript cleavage by *Drosophila* RNA polymerase II in a manner similar to that reported for DmS-II. However, the transcript cleavage mediated by RAP30 was 400-1000 times slower than that of DmS-II. Intact factor 5 mediated detectable transcript cleavage although to an even lower extent than RAP30. Consistent with this limited ability of the intact factor to mediate transcript cleavage F5a inhibited transcript cleavage mediated by RAP30. Both factor 5 and F5a also inhibited transcript cleavage mediated by DmS-II. Recombinant F5a truncations were used to determine the regions of F5a that are responsible for this inhibition. Although the level of transcript cleavage activity mediated by factor 5 is not kinetically significant with respect to the large effect that the factor has on stimulating elongation, we have begun to use this information to investigate the mechanistic details of factor 5 action and RNA polymerase II elongation.

L 421 OPERATOR SEQUENCE CONTEXT INFLUENCES AMINO ACID-BASE PAIR CONTACTS IN 434 REPRESSOR-OPERATOR COMPLEXES. Gerald B. Koudelka & Adam C. Bell, Department of Biological Sciences, SUNY at Buffalo, Buffalo, NY 14260.

The 434 repressor binds more tightly to O_R1 than it does to O_R3. The repressor makes several specific contacts with the symmetrically arrayed outer four base pairs of the 14 base pair site, and no specific contacts to the central six base pairs. The sequence of the outer base pairs of O_R1 and O_R3 differs only by an A->G substitution at position 4 in one half-site of O_R3, while that of central bases is very different.

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-G-T-T

As expected from sequence analysis of wild-type operators, the results of a mutational analysis of these operators show that repressor prefers an A·T base pair at position 4. Our results show, however, that the magnitude of this preference depends on the sequence context at the operator's center and solution conditions. Position 4 changes in the context of O_R1 have a greater effect on operator affinity for 434 repressor than do similar changes in O_R3. Although O_R1 and O_R3 display different affinities for 434 repressor, their repressor-operator complexes are similarly insensitive to changes in salt concentration and temperature. By contrast, complexes formed between repressor and position 4 mutant O_R1, bearing an A·T->G·C change, and O_R3, which bears a G·C->A·T change, are affected greatly, and to similar extents, by changes in ionic strength and temperature. Nuclease protection experiments show that 434 repressor protects the DNA phosphate backbone of wild-type operators from cleavage more efficiently than those of mutant operators. These data show that the biochemical and structural properties of a repressor-operator complex, while affected by position 4 base sequence, are independent of the identity of this base. The ability of repressor to recognize the base at position 4 depends on the sequence context at operator positions 5-7. We will present results which show that a poly dA-poly dT tract at the central positions in a 434 operator facilitates repressor's tight binding to operators bearing A·T->G·C substitutions at operator position 4. Taken together, our results indicate that there is an interplay between the bases at operator positions 4-7 which has a global effect on the structure and stability of the repressor-operator complex.

L 422 THE REGULATION OF TRANSCRIPTION AND DNA SUPERCOILING: A CORRELATION BETWEEN PROMOTER STRENGTH AND THE ANTITERMINATION ABILITY OF MUTANT *gyrA* PROMOTERS IN *Escherichia coli*. Regis Krah[1], Martin Gellert[1], Rolf Menzel[2], [1]Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892. [2]Bristol Myers-Squibb, P.O. Box 4000, Princeton NJ 08543

DNA relaxation increases the expression of the *gyrA* promoter in *E. coli*. A substantial fraction of transcription from this promoter can pass through the *rho* independent λ_{Toop} terminator, and this readthrough is enhanced following DNA relaxation. To determine the relationship between these two properties of the *gyrA* promoter we looked at the capacity of transcripts initiated at 13 different *gyrA* promoter mutants to readthrough the λ_{Toop} terminator. A strong correlation between the level of promoter activity and the degree of antitermination is observed when the promoters are induced by DNA relaxation *in vivo*.

The *gyrB* promoter, which is also stimulated by DNA relaxation, exhibits a similarly increased degree of readthrough at λ_{Toop} after DNA relaxation. However, transcription by purified RNA polymerase that initiates at either the *gyrA* or *gyrB* promoter on linear DNA terminates at λ_{Toop} with >90% efficiency. The antitermination capacity of the *gyrA* and *gyrB* relaxation-induced promoters apparently requires a factor(s) in addition to RNA polymerase.

L 423 **PROMOTER-PROXIMAL PAUSING OF POLYMERASE IN THE HUMAN C-MYC GENE,** **Anton Krumm[1], Tea Meulia[1], Linda Madisen[1] and Mark Groudine[1,2]. [1]Division of Basic Sciences, Fred Hutchinson Cancer Research Center; and [2]Department of Radiation Oncology, University of Washington School of Medicine, Seattle, WA (USA)**

A conditional block to transcriptional elongation is an important mechanism for regulating c-myc gene expression. This elongation block within the first c-myc exon was defined originally in mammalian cells by nuclear run-on transcription analyses. Subsequent oocyte injection and in vitro transcription analyses suggested that sequences near the end of the first c-myc exon are sites of attenuation and/or premature termination. More recently, the mapping of single stranded DNA in vivo with potassium permanganate ($KMnO_4$) and nuclear run-on transcription assays have revealed that polymerase is paused near position +30 relative to the major c-myc transcription initiation site. In addition, sequences upstream of +50 are sufficient to confer the promoter-proximal pausing of polymerases and to generate the polarity of transcription further downstream. Thus, the promoter-proximal pausing of RNA polymerase II complexes accounts for the block to elongation within the c-myc gene in mammalian cells. Currently, we are attempting to determine the sequence elements within the human c-myc promoter that contribute to the promoter-proximal pausing of RNA polymerase II in mammalian cells.

L 424 ANALYSIS OF TRANSCRIPTION FACTORS IN THE RNA POLYMERASE II INITIATION AND ELONGATION COMPLEXES, K.Prasanna Kumar, Leigh Zawel and Danny Reinberg, Dept. of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, N.J. 08854

Transcription initiation from class II promoters involves the concerted interaction between at least six different protein factors (TFIID, IIB, IIF, IIE, IIH and IIJ), referred to as the general transcription factors (GTF's), and RNA polymerase II. The GTF's assemble at the promoter in a highly ordered fashion, concomitantly loading the polymerase onto the DNA. The preinitiation complex can readily initiate the synthesis of mRNA in the presence of rNTP's. Once the transcription complex proceeds from the initiation to elongation mode, RNA synthesis occurs in a highly processive manner. The sequence in which the GTF's assemble at the promoter has been studied functionally by template competition assays, and the presence of individual factors in the preinitiation complex was studied by western blot after forming them on immobilized templates. Recent studies indicate that transcription elongation is a highly regulated process. To study the elongation complexes, we assembled preinitiation complexes on immobilized templates, then generated paused ternary complexes by adding select combination of rNTP's. A restriction site upstream of the ternary complex allowed the separation of the complex from the promoter-containing fragment. The viability of the ternary complexes was tested by different parameters. These complexes were then probed on western blots for the presence of different factors using specific antibodies. The results indicate that at least TFIIF remains tightly bound to the elongating polymerase. Furthermore, recent studies in our laboratory indicate that TFIIJ acts as an elongation factor. It has been found that Dr2, which is the same as topoisomeraseI, represses basal transcription by binding to TFIID, and this repression is relieved in the presence of TFIIA or some activators, probably because Dr2 translocates from TFIID to the polymerase. To test this interaction, we used the same approach of preparing the elongation complex and probed for the presence of Dr2. Preliminary results indicate that Dr2 is present in the ternary complexes.

L 425 **AMINO ACID SUBSTITUTIONS IN CONSERVED REGIONS OF THE ß AND ß' SUBUNITS OF *E. coli* RNA POLYMERASE REVEAL REGIONS IMPORTANT FOR TRANSCRIPTIONAL PAUSING AND TERMINATION.**

Robert Landick, Rodney Weilbaecher, Clarissa Hebron and Guohua Feng, Department of Biology, Washington University in St. Louis, MO 63130

The two largest subunits of cellular RNA polymerases, ß and ß' in bacteria, are highly conserved among prokaryotes and eukaryotes. In a previous study we mutagenized *rpoB*, the gene for the *E. coli* ß subunit, in discrete intervals and then screened for changes in termination when the altered subunits were expressed in cells. At least four distinct regions of ß, three with recognizable conservation to its eukaryotic homologue, appear to participate in pause and termination signal recognition: residues 368-418; 489-567, the region in which rifampicin- and streptolydigin-resistance substitutions occur; 788-822; and 1238-1342, the C-terminus. We now have extended this study to ß'. As for ß, we find that termination-altering amino-acid substitutions occur in discrete intervals, designated 2, 3, 4, and 5, that are highly conserved in the eukaryotic homologues of ß'. Region 2 (residues 311-386) is tightly clustered around a short sequence that is identifiably conserved at the floor of the DNA-binding cleft in the structure of *E. coli* DNA polymerase I. Region 3 contains two subclusters: A (residues 632-671) and B (residues 718-798), the latter of which corresponds to the segment of the largest subunit of pol II in which amanitin-resistance substitutions occur. Region 4 consists of three distinct subclusters between residues 930 and 1190, whereas Region 5 is tightly clustered between residues 1308 and 1356 in a section of high conservation between *E. coli* RNA polymerase and the segment of pol II immediately preceding the heptapeptide-repeat CTD. These discrete regions of ß and ß' probably reflect functional sites on the enzyme that are important for transcript elongation and termination.

Studies of purified RNA polymerases containing selected substitutions reveals that: (i) some substitutions consistently affect termination at 7 ρ-independent terminators, others exhibit terminator-specific effects and some show no defect in vitro; (ii) the P560S,T563I double substitution in ß prevents recognition of some pause sites completely, yet also causes recognition of new ones; (iii) some substitutions dramatically alter elongation without significant effect on substrate Km during abortive initiation. Together these results suggest that specific interactions between conserved regions of RNA polymerase and sequences surrounding pause and termination sites contribute to complex conformational changes in the pathways for pausing and termination.

L 426 LA RELEASES NASCENT TRANSCRIPT AND RNA POLYMERASE III FROM PAUSED TERMINATION COMPLEXES THEREBY PROMOTING MULTIPLE REINIATIONS: THE ROLES OF LA IN ALU EXPRESSION. Richard J. Maraia, Daniel Kenan and Jack Keene, Laboratory of Molecular Growth Regulation, NICHD, NIH, Bethesda, MD, 20892.
Using pure recombinant La protein and immobilized in vitro transcription termination assays we demonstrate three activities of La: i) La stimulates release of various pol III-synthesized RNAs (VA1, 7SL, Alu, B1) which otherwise remain paused at their termination signals, ii) La protects the 3' end of nascent B1 RNA from posttranscriptional processing, and iii) La stimulates multiple rounds of synthesis from stringently washed pol III-containing transcription complexes.
B1 and Alu short interspersed elements (SINEs) are endogenous to rodent and primate genomes respectively. Ample structural evidence indicates that these sequences were retrotransposed via RNA intermediaries synthesized by RNA polymerase III (pol III). A model of SINE transposition suggests that the terminal oligo-(U) tract of a pol III-terminated primary transcript may intramolecular basepair with an internal poly-(A) stretch to "self-prime" reverse transcriptase. Subsets of B1 and Alu sequences are expressed by pol III and undergo post-transcriptional 3' processing which removes their poly-(A) and terminal oligo-(U) to produce small stable RNAs that accumulate in the cytoplasm of cells. Subtle base changes around the pol III termination signal $dT_{(4)}$ dramatically inhibits the rate of post-transcriptional 3' processing of a B1 primary transcript in vitro and in vivo (Maraia et al., 1992, *Mol Cell Biol* **12**:1500-1506). Here we identified the protein factor that confers this pol III-dependent processing inhibition as La, an autoimmune antigen previously known to bind the 3' ends of all primary trasnscripts of pol III, and to be involved in transcription termination. In addition, the influence of base composition surrounding the termination signal on La-dependent post-transcriptional maturation of nascent pol III transcripts will be discussed.

L 427 **THE CHARACTERIZATION AND PARTIAL PURIFICATION OF *DROSOPHILA* P-TEF**, Nick F. Marshall and David H. Price, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.

We have previously shown that in nuclear extracts, most polymerases are incapable of efficient elongation after initiation at a promoter. The fraction of productively elongating polymerase complexes is controlled by the action of P-TEF (positive transcription elongation factor) and this activity is sensitive to the drug DRB (5,6-dichloro-1-b-D-ribofuranosylbenzimidazole). Fractionation of *Drosophila* K_c cell nuclear extracts has led to the discovery that at least three separate protein fractions must be added to the basal initiation apparatus in order to reconstitute DRB sensitive transcription. These three activities, in order of their elution from phosphocellulose, are termed P-TEFa, factor 2, and P-TEFb. P-TEFb is absolutely required while P-TEFa and factor 2 are stimulatory. P-TEFb has been purified to near homogeneity and its activity correlates with two polypeptides of 124 and 43 kDa.

The protein kinase inhibitor H-8 has been shown to inhibit the phosphorylation of the CTD of the largest subunit of RNA polymerase II. We have recently found P-TEF is completely inhibited by concentrations of H-8 as low as 25μM when assayed in nuclear extracts. As seen by others, H-8 does not inhibit transcription when it is added to a more purified initiation reconstruction system (which also does not reproduce DRB sensitivity). Using isolated elongation complexes it was found that complete inhibition of CTD phosphorylation by H-8 during initiation does not preclude P-TEF from acting on the resulting early elongation complexes. These results eliminate the possibility that P-TEF requires a highly phosphorylated CTD and show that abortive elongation is not controlled by the transition of RNA polymerase from the IIA to the IIO form.

L 428 RECOMBINANT HIV-1 TAT ENHANCES THE EFFECT OF TFIIF ON TRANSCRIPT ELONGATION BY RNA POLYMERASE II IN VITRO. Timothy I. Meier, Richard G. Keene, Michael J. DeVit, Daguang Wang, and Robert Landick. Department of Biology and Division of Biology and Biomedical Sciences, Washington University, St. Louis, MO 63130.

Purified and renatured recombinant transcription factor TFIIF[1] (a heterodimer of 30 Kd and 74 Kd subunits, also called Rap 30/74) and HIV-1 Tat were used to study effects on RNA polymerase II (pol II) transcript elongation *in vitro*. HIV-1 LTR/c-*myc* fusion templates were immobilized on magnetic beads, incubated with HeLa nuclear extract, pulse labeled and washed with 1% sarkosyl to remove loosely bound proteins. Addition of renatured recombinant TFIIF to washed elongation complexes increased the rate of elongation and at high concentrations relieved stopping at defined *c-myc* template sites. These effects were dependent on the addition of both the 30 and 74 Kd subunits. Addition of sarkosyl to 0.02% increased the concentration of TFIIF needed to stimulate transcript elongation. At suboptimal concentrations of TFIIF, addition of HIV-1 Tat protein to the washed elongation complexes further stimulated transcript elongation. However, the effect of Tat disappeared as the concentration of TFIIF was increased. Neither Tat alone nor Tat in the presence of Rap 30 or Rap 74 separately increased the elongation rate. In at least some conditions, Tat also stimulated the effect of TFIIF on transcript elongation on templates that do not encode the TAR RNA hairpin. These results suggest that stimulation of transcript elongation by HIV-1 Tat occurs at least partly by recruitment of TFIIF to the elongating pol II transcription complex, that recruitment is mediated by protein-protein interactions among pol II, TFIIF, and Tat, and that Tat is incapable of substituting for either of the TFIIF subunits.
1. Wang, B.Q. *et al. Protein Expr. Purif.*, **4**, 207-214 (1993).

L 429 COUPLING BETWEEN PRODUCT EXTRUSION AND TRANSLOCATION DURING ELONGATION. Evgeny Nudler, Alex Goldfarb, Mikhail Kashlev, Public Health Research Institute, New York, NY 10016
The relationship between parts of RNA polymerase during its inchworm-like propagation along DNA template was studied by footprinting of defined elongation complexes, using exonuclease III and transcript cleavage factor GreB as probes for DNA and RNA, respectively. Strict correlation was found between the leap-like translocation of the enzyme "front end" along DNA and the shielding of the nascent RNA from cleavage in the "tight product binding site". The front end of the enzyme and the front edge of RNA shielding are always separated by a constant distance of 18 nucleotides and are translocated synchronously. However, the length of each translocation leap is variable ranging from 1 to 12 nucleotides. The leaping pattern is determined by the nucleotide sequence reflecting the existence of specific "leap sites" in DNA. The unshielded 3'-proximal segment of RNA that is synthesized between the leaps varies in length and is equal to the span of the next leap; when the leap occurs this segment becomes shielded. The results suggest a model of elongation in which incremental RNA chain extension occurs internally in the non-moving complex and is followed by the extrusion of the nascent 3'-proximal segment though the product channel with concomitant leaping of the enzyme along DNA.

L 430 TRANSCRIPT CLEAVAGE FACTORS GREA AND GREB UTILIZE INTRINSIC ACTIVITY OF RNA POLYMERASE, Marianna Orlova[1], Sergei Borukhov[1], Janet Newlands[2], Alex Goldfarb[1], Asis Das[2], [1]Public Health Research Institute, New York, NY 10016, [2]University of Connecticut Health Center, Fazrmington, CT 06030.
RNA polymerase isolated from a double *GreAGreB* mutant displays low-level transcript cleavage activity which cannot be due to contamination with GreA or GreB, or the presence of an unkllnown cleavage factor. Upon long incubation, the cleavage activity is stoichiometric to the numebr of ternary complexes. The rate of intrinsic cleavage is dramtically enhanced by the addition of the exogeneous factors. It is also demonstrated that GreA and GreB proteins bind to the elongation complex near the active center of RNA polymerase since they can be photocrosslinked to the nascent transcript via 3'-terminal 8-azido-ATP.

L 431 **MOLECULAR FLEXIBILITY IN PROKARYOTIC COMBINATORIAL REGULATION: The CytR repressor binds DNA operators with variable half-site spacings even in cAMP-CRP/CytR/DNA nucleoprotein complexes.**
Henrik Pedersen and Poul Valentin-Hansen, Dept. of Molecular Biology, Odense University, Odense , DK-5230, Denmark.

Repression by CytR depends on the formation of nucleoprotein structures in which CytR contacts DNA as well as DNA bound cAMP-CRP complexes. This cooperative binding of CytR and cAMP-CRP stimulates binding of CytR 1000-fold, raising the affinity of CytR for its cognate promoters to a level adequate for repression. Specificity and affinity of CytR is therefore determined by a combination of protein-protein and protein-DNA interactions. By employing an in vitro selection procedure, we here demonstrate that the CytR dimer is a flexible protein whose DNA binding domains can contact operators with different half-site spacings, both in the presence and absence of cAMP-CRP.
From a pool of approximately 10^{10} different DNA fragments we have selected those that bind CytR most efficiently. Sequencing of these fragments shows that CytR can recognize two half-sites on the DNA whose spacings vary from 8-17 bp. This suggests that CytR and cAMP-CRP form repression complexes in which CytR is sandwiched between two fixed cAMP-CRP complexes, but still retains the freedom of its DNA binding domains to "walk around" on the DNA, searching for the two best possible half-sites. This flexibility may explain the observed low CytR-DNA affinity - it may simply be the result of a great loss of entropy upon binding of a flexible protein to a relatively stiff DNA molecule.

L 432 10Sa RNA AND GENE EXPRESSION IN *E. coli*, Diane Retallack and David Friedman. Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI 48109.
The *ssrA* gene of *E. coli* encodes a stable, relatively abundant 362 nucleotide RNA, 10Sa RNA. We find that mutations which alter or inactivate the *ssrA* gene appear to enhance the effect of certain DNA binding proteins. For example, there appears to be an increased repression of lactose operon gene expression, and of phage λ gene expression, in *E. coli* whose *ssrA* gene is insertionally inactivated. This host fails to support lytic growth of λ derivatives that express the *cI* repressor gene (λ*cI*$^+$), but support growth of λ mutants unable to express active repressor (λ*cI*$^-$) or λ that express a repressor with different binding characteristics, such as that of phage 434. Moreover, ~100% of the mutant bacteria infected with λ*cI*$^+$ survive but, surprisingly, are not stable lysogens. We ascribe the latter observation to premature repression, since derivatives that constitutively express integrase (λ*cI*$^+$*intH*) form stable lysogens in the *ssrA* mutant. The insertional inactivation of the *ssrA* gene also appears to influence expression of *lacZ*. When grown in the presence of low levels of IPTG (0.01 mM), an *E. coli* with a wild-type *ssrA* gene expresses 10 fold more β-galactosidase than an isogenic bacterium with an insertionally inactivated *ssrA* gene. Moreover, derivatives of these strains that do not express active *lac* repressor produce essentially equal levels of β-galactosidase. A model explaining how 10Sa RNA might influence binding of proteins to DNA will be discussed.

L 433 **THE *E. COLI RHO* ATTENUATOR CONTAINS A DYNAMIC TRANSCRIPTIONAL TERMINATOR,**
Elizabeth A. Roberts, Gregory T. Runyon, and David G. Bear, Department of Cell Biology and the Cancer Center, University of New Mexico School of Medicine, Albuquerque, NM 87131
The *rho* gene of *E. coli* is autoregulated by its gene product, transcription termination factor Rho protein. Autogenous control is accomplished through premature termination of transcription in the 5' untranslated region of the transcript referred to as the *rho* attenuator or tRA. We have characterized the *in vitro* transcription termination reaction and the Rho-transcript interactions in tRA using a linear restriction fragment that includes the *rho* promoter and the 255 nt attenuator region. Three sites of transcript release are observed in a purified transcription system; they occur at approximately -90, -1, and +40 nt relative to the AUG of the translation start site (+256). RNases were used to probe interactions between Rho and nascent tRA transcript, and several sites of RNA-protein contact were revealed. The strongest and most consistently observed segment of the transcript protected by Rho from RNase is +200-210. The importance of this region is supported by the observation that a complimentary oligonucleotide hybridized to +200-210 reduces efficiency of termination at both sites II and III. Oligonucleotides directed to segments upstream of +200 reduce termination efficiency at sites I and II, but not site III. A transcriptional blockade by the cleavage-defective EcoR1(Q111) mutant protein was engineered at various locations along the attenuator template, and transcript release was assayed by a nitrocellulose filter binding. Efficient Rho-mediated release from the blocked complexes requires sequences located upstream of +248-252. Together, these results suggest that the sites required for Rho attachment and transcript release within the rho attenuator are dynamic and that termination at each of the three principal sites has a different requirement for the various segments of attenuator RNA.

L 434 THE USE OF *ECO*RI(Q111) BLOCKADED TRANSCRIPTION COMPLEXES TO STUDY RHO-DEPENDENT TERMINATION, G. T. Runyon, E. A. Roberts and D. G. Bear., Dept. of Cell Biology and the Cancer Center, Univ. of New Mexico School of Medicine, Albuquerque, NM 87131.

The *E. coli* Rho protein catalyzes the ATPase-dependent release of nascent transcripts from ternary transcription complexes. We are studying the kinetics of the Rho-mediated transcript release reaction using blockaded transcription complexes (BTC's). These BTC's are generated by binding a cleavage-defective mutant *Eco*RI protein, *Eco*RI(Q111), to strategically installed *Eco*RI restriction sites. The bound protein acts as a physical block to transcription (Pavco & Steege, J. Biol. Chem. 265: 9960-9969 [1990]). The BTC's are stable during gel filtration and native gel electrophoresis. Rho-mediated dissociation of the nascent transcript is dependent on the position of the *Eco*RI blockade, suggesting that an intact Rho attachment site is necessary for release. The release reaction is dependent on ATP hydrolysis and release at each blockade has an individual dependence on [$MgCl_2$]. We've used the BTC attached to magnetic beads to demonstrate that the release reaction is not influenced by the *Eco*RI(Q111) protein. The *Eco*RI(Q111) BTC substrate simplifies our identification of essential signals and key intermediates in Rho-mediated reactions because RNA release is uncoupled from transcription elongation. Supported by NSF grant MCB-9106112 (D.G.B.) and NIH Postdoctoral Fellowships GM 15409 (G.T.R.) and GM 13895 (E.A.R.).

L 435 ANALYSIS OF THE BACTERIOPHAGE T4 ENHANCER: THE ROLE OF GP45 AND ATP HYDROLYSIS, Glenn M. Sanders, Jeffrey W. Winkelman, George A. Kassavetis, and E. Peter Geiduschek, Department of Biology and Center for Molecular Genetics, University of California San Diego, La Jolla, CA 92093

Transcription of T4 late genes is dependent on concurrent DNA replication. An *in vitro* system that reflects some aspects of this connection between replication and transcriptional regulation has been developed. The system requires *E. coli* RNA polymerase supplemented with the T4-encoded sigma factor gp55, and a DNA template carrying a T4 late promoter. Transcription is subject to enhancement by DNA polymerase accessory proteins gp45 and gp44/62, acting at a nick or gap in the template DNA. The nick can be located some distance upstream or downstream from the promoter and accordingly has some of the properties of an enhancer. *In vivo* the necessary nicks would be generated by the replisome itself and so the replication fork has been somewhat speculatively termed a mobile enhancer. Activation of transcription also requires ATP hydrolysis.

We have used the molecular crowding reagent polyethylene glycol (PEG) to alter the normal parameters of protein/protein interactions in this *in vitro* system, and have identified gp45 as the proximal effector of enhancement of transcription. In the presence of PEG, gp45 associates directly with RNA polymerase and activates transcription without the need for gp44/62 or ATP hydrolysis. The role of gp44/62 and ATP hydrolysis appears to be to deliver gp45 to promoters located on nicked DNA, thus serving to bias transcriptional enhancement toward newly replicated DNA templates.

L 436 MUTATIONS IN σ^E FROM *Bacillus subtilis* THAT AFFECT BINDING TO CORE RNA POLYMERASE, Magdalena F. Shuler and Charles P. Moran, Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322

Sigma factors are a family of proteins which associate with the core subunits of RNA polymerase to form the RNA polymerase holoenzyme and initiate promoter-specific transcription. Amino acid alignment of the bacterial sigma factors shows several regions of homology. We have examined the role of a highly conserved region in sigma factors by analyzing the effect of amino acid substitutions and deletions in σ^E from *B. subtilis*. Since σ^E is required for the production of endospores in *B. subtilis* and not vegetative growth, the function of the mutant forms of σ^E was determined by assaying their ability to complement for sporulation activity. The accumulation of the mutant forms of σ^E were studied in *Escherichia coli* and *B. subtilis* by western blot analysis with a monoclonal σ^E antibody. Glycerol gradient sedimentation of lysates containing wildtype and mutant forms of σ^E were analyzed for their ability to associate with the core subunits of RNA polymerase from both *E. coli* and *B. subtilis*. Interestingly, σ^E with a deletion of three amino acids, two of which are highly conserved, from amino acid positions 61 through 63 (Δ61-3) accumulates in both *E. coli* and *B. subtilis* but does not complement for sporulation activity. Sedimentation analysis of the Δ61-3 form of σ^E showed that it does not bind to *E. coli* core RNA polymerase. Normally in *B. subtilis*, σ^E is produced in a pro-form, known as P-31, and undergoes a stage-specific proteolytic processing to the active form. One model suggests that the processing of σ^E requires binding to core RNA polymerase. The Δ61-3 form of σ^E was not efficiently processed when compared to wildtype *B. subtilis* in western blot analysis. Our results suggest that these conserved amino acids in this region of σ^E are important for the association of sigma factors and the core subunits of RNA polymerase.

L 437 DIMERIZATION OF NTRC : HOW AND WHY?, Kenneth M. Stedman, Anne K. North, Karl E. Klose and Sydney G. Kustu.
Departments of Plant Biology and Molecular and Cell Biology, University of California, Berkeley, Berkeley, California, 74720.

NTRC is a prokaryotic transcriptional enhancer binding protein with an intrinsic ATPase activity which is required for transcriptional activation. NTRC binds to dyad symmetrical sites and is a dimer in solution. To study the dimerization of NTRC we characterized in vitro: (1) The isolated C-terminal domain of NTRC; (2) NTRC proteins with C-terminal deletions; (3) NTRC containing the amino acid substitution A410E in the C-terminal domain. Unlike those for other activators of σ^{54}-RNA polymerase, the major dimerization determinants for NTRC lie in and are inseparable from its C-terminal DNA binding domain. To investigate the role that dimer formation plays in the activity of NTRC we investigated the behavior of NTRC proteins containing the A410E amino acid substitution, which causes NTRC to be predominantly monomeric. We show that dimerization of NTRC is required for DNA binding and transcriptional activation, and we are currently investigating the oligomerization requirement for ATPase activity.

L 438 Hydrolytic Cleavage of Nascent RNA in RNA Polymerase III Ternary Transcription Complexes, Simon K. Whitehall, Carolyne Bardeleben and George A. Kassavetis, Department of Biology and Center for Molecular Genetics, University of California San Diego, La Jolla CA92093.

It has recently been demonstrated that a number of different RNA polymerases in ternary complexes have a hydrolytic chain retracting activity. In this process, retraction of the RNA polymerase along the DNA template occurs through hydrolytic cleavage of the growing end of the nascent transcript, generating short RNA products. It has been postulated that this hydrolytic retraction provides a mechanism for overcoming elongation arrest, and that the process in which the paused complex retracts and subsequently resumes elongation facilitates multiple approaches to the block in transcription. In this work highly purified yeast RNA polymerase III ternary complexes were found to possess a hydrolytic chain retracting activity that cleaves nascent RNA from its 3'-OH end. Most of the shortened transcripts were capable of resuming RNA chain elongation, indicating that they remain stably associated with the enzyme–DNA complex. Analysis of the products of cleavage indicated that retraction primarily occurred in dinucleotide increments, but that mononucleotides were also excised at lower frequency. The ribonuclease activity was totally dependent on the presence of a divalent cation and was stimulated by the addition of non-cognate ribonucleotides. The inclusion of ATP in the reaction enhanced both the rate and extent of transcript cleavage. Evidence strongly suggesting that the hydrolytic activity is intrinsic to pol III and factor-independent is also presented. Transcript cleavage by pol III ternary complexes appears to be more closely related to the intrinsic nucleolytic activity of vaccinia virus RNA polymerase ternary complexes than to TFIIS-dependent cleavage that has been described for RNA polymerase II ternary complexes.

L 439 PHOTOAFFINITY LABELING OF RNA POLYMERASE II SUBUNITS IN PAUSED TRANSCRIPTION ELONGATION COMPLEXES
Christine I. Wooddell and Richard R. Burgess
McArdle Laboratory for Cancer Research, University of Wisconsin-Madison.

Eukaryotic RNA polymerase II is a large enzyme with 10 to 14 subunits. We are using photoaffinity labeling to identify the subunits of calf thymus RNA polymerase II in close proximity to the DNA during transcription elongation. The photoaffinity crosslinker 5-[N-(p-azidobenzoyl)-3-aminoallyl]-deoxyuridine [Bartholomew, B., G.A. Kassavetis, B.R. Braun, and E.P. Geiduschek. 1990. EMBO J. 9:2197-2205] was synthesized and incorporated into a "tailed template". This double-stranded DNA template contains a 3' tail of poly dC on which the polymerase initiates transcription without a promoter. The sequence of the template allows the polymerase to transcribe an initial region before it is paused by the lack of CTP in the transcription reaction. This specific pause site allows labeling of subunits with respect to their orientation on the template.

Initially the crosslinker is being incorporated into the DNA in either the template or nontemplate strand, either upstream or downstream of the pause site, in order to identify the subsets of RNA polymerase subunits in spacial proximity to each of these four regions along the DNA template during transcription elongation. Later experiments will focus on finer mapping of each subunit's location along the template, as a means to determine the proximity of the subunits to each other as well as to the DNA. The identities of the RNA polymerase II subunits crosslinked will be discussed.

L 440 REGULATION OF EUKARYOTIC GENE EXPRESSION BY TRANSCRIPTIONAL ATTENUATION
Stephanie Wright, Wellcome/CRC Institute of Cancer and Developmental Biology, Tennis Court Rd., Cambridge, CB2 1QR, England (tel. 0223-334-088; FAX 0223-334-089)

The expression of variety of eukaryotic genes may be regulated via the modulation of transcriptional elongation through discrete sites of premature termination within the gene. The *degree* of transcriptional attenuation, which may be controlled in response to different physiological signals, thus determines the steady state level of transcript in the cell.

Our data support a model in which certain genes, notably those capable of responding rapidly to various physiological signals, are capable of assembling an "elongation -incompetent" transcription complex at the promoter at all times; efficient transcriptional initiation then occurs, but premature termination takes place at various downstream attenuation signals within the gene. We have characterised sequence elements and factors which mediate premature transcriptional termination events within eukaryotic genes. We have also defined elements within the c-myc gene which determine the *responsiveness* of the transcription complex to such attenuator signals, and thus control steady state levels of c-myc transcript in the cell.

L 441 MULTIPLE EFFECTS OF *DROSOPHILA* FACTOR 2 ON TRANSCRIPTION ELONGATION
Zhi Xie, David H. Price, Department of Biochemistry, University of Iowa, Iowa City, IA 522422

Factor 2 is a previously identified factor that we have partially purified from *Drosophila* nuclear extract. It suppresses the appearance of long abortive transcripts during *in vitro* transcription reactions. Using a pulse chase protocol we have found that factor 2 seems to act shortly after initiation by RNA polymerase II from a promoter by causing a greater percentage of polymerase molecules to terminate. This indicates that factor 2 may be a part of N-TEF (negative elongation transcription factor) activity. We have also found that factor 2 can increase the number of polymerase molecules that are affected by P-TEF (positive elongation transcription factor) and enter productive elongation.

Using isolated elongation complexes on dC-tailed templates, we have found that factor 2, paradoxically, stimulates the elongation rate of some polymerase molecules. In the absence of NTPs factor 2 can cause the disappearance of some of the 13 nucleotide long transcripts that arise during the transcription of a dC-tailed template. However, factor 2 contains no detectable nuclease activity when incubated with isolated transcripts generated from either promoter containing templates or dC-tailed template. Its activity seems to be dependent on the polymerase or the transcription complex. Our results suggest that factor 2 interacts with RNA polymerase II and may act as a 5' to 3' nuclease on short nascent transcripts.

L 442 REDUCED RHO-DEPENDENT TRANSCRIPTION TERMINATION AND NUSA-INDEPENDENT GROWTH OF *E. coli*, Chuanhai Zheng and David I. Friedman, Department of Microbiology & Immunology, University of Michigan, Ann Arbor, MI 48109-0620

We have constructed an *E. coli* strain totally defective for NusA synthesis by substituting an internal 533 base pair fragment of the *nusA* gene with a *cat* cassette (*ΔnusA::cat*). Construction of this strain followed from the observation that the *rhoE134D* mutation, a point mutation resulting in a glu to asp change at amino acid position 134 of the Rho protein, suppresses the thermo-sensitive growth caused by several *nusA* mutations. A *rhoE134D* mutant exhibits 8 fold reduction in Rho-dependent transcription termination. Although we are unable to construct a *ΔnusA::cat* strain that has a *rho*$^+$ allele, we can construct a *ΔnusA::cat* strain with another *rho* mutation, *rho1*. A *rho1* mutant exhibits a similar 8 fold reduction in Rho-dependent termination and our sequence analysis shows that the *rho1* mutation is a point mutation resulting in a lys to glu change at amino acid position 352 of the Rho protein.

Our results suggest that the essential role for NusA in *E. coli* is to antagonize some role of Rho. We suggest that by extending pauses of RNA polymerase, NusA facilitates coupling of transcription and translation. Ribosomes in close proximity to the transcribing polymerase could then impede access of Rho to paused polymerases and interfere with termination. Accordingly, the *rho1* and *rho134* mutations permit growth in the absence of NusA because these mutations severely reduce Rho-dependent termination.

Transcription by RNA Polymerase I and II

L 500 FURTHER MAPPING OF THE SUBUNITS OF TFIIIB AND RNA POLYMERASE III IN TRANSCRIPTION COMPLEXES, Blaine Bartholomew, Lei Zhong, and Jim Persinger, Medical Biochemistry, Southern Illinois University at Carbondale School of Medicine, Carbondale, IL 62901-6503.

Previously, the position of several of the subunits of RNA polymerase III (Pol III) and transcription factors TFIIIC and TFIIIB have been mapped to specific positions of the SUP4 tRNA gene using DNA photoaffinity probes. The DNA photoaffinity probes had the photoactive nucleotide, 5-[*N*-(*p*-azidobenzoyl)-3-aminoallyl]-dUMP (AB-dUMP) incorporated at different specific positions and immediately adjacent were incorporated α-^{32}P labeled nucleotide monophosphate. The labeled DNA photoaffinity probes were used to radioactively tag proteins located at these different positions of DNA. We present here a further extension of those earlier results with several new photoaffinity nucleotide analogs that have been synthesized containing an aryl azide attached to the C-5 position of deoxyuridine triphosphate using tethers of varied lengths. These nucleotides analogs place the aryl azide moiety farther away from the DNA helix and allow further probing of the RNA polymerase III transcription complex. The choice of tether has been examined by using either a flexible tether (alkyl chain) or a somewhat more conformationally restricted tether (peptide chain). Two of these nucleotide analogs (ABU-dUTP and ABG-dUTP) extend the aryl azide approximately an additional 4.5 Å as compared to the previous nucleotide analog AB-dUTP. DNA probes constructed with ABG-dUMP have shown specific photoaffinity labeling of the 67 and 90 kDa subunit of TFIIIB at nucleotide position -26 to -21 of heparin-treated complexes having only TFIIIB bound. DNA probes constructed with either ABU-dUMP or AB-dUMP at this same position have shown only labeling of the 67 kDa subunit of TFIIIB, presumably because AB-dUMP has too short of a tether and ABU-dUMP has too flexible of a tether in comparison to ABG-dUMP. Binding of Pol III eliminates photoaffinity labeling of the 90 kDa subunit with the DNA probe containing ABG-dUMP at this position. Initiation of transcription by the addition of ATP, CTP, and UTP results in the translocation of Pol III downstream and the restored photoaffinity labeling of the 90 kDa subunit at this position. Additional nucleotides have been made that potentially extend the aryl azide ~10 Å farther away from DNA than the nucleotide AB-dUMP and photoaffinity labeling experiments with these nucleotides are in progress.

L 501 THE ROLE OF TBP-ASSOCIATED FACTORS (TAFs) IN SPECIES-SPECIFIC TRANSCRIPTION BY RNA POLYMERASE I, Dirk Eberhard, Udo Rudloff, and Ingrid Grummt, German Cancer Research Center, 69120 Heidelberg, Germany.

Transcription of rRNA genes is highly species-specific. This specificity is brought about by a promoter selectivity factor which both in man (SL1) and mouse (TIF-IB) is a multiprotein complex consisting of TBP and three TAFs. To investigate the molecular mechanisms which govern species-specific Pol I transcription, we have compared the transcriptional properties and the subunit composition of the murine and the human factor, respectively, and show that both factors share two subunits (TAF$_I$48 and TAF$_I$70) but differ in their largest TAF. Thus, evolutionary changes of rDNA promoter sequences have been accompanied by changes in Pol I-specific TAFs. Data will be presented showing specific interaction of distinct TAFs with TBP and the rDNA promoter, respectively.

Furthermore, we have investigated whether rDNA selectivity is exclusively mediated by the TAFs or whether the variable amino-terminal domain of TBP contributes to Pol I transcription specificity. Cell lines have been established which stably express tagged human TBP, yeast TBP or N-terminally truncated TBP. The results demonstrate that the conserved "core" of TBP is sufficient to nucleate the assembly of active Pol I-specific TBP-TAF complexes.

L 502 GENETIC AND BIOCHEMICAL ANALYSIS OF A MULTI-PROTEIN COMPLEX INVOLVED IN THE SPECIFIC INITIATION OF RNA POLYMERASE I-DEPENDENT TRANSCRIPTION OF rRNA. Daniel A. Keys, Joan S. Steffan, Robert T. Yamamoto, Yas Nogi, Loan Vu, Jon Dodd, and Masayasu Nomura. Department of Biological Chemistry, University of California, Irvine, Irvine, CA 92717

The *Saccharomyces cerevisiae* genes *RRN3*, *RRN6*, and *RRN7* are among a group of genes required for Pol I-dependent rRNA transcription *in vivo*. We have partially purified Rrn3p, Rrn6p and Rrn7p proteins from wild type extracts based on complementation of *in vitro* activity in extracts prepared from mutant strains. We have evidence that Rrn6p and Rrn7p, along with the TATA-binding protein, TBP, are components of a single multi-protein complex required for the specific initiation of transcription by Pol I. In biochemical fractionation experiments, RRN6 and RRN7 activities co-fractionate, and Rrn6p, Rrn7p, and TBP proteins were detected together in a purified fraction by Western blot analysis. RRN6 and RRN7 activities co-purify in affinity chromatography experiments using antibodies specific for epitope-tagged Rrn6p, Rrn7p, or TBP. *In vitro* synthesized ^{35}S-labeled recombinant Rrn6p and Rrn7p were found to co-immunoprecipitate. Results of further biochemical and genetic experiments defining the structure of this complex and its function in rRNA transcription will be presented.

L 503 PROTEIN PHOSPHORYLATION INHIBITS TWO DIFFERENT STEPS DURING XENOPUS RIBOSOMAL GENE TRANSCRIPTION IN VITRO, Paul Labhart, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037

Protein kinase(s) and protein phosphatase(s) that are present in a Xenopus S-100 transcription extract strongly influence promoter-dependent transcription by RNA polymerase I. The protein kinase inhibitor 6-dimethylaminopurine (DMAP) causes transcription to increase, while the protein phosphatase inhibitor okadaic acid causes transcription to decrease. Repression is also observed with Inhibitor-2, and addition of recombinant Xenopus protein phosphatase 1 stimulates transcription, indicating that the endogenous phosphatase is a type 1 enzyme. The endogenous DMAP-sensitive kinase that represses transcription has functional characteristics of a DNA-dependent protein kinase: it represses transcription only on linear templates -- suggesting it needs DNA-ends for activity --, it cannot use GTP, and it is insensitive towards several specific inhibitors for other protein kinases. Partial fractionation of the system, single-round transcription reactions, and kinetic experiments show that two different steps during ribosomal transcription are sensitive to protein phosphorylation. Okadaic acid affects a step before or during transcription initiation, and a protein dephosphorylation step appears to be required for every round of transcription. On the other hand, DMAP stimulates a process "late" in the reaction, suggesting that the inhibition of the DNA-PK-like activity is required for long-term stability of the transcription complex. The present results are a clear demonstration that transcription by RNA polymerase I can be regulated by protein phosphorylation.

Supported by NIH grant GM46938.

L 504 **IDENTIFICATION AND ANALYSIS OF THE RIBOSOMAL RNA PROMOTER REGION OF *Entamoeba histolytica.***

Bertha Michel*+, *Alejandro Alagón, *Paul Lizardi and +Mario Zurita. *Depto de Bioquímica and +Depto de Biología Molecular, Instituto de Biotecnología, UNAM.

Little is known about the molecular organization of the genome of *Entamoeba histolytica*. The rRNA genes are located in an extrachromosomal circular molecule that is an interesting model to study DNA replication, transcription and gene organization in this parasite. We have cloned and sequenced the upstream region of the rRNA cistron. Different experimental approaches, such as nascent labelled RNA hybridizations, primer extension analysis and S1 mapping were used to locate the transcription initiation site upstream of the 17S rRNA structural gene. A computer analysis of the region containing the transcription start site showed the presence of repetitive elements in tandem, both upstream and downstream the putative core promoter, suggesting that UCE-like sequences could be involved in the transcriptional regulation of *Entamoeba histolytica* rDNA genes.

L 505 THE STRUCTURE OF THE XUBF-DNA COMPLEX, Tom Moss*, David Bazett-Jones+ & Benoît Leblanc*,

*Cancer Research Centre and Department of Biochemistry, Faculty of Medecine, Laval University, Québec, Canada and +Department of Medical Biochemistry and Anatomy, Faculty of Medecine, University of Calgary, Alberta, Canada.

We have previously shown that the ribosomal transcription factor xUBF interacts in a specific manner with the RNA polymerase I core promoter. It was found that xUBF positions itself symmetrically across the transcription initiation site and that C-terminal and N-terminal domains of xUBF interact. The data suggested extensive folding of the core promoter within the xUBF complex. We have now shown that xUBF can stabilise and to some extent induce negative supercoils in plasmid DNA and that within the xUBF-promoter complex the highly acidic C-terminal domain of xUBF interacts with the N-terminal HMGbox 1 domain. Electron spectroscopic imaging (E.S.I.) has been used to observe the xUBF-DNA complex, to visualise the path of the DNA within the complex and to put close limits on the size and molecular weight of both the protein and DNA moities. The data provide a detailed view of the novel xUBF-DNA structure and a compelling explanation of xUBF function and species specificity.

L 506 REGULATION OF RNA POLYMERASE I, II and III DEPENDENT TRANSCRIPTION AT THE BEGINNING OF MOUSE DEVELOPMENT, Jean-Yves Nothias, Miriam Miranda and Melvin L. DePamphilis, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110, USA.

In mice, the onset of zygotic gene transcription begins ~20 hours after fertilization, a time that normally coincides with formation of a 2-cell embryo. One approach to investigating the requirements for unique DNA sequences that control this developmentally regulated event has been to inject plasmid DNA into the nuclei of mouse oocytes and embryos. Results from these and other studies have revealed two important mechanisms that regulate transcription. The first is a time dependent "zygotic clock" of unknown detail that delays the onset of transcription, regardless of whether a 2-cell embryo is formed or a 1-cell embryo development is arrested. The second is a mechanism that represses the activity of promoters specifically in maternal pronuclei of oocytes and 1-cell embryos, and in all nuclei of 2-cell embryos, regardless of their parental origin or ploidy. This repression is linked to chromatin, but the striking ability to relieve this repression with specific enhancers first appears with formation of a 2-cell embryo. So far, all of these studies have been done with RNA pol II promoters. Experiments are now in progress to determine whether or not the same developmentally regulated mechanisms that govern pol II transcription also apply to pol I and III transcription. We measured the level of transcription by RNA polymerases II and III by RNase protection assays after DNA injection to compare their regulation during the 1- to 2- cell embryo transition. In addition, we studied the regulation of endogenous RNA polymerase I dependent rRNA transcription. Our results emphasize how important are the processes that occur during the first mitosis of the mouse embryo when the regulation of the transcription machinery is first established.

L 507 **TRANSCRIPTIONAL REGULATION OF A SUBUNIT COMMON TO YEAST RNA POLYMERASES (RNAPs) BY THE TRANSCRIPTION FACTOR ABF1,** SHAHRZAD NOURAINI[1,2], JIM HU[1], LINDA McBROOM[3], JAMES.D. FRIESEN[1,2], 1)Department of Genetics, Hospital For Sick Children, 555 University Ave. M5G 1X8, 2)Department of Molecular and Medical Genetics, University of Toronto, Canada, 3)Department of Endocrinology, Hospital for Sick Children.

Eukaryotic transcription is a highly regulated process. This regulation can be implemented at various levels, such as the initiation or elongation of transcription. Regulation could also be mediated by the amount of RNAPs present. Using yeast genetics we have been able to identify a promoter element, the binding site for ABF1, that is important for expression of RPO26, the gene encoding a subunit common to yeast RNAPs. Using purified ABF1 and band shift assays we have shown that mutational alterations in the ABF1 consensus sequence in the RPO26 promoter can abolish specific binding of this factor. Furthermore, although these mutations do not generate a discrenible phenotype in an otherwise wild-type yeast, they give rise to cell death in the presence of a temperature sensitive (ts) mutation in the largest subunit of RNAPII (rpo21-4). The ts phenotype of rpo21-4 is sensitive to the level of RPO26, since it is suppressed upon increasing RPO26 gene dosage by a small factor. RPO26 and TFIIIA, the transcription factor required for the expression of 5SrRNA by RNAPIII, are divergently transcribed; it is possible that these genes are co-regulated. We currently are investigating RPO26 and TFIIIA expression in the presence and absence of mutations in the ABF1 binding sequence. This work has been supported by the National Cancer Institute of Canada Studentship No.4241.

L 508 **Bead-Shift Isolation of Protein-DNA Complexes Using a Reversibly Immobilized DNA**

Lawrence J. Peck, Department of Biology, Sinsheimer Labs, University of California, Santa Cruz, Santa Cruz, CA 95064

We have recently developed a new method for examining protein-DNA interactions in vitro. Template DNA, biotinylated at one end and radioactively labeled at the other, is immobilized on streptavidin coated paramagnetic beads. Protein-DNA complexes formed in vitro can be rapidly and extensively washed and concentrated using a magnet to separate the beads from the solution. The protein-DNA complexes are then released from the beads via restriction enzyme digestion at a specific site. The soluble complexes can then be subjected to any of a number of enzymatic and chemical probing techniques, e.g. DNAse I footprinting. Probing is followed by band-shift gels to achieve the separation of free DNA and various protein-DNA complexes. The novel aspect of this method is to use a restriction enzyme to recover the protein-DNA complexes in a native form following affinity purification and extensive concentration using paramagnetic beads. We have applied this technique to the analysis of RNA polymerase III transcription complexes formed on 5S RNA genes in *Xenopus* oocyte extracts and have detected multiple complexes with footprints extending over nearly the entire gene as well as protein binding to the 5' and 3' flanking DNA. In addition, transcriptional activity is retained even though the complexes have been extensively washed in the presence of competitor DNA.

L 509 UBF-NUCLEIC ACID INTERACTIONS AND THE MOLECULAR EVOLUTION OF RIBOSOMAL GENE ENHANCERS, Craig S. Pikaard, Gregory P. Copenhaver, Christopher D. Putnam and Michael L. Denton.Biology Department, Washington University, Box 1137 One Brookings Drive, St. Louis, MO, 63130.

Upstream Binding Factor (UBF) is a protein implicated in ribosomal RNA gene promoter and enhancer function. We have investigated the protein and nucleic acid requirements for Xenopus UBF-enhancer interactions to better understand how UBF recognizes a binding site. The dimerization domain and first (of five) HMG-box DNA binding domain are required for UBF footprinting, but domains downstream of HMG-box 2 contribute to UBF positioning on multiple enhancers. Major groove methylations had no effect on UBF binding, but the minor groove specific drugs chromomycin A3, distamycin A and actinomycin D disrupted binding suggesting that UBF interacts with the minor groove. Following selection by UBF of binding sites among a population of enhancer oligonucleotides with randomized central sequences, no consensus emerged. UBF can bind tRNA and DNA cruciforms and probably bends DNA to produce disproportionately large DNase I footprints and to accelerate ligation of linear enhancer probes into circles. A structural role for UBF is proposed to reconcile its sequence-tolerant DNA/RNA binding properties with its function as a transactivator of RNA polymerase I.

RNA polymerase I enhancers are repetitive DNA elements tandemly arranged within the intergenic spacers of higher eukaryotic ribosomal RNA genes. In Xenopus and mouse, the repetitive enhancers and the ribosomal RNA gene promoter bind UBF, and in Xenopus, enhancers also share sequence similarity with an upstream domain of the promoter. This upstream Xenopus promoter domain can itself act as an efficient enhancer when polymerized, cloned upstream of an intact rRNA promoter, and injected into frog oocytes. A core promoter domain that lacks similarity with spacer sequences in X. laevis but which is analogous to a repeated sequence in X. borealis can also function as an enhancer. These data demonstrate functional relatedness between the Xenopus ribosomal gene enhancers and promoter, supporting the hypothesis that in Xenopus, at least, the enhancers could have originated by chance duplications of promoter domains that bind one or more essential transcription factors. The ability of UBF to bind enhancers inactivated by clustered point mutations suggests that UBF binding may be necessary, but is probably not sufficient, for enhancer function.

L 510 Targeting TBP to a non-TATA box cis-regulatory element present in snRNA promoters, Sadowski, C.L., Henry, R.W., Lobo, S.M., and Hernandez, N., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Human small nuclear RNA (snRNA) genes are different from other transcription units in that despite similarities in promoter structure some of these genes are transcribed by RNA polymerase II (pol II; e.g. U1, U2) while others are transcribed by RNA polymerase III (pol III; e.g. U6). Both sets of promoters contain an essential cis-acting element, the Proximal Sequence Element (PSE), which is involved in the determination of the start site of transcription. The U6 promoter contains in addition a TATA box at -25 that determines the RNA polymerase III specificity of this promoter.

We have previously shown that the TATA-box binding protein (TBP) binds to the U6 TATA box and that TBP is required for transcription of the U6 gene by pol III in vitro. We now show that TBP is also required for pol II snRNA gene transcription in the form of a TBP-containing complex (termed $SNAP_c$) that binds specifically to the PSE. $SNAP_c$ has been purified to near homogeneity. Highly purified fractions of $SNAP_c$ contain three prominent polypeptides in addition to TBP which are similar in molecular weight to three polypeptides specifically UV-crosslinked to the PSE. Efforts to clone these TAFs are currently underway. Fractions that are highly enriched in $SNAP_c$ are required for transcription of the U6 gene as well as the U1 and U2 genes suggesting that the same complex is involved in transcription by two different RNA polymerases. We have found that reconstitution of U6 transcription requires both TBP and SNAP. In addition, $SNAP_c$ and TBP can co-occupy a probe containing both wild-type PSE and TATA elements. These results imply that the U6 initiation complex contains TBP in two contexts: one bound to the TATA box and the other bound to the PSE as part of $SNAP_c$.

L 511 THREE-DIMENSIONAL MODEL OF YEAST RNA POLYMERASE I. P. Schultz, H. Célia, M. Riva*, A. Sentenac* and P. Oudet. Laboratoire de Génétique Moléculaire des Eucaryotes. 11, rue Humann, 67085 Strasbourg France. *Service de Biochimie et Génétique Moléculaire. Centre d'Etudes de Saclay, 91191 Gif sur Yvette France.

We studied the structure of yeast RNA polymerase I by electron microscopy using a two-dimensional crystallization method on positively charged lipid layers (1-3). Upon interaction with the lipid layer the local enzyme concentration is increased by a factor of about 1000-fold and the enzyme, which binds mainly as a dimer, is preferentially oriented. These properties, combined with the in-plane mobility of lipid layers, promote the self-assembly of the proteins into higher ordered structures. The highest degree of organization is found in two-dimensional crystals of molecular thickness which diffract to about 2.5 nm. The three-dimensional envelope of the enzyme was calculated from images of crystals tilted up to 50°. The structure shows an irregularly shaped molecule 11 nm x 11 nm x 15 nm in size characterized by a 3 nm wide and 10 nm long groove. The overall shape is similar to the E. coli holo enzyme (1) and the yeast RNA polymerase II Δ4/7 (2). The most remarkable structural feature is a finger-shaped stalk which partially occludes the entrance of the groove and forms a 2.5 nm wide channel. This peculiar region shares some homology with monomeric nucleotide polymerases and may contain the active site (4). Channel and groove have a size suitable to accommodate about 30bp of double stranded DNA, in good agreement with the DNase footprint of an elongation complex. This model is currently tested using a 3' polyC extended template labelled with an electron dense marker. The analysis of RNA polymerase-DNA co-crystals will help to define the path and the orientation of the DNA.

1-Darst, S.A., Kubalec, E.W. and Kornberg, R.D. (1989) *Nature*, **340**, 730-732.
2-Darst, S.A., Edwards, A.M., Kubalec, E.W. and Kornberg, R.D. (1991) *Cell*, **66**, 1-20.
3-Schultz, P. Célia, H., Riva, M., Sentenac, A. and Oudet, P. (1993) *EMBO J.* **12**, 2601-2607.
4-Moras, D. (1993) *Nature*, **364**, 572-573.

L 512 CHARACTERIZATION OF TRANSCRIPTION FACTOR IIIA FROM *SACCHAROMYCES CEREVISIAE*, Jacqueline Segall, Catherine Milne, Owen Rowland and Randall Willis, Departments of Biochemistry and Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada M5S 1A8

TFIIIA is an RNA polymerase III transcription factor that binds to the internal control region (ICR) of the 5S RNA gene as the first step in the assembly of a TFIIIB-TFIIIC-TFIIIA-DNA transcription complex. Yeast TFIIIA is structurally similar to the well characterized *Xenopus* TFIIIA in that both proteins contain nine zinc-fingers. A unique feature of yeast TFIIIA is, however, an 81-amino-acid domain which interrupts the repeating zinc-finger motifs between fingers eight and nine. Using yeast TFIIIA synthesized *in vitro*, we have shown that a truncated polypeptide containing only the three amino-terminal zinc-fingers retains the ability to bind to the ICR of the yeast 5S RNA gene and to recruit TFIIIC. Using bacterial-produced protein, we have found that the affinity of the amino-terminal three zinc-finger module of TFIIIA for the 5S RNA gene is only sightly less than that of wild-type TFIIIA. The TFIIIC-TFIIIA-DNA complex formed with this three zinc-finger module is, however, transcriptionally inactive. The 81-amino-acid domain of yeast TFIIIA appears to be essential for its transcription factor activity. We propose that both this domain and a surface of the amino-terminal zinc-fingers of TFIIIA are required to dock TFIIIC with the correct topography to position TFIIIB upstream of the 5S RNA gene. DNase I protection studies with truncated proteins have shown that TFIIIA is situated on the 5S RNA gene with its amino-terminal domain at the 3'-boundary of the ICR. The three amino-terminal zinc-fingers span the ICR and the carboxyl-terminal portion of TFIIIA lies upstream of the ICR. The region of the 5S RNA gene protected from DNase I by wild-type TFIIIA and TFIIIA lacking the carboxyl-terminal two zinc-fingers are indistinguishable, extending from +65 to +97. Similarly, there are only minor differences between the DNase I protection patterns generated by TFIIIA containing the amino-terminal four zinc-fingers and TFIIIA containing the amino-terminal three zinc-fingers. These truncated proteins provide protection from ~77 to 97. This suggests that not all zinc-fingers approach DNA in the same manner. Consistent with the *in vitro* properties of mutant TFIIIAs, *in vivo* studies using a plasmid shuffle system indicate that the carboxyl-terminal region of yeast TFIIIA, including the ninth zinc-finger, can be removed without affecting cell viability; TFIIIA lacking the first zinc-finger is, however, unable to support viability. (Supported by the Medical Research Council of Canada)

L 513 **REGULATION OF POL III TRANSCRIPTION DURING THE DIFFERENTIATION OF F9 CELLS, K.H. Seifart and W. Meißner, Institut für Molekularbiologie und Tumorforschung, Lahnstraße 3, 35037 Marburg, FRG.**

Mouse F9 embryonal carcinoma (EC) cells differentiate in culture to parietal endoderm (PE) cells, upon the induction with retinoic acid and cAMP. In the course of this process, the expression of pol III transcripts e.g. 5S rRNA, tRNA and U6 snRNA is dramatically reduced. This reduction of endogenous RNA content is accompanied by a loss of transcriptional capacity in cell free extracts from PE cells. Partial purification of such extracts reveals that the DNA binding activity of transcription factors TFIIIC and PBP are significantly reduced. This finding is supported by a loss in the transcriptional activity of these factors in reconstitution assays with partially purified pol III transcription factors. Moreover we could show, that the activity of TFIIA, also involved in the expression of pol III genes, is diminished in extracts from PE cells. In contrast, the activity of TFIIIA, TFIIIB and TBP remain unchanged during the differentiation process analysed here. These data show for the first time that the PSE binding protein PBP is essentially involved in the differential regulation of pol III genes governed by external promoters.

L 514 A SUBUNIT OF TFIIIC IS BOTH A POSITIVE AND A NEGATIVE REGULATOR OF RNA POLYMERASE III GENE TRANSCRIPTION, Indra Sethy and Ian Willis, Department of Biochemistry, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Ave, Bronx, NY 10461

Our laboratory has taken a genetic approach to study transcription by RNA polymerase III (pol III). Mutations affecting this process were isolated by selecting for extragenic suppressors of a mutation in the A box of a tRNA gene promoter. Transcription from this defective promoter is monitored by the expression of a cotranscribed downstream amber suppressor tRNA (*supS1*). So far we have reported the identification of two genes, *PCF1* and *PCF4*, in which dominant mutations activate transcription by this polymerase. *PCF1* encodes the 131 kD subunit of TFIIIC and *PCF4* encodes the 70 kD subunit of TFIIIB. We now present our work on the isolation and characterization of recessive mutations that increase pol III gene transcription.

Fourteen independent isolates containing recessive mutations have been obtained from the same mutagenesis that gave rise to the *PCF1-1* and *PCF4-1* dominant mutations. Complementation analysis shows that all of the recessive mutations are allelic and are located in the *PCF1* gene. A biochemical characterization of a strain carrying the *pcf1-3* allele has been initiated. *In vitro* studies which compare transcription in extracts prepared from wild-type and mutant strains demonstrate that the *pcf1-3* mutation causes a general increase in pol III gene transcription. Specific transcription activity in multiple round assays is elevated about 2-fold in mutant over wild-type extracts. This results from an increase in the number of transcriptionally competent preinitiation complexes formed in the mutant cell extract. The ability of both dominant and recessive mutations in the *PCF1* gene to increase pol III transcription suggests the protein is multifunctional. We hypothesize that different domains in the 131 kD subunit of TFIIIC are responsible for positive and negative regulation of transcription by this polymerase.

L 515 A NOVEL GENETIC SCREEN FOR MUTATIONS AFFECTING THE DNA-BINDING SPECIFICITY OF *XENOPUS* TRANSCRIPTION FACTOR IIIA, David R. Setzer* and Michael Bumbulis*#, Department of Molecular Biology and Microbiology* and Department of Genetics#, Case Western Reserve University School of Medicine, Cleveland, OH 44106

Transcription Factor IIIA (TFIIIA) is a zinc-finger protein that specifically binds to the internal control region (ICR) of 5S rRNA genes and is required for the synthesis of 5S rRNA by RNA polymerase III. Extensive biochemical analyses of TFIIIA and various site-directed mutant varieties of the protein have contributed to an improved understanding of its function in sequence-specific DNA recognition but have been limited by the necessity of using specific structural models to direct the design of mutant forms of the protein to be analyzed. Genetic screens of pools of randomly generated mutant proteins has not been feasible in *Xenopus*, where TFIIIA has been most extensively analyzed. To overcome this limitation, we have designed a system in which a TFIIIA-VP16 fusion protein is expressed in yeast and used to activate RNA polymerase II transcription from a yeast *cyc1* core promoter directing the synthesis of *E. coli* beta-galactosidase. We have demonstrated that the activity of the TFIIIA-VP16 fusion protein in this assay is dependent upon the proper positioning of a TFIIIA-binding site (the ICR of a *Xenopus* 5S rRNA gene) upstream of the core promoter and that mutations in the 5S ICR that reduce TFIIIA binding affinity in in vitro assays also reduce the ability of TFIIIA-VP16 to mediate elevated beta-galactosidase synthesis. The function of the *Xenopus* 5S ICR as an upstream activation sequence is also dependent on the expression of TFIIIA-VP16. We have used this system to screen for mutations in TFIIIA that reduce its binding affinity for the 5S ICR as well as to identify mutant forms of the protein that activate transcription from promoters containing mutant low-affinity TFIIIA-binding sites. These mutant proteins are good candidates for altered-specificity and/or high-affinity binding variants. The biochemical characterization of several of these mutant proteins will be described.

L 516 THE EXTRAGENIC PROMOTER ELEMENTS OF THE TRYPANOSOME U6 snRNA GENE COINCIDE WITH THE A AND B BOX OF A FUNCTIONAL tRNA GENE, Christian Tschudi, Valerian Nakaar and Elisabetta Ullu, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06510

Our understanding of transcriptional mechanisms in trypanosomes is extremely limited. To date only RNA polymerase I promoters have been characterized in detail. We have begun an analysis of RNA polymerase III-mediated transcription in Trypanosoma brucei and have uncovered a peculiar arrangement of tRNA genes and small RNA genes: three different genes (U6 snRNA, U-snRNA B, and 7SL RNA) have a divergently oriented tRNA gene in their 5'-flanking regions and the spacing between the tRNA and the small RNA gene (95 to 97 bp) is highly conserved in all three cases. Using transient DNA transfection of trypanosome cells, we have shown that deletion of the respective tRNA gene abolishes expression of the U6 snRNA, U-snRNA B, and 7SL RNA gene and point mutations in either the A box or the B box of the companion tRNA gene have the same effect. We have chosen the U6 snRNA gene and the upstream $tRNA^{thr}$ gene to further study the promoter arrangement at this locus. In addition to the intragenic A and B box of the $tRNA^{thr}$ gene, the U6 promoter consists of an element located close to the 5' end of the U6 coding region. This element conforms to the A box consensus sequence. Thus, it appears that two A boxes, divergently oriented and 120 bp apart, are involved in trypanosome U6 snRNA gene expression. By using two different strategies, namely the introduction of a synthetic intron in the tRNA gene and the creation of an amber suppressor tRNA, we have demonstrated that the $tRNA^{thr}$ gene is expressed in vivo. Moreover, mutations in the A or B box abolish expression of the $tRNA^{thr}$ gene. From this we conclude that the internal control region of this tRNA gene, and presumably of the other small RNA genes-associated tRNA genes, functions as a bidirectional promoter.

L 517 GENES FOR THE THIRD LARGEST SUBUNIT OF RNA POLYMERASE II AND ITS HOMOLOGS FROM RNA POLYMERASES I AND III FROM ARABIDOPSIS THALIANA. Tim Ulmasov and Tom Guilfoyle. University of Missouri-Columbia, 117 Schweitzer Hall, Columbia MO 65211.

Eukaryotic nuclear RNA polymerases are complex multisubunit enzymes consisting of about 10-12 different polypeptides. Knowledge of individual subunits structure is important for understanding of enzyme function and its protein-protein interactions. The genes for the first, second and fifth largest subunits of RNA polymerase II from Arabidopsis thaliana have been previously cloned in our lab. Here we report cloning of the genes for the third largest subunit and its homologs from RNA polymerases I and III. We have used affinity purified antibodies against 40 kD third largest subunit to isolate two closely related but distinct cDNAs and the genes for this subunit. The third largest subunit from RNA polymerase II is related in sequence to the subunit of similar size shared by RNA polymerases I and II in yeast and cross-reacts immunologically with corresponding subunits in plants. In order to prove that both genes encode for the RNA polymerase II subunit, we have cloned cDNAs encoding these polI/polIII subunits using expressed sequence tag from rice. Unlike the situation in yeast where both pol II and polI/polIII subunits are represented by single copy genes, both polII and polI/polIII genes for these subunits are duplicated in the Arabidopsis genome. This raises a question whether RNA polymerase I and RNA polymerase III share these subunits or employ closely related, but distinct proteins in Arabidopsis. Arabidopsis is the second organism after yeast from which these polI/polIII subunits genes have been cloned. In our antibody screening procedure for the 40 kD third largest subunit we have also isolated another cDNA encoding a protein homologous to the human TAT-binding protein (TAT-BP) and MSS1 gene product. These proteins are thought to regulate transcription elongation, and it is possible that our TAT-BP homolog from plants co-purifyies with RNA polymerase II (and co-migrates with the third largest subunit used for antibody production) and it also may be involved in some aspects of regulation of trnascription.

L 518 DNA-BINDING CHARACTERISTICS OF REGULATORY FACTORS FOR THE MOUSE rDNA TRANSCRIPTION

Kazuo Yamamoto, Yu-ichi Nishi, Yasuhisa Nogi and Masami Muramatsu, Department of Biochemistry, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-04, Japan

Eukaryotic ribosomal RNA is transcribed by RNA polymerase I and its production is regulated coordinately with cell growth and differentiation. An activator protein, UBF, which binds to the upstream control element of the rRNA promoter, was cloned previously. In addition, it has been demonstrated that a multi-complex factor SL1, which binds to the core promoter element of the rRNA gene, is essential for accurate transcription initiation and defines species-specificity. However, the molecular characteristics of SL1 is still unclear because of the small quantities in a cell and technical limitations for purification. Here, we improved an electrophoretic mobility shift assay system which is capable of detecting the protein complex specifically bound to the core promoter element. The addition of anti-TBP antibody in the binding reaction mixture resulted in the appearance of a super-shifted band, confirming that this complex includes TBP as an integral component. UV-cross linking experiment under the DNA-binding conditions suggest that 66 kDa protein confers direct DNA-binding activity. These assay systems will provide simpler and quicker detection suitable for identification of peak fractions containing SL1 during purification steps.

L 519 MULTIPLE OVERLAPPING FUNCTIONS IN TRANSCRIPTION OF A REGION OF THE RNA POLYMERASE III CATALYTIC SUBUNIT, Nick Zecherle, Salam Shaaban and Benjamin Hall, Genetics Dept. SK-50, University of Washington, Seattle WA 98195. We have intensively mutagenized the region of yeast *RET1* encoding amino acids 455-521. These correspond in position and sequence to *E.coli* β residues 512-582, spanning both major clusters of Rif^r mutations. To screen the library of *RET1* mutants for alterations in PolIII transcription at specific steps, we employed appropriate *in vivo* test templates: *SUP4UIV* and *SUP4Δ94* to identify, respectively, *RET1* alleles with decreased and increased termination ability; to assay *RET1* mutants affecting transcription initiation, we used two mutant *SUP4* alleles with altered initiation sequences. For each of these, mutant genes in the library gave increased function of the *SUP4* test gene, as seen by suppression of *ade2* and *lys2* ochre mutations. The mutant polymerases so identified were characterized in vitro as regards termination efficiency, elongation behavior and transcription initiation. Based upon criteria of mutational spectrum, degree of conservation and predicted protein secondary structure, we subdivide the region as follows: a) AAs 455-473 (predicted β sheet); b) AAs 474-486 (non-conserved hinge region); c)AAs 487-509 (highly conserved region) and d) AAs 510-521 (predicted α helix). Termination-altering mutations occur throughout the region, being in slight excess in region b) and in deficit in region a). Mutants in both regions a) and d) that have strongly increased termination show moderate decreases in elongation rate. Furthermore, allelic interactions occur between regions a) and d), suggesting that they are physically juxtaposed. Mutants in region b) are very strongly reduced in elongation rate, even at high [0.5mM] NTP concentration. Polymerases mutated at highly conserved residues in region c) have the ability to transcribe initiation-defective *SUP4* genes. The varied consequences of the closely linked mutations implicates this protein motif in transcript and/or DNA template interactions that are common to the initiation, elongation and termination steps in transcription.

Late Abstracts

CO-FACTORS INVOLVED IN REGULATION BY THE DROSOPHILA MORPHOGEN DORSAL, Josh Brickman, Norbert Leming and Mark Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

The Drosophilia morphogen Dorsal, a member of the rel protein family, has been shown to both activate and repress genes in the early embryo. Dorsal activity mediates repression of zerknüllt (zen) and activation of twist (twi). In yeast, Dorsal activates both genes. We have employed a yeast system to isolate proteins which might convert Dorsal into a repressor. Two of these proteins (R03 and R019) have been purified from E. coli and characterized further. RO19 is 55 Kd protein which belongs to the HMG1 family. In vivo, this protein represses Dorsal activity on the zen promoter and activate it from the twi promoter. In vitro, this protein binds to both the zen and twi promoters and isolated Dorsal binding sites. RO19 also binds to two sites within the minimal ventral repression (mVRE) element identified by Kirov et al (Nikolai Kirov et al, EMBO 12, 3193-3199, 1993). On this 55bp fragment containing a single Dorsal site RO19 binds cooperatively with various members of the rel family of DNA binding proteins. RO19 binds highly cooperatively with NF-kB heterodimers and p50 homodimers, but only weakly with p65 homodimers and not at all with the rel domain of Dorsal and the rel domain of p65.

RO3 is a 30 Kd protein without homology to any known family of DNA binding proteins. In vivo RO3 represses Dorsal activity from the Zen promoter and has no observable affect on Dorsal activity from the twi promoter. In vitro RO3 binds to multiple sites with in both promoters; competition experiments suggest that RO3 may bind these sites cooperatively. RO3 also binds to the mVRE and has a DNAse I footprint which overlaps those reported Kirov et al and Jiang et al (Jim Jiang et al, EMBO, 12, 3201-3208, 1993) for the factor which they refer to a AT1. An alternating pattern of protections and DNAse I hypersensitivity in the RO3 footprint suggest that it may bend DNA. RO3 also binds cooperatively with the NF-kB heterodimer on the 55 bp mVRE.

TATA-DEPENDENT ENHANCER STIMULATION OF PROMOTER ACTIVITY IS DEVELOPMENTALLY AQUIRED IN THE MOUSE, Sadhan Majumder and Melvin L. DePamphilis, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110, USA.

Herpes simplex virus (HSV) thymidine kinase (tk) promoter activity depends on four transcription factor binding sites, one of which is a TATA box element, and the presence of either a cis-acting enhancer sequence or a transactivator protein. Studies presented here show that the need for a TATA box element is a developmentally aquired property of the tk promoter that appeared only in differentiated cells and only when promoter activity was stimulated by either an enhancer or a transactivator. Early mouse embryos from the 1-cell to 8-cell stage in development as well as mouse embryonic stem cells did not require the TATA box element in order to activate the tk promoter either in the presence or absence of the embryo-responsive polyomavirus F101 enhancer. The TATA box element was required for activity only when the tk promoter was placed in differentiated cells and only when its activity was stimulated either by linking it to the F101 enhancer or by expression of its normal transactivator protein, HSV ICP4. Disruption of the TATA box by a linker scanning mutation did not reduce tk promoter activity in primary mouse embryonic fibroblasts (PMEF) or in immortalized 3T3 mouse fibroblasts; in fact, promoter activity was increased up to 2.6-fold. However, disruption of the TATA box prevented stimulation of the tk promoter by either the F101 enhancer or ICP4. The activity of the HSV tk promoter alone was also dependent on its TATA box in mouse oocytes, a terminally differentiated cell that contains its own transactivator. These results clarify the conditions under which a TATA box element is needed for promoter activity, provide an example of a basic transcriptional element that is developmentally acquired, and link TATA box function to enhancer function.

NEGATIVE AND POSITIVE COFACTORS INVOLVED IN REGULATION OF CLASS II GENE TRANSCRIPTION

Hui Ge and Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY10021

Regulation of transcription by RNA polymerase II in eukaryotic cells appears to require both basal and accessory factors which interact through specific protein-DNA or protein-protein interactions. During further characterization of negative and positive cofactors in the USA fraction, we have found that a negative component, which may relate to NC1, is identical to HMG1, a non-histone chromatin-associated protein. Purified human HMG1, either overexpressed in E.coli or from HeLa nuclear extracts, could interact with TBP in the presence of TATA box-containing oligonucleotide to form a specific HMG1-TBP-DNA complex. This complex prevents TFIIB binding to TBP directly and consequently blocks assembly of the preinitiation complex. In contrast, TFIIA can compete with HMG1 for binding to TBP. Using an in vitro reconstituted transcription system, HMG1 is able to inhibit transcription by RNA polymerase II over 30 fold. As expected, addition of TFIIA can partially reverse this repression in a concentration-dependent manner. Meanwhile, we have also highly purified (from USA) a positive activity which correlates with the presence of three tightly associated polypeptides. Although the functional involvement of two of these remains to be demonstrated, we show that one polypeptide, following expression in and purification from E.coli, can sufficiently support activator-dependent stimulation. The positive effect of this component on activated transcription can be accompanied by repression of basal (activator-independent) transcription, and thus mimics the properties of the original PC1 activity. A mutational analysis as well as the activator-specificity of this positive component will be discussed in detail.

SPECIFIC PROTEIN-PROTEIN INTERACTIONS OF TRANSCRIPTION FACTOR AP-2, Axel Imhof, Markus Moser, Armin Pscherer, Michael A. Tainsky* and Reinhard Büttner, Department of Pathology, University of Regensburg, 93053 Regensburg, Germany, * M.D. Anderson Cancer Center, Houston, Texas

Eukaryotic transcription factors are thought to mediate their transcriptional activation via specific protein-protein interactions. We have observed that overexpression of AP-2 in PA-1 teratocarcinoma cells leads to a strong inhibition of AP-2 transactivator function in CAT-assays. Furthermore the EMSA-pattern of nuclear extracts of PA-1 cells differs significantly as compared to the one that results from bacterially produced AP-2 protein. These two observations indicate that AP-2 may in vivo form a complex with other regulatory proteins. In order to characterize these AP-2 associated proteins we used two different approaches.

First we screened a 13.5 day old mouse embryo cDNA library with a full length AP-2 cDNA with low stringency in order to clone other AP-2 sequence-related genes which are important during mouse embryogenesis. The second approach was to purify AP-2 associated proteins with an affinity column using recombinant GST-AP-2 fusion protein as a matrix.

Resulting from these two approaches we cloned a new AP-2 related gene called AP2REL which differs from AP-2 mainly in its transactivation domain but is nearly identical in its DNA-binding and dimerization region. Bacterially expressed AP2REL protein has similar DNA-binding properties when compared to AP-2. We could also show that AP2REL heterodimerizes with AP-2 via binding of in vitro translated AP2REL to GST-AP-2 agarose. When using the affinity column to purify AP-2 associated proteins from ^{35}S-methionine labeled nuclear extracts, we demonstrated the specific interaction between AP-2 and a protein with an apparent molecular weight of 70 kDa.

ANALYSIS OF THE GLIAL CELL SPECIFICITY OF THE JC VIRUS CORE PROMOTER, G. Kumar*, C.J. Krebs, S. Chen and M. McAvoy, Department of Molecular Biology and Genetics, Center for Molecular Biology, Wayne State University, Detroit, MI 48201

The human papovavirus JC (JCV) causes a fatal demyelinating disease Progressive Multifocal Leukoencephalopathy (PML) in immunocompromised individuals. The glial cell specific transcription from the JCV promoter has been demonstrated to be due to the presence of both positive and negative regulatory elements upstream of the TATA region.

In order to investigate transcriptional regulation of JCV (Mad 11.3 variant), we cloned a series of JCV promoter deletion mutants adjacent to a CAT reporter gene and assayed their ability to support transcription in both C6 glial and HeLa non glial cells. These studies revealed that the JCV minimal core promoter possessing only the TATA box and a poly T region immediately upstream of the TATA box is sufficient to support transcription of an attached gene in glial cells.

Ectopic expression of a POU domain transcription factor Tst-1 in HeLa cells, due to the cotransfection of a Tst-1 expression vector, resulted in the activation of the JCV minimal core promoter. The examination of the binding of recombinant TBP and Tst-1 to the TATA box core promoter regions of both JCV and SV40 early promoter revealed that both Tst-1 and TBP can bind to both the JCV and the SV40 TATA region. However, Tst-1 can only transactivate the JCV, not SV40, core promoter in HeLa cells. We conclude that although Tst-1 can impart glial specificity to the JCV minimal core promoter, its binding to the TATA box region may be a necessary but not sufficient requirment for its function as a glial specific transcriptional activator.

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

CREB BINDING PROTEIN (CBP) ALSO BINDS TO TFIIB. Roland P. S. Kwok*, John C. Chrivia#, Stefan G.E. Roberts+, Michael R. Green+, and Richard H. Goodman*. *Vollum Institute, Oregon Health Sciences University, Portland, OR, 97201; #Department of Pharmacological and Physiological Science, St. Louis University School of Medicine, St. Louis, Missouri 63104; +Program in Molecular Medicine, University of Massachusetts Medical Center, Worcester, MA, 01605.

Phosphorylation of the CRE-binding protein CREB is required for activation of many genes by cAMP. Recently, we have identified a 265 kD nuclear protein, CBP, which binds specifically to phosphorylated CREB. We have suggested that CBP may act as a coactivator, bridging the CRE-CREB complex to general transcription factors. This model is supported by the finding that a GAL-CBP fusion protein activates expression of a GAL-CAT reporter in a PKA-dependent, but CREB-independent manner. To test our hypothesis further, we asked whether a 89 kD carboxy-terminal fragment of CBP, which we have also shown to be transcriptionally active when fused to a GAL4 DNA binding domain, interacts with TFIIB. This fragment of CBP includes a zinc finger motif which is highly homologous to a corresponding region in the putative yeast coactivator ADA2, a PKA phosphorylation site, and a glutamine-rich region. Full length TFIIB and TFIIB deletion mutants were fused to glutathione-S-transferase (GST). The GST-TFIIB fusion proteins were immobilized on glutathione-agarose beads and were incubated with in vitro translated , ^{35}S-labeled CBP fragment. After incubation and washing, the bound CBP was eluted, separated on a SDS-polyacrylamide gel, and visualized by autoradiography. The 89kD carboxy-terminal portion fragment of CBP, as well as a 28kD fragment which includes the zinc finger motif and PKA phosphorylation site, bound to the full length TFIIB protein. To determine which portions of TFIIB were responsible for the interaction, binding assays were repeated using TFIIB deletion mutants and the 28kD CBP fragment. Binding of the 28kD fragment of CBP to TFIIB was markedly reduced when the amino-terminus or the first of the two imperfect repeats at the carboxy-terminus of TFIIB was deleted. Binding was slightly reduced when the second imperfect repeat was deleted. These data support the model that CBP functions as a CREB coactivator.

ICSBP IS A CONDITIONAL REPRESSOR OF INTERFERON INDUCIBLE GENES, Ben-Zion Levi, Rakefet Sharf and Anat Weisz, Department of Food Engineering & Biotechnology, Technion, Haifa 32000, Israel.

Interferon consensus sequence binding protein, ICSBP, is a member of the interferon regulatory factor family of proteins which also include IRF1, IRF2 and ISGF3γ. ICSBP is expressed predominantly in cells of hematopoietic origin. Previously, we have demonstrated that ICSBP acts negatively on ICS-containing promoters. In addition, continuous treatment of cells with either IFN-β or IFN-γ is found to alleviate the negative effect of ICSBP.

In this presentation, we demonstrate that priming of different cell lines with either type I or type II interferons is sufficient to alleviate the repression effect of ICSBP on IFN-inducible promoters. Moreover, combined pretreatment of cells with both interferon and the synthetic dsRNA poly(I)·Poly(C), has a strong synergistic effect resulting in abrogation of the repression activity of ICSBP. The possible involvement of other interferon regulatory factors in this signaling pathway will be presented.

Analysis of the modular structure of ICSBP revealed that it is a "true" repressor. Swapping part of the DNA binding domain of ICSBP with that of the yeast transcription factor GAL4 resulted in the repression of promoters that contain GAL4 binding sites. In addition, this chimeric construct is capable of blocking the strong enhancement of such promoters by the GAL4-VP16 fusion factor. The results of the reciprocal experiments in which the repression domain of ICSBP is swapped with the activation domain of VP16 demonstrate that ICS-VP16 fusion factor is a strong activator of interferon inducible promoters. Thus, the repression activity of ICSBP can be separated from its DNA binding activity.

CHARACTERIZATION OF COLIPHAGE N4 SINGLE-STRANDED DNA BINDING PROTEIN: THE ACTIVATOR OF E. COLI RNA POLYMERASE AT N4 LATE PROMOTERS, Alita Miller[1], Mie Young Choi[2], and Lucia B. Rothman-Denes[2], Departments of [1]Biochemistry and Molecular Biology and [2]Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois, 60637

The 32 kDa single-stranded DNA binding protein (SSB) of coliphage N4, a product of middle transcription of the phage genome, was characterized as a phage gene product required for replication (1). N4 SSB is also essential for phage recombination. Surprisingly, it is also essential for late transcription of the phage genome which is carried out by E. coli σ^{70} RNA polymerase. The gene for this protein has recently been cloned. When produced from a T7 gene expression system, the cloned protein complements a N4 amber mutant for all three activites: recombination, replication, and late transcription. Initial functional analysis of N4 SSB using both truncated and internally deleted mutants indicated that the carboxy-terminal seven amino acids are essential for N4 SSB function in all three in vivo activites. In vitro analysis of these mutants revealed that the ability of the protein to bind to single-stranded DNA resides in the amino-terminal two-thirds of the protein. Extensive point mutational analysis of the C-terminal heptapeptide shows that separation of function can be achieved by changing some residues within this region. Two mutants that have wildtype ability to activate both recombination and replication, but are unable to stimulate late transcription in vivo have been identified and purified. Initial in vitro analysis suggests that these mutants have been altered in their ability to carry out protein-protein interactions. Characterization by deletion and substitution mutagenesis of the N-terminal portion of the molecule is in progress to assess the role of ssDNA binding in the activation of E. coli RNA polymerase at N4 late promoters.

(1) Lindberg, G. et. al. (1989) J. Biol. Chem. 264, 12700-12708.

ANALYSIS OF TRANSCRIPTIONAL INITIATION OF THE *S. POMBE* RIBOSOMAL RNA GENES, Louise Pape, Ling Chen, Zhao Liu and Zhihong Zhao, Department of Chemistry, New York University, New York, NY 10003

We are analyzing requirements for synthesis of the ~37S ribosomal RNA genes in *Schizosaccharomyces pombe*. To this end, we have established the first *pombe* transcription extract capable of supporting accurate initiation on an rDNA mini-gene in vitro. Transcription supported by the *pombe* rDNA promoter in vitro is sensitive to the action of a vertebrate rDNA enhancer which interacts with the nucleolar factor UBF (upstream binding factor).

Activation of the rDNA promoter in this S-100 extract is also responsive to regulatory elements in the *pombe* intergenic spacer (from ~-1800 to ~-850) that apparently act by stably binding RNA polymerase I transcriptional factor(s). This region of the spacer rDNA may function analogously to the vertebrate rDNA enhancer elements, known to contain binding sites for the nucleolar transcription factor UBF; this intergenic rDNA region stimulates transcription of a downstream *pombe* rDNA promoter in vitro in an orientation-independent manner. We are investigating recognition and interactions of a *pombe* UBF-like activity with these intergenic sequences and with the rDNA gene promoter. In addition, our analysis of *pombe* UBF activity includes isolation of *pombe* sequences homologous to the vertebrate UBF HMG box regions, partial purification of a *pombe* UBF-like activity, and characterization of *pombe* UBF-like activity. (Supported by NSF MCB-9219220 and in part by NYU Research Challenge Fund Grants.)

TAT OVERCOMES A NOVEL LBP-1 RESTRICTION OF HIV-1 TRANSCRIPTION AT THE LEVEL OF ELONGATION IN VITRO, Camilo A. Parada, Jong-Bok Yoon and Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021

The HIV-1 promoter is strongly activated by the HIV-1-encoded Tat protein. Although Tat overcomes a restriction of HIV-1 transcription at the level of elongation the mechanism of this transcriptional control is still unknown. A DNA element, Inducer of Short Transcripts, has been shown by others to specify non-processive transcription of short RNAs from the HIV-1 promoter. Since the high affinity LBP-1 binding sites on the HIV-1 promoter (-4 to +18) overlaps the IST element, we tested whether recombinant LBP-1 could effect the processivity of RNA polymerase. We used a "pulse-chase" assay that involves the isolation of a preformed preinitiation complex (PIC) on the HIV-1 promoter by gel filtration. LBP-1 had no effect either on initiation from preformed PICs or on elongation to position +13 (pulse). However, addition of LBP-1 just after stalling of RNA polymerase at +13 strongly inhibited elongation (chase) by reducing processivity of the RNA polymerase. Thus, LBP-1 has a novel activity, restricting the elongation of HIV-1 transcripts.

In a further investigation of the contribution of the high affinity LBP-1 binding sites (located between -4 to +18) to the block of HIV-1 elongation by LBP-1, using the "pulse-chase" assay, severe mutations in the high affinity LBP-1 binding sites were found not to relieve the LBP-1 block. This suggested that binding of LBP-1 to these sites might not be the sole mechanism by which LBP-1 blocks HIV-1 elongation. Using gel retardation and footprinting analyses, we found that LBP-1 can bind independently to the low affinity LBP-1 recognition sites under appropiate conditions. Taken together, these results argue for a model in which LBP-1, by binding to low and/or high affinity recognition sites, renders the elongation complex incompetent for its processivity. In this model LBP-1 might interact with RNA polymerase in a fashion analogous to the lambda Q protein interaction with E. coli RNA polymerase on the late lambda promoter. However, while in the latter case lambda Q enhances the processivity of E. coli RNA polymerase, LBP-1 decreases the processivity of RNA polymerase II.

Given our prior development of a cell-free transcription system that mediates Tat activation of the HIV-1 promoter, a possible relationship between LBP-1 restricted elongation and the action of Tat in enhancing transcription elongation was investigated. Under conditions where LBP-1 restricts HIV-1 transcription at the level of elongation, the presence of Tat was found to supresses the LBP-1 effect and to restore the inhibited level of HIV-1 transcription.

These findings strongly suggest that LBP-1 may provide a natural mechanism for restricting HIV-1 transcription at the level of elongation and that this may be a target for the action of Tat in enhancing transcription.

A NEW MOUSE FACTOR THAT INTERACTS WITH THE POLYOMA VIRUS LATE PROMOTER INITIATOR REGION, Lisa M. Rapp and Gordon G. Carmichael, Department of Microbiology, University of Connecticut Health Center, Farmington, Connecticut 06030

We have been interested in characterizing the *cis* elements and *trans* factors necessary for transcription from the polyoma virus late promoter. Previous analysis defined a 41bp minimal promoter fragment spanning the major late startsite in our system that consists of an initiator element (Inr). This minimal promoter contains no TATA box, is not GC-rich as are Inrs of the housekeeping type, and does not require upstream sites for the known polyoma nuclear factors for activity. Mobility shift assays and DNAse footprinting analysis demonstrated that extracts from NIH 3T3 cells contain a factor or factors that interact specifically with sequences flanking the major startsite of this promoter. UV crosslinking studies revealed two proteins, ~ 93kDa and ~106kDa, which bind the minimal promoter. To isolate the factors that interact with the minimal promoter, we screened an NIH 3T3 cell lambda ZAP II cDNA expression library with an oligonucleotide containing tandem repeats of the minimal promoter fragment. Two clones that demonstrated strong binding activity have been isolated. One of these clones has been sequenced and is 2480 bp in length, encoding a polypeptide with a calculated molecular weight of 86kD. The polypeptide has 13 zinc finger motifs conforming to the consensus ($CX_2CX_3FX_5LX_2HX_3H$). These zinc finger domains are separated by the characteristic H/C links of Krüppel-related proteins (consensus $HTGEKP(Y/F)XC$). The entire sequence was cloned into the His-tag expression vector pRSETC, creating a fusion protein under the control of a lac inducible promoter which can be purified on a nickel chelating column. Protein of the correct size was induced, and bound to a specific DNA probe in Southwestern analysis.

A NOVEL MULTISUBUNIT COMPLEX REQUIRED FOR ACTIVATED TRANSCRIPTION IN YEAST: IDENTIFICATION OF YEAST TAF_{II}s, Joseph C. Reese and Michael R. Green, Program in Molecular Medicine, University of Massachusetts Medical Center, Worcester MA 01605

In higher eukaryotes the RNA polymerase II transcription factor TFIID is composed of a TATA-box-binding protein (TBP) and a set of tightly bound polypeptides, designated TBP associated factors (TAFs). One or more TAFs are coactivators that are required to achieve activated transcription, but do not affect an activator-independent basal transcription reaction. The eukaryotic transcription machinery is highly conserved and it is therefore puzzling that yeast TAFs with coactivator activity have not been identified. Here we use TBP as a protein-affinity ligand to isolate a yeast multisubunit complex that is specifically required for activated transcription by RNA polymerase II. The 145kD subunit of this complex binds TBP directly. Microsequence analysis reveal that the 90kD subunit of this complex is the probable homologue of a known Drosophila TAF, $dTAF_{II}80$. These and other data indicate that this multisubunit complex contains yeast TAF_{II}s.