REGULATION OF TRANSCRIPTION ELONGATION AND TERMINATION

Organizers: Ronald Reeder and Caroline Kane

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Plenary Sessions Page

Poster Sessions

Regulation of Transcription Elongation and Termination

Biochemistry of Elongation

U 001 IDENTIFYING TRANSCRIPTION FACTOR INTERACTION SITES ON RNA POLYMERASE II, Arno L. Greenleaf and Andrea Skantar, Department of Biochemistry, Duke University Medical Center, Durham, NC 27710. We have begun attempts to identify sites on Pol II which interact with basal transcription factors, using the following "domain interference assay." We constructed a fusion between *E. coli lacZ* and *Drosophila* DNA encoding the C-terminal two-thirds of the second largest subunit of Pol II (*RpII140*), and we expressed the β-galactosidase/RpII140 fusion protein in *E. coli*. After solubilizing, renaturing and purifying the fusion protein, we tested its ability to inhibit accurate initiation in Kc cell nuclear extracts. At concentrations in excess of the endogenous Pol II, the fusion protein did inhibit specific transcription, whereas control β-galactosidase did not. These results suggested that a domain or domains in the RpII140 portion of the fusion protein might be sequestering a transcription factor(s), making it unavailable for productive interaction with intact Pol II. Since we had previously shown that one of the basal transcription factors, *Drosophila* factor 5 (F5), binds to purified Pol II and in addition can be detected by a relatively simple assay, we tested the possibility that the fusion protein was interfering with the action of F5.

We showed previously that *Drosophila* F5 (probably the counterpart of mammalian factor TFIIF), which is essential for accurate initiation *in vitro*, also stimulates the elongation rate of purified Pol II transcribing 3'-extended DNA templates (dC-tailed templates), apparently by decreasing the time polymerase spends at numerous pause sites [1]. In addition, purified F5 binds tightly to free Pol II but not to the elongating enzyme [1]. We now find that the above fusion protein inhibits the F5-dependent stimulation of elongation rate in the dC-tailed template assay, whereas it does not inhibit polymerase II alone. These results suggest that a domain contained in the C-terminal two-thirds of RpII140 interacts specifically with F5. We have prepared fusion proteins containing subfragments of this RpII140 region and are testing them for their ability to interfere with F5 stimulation of Pol II elongation rate. We hope ultimately to define as precisely as possible the F5 interaction site and to use that information as a basis for biochemical and genetic investigations into transcription factor•RNA polymerase II interactions.

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U 002 THE TRANSITION FROM INITIATION TO ELONGATION BY RNA POLYMERASE II Donal S. Luse, Dept. of Molecular Genetics, Biochemistry and Microbiology, Univ. of Cincinnati Col. of Medicine, Cincinnati, OH 45267-0524

We have shown that accurate initiation of productive RNA synthesis at the Ad 2 ML promoter is accompanied by abortive initiation of very short transcripts (1). Pausing after the synthesis of a single bond inevitably leads to abortive initiation; intact ternary complexes can be demonstrated after the synthesis of 2-7 bonds but these complexes are labile (2). At least 10 bonds must be made before the ternary complexes are sufficiently stable to allow purification in active form (2,3). We have recently extended these approaches in order to address the role of promoter clearance and early chain elongation in promoter function. We first asked how the proportion of initiations which are abortive varies with promoter strength. For this purpose we assembled a set of mutated Ad 2 ML promoters. These 8 promoters varied over a 40-fold range in their ability to direct productive RNA synthesis. The 6 strongest members of the set were found to have the same ratio of abortive to productive transcription, but for the two weakest promoters this ratio was reduced by 3-4 fold. Interestingly, these latter two promoters share a particular mutation (TATA to TATC) within the TATA box. We have also investigated the possibility that changes in transcription complex structure and properties might continue to occur as RNA synthesis proceeds past the point of elongation commitment, at about position +10. We prepared and purified two ternary complexes initiated at the Ad 2 ML promoter. These complexes, which were uniformly paused at either +15 or +35, remained fully active in chain elongation. Surprisingly, the +15 and +35 complexes do not have equivalent structures or properties- the +15 complex has a much larger DNase I footprint and is considerably more stable than the +35 complex. We have recently begun to explore promoter clearance at other RNA polymerase II promoters, including those which lack a TATA box. Current findings from these studies will be presented.

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Termination by RNA Polymerase I

U 003 MOLECULAR MECHANISMS OF MAMMALIAN RNA POLYMERASE I TRANSCRIPTION TERMINATION

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In earlier studies, we identified a sequence element AGGTCGACCAGT/AA/TNTCCG (the "Sal box") which mediates termination of mouse and rat rDNA transcription. This terminator sequence is repeated several times in the 3´ terminal spacer and is also present upstream of the rDNA promoter. The "Sal box" is specifically recognized by a nuclear factor (TTF I) which catalyzes both the stop of the elongation reaction of RNA polymerase I (pol I) and the release of the nascent transcripts. This factor represents a heterogenous population of proteins with apparent molecular weights of 100, 90, 80 and 65 kDa which form four specific DNA-protein complexes detectable in mobility shift assays. We show that the individual proteins contact the same bases within the "Sal box" target sequence and that TTF I binding induces bending of the DNA, suggesting that conformational alterations of the DNA may play a role in the termination process.

The human pol I termination signal is shorter encompassing only the proximal part of the mouse sequence (GGGTCGACCAG). A comparison of the physicochemical properties of the mouse and human factor indicates that changes in terminator sequences have been accompanied by compensatory changes in the DNA binding domain of the corresponding trans-acting factors. In order to investigate the molecular mechanisms which specify transcription termination an assay was developed which allows promoter-independent transcription initiation by any RNA polymerase. Using the tailed template assay we show that rDNA transcription termination is not brought about *via* a steric hindrance mechanism but by a very specific interaction between the murine termination factor and pol I. Heterologous polymerases (RNA polymerase II and III, E. coli or bacteriophage T7 RNA polymerase) bypass the complex consisting of TTF I and the "Sal box" termination signal. Interestingly, highly purified pol I from yeast stops at the murine termination signal in the presence of TTF I. The results demonstrate that (I) TTF I is both necessary and sufficient for termination of rDNA transcription, and (II) TTF I appears to interact specifically with a defined functional domain of the RNA polymerase I molecule, which has been conserved during evolution of pol I and which is not present in other RNA polymerases.

U 004 TERMINATION OF TRANSCRIPTION BY RNA POLYMERASE I IN XENOPUS LAEVIS, Ronald H. Reeder, Paul Labhart, and Brian McStay, Division of Basic Sciences, Hutchinson Cancer Research Center, Seattle, WA 98104

Termination of RNA polymerase I is directed by the DNA sequence GACTTGCNC. This sequence is located about 200bp upstream of the initiation site for ribosomal RNA precursor transcription (Site T3) where it appears to serve a dual function. T3 terminates any polymerase coming from the intergenic spacer and thus protects the adjacent gene promoter from occlusion. T3 also directly interacts with the adjacent gene promoter to stimulate transcription initiation. A related sequence, differing by one bp (GACTTGCNG) is located at Site T2 at the 3'end of the precursor coding region. This C to G change allows RNA 3'end formation to continue efficiently at T2 but abolishes its ability to cause polymerase release. Therefore, most of the polymerase reaching T2 continues on past into the intergenic spacer (and presumably terminates at T3). In a related species, X. borealis, there are two terminator elements at the T2 location, both of them with the canonical T3 sequence and both function to cause polymerase release. Thus, X. laevis T2 appears to be an allowable mutation.

S-100 extracts of X. laevis kidney cells contain a protein activity (Rib2) which footprints on T3 (but not on T2) and which requires the GACTTGCNC element for binding. Removal of Rib2 from extracts eliminates termination and adding it back restores termination. Elimination of the footprint (by competition with a specific oligonucleotide) destroys termination at the same rate as it eliminates the footprint. We conclude that termination of transcription by RNA polymerase I requires the stoichiometric binding of a site-specific protein (Rib2) to the GACTTGCNC sequence. Oligonucleotide competition experiments suggest further that binding of Rib2 is also required for T3 to positively interact with the adjacent gene promoter.

Termination by RNA Polymerase III, Organellar and Viral

U 005 TERMINATION OF RNA POLYMERASE III TRANSCRIPTION AND THE ROLE OF THE LA PROTEIN,
Nathalie Lin, Francoise Stutz, Daniel Scherly, Stuart G. Clarkson, Département de Microbiologie, Centre Médical Universitaire, 9 avenue de Champel, CH-1211 Genève 4, Switzerland.

Termination of transcription by RNA polymerase III (Pol III) usually occurs at a site corresponding to a cluster of T residues in the non-coding DNA strand. For *Xenopus* 5S RNA genes, an efficient site comprises 4 or more T residues surrounded by GC-rich DNA (1). DNA with these sequence features is found behind several *Xenopus* tRNA genes but in some of these cases termination efficiency at the T cluster is influenced by the surrounding nucleotides in a way that is exactly contrary to the simple "5S rules" (2). Although purified Pol III appears to be able to stop synthesis at T clusters (3), transcript release from certain sites may require additional factors.

A candidate for such a factor is a 48 kd autoantigen called the La protein that transiently binds to the 3'-U residues of newly synthesized Pol III transcripts. Indeed, it has been proposed that La is needed both to complete the synthesis of nascent Pol III transcripts and to cause their release (4,5). To investigate this further, we have cloned a full-length La cDNA from *X.laevis* into a bacterial expression vector, raised rabbit polyclonal antibodies against the cloned frog protein, and used the antibodies to progressively remove the La protein from S-100 extracts of cultured *X.laevis* kidney cells. Consistent with the results of Gottlieb and Steitz (4,5), such immunodepleted extracts generate a reduced number of nascent Pol III transcripts, many of which are shorter than usual, and addition of the bacterial-made La protein partially restores the number of normal length transcripts. But these changes in transcript number and length can be dissociated from Pol III activity because identical results are obtained when RNA generated in a prior reaction with T7 RNA polymerase is incubated in La depleted extracts. Hence, it appears that one function of La is to protect nascent Pol III transcripts from nucleolytic degradation.

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U 006 TRANSCRIPT TERMINATION AND PROCESSING IN MAMMALIAN MITOCHONDRIA,
David A. Clayton, Jeffrey L. Bennett, John F. Hess, Robert Karwan and Mark E. Schmitt, Department of Developmental Biology, Beckman Center, Stanford University School of Medicine, Stanford, CA 94305-5427.

Transcription of the heavy strand of mitochondrial DNA in mammalian cells terminates at the 3' end of the 16S rRNA gene and gives a 50-100 fold excess of promoter-proximal gene transcripts over promoter-distal transcripts. Previous work from this laboratory described an *in vitro* transcription system that faithfully reproduces the *in vivo* termination event. Use of this system permitted mapping the DNA sequences necessary and sufficient for termination and implied that transcription and termination were separate processes and that a DNA-binding protein was required for the termination process. Further experiments using mobility shift assays of DNA binding indicate that a specific protein binds to the DNA sequences essential for termination. The requirement of termination factor binding for termination was shown using *in vitro* transcription reactions programmed with RNA polymerase separated from mitochondrial termination factor. Identification of the mitochondrial termination factor polypeptide from partially purified fractions was demonstrated by several independent methods.

In addition, mammalian mitochondria contain a site-specific endoribonuclease, RNase MRP, that cleaves RNA involved in priming DNA replication. It is present in an active form as isolated from the nucleus, suggesting that RNase MRP is bipartite in cellular location and function. The relatively high abundance of nucleus-localized RNase MRP has permitted its purification to near homogeneity and in turn led to the identification of protein components of this ribonucleoprotein; the major species in a 40-kDa protein recognized as an autoantigen. Substrate cleavage is strictly dependent upon the presence of a previously described G-rich sequence element adjacent to the primary site of RNA processing. Additional, downstream cleavages can occur that are dependent on the integrity of the G-rich element.

U 007 INITIATION AND TERMINATION OF TRANSCRIPTION BY BACTERIOPHAGE RNA POLYMERASES, William T. McAllister, Youwen Zhou, Lynn Macdonald, Curtis Raskin, George Diaz, Ellen Jorgensen, Department of Microbiology and Immunology, SUNY-Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, New York 11203-2098

Due to their structural simplicity and high template specificity the single subunit RNA polymerases that are encoded by bacteriophage T7 and its relatives are particularly well suited for studies of polymerase structure and function. The specificity of the phage T3 and T7 promoters is determined largely by a single basepair at position -11 in the highly conserved 23 basepair consensus sequence. Methylation-interference experiments demonstrate that multiple specific contacts are made between the polymerase and residues in the major groove in this region of the promotor. Experiments involving synthetic heteroduplex promoters suggest that it is primarily the base on the non-template strand that provides the discriminatory contact. The construction of hybrid T3/T7 polymerases and site-directed mutagenesis of the polymerase gene have revealed that a single amino acid at position 748 is responsible for discriminating this base. A molecular model involving hydrogen bonding between amino acid side chains and the base are being tested.

With regard to termination, the efficiency of termination by the phage polymerase at a number of terminators has been assessed. The terminators studied include: the T7 late terminator (Tϕ), the lambda 6S terminator, the trpE terminator, and a weak terminator in PBR322 (P4). The efficiencies of termination at these terminators range from 25 to 65% but do not depend upon the predicted stability of the hairpin loop structure that could be formed in the RNA product. Previous results indicated that proteolytic nicking of the polymerase near the amino terminus resulted in a less processive enzyme; the termination properties of such nicked enzymes are being explored. The T7 RNA polymerase has been observed to terminate at extended runs of A in the template strand (A_{20}, A_{40}) especially under conditions of limiting UTP. Under certain conditions the polymerase appears to stutter at A-tracts, and to move both forward and backwards along the template strand of the DNA, resulting in the synthesis of run-off products that are both shorter and longer than expected. Genetic methods to select for polymerase mutants that are defective in termination are being developed.

Regulation of Termination by Bacterial Polymerases

U 008 INTERACTIONS BETWEEN TERMINATION FACTOR RHO AND RNA, John P. Richardson, James Graham, Asuncion Martinez, Timothy Opperman, Lislott Richardson and Lanling Zou, Department of Chemistry, Indiana University, Bloomington, IN 47405

The process of transcription termination that is mediated by rho factor in Escherichia coli is initiated by binding of the factor to the nascent RNA and is driven by the hydrolysis of NTPs. Productive binding is dependent upon special segments in the RNA encoded by the rut sequence in DNA that serve either as a factor entry site or as a primary anchor site for the subsequent interactions. The role of these segments and of particular cytidylate residues in the termination process will be analyzed using RNA binding and ATP hydrolysis activation measurements with variants of λ cro RNA, the transcript terminated by rho action at the λ tR_1 terminator. A further identification of important residues in the various domains of the rho polypeptides will be made from the results of mutational changes and of phylogenetic comparisons of rho genes from other Gram negative bacteria. The exceptional activation of rho action by 0.15 M potassium glutamate will be shown to be a consequence of the balance of two features of the glutamate ion, its ability to buffer the concentration of free magnesium ions and its relatively weak binding as a counterion to positively charged groups in the RNA binding site of rho protein.

U 009 DNA BINDING PROTEINS AS REGULATORS OF ELONGATION IN *E. COLI*, Deborah A. Steege and Pamela A. Pavco, Department of Biochemistry, Duke University Medical Center, Durham, NC 27710

Present evidence indicates that the response of elongating RNA polymerase to a protein bound in its path ranges from transient or partial displacement of the protein to a complete block that leads to termination. As a means of addressing this issue and determining the properties of elongation complexes, we have used *Eco*RI endonuclease mutants to block RNA polymerase upstream of the *Eco*RI recognition sequence. The Gln-111 endonuclease mutant, as a result of a single amino acid substitution at position 111, lacks appreciable cleavage function yet shows increased binding to the wild-type recognition sequence GAATTC (1). Its presence on linear and circular DNA templates being transcribed *in vitro* by *E. coli* RNA polymerase (2) or by the single-subunit enzymes specified by phages T7 and SP6 results in blockage of the ternary complex a constant distance upstream of the GAATTC. The 3' end of the nascent, blocked-length RNA in the *E. coli* ternary complex is 14 bp from the GAATTC, only ~7 bp upstream from the boundary defined for Gln-111 protein by exonuclease III footprinting. The corresponding RNA 3' end for T7 RNA polymerase is just 3 bp upstream of the GAATTC, encroaching upon the protected region defined for bound Gln-111 protein. This RNA 3' end is the same for each of a series of templates containing from 35-58 bp of DNA between the startpoint for T7 transcription and the GAATTC. *E. coli* ternary complexes are blocked efficiently and remain stable over time in an active form. They are fully capable of either resuming elongation once the blocking protein is displaced from the template by an increase in ionic strength, or interacting with rho and undergoing release. The phage polymerases, by contrast, show significant levels of read-through transcription beyond the protein block. Experiments further probing the interactions of T7 RNA polymerase and Gln-111 protein with the DNA template will be discussed. Overall, the results argue that these examples of polymerase blockage by template-bound protein reflect a simple roadblock mechanism. They also suggest that in the ternary complex, the sites for DNA unwinding and nucleotide addition lie at or near the leading edge of the polymerase.

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U 010 MULTIPLE CONTROL MECHANISMS FOR PYRIMIDINE-MEDIATED REGULATION OF *pyrBI* OPERON EXPRESSION IN *Escherichia coli*, Charles L. Turnbough, Jr., Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294.

The *pyrBI* operon of *Escherichia coli* encodes the subunits of the pyrimidine biosynthetic enzyme aspartate transcarbamylase. Expression of this operon is regulated over an approximately 300-fold range by pyrimidine availability primarily (~50-fold) through UTP-sensitive attenuation control. Characterization of numerous mutations that alter the rate or extent of transcription and translation within the *pyrBI* leader region provide strong support for a control mechanism in which the relative rates of transcription and translation in this region regulate transcriptional termination at a Rho-independent attenuator preceding the *pyrB* gene. In this mechanism, tight coupling of leader transcription and translation occurs due to strong transcriptional pausing at several uridine-rich sites when intracellular levels of UTP are low. The coupled ribosome, which is engaged in the synthesis of a 44-amino acid leader polypeptide, physically prevents the formation of the attenuator-encoded RNA hairpin necessary for transcriptional termination, resulting in readthrough transcription into the structural genes. When UTP levels are high, leader transcription and translation are uncoupled and transcription is efficiently (~98%) terminated at the attenuator. Additionally, strains containing a deletion inactivating the *pyrBI* attenuator were used to show that operon expression is regulated over a 6- to 7-fold range by pyrimidine-mediated, attenuation-independent control. Characterization of *pyrBI* transcriptional initiation *in vitro* and of a mutation that alters a run of 3 uridine residues near the 5' end of the *pyrBI* mRNA indicate that a large part of attenuation-independent control is due to a mechanism in which, in the presence of high UTP, reiterative transcription occurs just after the start of *pyrBI* transcript synthesis. This reiterative synthesis results in transcripts containing very long runs of uridines which are not extended into the *pyrBI* structural genes.

U 011 MECHANISTIC STUDIES OF TRANSCRIPT ELONGATION AND TERMINATION IN E. COLI, Peter H. von Hippel, Thomas D. Yager, and Stanley C. Gill, Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, OR 97403. Recent biophysical studies, dealing with the interactions of the transcriptional regulatory proteins, sigma^{70} and NusA, with core RNA polymerase in solution and within the transcription complex, will be reviewed. These interactions are used to probe the conformational states of the core RNA polymerase at various stages of the transcription cycle, and the results permit us to construct a partial free energy diagram for the overall process. As a consequence we can begin to define the magnitudes of the interactions between the protein and nucleic acid components of the transcription complex that may be important in regulation. Recent structural, thermodynamic, and kinetic approaches to the determination of the positions and efficiencies of intrinsic termination sequences will also be described, and ways of extending these approaches to permit a quantitative description of some aspects of factor-dependent termination (and anti-termination) will be considered. We will show that this overall framework can be used to rationalize physiological aspects of the transcription cycle and its regulation, as well as to suggest experiments to establish molecular mechanisms at points at which our knowledge is still incomplete. [Supported by USPHS Grants GM-15792 and GM-29158.]

Termination by RNA Polymerase II Downstream of the Processing Site

U 012 **MULTIPLE FUNCTIONS OF THE 3' END OF HISTONE mRNA: REQUIREMENTS FOR TRANSCRIPTION TERMINATION AND TRANSPORT OF mRNA TO THE CYTOPLASM.**

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Histone genes are the only class of genes encoding mRNAs which lack introns and which do not code for polyadenylated mRNAs. Two mouse histone genes, an H2a and H3 gene are located on chromosome 3 in the same orientation 800 nts apart. We have identified a potential transcription termination site between these two genes and have studied the role of the 3' processing signal in transcription termination and eventual function of the mRNA. Several lines of evidence suggest that there is a transcription termination site in this region and that it requires an intact processing site to function. 1. a non-polyadenylated transcript which has a defined end is present in nuclear RNA 2. measurement of transcription in isolated nuclei shows that transcription extends to the region of the end of this transcript but not beyond. 3. deletion of either portion of the 3' processing site results in transcripts which read-through into the adjacent histone H3 gene; 4. a 150 nt sequence containing the termination site can block usage of a cryptic 3' polyadenylation site only if it is preceded by an intact histone processing signal. These results strongly suggest that transcription termination of the histone H2a gene, like that of genes encoding polyadenylated RNAs, requires that the polymerase pass an intact 3' processing signal before it responds to a termination site. Another role of the histone 3' end is in directing transcripts to the cytoplasm after processing. Transcripts from which the histone 3' processing signal has been deleted end heterogeneously and are retained in the nucleus. Replacing the histone 3' end with a polyadenylation signal results in cytoplasmic localization of the transcripts suggesting that the histone 3' end and polyA tail are functionally similar. To express histone RNA with defined 3' ends which are not the normal histone 3' end, UHU genes were constructed which have a U1 snRNA promoter, complete histone coding region and U1 snRNA 3' end. Defined transcripts are formed from these genes which end at the U1 3' end. Although these transcripts contained the complete histone mRNA sequence and only 9 nucleotides of U1 snRNA sequence at the 3' end, they were not found on polysomes, but were primarily in the nuclear fraction. Placing a histone 3' end downstream of the U1 3' end to give UHUH genes, resulted in two types of transcripts. The transcripts ending at the U1 3' end were found predominantly in the nucleus and were not present in polyribosomes, while the longer transcripts ending at the histone 3' end were all present in the cytoplasm on polyribosomes. These results demonstrate that another function of the histone 3' end is to direct transcripts to the cytoplasm and to promote their association with polyribosomes. A protein which specifically binds the terminal stem-loop sequence and which may play a role in transport of histone mRNA from the nucleus to the cytoplasm has been identified by mobility-shift analysis and UV-crosslinking. This protein is a component of the histone mRNP and may play a role in transport of histone mRNA to the cytoplasm.

U 013 DIFFERENTIAL 3' END FORMATION OF YEAST MESSENGER RNAS IN VITRO. Terry Platt, Parag P. Sadhale, and Shwu-Yuan Wu. Department of Biochemistry, University of Rochester Medical Center, Rochester, NY 14642.

In whole cell extracts of S. cerevisiae, many mRNA 3' ends can be faithfully generated by endonucleolytic cleavage and polyadenylation, yet with considerable variation in efficiency among substrates corresponding to different genes. In vivo, the 3' end formation signals of the GAL genes respond differentially to the ts mutation prp1-1 (formerly rna1-1): under nonpermissive conditions the GAL1 and GAL10, but not GAL7, mRNAs are longer at their 3' ends. With extracts from prp1-1 cells shifted from 23°C to 37°C, GAL7 (and CYC1) but not GAL1 or GAL10 pre-mRNAs are susceptible to processing, mimicking the in vivo observation. Cutback experiments suggest that the signals for GAL7 premessage processing are confined to a much shorter region than those involved in processing of GAL1 and GAL10, though the relationship between this and the prp1-1 defect remains to be determined. As for CYC1, antisense transcripts for all three GAL genes are also processed in vitro at positions such that the processed RNAs overlap their sense counterparts by 20-50 nt. Though this suggests symmetry, its significance is unclear and no corresponding mRNAs have been reported in vivo.

In collaboration with Drs. T. Humphrey and N. J. Proudfoot (Oxford), we have found that the 3' end processing and polyadenylation signals of the CYC1 gene from S. cerevisiae (a budding yeast) function accurately and efficiently in S. pombe (a fission yeast), and an inactivating 38 bp deletion does so in both organisms. Moreover, synthetic pre-mRNAs encoding 192 or 336 nt of the 3' region of the S. pombe URA4 gene are accurately processed in S. cerevisiae whole cell extracts, suggesting that mRNA 3' end formation in S. pombe is functionally compatible with S. cerevisiae, and substantially different in the nature of its signal elements (if not the mechanism) from higher eukaryotes.

Anticipating that pol II transcription termination signals may be closely associated with 3' end regions, we examined the behavior of RNA polymerase II in vitro on DNA templates spanning the end of the S. cerevisiae CYC1 gene. Our partially purified cell extract that supports processing also displays polymerase II (α-amanitin sensitive) activity on dC-tailed linear templates. To date, we detect no yeast specific termination. Nonetheless, control experiments with the bacterial trp t' rho-dependent termination region inserted within these templates indicate that E. coli rho factor can cause the yeast RNA polymerase II to terminate transcription, albeit heterogeneously and not efficiently. This is mechanistically informative, and a search for functionally equivalent yeast factors is underway.

Processing of RNA Polymerase II 3' Ends

U 014 REGULATION OF HISTONE mRNA 3' PROCESSING AND OF NUCLEO-CYTOPLASMIC TRANSPORT OF HISTONE mRNA, Max L. Birnstiel, Richard Eckner and Ingrid Hoffmann, Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria. There are three factors acting *in trans* which, in conjunction with *cis*-acting RNA signals bring about 3' processing of histone pre-mRNAs: the heat-labile factor, the hairpin binding factor, and the U7 snRNP. During 3' processing, the 5' sequences of the U7 snRNA are thought to make basepairing contacts with complementary spacer sequences, the so-called CAGA-box sequence of the histone pre-mRNA. Cell cycle regulation of 3' processing is brought about in part by up-, respectively down-regulation of the activity of the heat-labile factor. We reported earlier that the 5' sequences in the U7 snRNP become occluded in G0, but are freely accessible during S phase and have imputed from this that the availability of the 5' sequences to histone pre-mRNA was an important factor during down-regulation of 3' processing during G0. Using Schümperli's decoration assay (unpublished results) we now demonstrate that the 5' sequences of U7 snRNA from G0 arrested C3H10T1/2 cells are not accessible to labelled oligonucleotides complementary to U7 snRNA 5' sequences, while snRNPs prepared from exponentially growing cells interact and bind efficiently to these nucleic acids. This confirms our previous conclusion that the making available of the 5' sequences of the U7 snRNP to histone pre-mRNA is an important aspect of regulated 3' processing of histone pre-mRNAs.

We have analyzed the nucleo-cytoplasmic export of mRNAs in mammalian cells with an aim to identifying signals promoting RNA transport. Our results demonstrate that mRNA 3' processing and cleavage / polyadenylation signals can stimulate RNA transport from the nucleus to the cytoplasm. We tried to elucidate the nature of this stimulation for the case of histone transcripts. For this, we engineered a series of histone mRNAs obtaining their 3' ends by the self-cleaving activity of a cis-acting ribozyme thereby circumventing the cellular 3' end processing machinery. These constructs allowed us to investigate how the cellular transport apparatus is capable of distinguishing between primary transcripts and mature mRNA. Does it recognize certain features specific for processed mRNA or does a coupling of processing and transport provide the molecular basis for specificity? Our analysis indicates that the terminal histone palindrome is a positively acting transport signal, but more importantly, that histone mRNA processing and RNA export are most likely interdependent events.

U 015 HOW DO MESSENGERS GET THEIR TAILS? RECONSTITUTION OF AAUAAA-DEPENDENT MESSENGER RNA POLYADENYLATION FROM PURIFIED COMPONENTS IN VITRO.Silke Bienroth, Elmar Wahle, and Walter Keller, Biocenter of the University of Basel, CH-4056 Basel, Switzerland.
Polyadenylation of mRNA depends on the sequence AAUAAA located upstream of the poly(A) site. We have reconstituted this reaction from purified components in vitro. Poly(A) polymerase was purified to homogeneity from calf thymus. The enzyme is a monomer in solution with a molecular weight of 60 kD. Poly(A) polymerase is nonspecific with respect to the RNA primer; its activity is much higher in the presence of Mn^{++} as opposed to Mg^{++}, due to a 100fold higher affinity for the primer terminus. In the presence of Mg^{++}, a second factor (cleavage and polyadenylation factor, CPF) specifically permits polyadenylation of AAUAAA-containing RNAs. Such RNA-substrates can be as short as 14 nucleotides. CPF was purified to near homogeneity as a complex of four polypeptides of 160, 100, 70, and 30 kD. CPF interacts directly with the AAUAAA sequence, as shown by mobility shift, RNA-modification exclusion, and UV-crosslinking experiments. Although poly(A) polymerase and CPF are sufficient for the reaction, AAUAAA-dependent polyadenylation is strongly stimulated by poly(A) binding protein. In the presence of this protein, a biphasic polyadenylation is observed: First an oligo(A) tail is added, which is subsequently rapidly elongated. Poly(A) binding protein allows CPF- and AAUAAA-independent elongation of an oligoadenylated RNA.

U 016 CIS AND TRANS-ACTING FACTORS INVOLVED IN THE FORMATION OF THE 3' ENDS OF YEAST mRNAS, Claire L. Moore, Jie Chen, Linda Hyman, Marco Kessler, and Anne Skvorak, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111.

We are interested in the molecular mechanisms which generate the 3' ends of yeast mRNAs, the contributions of transcription termination and precursor processing to this event, and the ways in which this reaction resembles or is different from the mammalian equivalent. We are pursuing the following projects, and we will report on our progress in each of these directions.

1. We are purifying the yeast poly(A) polymerase, and are currently characterizing a bank of monoclonal antibodies generated against our purest fraction. We are also fractionating yeast extracts to determine how many and what kind of components are involved in recognition and cleavage of precursor RNA.

2. We are isolating and characterizing mutations in trans-acting factors which cause the wild type ADH2 site not to be recognized, as well as suppressors which recognize an inactive site containing a single base change. We have three candidates of each type which give the appropriate RNA phenotype, and are characterizing these as to whether the phenotype segregrates in a Mendelian fashion, whether they are dominant or recessive, and whether the processing of other mutated ADH2 sites or sites other than ADH2 is affected in vivo and in vitro.

3. By random in vitro mutagenesis, we have identified a cluster of single base mutations upstream of the ADH2 poly(A) site which prevent utilization of this site. These mutations affect nucleotides in a potential stem loop structure determined by computer folding programs. We are testing the possible involvement of this structure by oligonucleotide-directed site-specific mutagenesis.

4. We are trying to set up an in vitro reaction for site-specific termination of RNA polymerase II transcription, using extracts competent for transcription initiation.

U 017 POLY(A) SITE EFFICIENCY REFLECTS THE STABILITY OF COMPLEX FORMATION INVOLVING THE DOWNSTREAM ELEMENT. Joseph R. Nevins, Elizabeth A. Weiss, and Gregory M. Gilmartin, Department of Microbiology & Immunology, Duke University Medical Center, Durham, NC 27710

A critical step in mRNA biogenesis is the generation of the mRNA 3′ end through an endonucleolytic cleavage of the primary transcript followed by the addition of a ~200 nt poly(A) tail. The efficiency of poly(A) site function can vary widely and for those genes with multiple poly(A) sites, the choice can be a regulated event. A functional poly(A) site is characterized by *cis*-acting RNA sequences including the well-conserved AAUAAA hexamer, located 10 to 30 nucleotides upstream of the cleavage site, and a highly variable downstream GU- or U-rich element. The gene-specific nature of the downstream sequence suggests that it may be a primary determinant of poly(A) site efficiency. Our recent studies have detailed the purification of factors that mediate the cleavage and polyadenylation reaction and that recognize the *cis*-acting signals. Two of these factors, termed PF2 (an AAUAAA specificity factor) and CF1 (a cleavage-specific factor that recognizes the downstream element) are responsible for the formation of a stable, committed ternary complex with the pre-RNA. In order to define the role of this stable complex in poly(A) site function, we have used several pre-mRNAs that differ in the efficiency with which they are processed. We show that ternary complex stability reflects both the *in vivo* and the *in vitro* efficiency of the poly(A) site and that the stability of this complex is dependent on the nature of the downstream sequence element. We conclude that the stability of these protein-RNA interactions, dictated by the interaction of the CF1 factor with the downstream element, plays a major role in determining the processing efficiency of a particular poly(A) site.

Antitermination Mechanisms in Prokaryotes

U 018 Transcript Elongation in Phage λ: Players, Ways & Means

Asis Das*, William Whalen, Samit Chattopadhyay, Joseph DeVito, Jaime Garcia, David Lazinski & Jason Sparkowski. University of Connecticut Health Center, Farmington, CT 06030.

Phage λ *N* gene product is an antiterminator that captures the host RNA polymerase and modifies it to a termination-resistant form, allowing transcription of all of the distal genes encoded within the leftward and the rightward operons of the phage. The formation of the antitermination complex requires a cis-acting site, *nut*, present between the promoter and the first terminator within each operon, and several protein factors, NusA/B/E/G, encoded by the host. We will describe recent genetic and biochemical studies addressing the mechanism by which N protein recognizes the *nut* signal, captures RNA polymerase, and allows termination suppression. The current working model emerging from these studies proposes the following:

1. The RNA sequence encoded by *nut* serves as the recognition site for at least two proteins. The N protein binds the hairpin element called boxB, and a host factor binds the conserved sequence called boxA. A conserved arginine motif present within the amino terminus of N is involved in binding boxB. **2.** Upon transcription of *nut*, N becomes anchored to RNA polymerase by binding to boxB and also the host factor that binds boxA. **3.** N, bound on *nut* RNA, contacts the β subunit of RNA polymerase, utilizing the adaptor protein NusA which binds both N as well as RNA polymerase. **4.** The capture of polymerase by N does not require a transcriptional pause within the *nut* region; capture can take place at distant sites by mRNA looping. **5.** The binding of N to β endows polymerase the ability to elongate RNA chain at an increased rate and ignore signals for pausing and termination. **6.** The number and type of terminators suppressed by N depends upon the stability of N-polymerase complex, which in turn is determined by the combined strength of all individual interactions that attach N and the N-binding factors to RNA polymerase. **7.** The host factors NusB, NusE and NusG which also interact with the N-polymerase complex increase the processivity of antitermination, most likely by stabilizing a "core" antitermination complex that can form with minimal components.

We do not know yet whether the *nut* RNA remains attached throughout transcription to maintain the antitermination complex and/or allow termination suppression. The host factor that recognizes boxA remains to be identified. It is likely to be a vital component of antitermination in E. coli.

Regulation of Transcription Elongation and Termination

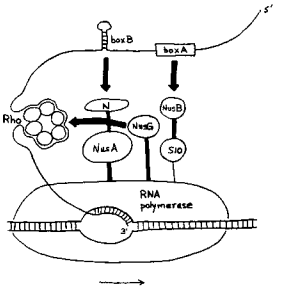
U 019 TERMINATION AND ANTITERMINATION IN COLIPHAGE HK022. Max E. Gottesman[1], Brent L. Atkinson[1], Renato Robledo[1], and Robert A. Weisberg[2]. [1]Institute of Cancer Research, College of Physicians and Surgeons of Columbia University, 701 West 168th Street, New York, NY 10032, [2]Laboratory of Molecular Genetics, NICHHD, NIH, Building 6, Room 320, Bethesda, MD 20892.

The nun gene product of coliphage HK022 terminates transcription at or near the nut sites of coliphage λ. The termination reaction is inhibited by mutations in the λ boxA or boxB sequences, or in the host nusA, nusB, nusE or rpoC genes. Some of these mutations invert Nun into a suppressor of transcription termination.

Despite similarities in sequence and chromosomal location, Nun does not serve as the N analogue of HK022. HK022 antiterminates early transcription in the absence of an intact nun gene. On the other hand, HK022 does encode a Q gene which, in most cases, is functionally exchangeable with λ Q. The exceptional case is that of the nusC60 mutation in the E. coli rpoB gene, which specifically blocks HK022 Q activity. The critical difference between the two Q genes lies two amino acids from the carboxyl terminus. A change in this residue from Thr to Ala restores HK022 Q activity in nusC60 mutants.

U 020 MECHANISMS OF TRANSCRIPTIONAL ANTITERMINATION BY THE N PROTEIN OF PHAGE LAMBDA Jack Greenblatt, Justin Nodwell, Steve Mason and Joyce Li, Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5G 1L6 CANADA.

We have isolated a new E. coli protein, called NusG, involved in antitermination by the lambda N protein. NusG is encoded by a gene in which a mutation suppresses the effect of the nusA1 mutation on antitermination by N. Although antitermination by N at a Rho-dependent terminator located just downstream from the N utilization site, nutR, requires only NusA in vitro, more processive antitermination through multiple Rho-dependent terminators located further downstream from nutR additionally requires NusB, ribosomal protein S10, and NusG. This enhanced processivity correlates with enhanced stability of the N-modified transcription complex: although NusA and S10 can bind to the RNA polymerase in transcription complexes in the absence of a nut site or any other elongation factor, the stable association of N, NusB, and NusG with the elongation complex depends on the nut site and all the factors. Protein affinity chromatography and other techniques were used to demonstrate several direct and specific protein-protein interactions among the antitermination factors, as illustrated in the accompanying diagram. These include: N-NusA; NusA-RNA polymerase; NusB-S10; S10 RNA polymerase; and NusG-RNA polymerase. RNA protection experiments were used to show that the nut site is made of RNA and coordinates the ordered assembly of the elongation factors onto the surface of RNA polymerase. There may be two mechanisms of antitermination by N: firstly, N inhibits pausing by polymerase; secondly, the presence of NusG in the elongation complex depends on N, and we have shown by protein affinity chromatography that NusG binds directly and selectively to Rho factor. This interaction is made temperature-sensitive by the rho026 mutation, which makes antitermination by N ts at Rho-dependent terminators. If NusG sequesters Rho factor as it approaches the transcription complex, as shown in the diagram, N may indirectly inhibit Rho-dependent release of the nascent RNA.



Elongation Control within Eukaryotic Genes

U 021 ELONGATION PROPERTIES OF PURIFIED RNA POLYMERASE II, Gretchen A. Rice, Michael J. Chamberlin, and Caroline M. Kane, Division of Biochemistry and Molecular Biology, University of California, Berkeley CA 94720.

Ternary transcription complexes of RNA polymerase II are intermediates in the synthesis of all eukaryotic mRNAs, and are implicated as regulatory targets of factors that control RNA chain elongation and termination. Structural information about such complexes is necessary for a full understanding of the catalytic and regulatory properties of the RNA polymerase during elongation. We have isolated ternary complexes containing purified RNA polymerase II halted at defined positions along the template. We have used nucleases to probe the footprint of the polymerase on both its template and the nascent transcript within each complex.

Deoxyribonuclease footprinting demonstrates that the structure of the ternary complex is dynamic during elongation; that is, the size and relative position of the footprint changes as the enzyme moves across the template. Thus, the complex may be more or less accessible to regulatory factors depending upon its position along the transcription unit.

Ribonuclease footprinting of the nascent transcript in the ternary complex shows that the RNA is sensitive to ribonucleases A and T1 at positions as close as 3 nucleotides from the 3' terminal growing point. Ribonuclease-treated ternary complexes containing transcript fragments 4 nucleotides or longer retain that fragment and are able to continue elongation. Since DNA:RNA hybrid structures are completely resistant to cleavage under our reaction conditions, these results suggest that if there is any DNA:RNA hybrid in the complex, it can extend for no more than three base pairs. Cleavage with lower concentrations of nuclease reveals a partially protected region of the transcript extending about 24 nucleotides from the 3' end. We suspect that this partial protection reflects an RNA binding site on the surface of the polymerase which binds the nascent transcript during elongation and separates it from the DNA template strand.

These studies provide a physical portrait of the polymerase itself during elongation. We can now examine changes in the ternary complex structure caused by factors which have been shown to regulate the elongation and termination properties of the polymerase.

U 022 THE ACCUMULATION OF TRANSCRIPTIONALLY ENGAGED RNA POLYMERASE II AT THE 5' END OF DROSOPHILA GENES John T. Lis, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853

A molecule of RNA polymerase II is present at the 5' end of the Drosophila hsp70 gene in uninduced cells. This was shown both by UV-crosslinking experiments performed in vivo (1) and by nuclear run on assays (2). We have also used this latter assay to demonstrate that this RNA polymerase is transcriptionally engaged but arrested approximately 25 nucleotides from the transcription start (2). Thus, the rate-limiting step in transcription under these conditions appears to be the movement of RNA polymerase out of this early elongation arrest and into the body of the hsp70 gene. We have proposed that heat shock factor may increase transcription of heat shock genes by accelerating the rate of RNA polymerase release from this arrested configuration (2). I will describe our recent studies of the generality of this polymerase arrest and our attempts to identify the DNA sequence elements and factors that modulate the formation and release of the arrested RNA polymerase on heat shock genes.

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2. A. E. Rougvie, J. T. Lis, *Cell* 54, 795-804 (1988).

U 023 ANALYSIS OF ELONGATION FACTOR S-II, Shunji Natori, Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

Transcription factor S-II was originally purified from Ehrlich ascites tumor cells as a protein stimulating RNA polymerase II. Subsequently, this factor was found to affect the elongation reaction by RNA polymerase II and to be important for nuclear RNA synthesis. Recent studies revealed that S-II influences the ability of RNA polymerase II to read through intrinsic premature termination sites in various eukaryotic genes. In this symposium, I will discuss two new findings about S-II. One is the multiplicity of S-II cDNAs. We have isolated two new cDNA clones for S-II from a mouse liver cDNA library. A cDNA clone termed pSII-L122 encodes a protein showing about 70% homology to the carboxyl terminal half of pSII-3, which is an S-II cDNA of Ehrlich ascites tumor cells isolated previously. However, the amino acid sequence of the amino terminal half of this new cDNA is completely different from that of pSII-3. Another clone, pSII-L121, showed even more striking differences. This clone contained two open reading frames. The downstream open reading frame was that of S-II. The amino acid sequence of this S-II was identical with that of Ehrlich S-II except for a difference in the 20 amino terminal amino acid residues. These results strongly suggest the existence of S-II families with very similar carboxyl terminal sequences but totally different amino terminal sequences. The upstream region of pSII-L121 contained an open reading frame for 89 amino acid residues, the sequence of which has not been reported previously. Possibly, this protein is also a transcription factor and functions cooperatively with S-II. The other point I would like to talk about is the ability of pSII-3 to transform NIH3T3 cells. When NIH3T3 cells are transfected with pSII-3, many transformed foci appeared. We characterized some transformants and found that transfected S-II cDNA was, in fact, transcribed in these cells. The mechanism of transformation by pSII-3 is unknown. Possibly unbalanced expression of transfected S-II genes alters the ratio of multiple S-II molecules in the cells, resulting in transformation.

U 024 TRANSCRIPTIONAL REGULATION OF HIV, Matija Peterlin, Alicia Alonso, Subir Ghosh, Mark Selby, and Sandra Tong, Howard Hughes Medical Institute, Departments of Medicine, Microbiology, and Immunology, University of California, San Francisco, San Francisco, CA 94143

The long terminal repeats (LTRs) of HIV-1 and 2 are transcriptionally activated following T cell or macrophage activation, growth, and proliferation. Cellular transacting factors that participate in this response include nuclear factors κB (NF-κB) and of activated T cells (NF-AT) in HIV-1 and NF-κB and AP-3 in HIV-2. Following activation, HIV LTRs are transactivated by their respective transactivators (tats). Tat interacts with an RNA stem-loop 3' to the site of initiation of viral transcription called TAR. Tat modifies an unstable to a stable (i.e. elongation competent) transcription complex. Evidence for efficient loading of RNA polymerase II at the HIV promoter by upstream transcriptional activators and utilization of tat at TAR will be presented. By fusing tat to the MS2 coat protein, a prokaryotic RNA binding protein, and replacing TAR with the MS2 operator, we were able to map activation and RNA binding domains of tat. While mixing and matching HIV LTR sequences reveal differences in activation and transactivation that might explain distinct clinical courses of HIV-1 and HIV-2 infections, studies on tat represent a new dimension of transcriptional control in eukaryotic systems.

Cell **62**: 769-776 (1990)
Genes Dev **3**: 547-558 (1989)
J Immunol **142**: 702-707 (1989), **45**: in press (1990)
Nature **330**: 489-493 (1987)
PNAS **83**: 9734-9738 (1986), **84**: 6845-6849 (1987), **85**: 8286-8290 (1988)

Regulation of Transcription Elongation and Termination

Polymerase Subunits Involved in Elongation and Termination

U 025 THE ROLE OF PHOSPHORYLATION IN THE TRANSCRIPTION CYCLE OF RNA POLYMERASE II, Michael E. Dahmus, Jon Chesnut and Joseph Stephens, Department of Biochemistry and Biophysics, University of California, Davis, CA 95616

The largest subunit of RNA polymerase II (RNAP II) contains at its C-terminus an unusual domain consisting of tandem repeats of the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. The extensive phosphorylation of this domain is thought to play an important role in the initiation phase of transcription and may be of regulatory significance. The observation that nascent transcripts are crosslinked almost exclusively to the phosphorylated form of RNAP II (IIO) in HeLa nuclei and in reconstituted transcription systems utilizing the major late promoter of adenovirus-2 (Ad2-MLP) indicates that elongation is catalyzed by the phosphorylated form of RNAP II. Considerable evidence also supports the idea that RNAP IIA, the unphosphorylated form of RNAP II, interacts with the promoter to form a stable preinitiation complex. Consequently, the phosphorylation of the C-terminal domain (CTD) is thought to occur after association of RNAP IIA with the promoter by a protein kinase that is an integral component of the preinitiation complex. According to this model, each round of transcription is associated with the reversible phosphorylation of the CTD of the largest polymerase subunit.

The state of phosphorylation of the CTD may, therefore, directly influence the ability of RNAP II to interact with the promoter. In an effort to determine the consequence of phosphorylation on complex formation, ^{32}P-labeled RNAPs IIA and IIO were prepared and examined for their ability to form a stable preinitiation complex on the Ad2-MLP in the presence of a reconstituted HeLa cell transcription extract. ^{32}P-RNAP IIA was prepared by phosphorylation of the terminal serine of subunit IIa with casein kinase II. ^{32}P-RNAP IIO was prepared by phosphorylation of labeled RNAP IIA with partially purified CTD kinase in the presence of excess cold ATP. Consequently, both RNAP IIA and IIO were labeled at the same position and had the same specific activity. Preinitiation complexes were formed in the absence of ATP and the state of phosphorylation of the largest subunit was monitored by SDS-PAGE. The observation that a significant fraction of the input RNAP IIO was converted to RNAP IIA during the course of the transcription reaction indicates that the partially purified transcription factors used to assemble the reaction contain a CTD phosphatase. Preinitiation complexes were purified from free RNAP II by chromatography on Sepharose CL-4B and the state of phosphorylation of RNAP II in each fraction was determined as described above. Purified preinitiation complexes contained predominantly RNAP IIA even in reactions initiated with RNAP IIO. The results of these experiments, therefore, support the idea that RNAP II containing an unphosphorylated CTD preferentially associates with the promoter. Consequently, upon completion of the transcript by RNAP IIO, the CTD may need to be dephosphorylated before RNAP II can interact with the promoter to initiate the next round of transcription.

The partially purified transcription factors used to reconstitute the polymerase dependent reaction were examined for CTD phosphatase by utilizing an assay based on the shift in electrophoretic mobility of subunit IIo to that of IIa. CTD phosphatase activity was associated with the fraction containing transcription factor IIA (TFIIA).

U 026 TERMINATION-ALTERING AMINO-ACID CHANGES IN BACTERIAL RNA POLYMERASE, Robert Landick, Department of Biology, Washington University, St. Louis, MO 63110

Some amino-acid substitutions in the ß subunit of *E. coli* RNA polymerase that confer resistance to rifampicin also result in increased or decreased transcriptional termination. We have investigated the role of the RNA polymerase ß subunit in transcriptional termination by directed mutagenesis of a plasmid-borne copy of the *rpoB* gene. To detect termination-altering mutations, we have screened the mutagenized plasmids using a strain in which both increased and decreased termination can be detected when the cloned gene is expressed and the resulting ß subunit is incorporated into RNA polymerase. Several of the altered RNA polymerases have been purified and show significant changes in pausing and termination during transcription in vitro. The major conclusions from our studies to date are (i) amino-acid substitutions in several regions of ß that are conserved between prokaryotic and eukaryotic ß-subunit homologs can significantly increase or decrease termination, (ii) whereas one of these clusters of mutations corresponds to the previously characterized Rif^r region (amino acids 500-575), many substitutions in this region alter termination without conferring resistance to rifampicin, (iii) none of the termination-altering mutations that were located outside of the Rif^r region confer resistance to rifampicin, and (iv) one particularly interesting altered RNA polymerase (a double substitution of $Pro_{560}\rightarrow$Ser and $Thr_{563}\rightarrow$Ile) exhibits a dramatic reduction in transcriptional pausing, lesser reductions in transcriptional termination, and termination at new sites at which wild-type RNA polymerase does not terminate.

We have tested the structure of paused transcription complexes containing another of the altered ß subunits that enhances both termination and pausing. Using RNase T1 to probe the conformation of the nascent transcript and $KMnO_4$ to probe single-stranded regions of the DNA template, we have detected no dramatic differences in the structure of transcription complexes that contain wild-type or altered ß subunit.

We now are engaged in the isolation of new termination-altering mutations in the interesting regions of *rpoB* by directed mutagenesis of a tightly regulated *lac* promoter - *rpoB* plasmid that allows recovery and characterization of lethal mutations. We also are testing for termination-altering amino-acid substitutions the ß' subunit of *E. coli* RNA polymerase.

U 027 MAPPING THE PATHS OF GROWING RIBONUCLEIC ACID MOLECULES BY PHOTOAFFINITY LABELING. Claude F. Meares and Thomas M. Stackhouse, Chemistry Department, University of California, Davis, California 95616.

We are interested in mapping the path that nascent RNA follows through the macromolecular complex that catalyzes its synthesis. Because of the large sizes and relative scarcity of the molecules involved, we have developed a strategy that uses the tools of chemistry and molecular biology to carry this out. Previously, we have synthesized dinucleotide conjugates containing photoreactive aryl azides at the leading (5') end.[1] Aryl azides are chemically unreactive until activated by photolysis; then they form nitrenes or other highly reactive species, which react indiscriminately with molecules in their vicinity. After verification of the probes' biological activity, enzyme•DNA•RNA complexes were photolyzed, then dissociated and fractionated by gel electrophoresis. To be labeled, an RNA polymerase subunit must have been contacted by a photogenerated reagent located at the 5' end of a nascent RNA molecule in a transcription complex.

The σ subunit of RNA polymerase is responsible for promoter recognition, and dissociates from the core polymerase after initiation. Photoaffinity labeling experiments performed using two different DNA promoters, λ P_R and T7A1, reveal that the σ subunit of RNA polymerase is contacted by the 5' ends of *different lengths* of nascent RNA. When transcribing from the λ P_R template the σ subunit is labeled by RNAs 8-14 bases long, with maximum 4% yield at 11 nucleotides (in comparison to 0.7% for either λ P_L or T7A1).[2] Thus photolabeling of σ is unmistakably dependent on the DNA sequence. To determine the nucleic acid region responsible for the different σ interactions, two recombinant DNA templates were constructed. One of these contained the λ P_R promoter and T7A1 transcript, while the other contained the T7A1 promoter and λ P_R transcript. Regardless of the transcribed region, significant labeling of the σ subunit is observed only on the templates containing the λ P_R promoter region. These data indicate that the major interactions leading to the release of σ from ternary transcription complexes involve the promoter sequence of the template DNA, upstream from the transcribed region. Further studies with λ P_R templates mutated at single positions may reveal the individual bases responsible for this effect. Supported by NIH Research Grant GM 25909.

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U 028 **YEAST RNA POLYMERASE C TRANCRIPTION SYSTEM,** Nuchanard Chiannilkulchai, Sophie Stettler, Sylvie Hermann, Rolf Stalder, Michèle Chablat, Christine Mosrin, Isabelle Treich, Michel Werner, Janine Huet, Michel Riva, Christophe Carles, Sylvie Labarre, Jean Marie Buhler, Pierre Thuriaux, Olivier Lefebvre, Christine Conesa, Robert Swanson, Christian Marck, Anny Ruet, Odd Gabrielsen and André Sentenac, Service de Biochimie et de Génétique Moléculaire, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette Cédex, France.

Transcription of tRNA genes can be performed in vitro using RNA polymerase C and transcription factors TFIIIB and τ/TFIIIC. Altogether, taking into account all the polymerase and factor subunits, an estimate of 20 polypeptide chains are involved in transcription complex formation. Several RNA polymerase C subunit genes have been cloned using subunit-directed antibodies and microsequence data : RPC160, RPC82, RPC53, RPC40, RPC34, RPC31 and RPC19. All these genes were shown to be unique and essential for cell growth. Conditional mutants have been constructed by in vitro mutagenesis to demonstrate the in vivo requirement for enzyme C subunits in transcription and to identify functional domains. In parallel, nucleotide and zinc binding domains were mapped and mutagenized in the large subunits of RNA polymerase B.

Transcription factor τ(TFIIIC) is a large (600 kD) DNA binding protein comprised of at least four polypeptides (τ145, τ135, τ95 and τ60). Subunits τ95 and τ145 belong to two DNA binding domains that bind to the A and B blocks of tRNA genes. Microsequence data have been obtained for subunits τ145, τ135 and τ95 and the corresponding genes have been cloned. Eventually, we hope to be able to describe enzyme-factor interactions in the pol C system.

Late Abstracts

REGULATION OF TRANSCRIPTION TERMINATION IN E. COLI BY RIBOSOMAL PROTEIN L4, Janice M. Zengel, Ping Shen and Lasse Lindahl, Department of Biology, University of Rochester, Rochester, NY

Ribosomal protein L4 of *Escherichia coli* functions not only as a component of the ribosome but also as a regulatory factor inhibiting both transcription and translation of its own operon, the eleven gene S10 operon. L4-medialec transcription control results in premature termination of transcription within the 172 base S10 leader, immediately downstream of a stable terminator-like hairpin structure. This attenuation control can be reproduced *in vitro* by addition of purified L4 to a cell-free transcription system containing RNA polymerase, but depends on the addition of transcription factor NusA. Kinetic experiments suggest that NusA is required for RNA polymerase to form a stable paused complex at the attenuation site. The half-life of this paused complex is increased by L4, and is also very sensitive to the UTP concentration. Surprisingly, we detect little or no release of the attenuated transcript, suggesting that the *in vitro* system is defective in the final step of the termination reaction. Our analysis of mutated S10 leaders indicates that most or all of the leader upstream of the attenuator hairpin is dispensable for NusA-dependent pausing/termination. However L4 stimulation requires upstream bases, in particular a small hairpin structure immediately upstream of the attenuator hairpin. This sequence/structure may represent the site of L4 interaction with the S10 leader, although we have not been able to demonstrate directly L4 binding to the leader RNA. The specific L4 recognition site on 23S rRNA is also not yet known. However we have recently observed that a fragment of 23S containing the proximal 610 bases can eliminate *in vitro* L4-stimulated attenuation, and hence, contains information sufficient for L4 binding to 23S rRNA.

MUTATIONS IN RET1, THE YEAST GENE ENCODING THE SECOND LARGEST SUBUNIT OF RNA POLYMERASE III, Philip James, Salam Shaaban, Linda K. Hardison, and Benjamin Hall, Dept. of Genetics, University of Washington, Seattle, WA. 98195

The RET1 gene was cloned by complementation of ret1-1, a recessive allele that reduces termination by RNA polymerase III. The ret1-1 mutation is a T315K change in a non-conserved region 150-200 amino acids N-terminal to sequences resembling the Rif^R region of E. coli RPOB. Mutations are being generated throughout this area by "spiked oligonucleotide" mutagenesis. Mutant screening for RET1 alleles with altered Pol III behavior utilizes SUP4-o alleles that score either for decreased termination (SUP4-UIV) or for increased termination (SUP4-Δ94). RET1 shares thirteen conserved sequence blocks with all other genes for RNA polymerase catalytic subunits. Situated between the last two conserved sequence blocks is the sequence 1092-VDVCDKCGLMGYSGWCTTCKSAENI-1116. A similar zinc-finger motif is found, with varying distance between the cysteine pairs, in all archaebacterial and eukaryotic nuclear RNA polymerases. Selected residues in this region of RET1 have been mutagenized; thus far, C1098A has proven to give a functional subunit, while G1099V lacks function. Because of the ability of the yeast Pol III system to carry out initiation, elongation, and termination correctly in vitro, transcription with RNA polymerase III from these and other ret1 mutant strains will make possible the assignment of specific functions to the conserved domains of the eukaryotic RNA polymerase III catalytic subunit.

Regulation of Transcription Elongation and Termination

Poster Session I

U 100 RHO-DEPENDENT TERMINATION ELEMENTS WITHIN CISTRONS. POTENTIAL SITES OF ELOGANTION CONTROL?, Pietro Alifano, Claudia Piscitelli, Anna G. Nappo, Flavia Rivellini, C. Bruno Bruni and M. Stella Carlomagno, CEOS of CNR, Dpt of Pathobiol Cell & Mol, University of Naples, via S. Pansini 5 80131 Napoli, Italy

We have studied and characterized a series of cryptic Rho-dependent termination signals located within the cistrons of the his operon of S. typhimurium and unmasked by polar mutations. The analysis of the transcripts produced under different growth conditions by polar mutants in four different cistrons of the operon led to the identification of several cryptic Rho-dependent transcription termination elements which are activated by the uncoupling of transcription and translation. We were able to identify a consensus motif common to all these elements. DNA fragments containing such motif, irrespective of their biological function, were both necessary and sufficient to cause Rho-dependent transcription termination both in vivo and in vitro. The presence of consensus elements for other transcriptional factors (Nus) in the regions where release of transcripts occurs suggests that Rho factor, in concert with other elongation (termination-antitermination) factors, participates in a control particle to regulate the elongation step during transcription of polycistronic messages.

U 101 THE ROLE OF THE MAMMALIAN TRANSCRIPTION FACTORS IIF, IIS AND IIX DURING ELONGATION BY RNA POLYMERASE II, Yosef Aloni[1], Eyal Bengal[1], Osvaldo Flores[2], Anat Krauskopf[1] and Danny Reinberg[2], Department of Molecular Genetics and Virology The Weizmann Institute of Science Rehovot, Israel (76-100)[1] and Department of Biochemistry Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey Piscataway, New Jersey, USA[2].

In the present study we have used a recently developed system that allows the isolation of RNA polymerase II-elongation competent complexes [Bengal, E., Goldring, A. and Aloni, Y. (1989) J. Biol. Chem. **264**, 18926-18932]. Pulse labeled transcription complexes were formed at the adenovirus major late promoter using HeLa cell extracts. Elongation competent complexes were purified from most of the proteins present in the extract, as well as from loosely bound elongation factors, by high salt gel filtration chromatography. We found that under these conditions the nascent RNA was displaced from the DNA during elongation. These column purified complexes were used to analyze the activities of different transcription factors during elongation by RNA polymerase II.

We have found that TFIIS, TFIIF and TFIIX affected the efficiency of elongation through the adenovirus major late promoter attenuation site and a synthetic attenuation site composed of eight T residues. These factors have distinct activities that depend on whether they were added before RNA polymerase has reached the attenuation site or at the time when the polymerase is pausing at the attenuation site. TFIIS was found to have anti-attenuation activity while TFIIF and TFIIX stimulated the rate of elongtion. In comparison to TFIIF, TFIIS is loosely bound to the elongation complex. We also found that the activities of the factors are dependent on the nature of the attenuator. The present results indicate that at least three general transcription factors play a major role in regulating gene expression during elongation by RNA polymerase II.

U 102 CONSTRUCTION AND CHARACTERIZATION OF SYNTHETIC RHO-DEPENDENT TRANSCRIPTION TERMINATION SITES, David G. Bear, Kenneth W. Escudero, David S. Peabody, Joseph Fazio and Elizabeth A. Roberts, Department of Cell Biology and the Cancer Center, University of New Mexico School of Medicine, Albuquerque, NM 87131

The nature of the E. coli Rho-dependent transcription termination signal that lies within the nascent RNA transcript is complex. No obvious structural features appear to be common to the sites that have been characterized thus far. Based on the observations that Rho binds tightly to pyrimidine-rich unstructured RNA, and that cytosine-rich RNA stimulates the Rho ATPase activity that is required for termination, we have attempted to construct synthetic Rho-dependent termination sites from large repetitive pyrimidine-rich tracts obtained from Drosophila melanogaster DNA. When fused to prokaryotic promoters and use as templates for in vitro transcription, some of these repetitive sequences function as Rho-dependent terminators; of several sequences tested, the repeats TCTTG and TCTTG are the most effective. Termination occurs within a 360 nt tract of TCTTC at a dispersed set of sites spaced at 5-base intervals beginning approximately 80-135 nucleotides into the sequence. Fusion of an 80 nt TCTTC tract with a strong RNA polymerase pause site from phage T5 yields a termination site that generates transcripts of more discrete lengths, with 3' termini that map proximal to the pause site. The TCTTC tract also functions as a Rho-dependent termination site in vivo.

U 103 In vitro trans-activation of the HIV-1 LTR by HIV-1 Tat. Cindy A. Bohan[1], Fatah Kashanchi[1], Barbara Ensoli[2], Robert C. Gallo[2] and John N. Brady[1], [1]Laboratory of of Molecular Virology, [2]Laboratory of Tumor Cell Biology, National Cancer Institute, NIH, Bethesda, Maryland 20892.

The HIV-1 Tat protein is essential for viral replication and is a potent trans-activator of viral gene expression. Tat function requires cis-acting sequences located downstream of the transcription initiation start site (TAR). We have utilized a cell-free in vitro transcription assay to investigate trans-activation of the HIV-1 LTR by Tat. The Tat protein used in these studies has been purified to homogeneity and is active in vitro in cell proliferation assays and activates transcription from the HIV-1 LTR in scrape-loading experiments in a variety of cell types. The addition of purified Tat to the in vitro transcription reaction significantly increased transcription from the HIV-1 LTR template. Tat trans-activation was dependent upon the concentration of HIV-1 template and Tat protein. In vitro Tat trans-activation required sequences downstream of -65 and inclusive of TAR. Promoter constructs containing TAR mutations which abolish Tat function in vivo did not support trans-activation in our in vitro assays. Experiments designed to elucidate the mechanism by which Tat affects transcriptional initiation and/or elongation will be discussed.

U 104 REGULATION OF TRANSCRIPTION ELONGATION BY DESIGNED CIS-ACTING UNUSUAL DNA STRUCTURAL ELEMENTS. Samir K.Brahmachari, Partha Sarathi Sarkar and Rajesh Bagga. Molecular Biophysics Unit, Indian Institute of Science, Bangalore, 560 012 INDIA.

Our earlier in vitro studies (Nucl.Acids Res.1990 18, 3363) have shown that a supercoil stabilized cruciform structure present within the gene could block the elongation of E.coli RNA polymerase. Waga et al have further corroborated these results by showing that this block could be abrogated by chromosomal HMG1 protein (J.Biol.Chem 1990 265, 19424). Taking advantage of the degeneracy of the genetic code we have developed a novel approach to introduce, within the gene, DNA sequences capable of adopting unusual structures and to investigate the role of such sequences in the regulation of transcription elongation in vivo. This approach was used to replace the N-terminal region of the β-galactosidase gene in pUC19 with DNA sequences capable of adopting cruciform structure or left handed Z-conformation in vivo. In the selection of newly designed sequences care was taken to include those codons which are favoured in E.coli mRNAs. E.coli DH5α cells harboring these newly designed plasmids showed drastic reduction in the expression of *β-gal* gene in vivo. Using oligonucleotide probes it has been possible to demonstrate that these unusual DNA structures present within the gene could block transcription elongation in vivo. This unique approach opens up the possibility to study the role of various unusual DNA structural elements present within the gene, acting in cis, in regulation of transcription elongation.

U 105 YEAST RNA POLYMERASE II STRUCTURE, Seth A. Darst, Aled M. Edwards, Caroline M. Kane*, Elizabeth Kubalek, R.A. Young† and Roger D. Kornberg. Department of Cell Biology, Stanford University, Stanford, CA 94305, *Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, †Whitehead Institute and Department of Biology, M.I.T., Cambridge, MA 02142.

RNA polymerase II (pol II) was purified from the yeast, *Saccharomyces Cerevisiae*. The purified enzyme is composed of 11 distinct polypeptides, two of which, the fourth and seventh largest (subunits 4 and 7), are loosely associated with the enzyme. A form of the enzyme lacking these subunits, pol II Δ4/7, was purified. Pol II Δ4/7 and pol II do not differ in many aspects of polymerase function, including the rate of elongation, the ability to synthesize RNA from denatured templates or the ability to recognize pause/termination sequences in double stranded templates. However, pol II Δ4/7 is deficient in promoter-directed initiation activity. Pol II Δ4/7 forms well-ordered, two-dimensional crystals on positively charged lipid layers. Using negatively stained pol II Δ4/7 crystals and electron crystallography, the structure of pol II Δ4/7 has been resolved at about 16 Å resolution. We find a structure in pol II Δ4/7 very similar to the 25 Å channel of the *E. coli* RNA polymerase holoenzyme. Additional features of the pol II Δ4/7 structure include an extension to the 25 Å channel, a deep groove connected to the 25 Å channel that is about half this diameter, and a tunnel that extends through the structure. These structural features are likely to be important to the enzymes' chain elongation activity.

U 106 **MULTIPLE DNA BINDING BY THE DROSOPHILA DEVELOPMENTAL PROTEIN PAIRED**

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The Paired protein (Prd) contains two conserved domains, also found in other developmental genes.

The Prd homeodomain (HD) is very divergent from most known HDs, such as Antennapedia (Antp). We have identified two modes of binding by the Prd HD. One mode appears quite different from that of prokaryotic HTH proteins. In this mode, the 9th amino acid of the recognition helix (RH) appears to determine the specificity of the HD. Crystal structure and NMR analysis of protein-DNA complexes have since shown that the HD binds as a monomer and that amino acid 9 of the RH is at a critical position for interaction with the DNA. Recently, we have found that the Prd HD can bind through a distinct mode to another completely different DNA sequence. Amino acid 2 of the RH appears important for this binding, as for the prokaryotic HTH proteins. Position 2 is of little importance for the first mode. This binding can be distinguished from the previous one because only molecules lacking the C-terminus of the Prd protein can bind to this sequence.

The paired box is another motif, first identified in Prd and other developmental genes. It encodes a 128 amino acid domain, the paired domain, which has since been found in other fly and mouse gene products, in association with the homeodomain or in its absence. We show that the paired box of the *prd* gene encodes a DNA binding activity, independent from the DNA binding activity of the Prd HD and with a different sequence specificity. The N-terminal region of the paired domain, including one of the three predicted helices, is essential for binding. We also show that a mutation in the paired box of Prd corresponding to the *undulated* developmental mutation in the paired box of the mouse Pax-1 gene destroys the ability of Prd to bind to its paired box specific sites.

U 107 **MISINCORPORATION KINETICS AND THE FIDELITY OF *E. COLI* RNA POLYMERASE**

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

We have been investigating the kinetics of misincorporation and the idling turnover kinetics of *E. coli* RNA polymerase stalled at a specific site on a DNA template. These experiments were performed using the DNA template, pDE13, which contains λP_R promoter and transcription sequence shown below.

pDE13: λP_R promoter—AUGUA*G*UAAGGAGGUUGUAUGGAA<u>C</u>AAC—

(+1 and +25 mark the first and last transcribed positions)

As can be seen from the transcript sequence shown above, stable stalled elongation complexes at position +24 (A_{24} complexes) can be made by performing transcription in the absence of CTP. Consequently, the rate of misincorporation at position +25, the first position in the transcript requiring CTP, can be measured. In addition, we can measure the rate of idling turnover of the stalled complexes by monitoring the production of ^{32}P-pyrophosphate ($^{32}PP_i$) from γ-[^{32}P]-ATP or γ-[^{32}P]-GTP. The idling turnover and misincorporation rates are measured as a function of $^{32}PP_i$ concentration as well as NTP concentrations. The rate of NTP turnover in these idling turnover experiments is 2000 to 10,000 times slower than the steady-state synthesis rate of ~30/sec. In addition, the observed rates appear to be a result of misincorporation and excision as opposed to correct incorporation and excision. The results of these experiments will be discussed in terms a kinetic mechanism for RNA polymerase fidelity.

U 108 TRANSCRIPTION-DEPENDENT COMPETITION FOR A HOST FACTOR: ROLE OF PROMOTER AND *boxA* SIGNAL IN TRANSCRIPTION ANTITERMINATION MEDIATED BY THE N PROTEIN OF PHAGE λ,

David I. Friedman, Linda L. Johnson and Karla Henthorn, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-0620. Eric R. Olson, Molecular Biology Research, Upjohn Co., Kalamazoo MI 49001.

The λ N and *E. coli* Nus proteins act through phage *nut* signals to modify RNA polymerase to a termination-resistant form. The *boxA* component of lambdoid phage *nut* signals has been conserved with slight variations. Although the consensus *boxA* signal (*boxAcon*), 5'CGCTCTTTA, is found only in P22, changing the nut_R region of either λ or 21 (from 5'CGCTCTTAC and 5'TGCTCTTTA) to the consensus creates more effective antitermination signals. An in vivo assay employing transcription of plasmid-based *nut* signals demonstrates: 1) A λ *nut* region with *boxAcon* outcompetes the wild-type signal for NusB. 2) Under conditions of limiting NusA activity, a weakly transcribed nut_R region with *boxAcon* enhances N action in trans at a wild-type nut_R signal. 3) Transcription of a nut_R region with the *boxA5* mutation (5'C<u>T</u>CTCTTAC), which eliminates *nut* antiterminator activity, acts in trans to partially suppress failure to support N action due to the *nusB5* mutation. Moreover, mutations increasing p_R promoter strength reduce antitermination effected by the wild-type nut_R signal, but additional mutations which increase the effectiveness of the interactions at nut_R restore antitermination. These results support a model in which the *boxA* signal is involved with both pro- and antitermination factors and suggest that unmodified polymerase interferes with successful transit of modifed polymerase.

U 109 TRANSCRIPTION ON NUCLEOSOMAL TEMPLATES BY RNA POLYMERASE II *IN VITRO*: INHIBITION OF ELONGATION WITH ENHANCEMENT OF SEQUENCE-SPECIFIC PAUSING **Michael G. Izban and Donal S. Luse, Dept. of Mol. Genetics, Biochemistry and Microbiology, Univ. of Cincinnati, Col. of Medicine, Cincinnati, Ohio 45267-0524**

The process by which RNA polymerase II elongates RNA chains in vivo, where the template is at least partially in a nucleosomal configuration, remains poorly understood. In order to approach this question we have partially purified RNA polymerase II transcription complexes paused early in elongation. These complexes were then used as substrates for chromatin reconstitution. Elongation of the nascent RNA chains on these nucleosomal templates is severely inhibited relative to elongation on naked DNA templates. Elongation on the nucleosomal templates results in a reproducible template-specific pattern of transcripts generated by RNA polymerase pausing. The RNA polymerases are not terminated because the large majority will resume elongation upon the addition of sarkosyl or 400 mM KCl. The effectiveness of known RNA polymerase II pause sites is enhanced by the presence of nucleosomes. For example, a pause site similar in sequence to the c-*myc* gene exon 1 terminator is used 4 to 7 times more effectively in reconstituted templates. A comparison of elongation on templates bearing phased nucleosomes and on reconstituted templates which show no predominant phasing pattern indicates that the locations of pause sites are not related to the positions of the nucleosomes. Rather, the major determinant of RNA polymerase pausing on the nucleosomal templates appears to be the underlying DNA sequence.

U 110 STRUCTURE AND EXPRESSION OF THE *NRU* / N-*Ras* GENE TANDEM, Hélène Jacquemin-Sablon, Sylvaine Cases, Oréda Boussadia, Gérard Triqueneaux and François Dautry, Laboratoire d'Oncologie Moléculaire, Institut Gustave Roussy, 94805 Villejuif, France.

We have characterized the genomic organization of the human N-*Ras* locus. A transcriptional unit that we have designated *NRU* (for N-*Ras* upstream), is located immediately upstream of N-*Ras*. Sequencing of *NRU* cDNAs shows the presence of an open reading frame of 767 aminoacids, with no significant homology to known proteins. Three mRNAs species are transcribed from the *NRU* gene in the same orientation as N-*Ras*, and differ by the location of their polyadenylation site. 150 nucleotides separate the 3' end of the *NRU* longest message from the cluster of N-*Ras* transcription initiation sites. The same organization has been recently reported in rodents (Jeffers et al, N.A.R. 18, 4891-4899, 1990). In human and rodent tissues, *NRU* and N-*Ras* are ubiquitously expressed. We have found examples of coordinated expression of the two genes, either as a direct correlation like during lectin stimulation of peripheral blood mononuclear cells, or as an inverse correlation, like in the course of skeletal muscle development, indicating a complex regulation of this gene tandem. To analyze the transcriptional regulation of these genes, we have undertaken the molecular cloning of the human *NRU* gene and the characterization of its promoter. We are also investigating the structure and function of the intergenic region. Preliminary experiments indicate that this region is transcribed and the nature of these transcripts is under study.

U 111 VARIABLE TRANSCRIPTION ELONGATION RATE IN *E. coli*, **Kaj Frank Jensen, Mogens Kilstrup and Ulla Vogel.**
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The transcription elongation rate in *Escherichia coli* is believed to be constant and independent of growth condition. However, all experiments made to estimate this parameter included rifampicin to block initiation of new RNA chain synthesis. This drug perturbs the growth and causes a rapid increase in the intracellular concentrations of nucleoside triphosphates. Therefore, the RNA chain growth rate may well have been overestimated in the previous experiments and differences between different media remained obscured. We have determined the RNA chain growth rate directly by probing the growth of the RNA chain from a gene with an inducible promoter: The RNA was extracted at 10 sec. intervals after the induction and quantitated by Northern hybridization with a probe complementary to the end of the RNA chain. The time lag between induction and the appearance of the hybridization signal was taken to represent the reciprocal of the RNA chain growth rate and was found to change considerably depending on the growth condition. The significance of this result for the understanding of the growth physiology of *E.coli* growth will be discussed.

Ref: Jensen, K.F. & S. Pedersen (1990) Microbiological Reviews **54**, 89-100.

U 112 ABORTIVE INTERMEDIATES IN TRANSCRIPTION BY WHEAT-GERM RNA POLYMERASE II: HYSTERETIC TRANSITIONS IN SELECTION OF THE ENZYME SYNTHETIC MODE

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At constant enzyme concentration and with the full set of nucleotide substrates dictated by template sequence, the chain-length distribution of polymeric product varies with template concentration in reactions catalysed by wheat-germ RNA polymerase II. Under the same conditions, but in the presence of a single triphosphate substrate, the rate of condensation of the triphosphate substrate to a dinucleotide primer also exhibits a complex dependence with the template concentration. This effect is observed using poly[d(A-T)] as a template. For both reactions there are two extreme types of behaviour in each of which transcription appears to involve a single enzyme synthetic mode, characterized by either a high (at low-template concentration) or a low (at high-template concentration) probability of releasing the transcripts. Quantitative analysis of the kinetic data suggests hysteretic properties of wheat-germ RNA polymerase II. Thus the basic requirement of the proposed model is a slow 'hysteretic' transition between two states of the polymerization complex, a state H and a state L, having distinct probabilities of releasing the nascent RNA chain. In the case of the poly[d(A-T)] template, the state H is involved in the formation of oligonucleotides shorter than 15 bases, while the L state catalyses the polymerization of chains longer than 40 bases. The proportion between these two states depends on several parameters such as the sequence (e.g. dA rich *versus* dT rich, palindromes) and conformation (e.g. right-handed B- *versus* left-handed Z-DNA) of the template, the nature and concentration of bivalent metal ions, the ionic strength, or the presence in the assay of a competitor template. The enzyme synthetic mode also varies during the progress of the polymerization reaction. Thus with poly[d(A-T)] as template, the form H predominates in the early time of transcription, and then evolves through a slow transient towards the L state.

U 113 NEGATIVE TRANSCRIPTION ELEMENT IN *XENOPUS C-MYC* GENE INTERACTS WITH *C-MYC* PROTEIN.

Michael W. King, Indiana University School of Medicine, Terre Haute Center for Medical Sciences, Terre Haute, IN. 47809.

The *Xenopus laevis c-myc* gene contains transcriptional regulatory elements similar to those identified in the mammalian *c-myc* genes. There are two positive and one negative *cis*-acting elements in the *Xenopus c-myc* gene within 1300bp of the major transcription start site. The activity of the negative regulatory element is only observed when assayed by transient transfection into cells expressing high levels of *c-myc* protein. The negative transcriptional element is located between -800 and -300 (relative to the major transcriptional start site). This region of the gene binds a single protein or complex of proteins as evidenced by gel-shift analysis. The ability of the DNA-binding complex to form is abolished by pre-treatment of nuclear extracts with affinity purified anti-*Xenopus-c-myc* antibodies. The complex can be regenerated by the addition of *in vitro* synthesized *Xenopus c-myc* proteins. These data are in support of the emerging evidence indicating a role for the *c-myc* protein in negative auto-regulation of transcription. This regulatory mechanism likely occurs via interaction of *c-myc* protein with elements of the *c-myc* gene.

U 114 HETERODUPLEX ANALYSIS OF THE RIBOSOMAL GENE TERMINATOR IN X.LAEVIS,

Paul Labhart, Research Institute of Scripps Clinic, La Jolla, CA 92037

The sequence GACTTGCNC directs termination of ribosomal gene transcription in *X.laevis*. The current model for termination suggests that RNA 3'ends are formed by processing 15 nt upstream from this signal, and actual release of RNA polymerase I occurs at as yet undefined sites further downstream (Labhart and Reeder, Genes & Dev. 4: 269-276, 1990). In order to address the question whether the termination signal exerts its function at the level of RNA or DNA, I have constructed heteroduplex templates in which mutations in the termination signal are present in either the transcribed or the non-transcribed strand. The results show that a mutation in the transcribed strand has the same phenotype as a mutation in the non-transcribed strand. In addition, those single-stranded mutations affect 3'processing and termination as strongly as the double-stranded mutation. The results therefore indicate that the non-transcribed DNA strand is also part of the 3'processing/termination signal and rule out any model in which the signal sequence GACTTGCNC is recognized solely at the level of RNA.

U 115 CHROMATIN STRUCTURE AT THE 3' FLANKING REGIONS OF THE XENOPUS rRNA GENES AND ITS RELATION TO TRANSCRIPTION TERMINATION.

R. Lucchini, Th. Koller and J. M. Sogo. Institute of Cell Biology, ETH Zürich, Hönggerberg, 8093 Zürich (Switzerland).

Using the psoralen crosslinking technique we have compared the chromatin structure of the rRNA coding and 3' flanking spacer regions of the two related frog species Xenopus laevis and Xenopus borealis. Isolated nuclei from tissue culture cells were photoreacted with psoralen and the extent of crosslinking in the different DNA regions was analyzed using a gel retardation assay. Both species showed basically the same following structures: Restriction fragments from the coding region were resolved into two bands indicating the presence of two distinct types of chromatin, one that contains nucleosomes which represents the inactive gene copies, and the other one which is free of nucleosomes and corresponds to the transcribed genes. The analysis of the spacer regions flanking the 3' end of the genes gave a different result. The majority of these spacer sequences are packaged in nucleosomes while a minor proportion, presumably those flanking the active genes, have an heterogeneous chromatin structure. These results suggest that not all the polymerases transcribing the upstream gene traverse these 3' flanking sequences and that those polymerases which pass the 3' end of the gene terminate at heterogeneous sites further downstream.

U 116 THE FORMATION AND STABILITY OF *DROSOPHILA* RNA POLYMERASE II ELONGATION COMPLEXES, David H. Price, Nick F. Marshall, Dan D. Kephart, Department of Biochemistry, University of Iowa, Iowa City, Iowa, 52242

The efficient transcription of eucaryotic genes requires the assistance of specific proteins which affect the elongation properties of RNA polymerase II. We are using a *Drosophila, in vitro* system to examine the properties of elongation complexes formed after initiation at specific promoters. The rate of elongation by such complexes is similar to the *in vivo* rate and the complexes do not exhibit the pausing characteristic of pure RNA polymerase II. When heparin, sarkosyl or moderately high salt are present during elongation the rate drops to 10% of the normal rate and numerous pause sites become apparent. The properties of the treated complexes are similar to those of pure RNA polymerase II, indicating that elongation factors have been inhibited. The results are consistent with the involvement of both DmS-II and factor 5 (TFIIF) during the elongation phase of transcription. We are using templates immobilized on paramagenetic beads to examine the association of elongation factors with transcription complexes. When preinitiation complexes formed on immobilized templates are washed to remove free proteins the resulting washed complexes initiate efficiently, but are unable to carry out efficient elongation. The elongation rate observed is much lower than that of pure RNA polymerase II which suggests the involvement of a negatively acting factor or an altered conformation of polymerase. This negative effect can be relieved by the same reagents which inhibit the elongation factors. We propose that initiating RNA polymerase II either enters productive elongation or becomes arrested early in elongation.

U 117 PURIFIED RNA POLYMERASE II ELONGATION COMPLEXES LACK TRANSCRIPTION INITIATION FACTOR α. Danny Reines, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.

The RNA polymerase II elongation complex is a nucleoprotein assembly which is a target for: 1) the enzyme systems which process primary transcripts and, 2) regulatory factors that control the ability of polymerase to complete primary transcript synthesis efficiently. This macromolecular structure is, however, poorly characterized. I have developed a novel approach for isolating active RNA polymerase II elongation complexes. A template containing a cloned transcription arrest site positioned downstream from a strong promoter is transcribed *in vitro* using partially purified rat liver RNA polymerase II and general transcription factors. Stable complexes of RNA, DNA, and protein are obtained when polymerase stops at the transcription arrest site. A monoclonal antibody that recognizes RNA is used to precipitate selectively RNA polymerase II molecules which have synthesized RNA chains and are still template-associated. These immunopurified elongation complexes remain functional and respond to the stimulatory action of elongation factor SII. Using this method I tested whether an *initiation* factor remained associated with the transcription complex after initiation. I show that rat liver factor α is not associated with the immunopurified elongation complex. Since α has been shown to associate stoichiometrically with RNA polymerase at the promoter prior to initiation, these data suggest that α departs the complex once RNA chain initiation has occurred.

U 118 EFFECT OF THE NUCLEOTIDE SEQUENCE ON THE IN VITRO RNA ELONGATION RATE BY E.COLI RNA POLYMERASE, Larissa P.Savotchkina, Tatjana V.Svirjaeva, Robert Sh.Beabealashvilli

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A model to estimate the RNA elongation rate depending on the sequence of the DNA-template has been developed. Based on the model, we constructed two recombinant DNA-templates containing the same promoter and remote transcribed regions but different nucleotide sequences of initial transcribed regions (ITR) - "fast" and "slow". Comparison of the ratios of abortive and productive RNA synthesis by E.coli RNA polymerase has shown that "fast" ITR yields more than 95%, but "slow" ITR less than 1% of productive synthesis.

U 119 OBSERVATION OF TRANSCRIPTION BY SINGLE MOLECULES OF *E. COLI* RNA POLYMERASE, Dorothy A. Schafer*, Jeff Gelles†, Michael P. Sheetz‡, and Robert Landick#,

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We have observed transcription by single molecules of *E. coli* RNA polymerase using video-enhanced light microscopy. The movements of 40 nm diameter gold particles that were attached to the upstream ends of DNA templates bound in transcription complexes were followed after the complexes were immobilized on a glass surface. In the absence of NTPs, the Brownian motion of the gold particles was consistent with that of particles attached by flexible tethers (the DNA) to fixed points on the glass surface (the immobilized RNA polymerase). Resumption of transcription by addition of 1 mM NTPs to the immobilized complexes often resulted in an increase in the range of Brownian motion of the tethered gold particles, consistent with a lengthening of the DNA tether. Under optimal conditions, this change in Brownian motion was followed by release of the gold particle from the surface, presumably after transcription proceeded to the end of the DNA template or to a downstream terminator. The change in the Brownian motion resulting from transcriptional elongation was analyzed quantitatively using digital image processing techniques. These analyses revealed a smooth increase in the extent of Brownian motion with time, reflecting elongation of the DNA tether. The rate was similar among several experiments and the average rate was consistent with that observed for transcription in solution. Detection of transcriptional pausing is at the limit of resolution obtained to date. However, analysis of pausing will by possible at lower NTP concentrations. Furthermore, refinement of the technology to visualize and analyze transcription by single RNA polymerase molecules should allow us to determine whether the enzyme moves in single monotonic steps after addition of each nucleotide or jumps in multiple-base-pair steps along the DNA template.

U 120 AN EARLY PERIOD IN ELONGATION BY PROKARYOTIC RNA POLYMERASES IS SPECIFIED BY THE REQUIREMENT FOR β,γ-PHOSPHODIESTER BOND OF ATP, N. Shimamoto, H. Terada, and M. Fujioka

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A linear DNA fragment containing an operator and a promoter was fixed at its ends to an acrylamide bead. Such immobilized DNAs are templates as good as free DNAs for E. coli, Bacteriophage T7, T3 and SP6 RNA polymerases, This technique enables us to interrupt elongation by rapidly diluting a reaction mixture without any changes in buffer components, and to recover transcription complex by brief centrifugation. The interrupted elongation is resumed upon a second addition of substrates. When ATP is replaced by a β,γ-unhydrolyzable analogue in the second addition, the products and their yield are similar to those obtained with the substrates containing ATP. However, the yield of long products is decreased to less than 1/5 when transcription is started with the analogue instead of ATP. In some case, the output of abortive products is increased. Thus the ATP analogue is a good substrate once elongation has been established, but a poor one at an early stage of elongation. We conclude that the β,γ-phosphodiester bond of ATP is important for efficient productive initiation by these RNA polymerases. These results are explained by a model which postulates two forms of transcription complex before elongation is established; one for further elongation and one for abortive initiation. These forms coexist in the early stage of elongation and ATP favors the former.

Poster Session II

U 200 REQUIREMENTS FOR TRANSCRIPTIONAL PAUSING IN THE *his* LEADER REGION

Cathy L. Chan and Robert Landick, Dept. of Biology, Washington University, St. Louis, MO 63130

Transcriptional pausing by *E. coli* RNA polymerase couples transcription and translation both in protein-coding genes and as a component of attenuation control in amino-acid biosynthetic operons. Both the DNA sequence downstream of the active site and formation of an RNA hairpin in the nascent transcript appear important for pausing, but neither the mechanisms underlying these effects nor the extent of RNA hairpin formation are known. We have been exploring these questions using as a model a strong pause that occurs during transcription of the *his* leader region following synthesis of the A:B hairpin and prior to G_{103}. We have examined the effects of neutral salts on pausing at this site. In the presence of elevated KCl, which is known to mask protein-nucleic acid interactions, pausing is reduced. In contrast, elevated potassium glutamate, increases the pause half-life at this site. These results support the idea that RNA polymerase-RNA hairpin or RNA polymerase-DNA interactions direct transcriptional pausing.

We also have investigated the RNA secondary structure requirements for transcription pausing by isolating base substitutions throughout the A:B hairpin. Of the changes examined to date, all on the B side of the hairpin decrease pausing, whereas, some, but not all of the changes on the A side of the hairpin decrease pausing. Interestingly, some of these substitutions reduce the fraction of transcribing RNA polymerase molecules that pause much more than the half-life of those that do pause. When the hairpin's secondary structure is restored by combining complementary substitutions, pausing is restored to near wild-type levels for several of the double substitutions, but not for the C_{71}:G_{93} base pair. Of those tested, this base pair is nearest the base of the hairpin. Furthermore, correction of mismatches in the A:B hairpin by base substitutions in the A side to form a "perfect hairpin" resulted in decreased pausing. These results suggest that not all of the A:B hairpin forms in the paused transcription complex and that features of the RNA hairpin in addition to its stability contribute to pausing.

U 201 SEQUENCES IN THE U5 REGION OF ROUS SARCOMA VIRUS LTR ARE REQUIRED FOR TRANSCRIPTION TERMINATION AND POLYADENYLATION

P. Cleavinger, J.C. Kandala and R.V. Guntaka, Department of Molecular Microbiology and Immunology, University of Missouri, School of Medicine, Columbia, MO 65212

The LTRs of retroviruses carry signals for transcription initiation and termination as well as enhancer sequences. In order to understand the nucleotide sequences that are involved in transcription termination, a vector containing a dominant selectable marker, *neo*^{R}, driven to expression by the RSV LTR, has been constructed. In this construct, LTR sequences from −299 to +44 flank the *neo* gene. Deletions (+44, +20, −23) were then introduced in the U5 (1 to +44) region of the downstream LTR and DNAs from appropriate deletions were introduced into QT6 cells by transfection. Stable G418-resistant transformants were selected, RNA from individual clones were isolated and assayed for transcription termination and processing by Northern blot analysis, S1 mapping, and by PCR amplification. As expected, deletion of the AATAAA sequence in the R region (clone 277) of the LTR eliminated termination and polyadenylation. Deletion of the sequence +20 to +44 (clone 319) resulted in the synthesis of larger precursor transcripts which are probably terminated and polyadenylated in the host cellular sites. In constructs with U5 sequence up to +44 (clone 343) allowed normal polyadenylation and the site is identical to the viral RNA in Prague C virus-infected cells. Southern blot analysis indicated that the downstream LTR sequence is not interrupted during integration. Individual wild-type virus producing clones were also examined for 3' processing properties. Prague C virus-infected and pAPrC transfected clones were isolated and total RNA was obtained from each. Polymerase chain reaction experiments using oligonucleotide primers were carried out to detect the presence of read-through transcripts. Only very small amounts of mRNA extending beyond the polyadenylation site could be detected. From these results we conclude that RSV polyadenylation is preceded by a termination reaction rather than read through and cleavage and that this reaction is probably mediated by the sequence +20 to +44 in the U5 region.

U 202 **Molecular Basis of *nut* Site Recognition.** ***Samit Chattopadhyay, Jaime Garcia,* David Lazinski & Asis Das.** Univ. of Connecticut, Farmington, CT 06030.

The *nut* sites of phage λ, composed of three sequence elements known as boxA, boxB and boxC, govern the interaction of host RNA polymerase with the phage N protein and several host Nus proteins, resulting in the formation of a termination-resistant transcription apparatus. We have recently shown that the RNA sequence encoded by *nut* acts in cis to deliver N to a downstream RNA polymerase. Gel mobility-shift assays reveal that N protein binds the boxB RNA hairpin with a high affinity and specificity, forming two distinct ribonucleoprotein complexes that differ in the relative content of protein. Complex I with lower N-content, which is formed with limiting amount of the protein, appears to be the precursor to complex II containing twice as much N protein. All mutant boxB sequences which show reduced affinity for binding to N in vitro, are defective in antitermination in vivo. The initial binding of boxB, presumably by a monomer of N, is dependent upon both the stem structure and the primary sequence. Specific recognition involves largely hydrophobic interactions between the arginine-motif of N and the 1st and the 3rd nucleotides of the pentamer loop 5'-GAPuAA. The formation of complex II involves recognition of the stem, presumably by a second monomer of N. Surprisingly, substitutions at 2nd, 4th and 5th positions of the loop which cause a severe defect in antitermination in vivo, do not affect N-binding in vitro. Perhaps, the boxB hairpin binds an additional protein - a host factor or the RNA polymerase. In vivo, the consensus boxA sequence can restore antitermination by mutant boxB sequences that are defective in interaction with N in vitro, even with a large excess of the protein. Since N does not bind boxA directly, we hypothesize that a boxA-binding factor binds N and delivers it to RNA polymerase independent of a specific N-boxB interaction.

U 203 **THE ROLE OF PROCESSING SIGNALS IN TRANSCRIPTION TERMINATION BY RNA POLYMERASE II**, Erik Falck-Pedersen and Gretchen Edwalds-Gilbert, Department of Microbiology, Cornell University Graduate School of Medical Sciences, New York, NY 10021.

Both a fuctional poly A site and a downstream termination region are required for efficient transcription termination at the 3' end of a Pol II transcription unit. To further characterize the relationship between RNA processing events and transcription termination we have studied the effects of 1) altered splice acceptor and splice donor sites on polyadenylation and transcription termination and 2) the relationship between poly A site strength and the efficiency of transcription termination. Recombinant adenoviruses were constructed which contain altered processing sequences, either splicing or polyadenylation, inserted into the E1A transcription unit upstream of termination sequences from the mouse beta-globin gene. In contrast to the poly A site alterations, splice site mutations do not impact on termination efficiency. The relationship between polyadenylation and transcription termination will be discussed.

U 204 THE ARGININE-RICH REGION OF LAMBDOID **N** PROTEINS IS CRUCIAL TO THEIR ANTITERMINATION FUNCTION. Naomi C. Franklin, Biology Dept., University of Utah, Salt Lake City, Utah, 84112.

The genomes of bacteriophage λ and its relatives are punctuated by transcription stop signals which prevent complete transcription in the absence of phage-coded **N** and **Q** antitermination proteins. Each phage type has antitermination functions specific to its own genome, apparently because recognition is required between each protein and a *nut* sequence in the transcript of each responding operon. Despite the commonality of function, comparison of the DNA sequences for *N* genes from λ, 21 and P22 showed similarity of size (each coding about 100 amino acids) but no homology of amino acid sequence, with the possible exception of an arginine-rich sequence near the amino-terminal of each protein (Franklin, J. Mol. Biol., 1985). Directed mutagenesis of λ's **N** now shows that arginine positions which could be drawn as conserved, as well as adjacent arginines and one glutamine, are indeed critical to N function and cannot be substituted without loss of function. Alterations have been made changing one codon at a time to nonsense, then using host suppressor mutants to generate 10 different amino acid substitutions. Alternatively, different sets of 3 codons have been scrambled by degenerate oligonucleotide cassettes. Changes in codons near to the critical codons may increase **N** function, but specificity for *nut* and for auxilliary host NusA and NusE proteins has not yet been altered. This mutational analysis is proceeding in vivo, using a 2-plasmid system to detect **N** function via *lacZ* expression (Franklin, Plasmid, 1989). Single base changes in the *boxB* loop of *nut* all diminish **N** function (Doelling and Franklin, NAR, 1989) and serve to test for relaxed specificity of mutant **N** proteins.

U 205 ROLE OF SPECIFIC CYTIDYLATE RESIDUES IN RHO-DEPENDENT TRANSCRIPTION TERMINATION AT LAMBDA *CRO* tRI. James E. Graham and John P. Richardson, Department of Chemistry and Program in Microbiology, Indiana University, Bloomington, IN 47405.

The enhancement of the RNA dependent ATPase activity of *Escherichia coli* transcription termination factor Rho by the presence of cytidylate residues in synthetic cofactor RNAs suggests a specific interaction between cytosines and the termination factor. Our studies have shown that sequences at the 3' end of the lambda *cro* RNA transcript flanking box B are involved in recognition of the RNA by Rho. These sites, designated *rut A* and *rut B* , define a region of approximately 40 residues of above average cytosine content. Site directed mutagenesis of this region has identified individual cytosine to uracil substitutions in the RNA which have significant, but not large, influences on transcription termination *in vitro*. Some substitutions of uracil for cytosine in the *rut* site transcript which alone do not reduce termination, contribute to decreased termination when combined with other substitutions. Although Rho apparently must recognize a variety of sequences to function in transcription termination, its function at lambda tRI appears to involve a cooperative interaction with several specific nucleotide residues.

U 206 TRANSCRIPTION TERMINATION REGION OF GALACTOSE OPERON FROM E. COLI, J. Jeffers, I. Dantas, K. Irmukhanova, S. Osmon, S. Schooley and S. Shell, Department of Chemistry, Ouachita Baptist University, Arkadelphia, AR 71923, and C. Debouck and A. Hsu, Department of Molecular Genetics, SmithKline and French Laboratories, Swedeland, PA 19401

The transcription termination region from the galactose operon of E. coli has been located some 1300 bp distal to the galactokinase gene, the last known gene of the operon. Data will be presented characterizing this transcription termination region.

U 207 **TERMINATION EFFICIENCY AT RHO-DEPENDENT TERMINATORS DEPENDS ON "KINETIC COUPLING" BETWEEN RNA POLYMERASE AND RHO,** Ding Jun Jin[1]*, Richard R. Burgess[2], John P. Richardson[3] and Carol A. Gross[1] Department of Bacteriology[1] and McArdle Laboratory for Cancer Research[2], University of Wisconsin-Madison, Madison, WI, 53706. Department of Chemistry[3], Indiana University, Bloomington, IN47405. * Present address: Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, MD 20892.

Rho dependent terminators constitute one of two major classes of terminators in *E. coli*. Termination at these sites requires the concerted action of RNA polymerase and rho protein. We present evidence that the efficiency of termination at these sites is governed by "kinetic coupling" of the rate of transcription of RNA polymerase and the rate of action of rho protein. Experiments *in vivo* show that slow moving mutant RNA polymerases are termination proficient while fast moving mutant RNA polymerases are termination deficient and that a slow moving RNA polymerase can suppress the termination deficiency of a slow acting mutant Rho protein. Termination experiments *in vitro* indicate that termination efficiency at a rho-dependent terminator is an inverse function of the rate of elongation of RNA polymerase, and each of the mutant phenotypes can be accounted for by the altered rate of elongation of the mutant RNA polymerase. Because of the close coupling of rho action with RNA polymerase, small changes in the elongation rate of RNA polymerase can have very large effects on termination efficiency, providing the cell with a powerful way to modulate termination and antitermination at rho-dependent terminators.

U 208 LAMININ B2 CHAIN GENE (LAMB2) EXPRESSION AND mRNA PROCESSING, Tuula Kallunki and Karl Tryggvason, Biocenter and Department of Biochemistry, University of Oulu, SF-90570 Oulu, Finland.

Laminin is a complex basement membrane protein which has numerous biological functions. The basic laminin molecule is composed of three chains: one A chain and two B chains. To date, three different B chains and two A chains have been described, which may give rise to several different laminin isoforms. Studies on the expression of individual laminin chain genes are important for understanding the nature of the different laminin forms. We have isolated the entire human LAMB2 gene, characterized its exon-intron structure and analyzed its promoter region and 3'-end untranslated region. Sequence analysis of the 5'-flanking region revealed absence of TATA and CAATT boxes and the presence of six GC-boxes and three potential AP-2 binding sites. The B2 chain has two mRNAs: 7.5 kb and 5.5 kb. Analysis of the 2797 bp of the 3'-end untranslated region revealed 68.8 % sequence identity with the corresponding mouse sequence. It also revealed five potential polyadenylation signals in two clusters separated by 2 kb, the clusters containing of two and three signals, respectively. Primer extension and S1 nuclease mapping analysis indicated the presence of two promoters separated by 200 bp each of which has 3-4 closely spaced transcription initiation sites. Northern analysis indicated that the first promoter drives transcription leading to a 5.5 kb mRNA and the second promoter is needed for the generation of the 7.5 kb mRNA. This suggests that the promoter has a role in the choice of polyadenylation site.

U 209 CONTROL OF THE HUMAN C-MYC BLOCK TO ELONGATION, Anton Krumm, Tea Meulia-Sossi, Sharon Plong, Charlotte Spencer and Mark Groudine, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Transcription of the human c-myc gene is regulated at both the level of initiation and elongation. Recent data from our laboratory indicates that polymerase molecules initiating transcription at the c-myc P1 promoter read-through the site of the elongation block at the end of exon 1, whereas transcipts initiated at the P2 promoter can be modulated to block or readthrough. We are using a combination of approaches to determine the sequences involved in regulating efficient c-myc promoter use and the block to elongation. These approaches include the analysis of activity and effect on the elongation block of linker-scanner mutations within the P2 promoter in an in vitro transcription assay, and in vivo after injection into Xenopus oocytes and after stable integration into mammalian cells. In addition, using genomic footprint analyses, we have monitored DNA/protein interactions in the c-myc promoter regions in vivo in proliferating and differentiated HL60 cells. Preliminary results of in vivo footprinting analyses reveal a correlation between the chromatin structure of the c-myc promoter region and the transcriptional activity. We are also examing the sequence elements within the immunoglobulin heavy chain region that may be involved in effecting a switch from P2 to P1 promoter usage in Burkitt Lympoma cells, which are characterized by translocations that juxtapose c-myc and the IgH sequences.

U 210 SINGLE-STANDED REGIONS OF DNA IN PAUSED AND STALLED TRANSCRIPTION COMPLEXES, Donna N. Lee and Robert Landick, Department of Biology, Washington University, St. Louis, MO 63130

Formation of an RNA hairpin in the nascent transcript is one determinant of transcription pausing by *E. coli* RNA polymerase. However, the effect of hairpin formation on the transcription complex is unknown. One model suggests that formation of an RNA hairpin in the nascent transcript disrupts an RNA:DNA heteroduplex and that the transcription complex pauses until the RNA:DNA heteroduplex is reformed. To test this model, we have isolated and determined the regions of single-stranded DNA in transcription complexes that either are paused after RNA hairpin formation or stalled by nucleotide limitation. Paused transcription complexes were formed on the *trp* and *his* leader regions. Stalled transcription complexes contained a 20 nucleotide transcript (A20 complexes). To form additional defined transcription complexes, both paused and stalled complexes were walked from these sites using an incomplete set of nucleotides. Using several chemical probes that detect the single-stranded DNA regions in these transcription complexes, we have determined that in both paused transcription complexes and stalled complexes (i) a 12-17 nt single-stranded bubble is cleaved by the probes; (ii) the active site is located near the downstream edge of the transcription bubble; and (iii) ~8 nts of the template strand upstream of the active site are protected from cleavage. These results are consistent with the presence of an ~8 bp RNA:DNA heteroduplex, although other explanations for template-strand protection are possible. If an ~8 bp RNA:DNA heteroduplex exists, it clearly is not disrupted in the paused transcription complexes. The presence of the transcription factor NusA or a mutant β subunit that increases pausing does not alter the single-stranded regions of DNA in the either stalled or paused transcription complexes. We now are determining the regions of DNA in these transcription complexes that are cleaved by DNase I and hydroxyl radicals.

U 211 A SYSTEM FOR THE *IN VIVO* ANALYSIS OF MUTATIONS IN THE TRANSCRIPTION TERMINATION FACTOR RHO. Asunción Martínez and John P. Richardson. Department of Biology and Department of Chemistry, Indiana University, Bloomington, IN 47405.

Rho is an essential Eschirichia coli protein involved in transcription termination. We have initiated a mutational analysis of the rho gene to identify those residues that are critical for function *in vivo*. We have set up a system in which the chromosomal copy of the rho gene has been interrupted and a wild-type gene is provided in a temperature sensitive plasmid. The mutagenized copy of the rho gene to be tested is then introduced in the ts strain in a compatible plasmid. When cells are plated at 42°C, the ts plasmid is lost and viability depends on the function of the gene being tested. We have tested in this system mutations created by oligonucleotide cassette mutagenesis in 12 of the first 13 amino acids of the amino-terminal RNA-binding domain of rho. Permissive mutations have been recovered at each of this positions indicating that none of these amino acids are absolutely required for rho function *in vivo*.

U 212 ON THE HEAT OF TRANSCRIPTION AND ITS DISPERSAL, Robert L. Metzenberg and Stan T. Metzenberg*, Department of Physiological Chemistry, University of Wisconsin, Madison WI 53706, *Intercampus Program in Molecular Parasitology, University of California, San Francisco CA 94143.

In a reinterpretation of existing physical and biochemical literature, we propose a new model for initiation and termination of bacterial transcription. In brief, since energy equal to roughly 24.3 kJ/mole must be lost as heat during phosphodiester bond formation, one can calculate an immediate local temperature rise of 5840^{0}C divided by the number of degrees of freedom. This energy pulse must, of course, be transmitted rapidly to the surrounding bath molecules. We suggest that, on the way to Boltzmann equilibrium, the energy pulse is respnsible for several phenomena: abortive initiation (due to limiting degrees of freedom in the nascent oligonucleotide), termination induced by rifampicin (binding of the drug may reduce the available degrees of freedom in the polymerase), factor-independent termination (RNA subterminal stem-loop structure limits the degrees of freedom in the nascent RNA chain), and the inability of RNA polymerase to incorporate 8-BrGTP and other rotationally-restricted analogs in the complete absence of their natural counterparts. We also propose that rho protein causes termination of transcription by hydrolyzing ATP and thus injecting additional enthalpy into the ternary complex. Although this model is frankly speculative, it is concordant with a large volume of published data on RNA polymerase. The model also makes predictions which can be tested experimentally.

U 213 THE ROLE OF RNA POLYMERASE β' SUBUNIT AND *NUS*A ARGININE DOMAIN IN TERMINATOR SELECTIVITY, Yoshikazu Nakamura and Koichi Ito, Institute of Medical Science, University of Tokyo, P.O. Takanawa, Tokyo 108, Japan

The *nusA11* mutation causes reduced transcription termination and Ts growth of *E. coli*. Suppressor mutations that restored growth of *nusA11* cells were isolated. One of them, *rpoC10*, was located in the *rpoC* gene which encodes the β' subunit of RNA polymerase. *rpoC10* compensated the defect in the t_{R1} termination by *nusA11* and, by itself, stimulated termination of transcription at the Rho-dependent terminators and, surprisingly, reduced termination at the Rho-independent sites. *rpoC10* is specific to the *nusA11* allele and unable to suppress Cs growth of the *nusA10* mutant. The *nusA* mutation sites were determined by nucleotide and peptide sequence analyses. *nusA10* carries two amino acid changes at position 104 and 212; *nusA1* and *nusA11* are located in an arginine-rich peptide region and substitute arginine and aspartate for leucine-183 and glycine-181, respectively. *nusA11* can be suppressed by replacing the mutated aspartate-181 to alanine or substituting aspartate-84 to tyrosine. The anti-arginine domain monoclonal antibody did not inhibit NusA-RNA polymerase binding; thereby we infer that the arginine domain of NusA is involved in recognition of RNA and *rpoC10* indirectly suppresses *nusA11* by a changed interaction with RNA at the termination site.

U 214 INACTIVATION OF RHO BY 8-AZIDO-ATP

InSug O and Barbara L. Stitt, Department of Biology, New York University, Washington Square East, New York, NY 10003

E. coli transcription termination factor rho is an α_6, 276 kDa protein which releases RNA polymerase and nascent RNA from transcription complexes. Rho hydrolyzes nucleoside triphosphates during transcription termination, but the molecular mechanism of coupling of the obligatory NTP hydrolysis is not understood.

To study the mode of action of rho, a photoaffinity analogue of ATP, 8-azido-ATP, has been used in our lab. When rho is irradiated with greater than 300nm light in the presence of 8-azido-ATP, the ATP hydrolysis activity of rho is inactivated. ATP, the normal substrate for rho, prevents the inactivation of rho by 8-azido-ATP. The site of 8-azido-ATP modification and the stoichiometry of inactivation of rho by 8-azido-ATP have been determined. The stoichiometry of the inactivation has been studied by correlating the ATP hydrolysis activity with the amount of labeling by α-^{32}P-8-azido ATP. Partial tryptic digestion of rho after inactivation, followed by polyacrylamide gel electrophoresis analysis shows that 8-azido-ATP covalently binds to a 15 kDa fragment of rho. The identification of a labeled fragment of rho after complete trypsin digestion has been performed by HPLC.

U 215 A PHYLOGENETIC COMPARISON OF PRIMARY AND PREDICTED SECONDARY STRUCTURE OF THE TRANSCRIPTION TERMINATION FACTOR RHO. Timothy Opperman and John Richardson. Department of Biology and Department of Biochemistry, Indiana University, Bloomington, IN 47405

We have cloned the genes for the putative homologs of the E. coli transcription termination factor Rho from Pseudomonas flourescens and Chromatium vinosum. The nucleotide sequence of these genes was determined and used to deduce the predicted amino acid sequences of the putative Rho proteins. The primary structure of both of these proteins appears to be highly conserved with approximately 81% identity overall when compared to E. coli Rho. When conservative amino acid substitutions were considered the proteins exhibit 91% similarity overall. The degree of similarity appears to vary between the different functional domains. The ATPase domains exhibit approximately 93% identity with E. coli Rho, while the N-terminal RNA binding domains exhibit approximately 77% identity. An analysis of the predicted secondary structures will be presented in an attempt to deduce conserved structural features of the Rho protein that may be related to function.

U 216 MOLECULAR ANALYSIS OF THE COMLPEX TRANSCRIPTION TERMINATION REGION OF E. COLI RIBOSOMAL OPERON rrnB, A., Orosz, I. Peres and P. Venetianer, Inst. of Biochemistry, Biol. Res. Ctr., Hung. Acad. of Sci., Szeged, POB 521, Hungary.

According to the sequence data the rrnB ribosomal RNA operon has two strong, factor independent transcription termination signals (T1,T2) separated by two inverted repeat structures (IR1,IR2) at the 3'end of the operon. We have analysed how each of these complex transcription terminator subregions, either alone or in combination, can contribute to the termination process by cloning them into a terminator probe vector where their efficiency can readily be measured by assaying CalK enzyme activity. Conclusions have been drawn concerning the essential sequence requirements of terminators and the importance of sequence elements, upstream and downstream of the terminators in the 3'end formation process. Terminator orientation dependence will also be discussed. The results also suggest promoter-terminator and translating ribosome-terminator interactions, that can markedly influence the stregth of a particular transcription termination signal.

U 217 TRANSITION OF POLYMERASE I FROM INITIATION TO ELONGATION: PROGRESSIVE DNA MELTING DURING INITIATION, Marvin R. Paule and Han Li, Department of Biochemistry and The Cell and Molecular Biology Program, Colorado State University, Fort Collins, CO 80523

We have been examining the transition of RNA polymerase I from the initiation complex into the elongation complex. The initiation complex consists of a single *trans*-acting factor, TIF, bound upstream of +1 and pol I bound downstream, directed by protein-protein contact with TIF (1987, **CELL 50**, 693). This complex is a closed promoter complex, as demonstrated by footprinting of melted DNA with diethylpyrocarbonate (DEPC) and by changes in supercoiling of covalently closed template (1988, **MCB 8**, 1940). Using a series of point mutants near +1, we have been able to move pol I down the DNA in controlled increments by depriving the system of a required nucleotide, and to analyze how melting occurs during these movements. We conclude that melting does not require energy coupling to ATP β-γ hydrolysis because nonhydrolyzable analogs can substitute for ATP. Binding of ATP alone does not lead to melting because a +2 mutant preventing formation of the first phosphodiester bond is not modified by DEPC. A +3 mutant allowing formation of only the first bond also is not susceptible to DEPC, while a complex stalled at +8 is modified at -1, -2, -3, +1, +2, +3, +6 and +7. Complexes stalled at +13 still show some modification of -1, -2 and -3, as well as at the downstream positions. We interpret these results in terms of a progressive melting of the DNA as pol I begins moving down the template, as opposed to a massive conformational change in which the entire melted bubble is produced in a single step. Supported by USPHS grant GM22580.

U 218 PURIFICATION AND CHARACTERIZATION OF A PROTEIN KINASE THAT PHOSPHORYLATES THE CARBOXYL-TERMINAL DOMAIN OF RNA POLYMERASE II, John M. Payne and Michael E. Dahmus, Department of Biochemistry and Biophysics, University of California Davis, Davis, CA 95616

The largest subunit of RNA polymerase II exists *in vivo* in two forms differing with respect to the extent of phosphorylation of a highly repetitive domain located at the carboxyl terminus (CTD). RNA polymerase IIA, the unphosphorylated form, associates with the adenovirus-2 major late promoter in the presence of transcription factors. RNA polymerase IIA complexed to the promoter is converted to the extensively phosphorylated IIO form upon the addition of ATP. Starting with a crude transcription extract from HeLa cells, we have partially purified an RNA polymerase II CTD kinase that is capable of converting RNA polymerase IIA to IIO. We have previously demonstrated that this kinase co-fractionates with the essential transcription factors TFIIE/F/G. Here we present the further purification of this kinase by Mono Q, Mono S, and RNA polymerase II affinity chromatography. Kinase activity in column preparations after Mono Q has been assayed by [^{32}P] incorporation into the IIa subunit. Kinase activity before Mono S chromatography has been assayed by mobility shift of RNA polymerase IIA labeled with [γ-^{32}P]ATP and casein kinase II. Chromatography over Mono Q yields two qualitatively different activities. The first, CTDK1, shifts the IIA substrate to a distinct band with a mobility between IIa and IIo on an SDS-polyacrylamide gel. The second, CTDK2, completely shifts the substrate to the position of IIo. CTDK1 chromatographs differently on an RNA polymerase II affinity column than TFIIF. Moreover, neither activity co-elutes with TFIIF on a Mono Q column as demonstrated by reactivity with an anti-TFIIF antibody. These results are consistent with the idea that the kinase is a component of TFIIE/G, and not TFIIF.

U 219 SEQUENCE-SPECIFIC 3' ENDS IN POXVIRUS EARLY AND LATE RNAS ARE PRODUCED BY TWO DIFFERENT MECHANISMS, David J. Pickup, Carol A. Ray, Dhavalkumar D. Patel, James B. Antczak, and Barbara S. Ink, Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710

The poxvirus DNA is transcribed by a virus-encoded RNA polymerase that resembles RNA polymerase II. Before viral DNA replication, transcription of viral genes is terminated about 50 nucleotides downstream of the signal UUUUUNU (Shuman and Moss, 1988, J. Biol. Chem. 263, 6220). After the onset of viral DNA replication, transcriptional termination of this type is discontinued. Although most late RNAs appear to have heterogeneous 3' ends, some late RNAs, such as the abundant transcripts of the *160K* gene of cowpox virus, have defined 3' ends. Sequence analysis of cDNA clones of the 3' ends of the late RNA transcripts of the *160K* gene has shown that most of these RNAs contain exactly the same sequence immediately upstream of their 3' poly(A) tails. A *cis*-acting signal lacking the UUUUUNU sequence that directs the termination of early transcription directs this precise RNA 3' end formation at late times during infection. This signal operates in an orientation-dependent manner. It operates in the context of the vaccinia virus genome, and it operates when placed downstream of the promoters of other late genes whose late RNA transcripts normally lack defined 3' ends. Deletion analyses have been used to define the minimum signal necessary to effect this RNA 3' end formation. Part of this signal is downstream of the sequence that constitutes the 3' ends of the mature RNAs. These results suggest that two distinct mechanisms are employed to effect the formation of the 3' ends of the viral RNAs during different stages of the viral replication cycle.

U 220 TRANSCRIPTION FACTOR IIF PREFERENTIALLY RECRUITS THE NON-PHOSPHORYLATED FORM OF RNA POLYMERASE II INTO THE PREINITIATION COMPLEX, Danny Reinberg, Hua Lu and Osvaldo Flores, Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854-5635.

The general transcription factor TFIIF was found to be required for the binding of RNA polymerase II to a pre-initiation complex containing the TATA binding protein, TFIID, and the general initiation factors IIA and IIB (DAB complex). The resulting DNA-protein complex (DABPolF) contained RNA polymerase II and the two subunits of TFIIF (RAP 30 and RAP 74). The two forms of RNA polymerase II that exist *in vivo*, phosphorylated and non-phosphorylated, were purified to apparent homogeneity and it was shown that the non-phosphorylated form of RNA polymerase II preferentially binds to the preinitiation complex. The non-phosphorylated form of RNA polymerase II was converted, after association with the preinitiation complex, to the phosphorylated IIo form. The conversion was catalyzed by a protein kinase, which could be separated from the general transcription factors. Addition of the partially purified protein kinase to a reconstituted transcription assay resulted in approximately 4-fold stimulation. The observed stimulation was specific for reactions reconstituted with the non-phosphorylated IIa form of RNA polymerase II. The protein kinase was without effect when added to transcription reaction mixtures reconstituted with the phosphorylated IIo form of RNA polymerase II.

U 221 TRANSCRIPTION TERMINATION FACTOR RHO FUNCTIONS AS A DIMER WHICH RELEASES POLYNUCLEOTIDE COFACTOR DURING THE ATPase REACTION CYCLE

Steven E. Seifried, Yan Wang, and Peter H. von Hippel.
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Transcription termination factor rho from *E. coli* has been shown to assemble as a trimer of dimers. Each dimer of identical protomers will function as an ATPase. The phosphohydrolase activity requires the occupation of a polynucleotide binding site on each monomer. Affinity for both cofactor sites in the dimer is determined by the base composition of the polymer; cytosines provide greatest free energy of binding, uracil provides moderate affinity, and purines do not contribute to binding. It has been stated that activation of the ATPase activity occurs if a 2' hydroxyl is contained within the nucleic acid bound in at least one of the adjacent cofactor sites.

ATPase functional assays with homopolymers, random copolymers, and designed oligonucleotides show that release of polymer from one of the two cofactor sites within the dimer is required for the continuation of the ATPase reaction cycle. Polymers of deoxycytosine can generate a low but significant ATPase rate. Competition experiments with ribo-polymers show the offrate of poly(dC) from a single polymer binding site to equal the measured poly(dC)-stimulated ATPase rate. Therefore the 2' hydroxyl of the ribose sugar increases the offrate of the polymer from an activated enzyme state. Additionally, base sequence can affect the offrate of an oligomer, and therefore the turnover rate of the ATPase cycle. The RNA bind-release cycle, driven by ATPase, is coupled to 3-D geometric elements to generate translocation of rho on nascent transcript.

U 222 MULTIPLE MECHANISTIC STEPS IN TRANSCRIPTION TERMINATION BY RNA POLYMERASE III: DEPENDENCE OF TERMINATION ON RNA STRAND DISPLACEMENT DURING ELONGATION, David R. Setzer and Frank E. Campbell, Jr., Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH 44106. We have studied transcription elongation and termination by purified RNA polymerase III using poly dC-tailed linear DNA molecules as templates. Purified pol III efficiently initiates transcription at two adjacent positions 3 and 4 nucleotides upstream of the single-strand/double-strand junction of these molecules in the absence of other transcription factors. Approximately 60% of the polymerase molecules that initiate on these templates read through the normally efficient *X. borealis* somatic-type 5S rRNA gene transcription terminator, while 40% correctly terminate at one of the two adjacent termination signals in this gene. We judge these to be authentic termination events based on kinetic analyses, determination of the 3′ ends of the transcripts, and demonstration of transcript release from the template. We have shown that terminated transcripts exist as free RNA molecules, whereas read-through transcripts exist as RNA/DNA hybrids, demonstrating that transcript displacement from the template coding strand during transcription elongation is required in order for termination to occur. Interestingly, kinetic analyses have shown that essentially all polymerases recognize and pause at the termination signals, even though only 40% actually terminate. We interpret these results in terms of a two-step model for transcription termination. In the first step, the elongating RNA polymerase recognizes the termination signal in the DNA template and pauses; this step is not dependent on RNA strand displacement during transcription elongation. In contrast, the second step, polymerase disengagement and transcript release, occurs only if the RNA strand has been displaced during elongation. We suggest that the efficiency of this second step is dependent upon the stability of the ternary complex at the termination signal, which in turn is dependent on the stability of the RNA/DNA hybrid just upstream of the termination site.

U 223 VACCINIA VIRUS mRNA CAPPING ENZYME IS A TRANSCRIPTION TERMINATION FACTOR.

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

Transcription of vaccinia virus early genes terminates downstream of the signal TTTTTNT in the nontranscribed DNA strand. Purified vaccinia RNA polymerase (a multisubunit enzyme with similarity to cellular RNA polymerase II) is itself unable to terminate appropriately, but is rendered competent to terminate in the presence of a viral transcription termination factor, VTF. VTF contains two subunit polypeptides of Mr 95,000 and Mr 31,000 and is, by the criteria of copurification and thermal coinactivation, seemingly identical to the vaccinia virus mRNA capping enzyme.The viral genes encoding the large and small subunits of vaccinia capping enzyme have been expressed in E. coli. Our results indicate that the Mr 95,000 and Mr 31,000 viral gene products are together sufficient to catalyze all three enzymatic steps in cap synthesis. The RNA triphosphatase and RNA guanylyltransferase domains are localized to the large subunit per se, while the small subunit is required (if not sufficient) for RNA (guanine-7)-methyltransferase. Expression of carboxy-deleted forms of the large enzyme subunit in E. coli further localized the guanylyltransferase domain to the amino two-thirds of the Mr 95,000 polypeptide. Significantly, we have demonstrated that the heterodimeric capping enzyme purified from E. coli can promote termination of transcription in vitro by purified vaccinia virus RNA polymerase, and that this termination requires the cis-acting termination signal TTTTTNT in the nontranscribed DNA strand. Thus, we have proven that capping enzyme and vaccinia termination factor are truly identical. Mechanistic studies of termination suggest that the termination signal is actually recognized at the RNA level as the sequence UUUUUNU in the nascent transcript.

U 224 RHO-RNA INTERACTIONS: RNA OFF-RATES AND ATP HYDROLYSIS STOICHIOMETRY, Barbara L. Stitt, Department of Biology, New York University, Washington Square, New York, NY 10003

E. coli transcription termination factor rho binds to ribosome-free nascent RNA in transcription complexes and then hydrolyzes ATP to effect transcript release. The role of ATP hydrolysis in transcription termination is uncertain, and the number of ATP hydrolyzed per transcript released is unknown. To obtain information concerning the molecular mechanism of rho action, we have used isotope-trap type experiments to learn whether the off-rate of RNA from rho•RNA complexes changes under ATP hydrolysis compared with nonhydrolysis condition. Our initial results indicate that the bacteriophage lambda *cro* mRNA is released from rho at least 200-fold faster when ATP is hydrolyzed by rho than it is in the absence of ATP. The experiments also indicate that only a single molecule of ATP is hydrolyzed per rho•*cro* RNA complex before the RNA is released. We discuss the significance of these findings in light of the use of poly(dC) as the trap component that prevents released RNA from rebinding to rho.

U 225 EFFECTS OF THE TEMPLATE TOPOLOGY AND TRANSCRIPTION FACTORS ON THE EFFICIENCY OF ELONGATION AND TERMINATION DURING RIBOSOMAL RNA TRANSCRIPTION *IN VITRO* Wagner, R., Krohn, M., Pardon, B., Klemens, A. and Theißen, G. Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstraße 1, D-4000 Düsseldorf 1

A complex regulatory network ensures the very efficient transcription of ribosomal RNA genes in bacteria. Efficiency and balance of synthesis are mediated by cis-acting sequence elements and trans-acting factors. Regulatory mechanisms involve RNA polymerase pausing or premature termination at specific sites within the transcription units. Here we describe the results of a series of *in vitro* studies taking advantage of a single round transcription system where the effects on elongation can be investigated independent from initiation (Levin, J. R., Krummel, B. and Chamberlin, M. J. 1987, J. Mol. Biol. **196,** 85-100). Our studies have shown that certain RNA polymerase pauses within the leader region of the *rrnB* operon are strongly affected by the superhelical density of the template. This was found for Nus A-dependent as well as for factor independent pauses. Unexpectedly, it turned out that even the readthrough of a Rho-dependent termination site within the 16S RNA gene was totally dependent on a superhelical template. In addition, some peculiar pauses induced by single base transitions up to 80 nucleotides downstream from the pause site were completely suppressed when a relaxed plasmid template was used. Consequences for the mechanism of transcription pausing, termination and the action of transcription factors Nus A and Rho are discussed.

U 226 CHARACTERIZATION OF *rho026*, Robert Washburn and Barbara L. Stitt, Department of Biology, New York University, Washington Square, New York, NY 10003

Rho factor in *E. coli* effects the release of RNA from transcription complexes. Rho also has an important role in controlling gene expression at the transcriptional level. One example is seen in T4 phage-infected *E. coli*. There are two classes of early T4 genes, immediate early and delayed early, which are separated by a rho-dependent terminator. It is thought that the delayed early genes are normally transcribed in part by readthrough, when an early viral gene product suppresses the action of rho. Rho mutants which block the expression of particular delayed early T4 genes have been isolated. The *E. coli* strain HDF026 contains such a mutation, *rho026*, in its *rho* gene.

In order to initiate a study of the participation of rho in T4 gene expression, and to gain more information about structure-function relationships in rho, we are characterizing the *rho026* protein. We have cloned *rho026* from the HDF026 genome, inserted it into an overproducing plasmid vector, and purified the mutant protein. We have determined the nature of the mutation in *rho026* from DNA sequencing, and have started to characterize the activity of the *rho026* protein.

U 227 MECHANISTIC STUDIES OF FACTOR-INDEPENDENT TERMINATION, A-Young M. Woody, Patricia A. Osumi-Davis, Marcela P. C. de Aguilera, and Robert W. Woody, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523. Termination remains the most poorly understood step in transcription. 33- and 46-base RNAs possessing all the features of the T7 early and late terminator (33W and 46W RNAs) and three 33-base mutant RNAs which lack the possibility of forming stem-loop structures, all bind to *E. coli* RNA polymerase with n=1 and K_d=20-60nM, when analyzed by nitrocellulose filter-binding. Competitive electrophoretic mobility-shift assays indicate that the mutant RNAs and 46W RNAs displace 33W RNA from *E. coli* RNA polymerase. Time-dependent electrophoretic mobility-shift assays show that a template containing T7A2 promoter and Te terminator displaces 33W and 46W RNAs in less than 10 seconds, but the mutant RNAs are only partially displaced after 3 minutes. 33W and 46W RNAs are not inhibitors of transcription, whereas the mutant RNAs are. If ternary complexes are preformed, none of the RNAs can function as an inhibitor for the elongation of the nascent RNA. The deoxyoligonucleotides which will produce the aforementioned five RNAs have been introduced into suitable cloning sites behind T7A2 promoter in pUC8 plasmid. Transcription on the templates containing T7A2 promoter and deoxy33W in supercoiled as well as linearized forms indicate stronger termination when the sequence down-stream of the release point is single-stranded. A working hypothesis of factor-independent termination mechanism will be presented based upon our data. (Supported by USPH GM-23697)

U 228 TRANSFER RNA GENE TRANSCRIPTION IN PLANTS, Michael G. Zwick, Scott E. Franklin, Julie M. Palmer and William R. Folk, Department of Biochemistry, 117 Schweitzer Hall, University of Missouri, Columbia, MO 65211

We are attempting to dissect transcription of plant tRNA genes by RNA polymerase III. A gel retardation assay has been used to detect a carrot nuclear protein which binds stably to a B box consensus sequence (a component of the internal control region of tRNA genes to which animal cell and yeast transcription factor IIIC binds). Two forms of this factor are seen in gel retardation assays and these can be partially separated by FPLC Mono Q chromatography. The two forms do not appear to be convertible by acid phosphatase treatment. Protein blots of extracts containing TFIIIC activity were probed with B box multimers and we identified a DNA binding subunit of this protein with a M_r of 90 kD. We are currently trying to identify the other components required for transcription in order to reconstitute an accurate RNA polymerase III transcription system.

Two $tRNA_i^{met}$ genes, one from soybean and another from *Arabidopsis*, have been examined for their transcription activity in human 293 cell extracts. These genes are identical in their coding regions but have no homology in their flanking regions, yet the transcription activity of the *Arabidopsis* gene is much greater than that of the soybean. A tRNA processing activity from carrot cell extracts is able to process the *Arabidopsis* $tRNA_i^{met}$ transcript, but not the soybean. Increasing the spacing between the A-B box promoter region abolishes transcription of both these genes. This contrasts markedly with observations made with animal cell $tRNA_i^{met}$ genes. We are now attempting to explain the differences in transcription and processing of these two genes.

Poster Session III

U 300 THE ROLES OF POLYADENYLATION AND TRANSCRIPTION TERMINATION IN THE REGULATION OF BPV-1 LATE GENE EXPRESSION, C.C. Baker and P.A. Furth, Laboratory of Tumor Virus Biology, NCI, NIH, Bethesda, MD 20892

The BPV-1 structural (late) genes lie immediately downstream of the early poly(A) site and, in productively infected cells, are expressed in the same transcriptional orientation as early genes. In nonproductively infected (transformed) mouse C127 cells, steady state levels of late region mRNAs are approximately 4 orders of magnitude lower than those of early region mRNAs. Analysis of BPV-1 transcription rates in BPV-1 transformed cells showed a 10-20 fold exponential decrease in the rate of transcription between the early and late poly(A) sites. A series of deletion mutations of various sizes have been made between the early and late poly(A) sites to determine if there are specific sequences which are required for transcription termination. Nuclear run-on analysis of cell lines harboring these BPV-1 mutants are in progress. However, analysis of steady state levels of late cytoplasmic mRNAs from these cell lines indicates that the amount of late mRNA shows an exponential relationship with deletion size. This data is consistent with transcription termination between the early and late poly(A) sites having a direct effect on late poly(A) site usage and suggests that there are no specific sequences between the two poly(A) sites which are required for transcription termination. An alternative interpretation is that deletions within the late region decrease the time that the early poly(A) site is the only poly(A) site available on the nascent transcript and that transcription termination between the two poly(A) sites is the consequence of polyadenylation at the early poly(A) site. Distinguishing between these two models is complicated by the fact that early poly(A) site mutants [E. Andrews and D. DiMaio, pers. comm.; Burnett et al., Plasmid 20:61-74, 1988] show a strong preference for use of cryptic poly(A) sites within the vicinity of the early poly(A) site rather than the late poly(A) site. Data will also be presented suggesting that the choice between use of the early or late poly(A) sites can be influenced both by the level of transcription and by specific splice site choices.

U 301 ANALYSIS OF THE BASEPAIRING INTERACTION BETWEEN MAMMALIAN U7 AND THE DOWNSTREAM ELEMENT OF HISTONE PRE-mRNAS. Ursula Bond, Therese Yario and Joan Steitz, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510.

The 3' ends of non-polyadenylated histone mRNAs are generated post-transcriptionally by a processing reaction which cleaves a 3' terminal extension from the pre-mRNA. Two sequence elements are required for correct 3' end processing: a highly conserved hairpin loop upstream of the processing site and a downstream element (DSE), which is highly conserved in sea urchins but less well conserved in higher eucaryotes such as mammals. Several lines of evidence argue that the U7 snRNP is an essential component of the processing reaction and interacts with the DSE via basepairing. Yet, the DSE is so loosely conserved in mammals that the exact contribution of it's basepairing with U7 remains unclear. We have therefore undertaken suppression studies using mutations in the DSE of a mouse H2A gene and in the 5' end of mammalian U7 snRNA. We have analysed the effects of these mutations on histone pre-mRNA 3' end processing both in vivo and in vitro in HeLa cells. Our results indicate that deletion or replacement of specific sequences within the DSE either abolish or significantly decreases 3' end processing in vivo and in vitro. Analysis of in vivo transcripts also revealed the presence of a second processing site further downstream which can be used when the proximal site of the H2A pre-mRNA is non-functional. To ask whether the lack of processing of the DSE mutants could be rescued by changes in U7 RNA, we introduced compensatory base changes into a co-transfected U7 'gene'. In the presence of the resulting suppressor U7 RNAs, processing at the correct 3' end was increased but not completely restored in vivo; the same effects were reproduced in vitro in processing extracts derived from cells carrying the suppressor U7 snRNP. Our results provide the first direct evidence for basepairing between the DSE of mammalian histone pre-mRNA ands U7. However, they also indicate an important role for the precise sequence of the base-paired region in the mechanism of histone pre-mRNA 3' end processing.

U 302 IDENTIFICATION AND CHARACTERIZATION OF A *SACCHAROMYCES CEREVISIAE* POLYADENYLATION MUTANT, J. Scott Butler, Daksha Patel and Songlin Liang, Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642

As part of our efforts to understand mRNA 3' end formation, we screened a collection of temperature sensitive mutants for defects in mRNA 3' cleavage and polyadenylation *in vitro*. Whole cell extracts from one isolate (ts148) show apparently normal cleavage, but little polyadenylation of synthetic pre-mRNAs. Polyadenylated RNA added to such extracts remains stable under these conditions suggesting that the defect does not accelerate poly(A) tail shortening. Genetic analysis shows that this defect *in vitro* results from a single recessive mutation which cosegregates with the temperature sensitive phenotype. Growth of ts148 strains at the permissive temperature followed by shift to the nonpermissive temperature results in a rapid decrease in the poly(A) content of the cells. Direct measurement of poly(A) tail lengths confirms the loss of poly(A) tails under these conditions. Interestingly, loss of poly(A) tails does not appear to have a drastic effect on the levels of several mRNAs monitored by Northern blotting. We have cloned the ts148 gene by complementation of the temperature sensitivity and its structure is being analyzed. We have also started to search for interacting gene products by isolating extragenic suppressors of the original temperature sensitive mutation.

U 303 REGULATION OF POLYADENYLATION IN RETROVIRUSES, Julie M. Cherrington and Don Ganem, Department of Microbiology and Immunology, University of California-San Francisco, SanFrancisco, CA. 94143

The synthesis of genomic RNA in hepadnaviruses, retroviruses and retrotransposons requires bypass of the polyA signal in the upstream position of the RNA (ie, the 5' terminal redundancy) but use of this site in the 3' redundancy. The mammalian hepadnaviruses contain a variant hexanucleotide (TATAAA) which upon first pass, poorly directs 3' end processing (20%). Previous work from our lab on hepadnavirus (GSHV) polyadenylation has identified two cis elements upstream of the variant hexanucleotide which increase its processing efficiency. Additionally, we have shown that the U3 sequences (sequences upstream of the transcription start site) of two retroviruses are able to functionally substitute for these hepadnavirus cis sequences to promote processing via the hepadnavirus polyA signal. Like GSHV, first pass 3' end processing in HIV is approximately 20%. However, in contrast to our findings with GSHV, our results with HIV indicate that the HIV hexanucleotide and downstream G/T cluster alone are sufficient to direct efficient 3' processing. When HIV sequences containing only 9 bp upstream of the polyA signal are placed downstream of a heterologous gene, polyadenylation occurs at the HIV polyA signal with 80% efficiency. HIV sequences extending 94 bp 5' to the polyA signal (contains sequences present during natural transcription from the HIV LTR) do not change this efficiency. Full use (100%) of the HIV polyA signal requires the addition of U3 sequences. Therefore, we conclude that the hexanucleotide and downstream G/T cluster function as an efficient polyA signal on their own and no 5' or 3' negative sequence elements exist that repress HIV polyadenylation upon first pass of the polyA signal. We are currently testing the model that the short distance between the HIV promoter and polyA signal is the determinant by which HIV mediates first pass readthrough of the 5' polyA signal yet allows efficient processing at the 3' polyA signal.

U 304 **REQUIREMENTS FOR 3'END FORMATION OF RNA POLYMERASE II TRANSCRIBED U-SNRNA GENES IN PLANTS.** Sheila Connelly and Witold Filipowicz, Friedrich Miescher-Institut, PO Box 2543, CH-4002 Basel, Switzerland.

RNA polymerase II transcribed snRNA genes in plants contain transcription elements that are distinct from those found in animal snRNA genes. Comparison of the structure of all characterized plant snRNA genes revealed a conserved sequence, CA--4-9nts--AGTNNAA, located directly downstream of the 3'end of the mature snRNA transcript. The role of this element in 3' end formation of Arabidopsis U2 snRNA transcripts was studied using a series of 3' end deletion and point mutants in transient expression assays. These studies showed that sequences 25 bps downstream of the U2 coding region, which contain the 3' consensus sequence, are sufficient to direct efficient and accurate 3' end formation. Deletion of this region, however, resulted in a greatly reduced accumulation of U2 RNA. Analysis of single and multiple point mutations showed that the AGTNNAA is highly tolerant to mutation, while the conserved CA dinucleotide is less tolerant. The role of sequences within the coding and promoter regions in 3' end formation is currently under investigation as well as whether the CA--4-9nts--AGTNNAA sequence is a signal for 3' end processing or transcription termination. In addition, we are in the process of determining if 3' end formation of plant snRNA precursors is coupled to transcription initiation from an snRNA promoter as has been shown in vertebrate systems.

U 305 REGULATION OF POLYADENYLATION SITE SELECTION, Jay Doniger, Division of Gynecologic Oncology, Lombardi Cancer Research Center, Georgetown University, Washington, DC 20007.

The molecular mechanisms for control of polyadenylation site selection are largely undefined. A human cervical epithelial cell line immortalized by human papillomavirus (HPV) type 16 (HCX16-5) has been established with properties that provide a unique opportunity for studying sequences that regulate polyadenylation site selection. cDNA analysis demonstrates that HCX16-5 produces predominantly 3'-truncated (400-500 bases shorter) as well as standard early HPV messages because of utilization of a series of alternate polyadenylation sites. The truncated mRNAs lack the viral E5 open reading frame. Even though HCX16-5 contains a single incomplete copy of HPV 16 integrated into the host genome, the upstream regulatory region, all the early genes, and the signals for cleavage and polyadenylation at the standard HPV 16 early mRNA polyadenylation site are intact while elements of the late viral genes downstream of this region have been deleted. No mutations were detected within 200 bases upstream or 500 bases downstream of the standard early polyadenylation signal. This suggests that either HPV 16 late gene sequences are required for choosing the proper early polyadenylation site or flanking host sequences influence the increased selection of alternate sites. Moreover, these regulatory sequences are at least 544 bases away from the standard early polyadenylation signal, because this is the minimum distance to the downstream viral integration site. The identification of sequences that affect polyadenylation site selection in the HPV 16 early transcription unit should provide insight into similar types of RNA processing mechanisms operating on cellular genes.

U 306 THE *suppressor of Hairy-wing* ZINC FINGER PROTEIN OF *Drosophila melanogaster* INCREASES UTILIZATION OF UPSTREAM POLYADENYLYLATION SITES, Dale Dorsett, Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Many spontaneous mutations in *Drosophila melanogaster* are insertions of the 7.5 kb gypsy retroposon. Phenotypes associated with most gypsy insertion alleles can be suppressed by mutations in the *suppressor of Hairy-wing* [*su(Hw)*] locus. The *su(Hw)* gene encodes a Zn finger protein that binds to a DNA sequence motif (YRYTGCATAYYY) in gypsy. In most gypsy elements, eight or twelve direct repeats of the motif are downstream of the 5' long terminal repeat (LTR), approximately 0.4 kb downstream of the LTR poly(A) site. When gypsy is situated in the transcribed region of a gene, the predominant gene transcript is polyadenylylated in 5' LTR. Mutation of *su(Hw)* or the *su(Hw)* protein-binding repeats reduces the LTR-polyadenylylated transcript level 3- to 10-fold. The level of polyadenylylated transcript increases with the number of downstream *su(Hw)* protein-binding sites. Although the *su(Hw)* protein-binding sequence is asymmetrical, both orientations potentiate upstream poly(A) sites. Downstream *su(Hw)* protein-binding sites also potentiate non-gypsy poly(A) sites. We envision that *su(Hw)* protein pauses transcription, allowing greater opportunity for a poly(A) site in the nascent transcript to be processed, although it is also feasible that *su(Hw)* protein interacts directly with the transcript or polyadenylylation apparatus.

U 307 SIGNALS FOR TERMINATION OF TRANSCRIPTION BETWEEN CLOSELY LINKED GENES: HUMAN GLOBIN α2--α1 AND HUMAN COMPLEMENT FACTOR CII--B,

Pita Enriquez-Harris, Rebecca Ashfield and Nick J. Proudfoot, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK.

The requirement of a functional poly(A) site for polII termination has led to the proposal that a second termination signal should exist in the 3' flanking region of polII genes[1]. This signal may function as a transcriptional pause site which slows down the RNA polymerase and thus allows nuclease and/or helicase activities to degrade or unravel the nascent transcript from the poly(A) site up to the paused polymerase. We have found such pausing signals between α2-α1 and CII-B. Based on nuclear run off analysis a short region of about 50bp causes an accumulation of polymerases over the pause region and then termination beyond it. As an alternative assay the relatively weak α2 or CII poly(A) sites have been placed upstream of a strong synthetic poly(A) site[2] (SPA) so that the SPA is used predominantly. Insertion of the α2 or CII pause sites between the adjacent poly(A) sites favours usage of the weak upstream poly(A) site. We are also investigating the behaviour of other putative pause sequences in this assay. We have shown that when the SPA is placed in an intron it is non functional due to the predominance of splicing over polyadenylation[2]. When the α2 or CII pause sites are placed downstream of the intronic SPA, the SPA is activated.

[1] Proudfoot N.J., TIBS 14:105-110 (1989)
[2] Levitt N., Briggs D., Gil A. and Proudfoot N.J., Genes & Develop. 3: 1019-1025 (1989)

U 308 TRANSCRIPTION TERMINATION IN THE MURINE IMMUNOGLOBULIN GAMMA 2b AND GAMMA 2a HEAVY CHAIN GENES, John Flaspohler and Christine Milcarek, Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Transcription termination in the immunoglobulin heavy chain genes, gamma 2b and gamma 2a, occurs over a 700 bp region located 500-1200 bp downstream of the respective promotor-distal membrane poly(A) sites. Termination occurs in this region regardless of the developmental stage of the gamma heavy chain-expressing B cell (Flaspohler and Milcarek, J. Immunol 144:2802).

Nuclear run-on analysis was performed on constructs containing combinations of the poly(A) site with or without the downstream termination region stably transfected into a myeloma cell line (J558L) lacking heavy chain expression. We have found that a DNA fragment containing the gamma 2a membrane specific poly(A) site and the downstream termination region is able to terminate transcription when inserted into the gamma 2b heavy chain gene in the IVS between the CH3 and MI exons. When placed in the same site in the IVS, a DNA fragment containing the gamma 2a membrane-specific poly(A) site without the downstream termination region or a construct lacking sequences 5' to the poly(A) site, which are important for efficient poly(A) site utilization, are able to terminate transcription but at lowered efficiencies. Results from constructs containing the termination region alone and inserted downstream of a secretory-specific poly(A) site will be presented.

U 309 IDENTIFICATION OF THE GENES FOR POXVIRUS POLY(A) POLYMERASE SUBUNITS, Paul Gershon, Byung-Yoon Ahn and Bernard Moss, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.
In the eukaryotic nucleus, transcriptional termination is closely linked to mRNA 3'-end formation. Processes involved in 3'-end formation include mRNA 3'-polyadenylation, and are catalysed by a protein complex shown to contain a poly(A) polymerase (PAP). Genes encoding a eukaryotic PAP have not previously been reported. Vaccinia virus transcripts are synthesized and modified within the cytoplasm of the infected cell, and a novel PAP is induced on vaccinia virus infection. The purified vaccinia virus-induced PAP is associated with two polypeptides, of molecular mass 55 and 39 kDa and these were shown, by peptide sequencing, to be encoded by open reading frames E1L and J3R respectively, of the virus genome. Homologs of the 55-kDa polypeptide were not found in sequence databases, and the only sequences with homology to the 39-kDa polypeptide were from the genomes of other poxviruses, with which the vaccinia virus polypeptide shares sizeable regions of three-way sequence identity. Neither PAP polypeptide possesses a recognizable nucleic acid binding structure, although the 55-kDa polypeptide contains a partial match to the cannonical nucleoside triphosphate binding motif. In addition there was some localized sequence identity between the two polypeptides. Antisera were raised to bacterially expressed products of the two open reading frames and to synthetic peptides. The 55-kDa and 39-kDa polypeptides were detected at 2 h after infection and appeared to accumulate throughout infection. Accumulation was not abrogated by inhibition of the intermediate and late phases of vaccinia virus transcription and, consistent with this, genes E1L and J3R were shown to be transcribed early during vaccinia virus infection. The 55-kDa and 39-kDa polypeptides were shown to co-chromatograph and co-sediment during extensive purification, suggesting a stable heterodimeric structure, with which PAP activity was associated. However, preliminary experiments indicate that PAP activity may be associated with one of the two subunits.

U 310 COMPONENTS REQUIRED FOR POLY(A) SITE RECOGNITION AND PROCESSING
Gregory M. Gilmartin[1] and Joseph R. Nevins[2], [1]Dept. of Microbiology and Molecular Genetics, Univ. of Vermont, Burlington, VT; [2]Dept. of Microbiology and Immunology, Duke Univ. Med. Ctr., Durham, NC
Four HeLa cell nuclear factors are required for specific pre-mRNA poly(A) site processing. Two factors are required for specific polyadenylation of the cleaved RNA: a poly(A) polymerase, termed PF1, and an AAUAAA specificity factor, termed PF2. The endonucleolytic cleavage of the pre-mRNA requires both PF1 and PF2, along with two additional factors termed CF1 and CF2.

PF2 forms an initial complex with the pre-mRNA dependent on the AAUAAA element. The PF2 activity is complex and consists of at least six polypeptides. UV cross-linking of the PF2/RNA complex specifically labeled a 170kd protein indicating that at least this protein is involved in RNA binding. This protein may also contain an epitope recognized by α-Sm sera.

The formation of the PF2/RNA complex is a pre-requisite for the interaction of CF1 at the poly(A) site. CF1 binding requires the downstream element in addition to PF2 binding. CF1 has been purified to apparent homogeneity and is composed of three polypeptides of 76, 64, and 48 kd. The 64 kd CF1 protein was UV cross-linked to the pre-mRNA dependent upon the downstream element. Since the binding of CF1 is dependent on the PF2-AAUAAA interaction, efficient cross-linking of the 64 kd CF1 protein is also dependent on the AAUAAA element.

Whereas the PF2/RNA complex is unstable and dissociates rapidly, the ternary complex formed by PF2, CF1, and RNA is stable. Moreover, the stability of this complex directly correlates with efficiency of processing of the poly(A) site. Thus, the interaction of CF1 with the processing complex can be viewed as a commitment of the poly(A) site for processing.

U 311 RANDOM, MUTLIPLE POLYADENYALTION IN TISSUE-SPECIFIC PROCESSING OF RAT γB mRNA SUGGESTS SPLICE SITE COMMITMENT AS A KEY REGULATORY STEP, Patricia J. Haidaris and Mary Anne Courtney, Department of Medicine, University of Rochester, Rochester, NY 14642.

Fibrinogen is secreted into circulation by the liver and delivered to the site of vessel injury from the α granule storage compartment of blood platelets. It is a dimeric molecule composed of pairs of three non-identical subunits, Aα and Bβ and γ, and its function in support of platelet aggregation or in fibrin clot formation is in part mediated through the γ chain carboxytermini. Fibrinogen γ chains differ in amino acid sequence at the carboxyterminus due to alternative 3' RNA processing resulting in two polypeptides which differ in their ability to support platelet aggregation in primary hemostasis. *In situ* hybridization was employed to demonstrate the expression of only γA transcripts in marrow, predominantly localized to megakaryocytes, while both γA and γB transcripts were localized to and co-distributed in liver hepatocytes, indicating that no subset of cells process γB mRNA. The results of RNase protection studies suggested that, in addition to the distal poly(A) signal thought to be the only signal used in γA and γB RNA processing, two additional poly(A) signals within the last intron (ATTAAA and AATAAA) were utilized in γB mRNA processing. The approximatly equal usage (37%, 36%, and 27%, in the 5' to 3' orientation) suggested that poly(A) site selection is random. Splicing of the last intron to produce γA mRNA appears to be the constitutive pattern of γ chain RNA processing that we have previously shown results in the ubiquitous expression of γA transcripts in other rat tissues. Retention of the last intron in γB RNA processing is tightly regulated in liver. These data support a general mechanism for tissue-specific splice site selection predominating over poly(A) site selection in genes subjected to 3' regulation, a mechanism observed for regulation of other complex transcription units. The expression of both fibrinogen γ chain transcripts in liver rather than mutually exclusive expression in liver and marrow provides a new model for studying alternative 3' RNA processing regulatory mechanisms.

U 312 MULTPILE UPSTREAM SEQUENCE DETERMINANTS FOR mRNA 3' END FORMATION IN A PEA *rbcS* GENE, Arthur G. Hunt, Brad Mogen and Margaret H. MacDonald, Plant Physiology/Biochemistry/Molecular Biology Program, Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091

We are characterizing the sequences that direct mRNA 3' end formation in a pea ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) gene. We have found that a sequence element located between 60 and 137 bases upstream from the multiple poly(A) addition sites in this gene is needed for functioning of these sites in tobacco. However, linker-scanning and fine deletion analysis indicates that there is no well-defined element in this region that is responsible for the phenotype seen with larger deletions. Moreover, these studies have identified other sequence elements, located between -1 and -60, that are involved in 3' end selection. In particular, there seem to be specific elements that correspond to each of the three polyadenylation sites in this gene.

Our studies indicate that there are multiple upstream sequence signals involved in mRNA 3' end formation in the *rbcS*-E9 gene. These include a "general" signal located relatively far from polyadenylation sites, and poly(A) site-specific signals located within 40 nt of specific sites. The "general" signal is similar in some respects to yeast polyadenylation signals, whereas the poly(A) site-specific signals may represent elements analogous to the mammalian AAUAAA motif. Since most plant transcription units give rise to mRNA populations with heterogeneous 3' ends, we suggest that our observations reflect general properties of plant polyadenylation signals.

U 313 REGULATION OF 3' END PROCESSING DURING ADENOVIRUS INFECTION Michael J. Imperiale, James D. DeZazzo, Jay Kilpatrick, and Susan Wilson-Gunn, Dept of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109

The major late transcription unit of adenovirus encodes five 3' co-terminal families of mRNA's, designated L1-L5. The relative amounts of these RNA's are regulated during viral infection by differential poly(A) site choice. Early in infection, the L1 poly(A) site is used predominantly, whereas late in infection its usage is down regulated. Using recombinant viruses, we have investigated the role of sequences flanking the AAUAAA and downstream elements at the L1 poly(A) site in regulating processing. We find that these flanking regions are required for both predominant early usage and regulation late in infection. We have also developed an in vitro combination transcription-processing system to study poly(A) site choice. The sequences upstream of AAUAAA which function in vivo to enhance L1 poly(A) site usage are also required in vitro. In addition, this requirement holds regardless of whether the RNA is transcribed and processed in the same reaction or just processed from an SP6-transcribed precursor. We have recently identified in vitro conditions which, in preliminary experiments, mimic the late infected cell. Using the in vitro system, we are attempting to elucidate the mechanism of action of these 3' end processing regulatory sequences.

U 314 POSTTRANSCRIPTIONAL PROCESSES INFLUENCE THE LEVEL OF GENE EXPRESSION IN TRANSGENIC PLANTS, Ivan L.W. Ingelbrecht, Marc C. Van Montagu and Ann G. Depicker

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We are investigating the functional role of a 3' end region on the expression of a reporter gene in plant cells. Recently we have shown that chimeric genes with 3' end sequences originating from different plant genes direct similar enzymatic activities in transient expression but display characteristic expression levels in stably transformed tissue. The construction containing the 3'-untranslated region of the chalcone synthase gene (3'CHS) was studied in more detail as its steady-state mRNA level was extremely low. This particular 3' end region contains all noncoding sequences upstream as well as 113 bp downstream from the poly-adenylation site. Now we have constructed a similar chimeric gene containing an additional 700-bp 3'-flanking sequence. In transient expression both genes were expressed at similar levels. However, the level of steady-state mRNA present in stably transformed cells was approximately 20-fold higher in case of the gene containing the longer 3'CHS. Nuclear run-off experiments and analysis of steady-state mRNA suggest that specific sequences downstream from the polyadenylation signals direct transcriptional termination and are essential for optimal expression in transgenic plants.

U 315 THE POLYADENYLATION SIGNAL OF THE CAULIFLOWER MOSAIC VIRUS IS RECOGNIZED IN THE YEAST SACCHAROMYCES CEREVISIAE, Stefan Irniger[1], Hélène Sanfaçon[2], Christoph M. Egli[1] and Gerhard Braus[1],
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The polyadenylation signal of a plant virus, the cauliflower mosaic virus (CaMV), was tested in vivo for its ability to direct 3'end formation when placed downstream of an efficient promoter and introduced into the yeast Saccharomyces cerevisiae. Transcript analysis showed that yeast can efficiently recognize the CaMV polyadenylation signal in an orientation dependent manner. Mapping of the CaMV transcripts 3'ends revealed that they were located at exactly the same position in yeast and in plant. These results suggest an evolutionary conservation for the mechanisms of mRNA 3'end formation between these organisms. In order to find out more about this, we compared the sequences required for CaMV mRNA 3'end formation in yeast and in plants by deletions and mutations and we investigated the recognition of other plant polyadenylation signals in yeast.

U 316 POLY(A) BINDING PROTEINS OF PROTOZOAN PARASITES,
Stan T. Metzenberg and Nina Agabian, Intercampus Program in Molecular Parasitology, University of California, San Francisco, CA 94143-1204.

The processing of mRNA transcripts in kinetoplastid protozoans is unusual in several respects. The precursor RNA molecules tend to be polycistronic, and are subdivided into constituent mRNAs by the processes of trans-splicing (addition of a 39-base capped leader RNA), 3' end cleavage and polyadenylation. The *cis*-acting sequences that direct the latter two events have not been identified, though with the advent of efficient DNA transfection in these organisms, they are processes that we may now study. In our attempts to find similarities between the mRNA-binding proteins of protozoans and higher eukaryotes, we have isolated and studied the poly(A) binding (PAB) proteins from several species of parasitic protozoans. Although PAB proteins are largely cytoplasmic, their presence in the nuclei of some species suggests that they could also bind to nascent poly(A) tails. We find that the kinetoplastids *Trypanosoma brucei* and *Leptomonas collosoma* express a predominant PAB protein species of 60 kdal, roughly 20% smaller than their counterpart in vertebrate organisms. The parasitic protozoan *Giardia lamblia* expresses a PAB protein that is smaller still, with a size of roughly 56 kdal. We have raised antibodies directed against the *Leptomonas* and *Trypanosoma* proteins, and have cloned a partial cDNA coding for the PAB protein of *T. brucei* . Our plans are to determine whether PAB proteins play a role in RNA processing in the nucleus. In kinetoplastid protozoans, for example, we speculate that a nuclear PAB protein might designate an RNA molecule as a suitable substrate for *trans*-splicing, thus triggering the assembly of a *trans*-spliceosome complex at the 5' end of the molecule.

U 317 IDENTIFICATION OF A TRANSCRIPTIONAL TERMINATION MOTIF WITHIN THE IMMUNOGLOBULIN GENE LOCUS, Bethany Beilue Moore, Philip Tucker, and Dorothy Yuan, Departments of Pathology and Microbiology and the Immunology Graduate Program, U.T. Southwestern, Dallas, TX 75235

The expression of IgM and IgD on the surface of B lymphocytes is developmentally regulated through alternative usage of a single V_H gene with either the Cμ or Cδ constant region genes. We have previously identified a region within the μ-δ intron where transcriptional termination occurs in immature B cells which synthesize very low amounts of δ mRNA. Termination within this intron is released in mature IgM+, IgD+ B cells allowing transcription to progress through to the δ locus. Termination maps to an approximately 1 kb region of the intron which contains a 162 bp unique sequence inverted repeat (USIR). We have analysed the elements which comprise this unique termination motif and have shown that part of this 1 kb region mediates transcriptional termination in a fashion dependent on orientation and linkage to a functional poly(A) site when assayed in a heterologous location.

U 318 The Efficiency of Coupling of the U1 Promoter with U1 3' End Formation Is Dependent on the Length of the Transcript. Duane R. Pilch and William F. Marzluff, Department of Chemistry, Florida State University, Tallahassee, FL 32306

RNA polymerase II transcribes all mRNAs as well as several of the small nuclear RNA (snRNA) genes. Among RNA pol II genes, the U1 snRNA genes and replication dependent histone genes are distinct in their lack of intervening sequences, their high level of expression and their lack of a polyadenylated 3'end. A unique feature of the U1 gene is that U1 3' end formation requires a complete snRNA promoter (Hernandez and Weiner, CELL 47,249,1986; Neuman de Vegvar, et al., CELL 47,259,1986). Chimeric histone/U1 genes transfected into CHO cells were used to show that transcripts initiated from histone promoters did not utilize a downstream U1 3' end signal, whereas transcripts initiated from U1 promoters formed normal histone 3' ends (Pilch and Marzluff, GENE EXPRESSION, in press). I combined these genes to make a UHU gene (U1 promoter, complete histone coding region, and U1 3' end). Using an S1 Nuclease assay I determined that 50% of the steady state UHU RNAs had U1 3' ends and 50% ended heterogeneously 3' of the U1 signal. All transcripts were localized in the nucleus. When I added the histone 3' end signal 3' of the U1 end signal to make a UHUH gene, most transcripts had histone ends, but the absolute number of transcripts with U1 ends remained the same, consistent with the U1 3' ends being formed by a transcription termination event. When I shortened the histone coding region (550 nt) to either 281 nt or 146 nt, more U1 ends were formed than histone ends, demonstrating that the efficiency of coupling the U1 promoter with 3' end formation depends on the length of the transcript.

U 319 **POLY A SITE USE IN THE ADENOVIRUS MAJOR LATE TRANSCRIPTION UNIT** John Prescott and Erik Falck-Pedersen, Department of Microbiology, Cornell University Graduate School of Medical Science, New York, NY 10021

Adenovirus undergoes multiple alterations in mRNA processing during the transition from early to late infection. In the major late transcription unit (MLTU), this early to late processing switch includes alterations in transcription termination, mRNA splicing, and polyadenylation. The early to late switch in polyadenylation involves a switch from nearly exclusive use of the upstream poly A site (L1) early in infection to use of all five poly A sites (L1-L5) late in infection. It has been shown previously that small (500 bp) DNA fragments containing the L1 and L3 poly A sites, when inserted in tandem into the E1a transcription unit and driven by the major late promoter (MLP), will undergo an early to late switch (Falck-Pedersen and Logan, J Virol. 63(2) 1989). We are currently using an in vitro polyadenylation system to further characterize this early to late polyadenylation switch. We find that L1 is used much less efficiently than L3 when the sites are assayed either separately or in tandem, regardless of their order. This L3 dominance persists even when processing is allowed to occur during an in vitro transcription reaction. This inefficient L1 usage is most likely due to low affinity binding of polyadenylation factors, as L1 is less able to compete for these processing factors than is L3. Dissection of the L1 and L3 poly A sites shows that there are multiple sequence elements that contribute to poly A site strength and that these sequence elements can function cooperatively to increase poly A site strength.

U 320 REGULATED POLYADENYLATION IN MAMMALIAN HEPATITIS B VIRUSES, Roland Russnak and Don Ganem, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143

In retroid elements, synthesis of terminally-redundant genomic RNA (the template for reverse transcription) requires differential use of a single polyA site. In the case of mammalian hepatitis B viruses, transcription begins 100 and 130 nt upstream of a variant hexanucleotide signal (UAUAAA) which is recognized inefficiently (less than 20%) upon first pass of RNA polymerase. Transcription continues on a circular DNA template until the same signal is encountered a second time where virtually all transcripts are processed (>95%). This regulation is mediated by two factors: (1) the presence of a variant hexanucleotide signal which, together with downstream sequences, is virtually inactive, and (2) the existence of at least two upstream signals (termed PS1 and PS2) which act to increase the efficiency of its use. Unexpectedly, PS2 is located between the 5' cap site and the polyA signal and probably contributes to the low level production of *short-stop* transcripts. Highly efficient processing on the second pass requires (in addition to PS2) that transcription proceed through sequences (PS1) located upstream of the genomic RNA start site. Both PS1 and PS2 function independently: when cloned singly upstream of the UAUAAA, each element can up-regulate its use. In both cases this effect is orientation-dependent. PS1 has been localized to an 80 bp region which can be further sub-divided into two distinct functionally redundant elements. For each element, critical residues have been located within 15 bp A/T stretches.

U 321 THE HIV-1 LTR CONTAINS AN UNUSUAL ELEMENT THAT INDUCES THE SYNTHESIS OF SHORT TRANSCRIPTS FROM VARIOUS mRNA AND snRNA PROMOTERS, Michael Sheldon ,Ratneswaran Ratnasabapathy, Lynn Johal and Nouria Hernandez, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

We describe an unusual element that activates the synthesis of short transcripts from a wide variety of mRNA and small nuclear RNA (snRNA) promoters, including the U6 RNA polymerase III promoter. This inducer of short transcripts (IST) is located between positions -5 and +82 relative to the cap site in the HIV-1 LTR. In the presence of IST, the total transcriptional activity of the different promoters is greatly increased, but the resulting additional RNA molecules are short, ending around position +60. IST is not TAR, the RNA target for transcriptional transactivation by the HIV-1 Tat protein; however, because it relies entirely on cellular factors for activity, IST may serve to provide abundant RNA targets for Tat binding without a requirement for full-length viral mRNA expression. We are currently engaged in mapping the boundaries of IST to the nucleotide by site-directed mutagenesis of the HIV-1 LTR region in which it is located.

U 322 MUTATIONS IN E. COLI RHO FACTOR THAT AFFECT RNA BINDING AND HELICASE ACTIVITY. Eric J. Steinmetz, Catherine A. Brennan, and Terry Platt. Department of Biochemistry, University of Rochester Medical Center, Rochester, NY 14642.

In rho factor, a ribonucleoprotein (RNP) consensus sequence, Gly-Phe-Gly-Phe, lies within the single peptide (residues 45-100) that can be covalently attached to either trp t' or short oligo(C) RNA by UV-irradiation. We have mutagenized both Phe residues to Leu or Ala. RNA binding by these mutant proteins is both weaker (6x and 160x, respectively) and more salt sensitive than wild-type. This suggests that the phenylalanines participate in a nonionic component of RNA binding, yet cannot themselves play a substantial role in the activation of ATP hydrolysis, because the mutants retain much of their RNA-dependent ATPase activity. In the normal helicase assay, the stoichiometry of release for a wide variety of substrates has approached, but never exceeded, 1:1. With the double Ala substitution, stoichiometry improves to 2-3 RNAs released per rho molecule, which we infer is due to more rapid release of RNA product after duplex disruption, because initial rates of DNA release are similar. Parallel results confirm that a slow off-rate from the RNA plus a surprisingly rapid inactivation of rho during incubation both contribute to the inability of wild-type rho to recycle in the helicase assay. Overall, results with various helicase substrates support an "anchored tracking" model in which rho retains contact with RNA to which it initially bound, while moving 5' to 3' along the RNA transcript towards the duplex region. These findings are also consistent with a "binding change" mechanism for harnessing ATP hydrolysis to directed molecular translocation.

U 323 **CHARACTERIZATION OF 3' TRANSCRIPTION TERMINATION SIGNALS IN THE MOUSE BETA-GLOBIN GENE.** J. Tantravahi and E. Falck-Pedersen, Department of Microbiology, Cornell University Graduate School of Medical Sciences, New York, N.Y., 10021

We are interested in determining how RNA polymerase II ceases transcription when it encounters specific DNA sequences downstream of a polyadenylation site.The gF region of the mouse beta-globin gene is an 809 bp DNA segment that has been shown to terminate transcription in an AATAAA poly A signal sequence dependent manner. Using both in vitro and in vivo techniques, we are defining the exact sequences required to terminate transcription. When the gF sequence is joined to the Ad major late promoter and transcribed in a nuclear extract, we detect mainly full length species. When we add Sarkosyl to the extract (at 0.25% final) and perform an identical transcription, we detect several paused species that map within the proximal part of gF. We compared this in vitro data to results generated from a nuclear run-on assay. We found, in contrast to our in vitro data, that the termination activity lies in the distal part of gF. To further define which sequences mediate termination, we made 5' and 3' exonuclease deletions of gF, and we tested their termination efficiency in vivo using nuclear run-on assays. Our preliminary observations indicate that sequences between +554 and +634 contain functional termination sequences; further characterization of this sequence and other putative termination sequences is in progress.

U 324 **THE HUMAN IMMUNODEFICIENCY VIRUS TYPE I POLYADENYLATION SIGNAL: A 3'-LTR ELEMENT UPSTREAM OF THE AAUAAA NECESSARY FOR EFFICIENT POLYADENYLATION.**

Alexandra Valsamakis, Steven Zeichner, Susan Carswell and James C. Alwine. 560 Clinical Research Building, 422 Curie Blvd., School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6142

Most higher eukaryotic mRNAs are posttranscriptionally processed at their 3' ends by cleavage and polyadenylation. The majority of characterized processing signals are com prised of two elements: AAUAAA and an element downstream of the AAUAAA. Recently a second class of polyadenylation (PA) signal has been identified. These signals have elements upstream and downstream of AAUAAA. We have recently characterized the upstream element (USE) of the SV40 late polyadenylation signal and are interested in identifying other PA signals with USE's. Using HIV as a model system, we have asked whether retroviral PA signals contain such USE's. HIV transcripts have AAUAAA and downstream elements (DSE's) at their 5' and 3' termini, however, processing occurs primarily at the 3' PA signal. We have asked whether sequences unique to the 3' end, namely U3 sequences upstream from AAUAAA, control utilization of the 3' PA signal. Using deletion and linker scanning mutations we show that the PA signal of HIV-1 contains USE's in the 3' LTR U3 region. The element which has the greatest effect on signal utilization lies between 77 and 94 nts. upstream of AAUAAA. Mutations in an adjacent region 59 to 76 nts. upstream of AAUAAA have a smaller effect. Mutations further upstream, 141-176 nts. upstream of AAUAAA also affect polyadenylation modestly. Functional similarity between upstream elements was indicated by the ability of the HIV upstream region to replace the upstream region of the SV40 late PA signal. The sequence of the major upstream element of HIV is U-rich, analogous to many defined downstream polyadenylation elements. This suggests USE's and DSE's share similar functions.

U 325 THE SEQUENCE DEPENDENCE OF RNA-STIMULATED ATPase ACTIVITY OF RHO PROTEIN, Yan Wang, Steven E. Seifried and Peter H. von Hippel, Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, OR 97403

Rho protein is a termination factor which causes the dissociation of *E. coli* transcription complex at termination sites. Nascent RNA transcript and the RNA-stimulated ATPase activity of rho are both essential for termination. In this study, the effects of RNA sequence and composition on the ATPase activity of rho are characterized by using synthetic ribo-oligonucleotides. We have demonstrated that the RNA-stimulated ATPase activity of rho is sequence dependent: (1) an octamer hetero-oligo(rU,rC_7) which has 5' terminal uracil residues stimulates the ATPase activity of rho to a higher level than does oligo($rC)_8$; (2) the enhanced stimulation is not observed if the uracil is replaced by adenine; and (3) the ATPase activity is significantly lower if the uracil residues are at the 3' terminus. In addition to the comparative ATPase study, the relative binding affinities of different oligomers to rho are determined. The results show the binding affinity of a ribooligomer to rho is determined by its ribocytidine content and is insensitive to the nucleotide sequence. We propose that, in addition to the binding of RNA to rho prior to ATP hydrolysis, the release of RNA from rho after the hydrolysis is also sequence dependent: (1) ribocytidine residues are required for the formation of a stable rho-RNA complex; and (2) the uracil residue at the 5' terminus either initiates or facilitates the release of the oligomer from the protein. Both binding and release of RNA are critical in increasing the catalytic turnover rate of ATP hydrolysis by rho, and for rho to function as a termination factor.

U 326 OCCLUSION OF THE HIV POLY(A) SITE,

Caroline Weichs an der Glon, Joan Monks and Nick J. Proudfoot, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK.

To investigate the selective use of poly(A) sites in the 3' long terminal repeat (LTR) but not 5' LTR of retroviruses, we have studied the poly(A) site of the human immunodeficiency virus (HIV1). Using hybrid HIV/α globin gene constructs we demonstrate that the HIV poly(A) site is inactive or occluded when adjacent to an active promoter, either the homologous HIV promoter or the α globin gene promoter. Furthermore this occlusion of the HIV poly(A) site occurs over a considerable distance of up to at least 500 bp. In contrast two non retroviral poly(A) sites (α globin and a synthetic poly(A) site) are active when close to a promoter. We also show that a short fragment of about 60 nucleotides containing the HIV poly(A) site is fully active when placed at the 3' end of the human α globin gene or within the rabbit β globin gene. This result rules out the requirement of more distant upstream elements for the activity of the HIV poly(A) site as has been suggested for other viral poly(A) sites. Finally we show that the GT rich downstream region of the HIV poly(A) site confers poly(A) site occlusion properties on a synthetic poly(A) site. This result focuses attention on this more variable part of a poly(A) site in retroviruses as a possibly general signal for poly(A) site occlusion.

U 327 Kinetic Barriers to Termination and Elongation of Transcription by *E. coli* RNA polymerase. *Kevin S. Wilson and Peter H. von Hippel*, Institute of Molecular Biology, University of Oregon, Eugene, OR 97405.

We are investigating the energetic interactions that determine the rates of termination and elongation of RNA polymerase at two representative rho-independent termination sites. Apparent activation energies are obtained through measurements of the termination efficiency of the λt_{R2} and the *trp t* terminators as a function of temperature. For comparison, we are also measuring the activation energy of transcript release from a specific and stable stalled elongation complex. Various approaches are underway to discover which nucleic acid interactions in the transcription complex are responsible for the observed activation energies. Specifically, we are concentrating on the exact role of the RNA hairpin structure in promoting termination. In a previous study, a thermodynamic analysis of transcription termination has led to the successful prediction of the *sites* where termination can occur *in vitro* in the absence of any protein factors. This thermodynamic analysis rests on the proposition that the polymerase is passive in the termination event and that termination depends principally on the stability of the RNA:DNA hybrid structure in the transcription elongation complex. In contrast, the *efficiency* of termination is more complex since it involves at least two competing kinetic pathways of elongation and termination. Our experimental studies on the λt_{R2} and the *trp t* terminators are extending the thermodynamic model to include the kinetic energy barriers to termination and elongation in rho-independent termination. Through kinetic studies, we hope to be able to partially elucidate the mechanism of the termination decision of RNA polymerase.

U 328 REGULATION OF THE MYC, MYB AND FOS PROTOONCOGENES BY TRANSCRIPTIONAL ATTENUATION. Stephanie Wright and J.Michael Bishop. Department of Microbiology and Immunology and The G. W. Hooper Foundation, University of California, San Francisco, CA 94143.

Modulation of transcriptional elongation through a "block" near the 5' end of the gene plays a role in determining the steady state level of c-myc RNA in the cell. We have previously identified a 180 nucleotide DNA fragment within exon 1 of the mouse c-myc gene which can cause attenuation when placed within a heterologous gene. We have partially determined the sequence requirment for attenuation within this fragment, and show that these sequences themselves are involved in regulating myc expression.

In order to gain insight into the mechanisms used to modulate attenuation within eukaryotic genes, we have localized the region of elongation blockage within the mouse c-fos and c-myb genes and have also identified another candidate attenuator within the c-myc gene. A DNA fragment containing the candidate c-fos attenuator is capable of replacing the c-myc attenuator when assayed by injection of cloned genes into Xenopus oocytes. We are currently investigating the role of a conserved DNA sequence within these attenuator fragments, and are determining how transcriptional elongation through these sequences is modulated in response to different physiological signals.

U 329 DIFFERENT TRANSCRIPTION TERMINATION AT VARIOUS MAMMALIAN GASTRIN GENES, Ook Joon Yoo, Sun J. Kim, Department of Biological Science & Engineering, Korea Advanced Institute of Science and Technology, P.O. Box 150, CheongRyang, Seoul 130-650, Korea

We have isolated genomic clones that encode monkey, mouse and sheep gastrin, and sequenced their 3' flanking regions. The terminator-like sequence of monkey gastrin gene for RNA polymerase II was identical to the previously shown human terminator, $A_2T_5AT_4AT_4AT_5$, except that the last nucleotide was changed from T to A. To identify the termination element in mouse and sheep gastrin genes, we employed an *in vivo* transiently expressing system. GT-repeat sequence at the 3' flanking region of mouse gastrin gene, acted as a transcription terminator. The sequences necessary for transcription termination functioned with orientation-dependent fashion. In the case of sheep gastrin gene, ten adenine residues appeared at the 3' flanking region. However the A rich sequence could not function as a terminator. It has been, therefore, noted that different mechanisms of transcription termination by RNA polymerase II could be employed from species to species even for the same eukaryotic gene.

U 330 TRANSCRIPTIONAL REGULATION OF IG HEAVY CHAIN GENE EXPRESSION.

Dorothy Yuan, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas. 75235.

The activation of B lymphocytes from a resting stage to that of high rate immunglobulin secretion is a multi-step process mediated by many intracellular molecular events. We have dissociated some of these events in an attempt to assess their effect on transcription across the Ig heavy chain loci. These experiments show that induction of cell proliferation alone does not measurably alter the level of transcription of μ or δ chain genes. In contrast, induction of the switch to μS mRNA production which precedes Ig secretion causes two changes in transcription which can be dissociated by the addition of a protein kinase C inhibitor, staurosporine. Addition of the drug 2 days after B cell activation with LPS inhibits the increased initiation of polymerases from the transcriptional unit. However, it does not inhibit the alteration in the site of termination of transcription to more upstream positions. These results suggest that increased promoter activity require signals affected by protein kinase C activity and is an event which occurs later than the induction of factors which can alter polymerase termination. These findings are consistent with our previous report showing that the lymphokine IL-5 can only induce alterations in polymerase termination but not increased initiation.

Poster Session IV

U 400 RNA POLYMERASE II TRANSCRIPTION ELONGATION IS BLOCKED IN VIVO BY POLYOMAVIRUS LARGE T ANTIGEN, Nicholas H. Acheson, John Bertin, and Noëlle Sunstrom, Department of Microbiology and Immunology, McGill University, Montreal, Quebec, Canada H3A 2B4.

Elongation by RNA polymerase II is blocked on both the early and late strands of polyomavirus DNA in the region where large T antigen binds to viral DNA (Skarnes et al., JMB **203**, 153-171, 1988). This leads to an accumulation of excess RNA polymerases on both DNA strands in this region during the late phase of infection, as detected by run-on assays using viral transcription complexes. Polymerases are blocked shortly downstream of the early promoter on the early DNA strand, and within 100 nucleotides of this site on the complementary late DNA strand. We have constructed a mutant virus which encodes a thermolabile large T antigen, and have shown that the elongation blocks on both DNA strands are reduced or absent after shift to the nonpermissive temperature (39 C). At this temperature, specific DNA binding by large T antigen was reduced by 10-fold. We are now constructing mutants containing altered large T antigen binding sites to determine whether these elongation blocks are mediated directly by binding of large T antigen to DNA. We conclude that polyomavirus large T antigen blocks elongation by RNA polymerase II at specific sites, and propose that repression of early transcription by polyomavirus large T antigen is due in part to a block in transcription elongation at the early promoter.

U 401 GENETIC ANALYSIS OF THE LARGEST SUBUNIT OF *SACCHAROMYCES CEREVISIAE* RNA POLYMERASE II, Jacques Archambault, Kim T. Arndt* and James D. Friesen, Department of Genetics, Research Institute, Hospital for Sick Children, Toronto, Canada, *Cold Spring Harbour Laboratory, Cold Spring Harbour, NY.

We have isolated and characterized mutations in the gene encoding the largest subunit of *Saccharomyces cerevisiae* RNA polymerase II (*RPO21*) with the aim of identifying important functional regions.

1) **Cloning and sequencing of *sit1* alleles.** Alleles of *RPO21* (*sit1*) have been isolated that can suppress the His$^-$ phenotype of a strain carrying deletions of three genes (*BAS1*, *BAS2* and *GCN4*) encoding transcription factors required for the expression of *HIS4* (Arndt et al. Cell **42**:527-537). Four *sit1* alleles so far sequenced carry a nucleotide change in a region of the largest subunit which is conserved amongst the largest subunit of eucaryotic and procaryotic RNA polymerases. In the mouse, a mutation that confers resistance to alpha-amanitin also lies in this region suggesting that this domain of the largest subunit is involved in transcription elongation.

2) **Pleiotropic phenotypes of *rpo21* mutant alleles.** Several mutations in *RPO21* (including *sit1*) impose a variety of phenotypes such as temperature-sensitvity and auxotrophy for inositol. We have constructed yeast strains that synthesize limiting amounts of *RPO21* gp by substituting the promoter of *RPO21* with the promoter of *PRP11*, a gene involved in pre-mRNA splicing or with the weak promoter of *LEU2*, a gene involved in leucine biosynthesis. Preliminary experiments suggest that decreased expression of *RPO21* is sufficient to confer temperature-sensitivity and auxotrophy for inositol. We are also investigating the effect of decreased expression of *RPO21* or the effect of mutations in *RPO21* on regulation of the cell cycle.

U 402 HK022 PHAGE MUTANTS THAT SUPPRESS A DEFECTIVE Q, Brent L. Atkinson and Max E. Gottesman, Institute of Cancer Research, College of Physicians and Surgeons of Columbia University, 701 West 168th Street, New York, NY 10032

The nusC60 mutation in the β-subunit of RNA polymerase prevents the growth of λ and its close relative, HK022. In the case of λ, nusC60 blocks N action, whereas for HK022, the site of inhibition is the phage Q function. We selected and characterized two HK022 phage mutants capable of growth on nusC60 hosts. Both suppressor mutations are located to the left of the HK022 att site, in a region analogous to the λ b region. The b^+ suppressors are recessive to HK022 b^+ expressed from a multicopy plasmid. HK022 b^+ does not inhibit the growth of λnin5 in nusC60 hosts. These results suggest that the b region of HK022 encodes a function that affects the synthesis or activity of HK022 Q-protein.

U 403 **An *In Vitro* RNA polymerase II Transcription System from Acanthamoeba.** Erik Bateman, Feng Liu and Jie-Min Wong, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, Vermont 05405.

We have prepared extracts from Acanthamoeba nuclei that accurately initiate transcription from the adenovirus major late promoter. The specifically initiated transcript is sensitive to amanitin, is resistant to RNase T and initiates close to the end of the G-free cassette. The reaction is not inhibited by chloride, but shows an unusual mono-, and divalent cation optimum. Acetate had no effect on the reaction.

A TFIID-like protein has been identified in nuclear extracts using band-shift assays. Several other DNA binding activities are also present; each of these are being tested in the *in vitro* system.

The gene coding for TFIID from Acanthamoeba has been identified using PCR methods. Approximately 490 base pairs of the product was sequenced. An open reading frame of 116 amino acids interrupted by a presumed intron was identified. The two exons show 88% identity to the yeast TFIID C-terminal portion and a correspondingly high identity to drosophila and human TFIID. Where amino acid changes occur, many are conservative, and are at the positions where yeast, drosophila and human TFIIDs differ. We are currently screening genomic and cDNA libraries to isolate full-length clones.

U 404 INTRON SEQUENCES ARE INVOLVED IN THE REGULATION OF MURINE C-*MYB* mRNA EXPRESSION BY A BLOCK TO TRANSCRIPTION ELONGATION. Timothy P. Bender, Katrina M. Catron, and Charles R. Toth. Department of Microbiology, University of Virginia, Charlottesville, VA. 22908.

The steady state levels of murine c-*myb* mRNA expression are down-regulated during hematopoietic maturation. Using murine B-lymphoid tumors and erythroleukemia cells (MEL) as model systems we have shown that much of this regulation is mediated by a block to transcription elongation (attenuation) that occurs in the first intron of the gene. We have begun to analyze the mechanism involved combining a search for protein/DNA interactions with deletion analysis in the first intron. Using gel shift analysis, a modified T7 gene 6 exonuclease assay and DNase I footprint analysis we detect two interactions in mature B cell lymphomas but not pre-B cell lymphomas or MEL cells and another interaction in immature B-lymphoid tumors and MEL cells. To search for functional sequences, we have made a vector, containing the murine c-*myb* exon I, intron I and exon II fused to a CAT reporter gene, that appears to faithfully reproduce attenuation when stably transfected into an MEL cell line which can be chemically induced to differentiate and down-regulate c-*myb* mRNA levels by attenuation. Stable MEL transfectants, containing either the parental or deleted constructs, have been used to identify a 700bp intron I fragment that is required for the transcriptional regulation of the c-*myb* gene during chemically induced differentiation. This fragment includes the tentative position of attenuation (mapped by nuclear run-on analysis) as well as one of the protein/DNA interactions that is correlated with attenuation in our panel of hematopoietic tumor cell lines. We are currently making vectors with fine-deletions and base substitutions to assay the relevance of this protein DNA interaction to transcriptional attenuation as well as examining promoter sequences for potential involvement.

U 405 ENHANCER EFFECTS ON TRANSCRIPTIONAL TERMINATION IN THE C-MYC GENE, David L. Bentley, ICRF, Lincoln's Inn Fields, London, WC2A3PX, U.K.

Several enhancer elements have been inserted into the 5' flanking sequence of the human c-myc gene and analysed by nuclear-runoff assay in stably and transiently transfected cells. The results imply that enhancers are able to suppress premature termination in exon 1. This observation suggests a possible explanation for the suppression of termination observed in the translocated c-myc gene of Burkitt's lymphomas.

U 406 CLONING AND CHARACTERIZATION OF C-*MYC* PROMOTER BINDING FACTORS, Steven A. Bossone*, Claude Asselin#, Amanda J. Patel# and Kenneth B. Marcu*#§, Depts. of Pathology*, Biochemistry# and Microbiology§, SUNY at Stony Brook, Stony Brook, NY 11794

The nuclear factor binding site, ME1a1 has been shown through deletional analysis to be critical for proper usage of the major c-*myc* promoter, P_2, and also for the premature termination of transcription within the gene's first exon (Oncogene,'89, 4:549; MCB, '89, 9:5340). This site, identified by DNase I protection and methylation interference, resides between +97 to +118 with respect to the P_1 start site. We have extended these studies by utilizing a site directed mutant, obtained by mutating four base pairs in the ME1a1 core binding sequence. Introduction of this mutant, which abolishes nuclear factor binding to this site as assayed by gel mobility shift, into CV1 cells, dramatically reduced P2 usage and shifted the P_2:P_1 ratio from that normally seen of 3:1 to 1:15. Mutation of the E2F binding site between +91 and +101 had no effect on the P_2:P_1 ratio. Replacement of the ME1a1 site by an Oct-1 binding sequence did not restore the normal P_2:P_1 ratio. The ME1a2 binding site is positioned upstream of the ME1a1 site at +58 to +78. ME1a2 mutants had a reduced P_2:P_1 ration of 1:1 due to a reduction in P_2 activity. These results indicate that proper binding of a nuclear factor to the ME1a1 site is essential for P_2 usage, while the ME1a2 site may contribute to but is not critical for P_2 usage, at least in CV1 cells.

Screening a λgt11 expression library with an ME1a1 binding site probe yielded one positive clones which binds specifically to the ME1a1 site and fails to bind the site directed mutant. Screening with an ME1a2 probe yielded one positive clone. The ME1a1 probe was able to bind the clone obtained with the ME1a2 probe while the ME1a1 clone did not bind to the ME1a2 probe. Southern analysis of the two clones showed no cross-hybridizing bands, indicating that both clones are unique. The Genbank data bank was searched with a partial nucleic acid sequence from these clones and revealed that they are heretofore unknown genes. Characterization of these novel DNA binding factors is in progress.

U 407 NOT ALL PROMOTERS ARE TRANSCRIBED BY RNAP IIB, Andrew B. Buermeyer and Peggy J. Farnham, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wi. 53706.

We have inhibited in vitro transcription reactions with monoclonal antibodies raised against the carboxy-terminal domain (CTD) heptapeptide repeat of eucaryotic RNA polymerase II (RNAP II) and examined the ability of various RNAP II promoters to be activated by purified calf thymus RNAP II lacking the CTD (RNAP IIb). Our results indicate that not all eucaryotic promoters can be efficiently transcribed by RNAP IIb. Some promoters were strongly stimulated (4-6 fold), others were weakly stimulated (2-4 fold), while others were not detectably stimulated. These results did not correlate with the in vitro promoter strength, the presence or absence of a TATA box or the presence of Sp1 sites. This is best illustrated by the bidirectional DHFR/REP locus. Both promoters are Sp1-activated and do not contain a TATA box. The REP promoter was transcribed by RNAP IIb, whereas, the DHFR promoter was not. We are investigating whether the ability to be transcribed by RNAP IIb is controlled at the level of elongation or initiation.

U 408 DEFINITION OF TRANSCRIPTIONAL ARREST SITES IN EXON 1 OF THE HUMAN ADENOSINE DEAMINASE GENE, Zhi Chen and Rodney E. Kellems, Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030.

Transcriptional arrest sites have been identified in exon 1 of the human adenosine deaminase (ADA) gene and this block to transcription elongation plays a role in ADA gene regulation (*Mol. Cell. Biol.* 10:4555). To define the template requirements of the transcriptional arrest, various ADA templates were constructed and their ability to confer transcription arrest has been determined in the *Xenopus* oocyte transcription system. We identified a 43 bp DNA fragment which is required for the major arrest site and it functions in an orientation–dependent manner. Insertions of *Eco*R1 linkers between the promoter element and exon 1 did not abolish transcription arrest, eliminating involvement of a short distance effect in this process. A dinucleotide mutation at the 5' splicing site did not affect the arrest process, indicating that RNA splicing does not play a role in the transcription arrest. The exon 1 transcriptional arrest signal functions downstream of several heterologous promoters, implying that the transcription arrest process in exon 1 of the human ADA gene is promoter–independent. Therefore, the *Xenopus* oocyte transcription system is very useful in delineating the boundary of the transcriptional arrest and its promoter dependence as well as distance requirements have been defined. We are now trying to understand the mechanism of the transcriptional arrest and the means by which it governs tissue–specific expression of the ADA gene in human cells.

U 409 REGULATION OF λ N GENE EXPRESSION, Donald Court,[1] Leonor Fernandez,[1] Luis Kameyama[1] and Gabriel Guarneros[2]
[1]ABL-Basic Research Program, NCI-Frederick Cancer Research & Development Center, P.O. Box B, Frederick, Maryland 21702 USA, [2]Department of Genetics & Molecular Biology CINVESTAV-IPN, Mexico City, Mexico.
The N gene is the first gene in the p^L-operon, and its AUG initiation signal is located at 223 nucleotides from the start of transcription. N regulates λ gene expression by transcription antitermination. The long N- mRNA leader contains the nutL region, with which N interacts to cause RNA polymerase to antiterminate. Downstream of nutL, in the N-leader, are RNaseIII processing sites.
To monitor N gene expression, we measured β-galactosidase from N-lacZ fusions. The p^L promoter, the N-leader and the N-lacZ (gene or operon) fusion construct was made and tested in multiple and single copy.
When processed by RNaseIII, N translation is stimulated. No effect was observed by RNaseIII processing upon expression of the operon fusion. When the RNase III processing site was deleted, the presence or absence of RNaseIII in the cell had no effect upon translation.
Expression of the N-lacZ gene fusion but not the operon fusion was inhibited several-fold in the presence of N protein. When the RNaseIII sites of the N-leader were deleted, N still inhibited. However, when part of the nutL site was deleted N inhibition was eliminated. We suggest that N inhibits its own expression at the translational level. This inhibition is dependent upon nutL, but the mechanism by which it occurs is not obvious. We note that N normally interacts with the transcription apparatus via nutL. Could it also interact directly with the translation apparatus, or is the effect on translation indirect?
Research sponsored in part by the National Cancer Institute, DHHS, under contract no. NO1-CO-74101 with ABL.

U 410 HIV-1 **TAT PROMOTES INITIATION AND STABILIZES ELONGATION OF TRANSCRIPTION IN A CELL-FREE SYSTEM**, Urvashi Dhingra, Maureen Holly, Liberata De Santis, Ming-Chu Hsu, Departments of Anti-Viral Research and Molecular Genetics, Hoffmann La-Roche Inc., 340 Kingsland Street, Nutley, New Jersey 07110
High level expression of the HIV-1 genome *in vivo* requires both HIV-1 tat and the trans-activation responsive element (TAR) present immediately downstream of the transcription start site. An *in vitro* cell-free system that faithfully reproduces the *in vivo* transcriptional trans-activation would render the process amenable for biochemical analyses. We demonstrate 7-13 fold trans-activation of HIV-1 LTR-directed transcription in nuclear extracts from a HeLa-tat cell line. Trans-activation *in vitro* required the cis-acting TAR sequence and was abolished by antibodies against tat. Exogenous TAR RNA, but not TAR DNA, competed for trans-activation. Tat increased both promoter-proximal and promoter-distal transcription from the HIV-1 LTR in this system, indicating increases in both initiation and elongation of transcription by tat.

U 411 **A PROTEIN BINDING SITE IN THE PROMOTER OF THE MURINE C-MYC GENE IS IMPORTANT FOR TRANSCRIPTIONAL BLOCK**, Daniel Dufort, Harvey Miller, and Alain Nepveu, Ludwig Institute for Cancer Research, Montreal Branch, Quebec, Canada, H3A 1A1.
The murine *c-myc* gene, like several eukaryotic genes, can be regulated at the level of transcription elongation. Deletion analysis of the murine c-*myc* gene has identified at least two cis-acting elements necessary for transcriptional block: a 3' element located close to the site of block, and a 5' element located within the promoter region between the P1 and P2 transcription initiation sites. Several protein binding sites have been identified in this region. Removal of one of these sites, ME1a1, results in the abrogation of transcriptional block while reducing initiation of transcription at P2. An oligonucleotide encoding the ME1a1 protein binding site can confer transcriptional block to a heterologous promoter when placed in conjunction with the *c-myc* 3' element. Oligonucleotides containing mutations within the ME1a1 protein binding site have been tested. Our results demonstrate that mutations in ME1a1 that reduce protein binding also affect the ability to confer transcriptional block. We are currently trying to characterize the protein(s) which interacts with the ME1a1 site.

U 412 TRANSLOCATION OF *E. coli* RNA POLYMERASE FOLLOWED BY DNA AND RNA FOOTPRINTING

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We have used hydroxylradicals as probe to determine the change of protection of the DNA and the RNA in different states of RNA synthesis. From these data a model was derived how RNA polymerase moves along the DNA.

U 413 PREMATURE TERMINATION OF TRANSCRIPTION OF THE MURINE ADENOSINE DEAMINASE GENE IN A CELL-FREE SYSTEM, Jeffrey W. Innis and Rodney E. Kellems, Institute for Molecular Genetics and Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030
An elongation block to transcription in exon one is a major step in regulation of mRNA production in the murine adenosine deaminase gene. To investigate the mechanism of transcription arrest, we studied transcription in vitro with HeLa nuclear extracts and purified ADA templates. Premature termination of transcription occurred predominantly 96 and 144 nucleotides(nt) downstream from the transcription initiation site. The 96 nt RNA is identical to that found in steady-state RNA from oocytes injected with ADA templates and is a product of a 108 nt precursor. The 108 and 96 nt RNA molecules are enriched in the supernatant solution after centrifugation of transcription complexes suggesting that these transcripts are terminated and released from the template. The use of supercoiled templates favors the formation of the 96 nt transcript. 108 nt transcripts purified from transcription reactions are processed to 96 nt suggesting that processing can be uncoupled from transcription. Longer ADA transcripts are not cleaved to 96 nt implying that processing requires a specific 3' end. Formation of the 108 nt precursor and processing to 96 nt are inhibited by low concentrations of Sarkosyl whereas the generation of the 144 nt RNA is unaffected over a wide range of concentrations tested. Transcription complexes which do not terminate at +108 are capable of terminating at +144. Therefore, the signals for termination at these two sites are different. The cell-free system will allow further characterization of the template and factor requirements for transcription arrest.

U 414 MUTATIONS IN RET1, THE YEAST GENE ENCODING THE SECOND LARGEST SUBUNIT OF RNA POLYMERASE III, Philip James, Salam Shaaban, Linda K. Hardison, and Benjamin Hall, Dept. of Genetics, University of Washington, Seattle, WA. 98195

The RET1 gene was cloned by complementation of ret1-1, a recessive allele that reduces termination by RNA polymerase III. The ret1-1 mutation is a T315K change in a non-conserved region 150-200 amino acids N-terminal to sequences resembling the Rif^R region of E. coli RPOB. Mutations have been generated throughout this area by "spiked oligonucleotide" mutagenesis. Mutant screening for RET1 alleles with altered Pol III behavior utilized SUP4-o alleles that score either for decreased termination (SUP4-UIV) or for increased termination (SUP4-Δ94). In the Rif^R-like region, the mutation Q490H slightly affects suppression of the two tester alleles in opposite directions, indicating that the mutant RNA polymerase has decreased transcription termination when yeast is grown at 38°C. The Gln to His change in this mutant is a change toward the consensus residue of the RPOB family at this position. RET1 shares thirteen conserved sequence blocks with all other genes for RNA polymerase catalytic subunits. Situated between the last two conserved sequence blocks is the sequence 1092-VDVCDKCGLMGYSGWCTTCKSAENI-1116. A similar zinc-finger motif is found, with varying distance between the cysteine pairs, in all archaebacterial and eukaryotic nuclear RNA polymerases. Selected residues in this region of RET1 have been mutagenized; thus far, C1098A has proven to give a functional subunit, while G1099V lacks function. Because of the ability of the yeast Pol III system to carry out initiation, elongation, and termination correctly in vitro, transcription with RNA polymerase III from these and other ret1 mutant strains will make possible the assignment of specific functions to the conserved domains of the eukaryotic RNA polymerase III catalytic subunit.

U 415 SEQUENCE REQUIREMENTS FOR PREMATURE TERMINATION IN THE MURINE ADENOSINE DEAMINASE GENE. Shera F. Kash, Jeffrey W. Innis and Rodney E. Kellems. Institute for Molecular Genetics and Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030.
Premature termination of transcription plays a major role in the regulation of murine adenosine deaminase (ADA) gene expression. In a cell-free system, short transcripts of 96 nucleotides (nt) have been shown to accumulate and are processed from a 108 nt precursor. The termination event at +108 occurs within the first exon following the 5' untranslated (5' UT) region and requires not more than 60 base pairs 3' to the termination site. To address potential sequence requirements within the promoter, 5' UT region and the region downstream of the termination site, we have constructed ADA gene fragments with mutations in these regions. To determine whether the RNA structure of the 5' UT region is important for termination, this region was inverted with respect to the promoter. To investigate the role of the downstream region, variable amounts of the 3' end of this region were deleted in constructs. To find out whether this downstream region can function in an orientation-independent manner, constructs which have this region in the opposite orientation with respect to the promoter were made. To address potential distance requirements between the promoter, 5' UT region and downstream region for the termination event, a series of insertion mutations was made between the latter two regions. To determine whether the 5' UT and downstream regions can function in the context of a different promoter, a construct which has these regions following the adenovirus major late promoter was constructed. To investigate whether the downstream element can function autonomously following a different promoter without the ADA 5' UT region, one to three copies of this downstream region were placed downstream of the adenovirus major late promoter. Following confirmation of the structure of these constructs by DNA sequencing, they will be analyzed in the *in vitro* transcription assay.

U 416 PHOTOAFFINITY LABELLING OF PEA CHLOROPLAST RNA POLYMERASE TRANSCRIPTIONAL COMPLEXES BY NASCENT RNA IN VITRO
Sujata Lakhani, Navin Khanna and K. K. Tewari, International Centre for Genetic Engineering & Biotechnology, NII Campus, Shaheed Jeet Singh Marg, New Delhi - 110 067, INDIA
We have used photoaffinity labelling to examine the chloroplast RNA polymerase components which come into contact with nascent transcripts during the in vitro transcription of plastid DNA. The transcripts were synthesized in the presence of a photoactive analogue (4-thio-UTP) and α-^{32}P-ATP, using a highly purified chloroplast RNA polymerase and a recombinant plasmid containing the plastid 16S rRNA promoter. Irradiation of the transcriptional complex crosslinked the photoactive nascent RNA to proximal proteins. The labelling of transcriptional complexes was dependent on 4-thio-UTP and template DNA which supported specific transcription. Two polypeptides of 60 and 56 kDa were consistently photoaffinity labelled by the nascent transcripts; about 60% of the total radioactivity of the crosslinked RNA was associated with these complexes. In some experiments, two additional polypeptides of 75 and 48 kDa were also found to be associated with about 17% and 13% of total crosslinked RNA radioactivity. The UV-crosslinked transcriptional complexes were stable to S1 nuclease hydrolysis but partially sensitive to RNase TI. Insensitivity of the complex to hydrolysis with either DNase I or RNase H suggested that the nascent transcripts were crosslinked to the proteins under our experimental conditions. The complexes were also partially hydrolysed by treatment with thermolysin. Irradiation of chloroplast RNA polymerase following incubation with purified transcripts containing the photoactive analogue did not result in the formation of a photocrosslinked complex. This suggested that only nascent transcripts were responsible for photoaffinity labelling. The results indicate that nascent transcripts contact at least two polypeptides of 60 and 56 kDa during transcription of ct DNA.

U 417 P4'S POLARITY SUPPRESSOR IS BOTH A TRANSCRIPTION ANTITERMINATION FACTOR AND A CAPSID STABILIZING PROTEIN, Nora A. Linderoth[1], Morten L. Isaksen[2], Richard L. Calendar[1] and Bjorn H. Lindqvist[2], [1]Dept. of Molecular and Cellular Biology, University of California, Berkeley, CA 94720, [2]University of Oslo, Institute of Biology and the Biotechnology Center of Oslo, Oslo, Norway.
The *p*olarity *su*ppression (*psu*) gene of bacteriophage P4 was cloned and its gene product overproduced. In vivo, cloned Psu protein suppressed the effects of mutational polarity in the late genes of helper phage P2 and in the host chromosome. Psu action required neither the presence of other phage proteins nor that transcription be initiated from a phage promoter. In characterizing the polarity suppression activity of Psu, we found that it caused efficient read-through by *E. coli* RNA polymerase at the rho-dependent terminators t_{R1} and T_{IS2}, either singly or in combination, but did not affect rho-independent events. The conserved antitermination sequence '*boxA*' was not required for polarity suppression. Psu protein promoted neither transcription nor translation initiation, nor the translation of a *lacZ* mRNA involved in secondary structure. Our data support a model in which Psu protein effects polarity suppression by inhibiting rho-dependent transcription termination, possibly by its direct interaction with RNA polymerase. The Psu protein is also found associated with P4 capsids. The presence of Psu on P4 capsids stabilizes the phage against certain kinds of stress. This stabilizing effect of Psu can be complemented in vivo and vitro. Psu is not able to bind to or stabilize the larger P2 capsids, whether they contain P2 DNA or P4 DNA. Psu is therefore thought to be an external stabilizing decoration protein specific for the P4 capsid.

U 418 EFFICIENT TERMINATION IN THE HUMAN c-*myc* GENE DURING TRANSCRIPTION IN VITRO. Lucille London, Richard G. Keene, and Robert Landick, Dept. of Biology, Washington University, St. Louis, MO 63130

Transcript elongation by RNA polymerase II in the c-*myc* gene is blocked near the exon 1-intron 1 junction in differentiated but not proliferating cells. The block to elongation has been mapped to T-rich sites (T1 and T2) on either side of the exon 1- intron 1 junction and appears to vary in vivo depending on the promoter used. We have investigated transcription of c-*myc* in vitro by testing transcription templates containing the native c-*myc* P2 promoter and downstream sequences or a precise substitution of P2 with the Adenovirus type 2 major late promoter (AdMLP). The use of these templates produces identical RNA transcripts. We found little, if any, intrinsic arrest in c-*myc* during transcription of either template in HeLa cell nuclear extracts using standard conditions. Addition of some agents known to perturb transcriptional elongation, such as heparin or sarkosyl, produced only low efficiency arrest near the exon-intron junction. However, addition of KCl to 400 mM during transcription caused a block to elongation at site T2 on both templates. The block to elongation resulted from transcriptional termination, as assayed by the retention of the transcript during filtration through Sepharose 4B. In addition, when an oligonucleotide that specifies the human c-*myc* site T2 was inserted in unrelated DNA sequence and transcribed from the AdMLP, we observed efficient termination in the presence of 400mM KCl. The efficiency of termination at site T2 varied significantly when different extracts were used and appeared to depend on the growth conditions of the cells from which the extracts were prepared. Thus, it appears that trans-acting factors may control termination at T2. Furthermore, when 400 mM potassium glutamate or potassium acetate were used in place of KCl, we observed a lesser, although still significant, level of termination at T2. Thus, termination does not result solely from chloride ion masking protein - nucleic acid interactions. We now are investigating the nature of the apparent regulatory factor and its target by fractionation of the nuclear extracts.

U 419 ELONGATION AND TERMINATION BY VACCINIA RNA POLYMERASE DURING A SINGLE ROUND OF TRANSCRIPTION IN VITRO. Yan Luo, Jeremiah Hagler, and Stewart Shuman, Molecular Biology Program, Sloan-Kettering Institute, New York, NY 10021

We have developed a system for analysis of discrete steps in vaccinia virus early mRNA synthesis during a single round of transcription in vitro. A synthetic early promoter is used to direct transcription by vaccinia RNA polymerase of a G-less cassette in linear duplex DNA. Omission of GTP from transcription reactions leads to the formation of ternary elongation complexes paused stably at the end of the G-less cassette. These complexes can be induced to elongate by provision of GTP. While initiation of transcription is sensitive to low concentrations of salt and sarkosyl, elongation is resistant to these agents. Termination can studied in a single synthetic cycle by forming transcription complexes paused just proximal to the termination signal TTTTTNT that can subsequently elongate and terminate. By selectively incorporating the termination-inhibiting analog BrUMP into proximal and distal portions of the nascent transcript, we localize the termination signal within or near the sequence UUUUUNU in the nascent RNA. We show that access of the termination factor (VTF/capping enzyme) to the transcriptional apparatus can occur subsequent to initiation and synthesis of a 390 nucleotide nascent RNA. Termination is considerably more sensitive to inhibition by salt and sarkosyl than is elongation. This sensitivity is not reversed by preincubation of VTF with the transcription complex.

A few early viral transcription units contain internal termination signals that are "ignored" in vivo. We have recapitulated this phenomenon in the in vitro system and have shown that antitermination is attributable to RNA secondary structure. When the signal UUUUUNU is positioned such that it may form part of a stem loop structure, termination is abrogated. This may have implications for the switch in termination mechanisms that occurs during the vaccinia replication cycle.

U 420 PROTEIN-PROTEIN INTERACTIONS WITHIN TRANSCRIPTION ELONGATION COMPLEXES CONTAINING THE N PROTEIN OF BACTERIOPHAGE λ, Stephen W. Mason and Jack Greenblatt, Department of Molecular and Medical Genetics, and Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

Transcription elongation complexes isolated from reactions containing the λ N protein, a *nut* site containing plasmid DNA template and the purified *E. coli* factors NusA, NusB, NusG, and ribosomal protein S10 contain all of these proteins, including S10. Mutations in the β-subunit of RNA polymerase, in NusA, and in the *nut* site that abolish antitermination *in vivo* also prevent the incorporation of N and NusB into the transcription elongation complex *in vitro*. Omission of any of the elongation factors from the transcription reaction reduces the amount of S10 and NusG in the elongation complex and prevents the incorporation of N and NusB into the complex. This suggests that the assembly of transcription elongation complexes containing the λ N protein is highly cooperative and involves multiple protein-protein and protein-nucleic acid interactions. It was previously shown that NusA binds N and RNA polymerase and presumably couples N to the RNA polymerase in N-modified transcription complexes. We have now used protein affinity chromatography with immobilized S10, GST-S10, and NusB to show that NusB binds directly and selectively to S10 with a K_d of about $5x10^{-6}$ M. In addition, S10 binds directly to a specific site on free RNA polymerase or the RNA polymerase in initation or elongation complexes. Therefore, S10 probably couples NusB to RNA polymerase in N-modified transcription complexes.

U 421 PREMATURE TERMINATION OF TUBULIN GENE TRANSCRIPTION IN *XENOPUS* OOCYTES

Garry T. Morgan, Kim G. Rabbitts and Kim M. Middleton, Department of Genetics, University of Nottingham, Queens Medical Centre, Nottingham NG7 2UH, U.K.

The *X. laevis* α-tubulin gene XαT14 is expressed during oogenesis, and shows accurate and efficient initiation of transcription when injected into oocytes over a wide range of template concentrations. However, at all but the lowest concentrations (i.e. 10-60 pg/nucleus) premature termination of transcription occurs frequently in the 5′ leader and results in the accumulation in the cytoplasm of an array of correctly-initiated transcripts of 45-76 nucleotides. Subclones containing as little as 200 bp upstream and 87 bp downstream of the initiation site exhibit this behaviour. Immediately downstream of the initiation site the transcript is apparently capable of forming a 36 bp stem/loop and a similar structure is conserved in the leaders of several mammalian tubulin genes. We have found, however, that removal of the stem/loop and the rest of the leader does not increase the production of "elongated" transcripts and that truncated transcripts are still present, presumably as a result of termination in flanking vector sequences. It seems possible that premature termination in XαT14 may result from a reduction in the processivity of many of the polymerases initiating at its promoter such that they terminate at any number of fairly non-specific sites in the transcribed DNA. Inefficient elongation of transcription through the injected gene is also suggested by nuclear run-on analyses measuring the relative amounts of transcription at various points along the entire 6.8 kb transcription unit. We have also found that as the amount of injected template is increased prematurely terminated transcripts represent a progressively greater proportion of the total transcripts initiated, and this is true even in oocytes in which the supply of polymerases able to produce elongated transcripts has not been exhausted. Thus, although premature termination may be due to a disruption of processivity, it seems to involve a template-dependent activity rather than simply one associated with RNA polymerase and we are attempting to determine if particular elements of the XαT14 promoter are involved in specifying the efficiency of transcription elongation.

U 422 PREMATURE TERMINATION OF MOUSE C-MYC TRANSCRIPTION

Sadia Roberts and David L Bentley, Imperial Cancer Research Fund Laboratories, P.O. Box 123 London WC2A 3PX, UK.

Premature termination of transcription within the mouse c-myc oncogene has been studied using the Xenopus oocyte microinjection system. A possible role of the promoter on the ability of polymerases to terminate has been investigated. A 200bp fragment comprising the termination site and flanking sequences confers termination from a number of diverse heterologous promoters- namely HSV TK, U1 snRNA and human alpha-globin- demonstrating no promoter specifity. Increasing the distance between the site of termination and the promoter has revealed that the efficiency of termination is distance-dependent. We believe that this may contribute to some of the observations of "promoter effects" reported in the literature.

U 423 TRANSCRIPTIONAL ATTENUATION IN THE DHFR GENE

Lynda J. Schilling and Peggy J. Farnham, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706.

Nuclear runon analysis suggests that there is greater transcriptional activity in the 5' end of the murine dhfr gene than in the 3' end. This result has been observed in NIH3T3 cells, F9 cells, and in a line derived from NIH3T6 cells which have been amplified for the dhfr locus by selection in methotrexate. In these logarithmically growing cells there was 15-30 fold higher transcriptional activity in the first exon than in the last exon. In fact, the high transcriptional activity declines before the end of the first intron and is thus contained within the first 300 bp of the gene. There are two inverted CCAAT boxes within this region. Interestingly, this motif has been shown to function as a transcriptional terminator in other systems. We are currently examining steady state RNA for the presence of an attenuated dhfr mRNA. Preliminary data suggests that attenuation may be regulated during density and/or serum arrest. We are monitoring the transcription rate throughout the dhfr gene during growth arrest and release from growth arrest to determine if there are modulations in the extent of attenuation in response to changes in growth conditions.

U 424 PROMOTER ELEMENTS PROGRAM THE c-myc BLOCK TO TRANSCRIPTION ELONGATION, Charlotte A. Spencer[1] and Mark Groudine[2], [1]Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7 and [2]Fred Hutchinson Cancer Research Center, Seattle, WA. 98104.

The block to transcription elongation in the c-myc proto-oncogene operates in both a promoter-specific and modulatable way, and contributes to c-myc regulation in normal and differentiating cells. Transcription from the P1 promoter reads through the exon 1 block region, whereas transcription from the major promoter, P2, can be modulated to block or to read-through. Burkitt's lymphoma cells show a shift to the P1 promoter, a loss of the transcription block and hence high or unregulated levels of steady-state c-myc RNA. Using in vitro mutagenesis and expression in Xenopus oocytes and mammalian cells, we have determined that c-myc promoter elements, rather than RNA structure per se, program the c-myc block to transcription elongation. We will also present data that support the hypothesis that RNA polymerase II elongation factors associate with transcription complexes at discrete sites within the P1 and P2 promoter regions. These factors modify transcription complexes to either recognize or read-through downstream transcription block signals.

U 425 FACTORS INVOLVED IN READTHROUGH OF THE ADENOVIRUS ATTENUATION SITE IN VITRO Debra Wiest, Daguang Wang, and Diane Hawley, Institute of Molecular Biology, University of Oregon, Eugene, 97403

Transcripts initiated from the adenovirus major late promoter in a nuclear extract pause or terminate at +184 in the presence of Sarkosyl. To address the mechanism of elongation arrest at this site *in vitro*, we have used two assay systems. First, we have analyzed the readthrough activity of purified RNA polymerase II at the site using a 3' extended poly(dC) tailed template. Second, we have attached DNA templates to agarose beads, incubated them with extract to allow preinitiation complexes to form, and washed these complexes free of unbound proteins. Elongation by both the washed complexes and purified polymerase is efficiently blocked at the attenuation site in the absence of Sarkosyl. These results suggest that the nuclear extract contains one or more proteins that modify the elongation behavior of RNA polymerase II at this site. Consistent with findings from other laboratories, addition of the elongation factor SII to either washed complexes or purified polymerase results in readthrough of the attenuation site. In addition, we have obtained a partially purified chromatographic fraction that when added back to washed complexes appears to function in combination with SII to promote readthrough of the site.

Late Abstract

CONTROL OF BACTERIOPHAGE P4 IMMUNITY BY TRANSCRIPTION TERMINATION, Gianni Dehò, Daniela Ghisotti, Sandro Zangrossi, Pierangela Sabbattini, Francesca Forti, Flavia Piazza, and Gianpiero Sironi, Department of Genetics and Microbiology, University of Milano, Italy.

In the prophage P4, expression of the early genes is prevented by premature termination of transcription from the constitutive promoter P_{LE}. This leads to the production of a transcript 300 nt long and of a more abundant family of 80 nt RNAs which may derive from the former by processing. No other phage coded products involved in the P4 immunity appear to be expressed by the prophage; we have obtained evidence that the transcripts produced by the prophage are directly involved in the maintenance of P4 immunity by promoting transcription termination. Two inverted repeats downstream of P_{LE} (*seqA* and *seqB*) appear to be relevant in this process: we suggest that pairing of these repeats favours transcription termination at immunity-specific termination sites. Transcription from a plasmid of an RNA containing only the P_{LE} proximal repeat (*seqA*) prevents the establishment of lysogeny, suggesting that an excess of *seqA* may compete with *seqA-seqB* pairing in the P4 transcripts, thus interfering with the establishment of immunity. A promoterless P4 fragment 170 nt long overlapping *seqB* cloned in a multicopy plasmid promotes in *trans* the establishment of immunity of a superinfecting P4 phage. We suggest that this fragment contains a binding site for the activation of a host coded transcription termination factor involved in the P4 prophage immunity. *Escherichia coli* mutants preventing P4 lysogenization have been isolated.