MOLECULAR BIOLOGY OF RNA

Organizer: Thomas Cech

April 4-10, 1988

Page

Plenary Sessions

Poster Sessions

Higher Order Structure of RNA

N 001 A PHOTOCROSSLINKING APPROACH TO RNA STRUCTURE, Samuel E. Lipson, Joseph A. Monforte and John E. Hearst, Department of Chemistry, University of California, Berkeley, CA 94720.
The RNA moiety of ribonuclease P from *Escherichia coli* (M1 RNA) has been photoreacted with 4'-hydroxymethyl-4,5'8-trimethylpsoralen and long wave UV light (320-380 nm) in a buffer in which the M1 RNA alone acts as a true catalyst of tRNA processing. Limited specific digestion followed by two dimensional gel electrophoresis yields fragments crosslinked by HMT. The positions of the crosslinks have been determined to within $\pm$15 nucleotides by photoreversal of the isolated crosslinked fragments and enzymatic sequencing of the resulting RNA. Assignments of the exact locations of the crosslinks have been predicted based on the known photoreactivity of the psoralen with different bases. A nucleotide resolution map of both crosslink and mono-adducted psoralen sites using AMV Reverse Transcriptase mapping methods is in progress. Psoralen crosslinked dimers of M1 RNA are observed. Seven intramolecular interactions and three intermolecular interactions have been identified. These interactions and new models for M1 RNA structure are discussed in detail.

N 002 3-D INTERACTIONS IN CLASS II CATALYTIC INTRONS AS REVEALED BY COMPARATIVE SEQUENCE ANALYSIS AND SITE DIRECTED MUTAGENESIS, François Michel and Alain Jacquier, Centre de Génétique Moléculaire du CNRS, 91190 Gif-sur-Yvette, and Institut Pasteur, 75724 Paris Cedex 15, France.
We are trying to identify those nucleotides of a class II self-splicing intron that interact with intron-exon junctions, with the goal of assessing the currently hypothesized relationship between class II introns and the nuclear premessenger splicing system. In a first step, we searched alignments and secondary structure models of the 67 known sequences of class II introns for nucleotides that covary with those ajoining intron-exon junctions. Five interactions have already been uncovered and proven by disrupting them through site-directed mutagenesis, followed by introduction of compensatory base changes that restore splicing. (1) Two single-stranded loops (EBS1 and EBS2) within the first structural domain of the introns interact with the last nucleotides of the 5' exon, forming two double-stranded segments of typically six base pairs each[1]. (2) Part of the GUGYG consensus sequence at the 5' end of the introns interacts with another single-strand loop within domain I. (3) The last nucleotide of class II introns must base pair with the one at the 'gamma' site, at the junction between domains II and III : by putting a C at the latter site we have shown it is possible to create a functional class II intron with GU...AG termini. (4) Many class II introns seem to possess an 'internal guide' for exon ligation, with the nucleotide immediately upstream of the EBS1 sequence interacting with the first nucleotide of the 3' exon: we have tested this possibility in Saccharomyces cerevisiae intron a5 by generating various combinations of bases at the two sites. Our data will be discussed both from a methodological point of view and for their implications concerning the plasticity and evolution of class II introns.
ref. 1: Jacquier, A. and Michel F. Cell 50 (1987) 17-29.

N 003 **HIGH FIELD NMR AND SITE-DIRECTED MUTAGENESIS AS PROBES OF THE STRUCTURE AND FUNCTION OF 5S RNA,** **Peter B. Moore, Daniel T. Gewirth, and Penghua Zhang, Departments of Chemistry, and Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511.**

The 5S RNA from E. coli is a small ribosomal RNA that interacts in a sequence-specific manner with three low molecular weight ribosomal proteins, L5, L18, and L25. It is an appropriate system in which to study RNA structure, and the interactions responsible for sequence-specific binding of proteins to RNA. NMR is a powerful method for obtaining structural information about such systems, but in order that it be used effectively, the molecular weights of the materials to be examined must be as low as possible, consistent with retention of function. Recently it has become possible to generate both point and deletion mutations of 5S RNA almost at will. Depending on the severity of the lesion, the mutant products can be produced off plasmids in vivo or synthesized in vitro using the RNA polymerase from bacteriophage T7. The structural consequences of these alterations in sequence can be monitored by NMR.

Both the protein binding properties and the spectroscopy of several 5S RNA variants will be discussed. It appears that the binding site for L25 is contained almost entirely within helices IV and V of 5S RNA (bases 70-106), consistent with earlier data from many sources. The deletion data suggest that the L18 interaction, on the other hand, depends strongly on interactions with sequences outside the region its presence protects from chemical attack. It is also clear that point mutations that do not disrupt the structure of the RNA to any large degree can disrupt protein binding significantly. Ribosomal proteins may recognize specific bases in their binding sites.

This work is supported by grants from the N.S.F. (DMB-8608283), and N.I.H. (GM-22778).

N 004 FACTORS DETERMINING RNA STRUCTURE AND FUNCTION, Douglas H. Turner, Naoki Sugimoto, John A. Jaeger, Alison Williams, Carl E. Longfellow, Department of Chemistry, University of Rochester, Rochester, NY 14627, and Michael Zuker, Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A OR6.

Studies on oligonucleotides have provided insights into the contributions of base pairing, stacking, Na^+, and Mg^{2+} to the stabilities of short double helixes (1,2). Comparisons with the crystal structures of transfer RNAs suggest stacking interactions in large RNA molecules are similar to those in oligonucleotides. A RNA structure prediction program incorporating these stacking interactions has been developed. When only the lowest free energy structure is considered, the program successfully predicts on average about 70% of the phylogenetically conserved helixes for a set of tRNA, 5S RNA, 16S RNA, and group I intron sequences. When the "best" structure within 10% of the lowest free energy is considered, about 85% of the phylogenetically conserved helixes are predicted. The results suggest inclusion of stacking interactions will be important for reliable predictions of structure from sequence.

Studies on reverse cyclization of the covalently closed, circularized form of the self splicing intron from Tetrahymena thermophila indicate binding of oligonucleotides and the effects of Na^+ and Mg^{2+} are not easily predicted from oligonucleotide studies. For example, the binding constant for the substrate CU is about 4 orders of magnitude larger than predicted. The effects of Na^+ and Mg^{2+} are not easily related to helix stabilization. In addition, the temperature dependence for reverse cyclization indicates the activation energy and entropy are both large and positive. Implications for the mechanism of reverse cyclization will be discussed.

1. D. H. Turner, N. Sugimoto, & S. M. Freier, Ann. Rev. Biophys. Biophys. Chem. 17, in press (1988).
2. D. H. Turner, N. Sugimoto, J. A. Jaeger, C. E. Longfellow, S. M. Freier, & R. Kierzek, Cold Spring Harbor Symp. Quant. Biol. 52, in press (1987).

RNA Catalysis

N 005 GENETICS OF GROUP I INTRONS IN THE T-EVEN PHAGES, Marlene Belfort[1], Dwight Hall[2] and David Shub[3], [1]Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany NY 12201; [2]School of Applied Biology, Georgia Institute of Technology, Atlanta, GA 30332; [3]Department of Biological Sciences, State University of New York, Albany, NY 12222.

The td intron in the thymidylate synthase gene of phage T4 lends itself favorably to a variety of mutagenesis strategies for studying the catalytic character of a group I intron. Genetic selections (for td^+ as well as td^-) and phenotypic screens have facilitated these approaches. A combination of non-directed (1,2) as well as site-directed mutagenesis studies and deletion analyses allow the following conclusions to be drawn: 1) Fewer than 250 residues of the 1016-nt intron are required for accurate and efficient cleavage-ligation activity; 2) Splicing-defective mutations in the group I core elements P1, and P3 through P9 (as per the td intron model of F. Michel) define these as functionally important structures; 3) Residues within the upstream exon that form part of the P1 stem are major specificity determinants in 5′ splice site selection; 4) Elements P3 through P9 appear to be involved in catalytic efficiency, but do not seem to play a major role in the specificity of the splicing reaction of td pre-mRNA.

Comparative analysis of the three homologous T4 introns in the td, nrdB (ribonucleotide reductase subunit B) and sunY (function unknown) genes (3,4) is fully consistent with the above genetic studies. A similar core structure, involving approximately 250 nt, can be drawn for the three intervening sequences (4). The striking sequence and structural similarities suggest a common ancester for these introns. Surprisingly, the occurrence of the three T4 intervening sequences in the closely related T-even phages T2 and T6 is highly variable (5). Both the innate similarities of the T4 introns, and their inconsistent distribution throughout the homologous T-even phage genomes argue in favor of the mobility of group I introns in prokaryotes.

1. Hall, D. et al. (1987) Cell 48, 63
2. Chandry, P.S. and Belfort, M. (1987) Genes and Devel. In press
3. Gott, J. et al. (1986) Cell 47, 81
4. Shub et al. (1988) Proc. Natl. Acad. Sci. In press
5. Pedersen-Lane, J. and Belfort, M. (1987) Science 237, 182

N 006 INVOLVEMENT OF AN AMINOACYL-tRNA SYNTHETASE IN SPLICING OF GROUP I MITOCHONDRIAL INTRONS, Alan M. Lambowitz, Robert A. Akins, Andrew D. Cherniack, Quinbin Guo, Robert L. Kelley, Arun L. Majumder, Amy J. Snook, Julie G. Ericson, Departments of Molecular Genetics and Biochemistry, The Ohio State University, Columbus, Ohio 43210.

We reported recently that mitochondrial tyrosyl-tRNA synthetase, which is encoded by the cyt-18 gene of Neurospora, is required for splicing the mitochondrial large rRNA intron and other group I introns in Neurospora mitochondria (1). Mutations in the cyt-18 gene result in defective splicing of a number of different group I introns, and mitochondrial tyrosyl-tRNA synthetase copurifies with a soluble activity that splices the mitochondrial large rRNA intron in vitro (1,2). We have continued to investigate the function of this aminoacyl-tRNA synthetase in RNA splicing using biochemical, immunochemical and genetic approaches.

1. Akins, R.A. and Lambowitz, A.M. Cell 50, 331-345 (1987).
2. Collins, R.A. and Lambowitz, A.M. J. Mol. Biol. 184, 413-428 (1985).

N 007 STRUCTURE AND CATALYTIC FUNCTION IN BACILLUS SUBTILIS RNASE P RNA.
Norman R. Pace, Claudia Reich, Bryan D. James, David S. Waugh, Gary J. Olsen, and Bernadette Pace. Department of Biology and Institute for Molecular and Cellular Biology, Indiana University, Bloomington, IN 47405.

The Bacillus subtilis RNase P, responsible for removal of 5' termini of tRNA precursors, has protein (ca. 14 kDa) and RNA (ca. 400 nucleotides) elements. Under physiological conditions, both components are required for activity. However, at high cation concentrations, the RNA alone is sufficient for catalysis. The high cation concentrations or the presence of RNase P protein provide electrostatic shielding so that the polyanionic substrate and enzyme RNAs may achieve the close contact required for catalysis. Although the holoenzyme reaction proceeds more rapidly overall than the RNA-alone reaction, kinetic analyses show this is not due to the binding or cleavage steps, rather, it is because enzyme and product RNAs dissociate slowly at high cation concentrations.

The secondary structure of the RNase P RNA is being elucidated using a phylogenetic comparative approach to test base pairing possibilities. The B. subtilis RNase P RNA is sufficiently different from that of Escherichia coli that alignment of the sequences is ambiguous over much of their lengths. Inspection of RNase P RNA sequences from organisms more closely related to both identifies substantial folded structure, but much remains to be determined. Addition or deletion of structural domains is a common theme, suggesting the RNA may have functions beyond RNase P activity.

The regions of the RNase P RNA structure required for processing activity are being sought by in vitro mutagenesis (linker scan and site-directed) and by RNA fragment reconstitution (using T7 polymerase run-off transcripts of cloned genes). Extensive portions of the RNA can be removed without abolishing catalytic activity.

N 008 REACTIVITY OF GROUP II INTRON RNA.
C. L. Peebles*, E. J. Benatan*, K. A. Jarrell#, R. C. Dietrich# and P. S. Perlman#; *Dept. of Biological Sciences, Univ. of Pittsburgh, Pittburgh, PA, 15260; and #Dept. of Molecular Genetics, Ohio State University, Columbus, Ohio, 43210.

We have investigated the self-splicing of several Group II introns from *S. cerevisiae* mitochondrial DNA. These introns have a secondary structure that has been modeled as a central wheel with six helical spokes ("domains", numbered 1 through 6, 5' to 3'). The boundaries of each domain are defined by inverted repeats that fold into the duplex stem at the base of the domain. These domains presumably interact by tertiary contacts to form the complete folded structure of the intron and to carry out self-splicing and other reactions catalyzed by this RNA. Our goal has been to determine the function(s) of each domain by analyzing mutants of the *oxi* 3 intron-5-gamma (al5g). These experiments make use of a set of high salt reaction conditions under which the wild-type form of the intron shows enhanced reactivity or altered product distribution. We have also investigated the reactions catalyzed by other group II introns under these conditions. Our results establish the generality of self-splicing and other reactions catalyzed by Group II introns.

Precursor RNAs lacking portions of the wild-type intron sequence have been investigated. Those lacking the 3'end of the intron (part or all of domain 6) catalyze a reaction related to step 1 of splicing -- cleavage of the 5'splice junction. This occurs without branch formation, since the site of branchpoint attachment is absent. Precursors lacking domain 5 cannot catalyze 5'junction cleavage. A bimolecular model for this reaction confirms that domain 6 is dispensible while domain 5 is required. Similarly, an *in vivo* mutant of al5g (C1067) with base changes in both domains 5 and 6 blocks step 1 of splicing*in vivo* and *in vitro*. A second mutant (C2116) with a base change in domain 3 can block splicing *in vivo* and under some conditions *in vitro* but permits reaction under other conditions.

Separate exons result from reactions conducted in KCl. Intron RNAs bind and cleave the spliced exon product at the spliced junction ("exon reopening"); this reaction accounts for the production of separate exons. Transcripts lacking domain 5 are fully active in this reaction; indeed, only domain 1 is required. This result distinguishes exon reopening from 5'junction cleavage and demonstrates that domain 1 contains the essential structures for bond scission. Two other point mutants (C1030 and C2159) in domain 1 block splicing *in vivo*. The former severely disrupts the reaction *in vitro*, confirming the importance of domain 1. In contrast, the later splices normally in high salt.

An efficient *trans*- splicing system has been developed using pairs of non-overlapping (or overlapping) transcripts interrupted within the intron sequence ("half molecules"). This system may serve as an *in vitro* model of the *trans* splicing found in chloroplasts, for example, the *rps* 12 gene of tobacco cpDNA. Products of the *in vitro* reaction include accurately spliced exons and *trans*- branched intron. Not all tested pairs of half molecules can splice. The most active pair is interrupted within domain 4 -- in fact, domain 4 can be entirely omitted. Interruptions within domain 1 or between domains 5 and 6 yield inactive half molecules. These results suggest that there are specific contacts between domain 5 and the 5'splice junction that activate the junction for attack.

RNA-Protein Interactions

N 009 ULTRASTRUCTURAL ANALYSIS OF RNA PROCESSING, Ann L. Beyer, Yvonne N. Osheim and Sally A. Amero, Department of Microbiology, University of Virginia, Charlottesville, VA 22908.

Based on analysis of the RNP structure and RNA processing patterns of Pol II transcripts as visualized in the EM via "Miller" chromatin spreads, we argue that splicing occurs with a reasonable frequency on nascent transcripts (~14% of Drosophila embryo transcription units). Evidence in support of this conclusion includes the identification of spliceosome intermediates and spliceosomes on nascent Drosophila chorion transcripts (Cell 43:143), and the analysis of a similar ultrastructure, including loop formation and removal, on many more unidentified transcription units. The details of the process we visualize are in close agreement with findings from in vitro splicing systems, and differ only in the more rapid completion of splicing in vivo (Beyer and Osheim, submitted). Different introns appear to be removed at different rates. The majority of genes (85%) display RNP particles on nascent transcripts; ~54% of these go on to display loop formation. We will present arguments that this particle deposition occurs at splice sites and occurs on the majority of nascent transcripts, although only a small percentage of introns are spliced co-transcriptionally. For this class, the only detectable rate-limiting step is the synthesis of the 3' splice site (ss), which initiates a series of events starting with RNP particle deposition at the 3' ss (within 48 sec) and ending with loop removal (splicing) within 3 minutes. The initiation of the process is correlated with 3' ss synthesis, but is independent of 5' ss synthesis, the position of the intron within the transcript, and the age or length of the transcript. Preliminary results also indicate that the time required for intron removal is independent of intron length, at least up to 8 kb. This observation suggests the rapid intramolecular scanning of sequences for cognate splice junctions as they are synthesized. We are continuing this investigation by analysis of very long, presumably "developmental loci" of Drosophila embryos, which are more likely to have very long introns. One observation to date is that co-transcriptional splicing is the rule, rather than the exception, on these long genes.

Evidence supporting the role of these particles in specific splice site selection comes from the observation of 2 cases of apparent alternative splicing, (specifically exon-skipping), in which particles are present at either end of the skipped exon only when the exon is used. One of these examples of alternative splicing (in the Ad-2 MLT leader) also supports a role for higher order RNP structure in exon skipping. Finally, visualization of (non heat shock) transcription units in heat shocked Drosophila embryos indicates that although RNP particles occur on these transcripts, intron loops form very infrequently, suggesting that the block to splicing during heat shock (Yost and Linquist, 1986, Cell 45:185) occurs at an intermediate stage of spliceosome assembly.

N 010 THE MOLECULAR STRUCTURE OF THE COMPLEX BETWEEN YEAST $tRNA^{Asp}$ AND ASPARTYL tRNA SYNTHETASE. M. Ruff, J. Cavarelli, A. Mitschler, A. Podjarny, B. Rees, J.C. Thierry, R. Giegé and D. Moras. I.B.M.C. du C.N.R.S., 15 rue Descartes, 67084 Strasbourg Cedex FRANCE.

The three-dimensional structure of the complex between yeast $tRNA^{Asp}$ and its cognate synthetase, an α_2 dimer of molecular weight 125 000 D, has been investigated in two different crystal forms.

I .Space group I432 a=b=c=354 Å with 1E and 2 tRNAs / a.u.
II.Space group $P2_12_12_1$ a=210.4, b=145.3, c=86 Å " " " "

The cubic crystals (I), the first to be analysed, have a limited diffraction power (7 Å). This study results in a 8.5 Å resolution map which leads to an accurate description of the topology of the complex and the contact area between the tRNA and the protein.
Much more experimental information is now available from the orthorhombic crystal form II, which diffracts up to 2.7 Å resolution. Diffraction data have been collected for the native crystals and two heavy atoms derivatives. Analysis is in progress.

N 011 FUNCTIONAL SITES IN rRNA IN RELATIONSHIP TO RIBOSOMAL ARCHITECTURE

Harry F. Noller, Danesh Moazed, Seth Stern, Ted Powers, James Robertson, Patrick Allen, and Peter Svensson, Thimann Laboratories, University of California, Santa Cruz, CA 95064.

We have mapped the sites of interaction of tRNA (bound at A, P and E sites), EF-Tu, EF-G and several antibiotics of known mode of action, to specific regions of 16S and 23S rRNA. We believe that these are functional sites that are directly involved in protein synthesis, because: (1) Almost without exception, they contain universally conserved nucleotide sequences, that are invariant in all eubacterial, archebacterial and eukaryotic cytoplasmic ribosomes so far examined; (2) they include the positions where tRNA has been crosslinked to rRNA at its anticodon and at its aminoacyl end; and (3) many sites of antibiotic interaction include nucleotides involved in mutations that afford resistance to the same antibiotics.

We have deduced a model for the 3-dimensional folding of 16S rRNA, by merging information on the sites of interaction between specific r-proteins and 16S rRNA with the neutron diffraction map for positions of the centers of mass of the 30S subunit proteins, obtained by Moore and co-workers. Most of the tRNA interaction sites in 16S rRNA cluster in our model, in a region recognizable as the "cleft" of the electron microscope model of the 30S subunit. Some sites, however, are mysteriously remote from the cleft region, providing evidence for at least two classes of tRNA-induced conformational changes involving 16S rRNA.

N 012 RECOGNITION OF YEAST $tRNA^{Phe}$ BY YEAST PHENYLALANYL-tRNA SYNTHETASE.

Jeffrey R. Sampson, Linda Behlen, Anthony DiRenzo,and Olke C. Uhlenbeck. Dept. of Chemistry and Biochemistry, Campus box 215, University of Colorado, Boulder, Co. 80309.

One goal of our laboratory is to determine the sequence and structural elements in yeast $tRNA^{Phe}$ which are required for the specific aminoacylation by its cognate yeast phenylalanyl-tRNA synthetase (PRS). We have developed an *in vitro* T7 transcription system for synthesizing $tRNA^{Phe}$ variants having nucleotide changes at any desired position. Using synthetic DNA oligonucleotides, recombinant plasmids are constructed with a T7 promoter directly upstream of the tRNA gene and a BstN 1 restriction site at the 3' terminus of the gene. Run off transcription of the wild type plasmid gives a 76 nucleotide RNA having the sequence of yeast $tRNA^{Phe}$ but lacking the 14 modified nucleosides. This unmodified $tRNA^{Phe}$ is aminoacylated by PRS with similar kinetics to that of the fully modified yeast $tRNA^{Phe}$. Using this method, we have assayed about 35 mutant tRNAs for aminoacylation by PRS. Because most of the nucleotides in tRNA are involved in either secondary or tertiary interactions, mutant tRNAs were generally designed with more than one nucleotide change in order to maintain the proper folding of the tRNA. For example, the A^{9} U^{12} A^{23} tertiary interaction in $tRNA^{Phe}$ was substituted with the isomorphic G^{9} C^{12} G^{23} present in other tRNAs. In every case where an isomorphic substitution could be made, the resulting nucleotide changes had no effect on the kinetics of aminoacylation by PRS. However, when structurally nonisomorphic substitutions were made, the disruption of the tertiary structure results in a lowered rate of aminoacylation. Thus, the nucleotides involved in the tertiary interactions are not involved in specific interactions with the synthetase but are required to maintain the proper folding of the tRNA. Nucleotide substitutions at five single stranded positions in $tRNA^{Phe}$ (G^{20}, G^{34}, A^{35}, A^{36} and A^{73}) result in a large decrease in the rate of aminoacylation by PRS. We suggest that nucleotide specific contacts at these positions are used to discriminate $tRNA^{Phe}$ from other tRNAs in yeast.

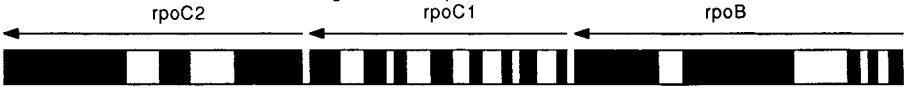
Pre-mRNA Splicing and Processing

N 013 U7 SNRNP AND A HEAT-LABILE FACTOR COOPERATE IN THE IN VITRO 3' PROCESSING OF HISTONE MESSENGER RNA, Matthew Cotten, Octavian Gick and Alain Vasserot, Max L. Birnstiel, Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria.
Histone mRNAs are of two kinds: a minority class which is polyadenylated and a majority which is poly A^- but terminates in a hairpin structure. They code for replacement and replication variant histone proteins, respectively. The 3' end of the poly A^- histone mRNAs is generated by endonucleolytic cleavage requiring the intervention of at least two transacting factors: the U7 snRNP and a heat-labile component of high molecular weight, which is resistant to micrococcal nuclease digestion and cannot be precipitated with anti-Sm antibodies. Both heat-labile factor and U7 snRNP exhibit closely similar chromatographic behaviour on DEAE, heparin Sepharose and Mono Q columns. The U7 snRNP isolated from Mono Q fractions contains an RNA which is 64 nucleotides long and has the typical m^3-G cap. It is related in sequence to the U7 snRNA isolated previously from sea urchin. Essentially, three features are conserved: (1) a 5' element capable of forming diffuse RNA-RNA hybrids with a histone messenger sequence element downstream of the cleavage site which is present in all known mammalian replication type histone mRNAs, (2) a sequence shown in the sea urchin U7 to mediate Sm-binding, and (3) a 3' palindromic structure. Oligonucleotides complementary to the 5' portion of the murine U7 RNA, in the presence of RNase H, inhibit in vitro processing of histone precursor RNA, suggesting that this RNA is indeed required for the 3' processing reaction. Furthermore, both oligonucleotides complementary to the histone element, and deletions in histone message precursors which interrupt this element are inhibitory to the processing reaction.

N 014 snRNPs AND SPLICING IN YEAST. C. Guthrie, Department of Biochemistry, University of California, San Francisco, CA 94143.
Nuclear pre-mRNA splicing is mediated by 5 small nuclear RNAs (snRNAs), found tightly associated with several proteins as ribonucleoprotein particles (snRNPs). We have recently identified the spliceosomal snRNAs in Saccharomyces cerevisiae. Surprisingly, the yeast RNAs range from 1X (U6) to 6X (U2) the size of their metazoan counterparts. Moreover, the extent of sequence conservation varies from virtually complete identity (U6) to fewer than a dozen nucleotides (U5). Curiously, in the case of U1 and U2, deletion of much or all of the "extra" domains can have only modest effects on growth. Conserved features of primary and secondary structure are now being subjected to site-specific mutagenesis and second-site reversion analysis. In this way we have shown that base-pairing between U1 and the 5' splice site dictates the efficiency, but not the specificity of the cleavage reaction. Recognition of the branch site region by U2 has, similarly, been genetically demonstrated. Finally, we have uncovered evidence for an extensive base-pairing interaction between U4 and U6; their cyclic disassociation during splicing must thus require a conformational "switch".

N 015 RNA PROCESSING REACTIONS IN CHLOROPLASTS, Richard B. Hallick, Don P. Bourque, David A. Christopher, Donald W. Copertino, John C. Cushman, Michel Herzog, Catherine S. Hibbert, Mark Hildebrand, Annie-Marion Poll, Carl A. Price, Catherine A. Radebaugh, Gloria M. Yepiz, Department of Biochemistry University of Arizona Tucson, AZ 85721.

We have been studying the processing of primary transcripts of higher plant and Euglena gracilis chlorplast operons in vivo and in vitro. Most chloroplast genes are encoded in polycistronic operons that may include combinations of rRNAs and tRNAs, multiple mRNAs, tRNAs and mRNAs, and multiple tRNAS. Many transcripts have introns. Most higher plant chloroplast introns are very similar to mitochondrial group II introns. Euglena pre-mRNAs have both group II introns, and a novel class of very small, unusual introns characteristically found in operons related to bacterial ribosomal protein and RNA polymerase operons. An example is the 11 kbp rpoB-C1-C2 gene cluster, which codes for polypeptides homologous to the E. coli rpoB and C loci (introns are shown as open boxes):



The following features of chloroplast RNA processing reactions will be addressed: (1) Chloroplast Intron Structure. The relationship of chloroplast introns and splicing mechanisms in vivo and in vitro to other known introns. (2) Trans-splicing. The tobacco chloroplast gene for ribosomal protein S12 contains three exons encoded on two separate transcripts. Formation of mature rps12 mRNA requires a bimolecular splicing in vitro. (3) Maturation of mono- and polycistronic pre-tRNAs. Enzymatic pathways for tRNA maturation have been characterized in vitro. (4) Maturation of Polycistronic mRNAs. The Euglena photosystem I and II genes psaA, psaB, psbE, psbF, two orf, and a tRNA are co-transcribed as a precursor with 12 group II introns. RNA processing includes non-ordered intron splicing to yield a 6.5 kbp polycistronic pre-mRNA, followed by multiple endonuclease cleavage events. (5) Maturation of RNA polymerase pre-mRNAs. The splicing sites for plant and Euglena rpoB-C1-C2 pre-mRNAs cannot be determined, but appear to be unusual. We will report on the use of cDNA cloning and sequencing to characterize transcripts from RNA polymerase operons.

N 016 TRANS-SPLICING IN NEMATODES. David Hirsh, Susan Bektesh, and Kevin Van Doren Synergen, Inc., 1885 33rd St., Boulder, CO 80301.

We have continued to study trans-splicing that we first described in the actin mRNAs of Caenorhabditis elegans. We have found that many different messages in C. elegans undergo trans-splicing to acquire the same leader sequence. The identity of some of these messages does not provide assignment of an obvious role for trans-splicing. We have found that the same trans-spliced leader sequence is present in a variety of parasitic and free-living nematodes but not in other organisms. In C. elegans the precursor splice leader is about 100 nucleotides long and encoded within the 5S DNA repeat. In some other nematodes the precursor is encoded elsewhere and not within the 5S repeat. Certain parts of the precursor sequence are conserved in other nematodes and may suggest functional domains for the molecule. The precursor can be precipitated from a C. elegans extract with lupus antibodies of the Sm type. On the other hand, U1 antibodies will not precipitate the spliced leader precursor RNA molecule.

Regulation of Gene Expression by RNA

N 017 ROLES FOR ANTISENSE RNA SYNTHESIS IN THE DEVELOPMENT OF BACTERIOPHAGES P22 AND LAMBDA, William McClure and Sha-Mei Liao, Dept. Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA 15213.
The P22 immI region includes an antirepressor gene (ant) and its regulators. One of the negative regulators is a small antisense regulatory RNA (sar RNA), which inhibits ant expression during the late phase of the P22 developmental program. The characterization in vitro of the Psar promoter and the sar RNA transcript has been published (1). This work and the characterization of mutants defective in repression (2) was performed in collaboration with M.M. Susskind and coworkers (USC). We have continued the collaboration by investigating the solution structure and RNA-RNA pairing properties of sar RNA. The sar RNA molecule is 68 or 69 nucleotides long and is complementary to the ant mRNA from a point immediately adjacent to the initiator AUG, extending through the ribosome binding site, and beyond. The results from partial digestion (with various RNAases) experiments suggest that sar RNA has two connected stem-loop structures and two short single-stranded termini. To identify target sites of sar RNA critical for inhibition of ant expression, RNAases were also used to detect accessible regions in the wild-type and mutant sar RNAs during the pairing process. A partially-formed duplex intermediate was detected in these studies. The structural and mechanistic results are compared to the inhibitory properties of sar RNA in vivo as determined by Susskind and coworkers.

1. Liao, S.-M., T.-H. Wu, C.H. Chiang, M.M. Susskind, and W.R. McClure (1987) Genes & Development 1: 197-203.
2. Wu, T.-H., S.-M. Liao, W.R. McClure, and M.M. Susskind (1987) Genes & Development 1: 204-212.

N 018 **REGULATION OF ALTERNATIVE RNA PROCESSING IN CALCITONIN/CGRP GENE EXPRESSION.** Michael G. Rosenfeld and Jeffrey W. Guise, Ronald B. Emeson, Andrew F. Russo, E. Bryan Crenshaw,III, Stuart E. Leff. Howard Hughes Medical Institute, University of California, San Diego. La Jolla, CA 92093.

Developmentally regulated cell-specific alternative RNA processing of the calcitonin/CGRP(calcitonin gene-related peptide), gene results in the production of calcitonin mRNA in thyroid C cells and CGRP mRNA in neurons. Calcitonin mRNA is produced by splicing the first three exons of the calcitonin/CGRP gene to the fourth exon, while for CGRP mRNA production, the first three exons are spliced to the fifth and sixth exons. Analyses utilizing transfected cell lines expressing the calcitonin/CGRP gene have shown that such lines exhibit mRNA processing choices analogous to those observed *in vivo*. Analyses of deletions within the calcitonin/CGRP transcription unit have suggested that a splice commitment regulatory machinery is required to produce CGRP RNA in transfected cell lines. Additional studies using transgenic mice expressing the calcitonin/CGRP transcription unit under the control of the mouse Mt-1 promoter show that cells that do not normally express the endogenous gene have the ability to make a clear RNA processing choice for either calcitonin or CGRP RNA. The majority of tissues studied produced greater than 90% calcitonin RNA. However both CGRP and calcitonin transcripts were produced in the brains of the transgenic mice. In situ RNA hybridization and immunohistochemical staining showed that CGRP was produced in a variety of neurons, while calcitonin was produced in glial cells and a few neurons. Taken together these studies suggest a model in which the splice commitment machinery required to produce CGRP mRNA is primarily restricted to neurons and cells that produce calcitonin mRNA are making the default RNA processing choice.

RNA in Translation

N 019 NATURAL UAG SUPPRESSOR GLUTAMINE tRNA IN MAMMALIAN CELLS, Y. Kuchino[1], F. Nemoto[1], H. Beier[2] and S. Nishimura[1], [1]Biology Division, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan, and [2]Institute für Biochemie, Baverische Julius-Maximilians-Universität, Würzburg, FRG.

We previously isolated three species of glutamine tRNAs from cytoplasmic fraction of *Tetrahymena*. Among them, two glutamine tRNAs that have CUA and UmUA as their anticodons were shown to recognize UAG and either UAG or UAA codons, respectively, in a rabbit reticulocyte cell-free protein synthesizing system. This is a strong supporting evidence that UAA and UAG are used as the glutamine codon in *Tetrahymena*, indicating deviation of genetic code (1). In order to know how extensively reading of the termination codons by glutamine occurs in different species of organisms, we searched glutamine tRNA species from mouse liver.

Two species of glutamine tRNA were isolated from mouse liver and their nucleotide sequences were determined (2). The minor glutamine tRNA ($tRNA^{Gln}_{UmUG}$) that possesses UmUG as the anticodon sequence showed suppressor activity for the UAG termination codon of TMV RNA. For recognition of UAG codon by $tRNA^{Gln}_{UmUG}$, there must be wobbling in both 1st and 3rd positions of the anticodon. The amount of this suppressor glutamine tRNA in mouse liver was 1-2% of the amount of the major glutamine tRNA ($tRNA^{Gln}_{CUG}$) that contains CUG anticodon sequence. However, it was makedly increased in NIH3T3 cells infected with Moloney murine leukemia virus (MoMuLV). This result supports the hypothesis that $tRNA^{Gln}_{UmUG}$ actually functions *in vivo* as a suppressor tRNA that recognizes the UAG termination codon located at the *gag-pol* gene junction of MoMuLV and results in the synthesis of the virus-encoded protease.

There is an extensive inverted repeat sequence in the region of the *gag-pol* junction, which may be important for readthrough of UAG termination codon by $tRNA^{Gln}_{UmUG}$. The activation of the $tRNA^{Gln}_{UmUG}$ gene expression may be dependent on the virus growth. In order to clarify the regulatory mechanism of the tRNA gene expression, an attempt was made to isolate genes for $tRNA^{Gln}_{UmUG}$ and $tRNA^{Gln}_{UmUG}$ from mouse liver DNA by using the synthetic oligonucleotides as probes. Although many clones containing $tRNA^{Gln}_{CUG}$ gene were isolated, only few clones for $tRNA^{Gln}_{UmUG}$ gene were obtained. 5'-Franking sequence of $tRNA^{Gln}_{UmUG}$ gene was quite different as that of $tRNA^{Gln}_{CUG}$. Possible regulatory domain in the 5'-franking region of $tRNA^{Gln}_{UmUG}$ gene will be presented.

(1) Hanyu, N., Kuchino, Y., Nishimura, S. and Beier, H. (1986) EMBO J. 5, 1307-1311.
(2) Kuchino, Y., Beier, H., Akita, N. and Nishimura, S. (1987) Proc. Natl. Acad. Sci. USA 84, 2668-2672.

N 020 **TRANSFER RNA INVOLVEMENT IN CHLOROPHYLL BIOSYNTHESIS.** Astrid Schön, Gary O'Neill, David Peterson, Robert Grumbles, Simon Gough#, C. Gamini Kannangara#, Michael Dalrymple, and Dieter Söll. Department of Molecular Biophysics & Biochemistry, Yale University, P.O. Box 6666, New Haven, CT 06511, USA and #Department of Physiology, Carlsberg Laboratory, DK-2500 Copenhagen Valby, Denmark.

One molecule of chlorophyll is synthesized from eight molecules of delta-aminolevulinic acid (DALA), the universal precursor of porphyrines. The biosynthesis of DALA in greening plant plastids, cyanobacteria and archaebacteria involves the reduction of glutamate to glutamate-1-semialdehyde and its subsequent transamination to DALA (see, e.g. 1). Studies on the in vitro biosynthesis of DALA in barley chloroplast extracts demonstrated that an RNA component is involved in this process. Purification and sequence analysis showed this RNA^{DALA} to be a chloroplast-derived $tRNA^{Glu}$ species (2). Glutamate is attached to the 3'-end of this tRNA via an aminoacyl bond by a chloroplast glutamyl-tRNA synthetase. In the subsequent reduction of glutamate to glutamate-1-semialdehyde the tRNA is required as "cofactor" for a NADPH-dependent dehydrogenase. In addition, this enzyme displays specificity for RNA^{DALA}, other $tRNA^{Glu}$ species of homologous or heterologous origin do not support this reaction. This represents a novel role for tRNA: participation in a metabolic conversion of its cognate amino acid into another low molecular mass metabolite, which subsequently is not used in peptide biosynthesis. Further studies showed (4) that RNA^{DALA}, this $tRNA^{Glu}$ species has a dual role: it functions in chloroplast protein biosynthesis in addition to its role in DALA formation.

The nucleotide sequences of the two other major glutamate-accepting tRNAs from barley chloroplasts (2) differ from each other only by nucleotide modifications; both contain a glutamine anticodon and have strong sequence similarity to chloroplast $tRNA^{Gln}$ genes from other organisms (4). A mischarging phenomenon, possibly similar to the one described for bacilli (3), is responsible for glutamylation of these $tRNA^{Gln}$ species. The amino acid (glutamate) attached to $tRNA^{Gln}$ is then converted to glutamine (still bound to $tRNA^{Gln}$) by a tRNA-dependent amidotransferase. No glutaminyl-tRNA synthetase can be detected in barley chloroplasts. Thus, normal protein biosynthesis in these organelles requires mischarged tRNA as an intermediate. This mechanism of $Gln\text{-}tRNA^{Gln}$ formation is widespread in the living world; it may be found in all organelles, in gram-positive bacteria and archaebacteria (4).

(1) Wang, W.-Y., Gough, S.P. & Kannangara, C.G. (1981) Carlsberg Res. Comm. **46**, 243-257.
(2) Schön, A., Krupp, G., Gough, S., Kannangara, C.G., & Söll, D. (1986) Nature **322**, 281-284.
(3) Wilcox, M., & Nirenberg, M. (1968) Proc. Natl. Acad. Sci. USA **61**, 229-236.
(4) Schön, A., Kannangara, C.G., Gough, S., & Söll, D. (1987) Nature, in press.

Plant Infectious RNAs and Enzymes with RNA Subunits

N 021 TELOMERE SYNTHESIS AND REGULATION, Carol Greider, Eric Henderson, Drena Larson, Janis Shampay, Dorothy Shippen-Lentz, Elizabeth Spangler and Elizabeth Blackburn. Dept of Molecular Biology, University of California, Berkeley, CA 94720.

Telomeres maintain chromosome integrity by stabilizing the linear chromosomal DNA and enabling complete replication of its ends. In all cases analyzed, eukaryotic telomeres consist of variable numbers of tandemly repeated, simple G + C-rich sequences. We have described a novel enzyme in the ciliate *Tetrahymena*, which synthesizes tandem repeats of the *Tetrahymena* telomeric sequence onto synthetic DNA oligonucleotide primers consisting of a few repeats of the G-rich strand of any of the known eukaryotic telomeric sequences [1,2]. Recognition of telomere primers by telomerase appears to involve a G.G base-paired structure common to telomeric G-rich strands. We have shown that such unusual base-paired structures form under physiological conditions[3]. The telomere terminal transferase enzyme (telomerase) is a ribonucleoprotein whose RNA and protein components are both required for activity[1,2]. Polymerization of telomeric repeats by telomerase occurs one nucleotide at a time and requires no external energy source *in vitro*. Telomerase synthesizes products up to ~10 kb in length *in vitro*, whereas increments of telomere length growth observed *in vivo* are much shorter[4,5]. Thus regulation of telomere length *in vivo* must involve other factors.

1. Greider,C. and Blackburn,E. (1985). Cell 43 405-413
2. Greider,C. and Blackburn,E.(1987). Cell, in press.
3. Henderson,E. , Hardin,C., Wolk. S., Tinoco, I., and Blackburn,E. (1987) . Cell, in press
4. Larson, D., Spangler,E. and Blackburn,E. (1987). Cell, 50 477-483.
5. Shampay,J. and Blackburn,E. (1987). Proc. Natl. Acad. Sci. USA, in press.

N 022 MITOCHONDRIAL ENZYMES WITH NUCLEAR RNA COMPONENTS. Jeffrey L. Bennett, David D. Chang, Robert P. Fisher, Lori L. Stohl, James N. Topper, and David A. Clayton, Department of Pathology, Stanford University School of Medicine, Stanford CA 94305-5324.

RNase MRP (for Mitochondrial RNA Processing) is a site-specific, single-strand endonuclease involved in primer RNA metabolism in mammalian mitochondria. Cleavage by RNase MRP occurs at one of the transition sites of primer RNA synthesis to DNA synthesis, located adjacent to a sequence block that is evolutionally conserved throughout vertebrate mtDNA. The activity of RNAse MRP is sensitive to either micrococcal nuclease or proteinase K treatment, suggesting that the functional enzyme is a ribonucleoprotein complex. Based on physical copurification and a complementary oligonucleotide-directed inhibition assay, we have previously demonstrated that a 136-nt RNA encoded in the nucleus is the most likely RNA moiety of mouse RNase MRP. Using complementary oligonucleotide probes we have identified and cloned a segment of mouse nuclear DNA that encodes this 136-nt RNA. Sequence analysis revealed that this 136-nt RNA constitutes the 3' portion of a larger 275-nt precursor RNA. The 5' end of the 275-nt RNA is not capped and retains a triphosphate group; the gene for this RNA contains features characteristic of both RNA polymerase II- and RNA polymerase III-dependent genes. The 138-nt RNA can form an extensive secondary structure and contains a region that can potentially base pair with the substrate RNA at the evolutionally conserved sequence block immediately adjacent to the cleavage site. RNase MRP from human cells shows similar features and the corresponding human gene and its basic RNA products have been characterized; in this case the larger RNA is 266 nt and the smaller is approximately 110 nt. Recent data suggest that the existence of RNase MRP may spread beyond the vertebrate species thus far examined and extend to yeast. (Supported by NIH grant GM-33088 and ACS grant NP-9.)

N 023 SELF-CLEAVAGE REACTIONS OF VIROID AND VIRUSOID RNAs, Robert H. Symons, Candice C. Sheldon, Alex C. Jeffries and Anthony C. Forster, Department of Biochemistry, University of Adelaide, South Australia 5000, Australia.

The ability of certain circular and linear plant pathogenic RNAs to specifically self-cleave *in vitro* is considered to be a property important in their replication *in vivo* by a rolling circle mechanism (1-3). Hammerhead-shaped secondary structures containing 17 conserved nucleotides were proposed as a basis for the formation of the active tertiary self-cleavage structures in the presence of Mg++ (2-4). Such structures have now been further characterized by site-directed mutations and by the ability of two or three separate RNA fragments to act catalytically in *trans*. Such approaches will help define the sequence and structural requirements for self-cleavage.

The hammerhead structure of the 324 nucleotide virusoid of lucerne transient streak virus (vLTSV) requires a minimum of 52 nucleotides for complete self-cleavage (4). Single base mutations and one or two base insertions had little effect, caused a significant decrease, or eliminated self-cleavage in synthetic RNA transcripts. Further information is being gained by the synthesis of separate RNA fragments which make up the three double-stranded stems of the hammerhead structure. The variation in self-cleavage found when these fragments were incubated either two or three at a time has provided information on the requirements for the stability of each stem. A DNA fragment can replace an RNA fragment but at very low efficiency.

1. Prody, G.A., Bakos, J.T., Buzayan, J.M., and Bruening, G. (1986) Science 231:1577-1580.
2. Hutchins, C.J., Rathjen, P.D., Forster, A.C., and Symons, R.H. (1986) Nucleic Acids Res. 14:3627-3640.
3. Forster, A.C., and Symons, R.H. (1987) Cell 49:211-220.
4. Forster, A.C., and Symons, R.H., (1987) Cell 50:9-16.
5. Forster, A.C., Jeffries, A.C., Sheldon, C.C., and Symons, R.H. (1987) Cold Spring Harbour Symposium Quant. Biol., in press.

Interactions with Nuclear Superstructures (joint)

N 024 HOMEOBOX GENES IN XENOPUS LAEVIS, Eddy M. De Robertis, Guillermo Oliver, Ken Cho, Thomas R. Burglin, Jane Hardwicke, Andreas Fritz, and Christopher Wright, Department of Biological Chemistry, University of California, Los Angeles, CA 90024.

Homeobox genes specify positional information in Drosophila. In vertebrates, although direct genetic proof is still lacking, the expectation is that they too play important roles in developmental decisions. We have isolated eight different homeobox genes from Xenopus. Studies with specific antibodies elicited against recombinant proteins show that some Xenopus homeobox genes encode abundant nuclear proteins, which remain bound to the entire length of meiotic and mitotic chromosomes. In vitro, fusion proteins containing a Xenopus homeodomain bind to Xenopus DNA promoter sequences containing a CAATTAAA motif, which is also conserved in Drosophila.

We will report on gene XlHboxl, whose isolation in 1984 led to the discovery of homeobox genes in vertebrates. XlHboxl has two transcripts in embryogenesis, and cDNAs have been isolated for both of them. Each transcript is transcribed from a different promoter which are 12 Kb apart. Promoter I codes for a short protein of 153 aa, and promoter II for a protein of 234 aa which has 81 extra aa at the amino terminus. We have prepared antibodies specific for the long version of the protein, or for the common parts, and have asked in which region of the embryo each protein is expressed. We used both mouse and Xenopus embryos for this analysis, because the antibody crossreacts among vertebrate XlHboxl proteins. Promoter I is expressed in a more anterior position than promoter II. At the CNS level promoter I is expressed in the hindbrain and promoter II in the cervical spinal cord. In the mouse somatic mesoderm, promoter I is expressed from the first cervical sclerotome and backwards, while promoter II is restricted to the thoracic region. Even in the internal organs of the mouse embryo the displacement is maintained: the mesenchyme of more anterior organs such as larynx, lung, oesophagus, stomach and duodenum expresses promoter I, while promoter II is expressed in the intestine.

The results suggest that the XlHboxI proteins are markers of the position that particular subsets of cells occupied during early embryogenesis, and not of particular germ layers or organs. Vertebrate development is now open for analysis by microinjecting into developing embryos specific antibodies, artificial mRNAs, fusion proteins or modified genes.

N 025 STRUCTURES OF THE NUCLEAR MATRIX INVOLVED IN PRE-mRNA SPLICING, Scott Zeitlin, Robert C. Wilson and Argiris Efstratiadis, Department of Genetics and Development, Columbia University, 701 West 168th Street, New York, N.Y. 10032

In vivo splicing of pre-mRNA might occur on the nucleoskeleton (in situ nuclear matrix). Because of possible experimental artifacts, a distinction should be made between the (mostly undefined) in situ matrix and a biochemical "nuclear matrix preparation" (MX) that is defined operationally as the insoluble pellet containing HnRNPs, remaining after high-salt extraction of interphase nuclei that have been treated with DNase I. To examine the possible involvement of MX-associated structures in pre-mRNA splicing by applying a functional criterion, we developed the following assay system: Plasmids containing the HSV tk gene promoter linked to an intron-containing segment of the rabbit β-globin gene are transfected into HeLa cells, and then the promoter is transactivated by infection with a tk^- HSV. Adequate amounts of precursor and splicing intermediates and products are obtained that can be assayed by Northern analysis. The majority of these species are associated with the pellet after a 0.4 M KCl wash (high-salt matrix; HS-MX). When HS-MX is incubated under in vitro splicing conditions with a HeLa cell extract, intact or pre-treated with micrococcal nuclease, the amount of globin precursor progressively decreases without a temporal lag, with a corresponding increase in free intron lariat. Although the final ligation of exons cannot be monitored because of the abundance of preexisting mature mRNA, these results suggested that parts of preassembled splicing complexes are present in the HS-MX and can be complemented with soluble extract factor(s), allowing at least three of the splicing events (endonucleolytic cuts and branching) to occur. The complementing activity is retained after heating the splicing extract for 30 min at 37°C. The reaction under standard in vitro conditions, but without Mg^{++}, stops after the first step (accumulation of lariat-exon) if the complementation is performed with extract dialyzed vs. low salt. Is the presence of complementable structures in the HS-MX an artifactual consequence of exposure to high salt? To resolve this issue, we prepared matrix by extraction with only 0.1 M KCl ("low-salt matrix"; LS-MX), and showed that a portion of the pre-mRNA is spliced rapidly in vitro in the presence of ATP and Mg^{++} and in the absence of splicing extract. We propose that at least a subset of the MX-bound HnRNPs, which are self-splicing complexes under in vitro conditions, correspond to in vivo spliceosomes (the 60 S in vitro spliceosomes are extract-complementable, but not self-splicing entities). Using the HS-MX as an assay system, we began fractionating the HeLa cell splicing extract through a series of FPLC chromatographic steps, in order to identify the complementing factor(s). Regardless of the method of fractionation, the activity is always present in one fraction, which suggests that there is a single complementing factor or a complex of factors.

N 026 ELEMENTS THAT ORGANIZE CHROMOSOMAL LOOPS IN THE INTERPHASE NUCLEUS, William T. Garrard, Veronica C. Blasquez, Barbara R. Fishel, Ming Xu, Robert E. Hammer, Steven C. Moses, Yasmin Mehrotra, and Sharon L. Jones, Department of Biochemistry, and the Howard Hughes Medical Institute, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235.

In both interphase nuclei and mitotic chromosomes DNA is organized into topologically constrained looped domains 5 to 200 Kb in length. Segregation of the eukaryotic genome into these looped domains is believed to facilitate the developmental regulation of linked genes triggered by interactions between *cis*-acting sequences and *trans*-acting factors. To gain insight into how this might occur, we have recently been interested in elucidating the organization, composition and function of the elements that anchor chromosomal loops. DNA binding assays have identified an evolutionarily conserved class of anchorage sequences, termed "matrix association regions"; *MARs* are about 200 bp long, AT-rich (ca. 70%), contain topoisomerase II consensus sequences and other AT-rich sequence motifs, often reside nearby *cis*-acting regulatory sequences, and their binding sites are abundant (>10,000 per mammalian nucleus) (1-3). The juxtaposition of *MARs* with the enhancers of the immunoglobulin genes has been conserved in rabbits, mice, and humans (1,3), suggesting that *MARs* may play a role in positive or negative transcriptional regulation. Interestingly, deletion of the *MAR* results in a 10-fold reduction in the level of steady-state κ mRNA as assayed by stable integration in plasmacytoma cells. The role of the *MAR* in determining the degree and timing of tissue-specific expression is also being tested in transgenic mice. Other studies suggest that the *MARs* adjacent to the heavy chain enhancer may participate in tissue-specific negative regulation (4). We have also taken advantage of the powerful genetic system of *Saccharomyces cerevisiae* to dissect chromosomal loop anchorage elements. Insertion of a mouse *MAR* into the yeast *GAL1* promoter between the UAS_G and TATA box of a *lacZ* fusion gene results in a 25- to 50-fold inhibition of β-galactosidase induction. We have exploited this observation to isolate *ts* conditional lethal mutants that are also *ts* inducible for *lacZ* expression, and are cloning genes that complement such mutations. Topoisomerase II *ts* mutants are also being employed in similar studies. Research supported by NIH and The Robert A. Welch Foundation.

References

1. Cockerill, P.N. and Garrard, W.T. (1986) *Cell* 44, 273.
2. Cockerill, P.N. and Garrard, W.T. (1986) *FEBS Letters* 204, 5.
3. Cockerill et al. (1987) *J. Biol. Chem.* 262, 5394.
4. Imler et al. (1987) *Mol. Cell. Biol.* 7, 2558.

RNA in Evolution

N 027 TEMPLATE-DIRECTED SYNTHESIS PRIOR TO RNA CATALYSIS

Gerald F. Joyce, The Salk Institute for Biological Studies, La Jolla, CA 92037

RNA, by virtue of its template properties and catalytic activity, is able to serve both a genotypic and phenotypic function. This has led to the notion that organisms based solely on RNA may have existed at a very early stage in evolutionary history, prior to the development of the translation apparatus. It should be emphasized, however, that it is very unlikely that RNA itself was the first genetic material. There are several major problems associated with the prebiotic synthesis of ribonucleotides. In addition, the two enantiomers of a mononucleotide form very close structural homologues when bound to a complementary template, such that one enantiomer inhibits the template-directed polymerization of the other. We have suggested [1] that RNA was preceded in the evolution of life by polymers constructed from simpler, more flexible, acyclic analogues of the nucleotides. Once these molecules developed the ability to catalyze the synthesis of one enantiomer of the ß-ribosides, the transition to an RNA genome would have become a more favorable event.

In the laboratory our concern is often directed toward constructing a chemical evolving system rather than toward determining how an evolving system arose from the prebiotic environment. For this purpose, commercially available supplies of pure ß-D-nucleotides do very nicely. A variety of preformed RNA templates have been used to direct the synthesis of complementary oligomers from a mixture of activated mononucleotides [2]. Self-replication has been demonstrated using chemically-modified dinucleotide substrates and a self-complementary tetranucleotide template [3]. However, these reactions are not general with respect to template sequence, and cannot be used to conduct an evolutionary search for RNAs that have novel behavioral phenotypes. Current studies involve attempts to extend the reaction system so as to include a broader range of self-replicating RNAs.

1 Joyce, G.F., Schwartz, A.W., Miller, S.L. and Orgel, L.E. (1987). *Proc. Natl. Acad. Sci. USA*, **84**:4398.

2 Joyce, G.F. (1987). *Cold Spring Harbor Symp. Quant. Biol.*, in press.

3 Zielinski, W.S. and Orgel, L.E. (1987). *Nature*, **327**:346.

N 028 The Genomic Tag Model for the Origin of Protein Synthesis

Nancy Maizels and Alan Weiner
Department of Molecular Biophysics and Biochemistry
Yale University, New Haven, CT 06510

A central problem in envisioning the evolution of modern protein synthesis has been that none of the individual components -- ribosomal RNAs and proteins, initiation and elongation factors, tRNAs and tRNA synthetases -- appears to be useful individually, yet the molecular apparatus for translation must have evolved stepwise. We propose that modern tRNAs derive from 3' terminal tRNA-like structures that tagged genomes for replication in the RNA world. In early genomes these tRNA-like structures would have provided an initiation site for the replicase, and would also have functioned as primitive telomeres to ensure that terminal nucleotides were not lost during replication. Such structures persist at the termini of modern viruses, including RNA phages and plant viruses, and as punctuation in the rRNA operons of E. coli.

This picture suggests that the CCA-adding activity was originally an RNA enzyme, that modern DNA telomeres with the repetitive structure C_mA_n descend from the CCA terminus of tRNA, and that the ancient precursor of the modern enzyme RNase P functioned to convert genomic into functional RNA molecules by removing the 3'-terminal tag. Such contemporary molecules may be considered "molecular fossils", since their structures or activities reflect evolutionary history as well as the requirements of function.

We propose, furthermore, that an early tRNA synthetase may have derived from an aberrant activity of the RNA replicase. If this is the case then tRNA synthetases would first have evolved as RNA enzymes, which may account for the remarkable diversity in structure of modern synthetases. Finally, we speculate that if early ribosomes evolved to facilitate polymerization of homopolymers of basic amino acids, then homopolymer synthesis by peptide-specific ribosomes may have provided a pathway for the origin of a rudimentary genetic code prior to the evolution of the first external templates, or mRNAs.

N 029 A RIBONUCLEASE ACTIVITY DERIVED FROM AUTOLYTIC PROCESSING SEQUENCES OF SATELLITE TOBACCO RINGSPOT VIRUS RNA, Paul A. Feldstein, Jamal M. Buzayan and George Bruening, Department of Plant Pathology, College of Agricultural and Environmental Sciences, University of California, Davis, California 95616

The nucleotide sequence of satellite tobacco ringspot virus RNA (STobRV RNA) has 359 or 360 nucleotide residues (nt) depending on the isolate. Multimeric forms of STobRV RNA have this "monomeric" sequence tandemly repeated. The phosphodiester bond at which the monomeric sequences join is designated as the junction. Autolytic processing occurs at a CpA junction in the encapsidated, or (+), polarity sequence, whereas in the complementary, or (-), polarity RNA the junction is in a specific ApG dinucleotide sequence. Previous work showed that the nucleotide sequences necessary for the autolytic processing of STobRV (+)RNA are confined to a region near the junction. Guided by the insertional mutagenesis data of others, we determined that, in contrast the result with (+)RNA, the nucleotide sequences that are responsible for the autolytic processing of STobRV (-)RNA are distributed into two regions which can function together even when incorporated into separate RNA molecules. The nucleotide sequences of the junction region that are necessary for this reaction number approximately 10. The other portion of the RNA thus is able to recognize this short sequence and to facilitate its cleavage in a manner similar to that of a ribonuclease.

RNA Structure

N 100 CORRELATION OF THE EXPRESSION OF THE micF RNA GENE WITH LEVELS OF ompF mRNA AND OmpF PROTEIN IN ESCHERICHIA COLI, J. Andersen*, K. Zhao*, S. Forst**, M. Inouye** and N. Delihas*, * SUNY Stony Brook, Stony Brook, N.Y. 11794, ** UMDNJ, Piscataway, N.J. 08854.

Expression of the gene coding for 4.5S micF RNA in Escherichia coli is thermoregulated and osmoregulated in a manner inverse to the expression of ompF. Under low osmolarity, the levels of micF RNA dramatically increase with increased temperature. Also, under low temperature (25°C), expression of the gene increases with increased osmolarity. Northern blot analyses were used to correlate relative in vivo levels of micF RNA with those of ompF mRNA in cells grown under different steady state growth conditions and also after sudden shifts in temperature or osmolarity. The kinetics of ompF mRNA decay and de novo synthesis of OmpF protein vary in response to the levels of micF RNA. The effect of multicopy inhibition of OmpF by micF is observed when the expression of the micF gene is highest. The results are consistent with the hypothesized function of micF RNA as a negative regulator of OmpF expression.

N 101 HIGH RESOLUTION IMAGING OF CONFORMATIONAL STATES AND INTERACTIONS OF RIBOSOMAL RNAs BY SCANNING TRANSMISSION ELECTRON MICROSCOPY, M. Boublik*, V. Mandiyan*, J.F. Hainfeld** and J.S. Wall**, *Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110 and **Brookhaven National Laboratory, Upton, NY 10021.

The high efficiency in collection of scattered electrons in the dedicated STEM dark-field mode, cold stage and low radiation dose (1e/Å^2) make it possible to visualize unstained freeze-dried molecules of nucleic acids free of structural distortion and resolution-limiting artifacts inherent in the conventional electron microscopy. The potential of STEM application to the structural analysis of nucleic acids is demonstrated on ribosomal RNAs (16S and 23S) from *E. coli*. Unlike conventional electron micrographs of nucleic acids contrasted by staining or shadowing with heavy metals the STEM images of free rRNAs show complex structures with reproducible branching of the major backbone consisting, on the average, of four strands when extended in low ionic strength. The extent of branching depends on buffer conditions. Increase of ionic strength induces intramolecular long-range interactions and coiling of rRNAs into more compact structures. These conformational transitions can be characterized by radii of gyration (R_G) calculated from the mass distribution in the digitized electron micrographs of individual rRNA molecules. R_G of the 16S rRNA in water is about 300 Å ± 50 Å and only about 80 Å ± 10 Å in the ribosomal reconstitution buffer. Conformational changes induced in rRNAs by interactions with ribosomal proteins can be monitored in similar way. The STEM technique can be conveniently applied to the conformational studies of any nucleic acid. The Brookhaven STEM Biotechnology Resource is supported by NIH Grant No. RR01777, J.S. Wall by USDOE.

N 102 CRYSTALLOGRAPHIC STUDIES OF THE XENOPUS LAEVIS TRANSCRIPTION FACTOR IIIA-5S RRNA COMPLEX, Raymond S. Brown, Howard Hughes Medical Institute, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138.

Previtellogenic oocytes of juvenile Xenopus laevis (South African clawed toad) store large amounts of a stable protein-RNA complex which consists of a single protein species, TFIIIA, bound to oocyte type 5S rRNA. This protein has attracted a great deal of interest since it has been found to be a zinc-finger protein whose function is the regulation of 5S rRNA genes. TFIIIA is thus an example of a dual nucleic acid-binding protein. The successful crystallization of the native complex and of a subcomplex obtained upon papain treatment, provides a unique opportunity to study the structural basis of specific protein-RNA interactions.

N 103 GROWTH RATE REGULATION OF TRANSCRIPTS ENTERING A BACTERIAL ORIGIN OF DNA REPLICATION, Anne Chiaramello and Judith W. Zyskind, Department of Biology, San Diego State University, San Diego, CA 92182. In *Escherichia coli*, transcriptional direction of the 16kd and *asnC* promoters is toward *oriC*, and termination of a portion of these transcripts is at RNA to DNA transitions mapped for the counterclockwise leading strand. We have demonstrated by S1 nuclease mapping that both transcripts reach *oriC*, with 87% coming from the 16kd promoter and 13% originating from the *asnC* promoter for *E. coli* cells growing with a generation time of 28 min. The 16kd promoter is stringently controlled in that it is inhibited by amino acid-starvation and stimulated by chloramphenicol. Also, using an in vitro transcription system, it has been demonstrated that ppGpp inhibits the 16kd promoter. We asked whether the 16 kd promoter was also growth rate regulated, because most stringently controlled promoters show growth rate control. RNA was isolated from cells grown at four different growth rates and the concentration of the 16kd and *asnC* transcripts was determined using a probe that contained the 5' ends of both transcripts. The ratio, RNA_{16kd}/RNA_{total}, increased five-fold as the generation time decreased from 109 min to 28 min, whereas the ratio, RNA_{asnC}/RNA_{total}, increased approximately two-fold. These results indicate that the 16kd promoter is growth rate regulated, with slight growth rate control observed for P_{asnC}. Implications of a growth rate regulated transcription event in the initiation process of DNA replication will be discussed.

N 104 STRUCTURE-FUNCTION STUDIES OF THE ROLE OF RIBOSOMAL RNA IN TRANSLATION BY SITE-DIRECTED MUTAGENESIS. Albert E. Dahlberg, H.Ulrich Goringer, William E. Tapprich, and Elizabeth A. De Stasio, Brown University, Providence, Rhode Island, 02912.

Studies on the functional role of E. coli rRNA indicate that it is involved in all stages of translation. At initiation there is base pairing between the Shine-Dalgarno sequence of mRNA and a polypyrimidine sequence at the 3' end of 16S rRNA. Base pairing may also occur between a putative translational enhancer sequence in certain mRNAs and the 460 region of 16S rRNA, as well as between complementary sequences in 16S and 23S rRNAs during subunit association. During elongation intramolecular rearrangements in rRNA have been proposed as well as interactions between tRNA and both 16S and 23S rRNA. There is evidence that one type of frameshifting involves base pairing between mRNA and the 3' end of 16S rRNA. Termination may involve intramolecular rearrangements within the 16S rRNA, followed by base pairing interactions between the stop codons and specific sequences in 16S rRNA. These projects are currently being studied by site-directed mutagenesis of plasmid-coded rRNA.

N 105 RNA IS NOT A RIGID MOLECULE : THE FIRST HIGH RESOLUTION STRUCTURE OF A SYNTHETIC OLIGORIBONUCLEOTIDE $U(U\text{-}A)_6A$.

A.C. Dock-Bregeon*, B. Chevrier*, A. Podjarny*, D. Moras*, G.R. Gough+, P.T. Gillam+ and J.J. Johnson+. I.B.M.C. du C.N.R.S., 15 rue Descartes, 67 084 Strasbourg Cedex, France. +Department of Biological sciences. Purdue University, West Lafayette, IN 47907.

The oligomer was synthesized chemically in solution, using a modified version of the phosphotriester method adapted for construction of oligoribonucleotides. Crystals diffracting to 2.3 Å resolution were obtained at 37°C. The structure was solved by a rotation-translation search method using the program ULTIMA[1] and refined by least squares to an agreement factor R of 14.5% for 2437 significant reflexions.

The molecule differs from a regular helical RNA and can be described as three blocks of 4, 6 and 4 base-pairs separated by two kinks. The propeller twist is particularly large with a mean value of 19.4 (2.4)°. Roll angles show an alternance of high values for U-A steps and low values for A-U steps. All but two riboses have a C3'-endo pucker. The two exceptions have C2'-exo conformations and are located at a terminal residue and at one kink position. At both kink positions the torsion angle around P-O5' shifts from *g*⁻ to *trans*, each kink being stabilized by one hydrogen bond with a symmetry-related molecule. The peculiar conformation of the RNA is probably related to the rather high temperature of crystallization and highlights the variability of RNA conformation.

(1) D. Rabinovitch & Z. Shakked, Acta Cryst., A40 (1984) 195-200.

N 106 TRANSCRIPT ALTERATION BY mRNA EDITING IN KINETOPLASTID MITOCHONDRIA, Jean E. Feagin[1], Janet M. Shaw[2], Larry Simpson[2] and Kenneth Stuart[1], [1] Seattle Biomedical Research Institute, 4 Nickerson St., Seattle, WA 98109, [2] Department of Biology, UCLA, Los Angeles, CA 90024.

Transcripts of a number of mitochondrial genes of the kinetoplastid protozoans Trypanosoma brucei, Leishmania tarentolae, and Crithidia fasciculata contain nucleotides, all of which are uridines, which are not encoded in the DNA sequence. Extra uridines are added within the 5' ends of some transcripts which lack genomically encoded AUG initiation codons, creating AUG initiation codons. The position of the created AUG and the predicted N-terminal amino acid sequences are very similar among the three species for each transcript, suggesting they are functional initiation codons. Transcripts from genes with encoded AUG initiation codons lack 5' uridine additions. In some transcripts, uridines are added internally, correcting frameshifts in the genes. In several cases, uridines which are predicted by the genomic sequence are missing from the transcript. These deletions, like the additions, have the effect of increasing the amino acid sequence similarity between the species. T. brucei, in which production of the respiratory system is developmentally regulated, exhibits developmental regulation of RNA editing. Uridines are added to some transcripts in all life cycle stages and to others only in specific life cycle stages. Thus the RNA editing activity acts in both a gene-specific and life cycle stage-specific manner.

N 107 REGULATION OF EXPRESSION OF HIV BY VIRAL FACTORS. Barbara K. Felber, Margarita Cladaras, Christos Cladaras, A. Tse, Connie M. Wright and George N. Pavlakis, NCI-Frederick Cancer Research Facility, BRI-Basic Research Program, Frederick, MD 21701.

Measurements of the expressed mRNAs and proteins of several HIV mutants have allowed the characterization of the trans acting factors and the cis acting elements necessary for the expression of HIV in human cells. The transactivator protein (tat) affects sequences in the R region of the LTR and increases the steady-state levels of the viral mRNAs. The second trans-acting factor (named trs or art) has been implicated in the regulation of translation and/or the regulation of splicing. We have shown that trs affects the stability of unspliced viral mRNAs and interacts directly or indirectly with a sequence within the env region of HIV. Therefore, trs affects the ratio of spliced versus unspliced viral mRNAs by an indirect mechanism. trs may also promote transport of the unspliced mRNAs to the cytoplasm and the efficient translation. We have characterized the functional protein domains of both tat and trs by mutagenesis. Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-74101 with Bionetics Research, Inc.

N 108 TANDEM REPETITIVE tRNA GENES AND PSEUDOGENES IN PHOTOBACTERIUM. Sylvie Giroux and Robert Cedergren, Département de biochimie, Université de Montréal, Montréal, Québec H3C 3J7 CANADA.

To evaluate the importance of genome fluidity in evolution, we are using the Arg T operon (genes for Arg, His, Leu and Pro tRNA) isolated from E. coli to probe related species. Hybridization of the E. coli probe was obtained with Photobacterium, Proteus, Serratia, Vibrio and Aeromonas DNA. Two clones of Photobacterium phosphoreum have been isolated and sequenced. The smaller clone contains two tRNA genes (His and Pro). The two genes are followed by a second $tRNA^{Pro}$ identical to the first, and a rho-independent terminator. The terminator and segments related to the $tRNA^{Pro}$ sequence are repeated eight times. These repeated tRNAs are thought to be bacterial pseudogenes, since they contain presumably deleterious deletions and insertions. In in vitro transcription, the tRNA genes are transcribed, although the pseudogenes are not. The second cloned DNA fragment contains six copies of $tRNA^{Pro}$, two copies of $tRNA^{His}$ and five copies of the rho independent terminator and the adjacent $tRNA^{Pro}$ pseudogene sequence found in the first cluster. A putative promoter is present 140 nucleotides upstream of the first $tRNA^{Pro}$. Extensive exchange of genetic information between the two clusters is strongly suggested by the fact that tRNA sequences in both fragments are identical and a 350 bp fragment which differs in only 3 positions is common to both loci. The two tRNA gene clusters are composed of the longest segments of tandem repetitive DNA so far reported in eubacteria. This work was supported by NSERC of Canada.

N 109 **A HIGHLY FRAGMENTED LARGE SUBUNIT RNA IN THE CYTOPLASMIC RIBOSOME OF *EUGLENA GRACILIS***, Michael W. Gray[1], James R. Cook[2] and Murray N. Schnare[1], [1]Dept. of Biochemistry, Dalhousie University, Halifax, Nova Scotia B3H 4H7 (Canada) and [2]Dept. of Zoology, University of Maine, Orono, Maine 04469.

In previous work [Spencer DF, Collings JC, Schnare MN & Gray MW (1987) **EMBO J. 6**: 1063-1071], we showed that the large subunit ribosomal RNA (LSU rRNA) of *Crithidia fasciculata*, a trypanosomatid protozoan, consists of 7 separate RNA pieces. This "fragmented" LSU rRNA is produced by the post-transcriptional removal of internal transcribed spacer sequences (ITSs) during processing of the precursor rRNA. To determine whether such a highly split LSU rRNA is more widely distributed than among the trypanosomatid protozoans, which diverged very early from the main line of eukaryotic evolution, we have analyzed the cytoplasmic LSU rRNA of *Euglena gracilis*, a flagellate that diverged from the trypanosomatids shortly after they diverged from other eukaryotes [Sogin ML, Elwood HJ & Gunderson JH (1986) **Proc. Natl. Acad. Sci. USA 83**: 1383-1387]. We find that *Euglena* cytoplasmic LSU rRNA is even more highly fragmented than that of *Crithidia*, with 14-15 major discrete RNA components detectable by gel electrophoresis. The 5'- and 3'-terminal sequences of these RNAs are being determined, in order to map them to the *Euglena* nuclear LSU rRNA gene, whose sequence we have also determined. Preliminary results suggest that some of the *Euglena* RNA species are novel; that the 3'-ends of some map close to the positions of ITSs in the *Crithidia* rRNA precursor; and that the 3'-ends of others map to the positions of variable regions in the mature LSU rRNA of *Crithidia*. Our results reinforce a previously suggested correlation between ITSs in rDNA and variable regions in LSU rRNA, and support the idea that "split rRNAs" are evolutionarily ancient. Supported by NSERC Canada (A8387 to MWG) and NSF U.S.A. (DCB-8408588 to JRC).

N 110 CELL CYCLE REGULATION OF THYMIDINE KINASE mRNA INVOLVES NUCLEAR PROCESSING EVENTS. J.M. Gudas, G.B. Knight, and A.B. Pardee. Dana Farber Cancer Institute, Boston, MA 02115.

As one approach to understanding regulatory events that occur at the G_1/S boundary of the cell cycle, we have chosen to examine the mechanism(s) that underlie thymidine kinase (TK) mRNA induction at the onset of DNA synthesis. Previous results have shown that the rate of TK gene transcription in S phase cells is about four fold greater than that detectable in quiescent cells. TK mRNA levels increase approximately 40-fold as cells enter DNA synthesis and therefore, posttranscriptional events are largely responsible for this accumulation. We examined nuclear RNA levels as serum-starved cells were released from quiescence and found that a dramatic change in the processing of TK mRNA occured at the onset of DNA synthesis. This change involved the appearance of a series of high molecular weight precursor bands that most likely correspond to various RNA processing intermediates. Evidence that the larger bands represent true processing intermediates derives from the fact that a TK cDNA probe hybridizes to the entire series of higher molecular weight precursors while an intron probe only hybridizes to a discrete subset of bands. Similar results were observed for dihydrofolate reductase mRNA. Taken together our results suggest that quiescent cells lack the ability to efficiently process certain mRNAs that are required during S phase and that this function is regained just prior to the onset of DNA synthesis.

N 111 STRUCTURE OF A PRE-mRNA BRANCH POINT/3' SPLICE SITE REGION. Kathleen Hall*, Alfred Redfield*, Michael Green+, *Brandeis University, Waltham, Ma 02254, +Harvard University, Cambridge, MA 02138.

We are studying the contribution of RNA structure to branch point selection and splicing efficiency. Using a combination of nuclease mapping and NMR data, we have proposed a structure for the branch point/3' splice site sequence of the human beta-globin IVS1 RNA. In the naked RNA, the adenosine used for branch formation appears to be in the loop of a stem/loop structure. The nuclease sensitivity of one portion of the polypyrimidine tract suggests that it too may be found in a loop. This RNA sequence adopts a unique structure which is present in the precursor RNA as well as in a short fragment consisting of only branch point/3' splice site sequences. NMR spectra of two RNAs containing the branch point sequences with or without the 3' splice site, indicate that one structural domain is present in both transcripts, as shown by a conserved set of resonances. To investigate the influence of structure and to test our model, we have begun to construct variants by site-directed mutagenesis. Preliminary data supports our model of a stem/loop structure near the adenosine used for branch formation.

N 112 CLONING AND PARTIAL SEQUENCING OF THE RIBOSOMAL RNA OF *MYCOBACTERIUM TUBERCULOSIS*, Karen E Kempsell, Iris CE Estrada-G, F Ian Lamb, M Joseph Colston and Robert A Cox, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA.

Little is known about the genomic organisation of pathogenic mycobacteria. As a first step, studies were initiated to determine the organisation and number of ribosomal RNA cistrons in *Mycobacterium tuberculosis*. Partial sequences of the ribosomal RNAs were also established from these studies.

The number of rRNA cistrons in *M tuberculosis* $H_{37}Rv$ was investigated by restriction analysis of chromosomal DNA and hybridisation with a number of probes. The probes used were (a) total (^{125}I)-labelled *Escherichia coli* rRNA; (b) (^{32}P)-labelled pUC8 clones containing fragments of *M tuberculosis* DNA corresponding to the 5'-end of the 16S rRNA gene and the 3'-end of the 23S rRNA gene; (c) (^{35}S)-labelled cDNA made by primer extension and corresponding to the 3'-end of the 16S rRNA gene.

These analyses revealed a distinctive pattern of restriction fragments containing portions of the *M tuberculosis* rRNA genes; other mycobacterial species gave different patterns. Five other strains of *M tuberculosis* produced patterns identical to that of $H_{37}Rv$, indicating extensive sequence homology. The results indicate that *M tuberculosis* probably possesses only a single rRNA cistron. A restriction map of the rRNA cistron of *M tuberculosis* was compiled.

Restriction fragments containing rRNA gene sequences were cloned into the vector pUC8, and partial sequences were obtained, which represent the 5'-end of the 16S rRNA gene and the 3'-end of the 23S rRNA gene. Sequence data corresponding to the 3'-end of the 16S rRNA were determined by direct primer extension on rRNA templates. Previously published data for bacterial 16S rRNA species show a pattern of conserved and non-conserved sequences that fit a common secondary structure. The data for *M tuberculosis* fit this general pattern.

N 113 p53 mRNA METABOLISM ALONG THE INDUCED DIFFERENTIATION OF MURINE ERYTHROLEUKEMIA CELLS, Saadi KHOCHBIN and Jean-Jacques LAWRENCE, Laboratoire de Biologie Moléculaire du Cycle Cellulaire, Unité INSERM 309, 85 X, 38041 GRENOBLE CEDEX, France

In a previous study (Journal of Molecular Biology, in press) we have investigated the control level of the accumulation of the transformation associated cellular protein p53 in murine erythroleukemia cells induced to differentiate by hexamethylene bisacetamide.

Looking for the regulation mechanism of the p53 decay, we found that the mRNA started to decrease as early as half an hour after the HMBA was put in the culture medium, and that the transcription rate of the gene itself could not account for the observed down-regulation of the mRNA, suggesting a post-transcriptional control for the mRNA accumulation. This control did not require the de novo synthesis of a protein component, as shown by cycloheximide experiments, but seemed to be governed by the induced synthesis of an RNA molecule. Our recent experiments gave evidence of an important degradation of p53 mRNA precursor, very early after addition of the inducer to the culture medium.

We suggest that, one of the early stages of the p53 pre mRNA splicing is inhibited by an induced activity after induction of differentiation and this leads to degradation of this precursor mRNA.

N 114 Pb^{2+} IONS INDUCED CONFORMATIONAL CHANGES IN $tRNA^{Phe}_{yeast}$.

T.Kulinski, A.J.V.G.Visser*, J.Ciesiolka, T.Marciniec, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12, Poznań, Poland. *) Dept of Biochemistry, Agricultural University, De Dreijen 11, Wageningen, Holland.

Crystallographic and solution studies show that the incubation of $tRNA^{Phe}$ with Pb^{2+} leads to a specific cleavage of tRNA , mainly at position 17. According to the already proposed mechanism Pb^{2+} binds to the specific site of the TΨC loop and acts as a metalloenzyme hydrolysing the phosphodiester linkage in the D loop [1]. The results of spectroscopic experiments will be presented (mainly fluorescence decay and anisotropy decay of the Wye base), which exhibits a drastic change when Pb^{2+} are present in the solution. These are evidences for important conformational change induced in the $tRNA^{Phe}$ due to the influence of Pb^{2+}. Possible mechanisms of Pb^{2+} complex formation with tRNA will be discussed in respect to experimental data.

[1].Brown,R.S.,Hingerty,B.E.,Dewan,J.C.,Klug,A.(1983) Nature 303,543.

N 115 **THREE CONFORMATIONS OF tRNA ANTICODON LOOP IDENTIFIED,** George Striker & Damian Labuda* (Max-Planck-Institut fur biophysikalische Chemie, 3400-Gottingen, FRG; * Génétique Médicale, Hôpital Ste-Justine, Université de Montréal, Montréal, P.Q. H3T 1C5, Canada).

In order to gain a better understanding of the complex conformational states of the anticodon loop of **yeast** $tRNA^{Phe}$, which we had previously studied with relaxation experiments by monitoring fluorescence of the naturally occuring **Wye-base,** the **time-resolved fluorescence** was measured dependent upon various counterion concentrations and temperature. As published lifetime measurements had failed to resolve the components of this fluorescence, a highly accurate apparatus and effective analysis procedures were necessary. The **synchrotron radiation** of the storage ring DORIS at DESY in Hamburg was utilized as a source of the 150 ps light pulses at 1 MHz. The emitted fluorescence, resolved as to time of arrival and polarization-orientation, was collected directly into an LSI 11/23 for analysis with modulating functions. Series of experiments with interrelated conditions were then reanalyzed using **global methods.**

Three conformations of the anticodon loop are detected, all three occuring in a wide range of counterion concentrations with and without Mg^{++}, each being **identified by its typical lifetime.** The fluorescence changes brought about by varying the concentrations, previously monitored by steady state fluorimetry and relaxation methods, are changes in the population of these three conformational states, in the sense of an **allosteric model,** where **the effectors are the three ions** Mg^{++}, Na^{+} **and** H^{+}. The population of the conformation with the longest lifetime of 8ns is seen to increase with Mg^{++} concentration. A conformation with subnanosecond lifetime is seen to vary with **pH** while the intermediate lifetime of 4ns is associated with a conformation most affine to the Na^{+}. At very high Mg^{++} concentrations an additional effect occurs which is not yet fully explained.

N 116 FUNCTIONAL SIGNIFICANCE OF CONFORMATIONAL FLEXIBILITY IN 5S RNA, Neocles B. Leontis, Bowling Green State University, Bowling Green, OH 43403.

5S ribosomal RNA sequences display the universally conserved potential to form two alternative secondary structures in the helix 2-helix 3 region of the molecule. It has been calculated that the two conformations are energetically equivalent and are likely to be in equilibrium (1). Such an equilibrium provides the basis for a conformational switch, which is presumed to be functionally significant (2). This hypothesis is being tested by a combined genetic, biochemical, and physical approach. Site-specific mutagenesis is being employed to introduce mutations which are expected to alter the relative stability of the alternative conformations. According to the hypothesis, mutations which alter the conformational equilibrium will also alter the functional properties of the molecule. The altered RNAs are being synthesized using an overproducing system as well as *in vitro* technques. Functional studies including protein binding, ribosome reconstitution, and protein synthesis assays are underway. High-field NMR techniques are being employed to elucidate the conformation favored under various solution conditions in the normal and altered RNA sequences. Preliminary NMR observations have been published (3).

(1) DeWachter, R., Chen, M.W., & VanDenBerghe, A. (1982) "Conservation of secondary structure in 5S ribosomal RNA: a uniform model for eukaryotic, eubacterial, archaebacterial and organelle sequences is energetically favored." **Biochimie 64**, 311-329.

(2) DeWachter, R., Chen, M.W., & VanDen Berghe, A. (1984) "Equilibria in 5S ribosomal RNA secondary structure." **Eur.J.Biochem. 143**, 175-182.

(3) Leontis, N.B. & Moore, P.B. (1986a) "NMR evidence for dynamic secondary structure in helices II and III of the 5S RNA of *E.coli*" **Biochemistry 25**, 3916-3925.

N 117 OVALBUMIN mRNA SECONDARY STRUCTURE, Charles D. Liarakos, Randolph P. Maddox and Randall A. Kopper, University of Arkansas for Medical Sciences, Little Rock, AR 72205. Secondary structure dependent chemical modifications have been used to identify the base-paired nucleotides in the region surrounding the initiation codon of ovalbumin mRNA. Modified bases were unambiguously identified by the specific DNA primer extension with reverse transcriptase. Base-paired adenine and cytidine nucleotides were identified by comparing native and denatured mRNA methylated with dimethylsulfate. Base-paired uridine nucleotides were identified by mRNA photoreaction with aminometyltrimethylpsoralen. The primer extension method was also used to analyze ovalbumin mRNA that had been digested with nuclease V1 from cobra venom - an enzyme that specifically cleaves double-stranded regions in RNA. Specific V1 cleavage sites were consistant with the secondary structure specific pattern of base modification. None of the local base-pairing relationships predicted from the ovalbumin mRNA sequence is consistant with this structural data. Furthermore, the region following the AUG initiation codon contains several base-paired nucleotides for which no local region of complementary sequence appears to exist. A computer search of the ovalbumin mRNA sequence has revealed the possibility of base-pairing between this AUG 3'-proximal region and nucleotides in the 3'-noncoding region. This possibility is intriguing given ability of chemical denaturation to enhance ovalbumin mRNA translation to levels approaching in vivo rates and is currently being evaluated by identifying the base-paired nucleotides and V1 cleavage sites in this 3'-noncoding sequence and by electron microscope visualization of psoralen-photocrosslinked ovalbumin mRNA.

N 118 A STUDY OF THE RIBOSOMAL DNA (rDNA) GAP SEQUENCE IN THREE SPECIES OF HUMAN SCHISTOSOMES, Prema M. Mertz#, Harry van Keulen*, Libuse A. Bobek*, Philip T. LoVerde* and David M. Rekosh#*, Departments of Biochemistry# and Microbiology*, SUNY at Buffalo, Buffalo, NY 14214.

We have determined the intragenic organization of the ribosomal RNA (rRNA) genes in Schistosoma mansoni, Schistosoma haematobium and Schistosoma japonicum, and found them to be similar to that of other eukaryotes. In addition, as in a wide variety of organisms within the phyla Protozoa, Mollusca, Annelida and Arthropoda, the 28S rRNA in schistosomes contains a "gap" which separates it into 2 subunits. We have defined the 5' and 3' boundaries of this gap sequence between the 28Sα and 28Sβ coding segments in all three species by S1 nuclease mapping and cDNA primer extension. The length of the excluded stretch in S. mansoni and S. haematobium is 54 bases and is identical. However, in S. japonicum the sequence is slightly different and is about 64 bases. In each case, irrespective of the species, the gap is located at the same positon within the 28S RNA. The possible secondary structures of the gap sequence were derived by computer analysis so as to predict the conformation with the minimum free energy. Strikingly, an UAAU tract in a hair-pin loop has been detected. This same sequence is found in all other known gap sequences and also seems to be involved in processing of dipteran 5.8S rRNA.

N 119 INTERNAL METHYLATION OF PROLACTIN mRNA IN VITRO, Prema Narayan, Edward Goodwin and Fritz M. Rottman, Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, Ohio 44106

Methylation of mRNA is a common post-transcriptional modification found in eukaryotic cells. In addition to the methylated cap structure at the 5' end, most mRNAs contain internal N^6-methyladenosine residues (m^6A). This modification occurs at the consensus sequences Gm^6AC or Am^6AC at an approximate frequency of 1 per 100 adenosine residues. In the mRNAs that have been characterized thus far, the distribution of m^6A within an mRNA is non-random. In the bovine prolactin mRNA, the majority of m^6A occurs in the 3' untranslated region within a 129 nucleotide sequence containing 3 potential m^6A sites. In order to more accurately localize the m^6A residue within this region, and determine the substrate requirements for this modification we have established a cell free methylation system with HeLa cell nuclear extracts. Full length capped bovine prolactin mRNA transcribed in vitro from a T7 promoter was used as the substrate in the presence of [^{3}H]-S-adenosylmethionine as the methyl donor. Analysis of the RNA by HPLC showed that the major methylated nucleoside was m^6A. Further analysis of the RNA indicated that m^6A methylation was occurring preferentially within the 3' 129 nucleotide sequence of prolactin mRNA. The specificity of the reaction in vitro was similar to that in vivo indicating that Hela nuclear extracts can be used as a valid system to further study RNA substrate requirements for methylation.

N 120 ABUNDANT 5 KB RNA OF HUMAN CYTOMEGALOVIRUS WITHOUT MAJOR TRANSLATIONAL READING FRAME, Bodo Plachter, Gerhard Jahn, Bernd Traupe and Bernhard Fleckenstein, Department of Clinical and Molecular Virology, University of Erlangen-Nürnberg, 8520 Erlangen, FRG.

Transcription in human cytomegalovirus (HCMV), a herpesvirus, is temporally regulated. The genes transcribed immediately after the entry of the virus into the cell, thus termed immediate early (IE), are clustered in a restricted area on the linear double stranded genome of 235 kb. One of the predominant transcripts from the IE region is a 5 kb RNA, which is clearly distinct from the other IE genes of HCMV. It is present within the infected cell throughout the whole lytic cycle of the virus. By nuclease anaysis, primer extension with synthetic oligonucleotides and sequence analysis the structure of the transcript was elucidated. No translational reading frame larger than 300 bp was detected in the whole of its AT-rich coding region. The RNA appears to be unspliced, and no signals such as TATA or CCAAT were found close to the 5'-end. However, the motifs known to be important for the 3'-end processing of eucaryotic RNA-polymerase II transcripts were found at the 3'-end of the coding region. Therefore this RNA presumably represents a thus far uprecedented case of a large non-coding polymerase II transcript present in cells that are lytically infected by an animal virus.

N 121 STUDIES ON THE SPECIFICITY OF THE RNA BINDING ACTIVITY OF THE *XENOPUS* TRANSCRIPTION FACTOR TFIIIA. Paul J. Romaniuk, Dept. of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada V8W 2Y2.

TFIIIA has a dual biological role in the *Xenopus* oocyte. It acts as a positive transcription factor, binding to an internal control region and modulating the expression of the *Xenopus* 5S RNA genes during oogenesis. The protein also forms a specific 7S RNP complex with newly synthesized 5S RNA, stabilizing the RNA for storage in the cytoplasm of immature oocytes. As a result of these functions, TFIIIA binds specifically to DNA and RNA, two nucleic acids which have distinctly different conformations.

We have determined the thermodynamic and kinetic parameters for the RNA binding activity of TFIIIA using a nitrocellulose filter binding assay. The protein binds *Xenopus* oocyte 5S RNA with a dissociation constant of 1 nM, and complex formation is favoured by both enthalpy and entropy. Approximately 5 ionic bonds are formed between the protein and the RNA, although *ca.* 70% of the free energy of complex formation is derived from non-ionic interactions. Several labs including ours have shown that TFIIIA binds to a variety of eukaryotic 5S RNA molecules with similar affinity, and footprinting experiments indicate that the protein protects helix II and the helix IV-V domain of the 5S RNA from modification.

In order to determine more precisely the structural features of the 5S RNA required for TFIIIA binding, we have been studying the interaction of site-directed 5S RNA mutants with the protein. Studies with deletion fragments have indicated that the helix II-loop B region of the 5S RNA contains the primary interaction site for the protein. *Xenopus* somatic 5S RNA binds TFIIIA with a higher affinity than the oocyte, and this enhanced affinity is the result of the somatic-specific nucleotide substitutions in loop B. Using a block mutagenesis strategy, we have tested the relative importance of twenty-eight highly conserved single-stranded nucleotides for binding TFIIIA. Only substitution of the nucleotides in loop A resulted in a significant decrease in TFIIIA binding affinity. This reduced affinity may be the consequence either of the loss of essential nucleotide-protein contacts, or a change in the co-axial stacking properties of the 5S RNA. The effects on TFIIIA binding of substituting bulged and base paired nucleotides are also being investigated.

N 122 Separation and Characterization of a Poly(A) Polymerase and Cleavage/Specificity Factor Required for Pre mRNA Polyadenylation: Lisa C. Ryner, Yoshio Takagaki and James L. Manley, Department of Biological Sciences, Columbia University, New York, NY 10027.

To study the mechanism and factors required to form the 3' ends of polyadenylated mRNAs, we have fractionated HeLa cell nuclear extracts that carry out the normally coupled cleavage and polyadenylation reactions. Each reaction is catalyzed by a distinct activity that can be separated from the other. The partially purified cleavage enzyme (at least 360,000 MW) retained the specificity displayed in nuclear extracts, as base substitutions in the AAUAAA signal sequence inhibited cleavage. In contrast, the poly(A) polymerase (300,000 MW) lost all specificity when separated from the cleavage activity. When fractions containing the cleavage and polyadenylation activities were mixed, the efficiency and specificity of the polyadenylation reaction were completely restored. Interestingly, the cleavage activity by itself functioned well on a precursor containing the SV40 late poly(A) site but not on precursors containing either adenovirus L3, E2a or SV40 early poly(A) sites. However, when mixed with the poly(A) polymerase-containing fraction, the efficiency of cleavage of the other precursors was enhanced so that all four were processed with comparable efficiencies. Mutational analyses indicate that sequences at or very close to the SV40 late cleavage site itself are responsible for the observed preference of the partially purified cleavage activity for this poly(A) site.

To analyze further the sequence requirements of the cleavage and polyadenylation reactions, several deletion mutants were constructed in adenovirus L3 and SV40 late poly(A) addition sites and their effects assayed in fractionated and unfractionated extracts. From the results of this analysis we conclude that sequences that lie far downstream of the cleavage site can influence the efficiency of both cleavage and polyadenylation and that sequences closer to the cleavage site direct cleavage to a specific site, as multiple sites were used when these sequences were deleted.

N 123 CHARACTERIZATION OF THE BINDING OF 5S RNA TO PROTEINS DURING XENOPUS laevis OOCYTE DEVELOPMENT. M.S. Sands and D.F. Bogenhagen, Department of Pharmacology, State University of New York at Stony Brook, Stony Brook, New York.
During Xenopus oocyte development, 5S RNA is synthesized prior to the synthesis of other ribosomal RNAs and ribosomal proteins. Therefore 5S RNA is stored in the form of ribonucleoprotein particles for subsequent ribosome assembly. In developing oocytes 5S RNA is complexed either with TFIIIA in a 7S particle, or with two other proteins and tRNA in a 42S particle. To characterize the interactions involved in the binding of 5S RNA to TFIIIA, transcripts derived from a series of linker substitution mutations encompassing the 5S RNA gene have been assayed for their ability to reconstitute 7S particles in vitro. A direct binding assay was developed which takes advantage of the fact that 7S RNPs migrate more slowly than 5S RNA under native polyacrylamide gel electrophoresis conditions. Reconstituted 7S RNPs have the same gel mobility as 7S RNPs purified from immature Xenopus oocytes. 5S RNAs with base changes in or around helices I, IV, and V, which would interfere with normal base pairing of that region, show a decreased ability to bind TFIIIA. Once assembled into ribosomes, 5S RNA is associated, in the large subunit, with the protein L5. We have isolated both the L5 protein-5S RNA complex from the ribosome as well as the 43kD protein-5S RNA complex from the 42S RNP present in immature oocytes. An RNA exchange assay is being utilized to study the binding of 5S RNA to the L5 protein. In an RNA exchange assay radiolabeled RNA can displace native RNA present in an isolated ribonucleoprotein particle. The radiolabeled RNP can then be analyzed by native gel electrophoresis.

N 124 RNA-PROTEIN INTERACTIONS IN THE RNA BACTERIOPHAGES, Peter G. Stockley, Gloria Medina and Simon J. Talbot, Leeds University, Leeds, LS2 9JT, U.K.

The translational repression complex between MS2(R17) coat protein and the intercistronic region of RNA between the coat and replicase genes is one of the best characterised RNA-protein interactions. The complex forms specifically even with a fragment of only 21 bases. During binding the RNA fragment does not change its conformation indicating that recognition involves the secondary and tertiary aspects of the RNA structure. Unfortunately, the complex has a half-life in solution of ~52s (commensurate with its biological role) and this precludes the application of many physical and biochemical techniques to probe the interaction. Because tight-binding protein mutants are expected to be lethal in vivo we have cloned the coat protein gene and used the random mutagenesis technique of Myers to create and identify point mutants. The interaction of these mutants with the RNA fragment is being studied by a variety of techniques.

N 125 UNIQUE RNA MOLECULES OF MALARIAL PARASITES ENCODED BY TANDEMLY ARRAYED GENE CLUSTERS, Kathleen Suplick, Rama Akella, Akhil B. Vaidya, Hahnemann University Philadelphia, PA 19102

We have recently reported that a 5.8 kb gene cluster cloned from a malarial parasite is tandemly repeated, highly conserved among all malarial species tested, and transcribed into poly A+ RNA's. Multicopy genes that are also tandemly organized include genes for tRNA, 5S RNA, and ribosomal RNA. However, the 5.8 kb gene cluster does not encode any of these RNA molecules as determined by Northern and Southern blot analyses using DNA and RNA from various invertebrate and vertebrate species; this gene cluster is unique to the members of the genus Plasmodium. Multiple transcripts varying in size from 0.2 to 1.6 kb are encoded by the gene cluster of Plasmodium yoelii, each from a discrete region within the 5.8 kb unit. Among these, the most abundant transcripts are 0.2-0.3 kb in size. Northern blot analyses of the erythrocytic stages of P. yoelii show that the 5.8 kb transcription occurs primarily during the trophozoite stage. Sequence data from the gene cluster reveal the absence of large open reading frames in regions transcribed into poly A+ RNA. Computer analysis of RNA from the sequenced regions of both P. yoelii and P. falciparum gene clusters reveals possible extensive secondary structures for the RNA. Since these gene clusters are highly conserved over a vast evolutionary period, and since the most abundant RNA transcripts are too small to be encoding for proteins, we propose that the RNA molecules play a vital role in the biology of Plasmodia as non-messenger, structural RNA's that are unique to these organisms.

N 126 ISOLATION OF RIBOSOMAL RNA MUTANTS IN *E. COLI*, K. Triman, E. Becker, S. Douthwaite*, J. Hagler, J. Katz, C. Yapijakis, S. Yoast and H. Noller, Thimann Laboratories, University of California at Santa Cruz, CA 95064 and *Molecular Biology Department, University of Odense, Denmark

We have constructed a plasmid pSTL102 containing *E. coli* rRNA genes carrying two selectable markers. In 16S rRNA the Spc^R allele (U1192) is used, and in 23S rRNA an Ery^R allele (G2058) is used. These antibiotic resistance mutations, originally identified by Morgan and coworkers, enable us to follow the *in vivo* expression of the cloned rRNA genes. Use of both markers together permits us to detect mutations in either 16S rRNA or 23S rRNA and exclude others, such as promoter mutations. Any mutation causing the loss of expression of the cloned 16S rRNA gene is seen as loss of ability of the organism to survive on media containing spectinomycin; likewise 23S rRNA mutations are detected as erythromycin-sensitive cells. We have generated a large number of Spc^S and a few Ery^S mutations by *in vitro* hydroxylamine mutagenesis of pSTL102 plasmid DNA. The mutations are localized by marker rescue *in vivo*, using specific rRNA gene restriction fragments cloned into pACYC184, a plasmid compatible with pSTL102. Finally, the mutations are identified by DNA sequence analysis.

N 127 A SECONDARY STRUCTURE MODEL FOR BACTERIOPHAGE RNA, J. van Duin, E. Skripkin, M. de Smit and M. Adhin, University of Leiden, The Netherlands.

We present a model for the secondary structure of the central domain of the RNA from bacteriophage MS2. The proposal is based on chemical and enzymatic modification and on phylogenetic comparison with several other phage RNA sequences. In addition, the phenotype changes resulting from base substitutions are used as an independent assay to probe the secondary structure. Among the four existing phage groups a few conserved structures have been found so far. Among these are the binding sites for the phage coat protein and for ribosomal protein S1.

N 128 YEAST MITOCHONDRIAL RNase P: STRUCTURAL CHARACTERIZATIONS OF TWO RNA MOLECULES NECESSARY FOR ENZYMATIC ACTIVITY. Carol A. Wise, Hsiao-Hsueh Shu, and Nancy C. Martin, University of Louisville, Louisville, KY 40202

5′ leaders of yeast mitochondrial tRNA precursors are removed by an RNaseP consisting of an unusual A+U rich mitochondrially encoded RNA subunit and a protein subunit(s) encoded by nuclear DNA. The RNA subunit isolated from different yeasts varies in size and nucleotide sequence. Northern hybridization experiments using RNA obtained from S. cerevisiae strain MH41-7B demonstrate that the most abundant product of the RNase P RNA gene is an RNA molecule of approximately 470 bases long. S1 nuclease mapping experiments demonstrate that neither end of this RNA is unique. There are 4 major 5′ termini which can differ by as much as 8 nucleotides and there are 3 major 3′ termini ranging over 30 bases. Identification of termini of a comparable RNA from a closely related S. cerevisiae strain, D273-10B is underway. We do know that this RNA contains insertions and deletions when compared to the RNA from MH41-7B. The smallest RNaseP RNA identified to date comes from the yeast T. glabrata. It is 186 nucleotides long and is 96% A-U rich. We are constructing transcription vectors to produce this RNA for enzymatic and structural studies. A combination of experimental approaches and phylogenetic comparisons may define a minimum structure sufficient for yeast mitochondrial RNase P activity.

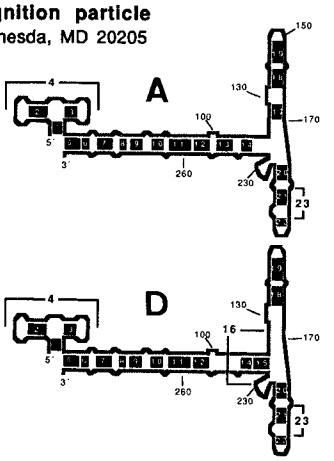
N 129 STUDY OF tRNA-RIBOSOME COMPLEXES USING PHOTOREACTIVE tRNA DERIVATIVES CONTAINING 2-AZIDOADENOSINE OR 8-AZIDOADENOSINE, J. Wower, L. Sylvers, S.S. Hixson and R.A. Zimmermann, University of Massachusetts, Amherst, MA 01003.

Cross-linking studies have been undertaken to determine the ribosomal components that contact the 3′ end of the tRNA molecule. The photoreactive analogs, 2-azidoadenosine ($2N_3A$) and 8-azidoadenosine ($8N_3A$),have been substituted for the 3′-terminal adenosine (A_{76}) of yeast $tRNA^{Phe}$ by ligation of their 3′,5′-bisphosphates to tRNA molecules lacking A_{76}. Replacement of A_{76} with $8N_3A$ rendered $tRNA^{Phe}$ inactive in the aminoacylation reaction. In contrast, incorporation of $2N_3A$ into the position 76 had no effect on aminoacylation. Both photoreactive tRNA derivatives were able to bind to the P-site of the *Escherichia coli* 70S ribosome programmed with poly(U) and became covalently attached to the 50S ribosomal subunit upon irradiation of the complexes at 300 nm. Non-aminoacylated $tRNA^{Phe}$ containing $8N_3A$ cross-linked exclusively to protein L27. When non-aminoacylated $tRNA^{Phe}$ containing $2N_3A$ was cross-linked to ribosomes, 69% of the label was incorporated into 23S RNA while 31 % was attached to protein L27. We have established that the site of cross-linking in 23S RNA is G_{1945}, a nucleotide within a highly conserved sequence for which no function has hitherto been suggested. The cross-linking of AcPhe-$tRNA^{Phe}$ containing $2N_3A$ at position 76 is currently under investigation.

N 130 PSEUDOKNOTTED RNA OLIGONUCLEOTIDES. Jacqueline R. Wyatt, Joseph D. Puglisi, Ignacio Tinoco, Jr., Department of Chemistry, University of California, Berkeley, CA 94720.
With the exception of tRNA, little is known about the structures and thermodynamic properties involved in RNA tertiary structure. A novel structural motif called pseudoknotting was postulated to occur at the 3' end of certain plant viral RNAs by Pleij and coworkers (1) and has since been proposed in other RNAs. A pseudoknot is formed when the dangling end of a hairpin pairs with bases in the loop to form a structure with two stem and loop regions (see figure). A continuous helix is formed by coaxial stacking of the two stem regions. In order to simplify physical studies on this type of structure and identify stem and loop length requirements, we have synthesized a series of oligonucleotides designed to form pseudoknots. The structure of each oligonucleotide has been probed using single-strand and double-strand specific nucleases. Additionally, the thermodynamic properties of oligonucleotides which form pseudoknots have been compared to those of hairpins which contain the individual stem regions (2).

1. Pleij, C. W. A., Rietveld, K., Bosch, L. *Nucleic Acids Res.* 13, 1717-1731 (1985).
2. Puglisi, J. D., Wyatt, J. R., Tinoco, I., Jr. *Nature* (in press).

N 131 **3D-modeling of the 7SL RNA of the signal recognition particle**
Christian Zwieb, NIH, Molecular Biology, Bldg 37, Rm 4B-04, Bethesda, MD 20205
Preliminary 3D models for the two alternative conformers of 7SL RNA were constructed by physical model building with plastic cylinders and wire and by using interactive computer graphics. Data derived from a variety of methods were used: 1)Secondary structure determination by the phylogenetic comparison of a variety of 7SL RNAs:The secondary structure is not altered except in areas for which alternative basepairing was found. 2)Sensitivity to RNases: Sensitive regions towards RNA-structure probing enzymes are located at the outside of the molecule, while unaccessible bases are placed into the inside of the structure. 3)Accessability to complementary oligonucleotides: Most of the bases being located in the dynamic core structure of the 7SL RNA are not accessible to complementary oligonucleotides. 4)Analysis of alternative conformers using linker insertion mutations: The switching from the A- to the D-conformer and *vice versa* does not change the overall strucure of the molecule. A simple swapping between helices 13 and 15, - having helix 14 fixed - is necessary for the switch. The switch is accompanied by a smooth transition from forming helix 16 and abolishing helix 17. 5)A longrange crosslink induced by mild irradiation with UV-light: A compact structure of the RNA is suggested. 6)A base pairing interaction: supported by the crosslink and phylogenetic comparison.



RNA Catalysis

N 200 A SYSTEMATIC SEARCH FOR RIBOZYMES IN RNA PROCESSING IN ESCHERICHIA COLI, David Apirion, Andras Miczak, Anil Chauhan and Ajit Srivastava, Washington University, St. Louis, MO 63110.

A number of observations suggest to us that ribozymes other than RNase P could be involved in RNA processing in Escherichia coli. 1. Studies on RNA metabolism in Escherichia coli mutants deficient in RNA processing indicate that RNA processing enzymes protect specific RNA molecules from degradation. 2. Mutant strains containing a thermolabile ribonuclease E accumulate at nonpermissive temperatures RNA precursors that are not substrates for ribonuclease E. 3. In extracts of strains deficient in RNase III or RNase E the ribozyme RNase P is inactivated.

The interpretation of observation 3 is that in the absence of RNase III or RNase E the RNA of RNase P is degraded and the extracts mimic a mutant deficient in RNase P. Likewise, we think that in vivo the inactivation of RNase E leads to the degradation of RNA from ribozymes, and their substrates remain unprocessed (observation 2).

One such RNA precursor, that accumulates in the RNase E mutant, is p10Sa RNA, a precurser of 10S RNA (a major small stable RNA of the E. coli cell). We are purifing the activity that processes this RNA precurser. It is a new RNA processing activity and interstingly, it requires obligatorily the divalent cation Mn^{+2} for its activity. This enzyme contains a protein and we want to find out whether or not it also requires an RNA moiety for its activity.

N 201 CHARACTERIZATION OF AN ABUNDANT RNA IN NEUROSPORA MITOCHONDRIA. Barry J. Saville and Richard A. Collins, University of Toronto. Toronto, Ont. Canada M5S 1A1.

Several natural isolates of Neurospora intermedia contain a previously-undescribed low-copy-number, double stranded DNA plasmid in their mitochondria. The plasmid population consists of an oligomeric series of head to tail multimers (monomer length = approximately 0.9 kb). Sequence analysis of the plasmid DNA reveals strong similarities to Group I introns. These mitochondria also contain two high-copy RNAs complementary to one strand of the plasmid. The more abundant of these RNAs is present at concentrations similar to those of the mt ribosomal RNAs. Upon agarose gel electrophoresis, the glyoxal-denatured RNAs migrate near a single-stranded size marker equivalent to the size of the plasmid DNA monomer. Neither the plasmid RNA nor DNA hybridizes with the mt chromosome. We are investigating the synthesis and structure of these RNAs, and their possible interactions with other mt components, to determine their relationship to one another and the reason for their very high stability.

N 202 *Tetrahymena* RIBOZYME CATALYZES TRANS-SPLICING OF MODEL OLIGORIBONUCLEOTIDE SUBSTRATES, J. B. Flanegan and T. R. Cech, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215

We have developed a system for studying splicing in *trans* at the 3' splice site. A shortened form of the *Tetrahymena* ribozyme (L - 21 Sca I IVS RNA) that starts at the internal guide sequence and terminates at U_{409} was used in this study. Five nucleotides preceding the 3' splice site including G_{414} are deleted from the L - 21 Sca I ribozyme. Oligoribonucleotides containing the 3' splice site (GGGAGUACUCG$\downarrow$UAAGGUA), the 5' exon (GGCUCUCU) or the 5' splice site (GGCUCUCUAAAAA) were prepared as T7 RNA polymerase transcripts of synthetic DNA templates. The ribozyme was shown to catalyze three specific reactions with these RNA substrates. In two component reactions (ribozyme and 3' SS RNA), the phosphodiester bond at the 3' splice site is specifically hydrolyzed. In a three component reaction (ribozyme, 3' SS RNA, and 5' exon), a specific transesterification reaction at the 3' splice site resulted in the ligation of the 5' and 3' exons. In a four component reaction (ribozyme, 3' SS RNA, 5' exon, and GTP), attack by GTP at the 5' splice site released the 5' exon which was then ligated to the 3' exon in a transesterification reaction at the 3' splice site. This system should be useful in determining kinetic parameters and sequence specificity for reactions at the 3' splice site.

N 203 PROCESSING OF the tRNA-LIKE STRUCTURE FROM TURNIP YELLOW MOSAIC VIRUS BY THE CATALYTIC RNA COMPONENT OF RNase P, Christopher J. Green and Barbara S. Vold, SRI International, Menlo Park, CA 94025; Marie D. Morch, Rajiv L. Joshi, and Anne-Lise Haenni, Institut Jacques Monod, C.N.R.S. and Université Paris VII, 2 Place Jussieu, 75251 Paris Cedex 05, France.

The 3'-end of the RNA genome of turnip yellow mosaic virus (TYMV) can form a pseudoknotted tRNA-like structure that can be recognized by several tRNA specific enzymes. We have found that the catalytic RNA component of Bacillus subtilis RNase P can cleave this structure in unusually low ionic strength buffers at a site analogous to the 5'-end of an aminoacyl stem. Other tRNA precursors can only be processed under low ionic strength conditions if the RNase P holoenzyme is used; processing by the catalytic RNA component alone requires a higher ionic strength buffer. The pseudoknotted TYMV tRNA-like structure may assume a conformation particularly suitable for recognition by catalytic RNA, even at low ionic strengths.

N 204 Characterization of the telomerase RNP from Tetrahymena, Carol W. Greider and Elizabeth H. Blackburn; University of California, Berkeley, CA. 94720

We reported earlier an activity in Tetrahymena extracts which synthesizes tandem repeats of the telomeric sequence TTGGGG without any apparent template. This enzyme may be involved in establishing a dynamic equilibrium of telomere length which protects chromosome ends from shortening during DNA replication. Characterization of the telomerase enzyme has shown that it is a ribonucleoprotein with several distinct primer recognition specificities. *In vitro*, telomerase recognized both structural and sequence characteristics of the oligonucleotides used to prime the elongation reaction. The telomerase activity was inactivated by treatment with both RNase A and Proteinase K. The addition of bulk RNA back to RNase treated extracts did not restore activity, suggesting that the enzyme requires a specific RNA component. Purification of telomerase over 6 different columns identified a small RNA approximately 150 nucleotides in length which consistently co-purified with the enzyme activity. We are currently characterizing this RNA component as well as determining what role RNA may play in the *de novo* telomere synthesis reaction catalyzed by telomerase.

N 205 CLUSTERING OF MUTATIONS AND A SECOND-SITE REVERTANT IN THE GROUP I *td* INTRON OF BACTERIOPHAGE T4, Dwight H. Hall[1], Michael D. Brown[1], Christine M. Povinelli[1], Deborah Bell-Pedersen[2,3], Karen Ehrenman[2,4], and Marlene Belfort[2]; [1]School of Applied Biology, Georgia Tech, Atlanta, GA 30332; [2]Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY 12201; [3]Dept. of Biological Sciences, SUNY Albany, Albany, NY 12222; [4]Dept. of Microbiology and Immunology, Albany Medical College, Albany, NY 12208.

The isolation and genetic characterization of a large collection of T4 thymidylate synthase-defective (*td*) mutants implicated the outer boundaries of the 1-kb intron (approximately 220 nucleotides at each end) as the domains functional in splicing (Hall et al., *Cell 48*, 63, 1987). The sequence changes of 17 mutations that map in or near the intron and have been shown to cause a splicing-defective phenotype have been determined. While the mutations lie scattered throughout the two domains, there is a marked clustering of mutations, each of independent origin, at specific sites. There are two mutations at the same residue in the consensus S element, three at the same site in the phylogenetically conserved P9 stem, and seven mutations in two adjacent residues that may form part of the P6 pairing. Isolation of spontaneous second-site suppressor mutations, using classical phage genetic strategies, is in progress. One false revertant of a mutation in P3[3'], HS9, has the following properties: 1) HS9 grows less well than td^+ in limiting thymidine at 37° and is temperature sensitive; 2) when HS9 is backcrossed to td^+, recombinants with the phenotype of the original *td* mutant are produced; 3) HS9 contains the original mutation by RNA sequence analysis; 4) HS9 is proficient in RNA splicing by dot blot hybridization analysis; and 5) HS9 has a second-site mutation in P3[5'] that suppresses the splicing defect of the original mutant and supports the secondary structure model of the *td* intron.

N 206 A MATURASE ENCODING GROUP II INTRON OF YEAST MITOCHONDRIA SELF-SPLICES IN VITRO, Sharda K. Hebbar and Philip S. Perlman, Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210.

The first intron of the oxi3 gene (aI1) is 2455nt in length and has a long open reading frame (ORF) that encodes a "maturase" protein essential for efficient splicing in vivo. We have shown that an in vitro transcribed RNA containing intron 1 plus flanking exon sequences undergoes a self-splicing reaction to yield spliced exons and excised intron lariat. The reaction occurs optimally at 37°-40°C with 0.1M $MgCl_2$ and 1M NH_4Cl.

Substitution of NH_4Cl with KCl produced a number of novel RNA species in addition to those observed in the NH_4Cl reaction. The linear intron, in KCl, is capable of cleaving itself at either of two sites near its 5' end yielding small 5' intron fragments and lariat RNAs that are smaller than the NH_4Cl lariat. These are the first examples of specific intramolecular transesterification reactions carried out by an excised group II intron. They may be analogous to some post-splicing reactions of group I introns.

Group II introns have a highly conserved core structure which is divided into six domains. A major portion of the aI1 ORF (1827nt) is sequestered within domain 4. Trans splicing experiments using pairs of non-overlapping transcripts interrupted within domain 4 have shown that most of that domain can be deleted with no effect on the formation of spliced exons. We have deleted 1103nt of domain 4 in cis and found that the intron retains the ability to self-splice in NH_4Cl and KCl. Interestingly, while the full-length intron is inactive in 1M $(NH_4)_2SO_4$, the deleted form is active. Experiments are now underway to delete domain 4 completely.

N 207 A SELF-SPLICING GROUP I INTRON IN THE DNA POLYMERASE GENE OF THE *BACILLUS SUBTILIS* BACTERIOPHAGE SPO1. David A. Shub[1], Heidi A. Goodrich[1], Jonatha M. Gott[1], Ming-Qun Xu[1] and Vincenzo Scarlato[2], [1]Department of Biological Sciences, State Univ. of NY Albany, Albany, NY 12222 and [2]Department of Biology, University of California San Diego, La Jolla, CA 92093.

The only examples of RNA splicing in prokaryotes are the three group I introns of the *E. coli* bacteriophage T4. Splicing of group I introns adds a non-coded G to the 5' end of the excised intron. We have used this reaction *in vitro* to identify an autocatalytic intron in RNA extracted from Gram-positive *B. subtilis* cells infected with phage SPO1. End-labeled intron was used as a probe for DNA blot hybridization, localizing the intron to the vicinity of gene 31, the gene for DNA polymerase. *In vitro* transcription of this region of SPO1 DNA gave products that were consistent with splicing. Sequencing of the ligated exon species confirmed the existence of an intron of 873 nucleotides. The predicted translation product of the ligated exons exhibits homology to DNA polymerase I of *E. coli*.

The nucleotide sequence of the intron can be folded into a secondary structure containing the phylogenetically conserved structures and sequences characteristic of group I introns. Thus, the molecular design of group I introns has been retained in the widely dispersed genomes of viruses of Gram-positive and Gram-negative bacteria, mitochondria, chloroplasts, and nuclei of primitive eukaryotes. The organization of the bacteriophage introns suggests models that involve splicing in regulatory networks.

N 208 Synthetic "tRNA Dimers": A Novel Approach to the Study of Transfer RNA Biosynthesis. Christopher K. Surratt, Barbara Carter and Sidney M. Hecht, Departments of Chemistry and Biology, University of Virginia, Charlottesville, VA

We have constructed a novel hybrid tRNA precursor analog consisting of the elements of yeast $tRNA^{Phe}$ and E. coli $tRNA^{Phe}$ connected in head-to-tail fashion. Construction was effected by the T4 RNA ligase-mediated coupling of E. coli $tRNA^{Phe}$ (acceptor oligonucleotide) and the 5'-half of yeast $tRNA^{Phe}$ (nucleotides 1-36; donor oligonucleotide). The derived ligated product was annealed to the 3'-half of the yeast $tRNA^{Phe}$ (nucleotides 38-76) and the "dimeric" product was tested as a substrate for E. coli RNase P and M1 RNA. The synthetic tRNA precursor was processed readily both by RNase P and M1 RNA, but only where the "missing" 3'-half of yeast $tRNA^{Phe}$ was present. Processing was shown to occur at the expected phosphate diester linkage, and to proceed essentially to completion under conditions comparable to those employed for the processing of the normal E. coli $tRNA^{Tyr}$ and B. subtilis $tRNA^{His}$ precursors. This approach to the study of tRNA processing allows the assessment of the effect of tRNA nucleotide modifications on the processing event, as well as the potential construction of precursors containing spacer oligonucleotides modified in mechanistically interesting ways.

N 209 3D STRUCTURE OF THE CATALYTIC DOMAIN OF GROUP I SELF-SPLICING INTRONS, Jack W. Szostak, J. Michael Cherry, Jennifer Doudna, Rachel Green, Maya Hanna and Sandra Couture. Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA 02114.

We are attempting to determine the three dimensional structure of the catalytic domain of the group I self-splicing introns by genetic analysis. The sequences of over 20 group I introns are all consistent with a common secondary structure, which must be held in a defined 3-dimensional structure by tertiary interactions. Interacting nucleotides can be identified by patterns of mutational suppression. We have obtained genetic evidence for a non-standard A:G base pair at the base of P8, a Watson-Crick G:C base pair separated from the right end of P7 by a bulged A, and a non- Watson-Crick A:U base pair separated from the left end of P7 by a bulged A. We have also identified 2 adjacent nucleotides with strong (50-fold) effects, and one base-pair with weak (2-5x) effects on the Km for guanosine. Studies of the RNA substrate indicate that the conserved U:G base pair is recognized by the enzyme, and determines the position of guanosine attack. We are using computer graphic molecular modeling to explore enzyme structures consistent with the genetic data, and to model potential guanosine and RNA substrate binding sites.

N 210 STRUCTURE OF YEAST MITOCHONDRIAL RNA POLYMERASE AND THE MECHANISM OF PROMOTER RECOGNITION, H.F. Tabak, A.H. Schinkel and M.J.A. Groot Koerkamp, Section for Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, AMC - Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.
Yeast mitochondrial RNA polymerase consists of two components that can be easily separated from each other by various chromatographic procedures: a 145 kDa protein which contains the catalytic center for DNA directed ribonucleotide polymerisation and a 43 kDa protein which confers the ability to specifically recognize the promoter. Interaction of RNA polymerase with promoters has been studied with a variety of techniques such as gelfiltration, competition experiments, gelretardation, DNase I footprinting and intercalation of methidium-propyl-EDTA. The results show that core RNA polymerase binds weakly to DNA while the specificity factor has no affinity for DNA at all. Core and factor together bind to DNA and locate a promoter by sliding along the DNA. Association with the promoter results in the formation of a specifc protein-DNA complex and the introduction of a bend in the DNA.

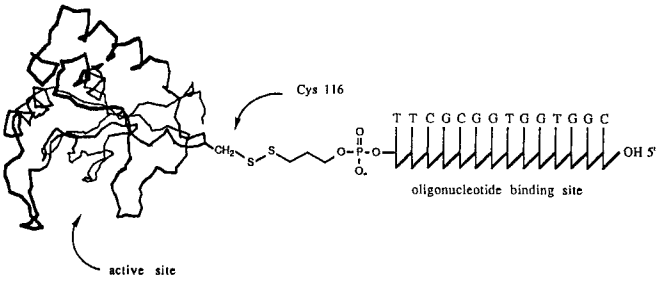
N 211 REPLICATION OF THE CIRCULAR RNA GENOME OF HUMAN HEPATITIS DELTA VIRUS, John Taylor, Mark Kuo, Lamia Sharmeen, William Mason and Gail Dinter-Gottlieb*, Fox Chase Cancer Center, Philadelphia, PA 19111 and *Drexel University, Philadelphia, PA 19104.
The structure and replication of the genome of hepatitis delta virus (HDV) are unique relative to all other known animal viruses. However, there are striking similarities to certain plant agents: the viroids, virusoids and satellite RNAs. Although initially isolated from human patients, HDV has been experimentally transmitted, by others, to both chimpanzees and woodchucks. We have shown that the virus can infect primary cultures of woodchuck hepatocytes (1) and this has made accessible an analysis of the molecular biology of viral genome replication. Results will be presented on the use of specific inhibitors of virus replication and on the application of cell fractionation of infected cells. We have also cloned and sequenced the HDV genome (2). The sequences have been combined into a single clone and results will be presented on the ability of these sequences to transfect cultured cells and to undergo processing in vitro by self-cleavage.

(1) Taylor et al., J. Virol. 61: 2891 (1987).

(2) Kuo et al., J. Virol. (in press).

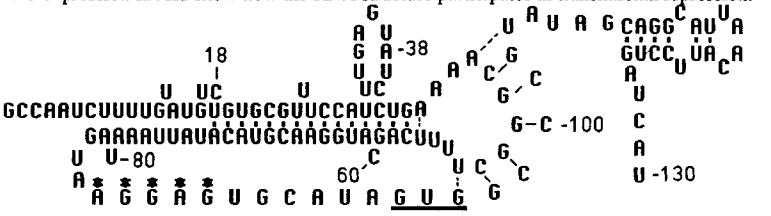
N 212 A MODEL FOR THE RNA CATALYZED CLEAVAGE OF A PLANT SATELLITE RNA, Arnold Hampel and Richard Tritz, Plant Molecular Biology Center, Northern Illinois University, DeKalb, Ill.
The (-) strand of the satellite RNA of tobacco ringspot virus (-sTRSV) has been shown to undergo both autocatalytic cleavage and ligation (Nature 323, 349 (1986); N.A.R. 14, 9729 (1986)). Cleavage of dimer to monomer, circular monomer to linear monomer and permuted monomeric and shorter fragments to the respective 5' and 3' halves was shown to occur. The products would also ligate. The monomeric form of this RNA is 359 bases long and the active site was hypothesized not to fit in the "hammerhead" configuration proposed for several of these small self-processing RNAs by Symons (Cell 49, 211, 1987). We now show that much shorter fragments of this RNA are minimally required for the cleavage reaction to occur. At present we have shown an RNA substrate 17 nucleotides long is cleaved by a ribozyme 26 nucleotides long. A model for the secondary structure will be presented.

N 213 SITE-SELECTIVE CLEAVAGE OF LARGE RNA MOLECULES, Ronald N. Zuckermann, David R. Corey and Peter G. Schultz, University of California, Berkeley, CA 94720.
A hybrid enzyme has been constructed by selectively fusing an oligodeoxyribonucleotide to the relatively nonspecific enzyme staphylococcal nuclease. The oligomer directs the phosphodiesterase activity of the nuclease to a specific target sequence of single-stranded RNA substrates. The hybrid enzyme has been shown to be site-specific, cleaving the phosphodiester bonds directly adjacent to the target sequence of both synthetic and natural RNA's.



RNA-Protein Interactions: Physico-Chemical Aspects

N 300 AN mRNA PSEUDOKNOT STRUCTURE IS RECOGNIZED BY A PROTEIN TRANSLATIONAL REPRESSOR, David E. Draper and Careen K. Tang, Department of Chemistry, Johns Hopkins University, Baltimore, MD 21218.
The ribosomal protein S4 binds to its own mRNA to repress translation. The mRNA folding (shown below) was suggested by structure mapping experiments. To confirm the structure we have made ≈20 site-directed mutations, including sets of compensating base changes in all the helical regions shown, and measured the RNA affinities for S4. The binding data show that S4 stabilizes the 'pseudoknot' base pairing (C48-C52 with G102-G98); formation of this helix should inhibit tRNA binding to the ribosome initiation complex, if not mRNA-ribosome binding. With this set of mutations we are also measuring i) ternary fmet-tRNA·30S ribosome·mRNA complex formation ± S4, and ii) translation and repression levels *in vivo* with lacZ fusions. Correlation of the *in vitro* binding data with *in vivo* expression should show how the RNA structure participates in translational repression.



N 301 NUCLEOLAR TRANSPORTING SIGNAL (NOS) IN HUMAN RETROVIRUSES HTLV-I AND HIV
Masakazu Hatanaka, Haruhiko Siomi, Hisatoshi Shida, Masatoshi Maki, Seok Hyun Nam and Akihiko Sato
Institute for Virus Research, Kyoto University, Kyoto 606 JAPAN
We have identified a nucleolar transporting signal (NOS)from human retrovirus HTLV-I and HIV. The signal peptide NOS consists of 9 to 19 amino acid residues and is included in trans-activating proteins such as p27X protein of HTLV-I (H. Siomi et al., submitted for publication), tat-III and art/trs gene products of HIV (M. Maki et al., submitted for publication). Since splicing of mRNAs is known to take place at splicesomes in nucleoplasm, the trans-activating gene products with the NOS signal peptide, act to prevent messenger RNAs from further splicing. By our new finding of the NOS signal, we are developing a new strategy for elucidation of the trans-activating mechanisms and also uncovering hitherto unknown functions of the nucleolus.

N 302 STUDIES ON RNA-PROTEIN INTERACTIONS IN THE YEAST 5S RNA-PROTEIN COMPLEXES BY FLUORESCENCE AND ENZYMATIC DIGESTION. Lee-Chuan C. Yeh and John C. Lee, Department of Biochemistry, The University of Texas Health Science Center, San Antonio, TX 78284-7760

To understand the molecular mechanism of action of the ribosome and its assembly requires a thorough knowledge of the nature of association between constituent RNAs and proteins. Ribosomal 5S RNA and its associated protein provides a convenient and amenable means for studying RNA-protein interactions in the ribosome. In the present investigation, the conformational states of the 5S RNA and protein L1a from Saccharomyces cerevisiae were determined using fluorescence with ethidium bromide (EtBr) and bis-anilinonaphthalene sulfonic acid (Bis ANS) as probes, respectively. Kinetic studies of EtBr fluorescence of free RNA and RNA in complex indicated that the RNA in the free form exhibited less secondary structure than in the complex. Digestion of the intact RNA-protein complex with RNase T1 resulted in a decrease in EtBr fluorescence but an increase in Bis ANS fluorescence. Concurrent to the exposure of hydrophobic sites on the protein was an increase in turbidity suggesting that the protein, once separated from the RNA, became less soluble in the aqueous environment. During the time course of RNase T1 digestion, discret RNA fragments were detected. Fragments generated from digestion of free RNA and of the complex were distinct implying that the RNA existed in a different conformation in these two states. The nucleotide sequence of the RNA fragments were determined and a model suggesting the structure arrangement in the complex was introduced. (Supported by a Public Health Service grant (GM 35851)

N 303 DOMAIN STRUCTURE OF THE A1 hnRNP PROTEIN. Barbara M. Merrill, Kathleen M. Keating, Samuel H. Wilson,* and Kenneth R. Williams, Yale University, New Haven, CT 06510, and *National Cancer Institute, NIH, Bethesda, MD 20892.

Newly transcribed heterogeneous nuclear RNA (hnRNA) is immediately associated with a discrete group of at least 6 proteins to form a ribonucleoprotein (hnRNP) complex that is thought to be involved in the packaging, processing and transport of the hnRNA. Since only the A1 hnRNP protein has yet been cloned and overexpressed in E. coli (Cobianchi et al., J. Biol. Chem. in press) and is thus available in sufficient amounts for detailed physico-chemical studies, we have begun our hnRNP structure/function studies with this protein. We had previously shown that the sequence of the first 184 amino acids in A1 form a 92 amino acid internal repeat. We now have used limited proteolysis studies and photochemical crosslinking experiments to show that the internal repeats in A1 correspond with two independent nucleic acid binding domains. The four sites of covalent photo-crosslinking of A1 to 32P-p(dT)8 occur at phenylalanine residues, which suggests that the mechanism of A1 binding to nucleic acids may, as is the case for several procaryotic ssDNA-binding proteins, involve the close approach of aromatic amino acids with the nucleotide bases. Although no cross-linking sites were found within the glycine-rich, COOH-terminal one third of A1 (residues 196-319), fluorescence studies on a limited cleavage product lacking this domain clearly indicate that it makes a significant contribution to the overall free energy of binding of A1 to nucleic acids (Cobianchi et al.). The multiple nucleic acid binding domains found in A1 seem to represent a common structural motif since homologous 80-90 amino acid long domains occur in several other eucaryotic RNA-binding proteins.

N 304 THIOPHOSPHATES ALTER SPECIFIC RNA-PROTEIN INTERACTIONS, John F. Milligan and Olke C. Uhlenbeck, Dept. of Chemistry and Biochemistry, University of Colorado, Boulder, Co. 80309-0215.

The interaction between the bacteriophage R17 coat protein and its RNA binding site is thought to include 4 or 5 ionic contacts. By transcribing synthetic DNA with T7 RNA polymerase and using one (α-thio) nucleoside triphosphate and three regular nucleoside triphosphates, RNAs with thiophosphates 5' to each of the four nucleosides can be synthesized. Twelve sequence variants, containing different α-thiophosphates, were tested for coat protein binding to deduce the contribution of each phosphate to the Ka of the interaction. Of the 23 phosphates in the molecule, three positions decrease the binding affinity from 3 to 10-fold each, while substitution at a fourth position causes a 10-fold increase in the Ka. Substitutions at any of the 19 other positions have no affect on the Ka. Thus, it appears that a possible consequence of replacing phosphates with thiophosphates is an altered specificity between an RNA and a protein.

N 305 **CRYSTALLOGRAPHIC STUDIES OF A TRANSFER RNA : AMINOACYL-tRNA SYNTHETASE COMPLEX:**
John J. Perona, Mark Rould, Dieter Söll and Thomas A. Steitz, Yale University, New Haven, CT 06511.

Aminoacyl-tRNA synthetases are a class of enzymes that are essential for protein synthesis; each synthetase specifically recognizes its cognate tRNA and, in a two-step reaction, attaches the proper amino acid to it. In this process the enzyme must discriminate successfully against noncognate tRNA species of very similar tertiary structure, making an investigation of the structural basis for specificity of recognition a complex and interesting problem (1).

We are investigating the interaction between the monomeric E. coli glutaminyl-tRNA synthetase enzyme (GlnRS) and the cognate $tRNA^{Gln}$ (2). Both protein and RNA have been obtained in abundant quantity via in vivo overproduction utilizing a high-expression vector system in E. coli. Each macromolecule was then readily purified, and crystallization of the complex was successfully carried out by the method of vapor diffusion using hanging drops. The crystals diffract to 2.8Å resolution and are of the orthorhombic space group $C222_1$ with cell dimensions a=240.5Å, b=93.7Å, c=115.7Å. Biochemical analyses of dissolved crystals indicate the presence of both GlnRS and $tRNA^{Gln}$, most probably in a 1:1 stoichiometric ratio. Data collection and a search for heavy atom derivatives are in progress.

References:
(1) Schimmel, P. (1987) Ann. Rev. Biochem. 56, 125-158.
(2) J. Perona et al. J. Mol. Biol. (submitted)

N 306 PROBING THE ASSEMBLY OF THE 3' MAJOR DOMAIN OF 16S rRNA (FURTHER STUDIES); INTERACTION WITH RIBOSOMAL PROTEINS S2, S3, S10, S13 AND S14,
[1]Ted Powers, [1]Seth Stern, [2]Li-ming Changchien and [1]Harry F. Noller, (1) Thimann Laboratories, University of California at Santa Cruz, CA 95064; (2) Laboratory of Molecular Biology and Department of Genetics, University of Wisconsin, Madison, WI 53706.

We have used rapid probing methods to follow the changes in reactivity of residues in 16S rRNA toward chemical and enzymatic probes as ribosomal proteins S2, S3, S10, S13, and S14 are assembled into 30S subunits. Effects observed are confined to the 3' major domain of the RNA and comprise three general classes:

(1) Monospecific effects, which may be attributed to a single protein. Proteins S13 and S14 each affect the reactivity of residues in the lower half of the domain, adjacent to regions previously found protected by S19. S10 effects are located in two distal segments of the domain, the 1120/1150 stem and the 1280 loop; these are near nucleotides previously found protected by S9. Both S2 and S3 protect residues at the top of the domain, between residues 1070 and 1112. In addition, S2 protects residues in the 1160/1170 stem-loop.

(2) Cooperative effects, which include four residues dependent on both proteins S2 and S3 for their reactivities to appear similar to that observed in native 30S subunits.

(3) Polyspecific effects, where proteins S3 and S2 independently afford the same protection and enhancement pattern in three distal regions of the domain: the 960 stem-loop, the 1050/1200 stem, and at the top of the 3' domain. Proteins S14 and S10 also weakly affect the reactivities of several residues in these regions.

We argue that protected residues of the first class are likely sites for protein-RNA contact while the third class is indicative of conformational rearrangement in the RNA during assembly. These results, in combination with the results from our previous study of proteins S7, S9, and S19, are discussed in terms of the assembly, topography, and involvement in ribosomal function of the 3' major domain.

N 307 NON-UBIQUITIN PRODUCT OF A NATURAL FUSION PROTEIN IS ASSOCIATED WITH RNA.
Kent L. Redman and Martin Rechsteiner, Department of Biochemistry, University of Utah Medical School, 50 N Medical Drive, Salt Lake City, UT 84132.
An 80 amino acid polypeptide synthesized as a carboxyl-terminal extension of ubiquitin has been shown to be cleaved from the cosynthesized ubiquitin. Structural and chromatographic properties of the protein suggest that it is a polynucleotide binding protein. The extension contains cysteine and histidine residues in patterns corresponding to the proposed "zinc finger" motif as well as a 30 amino acid region that is very basic (50% lysine and arginine). The isolated protein binds CM-Sephadex with high affinity in contrast to extension in cell lysates which fails to bind this resin, indicating that it is associated with an acidic molecule in vivo. Unlike the isolated protein, DEAE chromatography of cell lysates results in tightly bound extension which requires 0.2 to 0.4 M NaCl for elution. DEAE binding is abolished by prior treatment of lysates with RNAse A. High speed centrifugation of rabbit reticulocyte lysate and HeLa cytoplasm results in the sedimentation of the extension protein. Thus, the protein appears to be associated with ribosomes or other RNP particles. The authenticity of the RNA binding as well as the type of RNA molecules involved is being investigated.

N 308 INTERACTIONS BETWEEN NASCENT RNA AND RNA POLYMERASE I SUBUNITS IN ISOLATED NUCLEOLI, Michel Roberge and E. Morton Bradbury, University of California, Davis, CA 95616.

RNA polymerase I subunits that contact nascent RNA were studied in HeLa nucleoli using photoaffinity labeling with 4-thiouridine triphosphate. After incubation with the photoprobe, nucleoli were irradiated with near-UV light and digested with DNase I and RNase T1. Photoaffinity-labeled proteins were separated by electrophoresis and visualized by autoradiography. Restriction of nascent transcript length by short incubation times or by inclusion of chain-terminating nucleotides resulted in labeling of subunits Ia and Ib, the two largest RNA polymerase I subunits. When transcripts were allowed to elongate further by increasing incubation time or by decreasing chain terminator concentration, a Mr=52,000 polypeptide was also labeled which received up to 60% of the label. This polypeptide sedimented with subunits Ia and Ib at the position expected for RNA polymerase I in glycerol gradient velocity centrifugation. These data show that the Mr=52,000 polypeptide is part of the transcription complex and that it contacts RNA transcripts after they have left the Ia-Ib interface. It probably corresponds to subunit Id and it could serve to direct nascent RNA away from the RNA polymerase I core, to preribosome particles.

N 309 INSULIN EFFECT OF mRNA PROCESSING AND TRANSPORT, Dorothy E. Schumm, Ohio State University College of Medicine, Columbus, Ohio, 43210

A cell-free system containing only defined components has been developed to study the nuclear processing and nucleocytoplasmic transport of specific mRNAs. In this system, introns are removed from the pre-mRNAs coding for albumin and α_{2u}-globulin. Excised introns are retained in the nucleus and only fully processed mRNAs are released. Nucleocytoplasmic transport is dependent on a hydrolyzable nucleoside triphosphate and a 35-kilo dalton phsophoprotein. Intact insulin stimulates the transport of albumin and α_{2u}-globulin mRNAs 5 to 10 fold. Total mRNA transport is stimulated only 1.5 to 2 fold by insulin. Insulin has no effect on mRNA processing or on the processing or transport of rRNA or tRNA. The stimulatory effect of insulin is abolished by removal of the outer nuclear membrane by treatment with detergents.

N 310 ALPHA AND BETA TUBULIN mRNAs IN TRYPANOSOMA CRUZI ARE ORIGINATED FROM A COMMOM MULTIGENIC RNA PRECURSOR, Célia Maria de Almeida Soares, Edson Rondinelli, José Francisco O. Carvalho, Elizeu Fagundes de Carvalho, Rodrigo Soares de Moura Neto and Firmino Torres de Castro, Inst. Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, 21941,Brasil.

We have seen that the supra-optimal temperatures (37°C and 40°C) induces a gradual accumulation of high molecular weight tubulin RNAs of 12,0 and 8,0 Kb. At the same time a decrease in the accumulation of intermediate species of 6,0 and 4,8 Kb as well as in mature species of 2,4, 2,1 and 1,6 Kb is observed. These data is compatible with a precursor product relationship and suggest that at supra-optimal temperatures the processing of the 12,0 and 8,0 Kb RNA species is inhibited. The high molecular weight tubulin RNAs of 12,0 , 8,0 and 4,8 Kb are nuclear species as revealed by comparative northern blot analysis of total, cytoplasmic and nuclear RNA preparations. The tubulin RNA precursors hybridize with both, alpha and beta tubulin homologous cDNA probes. Thus, alpha and beta tubulin mRNAs are indicated to be originated from a common multigenic RNA precursor in T. cruzi. Supported by CNPq, FINEP and CEPG.

N 311 POST-TRANSCRIPTIONAL REGULATION OF T-CELL RECEPTOR mRNA EXPRESSION

Miles Wilkinson, Jay Rosenbloom and Carol MacLeod*, Oregon Health Sciences University, Department of Microbiology/Immunology and Vollum Institute, Portland, OR 97201; *U.C.S.D. Cancer Center, T012-C, San Diego, CA 92093. The T-cell receptor (TCR) for antigen is composed of two subunits, α and β, which are encoded by gene segments which are functionally rearranged and expressed during T-cell ontogeny. It is not known if TCR transcript accumulation is controlled at the level of: i) gene rearrangements; ii) gene transcription; or iii) post-transcriptional events. We examined this question using an "immature" murine T-lymphoma cell clone, SL12.4, which fails to accumulate significant amounts of mature TCR-α or -β mRNA. The SL12.4 cell clone possesses rearranged TCR genes as judged by Southern blot analysis. To determine whether these rearranged genes are transcribed, the nuclear run-off assay was employed. The results indicate that the rate of TCR-α and -β gene transcription in SL12.4 cells is similar to the rate in T-cells which accumulate large amounts of mature TCR-α and -β mRNA. Thus, post-transcriptional mechanisms are responsible for the lack of mature TCR mRNA in SL12.4 cells. Northern blot analysis revealed that the nucleus of SL12.4 cells contain precursor TCR transcripts. However, there is little or no evidence of mature TCR transcripts in the nucleus. Thus, mature TCR mRNA accumulation appears to be regulated by mechanisms which specifically control: i) the processing of precursor TCR transcripts to mature RNAs, or ii) by regulating the stability of mature TCR mRNAs in the nucleus. The protein synthesis inhibitor, cycloheximide (CHX), coordinantly induces the appearance of mature TCR-α and -β transcripts in SL12.4 cells. Thus, a labile protein(s) may be responsible for the nuclear post-transcripional regulation of these transcripts. Experiments are in progress to determine the precise post-transcriptional mechanisms which regulate the steady state levels of TCR-α and -β mature mRNAs.

Biological Aspects

N 400 STRUCTURAL CHANGES IN 16S rRNA FROM FIDELITY MUTANTS, Patrick Allen and Harry Noller, Thimann Laboratories, University of California at Santa Cruz, CA 95064

We have studied the structure of 16S rRNA from *E. coli* ribosomal fidelity mutants using chemical probes and reverse transcription. The chemical probes used were dimethyl sulfate (DMS), kethoxal and 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-p-toluenesulfonate (CMCT). Altered reactivity of individual bases to these probes reflect changes in the higher order structure. We find structural perturbations in 16S rRNA from two ribosomal ambiguity mutants (*ram*) D14 and D16 (containing altered ribosomal protein S4) and the streptomycin pseudodependent (Sm^P) UK318 (containing altered S12) when compared to the isogenic wildtype strain O17. At the 5′ end of the molecules residues A26 and A8 show enhanced reactivity to DMS in both *ram* (D14 and D16); both bases are proximal to the S4 binding site. The reactivity of residues A908, A909 and G1487 is perturbed in UK318. A908 is more accessible to DMS while A909 and G1487 are less accessible to DMS and kethoxal, respectively. The 910-915 and 1413/1487 regions are believed to be involved in streptomycin binding. The only altered reactivity common both to the *ram* strains and the Sm^P is enhanced reactivity at residue G6. Binding of S12 or S5 to 16S also affects the reactivity of G6.

When streptomycin is bound to the 30S subunit both the *ram* strains and the wildtype strain show strong protection at A909, A912-915 and G1487. In sharp contrast UK318 show a weak protection at these nucleotides, probably due to the mutant's low binding affinity for streptomycin. Alternatively this may suggest that UK318 binds streptomycin differently.

N 401 THE LOCALIZATION AND MOVEMENT OF 5S RNA THROUGHOUT OOGENESIS IN *XENOPUS LAEVIS*.

Lizabeth A. Allison and Aimee H. Bakken, Univ. of Washington, Seattle, WA 98195.

5S RNA and ribosomal RNA (rRNA) are both components of complete ribosomes which, in somatic cells, are assembled in the nucleolus. These RNAs are known to be present in equal ratios in somatic cells and in mature oocytes of *X. laevis*. However, the mode of regulation differs and during early oogenesis their synthesis is asynchronous. 5S RNA synthesis begins in Stage I oocytes, from many copies of the gene throughout the genome. The 5S RNA is stored in the cytoplasm. Synthesis of rRNA from amplified extrachromosomal rDNA begins in Stage III oocytes. It is not clear from prior studies when, or if, stored 5S RNA migrates back to the nucleus and associates with amplified nucleoli during ribosome biogenesis. Alternatively, the 5S RNA could wait in the cytoplasm for the migration of incomplete large ribosomal subunits from the nucleus. Data will be presented on the localization and movement of 5S RNA throughout oogenesis as studied by the following methods: 1) extraction of RNA from amplified nucleoli isolated from Stage I-IV oocytes, followed by Northern blot hybridization to a 5S DNA probe; 2) *in situ* hybridization of ^{3}H-5S RNA (riboprobe) to sections of Stage I-V oocytes; 3) microinjection of lucifer yellow-tagged 7S RNPs into the cytoplasm of mature oocytes to follow the movement of TFIIIA, by fluorescent microscopy of fixed sections; and 4) microinjection of ^{32}P-5S RNA and ^{32}P-7S RNPs (=TFIIIA protein + ^{32}P-5S RNA) into the cytoplasm of mature oocytes, followed by autoradiographic analysis for migration into the nucleus, incorporation into isolated ribosomes, and incorporation of injected 5S RNA into 7S RNPs. Results of these experiments will be discussed and a model for 5S RNA incorporation into oocyte ribosomes proposed.

N 402 SPECIFIC RNA BINDING PROTEINS IN X. LAEVIS OOCYTES, Paola Fragapane, Elisa Caffarelli, Francesca Cutruzzola', Alessandra Lucioli, Carlo Presutti and Irene Bozzoni, Dept. of Genetics and Molecular Biology, University of Rome.
The RNA precursor for the ribosomal protein L1 in X. laevis is under nuclear control: in the presence of excess amounts of free L1 protein its splicing is partially inhibited. As a consequence the L1 pre-mRNA is degraded by a mechanism which involves an endonucleolytic cleavage inside the non-processed introns. Experiments have been performed in order to test whether there are sequences and factors which mediate in cis and trans such specific regulation. The X. laevis oocyte is a suitable system for studying such a problem because it is possible to inject and follow the fate of many different macromolecules. In particular in order to verify whether specific proteins interact with the introns of the L1 pre-mRNA we have performed experiments of UV cross-linking: hot labelled in vitro transcribed RNAs have been microinjected in X. laevis oocytes, the nuclei were manually isolated and UV irradiated. After RNAse treatment, the indirectly labelled proteins have been analysed by gel electrophoresis. The injection of different template RNAs has allowed the identification of a protein which specifically interacts with the regulatory introns of the L1 pre-mRNA. Using this approach we have been able to identify other sequence-specific RNA binding proteins.

N 403 RNA-DNA HELICASE ACTIVITY OF TRANSCRIPTION TERMINATION FACTOR RHO, C. A. Brennan, E. Steinmetz and T. Platt, University of Rochester Medical Center, Rochester, NY 14642
Rho factor, in the absence of RNA polymerase, can catalyze the release of RNA from an RNA-DNA duplex in vitro. This unwinding activity requires energy supplied by the hydrolysis of NTPs, has 5' to 3' directionality, and is specific for an unhybridized region of RNA that binds well to rho and must be located 5' of the RNA-DNA duplex. The RNA-dependent ATPase activity of rho is probably utilized to unwind the RNA-DNA duplex in the transcription bubble, thus facilitating release of the transcript from the transcription complex. Parameters of the helicase activity under investigation include (1) the NTP requirement, (2) effects of sequences of varying structural potential between the rho binding region and the RNA-DNA duplex, (3) effects of distance between the rho site and the duplex, (4) effects of sequence deletion or alteration on RNA recognition, and (5) effects of duplex length (how long a region can rho unwind?). The unwinding is activated well by ATP and GTP, but poorly by CTP and UTP (in contrast, each NTP is a good substrate for rho's poly(rC)-dependent NTPase activity). Deletions into the trp t' rho-dependent termination region indicate that about 100 nucleotides of free RNA that is unstructured and cytosine-rich are required for efficient release of the RNA-DNA duplex; the extent to which a specific sequence is important is not clear. Sequences, varying in length and structure, inserted between the rho site and the duplex have little effect on rho's ability to unwind the RNA-DNA duplex, and the presence of sequences corresponding to the normally terminated 3' RNA endpoints also seems to be unimportant. We will discuss these results in the context of whether rho action from a distance may occur by translocation along the RNA as opposed to looping out to bypass the intervening stretch of RNA.

N 404 NUCLEOLIN, A RNA BINDING PROTEIN INVOLVED IN BIOSYNTHESIS OF PRERIBOSOMES IN EUCARYOTES, Henri Bourbon, Beatrix Bugler and Colette Mathieu, Centre de Recherche de Biochimie et de Génétique Cellulaires du C.N.R.S., Toulouse, France.
Nucleolin is a multifunctional nucleolar protein involved in the early steps of preribosome biogenesis. The complete aminoacid sequence of nucleolin (714 residues) presents 3 main structural domains : the acidic NH_2 terminus contains 7 serine giving rise to phosphorylation sites; the COOH terminus contains a glycin-rich region with regularly spaced phenylalanine - dimethylarginine residues and the central portion contains 4 repeats of 80 residues including the RNP consensus sequence founded in different RNA binding proteins (polyA binding protein, HnRNP core proteins, A_1-A_2, U_1 etc...).
Nucleolin, the first characterized RNA binding protein which is not included in the spliceosome, binds to nascent rRNA : 15 sites of interaction have been detected showing different affinities. Three sites of strong affinity characterized in vivo and in vitro have been founded in the 5' transcribed spacer and in the 3' ends of 18S and 28S rRNA.
Moreover each RNA binding domain presents a peculiar genomic organization : it is split into 2 exons by an intron which interrupts the consensus sequence suggesting that the RNA binding domains would be formed by two independent subdomains. Furthermore, in 3 species of rodents the left subdomains exhibited ten times more divergences than the right ones. We proposed that the RNP consensus sequence, that is conserved in different species and proteins could be a "linker", which would interact with the skeleton of the chain, as other RNA binding proteins, explaining its conservation between different proteins. Both left and right subdomains could be responsible for the specificity of the interaction with RNA.
It is suggested that nucleolin, controls pre rRNA synthesis by interacting with chromatin. Following initiation of rDNA transcription, nucleolin is actively synthesized and transported to the nucleolus. There it interacts with pre rRNA as a preribosome assembly factor.

N 405 **RNASE P ENZYMES FROM BACTERIA AND YEAST**, U. Burkard, I. Willis, M. Nichols, D. Drainas, S. Zimmerly and D. Söll. Yale University, New Haven, CT 06511

The ribonucleoprotein enzyme, RNase P, is responsible for removing 5' non-coding sequences from tRNA precursors. We are studying eubacterial (primarily E. coli) and lower eucaryotic (S. cerevisiae and S. pombe) RNase P enzymes to understand aspects of their catalytic mechanism, their specificity and, in the case of S. pombe, to define in molecular terms the protein and RNA components of the enzyme. The specificity of cleavage of the E. coli enzyme was examined using an E. coli $tRNA^{His}$ precursor. This precursor, unlike all non-histidine tRNAs from E. coli, is cleaved at position $^{-}1$ yielding a mature tRNA with an extra 5' guanylate residue. Point mutations at and around the RNase P cleavage site were generated and analyzed for their ability to be cleaved appropriately by the holoenzyme and M1 RNA from E. coli and P RNA from B. subtilis. Data describing the importance of the base at position $^{-}1$ and potential base pairing between nucleotides $^{-}1$ and 73 as well as the contribution of the protein component of the enzyme to cleavage specificity will be presented and discussed. Acceptor stem mutations in an S. pombe tRNA precursor and a gel retardation assay specific for RNase P/precursor tRNA complexes have been used to demonstrate a separation of catalytic and substrate binding functions for S. cerevisiae RNase P. Gel retardation assays performed with catalytically inactive RNase P suggest a possible role of the protein component of the enzyme in substrate binding. To examine more closely the role of the protein component in RNase P catalysis we are pursuing a biochemical purification of the enzyme from S. pombe. The current status of this work will also be described.

N 406 In vivo MAPPING AND CHARACTERIZATION OF rho-dependent TRANSCRIPTION TERMINATION SITES. M.S.CARLOMAGNO,S.CIAMPI,P.ALIFANO,A.G.NAPPO and C.B.BRUNI.CEOS of CNR,NAPLES, Dept of Pathobiol Mol & Cell,Univ of NAPLES and Inst of Genet, Univ of BARI

The polarity of nonsense and frameshift mutations within a polycistronic message is due to a premature termination of transcription mediated by the termination factor rho. In order to collect more information on the characteristics of the rho-dependent termination process, we have focused our attention on the mapping of cryptic rho-dependent sites unmasked by polarity. We have used as a system strongly polar mutants which map in the first three cistron of the his operon of S.typhimurium (genes hisGDC).The RNA produced in vivo by the polar mutants was extracted and analyzed by Northern blot and S1-nuclease mapping. Such an analysis enabled us to map at least one cryptic termination site within each cistron. Transposition of the polar mutation in a rho-ts background supported genetic evidence that the polarity of those mutations was due to the action of the rho factor. A more precise analysis of the RNA 3' ends was performed by high-resolution S1 mapping. It was possible to demonstrate that within each termination site transcription ends at several points and in addition it was shown that each site exhibits different levels of termination efficiency. The characterization at the molecular level of these intracistronic cryptic sites will be useful to gain a better understanding of the mechanisms required for in vivo rho-dependent termination.

N 407 **MOLECULAR CHARACTERIZATION OF THE RO scRNP.** Susan L. Deutscher and Jack D. Keene, Duke University Medical Center, Durham NC 27710.

Ro small cytoplasmic ribonucleoprotein particles (Ro scRNPs) were initially identified due to their reactivity with antibodies from autoimmune patients. Ro particles are present in low abundance and consist of a 60kD protein and a series of small RNAs designated hY1-hY5 in humans. We are currently using recombinant cDNA clones encoding the Ro protein as well as the Ro RNAs to investigate the structure and function of the Ro RNPs. Initial Ro protein cDNA clones were isolated by immunological screening of human brainstem lambda gt11 expression libraries. Purified recombinant Ro-B-galactosidase fusion protein was used to affinity-purify Ro-specific antibodies. These antibodies immunoprecipitated from HeLa cell extracts Ro RNPs containing hY1-hY5 RNAs and a 60 kD protein. Hybrid selection-in vitro translation experiments and peptide mapping studies verify that the cDNA encodes Ro protein. Full-length cDNAs encoding Ro protein were isolated from human placental libraries and contain an open reading frame which encodes a 539 amino acid protein with a predicted molecular weight of 60.6kD. The Ro DNA, when transcribed and translated in vitro, produced a 60kD protein which comigrated with authentic immunoprecipitated Ro protein. Northern blot analysis using various Ro probes detected multiple mRNA species of 3.2, 2.4 and 1.8 kb. The nature of these mRNA species is being analyzed.

We are using the Ro protein produced in vitro and in a B-gal fusion protein in binding studies with the Ro RNAs to determine the RNA binding-domain on the Ro protein. Expression studies with Ro protein and Ro Hy RNAs will hopefully elucidate the functions of the Ro scRNP.

N 408 MUTATIONS IN THE 5'-NONCODING REGION OF POLIOVIRUS RNA, Sandra L. Dildine, Bert L. Semler, University of California, Irvine, CA 92717.

Poliovirus is a positive strand RNA virus whose genome encodes a 5' untranslated region of 742 nucleotides. This region constitutes 10% of the entire genome and as yet has no well defined function. In order to determine the functions or functional domains of the 5'-noncoding region, we have introduced a number of short (2-4 bp) deletions and insertions into the full-length poliovirus cDNA at various locations in this region. A 4 nucleotide deletion at position 220 has yielded infectious virus, 220D1 (D1). D1 displays a small plaque phenotype, requires 4 days incubation for plaques to appear, and 1-step growth curves show dramatically delayed kinetics at 33°C and 37°C as well as a 3 log reduction in growth at 37°C. D1 also displays delayed kinetics for viral protein synthesis and host protein synthesis shut off at 33°C and no detectable viral protein synthesis as late as 15 hr post-infection at 37°C or 39°C. Viral RNA synthesis is sensitive to actinomycin D. Additional experiments suggest that at 39°C the viral RNA is either not an efficient template for protein synthesis or is unstable and degraded at this temperature. These data, along with data from a polio/coxsackie recombinant virus with one junction at position 220, suggest the presence of important secondary structures or interactions in this region which are disrupted by these mutations and that these structures play an important role in the poliovirus life cycle.

N 409 ROLE OF RHO-CRO mRNA INTERACTIONS IN TRANSCRIPTION TERMINATION OF λ CRO mRNA. Ignacio Faus and John P. Richardson, Department of Chemistry, Indiana University, Bloomington, IN, 47405.

In E. coli, rho-dependent transcription termination is mediated by interactions between rho factor and the nascent RNA chain. In order to further understand the molecular features of rho-mRNA interactions, we are studying the interaction between rho and λ cro mRNA, a transcript that is terminated by rho action at the λ tR1 terminator.

Using a nitrocellulose membrane retention assay, we have found that the binding energy for the interaction between rho and wild-type cro mRNA is 12.4 kcal/mole. Approximately 6 kcal of that total energy is from ionic interactions and 6 from non-ionic interactions. When the binding studies were performed using a mutant RNA, which was transcribed from a λ DNA template that was defective for rho action at tR1, the decrease in binding energy compared to that for wild-type RNA was accounted for by the loss of non-ionic interactions. The region of λ cro mRNA required for non-ionic interactions, which is near the 3' end of the RNA molecule, was found to be required for the activation of the RNA-dependent rho ATPase activity.

Using chemical probes and a reverse transcriptase primer extension assay, we have confirmed that the region of cro mRNA important for rho function is mostly unstructured. Specific nucleotides in this region of cro mRNA were found to be protected by rho from chemical attack.

The results indicate that the specificity of the rho-cro mRNA interaction is mediated by non-ionic interactions between rho and specific nucleotides near the 3' end of cro mRNA.

N 410 INFLUENCE OF tRNA CONTENT AND MODIFICATION ON THE TRANSLATION OF SYNTHETIC CODONS IN VIVO, B.T. French, E.F.Yamasaki, M.R. Grever, H. Grosjean and R.W. Trewyn, Ohio State University, Columbus, OH 43210.

Synthetic oligonucleotides containing 5 consecutive, identical codons have been subcloned into the EcoR1 site of the chloramphenicol acetyl transferase (CAT) gene in bacterial expression vectors. Orientation and reading frame have been confirmed by DNA sequencing for selected leucine [$(CUG)_5$ and $(CUC)_5$] and arginine [$(CGC)_5$ and $(AGA)_5$] codons among others. The modified CAT proteins have been detected in bacterial lysates by Western blotting analyses using antibodies specific for either the amino or carboxyl terminus, and the level and rate of expression are being correlated to the content of requisite tRNA isoacceptors in E. coli. The modified CAT gene sequences have also been subcloned into a eukaryotic expression vector and transfected into COS-7 and other mammalian cells. In this case, the influence of inosine and queuosine modifications in the anticodon wobble position of specific tRNAs is being assessed with regard to reading appropriate codons. The inosine modification is being evaluated in conjunction with the transient over-expression of cloned leucine and alanine tRNA genes. With the queuosine containing tRNAs, the modified (queuosine) and unmodified (guanosine) isoacceptors are being compared to species containing purine analogs (e.g., 6-thioguanosine). Supported by grants from ACS (CH-396 & IN-16Y), AFOSR (85-0003), NCI (5-T32-CA-09338 & P30-CA-16058), and NATO (RG.86/0140).

N 411 CHARACTERIZATION OF FACTORS RESPONSIBLE FOR CLEAVAGE AND POLYADENYLATION OF PRE-mRNA IN HELA NUCLEAR EXTRACT

Anna Gil, Anders Virtanen, and Phillip A. Sharp, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139

Specific ribonucleoprotein complexes which form upon incubation in HeLa nuclear extracts and in association with pre-mRNA substrates containing the Adenovirus L3 poly(A) site have been resolved by electrophoresis in low percentage nondenaturing polyacrylamide gels. These complexes have been characterized in detail by kinectic studies and by purification from nondenaturing gels. Furthermore, Northern blots of the polyadenylation-specific complexes were prepared and hybridized to probes containing complementary sequences to U1, U2, U4, U5, and U6 snRNA to determine whether these snRNPs are involved in mRNA 3'-end formation.

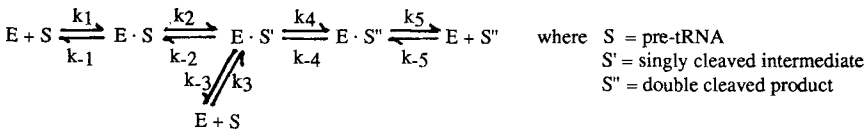
To further characterize and isolate the factors responsible for cleavage and polyadenylation of pre-mRNA, HeLa nuclear extracts were fractionated by affinity column chromatography and three activities required to restore accurate cleavage and polyadenylation have been identified.

N 412 THE ROLE OF PRE-mRNA SECONDARY STRUCTURE IN MAMMALIAN POLYADENYLATION, Edward R. Gimmi*, Ingrid C. Deckman• and Mitchell E. Reff* +, Temple University, Philadelphia, PA 19140*. University of Pennsylvania, Philadelphia, PA 19104•. Smith Kline & French Laboratories, Swedeland, PA 19479* +. Our earlier findings indicated that there may be a correlation between the computer predicted secondary structure of the poly(A) region in the pre-mRNA and the expression of the gene containing that region. Since the bovine growth hormone poly(A) region was predicted to form a major stem-loop and mediated the highest expression, we made mutants within this region to disturb its secondary structure. The secondary structures of these mutants have been determined using nucleases specific for single- or double-stranded RNA in conjunction with computer generated models. To create the mutants a synthetic linker complementary to a region including the wild-type site of cleavage was ligated upstream of the BGH poly(A) region which flanks a marker gene. This was done to compete-out any wild-type secondary structure. This mutant shows a drop in marker gene activity at the protein and RNA level. The linker, when inserted in reverse, does not effect the marker gene expression. A mutant with three linkers inserted, drives down the marker gene expression even further. To show that transcription was not being terminated by the linker constructs, the SV40 late poly(A) region was ligated downstream of the 'blocked' BGH poly(A) site and these mutants show restored expression. RNA mapping studies showing changes in the position and frequency of nuclease cutting at important regulatory sequences (AAUAAA, cleavage site, downstream sequence) will be discussed in relation to the the processing of each RNA species. These experiments revealed that the conformation of the pre-mRNA at or near the poly(A) site may be important in mediating efficient cleavage and polyadenylation.

N 413 YEAST PRE-tRNA CLEAVAGE BY THE tRNA SPLICING ENDONUCLEASE,

G. Knapp, University of Alabama at Birmingham, Birmingham, AL 35294.

There are several major questions to be answered about the tRNA splicing endonuclease. Among them is the elucidation of elements of the pre-tRNA structure that are necessary for recognition and binding by the endonuclease. This enzyme must recognize and cleave nine pre-tRNAs which have been shown to share certain common elements of 2^{o} and 3^{o} structure. Mutation of the most conserved element, the 3' splice site, has been done and has provided a battery of mutant pre-tRNAs which help in determining aspects of the pre-tRNA·endonuclease interaction. Analysis of the mutants is allowing dissection of the complexities of the kinetic pathway:



Permutations that alter the anticodon stem and size of the single-stranded loop that contains the 3' splice site have significant effects on the rate of this reaction. Some mutations appear to affect the cleavage rate(s) without an apparent effect on binding. Other mutations contribute to decreased cleavage rates; their effects on binding are under current investigation.

N 414 A CYTOPLASMIC PROTEIN BINDS IN VITRO TO A CONSERVED SEQUENCE IN THE 5'-UNTRANSLATED REGION OF FERRITIN H AND L SUBUNIT mRNAS, Elizabeth A. Leibold and Hamish N. Munro, MIT, Cambridge, MA 02139 and USDA Human Nutrition Center on Aging, Boston, MA 02111.
Ferritin is an iron storage protein consisting of 24 subunits of two kinds, one light (L), and the other heavy (H). Ferritin synthesis increases in response to iron by a translation mechanism in which stored cytoplasmic ferritin H and L mRNAs are activated, become polysome associated and are translated. This response to iron appears to involve the 5'-untranslated region of both L and H mRNAs, including a 28-nucleotide highly conserved sequence, which on computer analysis forms part of a stem-loop structure. To investigate the mechanism for the translational control of ferritin mRNAs a RNA-protein gel binding assay was used to identify cytoplasmic protein factors which bind to the rat ferritin L and H mRNAs. Two major RNA-protein complexes are specifically formed with the 5'-untranslated region of ferritin L and H mRNAs. The protein species protects 40-50 nucleotides within the 5'-untranslated region from RNAase T1 digestion. RNAase T1 mapping of the 40-50 nucleotide sequence show that the 28-nucleotide conserved region is present within this region. RNA-protein complexes are observed only with this 40-50 nucleotide sequence and not with other RNAs or other parts of the ferritin mRNAs. When rats or hepatoma cells are treated with iron, one of the two RNA-protein complexes disappears, suggesting loss of a specific protein factor sensitive to iron. Crosslinking by UV-irradiation showed that one of the complexes contains an 87 kd protein interacting with the conserved sequence. We propose that intracellular iron levels may regulate ferritin synthesis by changes in specific protein binding to the conserved sequence in the ferritin H and L mRNAs.

N 415 RIBONUCLEASE III PRODUCTION CORRELATES WITH INCREASED DNA REPLICATION IN COLE1 PLASMIDS, Paul E. March, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854. Our previous results have shown that the *rnc* operon of *E. coli* contains two genes. The upstream locus (*rnc*) produces RNase III, a double-strand specific ribonuclease. The downstream cistron (era) encodes a GTP-binding protein that is similar to the yeast RAS1 protein. A functional copy of *era* is required for cell viability. Several different subclones of these loci have been constructed in order to learn more about the function of these genes and their products. During such experiments it became obvious that ColE1 derived plasmids which over-express RNase III were present in higher copy number than control constructions. Over lapping clones from the *E. coli* chromosome, either expressing *rnc* or not, confirmed that elevated levels of RNase III correlated with an increase in the amount of plasmid DNA present. When these experiments were repeated in a *rnc*⁻ strain it was observed that plasmids unable to produce RNase III were present at a level only 16% of that of a plasmid which produced RNase III. Initiation of DNA replication in ColE1 plasmids is controled by both highly folded RNA and RNA-RNA hybrid formation. Since RNase III digests double stranded RNA, it is possible that either of these RNA molecules could be a RNase III substrate.

N 416 INTERACTION OF tRNA, EFG, AND EF-Tu WITH rRNA, Danesh Moazed, James M. Robertson and Harry F. Noller, Thimann Laboratories, University of California at Santa Cruz, Santa Cruz, CA 95064.
Protein synthesis involves the coordinated interaction of ribosomes with mRNA and a large number of other ligands. Most important among these are tRNA molecules, and elongation factors (EFG and EFT-Tu) which modulate the interaction of ribosomes with mRNA and tRNA. In these studies, we ask whether these ligands interact with specific sites in rRNA during protein synthesis and whether we can monitor the occurrence of such interactions at individually defined steps of the translational process. Using primer extension with reverse transcriptase, we identify bases in *E. coli* 16S and 23S rRNAs whose reactivity toward structure-specific chemical probes is perturbed by binding of elongation factors or tRNA to ribosomes.

We use antibiotics or non-hydrolyzable analogs of GTP to stabilize the normally transient interaction of EFG or EF-Tu with ribosomes. Further, we form complexes of mRNA programmed ribosomes containing deacylated tRNA in the P site, and either with aminoacyl-tRNA bound enzymatically to the A site, or with the A site unoccupied. Our footprinting results show that EFG, stabilized on the ribosome by fusidic acid, protects G2655, A2660, and G2665 against chemical attack. These bases are located in the highly conserved α-sarcin loop in domain VI of 23S rRNA. The only other protection caused by fusidic acid stabilized EFG is at A1067 in domain II of 23S rRNA. This base is located within the binding site for thiostrepton, a well known GTPase inhibitor of EFG. Binding of EFG to ribosomes using non-hydrolyzable analogs of GTP causes protection of the same bases in domain VI, but has little or no effect on the reactivity of A1067 in domain II.

Kirromycin prevents the dissociation of EF-Tu from ribosomes. In the presence of kirromycin, EF-Tu, in ternary complex with aminoacyl-tRNA and GTP, protects two of the same bases that are protected by EFG, G2655 and G2661 in the α-sarcin loop. These results show that EFG and EF-Tu interact with overlapping sites in 23S rRNA. In the absence of kirromycin, we observe other protections which can be assigned to the binding of aminoacyl-tRNA to the A site. These results confirm our previous prediction that class I and II tRNA protections in 16S rRNA result from binding of tRNA to A and P sites, respectively. Furthermore, they allow us to assign tRNA protections in 23S rRNA to A or P site binding.

N 417 RNA BINDING AND REPLICATION BY THE POLIOVIRUS RNA POLYMERASE, M. Steven Oberste, Gregory J. Tobin, and James B. Flanegan, Univ. of Florida, Gainesville, Fl. 32610.
We are studying RNA replication in vitro using purified poliovirus RNA polymerase and poliovirion RNA (vRNA). Previous work in our laboratory has led to a template-priming model for initiation of negative strand synthesis on a vRNA template, with synthesis beginning at the 3'-OH of the poly(A) tract. In the presence of a HeLa cell host factor, the poliovirus polymerase synthesizes a twice-genome-length product on a vRNA template in vitro. We have developed a sensitive filter binding assay to measure the binding of polymerase to RNA templates in vitro. Polymerase binding to radiolabelled vRNA can be inhibited by unlabelled ribohomopolymers to varying degrees (poly(G) >>> poly(U) > poly(C) > poly(A) = 0). Identical results are obtained from direct binding experiments with labelled homopolymers. Preference for poly(G) and poly(U) suggests that G and/or U residues may be important in replication initiation. To study the sequence and structure requirements for correct in vitro initiation by the polymerase, we have cloned full-length and subgenomic polio cDNA into a transcription vector. This allows production of large amounts of polio-specific RNA for use as template in binding and replication reactions. The polymerase and host factor can efficiently copy a synthetic RNA containing the 3' 313 bases of the genome, including the poly(A) tract. The labelled product RNA is covalently linked to the template and contains a long poly(U) tract, indicating correct initiation at the 3' end of the template. Some sequence or structure other than a 3'-OH must be required for initiation, as poly(A) alone is not copied by the polymerase in the presence of host factor. We are currently using deletion clones to determine which portions of the poliovirus genome are required for replication.

N 418 RHO FACTOR HAS SEPARATE BINDING DOMAINS FOR RNA AND ATP, T. Platt, P. Spear, and A. J. Dombroski, *University of Rochester Medical Center, Rochester, NY 14642*
E. coli rho protein was analyzed using oligonucleotide site-directed mutagenesis and chemical modification methods. The unique Cys-202, previously thought to be essential for rho function, was changed to serine or to glycine with no detectable effects on the protein's hexameric structure, RNA binding ability, or ATPase, helicase and transcription termination activities. A 151 amino acid N-terminal fragment (N1), generated by hydroxylamine cleavage, and its complementary C-terminal fragment of 268 amino acids (N2) were extracted from SDS gels and renatured. N1 binds to poly(C) nearly as well as the full length protein does, and appears to comprise a rho domain separate from that required for ATP binding; this should simplify our studies of specificity in RNA-protein recognition. Though uncleaved rho renatures readily to regain its RNA-dependent ATPase activity, neither the N1 nor N2 fragments, renatured separately or together, exhibit any detectable ATP hydrolysis. However, strong homology to consensus sequences shared by a large number of nucleotide binding proteins suggests that a structural domain for rho's ATP binding begins after amino acid 164. This is supported by preliminary crosslinking results with an ATP analog, and by conversion of Asp-265 (homologous to residues implicated in Mg^{++} binding) to Asn, which results in more than a 20-fold decrease in ATPase activity, with no reduction in RNA binding ability. We propose that amino acids 1-150 have the RNA binding specificity, 150-350 comprise the ATP binding domain, and 350-419 may be involved in oligomeric association. Characterization of other mutant proteins altered at Lys-181, Lys-184, Leu-285, Lys-347, Trp-381, and Lys-407, should clarify the interactions between rho domains, and the relationship between RNA binding, ATP hydrolysis, and transcription termination.

N 419 FORMATION AND CHARACTERIZATION OF PRE-SPLICEOSOME COMPLEXES, Ronald Pruzan, Piotr Lassota, Nilda Belgado, and Jerard Hurwitz, Sloan Kettering Institute, New York, NY 10021.
The splicing of pre-mRNA in vitro is preceded by the formation of a 55-60S 'spliceosome' complex. A smaller ATP dependent complex of 30-35S has also been observed during splicing reactions. We have labeled this putative pre-spliceosome, using subfractions of a nuclear extract which produce only the smaller complex. Upon addition of cold transcript and another subfraction, the pre-spliceosome complex can be 'chased' to the spliceosome complex, thus indicating that the smaller complex is indeed a precursor of the spliceosome.

We have focused our attention on the formation of the pre-spliceosomes as the first in a series of discernable partial reactions leading to splicing. CsCl density gradients were used to obtain two fractions which are reqiured for the formation of the pre-spliceosome complex. One of these fractions has buoyant density 1.44 and is sensitive to treatment by micrococcal nuclease. The second fraction, which is lighter in density (1.35) has been resovled into two additional components, by either gel permeation chromatography or by chromatography on heparin agarose. Our preliminary data indicates that U_2 SnRNP corresponds to the higher density component, while U_1 SnRNP, and an additional undefined protein represent the other two fractions derived from the lighter density CsCl fraction.

N 420 MONOCLONAL ANTIBODIES AGAINST 200S NUCLEAR RNP PARTICLES

Joseph Sperling[1], Daniel Offen[1], Gil Ast[1] and Ruth Sperling[2],
[1]The Weizmann Institute of Science, Rehovot 76100, Israel,
[2]The Hebrew University of Jerusalem, Jerusalem 91904, Israel.

In our attempts to isolate the native nuclear RNP complexes on which RNA processing occurs we have demonstrated that specific nuclear RNAs, that largely differ in size, can be released from nuclei of mammalian cells in an intact form as RNP particles that sediment at the 200S region in sucrose gradients. These findings raise the possibility of the existence in vivo of a unitary nuclear RNP particle on which processing of hnRNA presumably occurs.

To facilitate our studies on the structural and functional aspects of the large RNP particles, we have generated monoclonal antibodies against specific components of the large RNPs. Following fusion, the antibodies produced by the hybridoma cells were screened by RIA against nuclear RNP preparations from several mammalian sources. The final screen yielded 86 positive hybridomas producing antibodies against components of the 200S RNP particles. These hybrodoma cells were selected for further subcloning and characterization. The hybridomas tested thus far by immunoblotting produce antibodies against polypeptides in the molecular weight range of 34-45 and 83-100 kDa. Monospecific monoclonal antibodies against polypeptides of 36 kDa and 88 kDa, which have been shown to be integral components of the 200S RNP particles, inhibit splicing in vitro.

N 421 CONTROL OF SPINACH PLASTID GENE EXPRESSION: THE ROLE OF 3' END INVERTED REPEATS IN RNA PROCESSING AND STABILIZATION, David B. Stern, Xing-Wang Deng and Wilhelm Gruissem, Department of Botany, University of California, Berkeley, CA 94720

Post-transcriptional mechanisms play an important role in establishing mRNA levels during plastid development (1,2). We have used in vitro transcription and processing assays to identify mechanisms that influence mRNA stability, and to quantify RNA half lives and their changes during development of spinach. Inverted repeat (IR) sequences with the potential to form stem/loop structures flank the 3' ends of many plastid transcription units. We have demonstrated that these sequences do not effectively terminate transcription by the plastid RNA polymerase in vitro, but they do act as RNA stabilising elements, and barriers to exonucleolytic degradation that occurs upon incubation of synthetic precursors in a chloroplast protein extract. The processing activity yields 3' ends identical to those found in vivo (3). Gel mobility-shift and RNase protection experiments suggest that protein factor(s) associate with these synthetic RNAs in the region of the IR. These factors may play a role in determining the stabilities of different RNAs. Kinetic measurements of RNA degradation in protein extracts from different developmental stages demonstrates that mRNAs have distinct half lives that increase during leaf maturation, a result that is consistent with in vivo changes in transcription activity and steady-state mRNA levels. The magnitude of half life increase varies between genes, suggesting that selective stabilization of mRNAs via the 3' end IR may be an important regulatory mechanism in the chloroplast.

1. X. Deng and W. Gruissem (1987), Cell 49, 379.
2. J.E. Mullet and R.R. Klein (1987), EMBO J. 6, 1571.
3. D.B. Stern and W. Gruissem (1987), Cell, in press.

N 422 Non-contiguous poly(A)-head found at the 5' end of vaccinia virus late mRNA *in vivo* and *in vitro*, Beate Schwer and Henk Stunnenberg, EMBL, Heidelberg, FRG. Studies on the structure and synthesis of vaccinia virus late RNA transcripts have revealed that a non-contiguously encoded poly(A) stretch of approximately 35 A-residues is covalently linked to the 5' end of the late RNA in vivo (Schwer et al., 1987 Cell 50, 163). The poly(A)-head is preceded by a ^{7}m-G cap structure. The junction between the A-head and the coding body of the mRNA is located within a TAAATG-sequence which is highly conserved among vaccinia late genes. Mutational analysis revealed that the TAAATG-motif is essential for the transcription of the gene in vivo (Hänggi et al, 1986 EMBO J. 5,1071) as well as in vitro. We have established an in vitro transcription system derived from virus infected cells which is dependent on late vaccinia regulatory sequences. Furthermore, in vitro transcripts have a 5' poly(A)-head covalently linked to the coding body of the reporter gene. This poly(A)-head is synthesized de novo in our extract and its production appears dependent on the ATP concentration. We have not yet determined the exact mechanism of A-addition: the data suggest however, a primed initiation process or a slippage ("stottering") mechanism at transcription initiation. Alternatively, a nascent chain processing mechanism could explain our observations (Schwer and Stunnenberg, submitted to Cell).

N 423 RNA PROTEIN INTERACTION IN NEOPLASTIC TRANSFORMATION, DIFFERENTIATION AND EVOLUTION, Josée Espen and Karl-Heinz Westphal, Laboratorium für molekulare Biologie-Genzentrum, Am Klopferspitz, D-8033 Martinsried, FRG.

We have investigated the interaction of the Simian virus 40 T antigen-p53 complex with RNA. This complex binds RNA both in vivo and in vitro. The in vitro bound RNA is found in a size range from 200-2000 bases. We could demonstrate by Northernblot analysis that the eluted RNA contains B2 repetitive elements. These elements are highly enriched, compared to ribosomal RNA in the same eluate. A B2-SP6 run off transcript is specifically retained on the SV40 T antigen-p53 matrix. We are currently isolating specific c-DNA clones from SV40-p53 complex binding RNA by differential screening.

By Northwesternblot analysis we were able to identify B2 RNA binding proteins. The identified proteins are differentially expressed in nondifferentiated and differentiated mouse muscle cells, F9 embryonal carcinoma cells, human melanoma cells and SV40 transformed and nontransformed mouse fibroblasts. Furthermore, we identified a RNA binding protein of the same size present in human, mouse and Escherichia coli cells.

B2 repeats are expressed in a differentiation and proliferation dependent way. The interaction of RNA with the SV 40 T antigen-p53 complex might be an important regulation mechanism, which could have a negative or positive influence on the stability of RNA, especially on the B2 repeat RNA. Genes could be specifically switched on or off by RNA protein complexes. RNA protein interactions may play a key-role in evolution and in the important cellular processes of differentiation and neoplastic transformation.

N 424 RNA ESSENTIAL FOR PACKAGING OF BACTERIOPHAGE ϕ29 DNA, Jesdawan Wichitwechkarn, Suzanne Bailey, Bernard Reilly, James Bodley and Dwight Anderson, University of Minnesota, Minneapolis, MN 55455.

An RNA of approximately 170 nucleotides is a novel structural and functional entity essential in packaging of Bacillus subtilis bacteriophage ϕ29 DNA. The RNA, a transcript of the far left end of ϕ29 DNA, is a constituent of the portal vertex of the viral precursor shell (prohead) through which the DNA passes. The RNA can be detached and used with RNA-free proheads in the reconstitution of active proheads (1,2). Similar RNAs have been isolated from proheads of related phages. We have devised biological assays for a) analyzing the packaging of ϕ29 DNA into RNA-containing proheads of related phages and b) measuring the ability of heterologous phage RNAs to re-constitute RNA-free ϕ29 proheads. The structure of the prohead RNA is being examined by enzymatic digestion and chemical probing and by comparative sequence analysis. These approaches will facilitate the selection of additional cognate RNA molecules for sequencing and for analysis of RNA structure and function during DNA encapsidation.

1) Guo, P., S. Erickson, and D. Anderson, Science 236, 690-694 (1987).
2) Guo, P., S. Bailey, J.W. Bodley, and D. Anderson, Nucleic Acids Research, 15, 7081-7090 (1987).

N 425 INTERACTION OF hnRNP C PROTEINS WITH POLYADENYLATION SIGNAL CONTAINING RNAs REQUIRES THE DOWNSTREAM ELEMENT. Jeffrey Wilusz and Thomas Shenk, Dept. Mol. Biol., Princeton University, Princeton, NJ 08544.

We have analyzed RNA-protein interactions associated with the process of polyadenylation by using an *in vitro* system in conjunction with photochemical crosslinking. A 64KD protein was identified which required an intact AAUAAA signal for interaction with substrate RNAs. In addition, the hnRNP C proteins were found selectively associated with six independent polyadenylation signal containing RNAs. Deletion analyses of individual polyadenylation signals indicated that an intact downstream element was required for interaction with C proteins. C proteins interacted with sequences downstream of the cleavage site in the absence of upstream segments. Poly(A)$^+$ product RNAs did not interact with C proteins. These data identify sequence preferences for C protein - RNA interactions as well as a role for hnRNP proteins in efficient polyadenylation.

N 426 USING *IN VITRO* MUTAGENESIS OF *ESCHERICHIA COLI* RIBOSOMAL PROTEIN S8 TO STUDY PROTEIN - RNA INTERACTIONS. I. Wower and R.A. Zimmermann, University of Massachusetts, Amherst MA 01003.

Escherichia coli protein S8 binds directly to 16S rRNA and is essential for assembly of 30S ribosomal subunits. It also functions as a translational inhibitor by binding to a regulatory site on *spc* mRNA. Therefore, the interaction of S8 with both RNAs offers an interesting model for studying protein-RNA interactions. While structural elements of the RNA required for binding are well characterized, very little is known about the features of the protein involved in specific recognition of RNA. Since S8 binding domains on rRNA and mRNA are almost identical, it is probable that there exists a common RNA binding site on S8.The *rpsH* gene, coding for S8, has been cloned into a high-copy number plasmid under control of the *lac* promotor. Upon IPTG induction,the excess protein S8 produced inhibits translation of the *spc* mRNA causing the host cells to grow much slower.When certain mutations are introduced into the *rpsH* gene, however, the cells do not change their growth rate upon IPTG induction. We believe that the latter phenotype results from the failure of mutant S8 to bind *spc* mRNA and, by inference, to 16S rRNA. We have isolated mutants of this type with frame-shifts in the C-terminal part of S8. Chemical mutagenesis of the *rpsH* gene with nitrous acid and methoxylamine has also been performed. One such mutant with single amino acid change [Ser106 to Phe106] has been identified and a number of others are currently being analyzed.

Pre-mRNA Splicing

N 500 STUDIES ON THE T4 *nrdB* INTRON, Agneta Åhgren, Solveig Hahne and Britt-Marie Sjöberg, Department of Molecular Biology, University of Stockholm, S-106 91 Stockholm, SWEDEN.

The bacteriophage T4 *nrdB* gene contains a self-splicing 598 bp intron which divides the structural gene into two parts of 664 and 500 bp respectively. The T4 gene *nrdB* codes for the small subunit of the enzyme ribonucleotide reductase. It is preceded by the gene for the other subunit of ribonucleotide reductase, *nrdA*, and the intron containing *td* gene coding for thymidylate synthase, mapping around position 140 kb in the T4 map. Besides the two self-splicing introns mentioned at least one more group 1 intron containing gene has been localized in the T4 genome. This *sunY* gene maps at 45 kb of the T4 map.

The exon-intron boundaries in the *nrdB* gene can readily be postulated from a comparison with the known nucleotide sequence of the intron in the *td* gene. The splice-junction has been confirmed by direct sequence determination of the mature message (Pedersen-Lane et al., Science 237, 182-184, 1987). The splicing event results in the formation of a cysteine codon in the mature message by the fusion of a U residue at the end of exon 1 to a GU sequence at the start of exon 2. The presence of this cysteine residue in the mature protein has been confirmed. The predicted amino acid sequence has also been confirmed in several regions scattered throughout the protein.

A comparison between the *td* and the *nrdB* introns in T4 highlights several conserved areas, especially towards the 3'-end of the introns. Some specific sequences known to be common to the self-splicing group 1 introns are also conserved in the *nrdB* intron, e.g. the consensus sequences called boxR and boxS which are known to basepair in the group 1 introns. It is also possible to predict a folded structure of the *nrdB* intron which is analogous to the group 1 introns.

One common feature of the *td* and *nrdB* introns, which is not generally found in other group 1 introns is the presence of an in frame stop codon spanning the 5'-splice site. A stop codon at this position may be necessary for the proper folding and correct splicing of the intron within the bacterial cell. We are presently using site-directed mutagenesis to study the importance of the stop codon in the *nrdB* intron.

N 501 CIS AND TRANS ELEMENTS ARE INVOLVED IN SPLICING OF THE TROPONIN T MUTUALLY EXCLUSIVE EXONS. Athena Andreadis and Bernardo Nadal-Ginard, Laboratory of Molecular and Cellular Cardiology, Children's Hospital/Harvard Medical School, Boston, MA 02115.

The rat skeletal troponin T gene generates a single primary transcript, yet encodes for a variety of isoforms via alternative splicing: Five exons in its 5' region are spliced combinatorially while two exons near its 3' end are incorporated in a mutually exclusive manner in the mature mRNA. Of these two exons, the 5' proximal (α) is adult-specific, whereas the distal one (β) is embryo- and muscle cell line-specific. Scanning of the sequences within and around these exons has revealed no significant deviation of their donor/acceptor sites from the consensus sequences. Minigenes containing genomic subfragments that include α and β have been constructed and transfected into non-muscle (HeLa, COS) and muscle (YB30, C2) cells. Analysis of the minigene transcripts by S1 nuclease mapping has shown that, in non-muscle and growing muscle cells, the α (adult) exon is always absent, whereas the β (embryonic) is present. Deletion of β along with much of its two surrounding introns does not promote incorporation of α. A deletion which, in addition to β, eliminates the introns flanking α almost entirely results in inclusion of α in the construct mRNA. Reinsertion of β in the latter construct does not prevent α from being spliced into the final RNA product, whereas reconstitution of the intron 5' to α leads largely to its exclusion from the construct mRNA. As in the native gene, the two exons are never spliced together. The inclusion of β in non-muscle cells (the "default" splicing pattern) implicates cis regulatory elements in this selection mechanism, but the behavior of the deletion constructs indicates that it is not a simple case of splice site hierarchies. It is possible that sequences in the intron(s) represent binding sites for a trans factor. Additionally, the exclusion of α from spliced RNAs in muscle cells suggests that adult muscle-specific trans factors might be required for the generation of the full isoform spectrum.

N 502 958 OF 1175 NUCLEOTIDES OF YEAST U2 snRNA ARE DISPENSIBLE. Manuel Ares, Jr., and A. Haller Igel, University of California, Santa Cruz, Santa Cruz, CA 95064.

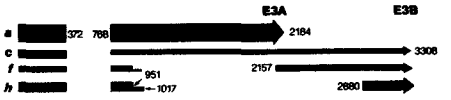
U2 is a highly conserved RNA found in the eukaryotic cell nucleus, where it plays a central role in the splicing of nuclear pre-messenger RNA. Although U2 RNA from most organisms is short (<200 nucleotides, nt), the yeast Saccharomyces cerevisiae contains a single essential U2 gene encoding an unusually large (~1175 nt) U2-like RNA. To test whether the extra RNA found in yeast U2 contained any essential function, we constructed a large deletion, leaving intact only the first 123 nt (containing the highly conserved U2 domain and "Sm-binding site"), and the last 90 nucleotides (containing sequences with weak homology to U2 RNAs from other organisms) of the yeast U2 coding region. The deletion was introduced by genetic techniques into yeast cells lacking any other functional U2 gene. Yeast cells containing only the deleted U2 gene are not obviously debilitated, and produce only a 220 nt U2 RNA. We are constructing pairs of strains with or without the U2 deletion (but otherwise isogenic) in order to determine whether there is any effect on growth or splicing efficiency.

N 503 CONTROL OF ROUS SARCOMA VIRUS RNA SPLICING, S.J. Arrigo and K.L. Beemon, Johns Hopkins University, Baltimore, MD 21218.

Unlike most eukaryotic primary transcripts, that of Rous sarcoma virus (RSV) is only partially spliced to subgenomic messages. Through deletion mutagenesis of a proviral RSV construct, we have localized a region within the gag gene which inhibits splicing from the normal splice donor. Removal of this region results in a dramatic increase in the ratio of spliced to unspliced RNA. We have determined that this element, located downstream of the splice donor, is both cis-acting and orientation dependent in its negative regulation of normal splicing. Experiments are presently being conducted to determine whether this element is capable of negatively regulating splicing from a heterologous splice donor and acceptor. We have further observed that translation of the gag gene is necessary for stabilization of the unspliced RSV RNA.

N 504 COMPETITION AMONG SPLICING FACTORS AND POLYADENYLATION FACTORS DETERMINES WHICH ADENOVIRUS REGION E3 OVERLAPPING mRNAs ARE MADE, Helen A. Brady and William S.M. Wold, St. Louis University Medical Center, St Louis, MO 63110.

The E3A poly(A) signal (forms 3' end of mRNA *a*) includes an ATTAAA and a GT-rich element 3' to the poly(A) addition site. The 2157 3' splice site (used by mRNA *f*) is only 4 nt upstream of the ATTAAA. We hypothesized that beause of this proximity, splicing factors and poly(A) factors could not bind to the same pre-mRNA, and that a competition among these factors for binding would determine whether mRNA *a* or *f* were made. To test our hypothesis we made (i) five virus mutants which destroy the E3A poly(A) signal but leave the 2157 3' splice site intact, and (ii) two insertion mutants which increase the distance between the 2157 3' splice site and the ATTAAA by 6 bp and 140 bp. All these mutants made almost exclusively mRNA *f*. This result, which is consistent with our hypothesis, strongly suggests that the 2157 3' splice is more efficient in these mutants than in wild type because splicing factors no longer have to compete with poly(A) factors.



N 505 USE OF ALTERNATIVE 5' SPLICE SITES DEPENDS ON THEIR SEPARATION.

S.A. CUNNINGHAM, A.J. ELSE and I.C. EPERON, Department of Biochemistry, University of Leicester, Leicester, LE1 7RH, U.K.

It has previously been demonstrated for the β-globin IVS-2 that the splicing preference of two closely situated 5' splice sites is dependent upon splice site sequence (Eperon et al.). In the assay system used a duplicated β-globin IVS-2 5' splice sequence (test site) is positioned from 25 to 231 nucleotides upstream of the authentic cleavage site (reference site) in order to investigate the influence of 5' splice site separation on splice site selection. Oligonucleotides ranging from 2 to 20 base pairs in length and fragments from a HpaII digest of λ DNA (24 to 206 base pairs) were inserted between the two sites. The splicing pattern was monitored in vivo by transient expression in HeLa cells and in vitro in a HeLa cell nuclear extract. Above a critical separation of approximately 24 nucleotides splicing can only be detected at the reference site. However, the stronger 'consensus' splice sequence can override this effect when incorporated into the test site. The importance of sequence between the two alternative 5' splice sites is implicated from mutagenesis experiments where the replacement of just one base can drastically alter the splicing pattern.

L.P. Eperon, J.P. Estibeiro & I.C. Eperon (1986) Nature 324, pp. 280-282.

N 506 CLONING AND CHARACTERIZATION OF THE SACCHAROMYCES CEREVISIAE RNA5 GENE.

Gloria Dalbadie-McFarland and John Abelson, California Institute of Technology, Pasadena, CA 91125.

Splicing of eukaryotic nuclear introns requires assembly of a spliceosome before covalent changes take place. The yeast RNA5 gene product is directly involved in pre-mRNA splicing (Lustig et al., 1986, Cell 47:953-696) and is required for spliceosome assembly (Lin et al., 1987, Genes & Development 1:7-18). Its specific function, however, is not known. We have cloned and characterized the yeast RNA5 gene in order to examine its products and their role in splicing.

The RNA5 gene was isolated from a yeast genomic library by complementation of a ts defect. Genetic analysis by integrative transformation showed that the cloned DNA is tightly linked to the RNA5 locus and not to an unlinked suppressor site, strongly suggesting that this is indeed the RNA5 structural gene. DNA sequence analysis revealed an open reading frame that encodes a 47 Kd predicted protein. A low abundance 1.4-1.6 Kb mRNA with at least three transcriptional starts upstream of the first AUG in the large open reading frame was detected. The properties of the RNA5 gene and the role of its products in splicing will be discussed.

N 507 CHARACTERIZING THE HETEROLOGOUS SPLICING CAPABILITIES OF TWO BUDDING YEASTS: SACCHAROMYCES CEREVISIAE AND KLUYVEROMYCES LACTIS.

James O. Deshler and John J. Rossi, Dept. of Microbiology at UCLA, and the Beckman Research Institute, City of Hope, Duarte, CA 91010.

We have cloned and sequenced the actin gene from K. lactis. This gene contains a single 780 base intron positioned three and a third codons downstream of the translational start. Exon I and exon II are 90% homologous to their S. cerevisiae counterparts, while intron sequences diverge completely, except at the 5' and 3' splice sites and the TACTAAC sequence. These regions are identical to the consensus sequences observed in all S. cerevisiae introns. C. Guthrie et al. have found that snRNA's from K. lactis are intermediate in size when compared to human and S. cerevisiae snRNA's. Also, K. lactis snRNAs' sequences diverge from S. cerevisiae at the same positions human and S. cerevisiae diverge. We have used primer extension analysis to demonstrate that K. lactis can splice S. cerevisiae actin transcripts in vivo and S. cerevisiae can splice K. lactis actin transcripts in vivo. An interesting feature of the K. lactis actin intron is a PyAG located 74 bases downstream of the branch site and 49 bases upstream of the 3' splice site. This PyAG represents a potentially "good" 3'splice site, yet it is not recognized as such by either K. lactis or S. cerevisiae. The process of 3' splice site selection is currently being addressed in this system and will be discussed.

N 508 STRUCTURAL CHARACTERIZATION OF THE INTRON CONTAINING RIBOSOMAL PROTEIN S25 GENES FROM TWO TETRAHYMENA SPECIES, Jan Engberg, Henrik Nielsen and Kirsten Bojsen, Department of Biochemistry B, University of Copenhagen, DK-2200 Copenhagen, Denmark.

The ribosomal protein S25 gene exists as a single copy gene in T. thermophila whereas two genomic copies are present in T. pigmentosa. Molecular characterization of these genes combined with primer extention analysis and c-DNA sequence data indicate that all three genes are functional and that they are interupted by a 979 bp large intron 68 bp downstream of the translation start site. No amino acid substitutions are seen in the coding region of the genes but several silent mutations exist. The TAA codon is used once for Gln in the T. pigmentosa genes whereas CAA is used in the analogous position in the T. thermophila gene. Sequence comparisons of the 5' flanking regions revealed regions of strong homology at either side of the putative transcription start site but no TATA-box is found at position - 35 of this site. Apart from the consensus dinucleotides GT/AG at the splice junctions very little homology was observed among the introns. The absence of a polypyrimidine stretch close to the 3' splice site of the S25 introns may indicate that the sequence requirements for pre-mRNA splicing in Tetrahymena are different from those of other eukaryotes studied thus far.

N 509 BIOTINYLATION OF RNA SPLICING SUBSTRATES, Barbara J. Fenn, Ning Li, Ki Tae Kim, and Timothy M. Herman, Medical College of Wisconsin, Milwaukee, WI 53226.

The affinity isolation of RNA splicing factors involves the use of biotin-labeled RNA splicing substrates. We are investigating different methods to affinity label RNA with biotin. As a first approach, we are randomly incorporating biotinylated nucleotides into RNA using T7 RNA polymerase. Kinetic analyses of this reaction reveal that the Vmax of the polymerase in the presence of Bio-4-UTP is 5-fold lower than in the presence of UTP (the Kms are not significantly different). We have used a label-transfer experiment to directly measure the number of biotinylated nucleotides incorporated into each RNA transcript. Transcription reactions run in the presence of 20 uM Bio-4-UTP / 380 uM UTP result in the incorporation of 6 biotinylated nucleotides per 500 nucleotide transcript. This RNA was efficiently bound to a streptavidin/biotin-cellulose affinity column. When the chemically-cleavable nucleotide Bio-19-SS-UTP was substituted for Bio-4-UTP, the RNA could be recovered from the affinity column following reduction of the disulfide bond by DTT.

As a second approach we have incorporated 5-(3-amino)allyl-UTP into RNA and chemically biotinylated the RNA post-transcriptionally with the hydroxysuccinimide ester of biotin. This reaction is specific for the amino group present on the C5 linker arm of allylamine UTP. In addition, we have recently synthesized a biotinylated 5',3'-uridine bisphosphate and are exploring its usefulness as a 3' end label for splicing substrates using T4 RNA ligase. This procedure provides a means of localizing the biotin moiety away from the splicing junctions, eliminating the possibility of steric hindrance by the biotin molecule during the assembly of splicing complexes.

N 510 ASSESSMENT OF THE POSSIBLE ROLE IN YEAST RNA METABOLISM OF TWO CLOSELY LINKED SMALL NUCLEAR RNA GENES, John Zagorski, David Tollervey[+], Haodong Li, and Maurille J. Fournier, Department of Biochemistry, University of Massachusetts, Amherst, MA 01003 and [+]Genie Microbiologique, Institut Pasteur, 75724 Paris, France.

The yeast *Saccharomyces cerevisiae* contains approximately two dozen small nuclear RNAs (snRNA). A small subset of these RNAs have been implicated in mRNA splicing and one species has been associated with the maturation of ribosomal RNA. Functions have yet to be ascribed to the other snRNAs, at least seven of which are dispensible for growth.

Two snRNA species under study by us consist of 128 and 190 nucleotides. These RNAs, designated snR128 and snR190, have tri-methyl G caps and are derived from genes that are separated by only 67 bps. The snR128 species is essential for growth while the snR190 species is not. The presence of intron-complementary sequences in snR128 suggests a possible role in mRNA splicing, by analogy with the metazoan U1 and U2 snRNAs. Inconsistent with this suggestion, however, is the finding that repression of snR128 synthesis has no effect on splicing of pre-mRNA for ribosomal protein 51. The splicing of other mRNAs in snR128-deficient cells is currently under study. Results from an examination of the subcellular condition of the snR128 and snR190 species suggest a possible role in ribosome biogenesis. Both RNAs exhibit extraction properties consistent with nucleolar localization and both co-sediment with deproteinized precursors to ribosomal RNA. The snR128 species sediments with 27S pre-rRNA while the snR190 species is associated with a 20S intermediate. New analyses are concerned with the effect of snR128 depletion on ribosomal RNA maturation. Supported by NIH grant GM19351.

N 511 **PROTEOLIPID PROTEIN GENE EXPRESSION IN JIMPY AND JIMPY^{MSD} MICE**

Minnetta V. Gardinier[1,2] and Wendy B. Macklin[1], [1]Dept. of Psychiatry and Biobehavioral Sciences, UCLA Medical Center, Los Angeles, CA 90024 and [2]Dept. of Biochemistry and Molecular Biology, LSU Medical Center, New Orleans, LA 70112.

The dysmyelinating mutants, jimpy (jp) and jimpy^{msd} (jp^{msd}), are allelic, and the jp mutation is a proteolipid protein (PLP) gene defect. We previously reported that PLP-specific mRNA expression is greatly reduced in jp mice and that these mRNAs are slightly smaller than wild type mRNAs as a result of a point mutation at an intron/exon junction. The present study characterizes PLP-specific mRNA expression in jp^{msd} mice as compared to jp and wild type mice. Southern analyses of jp^{msd} genomic DNA revealed no major rearrangements of the PLP gene. Unlike the shortened PLP-specific mRNAs seen in jp mice, these mRNAs are equivalent in size to wild type mRNAs by Northern and S1 nuclease analyses. Also, the related DM20 protein mRNA, an alternatively spliced PLP gene product, is present with no apparent aberrations. PLP/DM20 mRNA levels in affected jp^{msd} males are less than 20% of wild type mice, at a level slightly higher than age-matched jp males. The 3.2 and 2.4 kb PLP/DM20 mRNAs are seen; however, the 1.5 kb mRNA is either absent or below detection levels. Both PLP and DM20 protein are observed in jp^{msd} mice, albeit at drastically reduced levels. However, the "adult" jp^{msd} PLP:DM20 protein ratio reflects an immature expression pattern similar to that seen in very young wild type mice. Thus, the alternative splicing program for the PLP gene is affected in jp^{msd} mice. PLP gene transcription rates and PLP/DM20 mRNA stability are under investigation. This work was supported by the National Multiple Sclerosis Society and NINCDS.

N 512 INTRON RECOGNITION IN PLANTS: 3' SPLICE SITE SELECTION DIFFERS IN PLANTS AND ANIMALS, Gregory Goodall, Karin Wiebauer and Witold Filipowicz, Friedrich Miescher-Institut, 4002 Basel, Switzerland.

We have compared the splicing of pre-mRNA in plants and animals by transfecting the soybean leghemoglobin and human β-globin genes into both plant protoplasts and HeLa cells. Analysis of the splicing patterns obtained indicates that plants and animals differ in the mechanism of 3' splice site selection. A survey of animal and plant gene sequences indicated that the polypyrimidine tracts present at the 3' end of animal introns are not present in plant introns. Many plant introns, however, are AT-rich throughout the entire intron. To test the significance of the AT bias in plant introns we have chemically synthesized a hypothetical plant gene of mostly random sequence, but incorporating 5' and 3' splice site concensus sequences flanking an AT-rich "intron", embedded in a GC-rich sequence. This gene is efficiently spliced at the predicted sites in plant protoplasts. Using a series of unique restriction sites within the synthetic sequence, we have made a series of modifications to test the contribution of various sequence elements to intron recognition.

N 513 PHOSPHOROTHIOATE-SUBSTITUTED β-GLOBIN PRE-mRNA UNDERGOES A NOVEL SPLICING DEPENDENT CLEAVAGE THREE NUCLEOTIDES UPSTREAM OF THE NATURAL SMALL INTRON 3' SPLICE SITE, A.D. Griffiths*, B.V.L. Potter†, and I.C. Eperon*, Departments of Biochemistry* and Chemistry†, University of Leicester, Leicester LE1 7RH, U.K.

Phosphorothioate analogues of the rabbit β-globin pre-mRNA small intron and flanking exon sequences were prepared by in vitro transcription using T7 RNA polymerase in the presence of adenosine 5'-O-(1-thiotriphosphate) (ATPαS). When the above transcript was incubated in a HeLa cell nuclear extract splicing was inhibited, but a novel fragment of RNA was generated, which consisted of Exon 2 and the three most downstream nucleotides of the intron. This product possesses a 5' phosphate, strongly implying creation by an enzyme catalysed endonucleolytic cleavage. The product was formed with the same kinetics as the intermediates of a normal splicing reaction, and its formation, like normal splicing, was inhibited by oligonucleotide directed RNase H cleavage of U1, U2 and U6 snRNAs. We conclude that sequences proximal to the 3' splice site can be activated in the absence of exon ligation.

N 514 CRYPTIC SPLICE SITE SELECTION IN ALTERNATIVELY SPLICED CLASS I TRANSCRIPTS, Mary Lynne Hedley, Philip W. Tucker, and James Forman, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235.
Class I proteins are thought to bind peptides derived from viral and tumor antigens and present them to cytoxic T-cells which then initiate an immune response. Examples of differentially processed class I K and D gene transcripts have been identified, but their function remains an enigma. In order to facilitate the study of alternative splicing we used site-directed mutagenesis to alter the normal acceptor site bordering exon 2 of the D and K genes. The mutant genes were transfected into L-cells and cell surface expression of the cooresponding protein was assessed with D and K specific monoclonal antibodies. RNase and SI mapping experiments were performed to determine the structure of the alternatively spliced transcripts. Both genes have three potential alternative acceptor sites in the first intron. However, only one of these sites is used to generate the alternatively spliced transcripts from each gene. These experiments suggest that in addition to having sequences that match the concensus splice acceptor sites, other factors are important in determing which acceptor site will be used by a functional spliceosome.

N 515 A GENERAL MODEL FOR THE EVOLUTION OF NUCLEAR pre-mRNA INTRONS, Donal A. Hickey, Bernhard F. Benkel and Sumaia Abukashawa, University of Ottawa, Ottawa, CANADA; and W. Ford Doolittle, Dalhousie University, Halifax, Nova Scotia, CANADA.
We present an overview of the evolution of eukaryotic split gene structure and pre-mRNA splicing mechanisms. We have drawn together several seemingly conflicting ideas and we show that they can all be incorporated in a single unified theory of intron evolution. The resulting model is consistent with the notion that introns as a class are evolutionarily very ancient; it also supports the concept that introns have played a crucial role in the construction of many eukaryotic genes; moreover, it accommodates the idea that introns are related to mobile insertion elements. Essentially, our proposal is that exon mobility, i.e., exon shuffling, was facilitated by the presence of flanking mobile intron sequences. According to this hypothesis, the mobilization of exons by flanking intron sequences is analogous to the mobilization of bacterial antibiotic resistance-coding genes by flanking IS sequences. The attraction of the model is that, while acknowledging the potential long-term evolutionary benefits of exon shuffling, neither the evoltionary origin nor the maintenance of eukaryotic split-gene structure depends solely on these long-term benefits.

N 516 BIOCHEMICAL AND GENETIC ANALYSIS OF YEAST Sm SnRNPs. Michele Haltiner Jones and Christine Guthrie, UCSF, San Francisco, CA 14143.
The analogues of the mammalian UsnRNPs (U1, U2, U5, U4/U6) shown to mediate mRNA splicing have been identified in yeast. As with their mammalian counterparts, a hallmark of yeast spliceosomal UsnRNAs is their association with the highly conserved antigen designated "Sm", which we have demonstrated by immunoprecipitation of yeast UsnRNAs using human α-Sm antiserum (P. Siliciano, M.H. Jones, and C. Guthrie, Science (1987)237, 1484). Furthermore, yeast (and mammalian) UsnRNPs are maintained in highly salt resistant particles after equilibrium density centrifugation in cesium sulfate.

In an effort to identify the Sm protein constituents of yeast snRNP particles, we are purifying snRNPs from radiolabeled yeast extract by a combination of cesium sulfate centrifugation and immunoprecipitation. In a complementary approach, we have adopted a genetic strategy to enable a structure-function analysis of this highly conserved RNA:protein interaction. We have generated point mutations in the consensus binding site for the Sm protein (UAUUUUUUGG) in the yeast U5 snRNA to define more precisely the characteristics of a functional protein binding site. Mutations at one position are lethal; in contrast, mutations at three other sites have no apparent effect on viability. Mutations which result in a lethal phenotype are being used to isolate extragenic suppressors, which may permit the genetic identification of the Sm protein.

N 517 THE U2 SMALL NUCLEAR RNP: IN VITRO RECONSTITUTION AND ATP-DEPENDENT STRUCTURE. Ann M. Kleinschmidt, Jeffrey R. Patton and Thoru Pederson, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The small nuclear RNA U2, in the form of the U2 snRNP particle, is a cofactor for pre-mRNA splicing. Here we report the in vitro reconstitution of the U2 snRNP particle. SP6-transcribed U2 RNA assembles into a specific RNP complex in HeLa S100 extracts. This reconstituted particle has the same properties as endogenous U2 snRNP by the criteria of glycerol gradient sedimentation, RNA:protein mass ratio, reactivity with Sm (but not RNP) monoclonal antibody, and resistance of the Sm core region to micrococcal nuclease. Reconstitution occurs efficiently on a precursor length molecule and this particle has the same structure as one containing mature length U2. Pseudouridine formation does not occur appreciably until after snRNP assembly. ATP is required for U2 snRNP reconstitution, although a Sm antibody non-precipitable RNP complex forms in the absence of ATP. RNA-protein crosslinking of U2 snRNP assembled in the presence of ATP reveals a pattern reminiscent of known snRNP proteins. Most interestingly, we have discovered that the reconstituted U2 snRNP displays an ATP-dependent conformational switch, in which an additional ~25 nt region near the 3' end becomes nuclease-resistant in the presence of ATP. This ATP-mediated conformational state may be necessary for the interaction of U2 snRNP with pre-mRNA.

N 518 TISSUE-SPECIFIC DIFFERENTIAL EXPRESSION OF CHICKEN U4 SMALL NUCLEAR RNA GENES, Gina M. Korf, Ihab W. Botros, and William E. Stumph, San Diego State University, San Diego, CA 92182.

There are only two U4 small nuclear RNA genes per haploid genome in the domestic chicken (Hoffman, Korf, McNamara, and Stumph, MCB 6, 3910 [1986]). One gene, U4B, encodes the previously known chicken U4B RNA. The second gene, U4X, codes for a sequence variant of U4 RNA that was unknown prior to the cloning of the gene. By using U4X-specific and U4B-specific hybridization probes, we have found that both the U4B and U4X genes are expressed in all (seven) chicken tissues examined. However, the relative accumulation of the U4B and U4X RNA gene products can vary from tissue to tissue. Taking U4B RNA levels as a base line for normalization, U4X RNA is relatively underexpressed in adult gizzard, oviduct, breast muscle, and kidney, compared to U4X RNA levels in adult brain, heart, and liver. In each of three embryonic tissues examined, the U4X RNA to U4B RNA ratio is markedly higher than in the corresponding adult tissues, suggesting that U4X functions primarily as an embryonic U4 small nuclear RNA. A model consistent with our current data is that U4X RNA is synthesized at a relatively high level (approximately equal to U4B) in the early embryo, but its accumulation is gradually reduced as development proceeds. Since U4 RNA participates in the splicing of mRNA precursors, it is conceivable that U4X RNA (or changes in U4X/U4B ratios) could play a role in developmental or tissue-specific alternative splicing of pre-mRNAs.

N 519 THE USE OF HETEROLOGOUS IN VITRO SPLICING SYSTEMS TO IDENTIFY FUNCTIONAL COMPONENTS OF SPLICEOSOMES. Garry P. Larsen and John J. Rossi, Dept. of Microbiology at UCLA and Dept. of Molecular Genetics, Beckman Research Institute of the City of Hope, Duarte, CA 91010. Two intron containing genes (actin and RP59) have been isolated from the budding yeast Kluveromyces lactis. Both genes contain the conserved branch point sequence 'TACTAAC' found in S. cerevisiae introns and are over 90% homologous in their coding sequences. A comparison of the intron positions between the two organisms has demonstrated that the actin genes are identical in intron position, but the RP59 genes differ. The intron in S. cerevisiae RP59 interrupts codon three while in K. lactis codon four is interrupted. Currently we are developing heterologous in vitro splicing systems that can address the functional role of various components in the splicing process.

N 520 Characterization of Functional Spliceosomes and Extrinsic Splicing Factors in Yeast. R.-J. Lin, Department of Microbiology, University of Texas, Austin, Texas 78712.

We have shown that a whole-cell yeast extract is able to accurately splice synthetic pre-mRNA of yeast nuclear genes, and the splicing takes place on a 40S particle called the spliceosome. This in vitro mRNA splicing system has been used to study temperature-sensitive mutants (rna2-11) which accumulate intron-containing pre-mRNA at the restrictive temperature. We have found that extracts prepared from most of the rna mutants are heat sensitive for mRNA splicing activity in vitro. Furthermore, most of the heat-inactivated mutant extracts, with the exception of rna2, do not form the spliceosome, suggesting their gene products are involved in spliceosome formation. The spliceosome formed in the heat-inactivated rna2 mutant extracts is functional since, upon isolation from glycerol gradients, it can splice the associated pre-mRNA in the presence of complementing extracts. Currently, we are studying the so-called rna2Δ spliceosome and the activities required to complement it. We have found that splcing of the pre-mRNA on the rna2Δ spliceosome requires ATP and Magnesium ion as well as three extrinsic splicing factors (ESFs) including the RNA2 gene product. Interestingly, one of the ESFs, called the bn factor, is extremely heat stable yet sensitive to proteinase K digestion. Characterization of the rna2Δ spliceosome and the ESFs will be discussed.

N 521 LEADER RNA-PRIMED TRANSCRIPTION OF CORONAVIRUSES: AN ALTERNATIVE TO RNA SPLICING, Shinji Makino, Lisa H. Soe, Chien-Kou Shieh and Michael M.C. Lai, Department of Microbiology, University of Southern California School of Medicine, Los Angeles, CA 90033. Mouse hepatitis virus (MHV) is a member of Coronaviruses, which contains a 27-kb single-stranded RNA genome. The virus synthesizes six subgenomic mRNA species, which have a 3'-coterminal nested-set structure. Furthermore, all of the mRNAs contain a 72-nucleotide leader sequences derived from the 5'-end of the viral RNA genome. Evidence has been obtained that suggests a unique mode for the synthesis of these mRNAs. The leader RNA was apparently joined by a discontinuous transcription process, in which the leader RNA is synthesized independently, dissociates from the template and then rejoins the template at the distant downstream transcription initiation sites to serve as primers for transcription. The evidence in support of this transcription mechanism includes the detection of free leader RNA species, the presence of a temperature-sensitive mutant which synthesizes only the leader RNA at the non-permissive temperature, the demonstration of the high-frequency exchange of leader sequences between two co-infecting viruses, and the presence of homologous nucleotides between the 3'-end of leader RNA and the transcription initiation sites of various mRNAs. This leader-primed transcription apparently requires an endonuclease activity which trims the 3'-end of the leader RNA, and may also involve a proof-reading enzymatic activity to correct mismatches between the leader RNA and template RNA. We have also shown that the binding of the leader RNA to the template involves several UCUAA repeats, which leads to heterogeneity of mRNA species, containing different numbers of repeats. The amount of each mRNA synthesized paralles the extent of sequence homology between the leader RNA and intergenic sites. This mechanism provides a unique mechanism of regulation of mRNA transcription.

N 522 TISSUE-SPECIFIC REGULATION OF ALTERNATIVE SPLICING IN THE HUMAN FIBRONECTIN GENE REQUIRES EXON SEQUENCES. Helen J. Mardon, Susan Mallett, Giovanni Paolella and Francisco E. Baralle, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK.

Fibronectin (Fn) isoforms are generated by differential splicing of a single primary RNA transcript. Exon EDIIIA is either included (EDIIIA$^+$, cellular Fn) or omitted (EDIIIA$^-$, plasma Fn) in the mRNA via a process of exon skipping. We have investigated the tissue-specific regulation of splicing of EDIIIA in the human Fn gene during development and in different tissues and cell types. The level of expression of EDIIIA$^+$ mRNA is elevated in foetal liver and yolk sac, and hepatoma cell lines compared to adult liver. DNA sequences involved in alternative splicing of EDIIIA were investigated in transient transfection experiments using α globin/Fn minigene hybrids. We found that an 81 bp fragment, in the centre of the exon is required, in sense orientation, for alternative splicing of EDIIIA (1). We are further dissecting this region by deletion and mutational analysis to define more closely the minimum sequences required for alternative splicing of EDIIIA. Other features which may be involved in the alternative splicing pathway are also being investigated. The results so far would suggest that differential expression of EDIIIA may involve the interaction of tissue-specific cellular factors with sequences present in the primary RNA transcript.

1. Mardon H.J., Sebastio G. and Baralle F.E. (1987) Nucl.Acids Res. 15, 7725-7733

N 523 ANTISENSE RNA INHIBITS THE SPLICING OF PRE-mRNA IN VITRO, Stephen H. Munroe, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 and Marquette University, Milwaukee, WI 53233.

Antisense RNAs complementary to pre-mRNA substrates are shown to inhibit splicing in vitro. Efficiency of inhibition depends on the length, position and concentration of antisense RNA with respect to substrate. An activity in the HeLa cell nuclear extract promotes the rapid annealing of antisense and substrate RNAs. Annealing is blocked if antisense RNA is added after substrate to the splicing reaction. Antisense RNAs 100-200 nucleotides (nts) long complementary to the 5' splice site (ss) or the 3' exon strongly inhibit splicing. Antisense RNAs hybridizing to 3' exon sequences more than 80 nts from the splice site also inhibit efficiently, even though truncated substrates lacking sequences complementary to this RNA are efficiently spliced. Experiments with substrates containing duplicate 3' ss indicate that inhibition is localized to the closest splice site and that antisense inhibition does not activate alternative splice sites. Possible mechanisms for inhibition of splicing by antisense RNA include (1) disruption of pre-mRNA secondary structure; (2) blocking of scanning of pre-mRNA; (3) blocking of binding of factors to specific sites; and (4) disruption of RNA packaging required for assembly of active splicing complexes. The last possibility provides a hypothesis which is consistent with the inhibition observed with many different substrates and antisense RNAs.

N 524 SPLICING OF FIBRONECTIN RNA IN VITRO, Pamela A. Norton and Richard O. Hynes, Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Analyses of rat, human, and chicken RNAs have shown that the fibronectin primary transcript is alternatively spliced at three positions. At two of these, an entire exon can be skipped. These two exons, termed EIIIA and EIIIB, are characteristically absent from liver-derived fibronectin transcripts. Most other cell types include EIIIA and EIIIB, but to varying extents. When cells are transfected with short segments of the gene, the resulting transcripts exhibit alternative splicing, suggesting that the requisite cis-acting signals are present within the vicinity of the alternative exons. We are attempting to identify factors which govern these cell type-specific splicing events by utilizing an in vitro splicing assay. In vitro synthesized transcripts containing 5' and 3' splice sites derived from rat genomic clones of fibronectin have been tested in HeLa nuclear extracts. A number of boundaries, including one which is alternatively spliced, function in the HeLa extract, although in some cases the divalent cation concentration can be critical. Conditions have not yet been found which permit conversion of more than a few percent of the fibronectin precursors to spliced products. We are continuing to characterize both the extract and template requirements for the splicing of fibronectin, and are using these extracts and constructs to identify proteins which bind specifically to the RNA precursors.

N 525 CIS AND TRANS-ACTING ELEMENTS ARE INVOLVED IN THE ALTERNATIVE SPLICING OF THE HUMAN FIBRONECTIN mRNA, Giovanni Paolella, M. Vittoria Barone, Claire Henchcliffe and Francisco E. Baralle, Sir William Dunn School of Pathology, University of Oxford, OX1 3RE, U.K.

Fibronectin ED-B is a facultative type III domain present in about 5% of the human cellular fibronectin; it is produced by alternative splicing of the fibronectin mRNA. A 2.8 kb fragment from the human fibronectin gene, containing the ED-B and part of the flanking exons, inserted in the third exon of the human α1-globin gene and transiently expressed in Hela cells undergoes alternative splicing, accurately reproducing the splicing patterns observed in the fibroblast.

A set of deletion mutants was generated from this hybrid gene in order to evaluate the role played by the various splice junctions and other possible signal sequences in determining the alternative splicing. The analysis of the splicing patterns observed after transient expression in Hela indicates that at least two regions are involved, one of which, corresponding to the 3' junction of the intron in front of the ED-B, appears to be the main cause of the low level of expression of the ED-B exon.

Transfection of the α-globin fibronectin hybrid in several cell lines showed significant variations in the splicing patterns, which were not related to the total amount of RNA produced in different cells. The expression of this gene in SV40-transformed human fibroblasts produced higher levels of the ED+ RNA, with a ratio between the two forms similar to that observed in the endogenous fibronectin mRNA. The same clone, when expressed in a hepatoma cell line, only gives rise to the ED- form; which is also the only form produced in liver.

The present data indicate that both specific RNA sequences and trans-acting cellular factors are necessary to produce alternative splicing of the region. Further analysis of the splicing patterns of the deleted partial genes in other cell lines is now being carried out, to definitively clarify which RNA sequences are involved in determining the tissue specificity of the alternative splicing.

N 526 **RNA-BINDING PROPERTIES OF THE U1 snRNP 70K PROTEIN.** Charles C. Query, Rex C. Bentley, and Jack D. Keene, Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710.
In order to investigate the structure, assembly, and function of the U1 small nuclear ribonucleoprotein (snRNP), cDNA encoding a U1 snRNP-associated 70 kilodalton protein (70K) was cloned from a human brainstem expression library (Query and Keene, Cell 51, 211-220). The 70K-LacZ fusion protein selectively bound U1 RNA from total HeLa RNA and also bound U1 transcripts synthesized in vitro. Using deletion mutants of U1 RNA and transcripts truncated at various restriction sites, we have localized a region of U1 RNA required for binding the 70K fusion protein. The B, C, and D stem-loops of U1 RNA could be removed without decreasing the efficiency of binding. The A stem-loop (nt. 1-47) bound efficiently to the 70K-LacZ fusion protein, suggesting it contains the binding site for 70K protein. By protection from RNase and chemical modification, we are investigating the contact points between this binding site and the 70K protein. Using deletion mutants of the 70K-LacZ fusion protein, we have identified a 122 amino acid region which contains a U1 RNA-specific binding domain. We are currently further defining the RNA-binding domain(s) of the 70K protein and will be investigating possible protein-protein interactions in our efforts to understand the structure and activity of U1 snRNP in spliceosomal complexes.

N 527 PRE-MRNA SEQUENCES AND SPLICING COMPLEXES Robin Reed and Tom Maniatis, Harvard University, Cambridge, MA 02138
Lariat formation occurs at the adenosine residue in the weakly conserved sequence element, CURAC, that is located near the 3' splice site. However, this sequence element can be deleted without significant effects on splicing, and branch formation occurs at 'cryptic' branchpoint sequences. Thus, it is not known whether sequences at the site of lariat formation confer specificity in the splicing reaction. To examine this question, we employed a sensitive cis-competition assay in which we compared the use of tandemly duplicated 3' splice sites containing either normal or mutant branch point sequences. Our results indicate that the sequences at the branchpoint do indeed play a role in 3' splice-site selection.

To study the structure and function of splicing complexes generated during the in vitro splicing reaction, we developed a method for isolating splicing complexes on a preparative scale using gel filtration chromatography. In vitro complementation assays show that the spliceosomes isolated by this procedure are functional. Analysis of the protein and RNA content of the column fractions indicates that spliceosomes are highly enriched in these fractions. SnRNAs U1,U2, U4, U5, and U6 are present, and we are currently identifying the hnRNP and snRNP proteins in the spliceosome fractions. Examination of these fractions by electron microscopy, in collaboration with Jack Griffith (University of North Carolina), revealed a relatively homogeneous population of 300-400 Angstrom particles. Evidence that these particles are spliceosomes is provided electron microscopic examination of particles assembled on different sized pre-mRNAs.

N 528 GENERATION OF ALTERNATIVELY SPLICED TRANSCRIPTS FROM THE SACCHAROMYCES CEREVISIAE ACTIN PRE-MRNA.JohnJ. Rossi[1], Eduard Felder[1], Lance Ishimoto[2], and George Murakawa[2]. 1. Department of Molecular Genetics, Beckman Research Institute of the City of Hope, Duarte, Calif. 91010; 2. Department of Microbiology, Univ. of California at Los Angeles, Los Angeles, Calif. 90024. Alternative splicing is common among higher eukaryotes, although the mechanisms governing this phenomenon are poorly understood. In the yeast S. cerevisiae, there are no known examples of alternatively spliced transcripts amongst the 20 or so intron containing gene products analyzed thus far. In addition, and in contrast to higher eukaryotes, S. cerevisiae utilizes a highly conserved intron encoded **TACTAAC** sequence as the signal and site for branch formation. In the actin gene, there is a cryptic **TACTAAG** signal just upstream from the canonical **TACTAAC** signal. Utilizing site directed mutagenesis, we have inverted the relative positions of these two sequences. One of the consequences of this is the introduction of another AG inbetween the the TACTAAC signal and the normally used 3' splice junction AG(**TACTAAC**TCTCATG**TACTAAG**ATCGAT-------AG). This construct, when analyzed in an in vitro splicing extract, generates three lariat structures which appear in approximately equimolar amounts. Two of these species represent introns with different 3' termini, indicating that both the AG signals are utilized with an equivalent efficiency. Possible models by which this alternative splicing occurs will be presented.

N 529 INACTIVATION OF SPLICING ACTIVITY IN EXTRACTS FROM HELA CELLS SUBJECTED TO HEAT SHOCK OR SERUM STARVATION, Ryszard Kole and Tadeusz Zwierzynski, Department of Pharmacology and Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC 27599.
Nuclear splicing extracts prepared from HeLa cells which have been subjected to heat shock or serum starvation exhibit significant loss of splicing activity, suggesting that some splicing factor(s) have been damaged and/or depleted in these extracts. Supplementing the extracts with a chromatographic fraction of the nuclear extract from the cells grown under normal conditions preferentially restores the first step in splicing, cleavage at the 5' splice site. A cytoplasmic S-100 fraction from normal cells restores the complete splicing reaction in this assay. Lack of U-type RNAs in the S-100 fraction and their presence in the inactivated extracts shows that the reactivating factor(s) are proteins and not snRNPs. Experimental evidence suggests that both heat shock and serum starvation inactivate the same subset of splicing factors and that these factors differ from those identified previously.

N 530 TOWARDS A MINIMAL FUNCTIONAL GROUP I INTRON, Jill Galloway Salvo, Renée Schroeder and Marlene Belfort, Wadsworth Center for Laboratories and Research, N. Y. State Dept. of Health, Albany, NY 12201.

The phage T4 thymidylate synthase (*td*) gene contains a 1016-bp group I intervening sequence. Non-directed mutational studies allowed us to delineate two regions at the ends of the intron which are critical to splicing (Hall *et al.*, *Cell*, *48*, 63-71, 1987; Chandry and Belfort, *Genes Dev.*, *1*, 1028-1037, 1987). Removal of extensive sequences from between the two domains left a fully functional and efficient self-splicing intron of 393 nt (Belfort *et al.*, *CSH Symp. 52*, in press). These genetic studies and comparison of the secondary structure models proposed for *td* and other group I introns (Michel and Dujon, *EMBO J.*, *2*, 33-38, 1983; Shub *et al.*, *PNAS*, in press) suggested that there may be portions of the two functional domains which are not critical for accurate splicing. Utilizing oligonucleotide-targeted and processive nuclease deletion analysis coupled with phenotypic screens, we have begun to analyze the P2, P6 and P7.1-P7.2 structures for their dispensibility to splicing. Consideration of a deletion for its influence on secondary structure and analyses for function are helping to determine the role of the various intron structures in splicing. These approaches have thus far yielded a lower limit of 243 nt for a splicing proficient group I intron.

N 531 PLANT INTRON SEQUENCES: EVIDENCE FOR DISTINCT GROUPS OF INTRONS, Brian A. Hanley and Mary A. Schuler, University of Illinois, Urbana, IL 61801.
Over the past few years the number of available plant gene sequences has increased dramatically and enabled researchers to suggest that the conserved plant intron border elements are similar to those found in animals and yeast. In spite of these sequence similarities, a number of experiments have demonstrated that the intron splicing machineries are not interchangeable in all organisms. This is particularly evident in heterologous in vitro splicing experiments which have demonstrated that, although a few plant introns can be excised in HeLa cell nuclear extracts, many plant introns are not processed accurately. In addition, introns are not excised when human genes are introduced into transgenic tobacco plants. These differences between eukaryotic RNA processing systems extend to the monocot and dicot groups of plants and have prevented the efficient in vivo expression of monocot introns containing introns in dicot plants. Because the nucleic acid sequence within plant pre-mRNAs potentially account for some of these functional splicing differences, we have analyzed plant introns to determine if the intron splicing efficiencies can be accounted for by differences in conserved sequence elements. We have subdivided the available plant intron sequences into several groups depending on the purine or pyrimidine-richness of the sequences upstream from the 3' splice site and the monocot or dicot origin of the gene. These comparisons suggest that there are discrete differences in plant introns which account for the differential splicing of monocot and dicot introns in transformed plants and in mammalian splicing extracts.

N 532 THE YEAST U2 snRNA, snR20, HAS TWO FUNCTIONALLY IMPORTANT DOMAINS, Elizabeth O. Shuster and Christine Guthrie, UCSF, San Francisco, CA 94143.

The 5' 120 nucleotides (nte) of the essential SNR20 gene share ~80% identity with mammalian U2 RNA (Ares, Cell 47:49, 1986) and include a sequence which base pairs with the site of branch formation (TACTAAC box) during mRNA splicing (Parker et al., Cell 49:229, 1987). However, the S. cerevisiae RNA is 1175 nte long, in contrast to the 189 nte mammalian U2 snRNA. Ares (op cit) has suggested that snR20 contains domains analogous to mammalian U4, U5 and U6 snRNAs; alternate hypotheses are that the regions distal to the "U2 domain" perform either novel functions or no function. We have used deletion analysis to directly identify regions required for the essential function(s) of SNR20. Nearly 950 nte 3' of the "U2 domain", including the sequences with proposed homology to mammalian U4, U5 and U6, can be removed without noticeably affecting growth rate. This is consistent with other work from our laboratory which indicates that distinct, essential RNAs fill the comparable roles in yeast (Patterson and Guthrie, Cell 49:613, 1987; Siliciano et al., Cell, 50:585, 1987; D. Brow, pers. comm.). In contrast, deletions which enter the conserved "U2 domain" at the 5' end cause lethality. Also, deletions which remove sequences ~100 ntes from the 3' end cause instability of snR20 as well as a substantial decrease in growth rate. Intriguingly, we can generate a model for the 2° structure of this 3' terminal region of snR20 which resembles the 3' terminal stem/loop of mammalian U2. We conclude that SNR20 encodes two domains of functional importance; the first is likely to be directly involved in the splicing reaction while the second may only be required for the generation of mature, stable snRNA molecules.

N 533 THE FUNCTIONS OF snR19, THE YEAST U1 ANALOG. Paul Siliciano and Christine Guthrie. Dept. of Biochemistry and Biophysics, UCSF, San Francisco, CA 94143

The yeast analog of U1, snR19, differs from mammalian U1 in several unexpected ways. U1 recognition of 5' splice sites involves direct base pairing between the 5' end of U1 and the 5' splice site. While the yeast 5' splice site differs from the mammalian consensus sequence at two positions, the 5' ends of snR19 and human U1 are identical. Therefore, in contrast to the mammalian case, snR19 and the yeast 5' splice site are not perfectly complementary. Since the 5' splice site sequence is highly conserved in yeast, it was expected that the yeast U1 analog would be able to base pair perfectly with this sequence. These mismatches make it important to determine the role of base pairing in 5' splice site recognition in yeast. This will be done by making mutations in snR19 which restore base pairing to mutant 5' splice sites. Biological and biochemical assays will determine if these compensatory mutations in snR19 improve the splicing of mutant introns.

Another unexpected feature of snR19 is its large size (568 nucleotides), over 400 nucleotides longer than U1. The functions of this "extra" RNA have been investigated by deletion analysis. Deletions removing 150 nucleotides from the center of snR19 are not viable, but, suprisingly, larger deletions are viable, albeit with increased doubling times.

N 534 STRUCTURE AND FUNCTION OF THE HUMAN U1-70K snRNP PROTEIN, Richard A. Spritz and Carol S. Surowy, Laboratory of Genetics, University of Wisconsin, Madison, WI 53706.

During pre-mRNA splicing, recognition of 5' splice sites is mediated by U1 snRNP. Human U1 snRNP consists of U1 snRNA, several core snRNP proteins, and three U1-specific proteins. We have isolated human cDNA and genomic clones encoding the specific U1-70K snRNP protein. This protein contains three regions with similarity to regions in known nucleic acid-binding proteins; two arginine-rich segments are similar to protamines and one segment corresponds to the "RNP consensus" sequence found in many nuclear RNA-binding proteins. We have found that the hU1-70K protein binds RNA *in vitro*, and have mapped regions within the hU1-70K protein necessary for RNA binding. We are currently mapping sites in the hU1-70K protein necessary for interactions with other snRNP proteins, and are investigating the role of the hU1-70K protein in assembly of U1 snRNP and in recognition of 5' splice sites.

N 535 CIS-ACTING REGIONS AFFECTING SPLICING OF ROUS SARCOMA VIRUS RNA, C. M. Stoltzfus, S. J. Fogarty, and S. L. Berberich, Dept. Microbiology, U. of Iowa, Iowa City, IA 52242. A substantial portion of Rous sarcoma retrovirus RNA emerges from the nucleus as unspliced RNA. Two regions of the genome affecting splicing have been studied: 1) the 262 base non-coding sequence between the terminator of the *env* gene and the *v-src* initiation AUG containing the *v-src* 3' splice site at nucleotide 7054; 2) a region in the *gag* gene downstream of the 5' splice site at nucleotide 398. Various amounts of the *v-src* intron were deleted and relative levels of spliced and unspliced RNA were determined following transfection of chicken embryo fibroblasts (CEF) and a mouse fibroblast cell line (C127) with cloned DNA. CEF transfected with plasmid DNA deleted in viral sequences from nucleotides 1659 to 6574 demonstrated wild-type levels of spliced and unspliced viral RNA whereas CEF transfected with plasmids in which the regions from 1149 to 6579 or 630 to 5258 were deleted demonstrated dramatic increases in the levels of spliced vs. unspliced viral RNA. This pattern was not altered when the cells were cotransfected with a complete virus clone expressing all of the viral proteins. Mouse C127 cells were co-transfected with the deleted plasmids and a *neo* gene-containing plasmid and colonies of resistant cells were selected in the presence of G-418. Most of the RNA isolated from the cell lines was spliced to *src* mRNA and there was little or no difference in the ratios of spliced to unspliced RNA observed among the various deleted plasmids. The results suggest that the region between nucleotides 1149 to 1659 contain sequences which act in *cis* to alter the distribution of spliced and unspliced RNA in CEF but not C127 cells. We propose that element(s) in this region act negatively in CEF to inhibit splicing.

N 536 THE DEPENDENCE OF SPLICING EFFICIENCY ON THE LENGTH OF 3' EXON. A.D. Turnbull-Ross, A.J. Else and I.C. Eperon, Department of Biochemistry, University of Leicester, Leicester, LE1 7RH, U.K.

Oligonucleotide-limited transcription has been used to prepare a series of transcripts which allowed the positions of termination by T7 RNA polymerase to be characterized. The technique was used to prepare a set of transcripts from a rabbit β-globin gene which extend in intervals of two nucleotides from the 3' splice site of IVS-1 into the second exon. Splicing efficiency in HeLa cell nuclear extract decreased with decreasing length of the 3' exon, although both steps of the splicing reaction could still be detected with as few as four nucleotides in this exon. No evidence was found for a lower limit to the length of the 3' exon below which splicing would not take place. With longer substrates, the rate of the second step of splicing was increased substantially.

The mechanism of selection between polyadenylation and splicing of immunoglobulin M pre-mRNA is also being investigated.

N 537 CHEMICAL FOOTPRINTING OF SPLICING FACTORS ON PRE-mRNA, Xiaodong Wang and Richard A. Padgett, Department of Biochemistry, University of Texas Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235.

To define the factors that influence the choice of potential sites of pre-mRNA splicing, we have been investigating the interactions between the RNA substrate and components of the splicing machinery in cell free extracts of HeLa cells. We have adapted a high resolution chemical footprinting technique using hydroxy radicals to probe RNA-factor interactions in spliceosomes. Using 3' end labelled pre-mRNA we have detected protection near splice sites due to binding of specific factors. At the 3' splice site we can show separate protections at the 3' intron/exon junction, the polypyrimidine tract and the branch site. The complete set of protected regions is seen only under conditions where the first splicing complex is formed. The polypyrimidine protection is seen under all conditions while the branch site and junction protections can be distinguished by ATP and sequence requirements. The polypyrimidine protection appears to be produced by a non-snRNP factor(s).

N 538 TRANS-SPLICING IN TRYPANOSOMES: DETECTION OF A TRANS-SPLICEOSOME? Kenneth P. Watkins, Shulamit Michaeli, Richard G. Nelson, and Nina Agabian, Intercampus program in Molecular Parisitology, UCSF, Laurel Heights Campus, Suite 150, San Francisco, CA 94143.

All mRNAs of the parasitic protozoa *Trypanosoma brucei* share the same 39 base 5' leader sequence. Recent evidence suggests that the leader is added to messenger RNAs post-transcriptionally via a trans-splicing mechanism; the substrates of this reaction are the ~139 base SLRNA which contains the 39 nt leader at its 5' end and a separately transcribed pre-mRNA. A 'Y' branched RNA species consisting of the 3' 100 nt of the 139 base SL RNA covalently bound to heterogeneous poly A+ RNA via a 2'-5' phosphodiester bond has been detected *in vivo*. This structure is consistent with SL addition in *T. brucei* occuring in a manner mechanistically similar to cis splicing of pre-mRNAs of other eukaryotes; however, in a trans splicing reaction, a 'Y' structure rather than a lariat is generated as an intermediate. Recently, the cloning and sequencing of the U2, U4, and U6 *T. brucei* RNA homologues was reported. That these small RNAs may participate in spliced leader addition in *T. brucei* is suggested by the finding that the U2 RNA is unusual in possessing a striking complementarity to the SL sequence. This complementarity occurs over the same region of U2 RNA that in other eukaryotes is thought to interact with the branch point of pre-mRNAs. In order to assess the possibility that trans splicing utilizes machinery homologous to that used in cis splicing of pre-mRNAs, we have begun fractionating T. brucei extracts; assaying for the 139 nt SL RNA, the Y-branched intermediate, and U-RNAs. Sucrose gradient fractionation of SL RNA-enriched extracts shows that the SLRNA is found in a major 10S peak and a minor ~40S particle, while the majority of the Y-intermediate is found associated with the ~40S region. The U-RNAs follow the same pattern as the SL RNA, suggesting that trans splicing may take place on a spliceosome-like particle involving the *T. brucei* U-RNA homologues. Quantitative analysis of the stochiometry of the trans-splicing substrates and intermediates as well as the U-RNAs in these fractions will be presented.

N 539 GENETIC ANALYSIS OF SIGNAL RECOGNITION PARTICLE FUNCTION, Patrick Brennwald, Xiubei Liao, Diana Hamilton, and Jo Ann Wise; Department of Biochemistry, University of Illinois; Urbana, IL, 61801.

We have identified a small cytoplasmic RNA from Schizosaccharomyces pombe which has several structural properties in common with the 7SL component of signal recognition particle. As judged by sucrose gradient analysis and DEAE column chromatography, this fission yeast RNA is housed in a ribonucleoprotein similar in size (11S) and protein content to mammalian SRP. In addition, the S. pombe particle, like its higher eukaryotic counterpart, forms salt-labile contacts with microsomes. The gene encoding S. pombe 7SL, has been cloned and sequenced, revealing a rather low degree of primary sequence similarity with the mammalian RNA. However, secondary structure analysis shows a similar overall framework and exposes several conserved single-stranded regions which are thought to be important for function of the mammalian RNA. Disruption of the single copy gene demonstrates that 7SL is indispensable for growth in S. pombe, consistent with a role in secretion, as this has been shown to be an essential cellular process in bacteria and budding yeast. Using oligonucleotide-directed mutagenesis, we have recently isolated a set of point mutations in the region most highly conserved between fission yeast and human 7SL. This segment of the mammalian RNA is a binding site for a heterodimer composed of the 68 and 72K canine SRP proteins (V. Siegel and P. Walter, pers. comm.). Our mutant 7SL genes will be tested for their ability to complement the gene disruption at a variety of temperatures. Isolation of suppressors of the expected secretory defects should then uncover genes for S. pombe cognates of the mammalian SRP proteins.

N 540 DEVELOPING AN IN VITRO TRANSCRIPTION SYSTEM FOR TRYPANOSOMES, Joost C.B.M. Zomerdijk and Piet Borst, the Netherlands Cancer Institute H-8, Amsterdam, The Netherlands. To asses how the transcription initiation of genes for the trypanosome surface coat is controlled and to analyse the mechanism of discontinuous mRNA synthesis in trypanosomes, we are developing a cell-free transcription system for trypanosomes. The system consists of a dialysed and concentrated extract derived from Trypanosoma brucei nuclei, assayed with added DNA templates. With these extracts, alone or in combination with heterologous cell-free systems (HeLa extracts, SP6 and T7 RNA polymerase) we find only end-labeling (White, T.C. and Borst, P., 1987, Nucl. Acids Res. 15, 3275-3290), transcription of poly[dI-dC].poly[dI-dC] template but no transcription of any trypanosome DNA (5S RNA, rRNA and mini-exon genes). The trypanosome nuclear extracts contain minimal protease and nuclease activities but high ATP consuming activity. An ATP regenerating system can only partially prevent the ATP depletion. We think that a highly active glycerol kinase is responsible for this. In trypanosomes, glycerol kinase is contained in peroxisome-like glycosomes (Opperdoes, F.R. and Borst, P., 1977, FEBS Lett. 80, 360-364), that may be expected to contaminate nuclear preparations. Indeed, we found that RNA synthesis in trypanosome nuclei and nuclear extracts is inhibited by glycerol addition. Nuclear extracts prepared without glycerol, however, still did not show accurate transcription initiation, indicating that essential factors are missed or that there are other detrimental factors yet to be identified. [This work was supported in part by a grant from the Netherlands Foundation for Chemical Research (SON) with the financial aid from the Netherlands Organisation for the Advancement of Pure Research (ZWO)].

Polyadenylation and 3' End Formation

N 600 SPLICE SITE vs POLY A SITE CHOICE IN AD 5 RNA SYNTHESIS, Guy R. Adami and Joseph R. Nevins, Duke University, Durham, North Carolina 27710

The adenovirus late region is an example of a complex transcription unit in which the production of multiple RNAs is controlled by specific RNA processing events. Adding to the complexity is the fact that one of the early genes (E3) is wholly contained within the late transcription unit and within this overlapping region there are splicing events and poly A site choices specific to the primary transcripts made. The E3 gene which is expressed early after infection contains three poly A addition sites (L4, E3a and E3b) however only the E3a and E3b sites are used. Late after infection the L4 poly A site dominates. The E3 and the late genes are overlapping but differ in promoter site, splicing patterns and time of expression. Analysis of RNA processing of viral genes cloned in plasmids reveals that the L4 poly A site is not used in E3 RNA synthesis because of its location in the primary transcript. After substitution of the L4 poly A site by the E3a site in this system still no polyadenylation occurs at the substituted site. Deletion of splice sites confirms that the L4 poly A site is not used early after infection because it is an E3 intron. The level of control of poly A site choice in the E3 gene is at splicing. We go on to show that regulation of splicing is dependent on upstream sequences in the different primary transcripts early versus late after infection and is not dependent on virally induced trans acting factors.

N 601 IDENTIFICATION AND CHARACTERIZATION OF A REGULATORY DOMAIN IN THE c-Ha-ras ONCOGENE, Justus B. Cohen, Suzan D. Broz, Craig W. Crowley and Arthur D. Levinson, Genentech, Inc., South San Francisco, CA 94080

We are analyzing the human c-Ha-ras proto-oncogene for DNA sequences that control the levels of the gene's product, p21. A region has been identified that profoundly affects p21 expression as measured by the ability of position-12 activated versions to induce foci of transformed Rat-1 cells. The biological activity of this region is maintained upon transfer to a heterologous gene. Using site-directed mutagenesis and gene reconstruction experiments we are defining the precise boundaries of the active domain within this region and it's properties. Our results do not exclude a role for this element in RNA processing.

N 602 COMPLEXES INVOLVED IN PROCESSING AND POLYADENYLATION OF THYMIDINE KINASE mRNA. Fang Zhang, Cathie Heath, and Charles N. Cole, The Molecular Genetics Center and Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756.

We have characterized 3′ end RNA processing of tk pre-mRNA *in vitro*. Processing reactions produced 5′ and 3′ half molecules, and required ATP or an ATP analog. Cleavage, but not polyadenylation, occurred in the presence of 3′ dATP (cordycepin), AMP(CH_2)PP, or EDTA and was dependent on the presence of a poly(A) signal. A large set of linker scanning, internal deletion, and small insertion mutations were analyzed. *In vivo*, these mutants are processed with a range of reduced efficiencies and, with some mutants, at sites that differ from those used to produce wildtype tk mRNA [Zhang, Denome, and Cole, MCB, 6:4611-4623 (1986)]. The patterns and efficiencies of 3′ end RNA processing *in vitro* of mutant precursor RNAs paralleled, both qualitatively and quantitatively, those observed *in vivo*. Gel mobility shift analyses permitted identification of complexes involved in 3′ end RNA processing. Complex formation required the presence of a 3′ end processing signal, ATP or an ATP analog, and incubation at 30°C. Complex formation was analyzed in the presence of various competitor substrates. Excess unlabelled tk or adenovirus L3 precursor RNAs were efficient competitors, but RNAs lacking either AAUAAA or the GU-cluster second element were unable to compete. When the extracts were fractionated by glycerol gradient sedimentation, additional complexes with different mobility shifts were seen with some fractions. Other fractions produced the same mobility shift as was seen when substrate was incubated with the complete extract. Analysis of the components of these complexes is in progress.

N 603 REGULATION OF 3' END PROCESSING OF THE ADENOVIRUS-2 MAJOR LATE TRANSCRIPTION UNIT. K.H. Hales, S. Wilson-Gunn, J.D. DeZazzo and M.J. Imperiale. Dept. of Microbiology and Immunology, The University of Michigan, Ann Arbor, MI 48109.
The major late transcription unit of adenovirus 2 is subject to regulation at the level of RNA processing during the course of infection. Early in infection, mRNA 3' end cleavage at the L2 and L3 poly(A) sites occurs three times less frequently than at the L1 poly(A) site although transcription has been shown to be equimolar across the unit. Late in infection, cleavage at L1 is reduced three-fold relative to the other poly(A) sites. We have characterized the downstream sequence requirements for accurate and efficient cleavage at the L1 and L2 poly(A) sites using deletion, insertion and linker-scanning mutants. In addition, we have established that cleavage of L1 RNA in HeLa cell nuclear extracts requires more than the minimal downstream sequence requirement found in vivo. We are currently investigating the processing of L1 and L2 under infected conditions both in vivo and in vitro. It is possible that poly(A) site selection can be influenced by transcription termination as well as by splice site commitment. We are determining the relative usage of the two sites on single transcripts which have been constructed to allow for examination of the contribution of splicing and polyadenylation signals to regulation of the major transcription unit.

N 604 MESSENGER RNA 3' END FORMATION IN TRANSGENIC PLANTS, Arthur G. Hunt and Robert Graybosch, Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091
We have analyzed the polyadenylation signals from two transcription units from plants. Deletion analysis of the 3' region of a pea ribulose bisphosphate carboxylase/oxygenase (*rbcS*) gene has revealed the presence of a number of cryptic polyadenylation sites in this region. Sequences unusually distant from these sites (between 60 and 137 bases upstream of the normally utilized sites and between 37 and 76 bases upstream from the principal cryptic site) are required for functioning of these sites. Sequences between 13 and 156 bases downstream from the normal sites are required for polyadenylation at these sites. When placed in tandem arrays, the poly(A) sites from the *rbcS* gene direct mRNA 3' end formation at an inverted repeat located between 100 and 160 bases (depending on the construction) from the nearest poly(A) site. The polyadenylation signal from the cauliflower mosaic virus 35S/19S "genes" also directs mRNA 3' end formation at the same inverted repeat, located as far as 900 bases from the nearest known poly(A) site. Our results demonstrate that the RNA sequences that direct polyadenylation in plants differ, in both sequence and in position, from the analogous signals in animals. We are currently studying these two regions in greater detail, with the expectation of defining the rules that govern mRNA 3' end formation in plants.

N 605 INTERVENING SEQUENCES INTERFERE WITH FORMATION OF 3' ENDS OF HISTONE mRNAs. William F. Marzluff, Nunta Chodchoy and Niranjan Pandey. Department of Chemistry, Florida State University, Tallahassee, FL 32306.
Histone genes are the only major class of genes which lack intervening sequences and the histone mRNAs are the only major class of mRNAs which lack polyA. Histone mRNAs end in a stem-loop structure with a six base stem and four base loop. The 3' end is formed by an endonucleolytic cleavage which requires the U7 snRNP. We constructed histone-globin chimeric genes as part of our study of regulation of histone gene expression and introduced the genes into CHO cells by stable transfection. When the histone 3' end was attached to the intact human α-globin gene containing both intervening sequences, three mRNAs were formed. One ended at the histone 3' end and the others (>50% of the total) ended at cryptic polyadenylation sites and were polyadenylated. These cryptic sites are normally used only on 1% of the histone mRNA. The formation of these RNAs was independent of the promoter, since replacing the globin promoter with the histone promoter resulted in the formation of the same 3 mRNAs in similar amounts. A histone-globin chimeric gene constructed using the human α-globin cDNA, which lacked intervening sequences but encoded the same mRNA, gave rise to mRNAs containing only histone 3' ends. The presence of either of the two intervening sequences alone directed the formation of polyadenylated mRNAs. An intervening sequence as close as 150 nt to the 3' end altered 3' end formation. The transcripts were spliced efficiently and the mRNAs were all cytoplasmic. These results suggest that the spliceosome may form on a nascent transcript, interfering with the formation of the histone 3' end and directing polyadenylation.

N 606 TWO PROTEINS CROSSLINKED SPECIFICALLY TO RNA CONTAINING THE ADENOVIRUS L3 POLY(A) SITE, Claire Moore, Jie Chen, and John Whoriskey, Tufts University Medical School, Boston, MA 02111.

Precursor RNA containing the adenovirus L3 poly(A) site is efficiently and accurately polyadenylated when incubated with HeLa nuclear extract and ATP. If these reaction mixtures are exposed to ultraviolet light, proteins in close association with RNA can be crosslinked to the nucleic acid. When the RNA is digested by ribonuclease, fragments of radioactive RNA remain bound to the proteins and allow their detection by separation on SDS-polyacrylamide gels followed by autoradiography. Three proteins of 155kD, 68kD, and 38kD are crosslinked to precursor RNA after as little as two minutes of incubation at 30°. Point mutation of the AAUAAA polyadenylation signal sequence to AAGAAA prevents in vitro activity as well as binding of the 155kD and the 68kD species. Excess precursor competes for binding of these two proteins; excess RNA with the point mutation does not. All three proteins are found on RNA with the β-globin poly(A) site, but only the smaller two are found on RNA with the SV40 late site. Polyadenylation-specific complexes detected by electrophoresis on nondenaturing polyacrylamide gels contain all three proteins; nonspecific complexes contain only the 38kD polypeptide. The two larger proteins are not detected on RNA once it has been processed. By immunoprecipitation, the 38kD protein corresponds to the C protein of hnRNP particles. The 68kD and the 155kD proteins show reactivity with antibody to the Sm proteins of the U class of snRNPs. Ongoing studies are directed at determining the binding sites of these proteins and their possible role in the polyadenylation reaction.

N 607 POLYADENYLATION OF MURINE IgM TRANSCRIPTS IN NUCLEAR EXTRACTS PREPARED FROM LYMPHOID CELLS, Anders Virtanen* and Phillip A. Sharp, Massachusetts Institute of Technology, Cambridge, MA 02139; *present address: Department of Medical Genetics, University of Uppsala, Box 589, S-75123 Uppsala, Sweden.

We have developed an in vitro system for polyadenylation at the murine immunoglobulin membrane and secreted polyadenylation sites. We have shown that both polyadenylation sites and the adenovirus L3 site are active in nuclear extracts prepared from HeLa cells, three different kinds of murine B-cell lines (Wehi 231, AJ9, BCL-1) and in extracts prepared from a plasmacytoma cell line (P9.37.11). Using this system we addressed two questions. Will different RNA substrates show differences in polyadenylation activity if they are compared in the same nuclear extracts, and is the activity of a particular RNA substrate dependent on the origin of the nuclar extract? So far we have been able to show that the adenovirus L3 polyadenylation site is approximately 2-3 times more efficient than the two immunoglobulin sites in nuclear extracts prepared from HeLa and Wehi 231 cells. Our analysis indicated that this difference was caused by differences in the formation of an active polyadenylation complex and not at the subsequent cleavage and polyA-tail addition. A more drastic difference in polyadenylation activity between the different RNA substrates was observed in the nuclear extracts prepared from the plasmacytoma cell line. In these extracts the L3 site was 10 times more efficient than the two immunoglobulin sites. Thus, these data suggested that nuclear extracts prepared from different cell lines had a distinct composition of polyadenylation factors.

Regulation of Gene Expression by RNA

N 700 mRNA DECAY IN E. COLI K-12: STABILIZATION OF DISCRETE mRNA BREAKDOWN PRODUCTS. Cecilia M. Arraiano, Stephanie D. Yancey and Sidney R. Kushner, Dept. of Genetics, Univ. of Georgia, Athens, GA 30602.

The regulation of mRNA stability may be an important element in the post-transcriptional control of gene expression. In prokaryotes, mRNAs are in general rapidly degraded, but little is known about either what controls the rate of breakdown or which enzymes are actually involved in the process. Recently, it has become possible to study general mRNA decay as well as the degradation of specific mRNAs in E. coli through the use of multiple mutants carrying the pnp-7 (polynucleotide phosphorylase), rnb-500 (ribonuclease II) and ams-1 (altered message stability) alleles. The triple mutant stabilizes discrete mRNA breakdown products, which have chemical half-lives 2-4 fold slower than the wild type control. Using a high resolution polyacrylamide gel system, it has been possible to show that there are discrete intermediates in the decay of full length mRNAs. In the case of trxA (thioredoxin) mRNA, the decay process seems to proceed from both the 3' and 5' ends of the molecule. However, most of the breakdown products arise from processing at the 3' end. Work is in progress to resolve whether cleavages occur at unique nucleotide sequences and/or at sites of special secondary structures. (This work was supported in part by GM28760 to S.R.K.).

N 701 ALTERNATIVE RNA PROCESSING IS USED TO CONTROL THE EXPRESSION OF THE GENES REGULATING SEXUAL DIFFERENTIATION IN DROSOPHILA. B. Baker, K. Burtis, T. Goralski, M. Kuroda, W. Mattox, and R. Nagoshi. Dept. of Biological Sciences, Stanford University.

Among major developmental process in higher eukaryotes the regulatory hierarchy that controls sexual differentiation and dosage compensation in Drosophila melanogaster is one of the best understood, at both the genetic and molecular levels, . We have cloned several of the major regulatory genes in this hierarchy [doublesex (dsx), transformer (tra), transformer-2 (tra-2), and maleless (mle)]. All of the regulatory genes in the hierarchy analyzed to date have multiple transcripts. It has been shown (McKeown et al., 1987; Boggs et al, 1987; Baker and Wolfner, 1987; Burtis and Baker, 1987) that the expression of the tra and dsx genes is regulated by alternative RNA processing in the two sexes. Moreover, the effects of mutants at other regulatory genes in this hierarchy on the splicing of the tra^+ and dsx^+ transcripts are sufficient to account for all the functional interactions between these regulatory genes that have been seen in genetic studies (Nagoshi et al , 1987). Consistent with a role in RNA processing the tra-2 gene, which is thought to function immediately upstream of dsx, has a domain of amino acids with striking similarity to that which defines a family of single-stranded RNA binding proteins. P-mediated transformation is being used to determine the cis-acting sequences that are necessary for the alternative splicing of the dsx transcript.

N 702 PHYSICAL AND GENETIC CHARACTERIZATION OF THE MER OPERON: GENE EXPRESSION AND mRNA TURNOVER. B. Diane Gambill, Ike Whan Lee, and Anne O. Summers. University of Georgia, Athens, GA 30602.

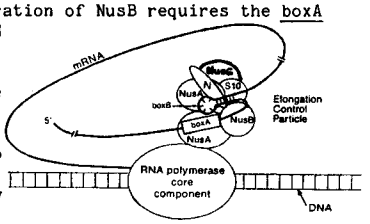
The regulatory and structural genes of the mercury resistance operon, mer, of Tn21 are transcribed from overlapping, divergent promotors. The 3.2 kb Hg(II) inducible structural gene transcript encodes proteins involved in the transport (merTPC) and reduction (merA) of Hg(II). **Using mung bean nuclease digestion and lacZ protein fusions, we have demonstrated** transcription and translation of an open reading frame, merD, located immediately 3' to merA. **Insertion and deletion mutants of merD result in mercury resistance indistinguishable** from wild type; thus, the role of merD in operon function remains unclear. Total mer mRNA is inducible 20-fold and the Mer T, P, C, and A proteins are present at approximately 4:10:1:1 respectively as determined by scanning laser densitometry. **The Mer D protein is** undetectable by conventional biochemical techniques. The half life of each gene of the transcript is ca. 3 minutes as determined by loss of specific mRNA following rifampicin inhibition; **thus, merTPCAD mRNA does not show differential decay which could account for** the very different **amounts of mer proteins. Analysis of the intercistronic DNA sequences** suggests Mer P will be the most efficiently translated of the mer genes. We are investigating differential protein synthesis and decay as parameters which effect the observed protein ratios. **The potential for mRNA stabilization by extensive secondary structure in the 3'** end of the mRNA is being assessed by deletion mutants in the region.

N 703 POST-TRANSCRIPTIONAL EFFECTS IN THE REGULATION OF GENE EXPRESSION BY INTERFERONS, D. Gewert, J. Rosen, G. Stark and I. Kerr, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, England.

The antiviral and growth inhibitory effects of interferons are mediated by the induction of specific mRNAs and proteins in sensitive cells. This is mainly due to an increased rate of transcription of these genes, but some lines of evidence suggest that post-transcriptional effects may also be important in the regulation of gene expression by interferons. We have begun to analyse these effects using two cloned, interferon-inducible genes, 6-16 and 2'-5' oligoadenylate (2-5A) synthetase. The 5' flanking DNA sequences which confer interferon inducibility have been identified and replaced with the promoter and enhancer regions controlling early gene expression in SV_{40}. We have transfected these recombinant genes into mammalian cells and obtained stable lines constitutively expressing high levels of both mRNA species in the absence of interferon. In transfected mouse L-929 cells (which lack an endogenous 6-16 gene) interferon does not detectably alter the steady-state levels of cytoplasmic 6-16 RNA. By actinomycin D chase experiments we have found that this RNA is very stable (t1/2 >16 hours) and interferons have no measurable effect on this stability. This is compatible with the high cytoplasmic levels of this RNA in untransfected cells several days after interferon treatment. In contrast, the 2-5A synthetase RNA is only transiently induced by interferons, and was found to be less stable (t1/2 ~ 4 hours). Interestingly, we have been able to detect a small increase in the half life of this RNA in response to interferons. We are currently extending these studies to investigate effects by interferons on the accumulation of nuclear RNA precursors.

N 704 PROTEIN-RNA INTERACTIONS IN TRANSCRIPTIONAL ANTITERMINATION BY THE N PROTEIN OF PHAGE λ, Jack Greenblatt, Joyce Li, and Robert J. Horwitz, University of Toronto, Toronto, Canada M56 1L6.

We have identified and purified a new E. coli transcriptional elongation factor, called NusG, that allows the N protein of phage λ to antiterminate transcription in the early operons of the phage in a reaction containing only purified proteins. Antitermination by N in vitro requires the E. coli proteins NusA, NusB, NusG, and ribosomal protein S10, as well as an N utilization site (nut site) on the DNA template. Transcriptional elongation complexes that had traversed a nut site in vitro contain near-stoichiometric amounts of N, NusA, NusB, and NusG. Incorporation of NusA into the elongation complex is sequence-independent; incorporation of NusB requires the boxA component of the nut site; and incorporation of N and NusG requires the boxB component of the nut site. Since T1 RNase causes the release of N and NusG, but not NusB, from the elongation complex, we hypothesize that an elongation control particle (ECP) containing nut site RNA and several proteins remains associated with RNA polymerase during chain elongation distal to the nut site (see diagram). The precise natures of the protein-RNA interactions in the ECP are currently under investigation.



N 705 Post Transcriptional Regulation of the Human Transferrin Receptor. David Koeller, John Casey, Bruno Di Jeso, Richard Klausner and Joe Harford. Cell Biology and Metabolism Branch, NICHD, National Institutes of Health, Bethesda,MD. 20892.

The level of transferrin receptor (TfR) mRNA varies with intracellular iron concentration. Addition of an iron source (hemin) to tissue culture cells is followed by a decrease in TfR mRNA level. In the presence of the iron chelator desferrioxamine the amount of TfR mRNA increases. Previously we have shown by nuclear run off assay that in both fibroblasts and erythroleukemia cells that the rate of TfR mRNA transcription varies only 2 to 3 fold in response to manipulations of iron levels. Treatment of the human plasmacytoma ARH-77 with hemin causes TfR mRNA levels to decrease greater than 20 fold within 6-8 hours. In addition to a decrease in the total amount of the full length 5 kb TfR transcript, a new shorter mRNA species appears as a result of hemin treatment. This RNA migrates at a size of approximately 4 kb and is non-polyadenylated. Treatment of the cells with the transcription inhibitor actinomycin-D blocks the iron dependant decrease in TfR mRNA levels normally seen following hemin treatment. The appearance of the truncated, non-polyadenylated species of Tfr mRNA is also blocked by actinomycin-D. Two other DNA transcription inhibitors, (camptothecin and DRB), showed the same inhibitory effect on iron regulation. No significant inhibition of iron regulation was observed following treatment with the protein synthesis inhibitors cycloheximide, emetin or puromycin. Possible mechanisms for these observations are currenly under evaluation.

N 706 ATTENUATION AS A PRINCIPLE FOR THE REGULATION OF CORONAVIRUS GENE EXPRESSION. D.A.M. Konings[1], P.J. Bredenbeek[2], P. Hogeweg[3] and W. Spaan[2] 1) EMBL, 6900 Heidelberg, FRG 2) Inst. of Virology and 3) Bioinformatics Group, Utrecht, The Netherlands.

We have studied the molecular basis which generates stable levels of subgenomic mRNAs increasing with the gene order from the 3' coterminal nested gene structure of the positive stranded coronavirus. Previous experiments have suggested mRNA synthesis to occur via a free leader RNA of about 60-70 bases, which through base-pairing at intergenic regions primes transcription at these sites. A computer-assisted analysis of the recently completed sets of sequences covering the leader encoding and intergenic regions (5 or 6) of the MHV-A59 and IBV-M42 strains shows that, in contrast to the previous theory, differences amongst stabilities of intermolecular base-pairings between the leader and the intergenic regions are not sufficient to determine the mRNA gradients in both MHV and IBV. Neither can the accessibility of the interacting regions on the leader and the negative stranded genome, as revealed by secondary structure analysis, explain the mRNA levels. The nested gene organisation itself, on the other hand, could be responsible for observed mRNA levels gradually increasing with gene order: A blockage of transcriptional elongation at a single site between or at each of the different gene borders causes more potential perturbances of the longer than of the shorter gene transcriptions. Newly and relatively slow initiation events at intergenic regions are proposed as likely candidates for the physical basis of these perturbances, which via temporary pausing can cause attenuation of passing transcripts. However, we expect the levels of mRNA to be further modulated by variations in base-pairing abilities between the leader and intergenic regions and in accessibilities of the base-pairing domains in the two separate molecules. By determining the frequency and rate of initiation, these structural differences will affect the number of newly initiating transcripts as well as the degree of blockage at each intergenic site.

N 707 EVIDENCE FOR A ROLE OF 7S NUCLEAR RNAs IN ONCOGENE TRANSCRIPTION, M.O. Krause and J. Kurz, University of New Brunswick, Fredericton, N.B., E3B 6E1, Canada.

Previous studies in our laboratory have identified a class III 7S nuclear RNA (7SK) in mouse and human cells which stimulates transcription initiation in vitro and shows sequence homology to the SV40 promoter (PNAS 80,7090, 1983). The amount of this RNA present in the cell was found to correlate with transformation and tumorigenicity (Canc. Res. 46: 1990,1986). We used SV40-TsA-transformed mouse cells to time the sequence of activation of cellular oncogenes and class III RNAs, following the switch to the permissive temperature. Among the oncogenes, only c-myc mRNA was found in substantially increased levels in Northern blots following induction of transformation. Class III B2 RNAs were also substantially increased, including a B2 species that hybridized to 7SK RNA. Run-on transcription assays indicated accelerated transcription rates for *myc*, *ras*, *fos* and *p53*. The highest increases, however, were found within 30 min. in class III B2 transcripts (15 fold) preceeding activation of the cellular oncogenes. Activation of 7SK with homology to SV40 Ori probe was also immediate and was followed by activation of c-myc transcription. As a preliminary test for a possible causal involvement of class III RNAs in oncogene activation, we dissected the regulatory regions of SV40 and c-myc genes to produce RNA probes for Northern hybridizations. Regions of homology with 7SK were found within the SV40 21 bp repeats and the c-*myc* cryptic promoter located in the first intron. A strong homology with another nuclear 7S RNA was located within the enhancer region of SV40 and the c-myc intron, but with opposite polarity to 7SK RNA. Results suggest that these RNAs are part of a complex system that ensures modulation of the expression of these genes.

N 708 SITE SPECIFIC ENDONUCLEOLYTIC CLEAVAGE REGULATE THE STABILITY OF E.COLI OMPA mRNA, U.Lundberg, G.Nilsson, O.Melefors, A.von Gabain, Dept.of Bacteriology, Karolinska Institute, Stockholm, Sweden.

mRNA degradation is not well understood compared to other systems involved in the gene expression. Previous work from our group have identified the 5'-non-coding region of the mRNA encoding ompA (outher membrane protein A) to be a determinant of stability. The results were obtained by analysing hybrid transcripts derived from gene fusions between ompA and bla (ß-lactamase). Additionaly it has been found that the ompA mRNA shows a growth rate dependent stability. The reduction of ompA protein synthesis at slow growth rate can be attributed to the altered half-life of the mRNA. More recent in vivo studies have identified cleavages in the 5'-non-coding region of ompA mRNA, using the Tac promoter to overproduce ompA transcript. These cleavages might control the growth rate dependent stability; the stability ot the transcript follows the rate of cleavages at different growth rates. The same cleavages have also been identified in an in vitro system, using SP6 RNA polymerase transcribed RNA and crude cell extract. Cleavages in the bla mRNA have also been identified in vivo and in vitro and they coincide also. We are now involved in purification of the protein causing the cleavages in vitro witch coincide with in vivo cleavages, to characterize and analyse further in which way the observed cleavages initiate the degradation of the entire transcript. The present results show that the endonucleolytic enzyme(s) is not identical to RNaseIII, RNaseE or RNaseP.

N 709 REGULATION OF IRON-INDUCED FERRITIN BIOSYNTHESIS IN K562 ERYTHROLEUKEMIA CELLS, Elena Mattia,Jan den Blaauwen,Jos van Renswoude,Dept.of Biochemistry,Univ.of Amsterdam,1018 TV Amsterdam,The Netherlands.

Ferritin biosynthesis in K562 erythroleukemia cells is dependent on the intracellular concentration of iron. Iron-induced ferritin biosynthesis is mainly regulated at the translational level but in some cell systems both transcriptional and translational control has been suggested. In this study we investigated whether, under a continuous iron supply ferritin biosynthesis is regulated at multiple levels and whether the choice of the operating regulatory mechanism is dependent on the intracellular "iron status". We show that upon incubation with iron the rate of ferritin biosynthesis reaches a maximum after a few hours without detectable variation in the amounts of mRNAs for H and L subunit of ferritin. Thereafter, it decreases while the concentration of ferritin subunits mRNAs gradually rises. After a 24 hr treatment with iron the levels of H and L subunits mRNA are respectively 2-5 fold and 2-3 fold over the control values. Nuclear run-off experiments performed on control and iron treated cells did not reveal differences in the rate of transcription of the ferritin genes. Incubation of cells with iron limiters did not affect the basal levels nor the increase of ferritin subunit mRNAs after exposure to iron. On the basis of these results we conclude that iron-induced ferritin biosynthesis is controlled at two different post-transcriptional sites. We propose that a short term response of cells to iron promotes the activation and translation of stored H and L subunit ferritin mRNA while a long term response is mediated by stabilization of the messages. Studies are in progress to determine the rate of turnover of the ferritin mRNAs as a function of the iron status.

N 710 THE USE OF cRNA PROBES IN THE STUDY OF CALBINDIN mRNA LEVELS AND IN VIVO REGULATION, S. Mayel-Afshar and D.E.M. Lawson, AFRC Institute of Animal Physiology and Genetic Resarch, Babraham, Cambridge CB2 4AT, UK.

The full length calbindin cDNA was inserted into a transcription vector with T_3 and T_7 promoters on opposite strands. In vitro synthesized ^{32}P-labelled transcripts in the anti-sense direction (cRNA cal) were used as probes to detect and quantify calbindin mRNA in various tissues of vitamin D deficient and dosed chicks. Comparisons between calbindin mRNA levels and translational activity in response to 1,25-dihydroxyvitamin D show that the hormone is not only necessary for the expression of the gene but also for its rate of transcription. In other studies, Northern analysis of intestinal total RNA using in vitro transcribed ^{32}P-calbindin mRNA as probe, have shown hybridization to a 300 base band. The nature of this fragment is currently under investigation.

N 711 LIGHT REGULATED TURNOVER OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE SMALL SUBUNIT RNA. Marin, D.L., Shirley, B.W., Senecoff, J.F. and Meagher, R.B. Department of Genetics, University of Georgia, Athens, GA 30602

The nuclear encoded small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase together with the chloroplast encoded large subunit comprise one of the most prevalent enzymes found in green plants. This enzyme is responsible for fixation of carbon dioxide. We have discovered a striking system of light regulated RNA turnover controlling SSU expression. In soybean and petunia, respectively, the SSU encoding genes are strongly transcribed in the light but are transcribed at 32 and 64 fold lower levels in darkness or after far-red light treatment. However, the steady state RNA levels are only 4 and 8 fold lower, respectively, under these repressed conditions. SSU RNA is turning over much faster in the light than in darkness or after far-red light treatment. We are continuing to examine the molecular physiology of this phenomenon and in particular the rate at which both transcription and steady state RNA levels respond to changes in the light regime. The level at which this turnover systems acts (e.g. splicing, polyadenylation, transport, translation) is being investigated. Possible RNA structures which could play a role in the regulation of this process are being determined by primer extension assays of both native and chemically modified RNAs and by computer analysis of RNA secondary structure.

N 712 CLONING OF ILV GENE(S) AND THE REGULATION OF ISOLEUCINE-VALINE BIOSYNTHETIC ENZYMES AND THEIR COGNATE AMINO ACID SYNTHETASES IN E. COLI K-12, Arun K. Misra, Arthur L. Williams and Luther S. Williams, Atlanta University, Atlanta, GA 30314.

The regulatory mutation ilvA538 causes a decrease in the expression of ilvGEDA operon and affects the activity of isoleucyl- and valyl-tRNA synthetases. The hyperattenuation and expression of the ilv biosynthetic operon is due to an increased rate of formation of a complex of valyl- and isoleucyl-tRNA synthetases and the altered form of threonine deaminase of the ilvA538 mutant strain. Wild type threonine deaminase gene (ilvA) was cloned onto pBR322 by employing HindIII insert. Strain PS1150 were subsequently transformed with pBR322 with ilvA gene(inserts). It was found that all isoleucine-valine biosynthetic enzyme levels and that of the branched chain amino acid t-RNA synthetase activities were higher than those of the parental strains. It has been concluded that cloned cells have twice the genetic potential for threonine deaminase ilvA gene on the chromosome and on the plasmid.Leucine's ability to feed-back and inhibit AHAS activity, valine limitation in transformed cells and the derepression of valine activity will also be discussed.

N 713 INHIBITION OF EXPRESSION OF MEMBRANE SKELETON PROTEIN 4.1 BY ANTI-SENSE RNA IN XENOPUS EMBRYOS, Dawn H. Giebelhaus, Douglas Eib, and Randy Moon, University of Washington, Seattle, WA 98195.

Structural proteins associated with the inner surface of the plasma membranes of cells restrict the lateral mobility of integral membrane proteins, support the lipid bilayer, and provide membrane attachment points for cytoplasmic proteins. Membrane skeleton protein 4.1 plays a key role in the membrane skeleton of erythrocytes by enhancing the binding of spectrin to actin. To investigate the role of protein 4.1 in nonerythroid cells, we have cloned Xenopus protein 4.1, examined its pattern of expression and used anti-sense RNA to inhibit its expression in Xenopus embryos. Briefly, cDNAs encoding all of Xenopus oocyte protein 4.1 have been cloned and sequenced, revealing a greater than 90% predicted amino acid identity with human erythroid protein 4.1. Nuclease protection assays reveal a single transcript which is expressed at all stages of development, in contrast to the non-constitutive pattern of expression of spectrin (Giebelhaus et al., *J. Cell Biol.* **105**, 843-845). Anti-sense protein 4.1 transcripts expressed in embryos lead to a drastic reduction in endogenous 4.1 transcripts. Although protein 4.1 transcripts become undetectable by nuclease protection assays, embryos develop normally. Higher levels of anti-sense RNA lead to high embryo mortality. We speculate that low levels of protein 4.1 are synthesized and enable survival in the former instance, whereas higher levels of anti-sense RNA reduce protein 4.1 expression below the minimum level necessary for development.

N 714 EXPRESSION OF HIV ENVELOPE PROTEIN COMPARED TO EXPRESSION OF HBV SURFACE ANTIGEN USING RECOMBINANT ADENOVIRUS VECTORS. J. E. Morin, B. M. Bhat, K. L. Molnar-Kimber, B. B. Mason, S. Dheer, P. K. Chanda, A. J. Conley, A. R. Davis, and P. P. Hung, Wyeth-Ayerst Research, POBox#8299, Philadelphia, PA 19101.

Cultured cells infected with recombinant adenovirus carrying the HBV surface antigen gene express this viral antigen at a level of approximately 0.5 to 1.0 micrograms per million infected cells. Although difficult to quantitate precisely, expression of HIV envelope protein by cells infected with a recombinant adenovirus carrying the HIV envelope protein gene appears to be less than expected. Examination of the mRNA isolated from cells infected with these recombinant adenoviruses suggests that they are of the correct size, with no unexpected splices, and that the relative abundance of messages for HIV envelope protein and for HBV surface antigen is approximately the same. These results are consistent with a post-transcriptional block to expression of HIV envelope protein.

N 715 ANTI-SENSE RNA REGULATES mRNA STABILITY IN DICTYOSTELIUM, Wolfgang Nellen, Hedi Kern and Uschi Saur, Max-Planck-Institut für Biochemie, Abt. Zellbiologie, 8033 Martinsried, West-Germany.

The prespore gene(s) EB4 expresses a 2 kb mRNA in the slug stage of Dictyostelium development, while in vegetative cells an approx. 1 kb mRNA is transcribed. When multicellular slugs are disaggregated, the 2 kb mRNA is rapidly degraded and the 1 kb mRNA reappears. At the same time an EB4 anti-sense RNA is synthesized. When transcription of the anti-sense RNA is inhibited by Daunomycin after disaggregation, the 2 kb mRNA remains stable for at least 3 hours indicating an involvement of the anti-sense transcript in the turnover of the 2 kb mRNA. Cells have been transformed with a 5'portion of the EB4 gene (covering part of the coding region, the 5'untranslated region and some 5'flanking region) fused in anti-sense orientation to an actin promoter. The transformants which contain RNA of the anti-sense hybrid gene throughout development mimick the effect of disaggregation in that the 2 kb mRNA is never accumulated in the slug stage but the 1 kb vegetative mRNA is found. Preliminary data suggest that specific degradation of the 2 kb mRNA can be achieved in-vitro using cytoplasm from cells that accumulate anti-sense RNA. The mechanism of differential RNA destabilization by endogenous anti-sense RNA will be discussed.

N 716 REGULATION OF IL-2 RECEPTOR EXPRESSION BY IL-1 AND TNF : MOLECULAR ANALYSIS. Plaetinck, G., Combe, M.C., Corthésy, P. and Nabholz, M., Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges s/ Lausanne, Switzerland.
In a mouse CTL x rat thymoma somatic cell hybrid, called PC60, IL-2 receptors can be induced by IL-1 and IL-2. Each factor alone already induces significant levels of IL-2Rα mRNA but a strong synergism is observed when both interleukins are added together. The kinetics of induction by IL-1 and/or IL-2 are different. Together these data suggest tha IL-1 and IL-2 operate at different regulatory levels. Induction of PC60 for 3 hours in t presence of cycloheximide shows that the effect of IL-1 is protein synthesis independent PC60 cells transfected with the human IL-2Rα cDNA in a SV40 expression vector were selected for stable constitutive expression of the human α chain. mRNA and protein level coded for by the transfected gene could still be strongly increased by IL-1 but not by IL-2. As it is unlikely that IL-1 regulates transcription from the SV40 promotor this predicts that the information for regulation by IL-1 is contained within the sequence of the IL-2α cDNA. Measurments of mRNA stability after treatment with Actinomycin D indicat that pre-incubation with IL-1 results in a twofold increase in the halflife of the mRNA. Because this is not enough to account for the over 10 fold increase in the steady state level , regulation by IL-1 probably involves additional mechanisms. TNF has the same effect as IL-1 on the expression of the resident and the transfected IL-2Rα gene suggesting that it works via a similar mechanism.
*the mouse homologue of the human "Tac" gene.

N 717 **CHARACTERIZATION OF AN mRNA DESTABILIZING ELEMENT IN THE 3' UNTRANSLATED REGION OF PROTO-ONCOGENE FOS**, Vincent Raymond, Jonathan A. Atwater and Inder M. Verma, Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, CA 92138.
The induced expression of proto-oncogene fos (c-fos), as well as that of several other genes containing AT-rich sequences with an ATTTA consensus motif in their 3' untranslated region, is characterized by rapid and transient mRNA accumulation. The c-fos gene can also transform fibroblasts provided two manipulations are carried out: (i) linkage of viral long terminal repeat (LTR) sequences, and (ii) removal of the AT-rich stretch of 67 nucleotides in the 3' non-coding region. To assess for a possible function of the 67 nt fragment at the post-transcriptional level, we first analyzed in a short-term expression system the decay rate of the mRNAs produced by deletion mutants in the 3' untranslated region and in the COOH-terminal coding domain of the c-fos gene under the control of the v-fos LTR. Using a sensitive RNase protection assay specific for the v-fos 5' end, quantitative analysis of cytoplasmic RNA revealed that the AU-rich sequences confer high instability to c-fos messenger RNA. The AU-rich region was responsible for reduced steady-state RNA levels and increased the rate of degradation of these messages by 8 to 10 fold. Experiments have been performed using various 3' specific probes to elucidate the mechanism of degradation of mRNAs containing the destabilizing element. Preliminary results indicate that the destabilizer region acts as a target for a specific nucleolytic cleavage event. To identify factors involved in mediating this mRNA degradation process, we are utilizing cell-free extracts derived from mouse L cells.

N 718 IN VITRO TRANSCRIPTION BY RNA POLYMERASE III OF CLONED SINE SEQUENCES, Valerie K. Slagel and Prescott L. Deininger, Louisiana State University Medical Center, New Orleans, LA 70112. Recent data suggest that very few SINES are actively transcribed in vivo, despite the activity of most of them in vitro. We chose to study the Monomer repeated DNA sequence from the prosimian, galago crassicaudatus. There are about 150,000 copies of the monomer repeat in the galago genome. This SINE contains an internal RNA polymerase III promoter, which consists of the canonical A and B box sequences and is ancestrally derived from a Met tRNA gene. SINES are believed to move via an RNA mediated process, termed retroposition. The Monomer repeat, gal39, was chosen based on its transcriptional activity in an in vitro RNA polymerase III transcription system. Since SINE family members are present in most RNA polymerase II transcribed genes, they are abundant in hnRNA. To date there is only one clear example of a primate SINE being expressed by its own pol III promoter, and that was in primate brain tissue. To avoid background transcripts, we chose to introduce the gal39 clone into the mouse Ltk- cell line. Cells transiently transfected with gal39 produce specific RNA pol III transcripts, as determined by S1 nuclease protection. However, when cells were cotransfected with gal39 and HSVtk and permanent cell lines were selected by HAT media, no RNA pol III or pol II directed transcripts containing this sequence were detected. This can be explained by either a "global" shutdown of repeated DNA transcription or integration of gal39 into transcriptionally inactive chromosomal locations. To test these hypotheses, we cloned the HSVtk gene next to the gal39 repeat and isolated permanent cell lines, both RNA pol III and II transcribed RNAs containing gal39 sequences were detectable.

N 719 RNA-EDITING IN TRYPANOSOME MITOCHONDRIA, P.Sloof,R.Benne,J.v.d.Burg and H.v.d.Spek. Lab.of Biochemistry,University of Amsterdam AMC, Amsterdam, The Netherlands.
A novel mechanism of gene-expression, RNA-editing, has recently been discovered in trypanosome mitochondria: during or after transcription of the reading frame shift containing gene for cytochrome-oxidase subunit II(coxII) 4 extra, non DNA-encoded, U-residues are included in the coxII mRNA at the frame shift position, thereby providing a continuous readingframe(1). In a first approach to elucidate this mechanism we searched for more RNA-editing events. A second example is provided by a *Crithidia fasciculata* reading frame shift containing mitochondrial gene: the overlapping frames C.URF 2 and 1 (2) are similar to different parts of the single ORF 392 from *Marchantia polymorfa* chloroplasts. At the RNA level the C.URF 2 and 1 reading frames are continuous because of the insertion of 5, non DNA-encoded, nucleotides at the frame shift position. We found no evidence for a second C.URF 2/1 gene version containing these extra nucleotides. A third example is found in the *Trypanosoma brucei*mitochondrial URF 2(T.URF 2). The 3'end of a T.URF 2 cDNA displays considerable sequence difference with that of the corresponding area of the gene which can be explained by insertion of extra U-residues at specific sites, whereas one genomic T appears to be skipped.Hybridization experiments proved the absence of a second T.URF 2 gene containing the extra U's and that the edited transcript is exclusively present in the bloodstream form of *T.brucei*, inwhich the mitochondrion is non-functional. The available data suggest that RNA-editing involves insertion of U's at specific sites in certain mitochondrial transcripts, 3' to purines and is developmentally regulated in a gene specific way.
(1). R.Benne et al, 1986,Cell 46,819-826; (2). P.Sloof et al, 1987, Nucl.Acids Res.15,51-65.

N 720 AN ACTIN ASSOCIATED 3' UTR IN DROSOPHILA CONTAINS A TRANSCRIPTIONAL UNIT, Ann Sodja, J. Papa Rao and Rasheeda S. Zafar, Wayne State University, Detroit, MI 48202.
In analyzing the transcriptional pattern of expression during Drosophila embryogenesis of one of the actin genes, that at 5C3-4 (act5C) on the polytene chromosome, we uncovered expression of a shorter transcript of 0.45 kb. Both this transcript as well as act5C hybridize with a 3' end probe derived from act5C gene, which contains some actin coding sequence as well as the 3' end transcribed and presumed untranslated region (3'UTR). It is unique on the genome and specific to act5C. Using this probe in developmental Northerns, we observe a co-accumulation of a 0.45 kb transcript with act5C mRNA over most of the developmental stages examined. Nucleic acid sequencing of the act5C 3'UTR reveals an open reading frame (ORF) with features typical of eukaryotic translation initiation and termination sequences. Its codon usage is qualitatively similar to that found in other Drosophila structural genes. The S_1 and primer extension experiments indicate a nested arrangement between act5C and the 0.45 kb transcript in that its 5' end resides within the act5C coding sequence, about 20 nucleotides upstream from act5C translation termination signal. In an in vitro translation system, this transcript appears to translate into a protein, the molecular weight of which as well as its pI correspond to that predicted from the size and the amino acid composition of the ORF. These and other data relating to the possible function of the 0.45 kb transcript found within the act5C 3' UTR will be presented and discussed in light of interesting observations made on actin associated 3'UTRs in other organisms.

N 721 ANTISENSE RNA-MEDIATED INHIBITION OF VIRAL INFECTION IN TISSUE CULTURE AND TRANSGENIC MICE, Kathy M. Takayama, Shigeki Kuriyama, Kiran Chada, Susan Weiss*, and Masayori Inouye, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854; *University of Pennsylvania, Phila., PA 19104. Antisense RNA has been employed in both prokaryotes and eukaryotes to regulate the expression of specific genes as well as to elucidate the functions of uncharacterized genes. A particularly interesting application of antisense RNA regulation is the complementary mRNA-mediated inhibition of virus production. Such an antisense RNA-immune system is characterized by the capability of the cell to produce antisense RNAs against viral RNAs, thus interfering with translation of the viral message. We have previously demonstrated, using a prokaryotic model, that the production of phage SP is effectively inhibited by antisense RNA produced in E. coli in the presence of inducer. We have hence attempted to examine the feasibility of an antisense RNA immune system in tissue culture cells and transgenic mice against mouse hepatitis virus (positive, single-stranded RNA virus). Portions of the viral genome were linked to the inducible mouse metallothionein promoter in the antisense orientation. The constructs were transfected into mouse L cells and also introduced into the mouse germline. In preliminary experiments virus was grown in either transfectant cells containing antisense sequences to viral genes 5 and 6 or in control L cells, and cellular extracts were subsequently titered on uninfected L cells. A decrease in plaque-forming titer was observed for virus grown in the transfectant line as compared to the titer for virus grown in control cells. The study of the tissue culture and transgenic mouse systems in parallel enables us to examine the antisense RNA effect both on a cellular level and in the whole organism.

N 722 THE ROLE OF mRNA TURNOVER IN β-INTERFERON GENE REGULATION
Lisa-Anne Whittemore and Tom Maniatis
Harvard University, Cambridge, MA 02138
We have been investigating the possibility that mRNA stability plays a role in β-interferon (β-IFN) gene regulation. β-IFN mRNA begins to accumulate within 1.5 hours after virus infection, reaches a peak within 4-6 hours and drops to a low level by 16 hours. This decrease in mRNA accumulation could be due to a decrease in the rate of transcription or mRNA turnover, or both. To test the possibility that mRNA turnover is involved we have analyzed the transcription of a hybrid gene in which β-IFN regulatory sequences have been placed upstream from a heterologous promoter driving human growth hormone (hGH) gene expression. We find that this hGH fusion gene is inducible by virus or poly(I)-poly(C) when introduced into mouse fibroblasts, but unlike β-IFN mRNA, the level of hGH mRNA remains high 30 hours after induction. However, when the 3' end of the hGH fusion gene is replaced with the 3' noncoding region of the β-IFN gene, the resulting hybrid mRNA displays the same induction kinetics as that of intact β-IFN mRNA. We are currently attempting to localize the sequences responsible for this change in induction kinetics.

N 723 GROWTH RATE DEPENDENT REGULATION OF *E. COLI GND* EXPRESSION MEDIATED BY AN ANTI-SHINE-DALGARNO SEQUENCE LOCATED WITHIN THE STRUCTURAL GENE, Richard E. Wolf, Jr. and Penelope Carter-Muenchau, University of Maryland Baltimore County, Catonsville, MD 21228.
Previous studies with *gnd-lac* operon and protein fusions showed that growth rate dependent regulation occurs at the posttranscriptional level and involves a negative control site between codons 48 and 118 of the *gnd* structural gene. To define the 3' boundary of the internal regulatory region a set of deletions extending from the fusion joint of a growth rate regulated fusion toward the start codon was prepared. Fusions with 78 or more codons of *gnd* showed normal growth rate inducibility whereas fusions with 71 or fewer *gnd* codons were growth rate derepressed. The region surrounding codon 71 is highly complementary to the ribosome binding site on *gnd* mRNA, including the Shine-Dalgarno sequence (SD). The core of this internal homology sequence (IHS) was synthesized, cloned and placed at the junction between the 3' deletion endpoints and *lacZ*. At codons 40, 53, 64 or 69 the core IHS conferred a growth rate inducible phenotype but at codon 13 gene expression was very low and growth rate uninducible. Mutations within the anti-SD confer phenotypes consistent with secondary structure predictions of an RNA folding program. Other mutations are being synthesized and a model for the growth rate dependent regulation will be presented.

N 724 DIFFERENTIAL mRNA STABILITY CORRELATES WITH 3' NON-CODING UnA SEQUENCES,
Daniel H. Wreschner and Gideon Rechavi, Tel Aviv University, Ramat Aviv, Israel 69978.
The stabilities of different mRNA species were analysed in a reticulocyte lysate system under protein synthesizing conditions. The experimental data presented indicate that according to their stabilities at least 3 major mRNA groups may be identified - (a) (Un)A poor mRNAs (e.g. globin) are essentially stable and are only slightly degraded by the 2-5A dependent endonuclease; (b) mRNA species with intermediate (Un)A levels (e.g. Igα and Igμ heavy chain mRNAs) are partially degraded by general ribonuclease activity and further degraded by the 2-5A dependent endonuclease and (c) (Un)A rich mRNA species (such as c-myc and non-skeletal actin mRNAs) are inherently unstable and are extremely sensitive to degradation by general ribonuclease activity. A comparison of non-coding region (ncr) sequences from 92 different mRNAs revealed that transiently expressed mRNAs such as the interleukins, nerve growth factor (NGF), epidermal growth factor (EGF) receptor, c-myc, c-fos, c-myb and several other oncogenes as well as interferon α, β and γ were exceptionally (Un)A rich. It is postulated that differential mRNA stability may be partly determined by the primary nucleotide sequence and in particular by (Un)A sequences within the 3'ncr.

RNA in Translation

N 800 **EVOLUTIONARY RELATIONSHIPS INFERRED FROM RIBOSOMAL RNA SEQUENCES**, Robert Cedergren, Michael Gray, Yvon Abel and David Sankoff, Département de biochimie, Université de Montréal, Department of Biochemistry, Dalhousie University, Halifax, N.S. and Centre de recherches mathématiques, Université de Montréal, Québec H3C 3J7 CANADA.

Sequence-based phylogenies are predicated on the assumption that the molecular evolution of one gene is representative of the evolution of the entire genome. In the absence of more extensive data, we have used the sequences of two genes to draw conclusions about relationships among different organisms. Our data set is composed of the most highly conserved core of both the small subunit and large subunit rRNAs; reliance on conserved elements of secondary structure minimizes problems in the alignment of sequences. The treeing algorithm uses the parsimony criterion and permits the evaluation of suboptimal trees. Robustness of the trees is ascertained by the use of a "bootstrapping" technique. With few exceptions, the trees constructed from the two data sets are congruent. Both trees support the notion of three primary lineages (archaebacteria, eubacteria and eukaryotes) and each is consistent with an endosymbiotic origin of chloroplasts from within the cyanobacteria and of mitochondria from within the purple bacteria. The major inconsistency between the two trees is in the nuclear lineage and concerns the position of the nematode worm, Caenorhabditis elegans; the incongruity may be related to the seemingly rapid rate of rRNA sequence divergence in the branch leading to this organism. In both trees, plant mitochondrial sequences occupy the same anomalous position near the mitochondrial root, which suggest a separate and more recent origin for these mitochondria (or at least the rRNA genes therein). This work was supported by the NSERC of Canada.

N 801 3' MATURATION OF YEAST MITOCHONDRIAL tRNAs, Jeou-Yuan Chen and Nancy C. Martin University of Louisville, Louisville, KY 40292

Maturation of the 3' end of mitochondrial tRNA in S. cerevisiae is achieved by the action of a 3' processing endonuclease and ATP(CTP):tRNA nucleotidyltransferase. Using in vitro synthesized substrates, we have shown that the 3' processing endonuclease correctly processes the 3' terminus of a 5' matured tRNA precursor at the site of CCA addition. It leaves a 5' phosphate on the trailer and a 3' hydroxyl group on the processed tRNA. The 3' endonuclease exhibits no detectable activity toward a precursor with a 5' leader sequence. The apparent requirement of the 3' endonuclease for a 5' matured tRNA precursor as substrate strongly suggests the existence of a favored pathway of mitochondrial processing events initiated with the removal of the 5' leader followed by the excision of the 3' terminus. To conclude the 3' maturation, CCA residues are added to the processed tRNA by a mitochondrial ATP(CTP):tRNA nucleotidyltransferase activity. The tRNA nucleotidyltransferase activity is mitochondrial because it is latent and only detected when matrix marker protein is released upon lysis of the organelle. A comparison of this mitochondrial activity with its cytoplasmic counterpart suggests that the two enzymes are extremely similar. Peptide sequencing and immunological experiments are underway to allow the isolation of the gene(s) encoding these activities derived from different cellular compartments.

N 802 FRAMESHIFTING AT RARE CODONS DURING HIGH LEVEL EXPRESSION OF FOREIGN PROTEINS IN E. COLI. Stephen P. Eisenberg, Synergen, Boulder, CO 80301.

Using a Tac promoter vector for the expression of foreign proteins in the E. coli strain JM109, I have expressed the 107 amino acid protein SLPI, an inhibitor of trypsin, chymotrypsin, and leukocyte elastase. The efficiency of expression is strongly dependent on the sequence of the SLPI gene. A synthetic SLPI gene which uses abundant E. coli codons is expressed efficiently, with SLPI accumulating to 8% of total cell protein (TCP). In contrast, when the SLPI gene is derived from the human cDNA clone which has many codons rarely used in E. coli, the expression level is 0.5% TCP. In an attempt to understand the difference in expression between the two genes, I constructed a SLPI gene in which the 5' half is derived from the synthetic gene, and the 3' half is from the cDNA. When this chimeric gene was induced, two forms of SLPI accumulated. One is the normal SLPI protein and the other is a smaller form of the molecule.

Mutants in SLPI derived by site-specific mutagenesis have been utilized to determine the structure and origin of this smaller protein. The results of expression experiments using these mutants show that this shortened protein is truncated due to a (+1) frameshifting event at codon #58 leading to a premature stop codon in the +1 frame. The frameshifting occurs in the cDNA-derived half of the gene at the second of two tandem AGG codons (very rare codons for E. coli), and appears to be due to the depletion of rare tRNAs (probably the AGG readers) since turning down the expression of the gene prevents framshifting.

N 803 TRANSLATIONAL ENHANCER SEQUENCES PRESENT WITHIN THE 5'-UNTRANSLATED LEADERS OF PLANT VIRAL mRNAs, Daniel R. Gallie[1,2], Michael Wilson[2] and Virginia Walbot[1], [1]Stanford University, Stanford, CA 94305 and [2]John Innes Institute, Norwich, U.K.
Many plant and animal RNA viruses possess an extensive untranslated leader at the their 5' end. In addition to their probable role in RNA dependent replicase recognition, they play an important part in determining the translational efficiency of viral mRNA expression. We have examined the effect that leader sequences from a variety of RNA viruses can have on the translation of reporter mRNAs in vivo. The leader from tobacco mosaic virus (TMV) was found to be the most stimulatory, enhancing translation up to several hundred fold. This enhancement has been observed in electroporated protoplasts from a variety of plants, in microinjected Xenopus laevis oocytes, and in prokaryotic systems. Deletion analysis of the 67 base TMV leader determined that the subsequence involved in disome formation was responsible for the enhancement observed in X. laevis oocytes. In contrast, mutations within this same subsequence had little effect on translation in protoplasts, suggesting that inherit differences do exist between plant and animal systems at the translational level.

N 804 *IN VITRO* TRANSLATION OF A BICISTRONIC mRNA ENCODING A HUMAN CYTOMEGALOVIRUS ENVELOPE GLYCOPROTEIN COMPLEX (gcII), D. R. Gretch and M. F. Stinski, Dept. of Microbiology, University of Iowa, Iowa City, IA 52242.
A gene family encoding the human cytomegalovirus glycoprotein complex II (gcII) was cloned into an SP6 expression vector. A monocistronic mRNA encoding the first open reading frame (ORF) and a bicistronic mRNA encoding the first two ORFs of the gene family were synthesized *in vitro*. The mRNAs were translated *in vitro* in rabbit reticulocyte lysates with or without canine pancreatic microsomal membranes. Non-glycosylated and glycosylated gene products were immunoprecipitated by monoclonal antibody 9E10, which is specific for the virion envelope glycoprotein complex gcII (gp47-52 complex), as well as human convalescent anti-CMV serum. Northern blot analysis with antisense RNA probes detected a predicted 1.62 kb mRNA transcript in the cytoplasm of infected human fibroblasts. Our results imply that gcII is synthesized predominantly from a bicistronic mRNA in infected cells.

N 805 TRANSLATIONAL REGULATION OF A HUMAN GENE via A DEFINED GENETIC ELEMENT: FERRITIN AND THE IRE, M.W. Hentze, S.W. Caughman, T.A. Rouault, J.B. Harford and R.D. Klausner; CBMB, NICHD, NIH, Bethesda, MD 20892
In eukaryotic cells, cytoplasmic mRNA is either polysome-associated and actively translated or stored as non-translated messenger-ribonucleoprotein particles (mRNPs). Little is known about the molecular mechanisms underlying the regulation of this distribution. The biosynthesis of the ubiquitous intracellular iron storage protein ferritin is highly regulated at the translational level by iron. Under conditions of low iron availability, the majority of ferritin mRNA is associated with free mRNPs. Increasing intracellular iron load leads to translational activation of ferritin mRNA and its shift into polyribosomes. By deletional and mutational analysis we identified the iron-responsive element (IRE) in the non-translated 5' leader sequence. The IRE is both necessary and sufficient for iron regulation. A synthetic 26 base oligodeoxynucleotide was shown to function as an IRE and transfer regulation to heterologous cDNAs of indicator genes. The translational control of these hybrid mRNAs is correlated with the regulation of the polysome/mRNP ratio in an iron-dependent fashion. This provides the opportunity to study the structural features of the IRE and to identify the trans-acting factor(s) that determine the cytoplasmic fate of these regulated mRNAs. In addition, the IRE will serve to develop a new class of translationally regulatable expression vectors.

N 806 MOLECULAR CLONING AND STRUCTURAL FEATURES OF THE RIBOSOMAL DNA OPERON FROM TOXOPLASMA GONDII, Ann Huot, Alain Tremblay, Françoise Boisvert, Gilles Richer and Roger C. Levesque, Laval University, Quebec, Canada, G1K 7P4.

Toxoplasma gondii is an ubiquitous parasite which infects humans and many other animal species. Studies on the ribosomal DNA genes of T.gondii were initiated by construction of genomic and cDNA libraries. Tachyzoïtes were cultivated in Vero cells and purified by centrifugation using a dextran-glucose density gradient. Total DNA and RNA were sequentially extracted from purified tachyzoïtes using standard biochemical techniques. The RNA sub-units were characterized by denaturing/non-denaturing gel electrophoresis and found as 3 major components of approximately 28S, 14-18S and 4-5S. Total DNA fragments between 10 Kb and 25 Kb partially restricted with Sau 3A were utilized to construct a genomic library in the phage λ2001. The packaged phages were plated on E.coli NM539 for genetic selection of Spi⁻ phenotype recombinants and 3,4 x 10^4 phages were calculated to encompass the parasite genome. Initial screening of the library for ribosomal DNA genes was plaque hybridization using total RNA. Parasite recombinant phages were isolated, insert DNA sub-cloned into pTZ18R and physically mapped using 7 restriction endonucleases. T.gondii RNA components were used as probes, specific homology was confined to a 12 Kb DNA unit and the genes localized by the Southern-type gel hybridization assay. Thus, we present evidence of the structural organization of the ribosomal DNA genes and efforts are under way for sequencing the 5' region of this regulon.

N 807 **Studies on the Mechanism of Cap Independent Translation of mRNA in Mammalian Cells: Randal J. Kaufman[Δ], Monique V. Davies[Δ], Jerry Pelletier[◊], Debra D. Pittman[Δ], and Nahum Sonnenberg[◊,Δ]Genetics Institute, Cambridge, MA and the[◊]Department of Biochemistry, McGill University, Montreal, PQ, Canada**

Poliovirus infection of primate cells induces rapid shutoff of host protein synthesis, whereas translation of poliovirus mRNA is not inhibited. This shutoff is thought to be mediated by a polio encoded polypeptide, p2A, which induces cleavage of the eukaryotic initiation factor 4F polypeptide p220. When cells are infected with adenovirus and then poliovirus, the adenovirus late mRNAs are efficiently translated, whereas host mRNAs are not. We have studied the requirements for the ability of adenovirus late mRNAs to be translated in polio infected cells. A dihydrofolate reductase expression vector that contains the adenovirus tripartite leader at its 5' end was transfected into COS-1 monkey cells. When the cells were subsequently infected with polio virus, host mRNA translation was shut off whereas the mRNAs derived from the transfected plasmid were efficiently translated. We are presently analyzing translation from mRNAs which have various portions of the adenovirus tripartite leader deleted.

To further investigate the role of the polio p2A protease in shut off of host protein synthesis, we have introduced the p2A coding region into an expression plasmid. COS-1 cells transfected with this plasmid exhibit cleavage of p220. At 48 hr post-transfection, the transfected cells look severely stressed and they do not translate dihydrofolate reductase when its mRNA is transcribed from a co-transfected plasmid. These results suggest that p2A mediated cleavage of p220 results on host protein synthesis shut off upon polio virus infection.

N 808 REPROCESSING OF HOST tRNA: A PHAGE T4 MODEL-SYSTEM OF RNA CLEAVAGE-LIGATION CATALYZED BY PROTEINS G. Kaufmann*, M. Amitsur*, D. Chapman*, M. J. Gait[@], R. Levitz*, L. Jorissen[&], I.Morad* and L. Snyder[&], Tel-Aviv University*, Israel 69978, Medical Research Council[@], Cambridge, CB2 2QH England, Michigan State University[&], MI 48824.

During T4-infection of E. coli strains carrying the prr gene, the host $tRNA^{Lys}$ is cleaved by anticodon nuclease (ACNase) 5' to the wobble base. The 2':3'-P> & 5'-OH cleavage termini are later repaired in consecutive polynucleotide kinase and RNA ligase reactions. ACNase is specified by two known genes: prr, which restricts T4 mutants lacking polynucleotide kinase (pnk⁻) or RNA ligase (rli⁻); and T4 stp, whose mutant allele suppresses prr-restriction. ACNase was reconstituted in vitro from the 29 residue stp polypeptide (pstp), itself inactive; and the prr encoded component(s) (pprr). By analogy with RNase A, pstp is proposed as a catalytic subunit of ACNase while pprr and perhaps other subunits impart protein folding environment and tRNA substrate recognition. ACNase depends on ongoing protein synthesis, suggesting its coupling to a translation-step in which the vulnerable tRNA partakes. prr-restriction is caused by depletion of vital host tRNAs in the absence of pnk and rli-mediated repair. Yet, stp, pnk and rli may have evolved to benefit T4 directly. Thus, in the prr-context, reprocessing may adapt host tRNAs to T4-codon-usage. In the absence of prr, the T4-reprocessing genes could exercise other roles, perhaps also connected with RNA cleavage-ligation. Reprocessing provides a model system for RNA-splicing pathways that ignore intron-structure and are driven by protein-enzymes. However, the utilization of pre-existing RNA substrates in response to a change in the cellular environment is a novel principle, perhaps a prevalent one.

N 809 EIGHT $tRNA^{TRP}$ AMBER SUPPRESSORS IN C. ELEGANS ARE EXPRESSED DIFFERENTIALLY, Kazunori Kondo, Betsy Makovec, Humaira Amaira and Robert H. Waterston, Washington University School of Medicine, St. Louis, Mo.63110.

The $tRNA^{Trp}$ gene family in C. elegans consists of 12 members. DNA sequencing revealed these genes encode identical tRNAs with 2 exceptions which contain each a single base change, G19 to A19 and G46 to A46, respectively. 8 of these $tRNA^{Trp}$ genes have so far been converted to amber suppressors: sup-5, sup-7, sup-21, sup-24, sup-28, sup-29, sup-33 and sup-34. These suppressors have been recovered in reversion analysis of amber alleles of a nervous system mutant, unc-13, a sex-determination mutant tra-3, or a morphological mutant dpy-20. Identification of the various suppressors used synthetic oligomers complementary to the anticodon region of the $tRNA^{Trp}$ gene to detect the single base change in the anticodon (CCA to CTA). To investigate the activity of each suppressor gene in different tissues, cross suppression tests were carried out against amber alleles of 4 genes presumably expressed in different tissues: unc-13, tra-3, dpy-20 and a muscle-structure-affecting gene, unc-52. Since molecular nature of these genes are not characterized yet, we compared the extent of rescue of mutant phenotype qualitatively or semi-quantitatively, relying on the assumption that the heterozygote sup/+ produces half as much suppressor as homozygote sup/sup. sup-29 is consistently the weakest, and is only detectable against tra-3. The other 7 suppressor activities vary depending on the gene tested; against dpy-20 or unc-52, they showed similar activities, while they vary widely against unc-13, suggesting that the expression of these $tRNA^{Trp}$ genes are regulated in tissue specific and/or temporally specific manner.

N 810 OPAL SUPPRESSOR PHOSPHOSERINE-tRNA TRANSLATES UGA CODON IN GLUTATHIONE PEROXIDASE mRNA, Byeong J. Lee*, Guy T. Mullenbach†, Nelly Avissar+, Harvey Cohen+ and Dolph Hatfield*, *Lab. Exp. Carcinogenesis, NCI, NIH, Bethesda, MD 20892, †Chiron Res. Lab., Chiron Corp., Emeryville, CA 94608 and +Dept. Pediat., U. Rochester Sch. Med. and Dent., Rochester, NY 14642.

We have been investigating the structure, expression and evolutionary origin of an opal suppressor phosphoserine tRNA (PS-$tRNA_{UGA}$) and its corresponding gene (see Hatfield, TIBS 10: 1, 1985, for review and Lee et al., PNAS 84: 6384, 1987, for subsequent work). Presently, we are examining the possible role of PS-$tRNA_{UGA}$ in the occurrence of selenocysteine [which is present in several enzymes and is coded by UGA (see Stadtman, FASEB J. 1: 375, 1987 for review)] in glutathione peroxidase (GPx). Our studies have focused on the direct incorporation of phosphoserine from PS-$tRNA_{UGA}$ into GPx. Bovine GPx cDNA was cloned into the Bgl II site of pSP64T [which is between the Xenopus globin gene leader and trailer sequences (Krieg and Melton, NAR 12: 7057, 1986)] for generating mRNA (XGPx mRNA). PS-$tRNA_{UGA}$ was prepared as described (Hatfield et al., PNAS 79: 6215, 1982). Synthesis of the readthrough protein product (which reacts with antibodies specific to cellular GPx) in rabbit reticulocyte lysates programmed with XGPx mRNA is dependent upon PS-$tRNA_{UGA}$. We are presently confirming the direct incorporation of phosphoserine into the GPx site coded by UGA by peptide analysis of the protein product and sequencing of the peptide corresponding to the UGA codon.

N 811 AUTOGENOUS CONTROL OF THE ESCHERICHIA COLI S10 RIBOSOMAL PROTEIN OPERON, L. Lindahl, J. M. Zengel, and P. Shen, Department of Biology, University of Rochester, Rochester, NY 14627

The S10 r-protein operon of E. coli is negatively regulated at both the transcription and translation levels by r-protein L4, the product of the third gene of this eleven gene operon. Translation control results from inhibition of translation initiation of the first gene. Transcription control is due to termination in the leader.

Genetic analysis of the translation control shows that, whereas the promoter proximal half of the 173 base leader is dispensable, a stem-loop structure located 50-65 bases upstream of the first initiation codon is absolutely required. This region may include the site for L4 binding to the S10 leader.

Secondary structure mapping suggests that an AU rich region immediately upstream of the Shine-Dalgarno sequence (SDS) alternates between two structures, being single stranded in form I and base paired in form II. The SDS itself is partially base paired in both forms. We suggest that L4 can shift the equilibrium between the two structures and that the base pairing of the AU rich sequence in form II is responsible for the L4-mediated inhibition of translation, perhaps because the AU-rich region must be single stranded to augment interaction between SDS and 16S rRNA. This notion is consistent with the observation that deletion of the AU-rich region reduces translation efficiency by about 10-fold.

N 812 TRANSCRIPTION OF A HUMAN TRANSPOSON-LIKE ELEMENT IS DIRECTED FROM UPSTREAM PROMOTERS: A MODEL FOR TRANSLATIONAL CONTROL, A. Gregory Matera and Carl W. Schmid, University of California at Davis, Davis, CA 95616.
The transcriptional activity of a highly-repeated human retrotransposon-like family, called THE-1, has been studied in human tissue and cell culture. Both strands of THE-1 are present in several discrete-length poly A+ RNAs. Primer extension, Northern blotting, and the structures of cDNA clones show that these THE-1 RNAs cosediment with polysomes, but are usually the products of upstream transcription units. The THE-1 LTR provides the polyadenylation processing site for two transcripts, which result from non-THE promoters. Another cDNA clone has a genomic locus that has been rearranged. Examination of clones of two genomic loci containing DNA that is homologous to a single-copy flanking region of the cDNA shows evidence for a retroviral insertion mechanism. A fourth transcript, containing an internal THE-1 member in the probable sense orientation, is greatly enriched in HeLa polysomal RNA fractions. Experiments are proposed to test the hypothesis that a THE-1 element could "stowaway" on another cistron in order to make its own gene products. Thus, mobile elements that lack their own promoters could still direct transposition translationally.

N 813 HOMOLOGOUS GENES OF MOUSE 4.5S hybRNA ARE FOUND IN ALL EUKARYOTES AND THEIR LOW-MOLECULAR-WEIGHT RNA TRANSCRIPTS INTERMOLECULARLY HYBRIDIZE WITH 18S rRNA, E. Stuart Maxwell and Joyce Liu, North Carolina State University, Raleigh, NC 27695-7622.
Previously we have reported the isolation and characterization of a new mouse lmwRNA transcript of 87 nucleotides which can intermolecularly base-pair with 18S rRNA and mRNA sequences [PNAS (1986) 83:7621]. Using synthetic DNA oligonucleotides complementary/homologous to this lmwRNA sequence, we have examined the conservation of its gene sequence and expression as a lmwRNA transcript across evolution. Southern blot analysis has demonstrated that 4.5S hybRNA-homologous genes (in single or low copy number) are found in yeast (*Saccharomyces cerevisiae*), *Xenopus laevis*, and man as well as the rodents mouse, rat, and hamster. Northern blot analysis revealed its expression in yeast and *X. laevis* as lmwRNAs of approximately 130 and 100 nucleotides, respectively, as compared with mouse/rat/hamster species of 87 nucleotides. Yeast and *X. laevis* 4.5S hybRNA homologs were isolated by hybrid-selection and tested for their intermolecular hybridization capabilities using Northern blot analysis. Both the yeast and *X. laevis* homologs specifically hybridized with eukaryotic 18S rRNA sequences. The ability of mouse 4.5S hybRNA as well as the yeast and *X. laevis* homologs to base-pair with homologous/heterologous 18S rRNAs demonstrated the conserved nature of this lmwRNA:18S rRNA interaction in eukaryotic evolution. The occurrence of 4.5S hybRNA-homologous genes in such evolutionarily divergent organisms as fungi, amphibia, and mammals, coupled with their expression as lmwRNA transcripts possessing similar lmwRNA:RNA intermolecular hybridization capabilities, strongly argues for a common, conserved, and required biological function for this lmwRNA in all eukaryotes.

N 814 TRANSLATIONAL EFFICIENCY AND mRNA STABILITY IN *E.COLI* , John E.G. McCarthy, Toni Belev, Birgit Schauder and Peter Ziemke, GBF, Braunschweig, West Germany.

1. The determinants of translational efficiency in the *E.coli atp* operon and in the bacteriophage λ morphogenetic region have been studied. Manipulation of the translational initiation regions of the various genes borne either on the chromosome or on plasmids has yielded information about the relationship between mRNA structure and translational efficiency.
2. The factors influencing mRNA stability have also been investigated. A system based on the *E.coli atp* operon has been developed for examining the effects of various RNA structures such as REP or RNase III sites upon physical and functional stabilities. The significance of these data in relation to the mode and control of mRNA degradation is discussed.

N 815 BINDING OF mRNA BY EUKARYOTIC INITIATION FACTORS: TENTATIVE RULES FOR 5' AND INTERNAL INITIATION, William C. Merrick, Richard D. Abramson, and Thomas E. Dever, Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106.
Early experiments by Kozak indicated that mRNAs were bound to ribosomes at their 5' caps and then "scanned" to their initiating AUG in an ATP-dependent manner. Recent studies in our laboratory combined with the reports of others indicate the mechanism for this process involves ATP and three mRNA specific protein synthesis initiation factors: eIF-4A, eIF-4B, and eIF-4F. eIF-4F binds to the m^7G cap structure in an ATP-independent manner, followed by binding of eIF-4B and ATP-dependent unwinding by eIF-4A. The function of these factors would suggest that steric availability of the m^7G cap and secondary structure in the 5' non-coding region are influential in the competitive selection of mRNAs for translation. Our most recent studies also indicate the possibility of efficient initiation of mRNAs in a cap-independent manner. Based upon the high efficiency of eIF-4A and eIF-4B binding to single stranded RNAs, we predict that these factors have the ability to bind to single stranded regions of an mRNA (minimum of 20 bases required) and allow ribosome binding at these points, perhaps followed by "scanning" to appropriate initiating AUGs. This mechanism would be appropriate for both uncapped mRNAs as well as those few viral examples of "internal initiation" which have been reported. A combination of both mechanisms could allow for quite complex patterns of translation control such as seen with yeast GCN 4 mRNA. (Supported by NIH grant GM 26796.)

N 816 DIRECT EVIDENCE FOR A TRANSLATIONAL ROLE FOR THE POLY(A) TRACT OF mRNA, David Munroe and Allan Jacobson, Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655.
We have proposed a model for the function of the 3'-poly(A) tract of mRNA which postulates that: a) an interaction between poly(A) and a cytoplasmic poly(A)-binding protein enhances the efficiency of translational initiation; b) mRNAs with relatively long poly(A) tails have a translational advantage over mRNAs with shorter poly(A) tails; and c) the regulatory mechanisms which ensure the efficient translation of poly(A)$^+$ and poly(A)$^-$ mRNAs may be quite different (**NAR** 11: 6353, 1983; **Cell** 36: 1017, 1984). The data to support this model are mostly indirect, including: a) a correlation between the adenylation status of mRNA and its translatability *in vivo* and *in vitro*, b) a demonstration that exogenously added poly(A) is a potent competitive inhibitor of the initiation of translation of A$^+$, but not A$^-$ mRNAs *in vitro*, and c) a correlation between the stability of the *Dictyostelium* poly(A)-binding proteins and the rate of translational initiation (**MCB** 7: 965, 1987; **PNAS** 84: 1858, 1987). To test this model directly we have constructed derivatives of pSP65 which direct the synthesis of synthetic mRNAs with different discrete poly(A) tail lengths and compared the relative efficiencies with which such mRNAs are recruited into polysomes in reticulocyte extracts. Using this assay, poly(A)$^-$ mRNAs are found to be recruited into polysomes less efficiently than poly(A)$^+$ mRNAs. The defect in poly(A)$^-$ mRNAs affects translational initiation, is distinct from the phenotype associated with CAP-deficient mRNAs, and appears to have a profound affect on the formation of mRNPs.

N 817 RIBOSOMAL RNA AND UGA-DEPENDENT PEPTIDE CHAIN TERMINATION, Emanuel J. Murgola[1], H. Ulrich Göringer[2], Albert E. Dahlberg[2] and Kathryn A. Hijazi[1], M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030[1], Brown University, Providence, RI 02912[2].
We have isolated a novel UGA-specific nonsense suppressor in *E. coli*. It corrects neither the other two termination codons (UAA and UAG) nor several UGA-related missense mutations, and its response to UGA mutations is context dependent. By genetic mapping procedures and cloning into a low copy number plasmid, the suppressor was localized to *rrnB*, one of seven ribosomal RNA (rRNA) operons. DNA sequence analysis revealed that the unique change in *rrnB* was a deletion of C1054 in 16S rRNA. This result suggests an important, direct role for rRNA in codon recognition, at least for accurate polypeptide chain termination. We propose that the 1054 region is a site of binding or action of release factor 2 (RF2). Proper interaction of RF2 with rRNA then allows the nearby triplet 5'-UCA-3' (nucleotides 1199-1201 or 1202-1204) to base pair with the mRNA termination codon UGA. Deletion of C1054 alters the structure of 16S RNA and changes this proposed sequence of events. Furthermore, the proximity of two conserved 5'-UUA-3' triplets, nucleotides 956-958 and 1211-1213, raises the possibility that the greater 1054 area may also be a site for release factor 1. Finally, it may be that "recognition" of termination codons is primarily a property of rRNA rather than the release factors.

N 818 EFFECT OF POINT MUTATIONS IN 16S rRNA AROUND THE DECODING SITE (C1400) ON THE ABILITY OF RIBOSOMES TO INITIATE POLYPEPTIDE SYNTHESIS. D. Negre, R. Denman, P. Cunningham, C. Weitzmann, K. Nurse, J. Colgan and J. Ofengand. Roche Institute of Molecular Biology, Roche Research Center, Nutley NJ 07110 USA.

Our previous work has shown that a tRNA bound to the ribosomal P site can be crosslinked via its 5'-anticodon base to C1400 of the 16S RNA. This base is located in a highly conserved single stranded sequence of the 16S rRNA for which no functional role is known.

In order to understand the role that is apparently played by C1400 and its surrounding nucleotides, functional 30S subunits were reconstructed from *E. coli* 30S r proteins and 16S rRNA synthesized *in vitro* by T7 RNA polymerase. Upon addition of 50S subunits, the particles were assayed for protein synthesis using a coupled transcription-translation system which measured the formation of fMet dipeptide and which is dependent on the presence of initiation factors. Under our conditions, the synthesis of dipeptide was proportional to the amount of ribosomes added and 30S plus 50S were as active as 70S. The synthetic particles were 58% as active as those reconstituted with natural 16S rRNA. In contrast, addition of 7 extra nucleotides at the 3' end of the synthetic 16S rRNA molecule decreased the activity to 11%. Changing C1400 to U did not affect the function of the mutant compared to the synthetic particles while C→A or C→G substitutions decreased the activity two-fold. Deletion of C1400 produced inactive particles as did deletions or insertions in the residues surrounding C1400. The precise steps of the initiation process which are affected by these mutations are currently under study.

N 819 THE ORGANIZATION AND FUNCTION OF TWO NOVEL BRAIN mRNAs, Felicia V. Nowak, Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06268.

Two previously undescribed cDNAs from the preoptic area of rat brain have been cloned and sequenced. Each contains an open reading frame (ORF) which encodes a hypothetical peptide similar to gonadotropin releasing hormone (GnRH). Additional ORFs of $\geq$63 nucleotides on both strands raise the possibility that a much greater percentage of the total number of nucleotides could be translated. Interestingly the same is true for the GnRH mRNA cloned by others. These messages are distinct from other neuropeptide mRNAs which give rise to multiple small peptides by proteolytic cleavage of large precursor molecules.

What determines which ORFs are translated and what is the function of the nontranslated segments? The mRNA and cRNA sequences have been analyzed for probability of translation of their multiple ORFs by inspection of AUG-neighboring nucleotides, calculation of nucleotide position and frequency, and analysis of codon usage. The putative peptides have been considered for compatibility of amino acid composition, charge, and predicted secondary structure with functionality. The predictive analyses have been correlated with Northern blot hybridization for multiple regions of both mRNA and cRNA strands. In situ hybridization, immunocytochemistry, and determination of the complete gene sequences will be used to extend these correlations as a model of translational control of peptide expression in eukaryotic brain.

N 820 *IN VITRO* SYNTHESIS, ASSEMBLY, AND FUNCTION OF 30S RIBOSOMES CONTAINING BASE CHANGES AND DELETIONS IN THE 16S RNA. J. Ofengand, P. Cunningham, R. Denman, C. Weitzmann, K. Nurse, and J. Colgan, Roche Inst. of Mol. Biol., Roche Research Center, Nutley NJ 07110.

The *E. coli* 16S rRNA gene, placed under control of the T7 promoter, was an efficient template for *in vitro* synthesis of full-length transcripts identical to natural 16S RNA except for the lack of all 10 methylated bases, 3 extra G residues at the 5' end, and an A2→G2 change. Upon addition of *E. coli* 30S ribosomal proteins, particles were formed which were physically indistinguishable from 30S ribosomes. Assembly required modified reconstitution conditions. Upon addition of 50S subunits, codon-dependent P-site binding of tRNA, EFTu-dependent A site binding, and codon-dependent but initiation-independent polypeptide synthesis were 1/2-2/3 of 30S reconstituted from natural 16S RNA and 1/3-1/2 that of isolated 30S. UV-induced crosslinking of P-site bound AcVal-tRNA to C1400 was preserved. Thus, modified bases and a correctly terminated 5'-end of 16S RNA are not essential for any of the tested partial reactions of protein synthesis.

In the decoding site region, identified by the tRNA anticodon crosslink at C1400, 11 mutants were made by single and double cassette mutagenesis. All of the mutants could form 30S ribosomes, and no specific proteins appeared to be absent. The C1400 residue was changed to U, A, or G, an additional C or U was inserted between C1400 and G1401, and single base deletions at or on either side of C1400 were constructed. The base changes at C1400 had only moderate effects. Certain of the deletion or insertion mutants were largely inactive in some assays but retained or even increased their activity in others. Thus far, only deletions of G1401 and C1402 block all reactions.

N 821 EPSILON, A PROKARYOTIC TRANSLATIONAL ENHANCER SEQUENCE, Peter O. Olins, Shaukat H. Rangwala & Catherine S. Devine, Biological Sciences, Monsanto Company, Chesterfield, Missouri, 63198.

The sequence determinants for efficient translation initiation in *E. coli* often extend into the proximal part of the coding sequence of an mRNA. Consequently, foreign coding regions are often poorly translated when introduced into *E. coli* expression vectors. We have evaluated various natural prokaryotic translation signals in an attempt to overcome this problem. One ribosome binding site (derived from gene 10 of the bacteriophage T7) was particularly useful. Embedded within this sequence is a novel element (denoted "Epsilon") which appears to be responsible for the effect. Moreover, Epsilon can function even when present in different parts of the mRNA, indicating that it is functioning as a translational "enhancer". A model for the mechanism of action of Epsilon will be presented.

N 822 **Identification of a third tRNA binding site on ribosomal RNA by chemical probing and primer extension.** James M. Robertson, Danesh Moazed and Harry F. Noller, Thimann Laboratories, University of California at Santa Cruz.

A third binding site for tRNA has been described for both eukaryotic and procaryotic ribosomes. The fact that the site is specific for non-aminoacylated tRNA distinugishes it from the acceptor (A) and peptidyl (P) sites. One of us (JMR) recently described the biochemical and topographical characteristics of the site on *E. coli* ribosomes (J.Mol.Biol. *196*: 525, 1987), and the evidence suggests that the site could serve as an exit (E) site for tRNA leaving the ribosome during the translocation step of elongation. Additional features of the E site are that the integrity of the CCA end of the tRNA is required for binding, whereas correct codon-anticodon interaction is not crucial.

Since the E site is still merely conceptual, the primary objective of the present study was to ascertain whether the tRNA could protect any residues of rRNA towards chemical modification. We have found that E site-bound tRNA shields G_{2112} and possibly U_{2111} in domain V of 23S rRNA in *E. coli* ribosomes from reaction with kethoxal. These residues could base-pair with the CCA end on tRNA; however, this conclusion must be tempered by the fact that these two nucleotides are not strictly conserved among the 23S rRNAs from various species. It is also known that the protein L1 binds to the same region of rRNA, so that the shielding could result from tRNA interaction with L1, if this led to an altered binding mode of the protein with the rRNA. Topographically, the presence of L1 in the E site allows us to draw limits to the location of the site, since the position of L1 on the 50S ribosomal subunit is generally accepted. This region is in the translational domain of the ribosome, consistent with existing data that tRNA passes through the E site during elongation.

N 823 PRIMARY EFFECTS OF ALLELOCHEMICALS: THE NUCLEAR ENVELOPE EXAMINATION, Matthew Ryuntyu, Department of Agronomy and Soil Science, University of New England, Armidale. N.S.W. 2351.

Transfer of minerals across membrane would be inhibited by any action that changes permeability or alters the energetics associated with the process (Einhellig, 1986; In Science of Allelopathy, Eds. A. Putnam & C. Tang, John Wiley & Sons, N.Y.:171). Scopolamine interference with mineral absorption is the result of effects on nuclear envelope (membranes). We suggest that these compounds caused an increase in membrane permeability to both cations and anions, allowing a non-specific efflux of ions. Moreover, sunflower root mitosis was slowed or prevented by scopolamine. The root cells had irregular nuclei and large internal globules, probably indicating food reserves were not properly utilized.

Balke (1985: ACS Symposium Series 330, Washington:161) confirmed that allelochemicals caused K^+ leakage from tissue through the drop in membrane potential or by structural alterations. How compound solubilize in the membrane remains unclear, but such changes infer other physiological effects.

N 824 TRANSLATIONAL COUPLING ACTIVATES A GENE START LACKING INHERENT INITIATION ACTIVITY, Deborah A. Steege and Mona R. Ivey, Duke University, Durham, NC 27710

Genes V and VII, immediately adjacent in the filamentous phage f1 genome, have properties of a translationally coupled gene pair. The initiation site sequences of the downstream gene (VII) show no activity in vitro in ribosome binding assays with the phage mRNAs or in vivo in a VII site-lacZ fusion, but they do drive β-galactosidase synthesis if all of gene V is present upstream of the VII site-lacZ fusion. Deletion analysis identifies the gene V translational initiation signal as the region required for VII site activity. The process of upstream translation, not the gene V initiation site sequence or the gene V product, is what activates the VII site: an amber mutation in gene V results in loss of activity, suppression partially restores it, and the VII site functions if placed downstream from a translated region other than gene V. VII site activity is proportional to the level of upstream translation, and extremely sensitive to the position at which it terminates. Although the VII site is predicted to be sequestered in structure involving f1 sequences immediately upstream, the isolated site is not activated if these sequences are deleted. Expression of the minor capsid protein encoded by gene VII thus results from strict translational coupling, with the start site itself apparently lacking the features required for independent recognition by ribosomes. The nature of mutations which confer independent activity, as well as the VII site's relationship to other initiation and reinitiation sites, will be discussed.

N 825 ANALYSIS OF CAPPING OF IN VITRO mRNA TRANSCRIPTS, Sue Ann Theus and Charles Liarakos, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The expression vector pSP65.OV was used to prepare ovalbumin messenger RNA (SP65.OV mRNA) by in vitro run-off transcription of the linearized plasmid DNA with SP6 Polymerase. The rate of protein synthesis directed by uncapped SP65.OV mRNA in the rabbit reticulocyte cell free translation system was approximately 10% that of hen ovalbumin mRNA. Synthesis of capped mRNA by including the cap analog 7-methylguanosine triphosphoguanosine (m7GpppG) in the transcription reaction. improved the translation rate of SP65.OV mRNA only slightly over the uncapped mRNA. This result suggested that only a small portion of the in vitro transcripts had become capped. We have developed a rapid and reliable assay using phenyl boronate agarose column chromotography (PBA:60) to measure the amount of cap incorporated into the mRNA transcripts. This assay enabled us to determine that the lower rate of capped SP65.OV mRNA translation was not due to low capping efficiency but may result from deletion of part of the ovalbumin mRNA 5' leader sequence from pSP65.OV.