TRANSLATIONAL CONTROL

Organizers: William Merrick, Albert Dahlberg and Olke Uhlenbeck

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Molecular Biology of RNA

K 001 CIS-DOMINANT MUTATIONS CAN BE USED TO TEST MODELS FOR LONG RANGE RNA-RNA INTERACTIONS IN THE GENOME OF COLIPHAGE QB, Mills, D., Khanna, R., Merz, P.A., Priano, C., and Jacobson, A.B., Department of Microbiology and Immunology, SUNY-Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, NY 11203 and Department of Microbiology, Life Sciences Building, SUNY at Stony Brook, Stony Brook, NY 11794

We are exploring the role(s) that long range RNA-RNA interactions may play in defining bacteriophage genome conformation and RNA virus gene expression.

Genetic systems have been developed for creating site-directed deletion and insertion mutations in the RNA genome of coliphage QB. By supplying the wild-type phage proteins(s) in trans from bacterial plasmids, we have been able to map many cis-dominant mutations that seem to affect only non coding RNA functions (1).

A model for the secondary structure of QB RNA which contains a 1500 nucleotide hairpin has been generated from electron microscopy (Jacobson, A.B. in preparation) and by solution conformation studies (Skripkin, E. and Jacobson, A.B. manuscript in press). We are now attempting to correlate and test mutations (many with RNA folding defects) against this model for QB genome structure. We are hopeful that these studies will enable us to elucidate the role(s) that RNA conformation plays in the mechanism of coliphage gene expression.

1. Mills, D., Priano, C., Merz, P.A., and Binderow, B. (1990) J. of Virology, 64, pp. 3872-3881.

K 002 STRUCTURE AND CATALYTIC FUNCTION OF RIBONUCLEASE P, A RIBOZYME, Norman R. Pace, James W. Brown, Alex B. Burgin, Sylvia C. Darr, Elizabeth S. Haas, Dirk A Hunt and Drew Smith, Department of Biology, Indiana University, Bloomington, IN 47405.

RNase P cleaves leader sequences from pre-tRNAs. In the eubacteria *Bacillus subtilis* and *Escherichia coli*, RNase P is composed of protein (119 amino acids) and RNA (ca. 400 nucleotides). *In vitro*, at high salt concentrations, the RNA alone is an efficient and accurate catalyst. The high salt (or RNase P protein) is thought to screen electrostatic repulsion between enzyme and substrate RNAs.

The secondary structures of the eubacterial RNase P RNAs are being elucidated using a phylogenetic comparative approach to test base pairing possibilities. Variation among known RNase P RNAs is substantially due to the presence or absence of discrete structural domains scattered in a highly conserved core of homologous sequence and secondary structure. It is clear that the conserved core contains the RNase P activity: a synthetic RNase P RNA, consisting of only the conserved structure (263 nt), has nearly native activity. Comparative analysis of RNase P RNAs from more diverse organisms provides further perspective on the conserved core of the RNA and identifies structural features useful for designing new synthetic RNase P RNAs.

A photoaffinity approach is being used to identify RNase P RNA residues that are located at or near the catalytically active site. A mature tRNA containing a photolabile azidophenacyl group on the 5' phosphate (the substrate phosphate) was bound to RNase P RNA under reaction conditions. UV-irradiation resulted in high-efficiency crosslinking of the tRNA to RNase P RNA. Cross-linked nucleotides in the RNase P RNA, potentially involved in the reaction, were identified by primer extension. The analysis has been carried out with RNase P RNAs from three disparate eubacteria: *B. subtilis*, *Chromatium vinosum* and *E. coli*. The same two discrete regions, of only a few nucleotides each, were crosslinked in each type of RNA. The crosslinked sequences are highly conserved and located in the core of the phylogenetic structure model.

The action of RNase P requires divalent cations: Mg^{2+}, Mn^{2+} or Ca^{2+}. There has been no evidence to distinguish whether divalent cations are required for the structure of the RNA, or for the catalytic mechanism. The possible structural role for divalent cations was tested by crosslinking experiments as described above. At high monovalent ionic strength, in the absence of divalent cations, RNase P RNA - tRNA crosslinks can form, in some instances nearly as efficiently as in the presence of Mg^{2+}. The sites of crosslinking are the same in the presence or absence of Mg^{2+}. These observations suggest that the global and local conformations of the enzyme and substrate RNAs are proper for catalytic function in the absence of divalent cations. The absolute requirement for divalent cations for catalysis by RNase P therefore indicates that divalent cations are intrinsic to the catalytic mechanism.

Substitution of deoxyribose instead of ribose at the pre-tRNA site cleaved by RNase P reduces the rate of cleavage by 10^3 - to 10^4 - fold. A model for the mechanism of RNase P action and the involvement of the substrate 2'-OH has been developed.

K 003 STRUCTURES AND STABILITIES OF RNA HAIRPINS, Ignacio Tinoco, Jr., Vincent Antao, Chaejoon Cheong, Marco Molinaro and Gabriele Varani, Department of Chemistry and Laboratory of Chemical Biodynamics, University of California, Berkeley, CA 94720.

Hairpin loops are ubiquitous in the structures of RNAs. They can provide nucleation sites to define the folding pathways of large RNAs with complex secondary and tertiary interactions. They are often recognized by regulatory proteins which help control translation and transcription. Hairpin loop sequences are conserved and regulated. We have used NMR to determine the conformations of several RNA hairpin loops, and we have measured absorbance melting curves to determine their thermodynamic stabilites. The main hairpin studied contained the sequence 5'...C(UUCG)G...3' with the loop nucleotides in parentheses. This is one of the most common loops found in ribosomal RNAs. A detailed structure was obtained for this hairpin (1). We have studied variants of this sequence to establish the role of the conserved nucleotides; we have also studied completely different sequences. General patterns are emerging. They include:

1. Most bases in a loop are base paired or otherwise hydrogen bonded. In the (UUCG) tetraloop the first U forms a reverse wobble pair with the G; the C amino group hydrogen bonds to a phosphate. Only the middle U is not hydrogen bonded (except to water). In a presumed nonaloop with sequence (AUUUCUGAC) there are actually three base pairs: an A•C, a U•A, and a U•G. Thus, only the central three bases of the nine bases in the loop are not paired.
2. Bases in a loop stack on the base pair at the top of the stem that closes the loop; the stack may continue in large loops.
3. Sugar conformations of some of the nucleotides in a loop are 2'-*endo*. Double helical RNA is usually in A-form with 3'-*endo* sugars; however, 2' *endo* sugars have a larger distance between phosphates. Thus the 2'-*endo* nucleotides can more easily bridge the distance between the closing base pair. Changing from 3'-*endo* to 2'-*endo* costs a negligible amount of free energy increase.
4. One of the phosphates in the loop will have a *gauche-trans* conformation instead of the usual *gauche-gauche* conformation. This allows the necessary reversal in direction of the polynucleotide chain; however, it does lead to a free energy increase. This may be compensated by base-phosphate hydrogen bonding.
5. Very small changes in conformation can lead to significant changes in the free energy of formation of a hairpin. For example, loss of one hydrogen bond in the loop can mean a gain of 1.5 kcal of free energy at 25°and a decrease of 10° in melting temperature.

1. C. Cheong, G. Varani & I.Tinoco, Jr., *Nature*, **346**, 680-682 (1990).

K 004 PROGRESS IN PREDICTING RNA STRUCTURE, Douglas H. Turner, L. He, J. A. Jaeger, A. Peritz, J. SantaLucia, Jr., Department of Chemistry, University of Rochester, Rochester, NY 14627, R. Kierzek, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland and M. Zuker, Division of Biological Sciences, National Research Council of Canada, Ottawa, ON K1A 0R6 Canada

In principle, free energy minimization provides a way to predict RNA structure. A simple nearest neighbor model currently predicts known secondary structures with roughly 70% accuracy (1). Little is known, however, about the sequence dependence of stability for non-base-paired motifs. Thermodynamic and spectroscopic results will be presented for helixes with internal loops. The results indicate current models for internal loops are oversimplified (2). Loop asymmetry and hydrogen bonding are two factors that contribute to loop stability, but others remain to be identified. Complete understanding of the molecular interactions determining structure should also help modelling of RNA structure in three dimensions.

1. J. A. Jaeger, D. H. Turner, and M. Zuker, Proc. Natl. Acad. Sci. U.S.A. **86**, 7706-7710 (1989).
2. J. SantaLucia, Jr., R. Kierzek, and D. H. Turner, Biochemistry **29**, 8813-8819 (1990).

Recognition of tRNA

K 005 YEAST $tRNA^{Asp}$-ASPARTYL tRNA SYNTHETASE: THE CRYSTAL STRUCTURE OF A CLASS II SYSTEM, Dino Moras, Marc Ruff, S. Krishnaswamy, Marcel Boeglin, André Mitschler, Alberto Podjarny, Arnaud Poterszman, Bernard Rees and J.C. Thierry, Laboratoire de Cristallographie Biologique, Institut de Biologie Moléculaire et Cellulaire du CNRS, 67084 Strasbourg, France.

Aspartyl-tRNA synthetase belongs to the subfamily of enzymes without the Rossman fold (Eriani et al., Nature 347 (1990) 203-206). The structure of the binary complex between yeast $tRNA^{Asp}$ and aspartyl-tRNA synthetase has been solved to 3 Å resolution, using MIR and solvent flattening techniques. The asymmetric unit of the crystals (orthorhombic space group $P2_12_12$) contains 1 molecule of the dimeric synthetase (125 kD) and 2 tRNAs (75 nucleotides each). A molecular two fold symmetry axis relates the two monomers and the two bound tRNA molecules. The resulting non crystallographic symmetry was used for phase improvement. The protein is rather globular (approximately 90x90x50 $Å^3$). The tRNA molecules interact with the enzyme on their variable loop side. Interactions between the two molecules involve two domains: the end of the CCA stem and the anticodon loop. Both parts are approached via their deep grove side. The angle between the two limbs of the L-shaped tRNA molecule is smaller in the complex than in the structure of the free tRNA. The difference is essentially due to a conformational change of the anticodon stem and loop. Despite sequence similarities, the CCA end of $tRNA^{Asp}$ adopts a helical structure at variance with the conformation observed for $tRNA^{Gln}$ in the complex with glutaminyl tRNA synthetase (Rould et al., Science 246 (1989) 1135). These results show the flexibility and the adaptability of the tRNA molecule to the protein, a good example of induced fit.The catalytic domain of the protein is formed by a antiparallel central sheet flanked by α helices. This domain contains the 3 sequence motifs characteristic of class II synthetases. Motif 2 includes a large loop which interacts with the discriminator base G. Another structural motif, barrel like, is in close interaction with the anticodon.

K 006 THE ROLE OF THE ANTICODON IN tRNA IDENTITY, LaDonne H. Schulman, Leo Pallanck, Heike Pelka and Shihong Li, Department of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, NY 10461

The fidelity of protein synthesis depends on highly specific interactions between tRNAs and aminoacyl-tRNA synthetases to produce properly aminoacylated tRNAs for translation. Using an *in vitro* approach whereby tRNA substrates containing base substitutions are tested for aminoacylation with purified enzymes, we, and others have shown that the anticodon contains sites which specify the amino acid acceptor identity of a number of tRNAs.[1] Ideally, these *in vitro* studies would be accompanied by parallel *in vivo* studies which establish the charging identity of a tRNA under normal physiological conditions where all 20 synthetases are in competition for the tRNA substrate. Studies with amber suppressor tRNAs have clearly shown the importance of the anticodon for the identity of many tRNAs *in vivo*,[2], however suppression assays require a change in the tRNA anticodon and are therefore not suitable for studies of tRNAs having important identity elements in this sequence. For this reason we have developed a new approach permitting *in vivo* analyses to be carried out without any constraints on the anticodon.[3] Our method involves constructing base changes in the anticodon of the *E. coli* initiator tRNA, thereby allowing it to read a new codon. Expression of the tRNA *in vivo* allows the effect of these base changes on its identity to be evaluated by exploiting the unique feature of the initiator tRNA to commence protein synthesis from a complementary initiation codon in a reporter gene. Subsequent purification and N-terminal sequencing of the reporter protein establishes the charging identity of the mutant initiator tRNA, indirectly. Conversion of the methionine anticodon CAU to the isoleucine anticodon GAU has been found to produce a switch in the *in vivo* identity of the tRNA from methionine to isoleucine. An additional single base change from GAU (Ile) to GAC (Val) eliminates the insertion of isoleucine into protein by the tRNA and leads to insertion of only valine. Similarly, conversion of GAU (Ile) to GAA(Phe) leads to insertion of mainly phenylalanine. Thus, single base changes in the anticodon of a tRNA are seen to have dramatic effects on aminoacylation specificity *in vivo*. Additional experiments have shown that both the middle and 3' anticodon bases are important to Val identity and all three anticodon bases specify Ile and Phe identity *in vivo*. The initiation assay is expected to be useful for determination of identity elements in a wide variety of tRNAs *in vivo*.

1. L. H. Schulman, Prog. Nucl. Acid Res. Mol. Biol. 41, in press.
2. J. Normanly and J. Abelson, Ann. Rev. Biochem. 58, 1029-1049 (1989).
3. R. Chattapadhyay, H. Pelka and L.H. Schulman, Biochem. 29, 4263-4268 (1990).

K 007 STRUCTURAL BASIS OF GLUTAMINYL-tRNA-SYNTHETASE ABILITY TO DISCRIMINATE AMONG tRNA'S, Thomas A. Steitz, Mark A. Rould and John J. Perona, Departments of Molecular Biophysics and Biochemistry and Chemistry and the Howard Hughes Medical Institute, Yale University, New Haven, CT

Crystallographic refinement of E. coli glutaminyl-tRNA synthetase (GlnRS) complexed with its cognate tRNA and ATP at 2.5 Å resolution has revealed details of a sequence specific interaction between the enzyme and the anticodon as well as the acceptor stem region of the tRNA. Complexation with the tRNA buries 2,700 Å of the GlnRS surface from solvent, far more than becomes buried upon formation of an antigen-antibody complex. Not all interactions, however, are sequence specific. Thus far, 3 to 5 bases in the anticodon loop and 7 bases in the acceptor stem are implicated by the structure to be involved in discrimination among tRNA's. The anticodon loop region of the enzyme-bound tRNA differs extensively from the crystal structures of the other unbound tRNA's. The protein stabilizes pairing of nucleotides 32 and 38, and 33 and 37, thereby extending the anticodon stem and leaving only the three anticodon bases unpaired and splayed out. Each of these three bases binds snugly into its own pocket in the protein, with single short polypeptide segments being responsible for most of the discriminating interactions. In all three cases, an arginine or lysine of the short segment "pulls" the base into its pocket through interaction with an adjacent phosphate. Peptide backbone atoms and side chains of these peptide segments form hydrogen bonds with Watson-Crick groups of the base. In addition, GlnRS makes discriminating interactions with base-pairs 3-70 and 2-71 via the minor groove and disrupts base-pair 1-73 (Rould et al., Science 246: 1135-1142 [1989]). Finally, other distortions in the $tRNA^{Gln}$ structure that occur upon complex formation may play a role in sequence specific binding.

A long loop of protein that extends from the anticodon binding domains to the active site region may be responsible for transmitting the signal that the correct anticodon bases are bound to the active site. This loop is seen to interact with the conserved KMSK sequence that in turn interacts with the ATP.

The Structure and Function of Ribosomes

K 008 THE DERIVATION OF 3-D MODELS FOR E. COLI 16S AND 23S RIBOSOMAL RNA, AND THE USE OF SITE-DIRECTED CROSS-LINKING TO STUDY BINDING OF mRNA AND tRNA, Richard Brimacombe, Max-Planck-Institut für Molekulare Genetik, Abteilung Wittmann, Berlin-Dahlem, Germany.

For a number of years our laboratory has been applying RNA-protein and intra-RNA cross-linking techniques to the study of the three-dimensional folding of ribosomal RNA in situ in the ribosomal subunits. Up to now over 60 sites of cross-linking to individual ribosomal proteins have been localized on the 16S or 23S RNA, and a similar number of intra-RNA cross-link sites has also been analysed. By incorporating these data into the phylogenetically established secondary structures of the RNA molecules, and combining them with other topographical information, we have been able to construct models for both the 16S and 23S RNA. Our current experiments are taking two directions. First, we are extending our cross-linking studies to improve the resolution and accuracy of the models. Since so little is known about the shapes of the individual ribosomal proteins, the further study of the RNA-protein topography is unlikely to be of much help in this context, and we are therefore concentrating on obtaining more intra-RNA cross-linking data. Secondly, we have begun a programme to correlate the RNA models with data relating to the binding of ribosomal ligands, using a site-directed cross-linking approach. In this method, a photo-reactive label is introduced into the ligand concerned at a desired position, the ligand is bound to the ribosome under appropriate conditions, and the ribosomal proteins or sites on the RNA which subsequently react with the photo-label are analysed. This approach is being applied to the study of the path followed through the ribosome by mRNA, the binding of tRNA to the A, P or E sites, and the path followed by the growing peptide chain. Computer graphics is being used to correlate the information, and the latest status of these projects will be presented.

K 009 STRUCTURE-FUNCTION RELATIONSHIPS OF RIBOSOMAL RNA, Albert E. Dahlberg, Section of Biochemistry, Brown University, Providence, RI 02912

The evidence is now quite convincing that ribosomal RNA plays a central role in the functional activity of the ribosome. A number of approaches have been used to explore the function of ribosomal RNA during the different stages of protein synthesis. In our laboratory, we have utilized the technique of site-directed mutagenesis focusing primarily on phylogenetically conserved single-stranded sequences in both 16S and 23S rRNA of *E. coli*. Mutations in 16S rRNA have been constructed at the anti-Shine-Dalgarno sequence and the 790 loop, important for IF3 binding (initiation); at the 530 loop and 1400 region, important for proper decoding (elongation); and in helix 34 which is thought to be involved in base pairing to UGA stop codons (termination). The 23S rRNA has been mutagenized at the binding sites for EF-G and EF-Tu (the 1067 and 2660 loops) and at the peptidyl transferase region in the central loop of domain V. Recently, considerable attention has been given to the possibility of base pair interactions between enhancer sequences in messenger RNAs and specific sequences in 16S rRNA to selectively increase the production of particular polypeptides. We have used site-directed mutagenesis to investigate the possibility that certain regions of 16S rRNA are involved in this type of regulation of translation. The results of studies on these and other mutants will be discussed.

K 010 HIGH RESOLUTION ANALYSIS OF RIBOSOMAL RNA FRAGMENTS, Peter B. Moore[1]; Susan M. White[1]; Alexander A. Szewczak[1]; Yuen-Ling Chan[2] and Ira G. Wool[2], [1]Departments of Chemistry & Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511, and [2]Department of Biochemistry & Molecular Biology, University of Chicago, Chicago, IL 60637.

Two fragments of ribosomal RNA are being characterized at high resolution by NMR techniques: helix I from 5S RNA of E. coli, and the α sarcin stem/loop from rat liver 28S RNA. The proton spectrum of helix I has been completely assigned, and a three-dimensional model has been derived from it using the usual two-dimensional NMR techniques. This model provides information about the structure of GU base pairs and unpaired terminal bases that may be applicable to RNA molecules in general.

The α sarcin loop contains a highly conserved, 17 base sequence that is important for the elongation activity of ribosomes in all species. It is the target of the antiribosomal agents α sarcin and ricin. Alpha sarcin cleaves it at a single position and ricin depurinates it at an adjacent residue. Both events are lethal to ribosomal activity. The NMR data indicate that the loop is highly structured, albeit less stable than the stem to which it is attached. The progress made in analyzing the structure of this fragment will be discussed.

K 011 A DOMINANT COLD-SENSITIVE MUTATION IN 16S rRNA SUGGESTS HELIX SWITCHING DURING RNA PROCESSING. Carol S. Dammel and Harry F. Noller. Sinsheimer Laboratories, UCSC, Santa Cruz, CA 95064.

We mutagenized a 16S rRNA gene randomly with hydroxylamine and screened for conditional dominant phenotypes. Out of several that turned up, all but one were found to be due to a C to U transition at position 23 of 16S rRNA, in the middle base pair of the 5'-proximal helix. Second-site suppressors were found, the most straightforward of which restored Watson-Crick base pairing by a G to A transition at position 11. More intriguingly, suppressors were also found in the 5' leader region. The upstream suppressors disrupted potential base-pairing interactions between the upstream sequence and one of the strands of the 5' helix. Although the 5' terminus of most of the mutant 16S rRNA is processed correctly, we observed two processing anomalies: (1) some of the mutant 16S rRNA has a truncated 5' terminus, and (2) extra fragments, originating from the 5' leader region, are associated with mutant ribosomes. We propose that the C23U mutation causes an imbalance between the competition between formation of the 5'-proximal helix and the competing upstream helix at low temperature, and that persistence of the competing helix interferes with late events in maturation of the structure of the 5' region of 16S rRNA. Pairing of the upstream region with internal sequences of 16S rRNA may be part of a transient structure that forms during rRNA processing.

Prokaryotic Protein Synthesis

K 012 **INITIATION OF mRNA TRANSLATION,** **Claudio O. Gualerzi, Anna La Teana, Roland Brandt, Roberto Spurio, Manuela Severini and Cynthia L. Pon,** Laboratory of Genetics, Dept. of Biology, University of Camerino, 62032 Camerino (MC) Italy and Max-Planck-Institut für Molekulare Genetik, 1000 Berlin 33, Germany

Knowledge of the mechanism of mRNA selection by ribosomes during initiation and identification and control of the molecular determinants of the efficiency and fidelity of this process are of utmost importance for both theoretical and practical reasons. A brief outline of the current views concerning these topics will be presented. To test the influence of various elements of the mRNA on the efficiency and fidelity of translation and to study the role of the initiation factors in these processes, we synthesized some defined, simple, modular genes specially designed for these purposes. Thus, a large number of model mRNAs have been prepared by in vitro transcription of these genes and their activity tested in the individual steps of initiation. Since, in addition to the best-fit AUG initiation codon, other wobbling initiation triplets (GUG, UUG, AUU) are found in nature, we have investigated the influence that these rare initiation triplets have on translation and clarified the mechanism by which IF3 autoregulates its own expression by selectively destabilizing the AUU-containing initiation complexes. Our results suggest that the cell uses the rare initiation triplets not so much to attain lower levels of translational expression but rather as targets for regulatory mechanisms aimed at select genes. Using similar model mRNAs, we were able to determine the function of the Shine-Dalgarno sequence in the initiation process. In addition, by binding, competition and crosslinking experiments we were able to show that while the initiation factors do not significantly affect the K_a of the mRNA-ribosome complexes and of the Shine-Dalgarno interaction, they can influence the position and the topographical neighborhood of the mRNA within its ribosomal binding site, presumably in preparation for the decoding of the initiator tRNA in the ribosomal P-site. Finally, evidence for a domain structure in IF2 and some new aspects of its function in the initiation process will be discussed.

K 013 POST - TRANSLATIONAL MODIFICATIONS AFFECT INITIATION FACTOR FUNCTION. John W. B. Hershey, Hubert Schwelberger, Hyun Ah Kang, Zeljke Smit-McBride, Joachim Schnier, Sang-Yun Choi and Randal J. Kaufman*, Dept. of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616, and *Genetics Institute, 87 CambridgePark Drive, Cambridge, MA 02140.

Protein synthesis is affected by post-translational modifications of numerous proteins comprising the translational machinery. Some modifications are irreversible and may be required for activity; others, like phosphorylation, are reversible and may be involved in regulation. The conversion of a Lys residue to hypusine (Hpu) in eIF-5A (formerly called eIF-4D) is an example of the former. The unmodified protein, obtained by expression of eIF-5A cDNA in *E. coli*, lacks activity in an *in vitro* assay for Met-puromycin synthesis, but gains partial *in vitro* activity when the Lys is converted to deoxy-Hpu by reaction with spermidine (in collaboration with E. Wolff and M.-H. Park, NIH). To study the requirement of Hpu in eIF-5A *in vivo*, two genes were cloned in yeast that encode similar (90% amino acid identity) proteins homologous (ca. 60% identity) with mammalian eIF-5A. Disruption of both genes indicates that eIF-5A is essential for growth. A mutant protein, in which the site of hypusination is altered to Arg thereby preventing the modification, does not support growth, reinforcing the *in vitro* finding that Hpu is essential. Phosphorylation of eIF-2 on the α-subunit correlates with translational repression *in vivo* and prevents eIF-2B-catalyzed GDP/GTP exchange *in vitro*. Possible sites of phosphorylation at Ser^{48} and/or Ser^{51} were evaluated by transient expression of mutant forms of eIF-2α cDNA transfected into COS-1 cells, where Ser residues at positions 48 and 51 were substituted with Ala and/or Asp. Evidence indicates that only Ser^{51} is phosphorylated in these cells, but replacement of Ser^{48} with Ala reduces the effect of phosphorylation at Ser^{51}. The overexpressed mutant forms of eIF-2α readily exchange into the endogenous eIF-2 complex and affect translation rates by interfering with the phosphorylation mechanism. Besides stimulation by the mutant eIF-2α(Ser^{51}Ala) of the translation of vector-derived mRNAs and adenovirus mRNAs, the mutant eIF-2α protein reduces repression of protein synthesis caused by heat shock. *Supported by NIH grant GM22135.*

K 014 MECHANICS OF RIBOSOME BYPASS ACROSS THE GENE *60* CODING GAP, Robert Weiss, Karen Herbst, Diane Dunn and Ray Gesteland. Department of Human Genetics and Howard Hughes Medical Institute, University of Utah Medical School, Salt Lake City, UT 84132. Within the 160 amino acid coding sequence of bacteriophage T4 DNA topoisomerase gene *60*, codon 46 is separated from codon 47 by a gap of 50 non-coding nucleotides. This 50 nt. gap persists in gene *60* mRNA and is bypassed with near unitary efficiency in vivo by translating ribosomes[1]. Mutational analysis of gap bypass in a gene *60-lacZ* reporter fusion has revealed several sequence elements necessary for full bypass. Essential features include a *cis*-acting nascent peptide, a codon duplication flanking the gap, and a stop codon within a short stem at the 5′ junction[2]. A proposed model of gap bypass postulates three discrete stages during the bypass event: dissociation of the peptidyl-tRNA from the mRNA at the 5′ junction (takeoff stage), passage over the gap nucleotides (migration stage) and re-association via codon:anticodon pairing at the 3′ junction (landing stage). The contributions of the different sequence elements to each stage of the bypass event is being investigated by further mutational analysis, especially for the instances of alternate landing site choice. A positive selection scheme for host mutation unable to bypass the gap is also being employed to identify other components of the bypass mechanism.

1. Huang et al., (1988). A persistent untranslated sequence within bacteriophage T4 DNA topoisomerase gene *60*. Science *239*, 1005-1012.
2. Weiss et al., (1990). A nascent peptide is required for ribosomal bypass of the coding gap in bacteriophage T4 gene *60*. Cell *62*, 117-126.

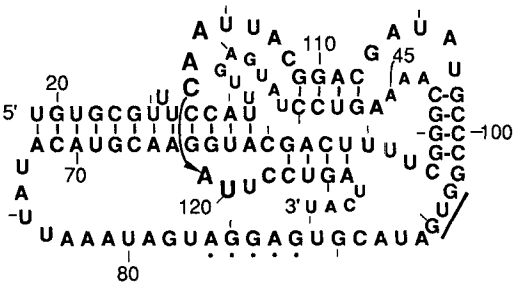
Translational Control in Bacteria

K 015 BIOSYNTHESIS OF SELENOCYSTEINE CONTAINING PROTEINS. August Böck, Karl Forchhammer, Walfred Leinfelder, Barbara Veprek, Franz Zinoni, Lehrstuhl für Mikrobiologie der Universität, D-8000 München 19, Federal Republic of Germany

The incorporation of selenocysteine into selenoproteins is directed by an in-frame UGA codon. In E. coli four genes have been identified whose products (SELA, SELB, SELC, SELD) have a function in the biosynthesis or insertion of this non-standard amino acid. The product of selC is a tRNA-species ($tRNA^{Sec}_{UCA}$) which is charged with L-serine by seryl-tRNA-ligase. SELA and SELD are required for converting seryl-$tRNA^{Sec}_{UCA}$ into selenocysteyl-$tRNA^{Sec}_{UCA}$, where SELA has the function of a selenocysteine synthase which by pyridoxal 5-phosphate-mediated catalysis dehydrates L-seryl-$tRNA^{Sec}_{UCA}$ into the aminoacrylyl-tRNA intermediate. SELD, on the other hand, under hydrolysis of ATP, generates a reactive derivative of reduced selenium which adds HSe^- to the aminoacrylyl double bond generating selenocysteyl-$tRNA^{Sec}_{UCA}$. An aminoacyl-tRNA specific translation factor, SELB, stoichiometrically binds selenocysteyl-$tRNA^{Sec}_{UCA}$ and GTP and is alternate in its function to EF-Tu. Decoding of the UGA requires an mRNA context involving sequence and secondary structure elements downstream of the UGA.

K 016 THE ROLE OF AN mRNA PSEUDOKNOT IN MEDIATING TRANSLATIONAL REPRESSION, David E. Draper and Gary Spedding, Department of Chemistry, Johns Hopkins University, Baltimore, MD 21218.

Most E. coli ribosomal protein operons are translationally repressed by one of the encoded proteins. In the case of the α operon, S4 represses the synthesis of itself and three other r-proteins. We have shown that S4 specifically binds to an RNA fragment containing the 5' 139 nts of the α transcript. By examining the behavior of a number of compensatory base changes *in vitro* and *in vivo*, we established that an unusual pseudoknot secondary structure (see figure) is required for S4 recognition and translational repression (1). The ribosome binding site is located on a single stranded linker region within the pseudoknot structure. Unexpectedly, some mutations bind S4 normally but do not repress translation. To account for this, we proposed that S4 and ribosomes bind to distinct sites on the mRNA, with S4 acting as an allosteric effector of initiation (2).



To further define the exact mechanism of translational repression in this system, we are examining ribosome binding to the α mRNA. Formation of the 30S·mRNA·$tRNA_{fmet}$ ternary complex with α mRNA becomes much more efficient at high temperatures; the data suggest that an mRNA conformational change takes place with an apparent Tm of ~40°. The transition is shifted in temperature or reduced in amplitude with different mRNA mutants which disrupt the pseudoknot secondary structure. A simple model in which ribosomes melt the pseudoknot structure is not consistent with the available data. It is possible that the transition is related to the proposed allosteric effect of S4 binding.

1. Tang, C. K., and Draper, D. E. (1989) Cell **57**, 531-536.
2. Tang, C. K., and Draper, D. E. (1990) Biochemistry **29**, 4434-4439.

K 017 EVOLUTION OF RNA LIGANDS: SITES RECOGNIZED BY PROTEINS, Larry Gold and Craig Tuerk, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309-0347
We have studied several proteins that bind to RNA and, as a result of that binding, regulate translation. In general the binding sites on target messages must overlap, directly or indirectly, the translation initiation region. Well understood proteins (and their respective target RNAs) include the R17 coat protein, the bacteriophage T4 regA, ssb, and DNA polymerase proteins, and the bacteriophage T4 regB "Shine and Dalgarno" nuclease. The target sites for these proteins illustrate, collectively, most if not all of the sequence and structural motifs available to small RNA molecules.

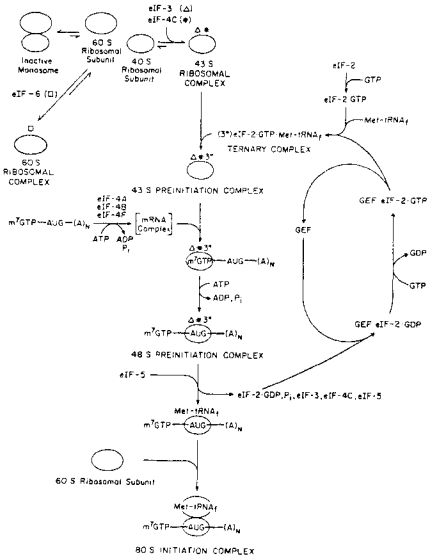
Our interests now include RNA sequences not found in nature. Solutions to a sequence/structure problem can be studied independently of a specific (and usually idiosynchratic) biological solution. From such work one derives new respect for those biological solutions, coupled with a deeper understanding of the narrow experimentation in "sequence space" that took place during evolutionary history. One sees as well new homologues and chemalogues (or "aptameres"), widening the appreciation for the flexibility of nucleic acids in "filling" a binding pocket in a target.

Eukaryotic Protein Synthesis

K 018 TRANSLATIONAL PARAMETERS ALTERED BY *IN VITRO* MANIPULATION OF THE CELLULAR LEVELS OF INITIATION FACTORS. John W. B. Hershey, Christine L. Olsson, Azhar Alvi and Helen S. Cummings, Dept. of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616.

Initiation of protein synthesis is promoted by three initiation factors called IF-1, IF-2 and IF-3. Their involvement in translation has been demonstrated by their stimulation of various *in vitro* assays of initiation of protein synthesis, but *in vivo* studies of their function generally are lacking. Conditional mutations in the genes for IF-2 and IF-3 suggest that these proteins are essential for cell viability and growth. The genes for all three factors have been cloned, and thereby provide a route for controlling cellular levels of these proteins. In collaboration with Drs. James Cole and Masayasu Nomura, we previously showed that IF-2 levels can be reduced by placing the *infB* gene under control of the *lac* promoter and regulating its expression by varying the IPTG inducer concentration in the medium. Low levels of IF-2 cause a decreased rate of protein synthesis and a reduction in the size of polysomes, consistent with the view that IF-2 promotes the initiation phase. The strain also is being used to isolate conditional and lethal mutations in *infB* expressed from a low-copy plasmid. In order to evaluate the effect of lowering the cellular concentration of IF3, a similar construct with *infC* has been made. The strain grows more slowly at low IPTG concentrations. However, in this case, regulation of the transcription of *infC* also causes changes in the expression of two closely linked downstream genes, *rpmI* and *rplT*, encoding ribosomal proteins L35 and L20, respectively. To attribute the growth phenotype to one or more gene products, strains have been constructed where the chromosomal genes for IF3, L35 and L20 are under *lac* promoter control, but two of the three genes are complemented by a lysogenic lambda phage that expresses them. The effects of limiting amounts of either IF3, L35 or L20 are presently under investigation. *Supported by NIH grant GM40082.*

K 019 INITIATION OF PROTEIN SYNTHESIS IN EUKARYOTES, William C. Merrick, Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, OH 44106.



The diagram to the left represents a "traditional" view of the initiation pathway in eukaryotics. While this is a useful format to indicate the function of a number of the factors, recent data indicates portions of this scheme are probably not correct. This reflects in large part an improvement in the ability to detect intermediates by methods which are less harsh than sucrose gradients.

A second element not considered is the rare, alternate initiation schemes which are referred to as internal initiation and re-initiation. Third, from the protein factors characterized to date, it is clear that these factors are highly conserved and that about half of the factors are expressed from more than a single function gene. Finally, the growing role of translation factors in other cellular events will be discussed. (Supported in part by NIH grant GM26796).

K 020 MECHANISM OF REGULATION OF INDIVIDUAL mRNA TRANSLATION RATES, R.E. Thach, J.J. Lin, S. Daniels-McQueen; M.M. Patino, L. Gaffield, and W.E. Walden, Washington U., Dept. of Biol., St. Louis, MO 63130, USA; U. of Illinois, Dept. of Micro. and Immunol., Chicago, IL 60612, USA.

Various mechanisms which allow individual mRNAs to be selectively translated at rates different from the average or "global" translation rate will be considered. The advantages of using kinetic models to distinguish among different selective mechanisms will be evaluated. The application of kinetic analysis to the regulation of ferritin mRNA translation will be considered in detail.

Three or more elements regulate ferritin mRNA translation in response to the iron supply. The first identified was a 28 nucleotide sequence in the 5' untranslated region (UTR) of ferritin mRNAs. This sequence confers iron responsiveness to a downstream open reading frame (ORF). It has been named the "iron responsive element" (IRE). Similar sequences occur in the 3' UTR of transferrin receptor (TfR) mRNA. These have been shown to correlate TfR mRNA stability with the iron supply. The second element found was a 90 kDa protein that binds specifically to the IRE, where it prevents translation of a downstream ORF. It has been named the "ferritin repressor protein" (FRP), and has recently been purified to homogeneity. It is thought to be similar or identical to the protein that binds to TfR mRNA IREs.

A third element, "the ferritin inducer," has recently been investigated. This is a metabolite of iron which interacts with FRP to relieve the repression of ferritin mRNA translation. It may accomplish this by changing the redox state of one or more sulfhydryl groups of FRP. Hemin can induce ferritin translation in vitro. It causes the FRP/IRE complex to dissociate, and thereby allows normal translation of ferritin mRNA. It seems likely that this is a result of the binding of hemin to a specific site on FRP, to which it can spontaneously crosslink in vitro. Both the crosslinking to, and inactivation of, FRP by hemin are highly specific events: no other proteins have been identified which are affected similarly by hemin under comparable conditions. (The inhibition of two unusually sensitive restriction endonucleases by hemin has been found to be due to the ability of hemin to act as a detergent and/or to generate free radicals. These non-specific side effects of hemin are avoidable by the selection of appropriate reaction conditions.) No other iron salt or complex has yet been found which mimics the derepressive effects of hemin. Taken together, these results suggest that hemin may be a physiologically significant ferritin inducer. (Supported by NSF, NIH, Monsanto Co., and the Schweppe Foundation.)

Post-Transcriptional Control of Gene Expression in Eukaryotes

K 021 CO-TRANSLATIONAL DEGRADATION OF TUBULIN mRNAs. D.W. Cleveland, Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

For an increasing number of eukaryotic genes, regulated stability of cytoplasmic mRNAs is now known to play an important role in establishing the appropriate levels of expression. This is clearly true for the mRNAs encoding α and β tubulin, the principal subunits of microtubules. In this instance, stability of these mRNAs is established by the concentration of their own translation products. The sequences that are necessary and sufficient to specify β tubulin mRNAs as substrates for this autoregulated instability reside within the first 13 translated nucleotides (that encode the first four β tubulin amino acids Met-Arg-Glu-Ile). Site directed mutagenesis has shown that β tubulin mRNAs are selectively targeted for degradation not through recognition of specific RNA sequences, but rather through co-translational recognition of the amino-terminal β tubulin tetrapeptide after it emerges from the ribosome. This co-translational interaction with the nascent polypeptide is similar to that found earlier for signal recognition particle binding to hydrophobic leader peptides and for codon skipping in the T4 gene 60, except that for tubulin recognition of the nascent polypeptide leads to RNA degradation. Co-translational mRNA instability has also been documented in other eukaryotic examples, including histone mRNA degradation at the cessation of DNA synthesis. The sum of these findings suggests the existence of a common pathway for co-translational degradation of some eukaryotic mRNAs.

K 022 POSITIVE CONTROL OF TRANSLATION IN YEAST MITOCHONDRIA, T.D. Fox, P. Haffter, T.W. McMullin, M.C. Costanzo and L.S. Folley, Section of Genetics and Development, Cornell University, Ithaca, NY 14853-2703 USA

The mechanism of translation initiation in mitochondria of the budding yeast *Saccharomyces cerevisiae* is poorly understood, but several nuclear genes exert *positive* control over the translation of *specific* mitochondrially coded mRNAs. We are studying the action of three such nuclear genes, *PET494*, *PET54* and *PET122*, on translation of the mitochondrial mRNA for cytochrome oxidase subunit III (coxIII). The 600 base coxIII mRNA 5'-leader contains the site(s) of action for the three nuclear genes (whose products are located in mitochondria), at least 170 bases upstream of the initiation codon. While this region of the coxIII leader contains a short upstream ORF, removal of the ORF by mutation and mitochondrial gene replacement has no effect on coxIII translation. To identify interactions of the mRNA-specific factors with the general translational machinery, we have analyzed allele-specific suppressors of a *pet122* mutation. Two such suppressor genes (nuclear) have been characterized and shown to code for ribosomal proteins of the mitochondrial small subunit. Both ribosomal proteins (termed PET123 and MRP1) are required for all mitochondrial translation, in contrast to the mRNA-specific translational activator with which they functionally interact. Neither protein is closely homologous to known ribosomal proteins in other organisms. Our results are consistent with models in which the mRNA-specific proteins mediate interaction between the mRNA and the ribosomal small subunit to activate translation. To investigate general features of mitochondrial translation initiation we altered the coxIII initiation codon (AUG) to AUA in an otherwise wild-type strain. The mutation reduced coxIII translation and produced a leaky non-respiratory growth phenotype. Among the nuclear mutations selected that improved translation of the mutant coxIII mRNA in diploids, were some that exhibited recessive defects in mitochondrial gene expression and, surprisingly, others that blocked cell growth.

K 023 TRANSLATIONAL CONTROL OF THE YEAST TRANSCRIPTIONAL ACTIVATOR GCN4. A. Hinnebusch, J.-P. Abastado, M. Cigan, E. Hannig, M. Foiani, B. Jackson, P. Miller, C. Paddon, M. Ramirez, R. Wek, N. Williams, Laboratory of Molecular Genetics, NICHD, National Institutes of Health, Bethesda, MD 20892.

GCN4 is a transcriptional activator of amino acid biosynthetic genes in yeast whose expression is regulated at the translational level by short open-reading-frames in the leader of GCN4 mRNA (uORFs). uORF3 and uORF4 (counting from the 5' end) inhibit initiation at GCN4 under conditions of amino acid abundance; uORF1 allows ribosomes to traverse uORFs 3 and 4 and translate GCN4 in amino acid-starved cells. Sequences surrounding the uORF1 stop codon allow ribosomes to resume scanning and reinitiate following termination at this site. The fact that altering the length and termination site of uORF4 has no effect on GCN4 expression, even when uORF4 is made to overlap GCN4 by 130 nt, suggests that ribosomes scanning downstream from uORF1 reach GCN4 by ignoring the AUG codons at uORFs 3 and 4. Increasing the distance between uORF1 and uORF4 impairs derepression, suggesting that uORF4 is too close to uORF1 for efficient reinitiation under starvation conditions, but that scanning the additional sequences between uORF4 and GCN4 allows ribosomes to recover the ability to reinitiate at GCN4. In this view, reducing the efficiency of reinitiation under nonstarvation conditions is responsible for the ability of ribosomes to bypass uORFs 3 and 4 and reach the GCN4 start site. GCN1, GCN2 and GCN3 are required for increased GCN4 translation under starvation conditions. Mutations affecting the general initiation factor eIF-2 and in multiple GCD genes overcome the requirement for these GCN factors for derepression. gcd mutations additionally lead to temperature-sensitive growth under nonstarvation conditions. These findings, and the results of recent biochemical studies on GCD1 and GCD2, suggest that GCD proteins are essential factors involved in general translation, and that GCN1-GCN3 antagonize these factors in starved cells in order to stimulate GCN4 translation. Genetic interactions between GCN3, GCD genes and eIF-2 structural genes suggest that GCN3 interacts directly with these negative-acting factors. This notion is consistent with the isolation of GCN3 mutations that cause constitutive derepression of GCN4 in the absence of GCN1 and GCN2. GCN2 is a protein kinase containing a large domain related to histidyl-tRNA synthetases (HisRS). Point mutations in the HisRS sequences inactivate GCN2 function *in vivo* without lowering its protein kinase activity *in vitro*, suggesting that this region is a positive regulatory domain. In accord with this idea, dominant mutations mapping just downstream from the HisRS sequences cause constitutive activation of GCN2 regulatory function. We propose that the HisRS sequences monitor the level of uncharged tRNA and activate the GCN2 kinase moiety in amino acid-starved cells in which charging is reduced. Phosphorylation by GCN2, in conjunction with the regulatory functions of GCN1 and GCN3, would lower the activities of certain initiation factors, allowing ribosomes scanning downstream from uORF1 to ignore the uORF3 and uORF4 start codons and reinitiate at GCN4 instead.

K 024 **TRANSLATIONAL CONTROL MECHANISMS IN EARLY SEA URCHIN DEVELOPMENT,** Mark A. Wilson[1], Zhe Xu[2], Merrill B. Hille[2], William C. Merrick[3], and Rosemary Jagus[1], [1]Center of Marine Biotechnology, University of Maryland, Baltimore, Maryland, 21202, [2]Department of Zoology, University of Washington, Seattle, WA 98195, [3]Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Studies using cell-free translation systems from sea urchin eggs and early embryos point to a multifactorial mechanism for the activation of protein synthesis during early development. At least two initiation factors, eIF-2B and eIF-4, show reduced activity prior to fertilization and increased activity following fertilization. These changes in initiation factor activity occur in coordination with "unmasking" of maternal mRNA by a process that remains elusive. The activation of eIF-2B following fertilization reflects changes in redox potential, and follows the fertilization-induced increase in NADPH levels.

eIF-4 activity rises slowly after fertilization reflecting the inactivation of a protein inhibitor of eIF-4 function. Inactivation of the inhibitor is dependent on the fertilization-induced changes in CA^{2++} concentrations. There is an interesting contrast between the slow release from inhibition of eIF-4 compared with the more rapid change in maternal mRNA availability. This may be important to allow changes in the relative translation of different mRNA species with time after fertiliation, since eIF-4 has been reported to have mRNA discriminatory activity. In starfish oocytes, completion of meiosis is followed by increases in protein synthetic rate, triggered by changes in intracellular Ca^{2++}, and accompanied by dramatic changes in the patterns of proteins synthesized. Immature starfish oocytes also contain an inhibitor of eIF-4 activity, which is progressively inactivated following completion of meiosis.

K 025 COORDINATE POST-TRANSCRIPTIONAL REGULATION OF FERRITIN AND TRANSFERRIN RECEPTOR EXPRESSION. Richard D. Klausner & Joe B. Harford, Cell Biology & Metabolism Branch, NICHD, NIH, Bethesda, MD 20892.

Recent work on the regulated expression of the proteins of cellular iron metabolism in higher eukaryotes has provided a relatively detailed picture of the control of the fates of mRNA molecules that encode ferritin and the transferrin receptor (TfR). The expression of these genes is highly regulated by the amount of iron available to the cell. Limiting iron results in an decrease in the biosynthesis of ferritin whereas synthesis of the TfR is regulated in the opposite direction. In contrast to the regulation of ferritin expression which occurs without a change in ferritin mRNA level, regulation of TfR protein synthesis is achieved through iron-dependent alteration of the level of TfR mRNA. When the 5' untranslated region (UTR) of the ferritin mRNA is deleted, all acute iron regulation is lost. Further deletion analysis and expression of chimeric mRNA's containing the ferritin IRE have identified 35 nucleotides as being necessary and sufficient for iron-dependent translational control of ferritin synthesis. These bases are capable of forming moderately stable stem-loop structures that we have referred to as an *Iron Responsive Element* (IRE). Post-transcriptional regulation of TfR mRNA levels by iron is mediated by a portion of the 3'UTR of the mRNA. Within this region are five RNA structures which resemble the single IRE found in the ferritin mRNA 5'UTR. Each of the TfR IRE's compete with a ferritin IRE for interaction with a cytoplasmic protein that we have called the *IRE-Binding Protein* (IRE-BP). Collectively, our data support a model for TfR regulation in which the interaction between the IRE-BP and the TfR mRNA can protect the transcript from rapid degradation mediated by a rapid turnover determinant within the 3'UTR. The activity but not the amount of the IRE-BP is dependent upon the iron status of the cells. Increased IRE-BP activity in response to iron chelation does not require protein synthesis. The biochemical basis for the altered activity of the IRE-BP appears to be the reversible oxidation-reduction of one or more disulfides in the IRE–BP. The IRE-BP population of cells treated with an iron chelator is relatively reduced and has higher affinity for the IRE. Thus, the iron status of the cells appears to set the redox state of the IRE-BP. We have termed this novel biochemical regulatory mechanism a sulfhydryl switch. By altering the redox state of the IRE-BP through the sulfhydryl switch, iron can coordinately mediate a decrease in the biosynthesis of ferritin (by translational repression) and an increase in the biosynthesis of the TfR (by repression of mRNA degradation). The IRE-BP is a 90,000 Dalton cytosolic protein that has been purified to homogeneity by RNA affinity chromatography. The cDNA corresponding to the IRE-BP has now been molecularly cloned and sequenced.

Synthesis, Structure and Stability of mRNA

K 026 ULTRASTRUCTURAL ANALYSIS OF EARLY RNA PROCESSING EVENTS, Ann L. Beyer, Martha S. Farrell*, Oscar L. Miller, Jr.* and Yvonne N. Osheim, Departments of Microbiology and *Biology, University of Virginia, Charlottesville, VA 22908

By EM analysis of Drosophila and Adenoviral genes using the Miller chromatin spreading method, we have shown that the splicing machinery typically assembles on nascent transcripts, and that splicing of a given intron is frequently initiated within about 3 min of synthesis of its 3' splice site, even for introns of 20 kb or greater. The observation that the time required for splicing is independent of intron length suggests that the native structure of nascent transcripts allows cognate splice junctions to find each other in a rapid non-rate-limiting process. In attempts to characterize a "more native" transcript structure, we have used physiological pH conditions in our chromatin spreads, instead of pH 9 used in the typical highly dispersed preparations. As the pH is gradually lowered from 9 to 6.5, a continuous transition is seen from fully extended transcripts with spliceosome particles and intron loops to a compact structure with a large central granule (70-100nm) and extending loops, resembling a "rosette". Analyses are consistent with the interpretation that these structures are a conglomerate of existing spliceosome particles, splice sites, and the relatively short exon sequences, with intron loops extending. Such a configuration in which all splice junctions, and possibly exons, are packaged as they are made into a compact structure that excludes introns has implications for the kinetics and orderliness of splicing, for alternative splicing options, and for the alternative fates of the central (exon) sequences vs the peripheral introns. In another study, we were interested in investigating if cleavage at poly(A) sites typically occurs on nascent transcripts, because our observations suggest that co-transcriptional splicing is the rule and there are several examples of gene regulation which involve a competition or interaction between splicing and polyadenylation. In a large sample of Drosophila Pol II genes, (N=109), we find that the majority event (50-60%) at the 3' end is an abrupt loss of transcripts, with no change in polymerase density and no evidence for transcript cleavage. A lower polymerase density is the second most frequent event observed (~30% of genes), although again the 3'-most transcripts are typically not cleaved. Since other studies have shown that transcription termination is dependent on a functional poly(A) site, we conclude either that the 2 events (poly(A) site cleavage and termination) are very efficient and closely-linked temporally and spatially, or that termination is efficient after a poly(A) site is recognized and cleavage at the poly(A) site occurs post-transcriptionally.

K 027 RNA-BINDING PROTEINS IN THE FORMATION AND FUNCTION OF mRNA, Serafin Piñol-Roma, Michael Matunis, Erika Matunis, Christopher Burd, Matthias Görlach, Megerditch Kiledjian, Matthew Michael, Haruhiko Siomi and Gideon Dreyfuss, Howard Hughes Medical Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6148, USA.

HnRNAs are bound in the nucleus with proteins, the hnRNP proteins. HnRNP proteins bind to hnRNA molecules as they are transcribed. Most of the hnRNP proteins bind the hnRNA directly and many of them belong to a large family of proteins that have (one or more) conserved ~90 amino acid RNP consensus sequence-containing RNA-binding domain (CS-RBD). In the cytoplasm, mRNAs are bound with mRNP proteins. Many of the mRNP proteins, including the poly(A) binding protein, are also of the CS-RBD family. The exchange of hnRNP proteins for mRNP proteins accompanies the nucleo-cytoplasmic transport of the mRNA. Recent progress in determining the structure of hnRNP and mRNP proteins will be described. The individual RNP proteins have different RNA-binding preferences. Several of the hnRNP proteins have RNA-binding specificities for sequences that are important for pre-mRNA splicing and for polyadenylation and it is thus likely that they influence mRNA formation. HnRNP complexes, which can be purified from nucleoplasm with antibodies to hnRNP proteins, are composed in humans of at least 20 major polypeptides in the range of 34,000-120,000 daltons, and probably many more proteins that are less abundant. To examine the generality of this picture and to facilitate the investigation of the role of hnRNP proteins in pre-mRNA processing, we have isolated and characterized hnRNP and mRNP proteins from several organisms including *Drosophila melanogaster.*

K 028 The poly(A)-binding protein and post-transcriptional gene regulation in Saccharomyces cerevisiae. Alan Sachs, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142.

Messenger RNA expression is controlled by both its degradation and translation initiation rates. An important regulatory element on mRNA for this control is the poly(A) tail-poly(A) binding protein (PAB) complex (1). For instance, depletion of PAB in vivo leads to the inhibition of poly(A) tail shortening (2). This reaction has been reconstituted in vitro by adding purified PAB and ^{32}P-poly(A) to PAB-depleted extracts. Using this assay, the PAB-dependent poly(A) nuclease activity (PAN) has been purified to near homogeneity. Information about PAN's structure and biochemical properties will be presented

PAB is also required for translation initiation, and this requirement is mediated through the 60S ribosomal subunit (2). Alterations in the 60S ribosomal subunit created by the *spb* mutations allow translation and cell growth in the absence of PAB. These mutations include a null mutation in the ribosomal protein L46 gene (*RPL46=SPB2*) and a modification of a putative rRNA helicase (*SPB4*) involved in the maturation of 25S rRNA (3). I have recently found that the *spb* mutations can also suppress lethal mutations in at least 150 other genes. A subset of these gene products appear to be interacting with each other, suggesting that they are part of a particle. A preliminary analysis of this subclass of mutations will be discussed.

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3. Sachs, A.B., and R.W. Davis. Translation initiation and ribosomal biogenesis: Involvement of a putative rRNA helicase and RPL46. Science 247:1077-1079 (1990).

Protein Trafficking

K 029 A NASCENT POLYPEPTIDE CHAIN'S VIEW OF PROTEIN SECRETION AND MEMBRANE PROTEIN INTEGRATION. Arthur E. Johnson, Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019.

The immediate environments of nascent secretory proteins undergoing translocation across the ER membrane, and of nascent membrane proteins undergoing integration into the ER membrane, were investigated by photocrosslinking. Nascent polypeptides of different lengths were synthesized in vitro by the translation of truncated mRNAs of secretory or membrane proteins in the presence of N^{ε}-(5-azido-2-nitrobenzoyl)-Lys-tRNA, signal recognition particle, and microsomal membranes. These samples, containing nascent protein chains functionally engaged with active components of the translation, translocation, and integration machinery, were then photolyzed. In each case the nascent chains reacted covalently with ER membrane proteins, showing that ER membrane proteins are in close proximity to nascent chains during translocation and integration. A nascent secretory protein, preprolactin, was crosslinked primarily to a 39 kDa integral transmembrane glycoprotein of the ER termed mp39. Since this crosslinking occurred via photoreactive moieties in either the signal sequence or the mature prolactin sequence at various locations within the bilayer, mp39 appears to constitute a guide for the translocating chain or part of a translocation tunnel. Two membrane proteins, each containing a single IgM transmembrane sequence, were synthesized in vitro to form polypeptides that contained photoreactive probes at one end of the transmembrane sequence where two lysine residues are located. When irradiated, the nascent chains reacted covalently with several ER membrane proteins. One of the two most prominent crosslinking targets of the photoreactive nascent chains was a glycoprotein similar in size to mp39, which suggests that nascent secretory and membrane proteins utilize the same or similar *sites* (translocons) on the ER membrane for translocation and integration. Changing the orientation of the transmembrane domain in the bilayer, or making the transmembrane domain the first topogenic sequence in the nascent chain instead of the second, did not significantly alter the identities of the primary ER crosslinking targets. Interestingly, the transmembrane sequence remained in the vicinity of these ER membrane proteins even after the cytoplasmic tail of the nascent chain had been lengthened by nearly 100 amino acids. Yet when the nascent chain was allowed to terminate normally, the major photocrosslinks were no longer observed. These results indicate that the transmembrane segment of a nascent membrane protein is located adjacent to ER membrane proteins during the integration process, and is prevented from lateral diffusion until protein synthesis has been terminated.

K 030 IDENTIFICATION OF PROTEINS CLOSELY ASSOCIATED WITH A SECRETORY PROTEIN TRANSLOCATION INTERMEDIATE. Sylvia L. Sanders and Randy Schekman, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

The molecular mechanism by which secretory proteins traverse the endoplasmic reticulum (ER) membrane is mysterious. The targeting of secretory proteins to the ER membrane by the signal recognition particle (SRP) and its receptor (SRP receptor or docking protein) has been studied in detail, but the subsequent event of ER pentration remains obscure. To determine which membrane proteins mediate this reaction, we have created an intermediate in the process of secretory protein translocation into the yeast ER in vitro. This intermediate is reversibly trapped in a partially translocated state at those sites where translocation occurs. Through crosslinking studies we have determined that three proteins with the molecular weights of ~43kD, 23.5kD, and 20kD are closely associated with the translocation intermediate. Through genetic selections, several loci have been identified which are required in yeast for proper secretion and viability. Though their molecular functions are unknown, Sec61p, Sec62p, and Sec63p are integral ER membrane proteins required for translocation. When analyzed by polyacrylamide gel electrophoresis, the 23.5kD polypeptide comigrates with a protein found in a complex with the Sec61, Sec62, and Sec63 proteins. Interestingly, the 43kD associated protein is identical to the authentic Sec61 protein. Experiments to determine some characteristics of these associated proteins, as well as the requirements for their association with the intermediate, are in progress.

Viral Regulation of Protein Synthesis

K 031 REGULATION OF THE INTERFERON-INDUCED, DOUBLE-STRANDED RNA ACTIVATED PROTEIN KINASE DURING VIRAL INFECTION, M.G.Katze(1), A. Hovanessian(2), S. Roy(3), N. Sonenberg(3), J. Tomita(1), T. Black(1), and T.G. Lee(1). 1-Department of Microbiology, University of Washington, Seattle, USA. 2-Unit of Viral Oncology, Institut Pasteur, Paris, France. 3-Department of Biochemistry, McGill University, Montreal, Canada.

We have been studying the mechanisms by which animal viruses downregulate the activity of the interferon-induced, dsRNA activated protein kinase (referred to as P68). This control is essential since, when activated, the P68 kinase phosphorylates the alpha subunit of eIF-2 resulting in a limitation of functional eIF-2 for protein synthesis initiation. We will present results on the regulation of the P68 protein kinase in three viral systems: (i) Influenza virus; (ii) Poliovirus; and (iii) Human Immunodeficiency Virus or HIV-1. (i) Influenza Virus: we have shown that P68 kinase activity and eIF-2 alpha phosphorylation is repressed during influenza virus infection. Utilizing an in vitro assay for P68 inhibition, we have purified, to near homogeneity, the P68 inhibitor from influenza virus-infected cells. The purified protein inhibited both the autophosphorylation of purified P68 as well as the phosphorylation of eIF-2 alpha by the kinase. The purified inhibitor, which has an apparent molecular weight of approximately 58,000 daltons, was determined to be a cellular protein and not an influenza virus encoded protein. In confirmation of these results, we have purified a protein with identical chromatographic properties and kinase inhibitory activities from uninfected cells. We also have raised peptide antibody against the 58 Kda inhibitor which recognizes the protein in extracts of both uninfected and influenza virus-infected cells. Experiments regarding the cloning of the 58 Kda inhibitor as well as its possible mode of regulation will be presented. (ii) Poliovirus: in contrast to the influenza system, poliovirus does not block activation of P68 during infection. To minimize the deleterious effects of kinase activation, poliovirus induces the degradation of P68 as revealed by pulse-chase analysis. These results were confirmed by development of an in vitro assay in which P68 is specifically degraded by poliovirus-infected extracts. Utilizing this assay we are purifying the responsible protease from virus-infected cells using FPLC and conventional chromatography, the results of which will be presented. (iii) HIV-1: we have previously shown that the physical levels of P68 declined in HIV-1 infected lymphoid cells and in HeLa cell lines stably expressing the HIV-1 tat regulatory protein suggesting HIV-1 may mediate the necessary downregulation of P68 via tat. Recent analysis has shown that such a decrease in P68 levels may be essential due to the presence of TAR sequences present at the 5' end of HIV RNAs. TAR containing RNAs were found to efficiently bind to and activate the protein kinase. Further, both these activities were dependent on the intact TAR stem structure. Efforts are now directed at defining the mechanisms of tat and TAR regulation of P68. Finally, recent results on the expression of the cDNA encoding the P68 protein kinase and the interaction of the expressed P68 with viral inhibitors and activators will be discussed.

K 032 **CONTROL OF INITIATION BY RNA REGULATORS**, Michael B. Mathews, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724.

The interferon-induced protein kinase DAI, also known as p68 kinase, plays an important role in controlling protein synthesis in infected cells and in establishing the anti-viral state. Viruses have developed mechanisms to counter and even exploit the enzyme. DAI is activated by double-stranded RNA (dsRNA) in a reaction that involves autophosphorylation. Once activated, DAI phosphorylates the initiation factor eIF-2, preventing it from being recycled by GEF (the guanosine nucleotide exchange factor, eIF-2B) and bringing initiation to a halt. The activation of DAI is tightly regulated: it follows second-order kinetics, suggesting that two molecules of DAI are involved, and it exhibits a rigorous and complex dependence on RNA. Low concentrations of dsRNA activate, while high concentrations of dsRNA prevent activation. Small effector RNAs, including adenovirus-2 VA RNA and a short RNA transcribed from the HIV-1 leader region, also prevent activation. The structural features responsible for RNA interactions with DAI and consequences for the viral life cycle will be discussed.

K 033 REOVIRUS-MEDIATED TRANSLATIONAL CONTROLS, Aaron J. Shatkin, Eduardo Fajardo, Ramona M. Lloyd and Lucia E. Tillotson, Center for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, NJ 08854

The reovirus S1 gene in all three viral serotypes contains two overlapping open reading frames. Both are translated in virus-infected cells, apparently from a single mRNA. In type 3 virus, the cap-proximal AUG is utilized for initiation of the 49 Kd viral hemagglutinin (σ1), and the next AUG, 58 nucleotides downstream and in a different reading frame, corresponds to the start of a 14 Kd nonstructural protein, p14. Mouse C127 cells that were stably transformed with a complete DNA copy of the S1 gene produced σ1 and p14 by translation of a full length S1 mRNA, demonstrating that uninfected cells have the capacity to express bicistronic mRNAs. In S1 gene transfected COS cells, p14 synthesis was increased 3-4 x by mutating the σ1 AUG to UUG or by inserting a terminator codon 10 triplets downstream of the σ1 AUG. Alteration of the consensus around the σ1 start site also increased p14 synthesis but without decreasing the production of σ1. The results indicate that σ1 synthesis is limited at the level of elongation and suggest a model for cis-regulation of internally coded proteins produced from bicistronic mRNAs.

The reovirus S4 gene codes for polypeptide σ3, a dsRNA binding protein which has been implicated in the serotype-specific alteration of host translation in infected cells via DAI, the dsRNA-activated eIF-2α kinase. Consistent with this role for σ3, the S4 gene effectively substituted for adenovirus VA1 in COS cells transiently expressing dihydrofolate reductase under conditions that depend on inhibition of DAI activation for efficient expression. In addition, DNA copies of the type 1 or type 3 S4 gene stimulated CAT mRNA translation by >10-fold in co-transfected COS cells, presumably also by blocking dsRNA activation of DAI. Reovirus M2 gene co-expression resulted in complexes between the S4- and M2-encoded polypeptides and prevented CAT stimulation by σ3. Lysates of type 3 infected mouse L cells contained more σ3 than type 2 extracts and required addition of 5-10 X more dsRNA to obtain maximal DAI phosphorylation, consistent with higher levels of activated DAI and greater host translational shut off in type 2 infected cells. The results suggest that host translation is modulated in reovirus infected cells by σ3 and interactions that influence the phosphorylation state of DAI and eIF-2α.

K 034 MECHANISM OF TRANSLATION INITIATION OF POLIOVIRUS mRNA, N. Sonenberg, R. Nicholson, and K. Meerovitch, Department of Biochemistry, McGill University, Montreal, Quebec, Canada.

Translation of poliovirus RNA occurs by binding of ribosomes to an internal region of the mRNA 5' UTR, without scanning from the 5' end. This region is termed ribosome landing pad (RLP). To study the molecular mechanism of internal initiation, we have performed deletion and site directed mutagenesis of the poliovirus 5' UTR, and identified a trans-acting protein that interacts with the poliovirus 5' UTR. The deletion experiments defined a 3' boundary of the poliovirus RLP. This region contains two elements that play a role in translation. (1). A conserved polypyrimidine stretch is critical; point mutations in this region abrogate translation in vivo and in vitro. (2). An AUG triplet (AUG#7 in the 5' UTR of poliovirus type 2) modulates translation both in vivo and in vitro. A stem-loop structure 3' to AUG7 that is conserved amongst enteroviruses and rhinoviruses is not required for translation. A 52 kDa protein (p52) binds specifically to this region, and its distribution in different tissue extracts positively correlates with the efficiency of poliovirus translation. We have purified p52 to near homogeneity and the purified protein could stimulate translation of mRNAs containing the poliovirus 5' UTR in a reticulocyte lysate. We are currently studying the mechanism by which ribosomes recognize the RLP and translocate to the initiator AUG to commence translation.

K 035 CAP-INDEPENDENT TRANSLATION OF PICORNAVIRUS RNAs: STRUCTURE AND FUNCTION OF THE INTERNAL RIBOSOMAL ENTRY SITE. Sung Key Jang, Tatyana Pestova, Gary Witherell, and Eckard Wimmer, Department of Microbiology, School of Medicine, SUNY at Stony Brook, Stony Brook, NY 11794.
mRNAs of mammalian picornaviruses are identical with genomic RNA in nucleotide sequence (on the average 7500 nt in length). These mRNAs are unique in that they are not capped with m^{7}GpppN but are instead 5' terminated with pU... Moreover, they contain a 5' non-translated region (5'NTR) unusually long (700 - 1500 nt) for self-replicating RNA molecules. We have discovered a 400 nt long segment within the 5'NTR of encephalomyocarditis virus (EMCV) called internal ribosomal entry site (IRES), that serves as positive translational control element. The function of the IRES renders the mRNA cap-independent, and initiation of translation occurs without scanning of the ribosomal subunits (for ref. see 1). As shown by Agol and his colleagues (see ref. 2) the IRES is of complex higher order structure. Mutations within the IRES impair or abolish cap-independent translation. We have identified the novel ribosome-associated RNA binding protein p57 whose binding to a specific stem-loop is required for IRES function (1). We have also found other cellular proteins that bind to sequences within the IRES, a sequence of which is an essential pyrimidine-rich segment located upstream of the initiator AUG (1). Several polypeptides may attach to IRES to form an RNP complex ("iresome"), a hypothesis supported by preliminary experiments. The IRES elements of 4 genera of picornaviruses fall into 2 structural classes: that of the entero- (polio) and rhinoviruses, and that of the cardio- (EMCV) and aphthoviruses [for ref., see (2)]; their function is identical but their ability to interact with specific host factors may differ, a phenomenon by which the IRES may confer special phenotypes to the viruses such as host range and pathogenicity.

(1) S.K. Jang and E. Wimmer, Genes Dev. 4: 1560-1572 (1990)
(2) E.V. Pilipenko et al., Nucl. Acids Res. 17: 5701-5711 (1989)

Late Abstract

INVOLVEMENT OF THE SIGNAL SEQUENCE RECEPTOR IN PROTEIN TRANSLOCATION ACROSS THE ER-MEMBRANE, T.A. Rapoport, Zentralinstitut für Molekularbiologie der Akademie der Wissenschaften, 1115 Berlin-Buch
The transport of proteins across the ER-membrane can be divided into two phases: 1. a fairly well characterized targeting step, involving the function of the signal recognition particle (SRP) and its membrane receptor (docking protein) [1], and 2. the actual translocation process, the mechanism of which is still unknown. The signal sequence receptor protein (α-SSR), previously identified by its close proximity to translocating nascent polypeptide chains [2-5], appears to play a role in the translocation process. It is a major integral glycoprotein of the ER-membrane restricted to its rough portion [6]. The protein is part of a complex consisting of stoichiometric amounts of four polypeptides (α-, β-, γ-, δ-SSR; molecular weights 35, 22, 20, 18 kDa, respectively). By the use of proteoliposomes reconstituted from detergent-solubilized ER-membranes, it could be demonstrated that the SSR-complex is essential for translocation, but not for the initial targeting step (D. Görlich and T.Rapoport, unpublished results). On the basis of these results and evidence that at least two copies of the tetrameric complex come close to each other in the membrane, it appears that the SSR-complex is part of a postulated protein-conducting tunnel.

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Chemistry of RNA

K 100 INTERACTIONS FORMED BY GLUTAMINYL-tRNA SYNTHETASE (GlnRS) WITH THE ACCEPTOR STEM OF $tRNA^{Gln}$: CRYSTAL STRUCTURES OF GlnRS MISCHARGING MUTANTS COMPLEXED WITH $tRNA^{Gln}$ AND CONSTRUCTION OF $tRNA^{Gln}$ VARIANTS IN THE ACCEPTOR STEM. John G. Arnez and Thomas A. Steitz, Department of Molecular Biophysics and Biochemistry and Howard Hughes Medical Institute, Yale University, New Haven, CT 06511.

Three mutants of glutaminyl-tRNA synthetase isolated by D. Soll *et al.* (GlnRS7 (D235N), GlnRS10 (D235G), GlnRS15 (I129T)) that mischarge the amber-suppressing *sup*F $tRNA^{Tyr}$ were cocrystallized with $tRNA^{Gln}$ and diffraction data to 3 Å collected. A difference electron density map between the mutant GlnRS*$tRNA^{Gln}$ complex and the wild-type complex was calculated in each case. The GlnRS7*$tRNA^{Gln}$ map shows not only a conformational change in the mutated residue 235, but a substantial shift in α-helix H which propagates into the active site. This result is surprising in light of previous kinetic studies (Perona *et al.*, Science 246, 1152 (1989)) indicating little change in the kinetic parameters for the charging of cognate tRNA by this mutant. The GlnRS10*$tRNA^{Gln}$ map shows the loss of the Asp 235 side chain and substantial local shifts that are not as extensive as those in the GlnRS7 complex. The GlnRS15*$tRNA^{Gln}$ map indicates little conformational change as a consequence of replacing the wild-type Ile 129 residue with Thr.

To test hypotheses on the structural mechanisms of tRNA discrimination by GlnRS, we have synthesized $tRNA^{Gln}$ variants at base pairs 3-70 and 2-71 by *in vitro* transcription using T7 RNA polymerase. Co-crystals of GlnRS with two variant tRNAs, G1-A72 and G1-C72 (from O. Uhlenbeck) have been grown and the structure of the G1-A72 variant determined. The *in vitro* and *in vivo* made $tRNA^{Gln}$ structures are identical except at the sulphur of thiouridine 8 and the water structure around the pseudouridine residues.

K 101 DELETION OF VARIABLE STRUCTURAL ELEMENTS IN THE RNA ENZYME RIBONUCLEASE P, Sylvia C. Darr, Karen Zito, Drew Smith, and Norman R. Pace, Department of Biology, Indiana University, Bloomington, IN 47405 USA

Ribonuclease P is an endonuclease which catalyzes removal of the 5' leader from precursor tRNAs. The catalytic subunit of RNase P is an RNA approximately 375 nucleotides in size. A secondary structure model of the RNA has been proposed based on comparisons of the sequence of RNase P RNAs from several different organisms. We are defining the minimum structure required for catalysis by deleting variable domains in the RNA. Four domains were deleted from the *E. coli* RNase P RNA to create *min1* RNA. The *min1* RNA is active, however it is defective in several properties. The *min1* K_m for substrate $tRNA^{Asp}$ is 100-fold greater than the K_m of the native RNA, and much more monovalent salt is required for maximum activity (D.Waugh et. al. Science 244:1569). We are analyzing the four *min1* deletions individually and in greater detail in order to identify the deletions that are responsible for these defects. We find that one of the deletions (helix 260-290) causes the increased K_m. We are attempting to repair the K_m defect by altering the sequence in the region of the deletion. Two of the other deletions confer the increased salt dependence of *min1*. We are testing the possibility that this high salt dependence is due to structural instability by assaying the stability of the deletion mutants at high temperatures.

K 102 REGULATION OF ompF mRNA LEVELS BY micF RNA, N. Delihas, J. Andersen, M. Schmidt and C. De Loughery, Dept. of Microbiology, SUNY, Stony Brook, N.Y. 11794.

micF RNA, a 93 nt transcript, negatively regulates the synthesis of outer membrane protein F (OmpF) in response to growth of Escherichia coli under conditions of stress. In vivo studies show that during the regulation of ompF expression, micF RNA participates in the destabilization of OmpF mRNA. The repressor RNA is essential but not a sufficient factor in the decrease of mRNA levels. In vitro studies show that a stable duplex forms between micF RNA and the 5' end of ompF mRNA with incubation at 37°C (but not at 0°C). A probe of ompF mRNA 5' end UTR secondary structure with ribonucleases shows that it is highly structured. This may account for the lack of duplex formation at 0°C. The melting curve of the duplex between micF RNA and the 5' end of ompF mRNA is characteristic of an imperfect double helix which is predicted in a proposed duplex model. A micF RNA binding protein has also been detected in vitro by gel mobility shift and UV cross-linking and this protein has some affinity for the 5' end of ompF mRNA. Furthermore, the micF RNP confers protection of micF RNA from degradation by nucleases. The elution position of micF RNA after the addition of cell extracts to a DE52 chromatography column suggests that micF RNP is present in vivo. Destabilization of ompF mRNA in vivo may be a consequence of micF RNP binding to the 5' end of ompF mRNA.

K 103 RECOGNITION OF RNA HELICES BY AMINOACYL TRNA SYNTHETASES.

Christopher Francklyn and Paul Schimmel. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA. 02139

Recent work in several systems has identified nucleotides in tRNAs which modulate recognition by aminoacyl tRNA synthetases. Among these, the G3:U70 base pair in the acceptor stem of alanine tRNA has been shown to be an important element for recogntion by the cognate synthetase. The alanine tRNA synthetase enzyme can accept as substrates short helical fragments that comprise the amino acid acceptor stem. We show that histidine tRNA synthetase is the second member of the class of aminoacyl tRNA synthetases that recognize determinants in the tRNA acceptor stem and can utilize short helical fragments as substrates. Both a mini- and microhelix based on the acceptor-TψC and acceptor domain of $tRNA^{His}$ are efficiently aminoacylated with histidine. Alanine and histidine tRNA synthetases retain specificity for their cognate substrates. We have constructed a set of sequence variants of the alanine and histidine microhelices in which substitutions have been introduced at the first three base pairs proximal to the acceptor end. Comparison of the aminoacylation of these substrates by their cognate and non-cognate enzymes suggests that alanine synthetase requires the G3:U70 and A73 nucleotides for efficient charging in vitro, while histidine synthetase requires G-1:C73 and U2:A71. The overlapping nature of these determinants illustrates how the nucleotide specificities of these enzymes have evolved to prevent aminoacylation of the non-cognate substrates.

K 104 WHAT DETERMINES THE RECOGNITION OF tRNA BY *E. COLI* GLUTAMINYL-tRNA SYNTHETASE?

Martina Jahn, M. John Rogers and Dieter Söll, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

The precision of aminoacylation depends on two principles; the identity elements (a limited set of nucleotides that are responsible for specific aminoacylation by the cognate synthetase) and competition between synthetases for tRNAs in the cell. The former can be determined by *in vitro* kinetic measurements while *in vivo* experiments evaluate the contribution of competition. For detailed analysis two sets of mutant tRNA genes were constructed; a set with the suppressor (CUA) anticodon for *in vivo* expression and a set with the wild type anticodon including a change (G1) for *in vitro* transcription by T7 RNA polymerase. The unmodified transcript can be aminoacylated by glutaminyl-tRNA synthetase (GlnRS) with similar kinetic parameters as the wild type tRNA. Approximately 25 mutants were made at positions inferred from biochemical and genetic data (Rogers, M.J. and Söll, D. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 6627-6631) and from the structure of the GlnRS:$tRNA^{Gln}$ complex (Rould, M.A., Perona, J.J., Söll, D. and Steitz, T.A. (1989) *Science* **246**, 1135-1142). The specificity constant (K_{cat}/K_M) determined *in vitro* is different at different positions and varies by more than a factor of 10^5. Mutations at the discriminator nucleotide (73) are about 0.1 relative to wild-type (= 1.0); mutations affecting the 3-70 base pair are 0.01 and the most severe mutations are in the anticodon (e.g. A36) which is 10^{-5}. Despite the impairment to aminoacylation of the suppressor anticodon (CUA), mutants of the suppressor tRNA gene can be evaluated by suppressor efficiency with, for example, mutations of the first base pair affecting aminoacylation *in vivo* about 5-fold. The effect of mutations on suppressor efficiency and possible mischarging by non-cognate synthetases was also evaluated. By correlation with the *in vivo* results, we hope to build a picture of the individual contribution *in vitro* and *in vivo* of elements in the recognition of the tRNA by GlnRS.

K 105 MUTATIONS AT METHYL-MODIFIED SITES IN 16S rRNA IN E. COLI.

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We are studying post-transcriptional modifications of the small subunit ribosomal RNA. Point mutations were constructed at three methyl-modified sites in a cloned 16S rRNA gene in E. coli by oligonucleotide-directed mutagenesis and expressed in wild type cells. The mutations are located at positions C966, G967 and G1207. At the first two positions we constructed three substitutions at each site in addition to a deletion of G967. Mutations at C966 were cloned into pKK3535, a plasmid with wild type promoters regulating rRNA gene expression. The mutations at G967 including the dominant lethal, deletion mutation were cloned into the plasmid pNO2680 where expression of mutant genes is directed by the lambda promoter pL and can be regulated by the lambda repressor protein. Using a modified maxicell procedure we found that mutations at both C966 and G967 are processed normally and are incorporated into 30S subunits and 70S ribosomes. With the exception of the G967 deletion, a dominant lethal mutant, no effects on cell growth rate are observed. Using pNO2680, we constructed three mutations at G1207 in the 16S rRNA gene and found that the transversions to either T(U) or C have dominant lethal phenotypes while the transition to A1207 shows no effect on growth rate. By maxicell analysis we found that these mutations are incorporated into ribosomes. Finally, we have used the dominant lethal mutation, U1207 cloned in pNO2680, as a target for *in vitro* chemical mutagenesis in order to produce second site mutations that abolish the lethal phenotype. We have isolated several revertants with a range of growth rates and we are currently analyzing them to determine the location of second site mutations.

K 106 ANALYSIS OF THE AMINOACYL-tRNA BINDING SITES OF EUKARYOTIC ELONGATION FACTOR 1α (EF-1α). Terri Goss Kinzy and William C. Merrick, Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio, 44106. EF-1 binds both GTP and aminoacyl-tRNA. This factors consists of multiple subunits, and for EF-1 the aminoacyl-tRNA and GTP binding activities have been assigned to the α subunit. This study focuses on further delineating the aminoacyl-tRNA binding site on EF-1α. Three aminoacyl-tRNA species (cys, phe and val) have been crosslinked to EF-1α using two different reagents. The first, trans-diaminedichloro Pt(II) (trans-Pt), crosslinks the N7 of guanine to a cys, his or met residue in the protein, and spans approximately 7 Å. The second, diepoxybutane (DEB), crosslinks the N7 of guanine to a cys or lys residue in the protein and spans a shorter distance. The two crosslinking reagents give different sets of peptides for the three different aminoacyl-tRNA species. By comparison to the proposed structure for *E. coli* EF-Tu (Nyborg and la Cour *in* The Guanine-Nucleotide Binding Proteins, Ed. Bosch, Kraal and Parmeggiani, Plenum Press,p. 3-14), tha approximate domains of EF-1α can be assigned by sequence homology. Both val-tRNA and phe-tRNA crosslink with trans-Pt to yield the same peptides found in domains II (residues 195-323) and III (residues 324-463), while cys-tRNA gives two peptides in domain I (residues 1-194) (GTP binding domain) and the same peptide as the other two from domain III. Only val-tRNA was found to crosslink with DEB, yielding a single peptide from domain III, different from those obtained using trans-Pt. Taken together all are consistent with the binding site being on a single face of the protein. To further support this model aminoacyl-tRNA protease protection studies are being performed using three proteases with different specificities (arg C [R], trypsin [K,R], and V8 [E]) The sites of cleavage and protection are being determined by amino acid sequencing and molecular weight determination.(supported by NIH training grant AM-07319)

K 107 ANALYSIS OF RIBONUCLEASE III - NUCLEIC ACID INTERACTIONS BY INTRINSIC FLUORESCENCE EMISSION SPECTROSCOPY, Paul E. March, Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

Ribonuclease III is a 26,000 molecular weight double strand specific endoribonuclease present in Escherichia coli. RNase III will digest double-stranded genomic viral RNA, rRNA precursors, and mRNA precursors from E. coli and some of its phages. Cleavage of target RNA by RNase III is known to be an important post-transcriptional regulatory event. In an earlier report, protein-protein cross-linking studies demonstrated that RNase III forms a dimer in solution which is capable of adopting more than one conformation state. In the absence of substrate RNA a protein dimer of molecular weight 50,000 was detected. A specific response to the inclusion of substrate RNA was that an additional protein dimer was detected at molecular weight 48,000 [P.E. March and M.A. Gonzalez (1990) *Nucleic Acids Res.* 18, 3293-3298]. These data indicated that an alternative structural state of the dimer occurs upon interaction with substrate. Experiments were carried out to measure the intrinsic fluorescence of RNase III since it is known that fluorescence emission from tryptophan can be a sensitive probe for changes in protein structure. In addition, interpretation of emission spectra is greatly simplified in proteins, such as RNase III, containing a single tryptophan. Tryptophan-139 of RNase III gives rise to an emission spectra with a blue shifted peak (at 328 nm), and a broad shoulder. This signal is quenched by addition of substrate RNA. Quenching also shows that the tryptophan is present in only one environment located at the surface of the protein. Furthermore, the tryptophan is apparently at, or near, a site that interacts with guanine and cytosine preferentially over adenine or uracil. Tryptophan-139 is followed by a tyrosine at residue 140. The positioning of two aromatic side chains at the surface of the molecule may have important implications for the molecular mechanism of RNase III action, and may define a site that contributes to the recognition of double-stranded RNA.

K 108 **THEORETICAL ANALYSIS OF RNA FOLDING**, Andrei A.Mironov, *All-Union Institute of Genetics & Selection of Industrial Microorganism, 113545, Moscow, USSR*

A kinetic approach to the problem of prediction of RNA secondary tsructures based on the analysis of self-organizing molecules is proposed. Structural rearrangements taking place during chain elongation and secondary structure formation are described in terms of Markov process. The elementary transition in this process is chain growth or the new helix formation, maybe after disruption of some old helices. The transition probabilities (speed constants) depend on loops and helices free energy. The Monte-Carlo modelling of this Markov process allow us to see the RNA folding in time. The result of analysis is a kinetic ensemble - a set of secondary structures, with statistical weights as a function of time.

K 109 MESSENGER RIBONUCLEOPROTEIN COMPLEXES FROM CANDIDA UTILIS – PHOSPHORYLATION STATUS AND THEIR TRANSLATION, Sucheta G. Pai, Shaila M. S and Ramananda G. Rao. Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 01 2, India.

Polysome-bound messenger ribonucleoprotein complexes (PmRNPs) and free (not associated with ribosome) messenger ribonucleoprotein complexes (FmRNPs) have been isolated from C. utilis and characterized. The nucleoprotein nature of both the classes of mRNPs was established by their buoyant densities, ratio of protein to RNA and filter binding assay. The complement of proteins associated with the PmRNPs does not coincide with proteins in FmRNPs. Included among the proteins common to both PmRNPs and FmRNPs are the 66 KD poly (A) binding protein and 38 KD protein. The PmRNPs are translatable in vitro whereas the FmRNPs are not. Examination of the in vivo phosphorylation states revealed a 38 KD major and 66 KD minor phosphorylated FmRNP proteins. These are not phosphorylated in case of PmRNPs. In vitro phosphorylation product of PmRNPs/FmRNPs is a 33 KD protein. The specific activity of FmRNP–associated protein kinase is three times higher than that found in PmRNPs. The phosphorylation levels and the difference in translation efficacies suggests that phosphorylation–dephosphorylation may play a role in mRNA translation.

K 110 THE ROLE OF CONTEXT IN tRNA IDENTITY, Kelley Rogers and Dieter Söll, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

Transfer RNA identity has been examined by searching for a specific limited number of nucleotides on a tRNA which are recognized by its cognate aminoacyl-tRNA synthetase. However, it has been speculated that discrimination could also involve the requirement for subtle structural contexts in which these identity nucleotides must exist for proper recognition leading to error-free aminoacylation. If this idea is correct, identity "switches" between tRNAs with similar context should require fewer nucleotide changes than those between tRNAs with different contextual backgrounds. An evolutionary relationship proposed for the glutamine and glutamate aminoacylation systems is supported by the high degree of amino acid homology found between glutamyl-tRNA synthetase (GluRS) and glutaminyl-tRNA synthetase (GlnRS). Since it has been observed in Gram-positive eubacteria and chloroplasts that a single aminoacyl-tRNA synthetase charges both $tRNA^{Glu}$ and $tRNA^{Gln}$ species, it is reasonable to propose that the two tRNAs may be contextually similar. To investigate this hypothesis, we are constructing mutants of two *E. coli* tRNAs to confer glutamine acceptance: $tRNA^{Glu}$ which shows detectable levels of glutamine-acceptance *in vitro* and *in vivo* as an amber suppressor, and $tRNA^{Arg}$ which has not been shown to have glutamine acceptance *in vitro* or *in vivo*. This study should also reveal elements important for recognition by GluRS.

K 111 COMPETITION OF AMINOACYL-tRNA SYNTHETASE FOR tRNA ENHANCES THE ACCURACY OF AMINOACYLATION IN VIVO AND IN VITRO, Joyce M. Sherman, Kelley Rogers, M. John Rogers, Elizabeth Scharl and Dieter Söll, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

Accurate aminoacyl-tRNA formation depends on at least two factors: 1) the specific recognition by an aminoacyl-tRNA synthetase of its cognate tRNAs and 2) competition between synthetases for a given tRNA. It is known that the relative levels of the synthetase and tRNA affect the accuracy of aminoacylation *in vivo* (Swanson *et al.* (1988) Science **242**, 1548-1551; Hou and Schimmel (1989) *Biochemistry* **28**, 4942-4947). We wanted to confirm *in vitro* that competition directly affects the accuracy of Gln-tRNA formation. For this purpose, we have established an *in vitro* competition system which allowed us to study the mischarging of $tRNA^{Glu}$ and *supF* $tRNA^{Tyr}$ and their mutants by *E. coli* glutaminyl-tRNA synthetase. Evidence will be presented which suggests that the *in vitro* competition assay is a novel method for evaluating the relative affinities of synthetases for a given tRNA or tRNA mutant. Results from our *in vitro* competition studies suggested further *in vivo* experiments to examine the role of competition in the aminoacylation of the above-mentioned tRNAs and their mutants. These aminoacyl-tRNAs were analyzed by suppression of an amber mutant in the *E. coli* dihydrofolate reductase gene followed by N-terminal protein sequencing of the gene product.

K 112 SERYL tRNA SYNTHETASE IS ASSOCIATED WITH mRNA AND IS A CONSTITUENT OF RIBONUCLEOPROTEIN COMPLEXES IN MAMMALIAN CELLS. Lawrence I. Slobin[1], Atilla Miseta[2], and Charles L. Woodley[1]. [1]Department of Biochemistry, The University of Mississippi Medical Center, Jackson, MS 39216-4505 and [2]Department of Clinical Chemistry, Medical University of Pecs, Pecs Hungary

Cross-linking by ultraviolet irradiation is a widely employed technique to demonstrate the association of specific proteins to RNA in vivo and in vitro. Using this technique it was demonstrated that a polypeptide of approximately 62 kD is a constituent of messenger ribonucleoprotein particles (mRNP's) from a variety of mammalian cell types [Greenberg, J.R. and Carroll, E.C. (1985) Mol. Cell. Biol. 5:342-351]. Using an antibody to a peptide derived from the structure of EF-1 we detected a positive reaction to a 62 kD polypeptide on Western blots [Slobin, L.I. and Greenberg, J.R. (1989) Eur. J. Biochem. 173:305-310]. Using a sequence derived from the amino-terminal region of the purified 62 kD polypeptide, a cDNA clone was isolated and sequenced. By sequence comparison with the yeast enzyme, as well as functional studies, we demonstrate here the previously reported constituent of mammalian mRNP's is seryl-tRNA synthetase. Studies on the role of this enzyme in the initiation of protein synthesis in rabbit reticulocyte lysates and in the structure of RNP's will be presented.

K 113 THE RNASE P REACTION MECHANISM: USE OF MG^{++} AND 2'-OH IN CATALYSIS. Drew Smith, Alex B. Burgin, Elizabeth S. Haas, and Norman R. Pace, Dept. of Biology and Institute for Cellular and Molecular Biology, Indiana University, Bloomington IN 47405

RNase P is a ribonucleoprotein enzyme which cleaves leader sequences from pre-tRNAs. The RNA is the catalyst, but requires monovalent and divalent cations to function in the absence of the protein. We used a recently developed photoaffinity cross-linking reaction between the enzyme RNA and the substrate phosphate to assay the cation requirement for substrate binding. We find that divalent cation is not required for the specific association of enzyme and substrate: cross-linking occurs at the same sites, and in comparable yield in the presence or absence of divalent cation. We interpret this result to mean that divalent cation is not required for substrate binding, but for cleavage, and so probably acts as a cofactor in catalysis.

To further investigate the reaction mechanism, we constructed a substrate pre-tRNA lacking the single ribose 2'-OH vicinal to the cleavage site. Cleavage of this substrate occurs at a rate at least 1000-fold slower than that of a comparable substrate which contains the vicinal 2'-OH. The 2'-deoxy substitution also increases the requirement for divalent cation: the [Mg^{++}] optimum for the 2'-deoxy cleavage reaction is greater than 100mM, while the optimum for the normal ribo cleavage reaction is 10-15mM. We conclude that removal of the vicinal 2'-OH from the site of cleavage reduces the affinity of the enzyme-substrate complex for Mg^{++}.

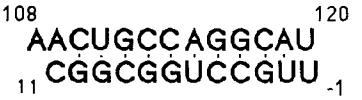
We combine these observations to propose a mechanism for the reaction of RNase P RNA, in which Mg^{++} coordinates directly to the substrate phosphodiester bond, and through water to the vicinal 2'-OH. This complex provides the proper stereochemistry for nucleophilic in-line attack of water on the substrate phosphate, and makes the 3'-O of the precursor phosphodiester linkage a better leaving group.

K 114 **NMR Structural Studies of the Alpha Sarcin-Sensitive Loop from Rat 28S rRNA,** Alexander A. Szewczak[1], Yeun-Ling Chan[2], Ira G. Wool[2], and Peter B. Moore[1], [1]Departments of Chemistry and Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, 06511, USA, [2]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637, USA

The alpha sarcin-sensitive loop contains a highly conserved sequence found in the large ribosomal RNA of both prokaryotes and eukaryotes that serves as the reactive site for the toxins alpha sarcin and ricin. A number of studies suggest that this region plays an important role in elongation during protein synthesis. Evidence indicates that the structure, not merely the sequence of the loop, is important for recognition by the toxins. Accordingly, an NMR study of a 29-mer T7 transcript which models this region in sequence and in reactivity to both toxins has been undertaken. Imino proton spectra support the hypothesis that the loop has a well-defined structure that is stable at 37°C despite an apparent lack of Watson-Crick hydrogen bonding. The ^{31}P NMR spectrum of the loop is rather well-resolved for a molecule of its size, although no peaks fall far outside the range expected for an A type helix. Preliminary NOESY and COSY experiments similarly suggest that the molecule has a well-defined, albeit somewhat unusual, structure. The most recent NMR assignments and structural conclusions regarding the molecule will be discussed.

K 115 **AN NMR STUDY OF HELIX I OF *E.COLI* 5S RIBOSOMAL RNA**, Susan A. White[1], Annabelle Huang[1], Michael Nilges[2], Axel T. Brünger[2], and Peter B. Moore[1], Department of Chemistry[1], Howard Hughes Medical Institute and the Department of Molecular Biophysics and Biochemistry[2], Yale University, New Haven, CT 06511

The goal of this NMR study is to determine the structure of a moderate sized RNA helix which contains two common RNA motifs, a G-U base pair and unpaired terminal bases. Helix I was prepared from 5S rRNA by enzymatic digestion and most of the base and sugar proton resonances have been assigned by COSY and NOESY experiments. Preliminary results indicate that Helix I is an A-form helix and that both the G-U base pair and the dangling ends are stabilized by stacking interactions. In addition, the ribose sugars of the terminal residues have conformations intermediate between 2' and 3' endo as shown by the COSY and DQF-COSY experiments. The structure of this molecule, obtained by using an extended version of X-PLOR which takes into account the full relaxation treatment of spin systems, will be discussed.



Gene Expression in Prokaryotes

K 200 **ARE SITES OF RIBOSOME STALLING DETERMINED BY rRNA-mRNA PAIRING?,**

Nicholas P. Ambulos, Jr., Elizabeth J. Rogers and Paul S. Lovett, Department of Biological Sciences, University of Maryland, Catonsville, MD 21228.

cat **and** ***erm*** **genes are inducible by chloramphenicol and erythromycin respectively, and both are regulated by a mechanism termed translational attenuation. Induction results from antibiotic-dependent stalling of a ribosome at a precise location in the regulatory leader of** ***cat*** **and** ***erm*** **transcripts. A ribosome stalled at this location is thought to destabilize a downstream region of RNA secondary structure which sequesters the ribosome binding site for** ***cat*** **and** ***erm*** **coding sequences. The ribosome stall sequences in the** ***cat*** **and** ***erm*** **leaders are designated** ***crb*** **and** ***erb*****.** ***crb*** **and** ***erb*** **are complementary to a sequence within** ***Bacillus subtilis*** **16S rRNA. An** ***in vivo*** **assay is described to test whether 16S rRNA of a translating ribosome can interact with** ***crb*** **in an inducer-dependent manner. The assay compares growth rates of** crb^+ **and** crb^- **cells in minimal levels of the inducing antibiotic. In the presence of inducer** ***crb*** **retards cell growth. This effect is abolished by insertion of ochre mutations into** ***crb*****, by synonymous codon changes in** ***crb*** **which decrease its complementarity with 16S rRNA or by deleting** ***crb*****. We suggest that inducers of** ***cat*** **permit the** ***crb*** **sequence in mRNA to base pair with sequences in 16S rRNA of a translating ribosome. When base pairing is extensive, ribosomes become transiently trapped on the mRNA and are temporarily withdrawn from cellular protein synthesis to the extent that growth rate declines. Translational attenuation is dependent on site-specific positioning of an inducer-stalled ribosome. The proposed rRNA-mRNA interaction may function to precisely position the ribosome on the mRNA.**

K 201 RNA IN *ESCHERICHIA COLI*: RNase E AND A SMALL STABLE RNA,

David Apirion, Evgeny Makarov, Andras Miczak, Bong-Kyeong Oh and Laimute Taraseviciene, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110

RNase E is a processing endoribonuclease that participates in the processing of 5S rRNA as well as RNAI, a small RNA that controls copy number of plasmid colE1. RNase E plays, probably, an indirect roll in the stability of a large number of messengers. The gene for this enzyme has been cloned and sequenced. It suggests a relatively small protein, but the expressed gene shows a product that migrates as an 110,000 Da polypeptide. This and a number of other peculiarities of the RNase E gene product led to a series of experiments that show that RNase E is found in the cell as a very tight complex of protein and RNA. At least some of the preculiarities connected with RNase E can be accounted for by these findings.

10Sa RNA is a major small RNA of *E. coli*. It is processed from a larger precursor, that accumulates in an RNase E mutant. The processing is a stepwise event, the first step is carried out by RNase III in presence of Mn^{2+}. The last intermediate is a conformer of the mature 10Sa RNA, and it seems that the cell contains a factor (protein) that can catalyze this reaction. By inactivating the 10Sa RNA gene in the chromosome, we found that it is a functional molecule. Since 10Sa RNA is not a messenger and since it is not complexed with any protein in the cell, it could be functioning as a ribozyme.

K 202 RIBOSOMES WITH FRAGMENTED RNA: A NEW APPROACH TO INVESTIGATION OF A FUNCTIONAL ROLE OF rRNA

Alexei A. Bogdanov, Nina V. Chichkova, Elena I. Afonina, Susanna L. Bogdanova, A.N.Belosersky Laboratory, Moscow State University, Moscow 119899, USSR

We offer a new approach to investigation of a functional role of rRNA in the ribosome. It consists of specific degradation of definite sites of 16S rRna of E.coli, reconstitution of 30S subunits from these fragmented RNAs and testing their biological activity. A set of E.coli 16S rRNAs with unique breaks was prepared using the method of oligodeoxyribonucleotide-directed RNA fragmentation with RNAse H. 16S rRNA with unique breaks or its isolated fragments were used for reconstitution of 30S ribosomal subunits. Their activity was assessed in the poly(U)- and MS2 RNA-directed cell-free systems of translation.

This approach was applied for the probing of eight different sites of 16S rRNA. The level of activity was high enough in all but two cases: when the cleavage occurred near the the position 1400 and near the center of the 16S rRNA molecule. Two types of the particles with the deletions in the region 995-1045 and 995-1210 were obtained. The former proved to be almost fully active, the activity of the latter has not been detected.

K 203 EFFECTS OF MUTATIONS IN 4.5S RNA ON PROTEIN SYNTHESIS IN *Escherichia coli*. D.B. Bourgaize and M. O'Loughlin Department of Chemistry, Colby College, Waterville, Me. 04901

4.5S RNA of *E. coli* is a small, stable RNA which associates transiently with the ribosome while performing an essential function in the protein synthetic process. A series of mutations was created within the gene for 4.5S RNA in order to determine more precisely its role within the cell. The ability of these altered genes to function *in vivo* was assayed by constructing strains in which the mutated gene provided the only 4.5S RNA molecules within the cell. Mutations which yield non-functional molecules did not survive. Strains which survived with altered forms of the molecule were examined to determine the effect of the changes in 4.5S RNA on protein synthesis. Results obtained thus far indicate that there are at least two regions of the molecule which can be altered without eliminating the essential function of the molecule. Mutations in both of these regions result in changes in the rates of synthesis of some proteins.

K 204 MUTATIONS AT POSITION 913 OR 915 OF ESCHERICHIA COLI 16S RNA INTERFERE WITH THE BINDING OF STREPTOMYCIN TO THE RIBOSOME, Léa Brakier-Gingras, Daniel Leclerc and Pierre Melançon, Département de Biochimie, Université de Montréal, Montréal, Québec, Canada H3C 3J7.
Footprinting studies (Moazed and Noller, Nature, 1987, 327, 389) have shown a strong protection of the 913-915 region of 16S rRNA by streptomycin. The nine possible single-base substitutions were introduced at position 913, 914 or 915 of E. coli 16S rRNA, in plasmid pKK3535-encoded rrnB operon, with the hypothesis that these changes would alter the response to streptomycin. Only two of the mutations (A913->G and A915->G) were viable in E. coli. None of these mutations affected the growth rate of the transformed cells nor did they confer resistance to streptomycin. A mutation in the plasmid-coded rRNA which could change the response to streptomycin cannot be detected in vivo because of the dominance of sensitive ribosomes with rRNA transcribed from the host genome (they account for about 30% of the total ribosome population). In vitro studies were then performed with ribosomes extracted from the transformed cells to assess the effect of the mutations. Protein synthesis assays under the direction of poly(U) or MS2 RNA did not show any significant change in the activity of the ribosomes. However, as expected, they bound less streptomycin and were restrictive to the stimulation of misreading by the drug. We propose that the mutations interfere with a conformational change of 16S rRNA which is required for the tight binding of the drug (Supported by MRC of Canada).

K 205 INTRODUCTION OF POINT MUTATIONS IN THE SPECTINOMYCIN BINDING REGION AND PSEUDO-KNOT HELIX OF 16S rRNA OF SPECIALIZED RIBOSOMES. Marcel F. Brink, Martin Ph. Verbeet & Herman A. de Boer, Department of Biochemistry, Gorlaeus Laboratories, University of Leiden, Leiden (The Netherlands).

We have studied the effects of several point mutations in 1) the spectinomycin binding region (centered around position 1192 of 16S rRNA), which may be involved in the translocation process and 2) the pseudoknot structure at the 5'end of the 16S rRNA, which has been suggested recently, to be part in a conformational switching mechanism of the 30S subunit.
In the specialized ribosome system, a distinct pool of mutated ribosomes is dedicated to the translation of one particular mRNA species coding for chloramphenicol acetyl transferase (CAT). This has been accomplished by an alteration of the Shine-Dalgarno sequence on the CAT-mRNA and a complementary change of the anti Shine-Dalgarno sequence in the 16S rRNA gene of the plasmid borne rrnB operon. Using the polymerase chain reaction (PCR), we have introduced point mutations in several regions of the 16S rRNA gene and subsequently we have studied their effect on the translation of the CAT-mRNA by specialized ribosomes.
Previously,it has been shown that mutating the C-residue at position 1192 of the 16S rRNA into any of the other nucleotides, resulting in a disruption of basepairing between residues C_{1192} and G_{1064}, renders ribosomes resistant to spectinomycin. Changing this C-residue also had a restorative effect on a set of otherwise deleterious mutations in the stem structure adjacent to the C_{1400} region. By changing the G_{1064} into any of the other nucleotides, we have examined whether the disruption of basepairing results in spectinomycin resistance and in restoration of translational activity of the C_{1400} stem structure mutants.
We also have disrupted the postulated pseudo-knot helix at the 5'end of 16SrRNA, by changing the C-residue at position 18, which can form a basepair with the G residue at position 917. We will present effects of disrupting this pseudo-knot on the translational activity of the specialized ribosomes.

K 206 **ANALYSIS OF THE ANTICODON NUCLEASE GENE CLUSTER,** Daphne Chapman-Shimshoni, Ilan Morad, Michal Amitsur and Gabriel Kaufmann, Department of Biochemistry, Tel Aviv University, Tel Aviv 69978 Israel

Anticodon nuclease (ACNase) is encoded in latent form by the optional E. coli locus prr. Latent ACNase is activated upon T4-infection by the polypeptide product of the phage stp gene. ACNase cleaves host $tRNA^{Lys}$ molecules, presumably during a step in translation and functions as a phage restriction system. However, additional roles in regulating protein synthesis can not be excluded. Three prr-encoded ACNase polypeptides have been identified. PrrC elicits ACNase activity when expressed in uninfected E. coli from a multicopy plasmid in the absence of other Prr elements. When PrrC is expressed with PrrA and a C-truncated PrrD, ACNase is not detected. Both PrrA and truncated PrrD are needed to mask ACNase. The masking must be executed at the enzyme level, as indicated by in vitro ACNase complementation assays but the masking elements could regulate PrrC in additional ways. Intact PrrD masks poorly, suggesting separate roles for its C- and N-domains in auto-regulation and ACNase masking, respectively. ACNase is not detected when PrrC is expressed from its own promoter at low plasmid copy number, both with or without expression of the masking elements. These data suggest the existence of a PrrC-activator encoded by a yet unidentified prr gene.

K 207 **INVOLVEMENT OF RIBONUCLEASE E IN BACTERIOPHAGE T4 MESSENGER RNA PROCESSING AND DEGRADATION,** Agamemnon J. Carpousis, Claude Ehretsmann, Griet Van Houwe and Henry M. Krisch, Department of Molecular Biology, 30 Quai Ernest-Ansermet, University of Geneva, CH1211 Geneva 4, Switzerland

Rne$^-$ N3431 (ribonuclease E) and *ams*$^-$ HAK117 (affects message stability) are independently isolated *E. coli* temperature-sensitive mutants that fail to make 5S ribosomal RNA, exhibit a six-fold increase in the stability of bulk *E. coli* mRNA, and show striking increases in both the chemical and functional stability of many bacteriophage T4 transcripts. Recently, these mutations were shown to be very tightly linked and probably within the same gene (Mudd et al., *Molecular Microbiology*, in press). *Rne* is believed to be the structural gene for ribonuclease E, an endonuclease implicated in the maturation of 5S ribosomal RNA. The bacteriophage T4 gene 32 transcription unit has RNase E dependent processing sites at -71 and -1340 relative to the gene 32 transcription initiation codon. In addition, messages encoding gene 32 show a dramatic increase in chemical and functional mRNA stability in the *rne*$^-$ strain at the non-permissive temperature. These *in vivo* results suggest that RNase E is responsible for the processing of the gene 32 transcript and may also be the limiting enzyme in the initiation of its degradation. We have prepared extracts from wild-type, *rne*$^-$ and *ams*$^-$ *E. coli* and partially purified the RNase E activity. These preparations will be used to study gene 32 mRNA processing and degradation *in vitro*. The aim of these experiments is to attempt a direct demonstration of RNase E processing *in vitro*, and to try to identify the RNase E target(s) in the gene 32 mRNA that is responsible for the initiation of degradation.

K 208 A STRUCTURAL EXPLANATION OF THE FUNCTION OF ELONGATION FACTOR TU

B.F.C. Clark, M. Kjeldgaard, J. Nyborg, S.S. Thirup, C.R. Knudsen, B. Degn, D.E. Otzen, J.N. Pedersen & O. Wiborg
Division of Biostructural Chemistry, Department of Chemistry
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Our research programme applies protein engineering techniques in studies of how alterations in a protein's structure can modify its biological functions. The protein selected for our studies is the bacterial elongation factor EF-Tu, particularly because of its multi-functionality and its possible use as a model for the GTP/GDP-binding proteins.

As a basis for protein engineering experiments the structure of EF-Tu:GDP has been solved to 2.6Å resolution with an R-factor of 19.2% by X-ray crystallography. This structure has generated logically designed experiments to elucidate the GTP-hydrolytic centre and the aminoacyl-tRNA binding sites. In addition to mutants VG20 and PT82, recent progress will be discussed.

Furthermore, EF-Tu has been used as a model for other translational factors, including the eucaryotic analogue EF-1α, to define surface features.

K 209 ONE OF TWO PROMOTERS THAT EXPRESS *infA* ENDODING IF1 IS GROWTH-RATE REGULATED, Helen S. Cummings and John W. B. Hershey, Dept. of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616

The IF1 gene, *infA*, is located at 20 minutes on the *E. coli* chromosome. S1 nuclease mapping, Northerns, and *infA-lacZ* fusions indicate that *infA* is expressed as monocistronic mRNAs by a classical promoter (P_1) lying about 250 bp upstream from the AUG initiator codon and by a second promoter (P_2) located 39 bp from the structural gene. A strong rho-independent terminator lies 65 bp downstream of the stop codon. To address the regulation of *infA* expression, a series of promoter and protein fusions with *lacZ* was constructed which contained the isolated P_1 or P_2 promoter with variable amounts of the 5'-UTR (untranslated region) and structural gene of *infA*. The ß-galactosidase activity from these fusions suggests that the expression of P_2 is 2-fold greater than that of P_1. The 5'-UTR apparently plays a role in the regulation of the P_1 promoter because a construct containing P_1 and the first 18 bp of the 5'-UTR gave maximal expression of P_1 while other constructs with P_1 and increasing lengths of the 5'-UTR decreased P_1 expression by 10-fold. The strains carrying the promoter and protein fusions were grown in different media in order to achieve a range of growth rates. The protein fusions exhibited increased expression at higher growth rates comparable to that of the fusion carrying just the P_2 promoter. Overproducing IF1 in the protein fusion strains did not affect ß-galactosidase activity. These data indicate that the expression of *infA* is not autogenously regulated and that the P_2 promoter is growth rate regulated whereas P_1 is constitutive.

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K 210 INTERACTION BETWEEN TWO HIGHLY CONSERVED, SINGLE-STRANDED REGIONS OF 16S RNA IS REQUIRED FOR RIBOSOME FUNCTION, Philip R. Cunningham, Michelle Pflumm, Carl J. Weitzmann, Kelvin Nurse and James Ofengand, Department of Biochemistry, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

Protein synthesis in all organisms is catalyzed by ribosomes, an organelle whose basic structure and function have been conserved throughout phylogeny. In *Escherichia coli* the small subunit RNA (16S) contains three sequence-conserved, single-stranded regions. Two of these sequences are in the 3' minor domain and extend from position 1397 to 1408 and from 1492 to 1505. We have constructed several mutations in these two regions and determined their effects upon many of the partial reactions of protein synthesis *in vitro*. In one set of mutations, single and double substitutions of the G1401 and C1501 residues were constructed. The results indicate that base pairing between G1401 and C1501 is essential for proper ribosome function. In another set of substitutions U1498 was replaced by an A, C, or G. While all other functions were unaffected, the C1498 and G1498 mutations specifically inhibited initiation-dependent dipeptide formation by approximately 50% and 80% respectively. The A1498 mutant was normal in all assays.

K 211 SITES OF CONTACTS OF mRNA WITH 16S RNA AND 23S RNA IN THE *E. COLI* RIBOSOME. Alain Expert-Bezançon*, Paul Wollenzien** and Alain Favre*.
*Institut Jacques Monod CNRS Université Paris VII , 2 Place jussieu 75251 Paris, France.
**Department of Biochemistry and molecular Biology, St. Louis University Medical Center, St. Louis Missouri 63104.

The location of interactions between ribosomal RNA (rRNA) and messenger RNA (mRNA) were determined by photochemical crosslinking of an artificial mRNA, 51 nucleotides long containing 14 U residues. Uracil residues were substituted randomly by one to four 4-thiouridine (S^4U) residues. The mRNA was bound to 70S ribosomes and 30S subunits and S^4U residues were specifically photoactivated at 366 nm. Crosslinking occurs to both 16S and 23S RNA. The sites of crosslink were determined by reverse transcriptase analysis. Twelve sites were detected on 16S RNA and two sites on 23S RNA. The distribution of these sites specifies an mRNA track that is consistant with other informations on the rRNA three-dimensional structure models. Some of the sites defines new constraints for the three dimensional folding of the rRNA.

K 212 16S rRNA Methylation, AppppA and Proper Induction of the Heat Shock Response in *E. coli*. Spencer B. Farr, Dept. of Toxicology, Harvard School of Public Health, Boston, MA., 02115.
The 'heat shock' response is induced when the translational machinery of the cell is damaged. The *ksgA* gene in *E. coli* encodes a dimethyltransferase whose function is to methylate two adjacent adenosines near the anti-S.D. site of the 16S rRNA. *E. coli ksgA* mutants do not methylate these adenosines. The *apaH* gene encodes AppppA hydrolase and is part of the *ksgA* operon. We have found that while *ksgA* mutants are hypersensitive to killing and do not show inducible thermotollerance, they show normal induction of heat shock proteins. They are very slow to resynthesize house-keeping proteins after heat shock, suggesting that methylation of 16S rRNA is required for correct resynthesis of non-heat shock proteins. *apaH* mutants are also sensitive to killing by heat and cannot be habituated to heat. They show prolonged synthesis of heat shock proteins. The hypersensitivity of *ksgA* mutants to thermal killing is ameliorated to some extent in an *apaH*$^-$ background, suggesting a connection between AppppA, 16S rRNA methylation and heat shock regulation. Recent results will be presented.

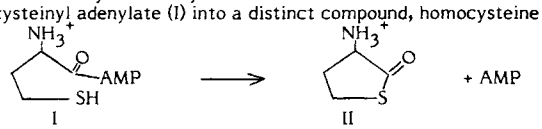
K 213 UNCHARGED tRNA, PROTEIN SYNTHESIS, AND THE BACTERIAL STRINGENT RESPONSE, Emanuel Goldman and Hieronim Jakubowski,
Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, N.J. 07103
Uncharged tRNA has been shown *in vivo* to have an active role in both the stringent response, and in modulating the rate of translational elongation. Both of these effects appear to be mediated by codon-anticodon interactions on the ribosome. Although the involvement of uncharged tRNA in the stringent response was expected from *in vitro* experiments, it has only recently been confirmed *in vivo*. Inhibition of translation by cognate uncharged tRNA was not expected, and a model is proposed in which excess uncharged tRNA completes with charged tRNA (in ternary complex) for the 30S component of the ribosomal A site. When uncharged tRNA is in sufficient excess over charged, interaction of uncharged tRNA with the 50S component of the A site occurs as well, leading to a stringent response. The cell has a continuum of responses to decreasing aminoacyl-tRNA levels: at moderate limitations, the proportion of uncharged tRNA increases, and the translation rate is slowed; under more severe limitations, uncharged tRNA provokes a stringent response, with pleitropic consequences for the cell.

K 214 ISOLATION AND CHARACTERIZATION OF THE *BACILLUS SUBTILIS rpsD* GENE, ENCODING RIBOSOMAL PROTEIN S4, Tina M. Henkin and Frank J. Grundy, Department of Biochemistry and Molecular Biology, Louisiana State University School of Medicine, Shreveport, LA 71130

Ribosome biosynthesis is tightly controlled in bacterial cells. The regulation of ribosomal gene expression has been analyzed in depth only in *E. coli*, where posttranscriptional control mechanisms have been found to predominate. The goal of this study is the analysis of ribosomal protein gene regulation in *B. subtilis*. The *B. subtilis rpsD* gene, encoding ribosomal protein S4, was isolated by hybridization with oligonucleotide probes derived from the S4 amino-terminal protein sequence, and its DNA sequence and transcriptional signals were determined. In *E. coli*, *rpsD* is in the α operon, and S4 is the translational repressor for that operon, binding to a target site on the α operon mRNA. In contrast, the *rpsD* gene in *B. subtilis* is monocistronic. Transcription of *rpsD* initiates at a site 180 bp upstream from the start of the coding sequence. This leader region is predicted to be rich in secondary structure, resembling structures found to be important in α operon regulation in *E. coli*. The biological significance of the leader region structure, and the possibility that S4 binds to this structure to regulate translation of the mRNA, are currently under investigation in our laboratory.

K 215 PROOFREADING in vivo: EDITING OF HOMOCYSTEINE BY METHIONYL-tRNA SYNTHETASE IN Escherichia coli AND Saccharomyces cerevisiae, Hieronim Jakubowski, Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, N.J. 07103

Previous in vitro studies have established a pre-transfer proofreading mechanism for editing of homocysteine by several aminoacyl-tRNA synthetases. The unusual feature of the editing is the transformation of homocysteinyl adenylate (I) into a distinct compound, homocysteine thiolactone (II).



I II

The thiolactone is also present in cultures of Escherichia coli and Saccharomyces cerevisiae. Both in E. coli and S. cerevisiae, the thiolactone is made from homocysteine in a reaction catalysed by methionyl-tRNA synthetase. One molecule of homocysteine is edited as thiolactone per 109 or 500 molecules of methionine incorporated into protein in E. coli or S. cerevisiae, respectively. Homocysteine, added exogenously to the medium or overproduced by some yeast mutants, is detrimental to cell growth. The cost of homocysteine editing in yeast is minimized by the presence of a pathway leading from homocysteine to cysteine, which keeps intracellular homocysteine at low levels. These results not only directly demonstrate that editing of errors in amino acid selection by methionyl-tRNA synthetase operates in vivo both in E. coli and in yeast but, in general, establish the importance of proofreading mechanisms in living cells.

K 216 TRANSLATION OF LEADERLESS TRANSCRIPTS IN EUBACTERIA

Gary R. Janssen, Robert L. Jones, and Chi-Ju Wu. Department of Biology, Indiana University, Bloomington, IN 47401.

Translation initiation is a critically important step in gene expression. The vast majority of eubacterial mRNA's interact with ribosomes via complementarity between a region on the ribosomal RNA and a sequence contained in a leader region upstream to the transcripts coding sequence. However, several prokaryotic transcripts have been reported that lack upstream leader regions but are still translated.

We have used a leaderless neomycin resistance gene (aph) from the bacterium Streptomyces to investigate translation of leaderless transcripts. Various alterations have been introduced to the transcripts' extreme 5' end to characterize the constraints for AUG codon placement in translation initiation and reading frame specification. Addition of short leaders to the aph transcript results in dramatic affects on its translatability. Placement of two AUG initiation codons at the 5' end, but in different reading frames, suggests that ribosomes initiate most frequently, perhaps exclusively, at the AUG nearest to the 5' end. Gene fusion techniques have been used to engineer two genes such that their normal upstream leader regions, containing conventional ribosome binding sites, are deleted; translation of these leaderless transcripts suggests that ribosomes are capable of translating a variety of leaderless transcripts provided an in-frame AUG is at, or near, the 5' end.

K 217 STABILIZATION OF puf mRNA SEGMENTS BY A HAIRPIN LOOP STRUCTURE IN VIVO AND IN VITRO, Gabriele Klug, Rüdiger Rothfuchs, Jürgen Fritsch, Zentrum für Molekulare Biologie Heidelberg, Im Neuenheimer Feld 282, D6900 Heidelberg, Germany

An intercistronic secondary structure located in the puf operon of the photosynthetic bacterium Rhodobacter capsulatus is responsible for segmental differences in puf mRNA stability and consequently for the stoichiometry of light harvesting (LHI) I complexes and reaction center complexes in wild type cells.

Here we report that an mRNA segment transcribed from a synthetic DNA sequence and identical to the wild type sequence that forms the intercistronic hairpin loop structure stabilizes the upstream puf mRNA, when inserted at various sites 5' to a region containing sites for rate-limiting endonucleolytic cleavage. When the inserted RNA sequence is translated, it stabilizes the 5' mRNA segment not to the same extend as an untranslated segment, suggesting that ribosomes interfer with the formation of a stable secondary mRNA structure. The LHI specific puf mRNA that is normally stabilized by the intercistronic hairpin loop structure can not be stabilized further by introducing two copies of this structure. The hairpin-loop structure also stabilizes LHI specific in vitro transcripts against degradation by cell-free R.capsulatus extracts or polynucleotide phosphorylase in vitro.

K 218 TRACE AMOUNTS OF FLUOROALUMINATES INHIBIT PROTEIN BIOSYNTHESIS VIA ELONGATION FACTOR-RIBOSOME INTERACTIONS, Barend Kraal, J. Martien de Graaf and Jeroen R. Mesters, Department of Biochemistry, Leiden University, Leiden, The Netherlands

Fluoroaluminates perturb the functioning of a number of different guanine-nucleotide binding proteins, such as membrane-associated signal-transducing G-proteins. This is thought to be due to their mimicking of the γ-phosphate in the GTPase centre of these proteins. We unambiguously demonstrated (Eur. J. Biochem., in press) that this is not true for the elongation factor EF-Tu. The latter is often referred to as a model for these regulatory G-proteins on account of the detaild X-ray data for the 'consensus' amino acid sequences in its GTP-binding domain. On the other hand, we indeed observe a drastic inhibition of poly (Phe) synthesis in an E. coli system in the presence of micromolar amounts of fluoroaluminates. The inhibition does not take place at the peptidyl-transferase centre of the ribosome as judged by the puromycin reaction. It is neither due to interference with the enzymatic binding of Phe-tRNA. It can be totally explained, however, by the observed blockage of the ribosome-induced activation of the GTPase centres of both the elongation factors EF-Tu and EF-G. This opens new perspectives for a better understanding of the drastic effect of fluoroaluminates on the interaction between GTP-binding proteins and effector complexes in general.

K 219 **Isolation and Characterization of the *B. subtilis infB* and *infC* Genes Encoding Protein Synthesis Initiation Factors IF2 and IF3:** K. Shazand*°, P. Hwang°, J. Tucker°, J.C. Rabinowitz°, T. Leighton° and M. Grunberg-Manago°, °Institut de Biologie Physico-Chimique; 13, Rue P.&M. Curie, 75005 - Paris, France, °Depts. of Biochemistry and Molecular Biology University of California, Berkeley, Ca. 94720 - USA.

B. subtilis genes encoding protein synthesis initiation factors IF2 and IF3 have been cloned in *E. coli*, using *E. coli* of *B. stearothermophilus infC* gene probes. Protein sequence comparisons between the *B. subtilis* IF2 gene and other bacterial IF2 homologs revealed sequence conservation of 40% to 70%, extending over all but the extreme N-terminal regions of IF2. The *infB* gene has been mapped at 145°, situated between *spcB* and *polC*. The *infB* gene encodes two forms of IF2, IF2 α and IF2 β, as does the *E. coli infB*. Expression of *B. subtilis* IF2 α or IF2 β in *E. coli* allows complementation of a deleted *infB* gene. Overlapping genomic restriction fragments spanning 6 kb of the *infB* locus have been isolated from random λ gt11 libraries. DNA sequence analysis of these flanking regions has identified open reading frames homologous to the *E. coli* proteins P15A, NusA, P15B, P35 and PNP which flank the *E. coli infB* region. Approximately 2 kb of genomic DNA surrounding the *B. subtilis infC* region has been cloned in M13 tg131. DNA sequence analysis of the *infC* gene revealed 50% aminoacid homology with *E. coli* and 75% with *B. stearothermophilus* IF3. Western blots of *B. subtilis* cell free extracts, using a monospecific *E. coli* anti-IF3 antibody detected an 18 kD cross-reacting protein.

K 220 THE hok mRNA FROM PLASMID R1 REQUIRES BOTH ENDS FOR ITS EXTREME STABILITY. Jan Martinussen and Kenn Gerdes, Institute of Molecular Biology, Odense University, Campusvej 55, DK-5230 Odense M, Denmark.

The hok/sok locus of plasmid R1 codes for two RNAs: the hok mRNA and the sok antisense RNA. The sok RNA is very unstable, whereas the hok mRNA seems to be totally stable. Two hours after the addition of rifampicin, no degradation of the hok mRNA was seen.

In order to determine the structures of the RNA, responsible for this extreme stability, two experiments were performed: First a synthetic linker was introduces into the middle of the mRNA, destroying its two potential reading frames. This did not change the stability of the message. Second, the 3´ and 5´ ends were deleted, and it was shown, that both ends must specify stability determinants.

From these observations we conclude: 1. Mature proteins encoded by the hok mRNA, are not involved in stabilising the message. 2. Sequences in the middle of the hok mRNA does not specify stability determinants. 3. The presence of both ends are a prerequisite for stability. 4. It is possible to insert foreign bases in the middle of the molecule without affecting its stability.

K 221 **USE OF THE "TRANSLATIONAL COUPLING" FOR OPTIMIZATION THE FOREIGN GENE TRANSLATION IN *E.COLI* CELLS AND CONSTRUCTING THE NEW TYPE OF EXPRESSION VECTORS,** Sergey V. Mashko, *All-Union Research Institute of Genetics and Selection of Industrial Microorganisms, 113545, Moscow, U.S.S.R.*

A "TGATG" expression vector system was developed that allows for the construction of hybrid operons with partially overlapping genes, employing the effects of translational coupling to optimize expression of cloned cistrons in *Escherichia coli*. In this vectors the coding region of a foreign gene is attached to the ATG codon situated on the recombinant plasmid, to form the hybrid operon transcribed from the phage λ p_R-promoter or from the T7 RNA polymerase-controlled T7 late promoter ϕ10. The cloned gene is the distal cistron of this hybrid operon ("overlappon"). The efficiently translated derivates of *E.coli cat* gene are the proximal hybrid cistrons to the promoter. At the 3'-end of these artificial genes there is a region for efficient translation reinitiation with overlapping stop and start codons, TGATG, where the last G is the first nucleotide of the *Sac*I-recognition site. Several dual--origin temperature-controlled amplifiable TGATG expression vectors was successfully used for accumulation of human or animal interferons, human interleukins, angiogenin and some prokaryotic proteins in *E.coli* cells after the thermal inactivation of CIts857 repressor and/or chemical inducing the synthesis of T7 RNA polymerase.

K 222 TRANSLATIONAL SUPPRESSION AND DECODING DETERMINANTS IN tRNA, mRNA, AND rRNA, Emanuel J. Murgola, Betsy H. Mims, Frances T. Pagel and Kathryn A. Hijazi, Department of Molecular Genetics, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.

We have been characterizing mutants, isolated by classical selection methods as well as with site-directed mutagenesis, of tRNA, mRNA, and rRNA that cause or influence translational suppression. Such mutants are being used to study the roles and interactions of those macromolecules in the specificity, accuracy, or efficiency of decoding. In our poster, information will be presented that bears upon the following: (a) interaction between decoding determinants and identity determinants in a tRNA; (b) codon-specific and tRNA-specific codon context effects caused by three nucleotides before and after the codon being read; and (c) rRNA determinants of ectopic termination (at a sense codon during elongation). Our results underscore the interconnectedness of translational macromolecules in the decoding of genetic information.

K 223 RIBOSOMAL PROTEIN PATH OF mRNA IN THE 30S RIBOSOMAL TERNARY INITIATION COMPLEX OF *E.COLI*, Kirill V.Rosen, Olga A.Dontsova, Susanna L.Bogdanova, Eugene A.Skripkin, Alexei M.Kopylov and Alexei A.Bogdanov, Department of Chemistry, Moscow State University, Moscow 119899, USSR.

The topography of mRNA within the translating ribosome is poorly understood. To study the path of mRNA within the small ribosomal subunit during initiation (mRNA•30S subunit•$tRNA_f^{Met}$) we used an affinity cross-linking approach in which photoactivated groups are attached to different positions along the mRNA. A series of mini-genes originating from the *cro* gene of lambda phage was constructed. 5-azidouridine was randomly incorporated into these test transcripts using T7 polymerase. Two messages were used that have U residues at either -4, -3, -1, +2 and +14, +19, +20 or only -4, -3, -1, +2 (A from *cro* AUG is +1). Two different *in vivo cro* transcripts were derivatized at there 5'-end with a photoinducible diaziril group. One of these messages allowed localization of the 5'-end of the Shine-Dalgarno sequence while the other allowed for labeling of the 5'-end of the start codon. Whereas cross-linking with the azidoU transcripts is essentially zero-length, the 5'-derivatized transcripts label ribosomal components 14Å from the 5'-end. From the cross-linking patterns obtained, we conclude that nucleotides +19 and +20 are close to S3 and nucleotides from -4 to +2 neighbour S7 and S5. The 5'-end of the start codon may be within 14Å from S7 while the 5'-end of the Shine-Dalgarno is located 14Å from S3.

K 224 FACTORS INFLUENCING THE TRANSLATIONS OF THE recF GENE OF E. COLI K-12, S. J. Sandler and A. J. Clark, Dept. of Molecular and Cell Biology, GPBB, University of California at Berkeley, Berkeley, CA 94720

recF is a nonessential gene located at 83 minutes on the chromosome in a group of genes (dnaA, dnaN, recF and gyrB) involved with DNA metabolism. Single recF mutant phenotypes include deficiency in some aspects of DNA repair, recombination and mutagenesis. Sequences in the first 543 bp. of the recF gene have been found to inhibit completion of translation 99% of the time (Gene **86**: 35-43) due to mechanisms which act after initiation of translation. There are two main regions of regulation, 8-140 bp. (Region I - 13 fold effect) and 300 to 477 bp. (Region II - 4 fold effect). Mutations in Region I have been isolated and increase expression 10 fold. These sequences affect a "Translational Enhancer" and or the potential for the RNA to form a psuedoknot. We hypothesize that interactions between the ribosome and this sequence after initiation stall the ribosome and lead to either premature termination or frameshifting.

K 225 3D-MODELS OF RNA AND PROTEIN IN RIBOSOMAL SUBUNITS AND THEIR COMPARISON WITH EXPERIMENTAL X-RAY AND NEUTRON SCATTERING DATA, Igor N. Serdyuk and Mikhail Yu. Pavlov, Institute of Protein Research, Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR

Theoretical scattering curves for different models of 3-D arrangement of protein in 30S subunits were calculated in the region of Q up to 0.2 $Å^{-1}$ ($Q=4\ \pi/\lambda\ \sin\ \theta$, where λ is the wavelength, $2\ \theta$ the scattering angle). Analogous curves were calculated for different models of tertiary structure of 16S and 23S RNA. These curves are compared with experimental SAXS and SANS data for 30S and 50S subunits at different contrast in the same region of Q. Complete accord between the experimental and theoretical curves were not observed for any of these models. The possible reason for the discrepancies are discussed.

K 226 A RETROVIRUS-LIKE ZINC DOMAIN IS ESSENTIAL FOR TRANSLATIONAL REPRESSION OF BACTERIOPHAGE T4 GENE 32, Yousif Shamoo, Kevin R. Webster, Kenneth R. Williams and William H. Konigsberg, Department of Molecular Biophysics and Biochemistry, Yale Univ. School of Med., New Haven, CT 06510

Gene 32 protein (gp32), a single-stranded DNA binding protein from bacteriophage T4, contains a zinc binding subdomain with sequence homologies to the 3 cysteine/1 histidine zinc binding motif found in a variety of retroviruses and plant viruses. In vitro studies suggest that autoregulation of gp32 occurs at the level of translation by specifically binding gene 32 mRNA at an unusual stem-loop structure that can be modelled as an RNA pseudoknot located 40 nt upstream from the gene 32 initiation codon. Nucleation of gp32 binding via this pseudoknot is thought to be needed to facilitate cooperative binding of gp32 through a largely unstructured region that overlaps the ribosome binding site [McPheeters, D.S., Stormo, G.D., and Gold, L. J. Mol. Biol., 201, 517-535]. Removal of Zn(II) from gp32 results in a protein that retains the ability to bind single-stranded RNA with high affinity but is unable to specifically autoregulate itself at the level of translation. Deletion of the pseudoknot sequences from the gene 32 autoregulatory region results in an mRNA that cannot be repressed by gp32. These results suggest that the zinc binding subdomain of gp32 plays an essential role in autoregulation by providing a critical element that is necessary for nucleating cooperative binding at the gene 32 mRNA pseudoknot.

K 227 pCIE; A COLD SENSITIVE INTEGRATION-EXPRESSION VECTOR, J. Daniel Sharer and Paul E. March, Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

A key event required for ColE1 plasmid replication is the production of primer RNA (RNA II). During normal plasmid DNA synthesis the amount of RNA II available for priming is regulated by a second RNA (RNA I). Plasmid copy number is controlled by the competition between RNA I : RNA II hybridization, and RNA II : primer-site hybridization. The plasmid pCIE was constructed from pUC19. A DNA fragment containing the phage λ cI857 gene and λ promoter P_R was inserted into pUC19 such that the synthesis of RNA II was controlled by the λ elements. In addition the RNA I locus was disrupted by this insertion. Therefore, in the absence of RNA I production, plasmid copy number is regulated only by the amount of RNA II produced from P_R. We show that the copy number of pCIE is dependent on growth temperature and can be adjusted to levels between 0 copies per cell (at 28°C) to > 10^3 copies per cell (at 42°C). Furthermore, with appropriate selection and screening procedures pCIE can be employed in genetic analysis via homologous recombination at 28°C, or to produce enormous amounts of plasmid DNA at 42°C (12 mg pure DNA/liter culture). The features of the pUC19 cloning vector have been retained (convenient multiple cloning site and blue-white selection for inserted DNA).

K 228 Measurements of *in vivo* translation rates in *E. coli.* Michael A. Sørensen and Steen Pedersen. Institute of Microbiology, University of Copenhagen, Denmark.

The translation rate in *E. coli* is codon specific. This has been shown by insertion of short sequences (90 bp) in a plasmid-borne *lacZ* gene and using a refined pulse-chase labeling technique to measure the translation time of the inserts. Measuring rates on inserts encoding multiple GAA or GAG glutamic acid codons, shows that the GAA codon is translated with a rate of about 25 aa/sec., whereas the GAG codon is translated with a rate of only 6.7 aa/sec. The determination of these exact rates also comprised measurements on the Pro codon CCG and the Arg codon CGA for which we find the rates of 6.7 and 3.1 aa/sec., respectively. The rarely used Arginine codon CGA is translated by the major $tRNA^{Arg}_2$. Both Glu codons are translated by the same tRNA ($tRNA^{Glu}_2$). The fourfold difference in translation rate can therefore not arise from differences in tRNA concentrations. From this we conclude that the tRNA concentration is not the sole determinant of translation rate. In these experiments we can not detect any significant effects of context on the codon specific translation rates. Previous experiments have shown that mRNA secondary structures do not affect the translation rate. We therefore suggest that the nature of the codon:anticodon interaction significantly affects the speed of the translating ribosome through one or more of the following mechanisms : initial recognition, proof reading or even translocation.

K 229 GENETIC AND BIOCHEMICAL APPROACHES TO THE ANALYSIS OF THE DOMAIN STRUCTURE OF THERMOPHILIC IF2, **Roberto Spurio, Claudio O. Gualerzi, Anna La Teana, Manuela Severini and Cynthia L. Pon**, Laboratory of Genetics, Dept. of Biology, University of Camerino, 62032 Camerino (MC) Italy and Max-Planck-Institut für Molekulare Genetik, 1000 Berlin 33, Germany

Prokaryotic IF2 contains at least four active sites: the binding sites for GTP, 30S ribosomal subunit, 50S subunit and fMet-tRNA. We have shown that IF2 contains at least two structurally and functionally distinct domains: (a) the G-domain (41K dalton), which is located in the central part of the molecule, contains the GTP- and 50S- binding sites and interacts weakly with the 30S subunit; this domain is relatively resistant to proteolysis in the presence of GTP or GDP and also retains the ribosome-dependent GTPase activity of IF2 displaying the same Km for GTP but a lower Vmax. (b) The C-terminal domain (~24K dalton), which constitutes a very compact domain, contains the fMet-tRNA binding site; in spite of its negligible affinity for ribosomes, the C-domain weakly stimulates the ribosomal binding of fMet-tRNA, probably affecting the conformation of the anticodon. We also present evidence that, as in the case of EF-Tu and EF-G, the GTPase catalytic center activated by the IF2-ribosome interaction resides in the IF2 molecule.

K 230 TARGETS FOR ENDONUCLEOLYTIC CLEAVAGE IN FILAMENTOUS PHAGE mRNA TRANSCRIPTS, Deborah A. Steege, Kendall J. Blumer, Mark D. Stump, Susan Madison-Antenucci and Robert J. Kokoska, Department of Biochemistry, Duke University Medical Center, Durham, NC 27710

In *E. coli* hosts infected with the filamentous phage f1, a number of the abundant mRNAs used as templates for synthesis of key phage proteins are products of post-transcriptional cleavage by host activity. Through RNA endpoint mapping and deletion analysis, the processing signals have been mapped to relatively local domains of 70-130 nucleotides near the point of cleavage. Data from nuclease mapping, RNA folding, and studies of the related filamentous phage IKe have been combined to develop working models for the secondary structures of these domains. The processing sites all occur in translated regions of the RNA transcripts, but they have been shown to function in the absence of translation. The nuclease activities of interest appear to be endonucleolytic in nature, since S1 nuclease mapping methods visualize upstream RNA fragments whose 3' ends map to the same positions as the 5' ends of the processed RNA species. By examining processing in a phage-infected host bearing a temperature-sensitive allele of the altered message stability locus (*ams*), we have found that production of the major processed species requires a component of the host cell which functions in a messenger RNA decay pathway; others appear to be generated by a distinct pathway.

K 231 HETEROGENITY OF RIBOSOMES ESTABLISHED BY SCANNING TRANSMISSION ELECTRON MICROSCOPY, Santa J. Tumminia and Miloslav Boublik, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

Quantitative mass and image analysis of ribosomal particles by scanning transmission electron microscopy (STEM) provided direct evidence that presumably homogeneous preparations of ribosomes, both prokaryotic and eukaryotic, are, in reality, populations of heterogeneous particles. Variations in composition, molecular mass and shape, as monitored by values of radius of gyration (R_G in Å), were observed both in the monosomes and in the ribosomal subunits. Although the origin of ribosome's heterogeneity may reflect the state (which already exists) in the cell, its extent is enhanced by the preparative conditions, shearing forces and ribosomal buffers, salt "washes" in particular. The variations in mass (Mr) and shape attributed to loss of ribosomal proteins and/or factors were correlated with the changes in ribosome composition and biological activity. For the 70S ribosome of *E. coli*, the highest activity was found in monosomes prepared from the standard 0.5 M NH_4Cl wash. The activity decreased slowly with increasing concentration (up to 1.5 M) of NH_4Cl in the wash buffer and dropped rapidly to about 2 M NH_4Cl. However, the most striking effects were observed in ribosomal particles washed with 0.1 M NH_4Cl. The Mr and R_G values of the 70S monosomes and the 30S subunits reached a maximum (2660 kDa and 76Å, and 990 kDa and 75Å, respectively), greater than the theoretical values, while the activity was minimal (~12%). The Mr and R_G parameters of the 50S subunits remained unchanged (~1600 kDa and 68Å). None of these changes can be resolved visually; they can be evaluated only by computer processing. The option of image selection of individual particles with the "complete" set of components makes it feasible to use their molecular parameters for selection of "native" ribosomal particles and apply these data for the 3-D reconstruction of a model of the native (and perhaps the most active) ribosome.

K 232 THE OPTIMIZATION OF HETEROLOGOUS GENE EXPRESSION IN *BACILLUS SUBTILIS*, Anita van Kimmenade, Louann Carlomagno, and Scott D. Power, Departments of Protein Chemistry and Molecular Biology, Genencor International, South San Francisco, CA 94080.

Bacillus species are known for their ability to secrete very high levels (>1gm/L) of various degradative enzymes including alkaline protease (subtilisin, **aprE**), neutral protease and amylases. Despite this capacity for high level secretion, it has proven difficult to tap this capability for the expression of heterologous genes. In this work we have taken a deliberate approach to optimizing the expression of a fusion of the aprE gene to a *Pseudomonas* cutinase (lipase) gene. Using PCR-mediated direct fusion techniques, we have systematically fused the **aprE** signal and pro sequence to the mature coding sequence of the cutinase gene at each of the 15 amino acids following the aprE signal cleaveage site. The dramatic effects on overall expression as well as on the steady state mRNA levels will be presented.

K 233 GROWTH RATE DEPENDENT REGULATION OF *ESCHERICHIA COLI gnd* EXPRESSION, R. E. Wolf, Jr., A. J. Pease, J. T. Chang, P. C. Muenchau, and C. B. Green, Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, MD 21228

Growth rate dependent regulation of *E. coli gnd* expression is exerted at the posttranscriptional level and requires the "internal complementary sequence" (ICS), a negative control site in the codon 71 to 74 region of the structural gene that is complementary to the Shine-Dalgarno sequence. The ICS is proposed to mediate regulation by forming a long-range mRNA secondary structure that sequesters the ribosome binding site (RBS). Here we report that functional decay of *gnd* mRNA is about 5 min and growth rate independent, which indicates that growth rate control is of translation efficiency, not mRNA half-life. Moreover, after rifampicin treatment, the synthesis capacity of the mRNA increases about twofold before functional decay begins. This mRNA utilization pattern is consistent with translation initiation frequency on *gnd* mRNA being dependent on ribosome concentration. As evidence for formation of the long-range RBS-ICS secondary structure, we report that the derepressed expression conferred by RBS mutation C45G is suppressed by the complementary ICS mutation G269C. Genetic analysis has also indicated that maximal translation efficiency and growth rate dependent regulation depend on the extensive secondary structure of the leader.

K 234 LOCATIONS OF UV-INDUCED RNA CROSS-LINKS IN ACTIVE E. COLI 30S RIBOSOMAL SUBUNITS, Paul Wollenzien, Patrick Hamblin, and Degang Zhong, Department of Biochemistry and Molecular Biology, St. Louis University Medical Center, St. Louis, MO 63104.

Active 30S ribosomal subunits were irradiated with levels of 300 nm light that would induce one or less long range crosslink per molecule. After removing proteins with proteinase K and phenol extraction, purifying 16S rRNA and 3' endlabeling it, the RNA was electrophoresed on a denaturing low concentration polyacrylamide gel to separate the crosslinked species according to loop size. Fractions from this gel were then subjected to reverse transcription with a series of synthetic DNA primers. Eight long range crosslinking sites have been determined by this method. They are: (1) U1126 x C1281, (2) G38 x G402, (3) C582 x G760, (4) C934 x U1345, (5) U991 x U1212, (6) C1109 x U1189, (7) C54 x C352 and (8) G38 x A397. Crosslink (1) occurs in a long range tertiary structure crosslink interaction. The remaining crosslinks occur at sites of interaction at the edges of base-paired regions (2-6) or at sites of close contact across half a turn of a helical duplex region (7 and 8). Crosslinks 1, 3, and 5 confirm and refine crosslinks previously reported by Atmadja et al. (Nuc. Acids Res. 13, 6919, 1985). Since G38 is involved in two very different types of interactions, this region must be a site of conformational flexibility in the subunit. Because it is possible to readily determine the frequency of a particular crosslinked species by electrophoresis on the low concentration polyacrylamide gel, this system will be useful in determining changes in the geometry of the 16S rRNA in the ribosome during its function.

K 235 THE STRUCTURE AND FUNCTION OF THE EFFECTOR DOMAIN OF ELONGATION FACTORS, Edward S. Yaskowiak and Paul E. March, Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

A large class of proteins capable of binding and hydrolyzing GTP possess invariant amino acids at certain characteristic positions. The nucleotide binding domain is encoded by approximately 150 amino acids usually present at the amino terminal portion of the protein. It has been demonstrated that the GTP-binding domains from RAS proteins are functionally interchangeable since yeast-mammalian protein chimeras can compliment yeast RAS mutations. It is thought that the information specifying function in specific RAS pathways lies outside of the GTP-binding domain.

Elongation factors required for protein synthesis comprise another important subgroup of this large class of GTP hydrolyzing proteins. EF-Tu and EF-G from E. coli, and their eukaryotic analogs, possess extremely similar GTP-binding domains; not only when comparing within a species, but when comparing between any and all species that have ever been examined. The effector region of elongation factors represents a highly conserved element amongst elongation factors, but it is not similar to the corresponding region of other GTP-binding proteins such as the RAS or G-protein subgroups. By constructing EF-Tu-EF-G chimeras in E. coli, we show that unlike the situation with RAS GTP-binding domains, neither the entire domain, nor just the effector region of one elongation factor can functionally replace that of another. Information defining specific function in protein synthesis occurs within the first 90 amino acid residues of the GTP-binding domain, despite extensive amino acid similarity in this region.

Gene Expression in Eukaryotes A

K 300 Differntial induction of IgM secretion at mRNA level by phorbor myristate acetate(PMA) and IL-2: Post-transcriptional control of IgM production by IL-2. Shungo Abe, and Osamu Saiki. Dept. of Microbiology, Ehime University School of Medicine. Shigenobu, Ehime, Japan. 791

Several stimulants, IL-2, IL-4, IL-6, and PMA (phorbol myristate acetate) involve in terminal differentiation of B cells including human B lymphoblastoid line, SKW6-4 cells. Induction of IgM secretion in SKW 6-4 cells by IL-2 and PMA resulted in an additional effect at the concentration which provide the maximum IgM induction by itself.

Northern blots analysis demonstrated that PMA induced a 2.5-fold preferential accumulation of mu-mRNA whereas the expression by IL-2 did not change significantly. The peak of mu-mRNA expression by PMA stimulation was about 10 hrs. Similarly, kappa-mRNA expression was increased by PMA but not by IL-2. In the presence of Actinomycin D to inhibit further mRNA synthesis, it was revealed that IL-2 treatment caused significant prolongation of mu-mRNA half-life rather than PMA.

These results suggest that IgM production in SKW 6-4 cells treated by IL-2 is regulated predominantly by steadystate modification of mu-mRNA, while PMA controlled mu-gene at transcriptional levels.

K 301 A FAVOURABLE UNTRANSLATED REGION AND INITIATION SITE BOTH CONTRIBUTE TO EFFICIENT INITIATION OF TRANSLATION IN VITRO, David Andrews and Mina Falcone, Department of Biochemistry, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada.

The role of the untranslated region (UTR) between the cap site and the initiation sequence in mediating translation was examined in vitro. Hybrid mRNAs were synthesized in which the cognate leader sequence was replaced with either optimized or compromised leader sequences and translational efficiency was measured for 6 different coding regions. The most efficient leader was composed of the UTR from Xenopus β-globin and an optimized initiation sequence. Compared to the cognate leaders the hybrid was observed to increase translation of the various coding regions as much as 300 fold. Moreover, the hybrid leader was shown to perform as well or better than a highly efficient cap independent viral leader. Nevertheless translation of the different coding regions varied by two orders of magnitude suggesting that this region can also contribute to translational efficiency. In contrast to earlier suggestions that increased leader efficiency was due to higher affinity for a limiting factor our experiments suggest increased translation from the hybrid leader sequence results from more rapid utilization of an abundant but labile factor.

K 302 EXTENSION INHIBITION ANALYSIS OF THE EUKARYOTIC RIBOSOME-mRNA INTERACTION.

Donald D. Anthony and William C. Merrick, Dept. of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106. We have applied a variation of the technique of extension inhibition analysis of translation initiation complexes, described by Hartz et al. (Methods in Enzymology 164, 419-425, 1988), to allow for the analysis of the eukaryotic ribosome-mRNA interaction. An mRNA-oligodeoxynucleotide hybrid is formed and then incubated with a ribosome source (micrococcal nuclease treated rabbit reticulocyte lysate). The efficiency of ribosome-mRNA complex formation and/or the stability of this complex is prohibitive of direct primer extension analysis. Instead, the ribosome-mRNA complex is isolated first by centrifugation through sucrose gradients, and then primer extension analysis is performed. Because sucrose gradient fractionation allows the separation of 80S-mRNA complex, 40S-mRNA complex, and free mRNA we have analyzed, by primer extension, each of these mRNA populations for each experimental condition. An 80S-mRNA complex (beta globin mRNA) as well as a minor 40S-mRNA complex can be isolated when the elongation inhibitor, anisomycin, is added to a translationally competent rabbit reticulocyte lysate. Similarly, when the non-hydrolyzable analog of GTP (GDPNP) is added to the system, 80S-mRNA and 40S-mRNA complexes can both be isolated in roughly equimolar quantities. The primer extension inhibition stop sites for all of these complexes indicate that the leading edge of a ribosome interacting with the initiation codon protects 13 to 15 nucleotides 3' to the first nucleotide of this codon (13 nucleotides is the distance described for prokaryotic ribosomes). The formation of all of these complexes is inhibited by the addition of cap-analog (7-methylguanosine 5'-triphosphate) to the system, indicating that these ribosome-mRNA complexes are formed in the expected cap-dependent manner. (supported by NIH grant GM-26796 and NIH training grant T32-GM-07520)

K 303 CHARACTERIZATION OF P40, A NOVEL COMPONENT OF THE PROTEIN-SYNTHESIZING MACHINERY.

D. Auth and G. Brawerman, Dept. of Biochemistry, Tufts Univ., Boston, MA. 02111

Studies in our laboratory have led to the identification of a major 33kD polypeptide that is under translational control in a wide variety of mammalian cells. This polypeptide is termed P40 on the basis of its mobility on a SDS-PAGE. We have characterized the P40 protein and find it to be involved in the translation process. It is found to a large extent as a 50-55S particle in a variety of mammalian cells. A considerable portion of the P40 is associated with polysomes as judged by co-sedimentation with these structures and by release as small particles by RNase treatment. In reticulocyte lysates, translational runoff leads to loss of the polysomal form of P40, and CHX prevents the release of the polysomal form. The P40 protein in the 50S particles is apparently associated with the small ribosomal subunit, as indicated by the fact that it co-sediments with this subunit after treatment with EDTA. The P40 released from polysomes sediments mostly as 60-80S particles, suggesting there is yet another component associated with the polysome-released P40. P40's association with the translation machinery appears to be dynamic. In rapidly growing cells, P40 is found in three forms; the polysome-associated form, the 50S form and as soluble protein. However, in stationary cells, P40 exists primarily in the 50S particle form, with less P40 associated with polysomes and none in the soluble form. The P40-containing particles may represent a novel intermediate in polypeptide chain initiation. It may be significant that P40 synthesis is under translational control. Other components of the protein-synthesizing machinery, such as some ribosomal proteins and EF-I, are also controlled in this fashion. This may represent a general mechanism for controlling the production of factors involved in the translation process.

K 304 TRANSLATIONAL REGULATION OF THE HYPOXIC STRESS RESPONSE OF MAIZE SEEDLING ROOTS,

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University of California, Riverside, CA 92521

At present, little is known about translational control mechanisms which occur in plants. Translational control has been implicated in the response to light, growth regulatory substances, heat shock and low-oxygen stress. Our research focuses on the post-transcriptional and translational regulation of gene expression in maize under hypoxic and anoxic stress. Anoxic stress of maize roots results in a shut-down of normal protein synthesis. After five hours of anoxia, translation is restored at low levels but is limited to a sub-set of the total cellular mRNAs. Both mRNAs encoding anaerobic and certain normal cellular proteins are located on polyribosomes in stressed root cells. Anoxia stimulates a rapid, dramatic decrease in the level of large polyribosomes and modifications in the protein component of polyribosomes (Bailey-Serres and Freeling (1990) Plant Physiol. 94: in press). Messenger RNAs encoding anaerobic proteins, such as alcohol dehydrogenase-1 (ADH1), are translated at high levels under hypoxia and anoxia. Current experiments are designed to determine whether specific mRNA sequences are required for efficient translation by normal and hypoxic ribosomes. Gene constructs of the 5' untranslated region (UTR) of *Adh1* linked to the beta-glucuronidase reporter, and the 3' UTR of *Adh1* have been made. These constructs are used to *in vitro* synthesize capped and polyadenylated mRNA. The mRNA is electroporated into maize protoplasts and the level of translation is examined, relative to a co-electroporated DNA construct, under normal and hypoxic conditions. We will report on the expression of mRNA constructs which have been systematically altered in nucleotide sequence and length of the UTRs.

K 305 A RIBONUCLEIC ACID REGULATOR OF C-MYC MESSENGER RNA STABILITY

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Previous work from this laboratory identified a cytoplasmic destabilizer of c-myc mRNA. The destabilizer, which is assayed in a cell-free mRNA decay system, is detectable in extracts from exponentially growing cells but not in those from cells exposed for one hour to cycloheximide. Its disappearance could explain why c-myc mRNA levels increase 4-fold in cycloheximide-treated cells. The c-myc destabilizer contains an essential nucleic acid component and might play an essential role in regulating c-myc mRNA metabolism.

These observations implied that cytoplasmic RNAs affect mRNA stability. We reasoned that regulatory RNA might interact directly with c-myc mRNA or with other factors that bind to c-myc mRNA, thus affecting its stability. To test this we incubated polysomes from human erythroleukemia (K562) cells with in vitro synthesized RNAs and monitored their effect on the decay rate of polysome-associated c-myc mRNA. c-myc anti-sense sequences had no effect. However, a 250 nucleotide c-myc sense mRNA fragment beginning 175 nucleotides upstream and ending 75 nucleotides downstream of the translation termination codon destabilized c-myc mRNA by at least 4-fold. When polysomes are incubated with the ^{32}P-labeled 250 nt RNA destabilizer a complex is formed that renders a portion of the RNA resistant to RNase A.

One model consistent with these data is that a stabilizer factor is normally associated with the 250 nt region of c-myc mRNA. The 250 nt RNA destabilizer extracts a factor from c-myc rendering it susceptible to decay. The nature of the factor and the way in which it interacts with the competitor RNA and the mRNA itself are being investigated. The 250 nt RNA destabilizer may have homology to the essential nucleic acid component of the previously identified cytosolic destabilizer. Hybridization experiments to the cytosolic destabilizer are being done to test this possibility. Taken together these data imply that several factors may interact with various regions of c-myc mRNA in determining the regulation of its stability.

K 306 CHARACTERIZATION OF APOLIPOPROTEIN II mRNA TURNOVER IN AN ESTROGEN-RESPONSIVE CHICKEN LIVER CELL LINE, Roberta Binder and David L. Williams, Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, NY 11794

Using the rooster liver as a model system, we have previously shown that turnover of the estrogen-induced apolipoprotein (apo) II mRNA is altered depending on the length of induction prior to hormone withdrawal (Gordon *et al.*, J. Biol. Chem. *263*, 2623-2631, 1988). Characterization of apoII mRNA degradation intermediates demonstrate specific endonucleolytic cleavages at 5′-AAU-3′/5′-UAA-3′ sequences within loop structures of the 3′ untranslated region (Binder *et al.*, J. Biol. Chem. *264*, 16910-16918, 1989). These cleavages occur in two larger domains of secondary structure which have been shown to bind specifically and independently to two different cytosolic proteins (Ratnasabapathy *et al.*, J. Biol. Chem. *265*, 14050-14055, 1990). Localization of endonucleolytic cleavage sites to protein binding domains suggests a possible role for both in the degradation of apoII mRNA. Further analysis of apoII mRNA stability is now being addressed in a chicken liver cell line, LMH. Recently, we have reported that LMH cells are estrogen-responsive and synthesize a low amount of apoII mRNA in response to 17ß-estradiol (Binder *et al.*, Mol. Endocrin. *4*, 201-208, 1990). In order to increase hormone-induced levels of apoII mRNA, we have established an LMH cell line, LMH/2A, that expresses a stably transfected chicken estrogen receptor. LMH/2A cells synthesize apoII mRNA in response to the synthetic estrogen, moxestrol, at about 10% of the level seen in estrogenized rooster liver. Preliminary results suggest that LMH/2A cells secrete a factor(s) that increases induced apoII mRNA two-to three-fold. Under these conditions, apoII mRNA half-life is 10.5 hours, either in the presence of moxestrol or upon hormone withdrawal. This is similar to the 13 hour half-life seen in rooster liver. ApoII mRNA in LMH/2A cells displays the same degradation intermediates that are seen in liver. Similar half-life and degradation intermediates in LMH/2A cells and liver are suggestive of a common degradation mechanism in both systems and attest to the value of this cell line as a model system for investigating estrogen-regulation of apoII mRNA stability.

K 307 THE CONTEXT OF TRANSLATIONAL TERMINATION AFFECTS MESSENGER RNA TURNOVER, Sharon Bowen, Stuart W. Peltz, and Allan Jacobson
University of Massachusetts Medical School, Worcester, MA 01655

We are interested in studying the role of translation in determining mRNA stability in the yeast S. cerevisiae. Previous studies have indicated that premature termination of translation increases the turnover rate of transcripts. One interpretation of these observations is that there may be a minimal distance that the ribosome must traverse on a given transcript in order for the transcript to be stable. Using the yeast HIS4 gene, our findings confirm that nonsense mutations have a destabilizing effect on mRNA stability. Furthermore, we find that when an in-frame deletion is made within the yeast HIS4 gene such that the normal stop codon is brought into close proximity of the site of translational initiation, there is a five-fold increase in the turnover rate of the resulting mRNA. Expression of this deleted HIS4 gene in a strain in which suppression of nonsense mutations occurs via stabilization of transcripts results in a decay rate comparable to that of wild type HIS4 mRNA. Further work with other in-frame and out-of-frame deletions is currently underway and will hopefully aid in the elucidation of the mechanisms by which mRNA turnover can be regulated during translation.

K 308 ARRANGEMENT OF E74 TRANSCRIPTS IN THE NUCLEI OF LARVAL SALIVARY GLANDS, Lynn Boyd and Carl S. Thummel, Department of Human Genetics, Howard Hughes Medical Institute, University of Utah School of Medicine, Salt Lake City, UT, 84132

The E74 gene of *Drosophila* encodes two related DNA binding proteins that are thought to be important regulators of gene activity at the onset of metamorphosis. Transcription of E74A mRNA is induced by ecdysone, the steroid hormone that triggers metamorphosis. Transcripts from this gene have been detected in the nuclei of salivary gland cells. The DNA of these larval glands is polytenized and the nuclei are quite large (~18-25 micrometers). When an antisense oligo tailed with digoxigenin-labelled nucleotides is used as a probe on whole salivary glands a distinct band of signal appears in the nucleus after incubation with an anti-digoxigenin antibody. This band is associated with the polytene chromosomes and is almost always seen to adjoin the edge of the nucleus. Currently, our experiments are aimed at determining whether the mRNA molecules are exiting the nucleus at this point of contact with the nuclear membrane.

K 309 ANALYSIS OF THE TRANSLATION INITIATION FACTOR 4A FROM S. CEREVISIAE. Peter Buser, Stefanie R. Schmid, Annik Prat, Carsten Elke and Patrick Linder, Dept. of Microbiology, Biozentrum, CH-4056 Basel, Switzerland

The eukaryotic translation initiation factor 4A (eIF-4A) is encoded in yeast by two genes, TIF1 and TIF2, which code for exactly the same protein but have highly divergent 5' and 3' flanking sequences. The eIF-4A protein is a member of a family of putative RNA helicases (D-E-A-D proteins). The mammalian factor shows RNA-dependent ATPase activity and functions together with eIF-4B as a RNA helicase.

Using a conditional system with the TIF gene under the control of the GAL10-CYC1 hybrid promoter we have shown that eIF-4A$_y$ is needed in a in vitro translation system. With the same conditional system we analyzed a number of mutants in the TIF genes in respect to growth rates and in vivo protein synthesis. We also investigated the expression of both genes. We have shown that in a wild-type cell under standard conditions (full media, 30°C) TIF2 is expressed 5 times more than TIF1 on the translational level. In another approach we tested the function of the two highly homologous mouse 4A proteins in yeast. Expression of the mouse eIF-4AI cDNA cannot complement a temperature-sensitive mutation in yeast eIF-4A.

K 310 CHARACTERIZATION OF *GCD6* AND *GCD7*, TRANSLATIONAL REPRESSORS OF THE YEAST TRANSCRIPTIONAL ACTIVATOR GCN4
Janet Leatherwood Bushman and Alan G. Hinnebusch
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In yeast, one of the first responses to amino acid starvation is to increase expression of the transcriptional activator GCN4 at the translational level. This regulation depends on four short open reading frames (uORFs) in the leader of *GCN4* mRNA. It has been proposed that *GCN4* translation is increased when ribosomes which have translated the first uORF are unable to reinitiate rapidly, allowing them to scan past the more inhibitory third and fourth uORFs and initiate at *GCN4* instead. Mutations affecting the general initiation factor eIF-2 have been isolated that constitutively derepress *GCN4* expression. We have shown that mutations in *GCD6* and *GCD7* have the same phenotype and that this effect requires the uORFs. These results, along with the fact that the *gcd6-1* and *gcd7-201* mutations lead to slow growth on rich medium suggests that GCD6 and GCD7 are essential factors important for reinitiation by scanning ribosomes. We have cloned *GCD6* and *GCD7* and begun the molecular characterization of their gene products.

K 311 ANALYSIS OF A CIS-ACTING SEQUENCE PROMOTING mRNA DECAY IN YEAST. Giordi Caponigro[1], Denise Muhlrad[1], Allan Jacobson[2], and Roy Parker[1]. [1]Dept. of Molecular and Cellular Biology, Univ. of Arizona, Tucson, AZ 85721; [2]Dept. of Molecular Genetics and Microbiology, Univ. of Mass. Medical School, Worcester, MA 01655.

The degradation of mRNA is an important step in the regulation of gene expression, but the features within individual mRNAs which dictate their respective half-lives are largely unknown. We have begun to utilize the powerful approaches possible in *Saccharomyces cerevisiae* to address this question. Previously, we have shown that a 363 nucleotide region within the unstable mRNA encoded by the yeast MATα1 gene ($t_{1/2}$=5') can promote rapid mRNA decay when transfered to stable reporter mRNAs provided that translation proceeds through the MATα1 sequences. Deletion analysis of this region has defined a 65 nucleotide region that is sufficient to promote rapid decay ($t_{1/2}$=8.2+/-0.9'). Included within this sequence is a region rich in rare codons (6/7 codons) that is required for rapid decay. Replacement of 33 nucleotides, encompassing the rare codons, with a run of four rare AGG codons restores rapid mRNA decay, suggesting that these rare codons may function in stimulating decay. Interestingly, this rapid decay requires the MATα1 sequence normally found 3' of the region of rare codons. One hypothesis to explain these observations is that the rare codons function as a translational pause site which exposes downstream regions for recognition by mRNA turnover factors. Experiments in progress are designed to address the possible role of a translational pause in mRNA decay and to identify the sequences 3' of the rare codons that are required for rapid decay.

K 312 POST-TRANSLATIONAL MODIFICATIONS AND SEQUENCE HOMOLOGIES OF EUKARYOTIC ELONGATION FACTOR 1α, Jens Cavallius and William C. Merrick, Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, OH 44106

Elongation factor 1α (EF-1α) is a very abundant protein. It has been characterized in a number of different species. It not only plays a very central key role in protein biosynthesis, but it has also been shown to have determining roles in protein synthesis rates during aging. Recently its gene dosage has been found to be critical in translational fidelity and have a life prolonging effect. Correlation between the specific activity of EF-1α and its post-translational modifications have been shown in several independent systems. We have sequenced to date 85% of yeast EF-1α protein. Two lysine residues (positions 79 and 316) which are modified in the corresponding positions in rabbit and brine shrimp are also observed to be modified in yeast. However, the modifications at three other lysine positions in rabbit EF-1α were not noted in yeast. The other modification of rabbit EF-1α is glycerylphosphorylethanolamine observed at two positions which are in the cDNA sequence of mouse and human EF-1α found to code for glutamic acid. To verify this sequence in rabbit EF-1α mRNA, the cDNA for EF-1α from rabbit has been *cloned* and sequenced. Glutamic acid was found in the coding sequence at residues 301 and 374. Apart from the very highly conserved coding sequence of EF-1α, striking sequence homologies have been found in the 3' non-coding region both between rabbit and human and rabbit and mouse mRNA. Despite the lack of the glycerylphosphorylethanolamine modification in yeast EF-1α, so far, preliminary results indicate that yeast EF-1α functions as well as rabbit EF-1α in poly(Phe) synthesis in the rabbit reticulocyte system.

K 313 SEQUENCES INFLUENCING TRANSLATION INITIATION AND TERMINATION IN EUKARYOTES: A STATISTICAL AND GENETICAL ANALYSIS, Douglas R. Cavener, Stuart C. Ray, and Yue Feng, Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235.

Sequences flanking start and stop codons were compiled for all major eukaryotic groups using a newly developed computer program. Considerable inter-taxon variation exists for the preference of nucleotides at positions flanking start and stop codons. However, all taxa exhibit a strong preference for purine nucleotides at position -3 upstream of start codons and at +4 immediately downstream of stop codons. After correcting for normal genomic biases, strong di, tri, and quadri-nucleotide biases are still observed among positions upstream of start codons. To a large extent, these multipostional biases are taxon specific. In order to determine the importance of sequences immediately upstream of initiation codons in *Drosophila,* two site-directed mutants were constructed in the *Adh* gene and transduced into the genome. An A to T substitution at the -3 position lead to a 2.4 fold reduction in relative translation at the second instar stage but did not reduce translation at the adult stage. The second mutant contained multiple substitutions designed to produce an anti-consensus sequence. This mutant lead to 12.5 fold and 5.9 fold reduction in translation at the second instar larval and adult stages, respectively.

K 314 ANALYSIS OF THE NUCLEOTIDE-BINDING DOMAINS OF THE YEAST TRANSLATIONAL ELONGATION FACTOR 3: Kalpana Chakrabortty, Mark Sandbaken, Richard Scopp, & Scott Snyder, Department of Biochemistry, Medical College of Wisconsin, Milwaukee. The yeast translational elongation factor 3 (EF-3) is an essential component of the fungal translational machinary. EF-3 catalyzes ATP and GTP hydrolysis in a ribosome-dependent manner (Chakrabortty & Kamath, Int. J. Biochem. 20, 581-590, 1988). The nucleotide hydrolytic activity of EF-3 is essential for its function in translation. Photo-crosslinking of EF-3 with azido-ATP inhibits the hydrolytic activity of EF-3. Inactivation of the protein can be overcome in the presence of purine nucleoside triphosphates but not by nucleosides. A complete inactivation of EF-3 by azido-ATP shows binding of 2 mols. of nucleoside triphosphates per molecule of EF-3. These results are consistent with the existence of two bipartate nucleotide-binding domains in the amino acid sequence of EF-3 (Sandbaken et al., J. Biol. Chem. 265, 15838-15844, 1990).

In an attempt to further explore the role of nucleotide hydrolysis in the EF-3 function, the nucleotide-binding domain distal to the N-terminal end of EF-3 was eliminated by an in-frame deletion in the YEF3 gene. Plasmid-mediated expression of the deletion mutant indicates that the truncated protein is stable but inactive in ATP hydrolysis. Site-directed mutational analysis is currently being conducted to replace the lysine residues in the conserved nucleotide-binding domains in the EF-3 structure in order to define the function of the conserved elements in ATP binding and hydrolysis.

K 315 POSITIVE AND NEGATIVE REGULATORS OF GCN4 EXPRESSION FORM A HIGH MOLECULAR WEIGHT COMPLEX THAT ASSOCIATES WITH YEAST eIF-2, A. Mark Cigan, Marco Foiani, Ernest Hannig and Alan Hinnebusch, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD 20892

GCN4 is a transcriptional activator of amino acid biosynthetic genes in S. cerevisiae whose expression is regulated by amino acid availability at the translational level. GCD1 and GCD2 are negative regulators required for repression of GCN4 translation under nonstarvation conditions by the upstream open reading frames (uORFs) in the leader of GCN4 mRNA. GCD factors are thought to be antagonized by the positive regulators GCN1,GCN2 and GCN3 in amino acid-starved cells to allow for increased GCN4 protein synthesis. Previous studies suggested that GCD1, GCD2 and GCN3 have closely related functions in GCN4 translational control and in general protein synthesis involving translation initiation factor 2 (eIF-2). In agreement with these predictions, we show that GCD1, GCD2 and GCN3 are integral components of a high molecular weight complex of approximately 600,000 Daltons. The three proteins co-purified through several biochemical fractionation steps and could be co-immunoprecipitated using antibodies against GCD1 or GCD2. Interestingly, a fraction of the α and β subunits of eIF-2 present in cell extracts also co-purified and co-immunoprecipitated with these regulatory proteins, but was dissociated from the GCD1/GCD2/GCN3 complex by 0.5M KCl. Incubation of a temperature-sensitive gcd1-101 mutant at the restrictive temperature led to a rapid reduction in the average size and quantity of polysomes, plus an accumulation of inactive 80S couples and free 40S ribosomal subunits containing excess eIF-2α, GCD1, GCD2 and GCN3. These results suggest that GCD1 is required for an essential function involving eIF-2 at a late step in the translation initiation cycle. We propose that attenuation of the function of this high molecular weight complex in amino acid-starved cells alters the amount of active eIF-2; this leads to reduced ribosomal recognition of the uORFs and increased translation initiation at the GCN4 start codon. Therefore, these studies in yeast may provide new insights into how initiation factors that associate with eIF-2 mediate the regulation of protein synthesis in eukaryotes.

K 316 TRANSLATIONAL INHIBITION OF CREATINE KINASE B BY A CYTOPLASMIC FACTOR.

Lisa E. Clark, J. Lai Ch'ng*, Edward W. Holmes, Department of Biochemistry and Medicine, Duke University, Durham, NC, 27710 * present address:Department of Medicine, Vanderbilt University, Nashville, TN,37232

Prior studies have demonstrated that creatine kinase-B (CK-B) mRNA is present in a subline of U937 cells which do not express detectable CK activity. When these cells are transformed with a retroviral vector containing conserved 3' UTR sequences of CK-B, the following results are obtained: 1) CK activity becomes readily detectable 2) CK-B mRNA abundance is not altered 3) A cytosolic protein present in control cells binds to the 3' UTR of CK-B mRNA in a gel-shift assay, but this protein is not detectable in transformed cells expressing CK-B. The correlation between this in vitro binding activity and in vivo inhibition of CK-B mRNA translation suggests this activity may be a translational repressor of CK-B. To directly assess this hypothesis, cytoplasmic extract prepared from U937 cells was added to an in vitro translation system containing in vitro transcribed CK-B mRNA with the following results: 1) CK-B peptide synthesis can be decreased by up to 95% 2) In the same system, inhibition of ß-globin peptide synthesis from endogeneous ß-globin mRNA ranges from 0-20%. We conclude from these experiments that U937D cells contain a factor(s) capable of repressing translation of CK-B mRNA with a minimal effect on translation of ß-globin mRNA. Experiments are in progress to further characterize this translational respressor and determine the mechanism for its selective effects on CK-B.

K 317 **INDUCTION AND TRANSLATIONAL CONTROL OF RNA IN *SOLANUM TUBEROSUM* IN RESPONSE TO WOUNDING AND HYPOXIC STRESS,**

John S. Crosby and Michael E. Vayda, Department of Biochemistry and Microbiology, University of Maine, Orono, Maine 04469 USA.

Potato tubers respond to environmental stress by the induction and accumulation of specific stress response RNA's within the cell. We have found that newly synthesized wound response messages such as PAL, EXT and GRP are polysome associated in tubers, but that the sucrose-induced tuberization messages (Patatin and PI-2) are quickly degraded or released from the ribosomes immediately after wounding. Further, upon the onset of hypoxic conditions (subsequent to wounding), newly synthesized and hypoxically induced RNA's (ADH and ALD) also accumulate within the tuber and are polysome associated. The wound response RNA's already present remain polysome associated, but are not translated. The cell therefore exhibits a preference to the translation of hypoxically induced message over wound response message even though both are associated with ribosomes.

Efforts are in progress to determine by what mechanism(s) this selective translation takes place. Current experiments indicate that translational competency of wound induced polysome associated message correlates with the association of that complex with cell membranes.

K 318 YEAST GENES REQUIRED FOR RAPID TURNOVER OF mRNAS CONTAINING PREMATURE STOP CODONS, M.R. Culbertson, B. Lee, and P. Leeds, Laboratories of Genetics and Molecular Biology, University of Wisconsin, Madison, Wi 53706.

In yeast and animal cells cis-acting elements have been identified that modulate mRNA decay rate. However, little is known about the trans-acting factors responsible for mRNA degradation. We have identified two genes, *UPF1* and *UPF3*, that potentially code for such factors. *UPF1* and *UPF3* mutations were isolated by selecting for improved expression of the yeast *his4* gene in a strain carrying *his4-38*, a frameshift mutation that causes an increased rate of *his4* mRNA turnover. *UPF1* and *UPF3* mutations improve expression in *his4-38* strains by amplifying the level of functional HIS4 product through stabilization of the *his4-38* transcript. This stabilization is not an indirect effect due to increased read-through of the *his4-38* mutation. Loss of *UPF1* function also stabilizes mRNA's coded by other genes in which translation is prematurely terminated due to a nonsense mutation. In collaboration with S. Peltz and A. Jacobson, we have also shown that the *UPF1* gene product affects the decay rate of wild-type *PPR1* mRNA, which codes for a positive transcription factor that regulates genes in the yeast uracil pathway. Since all the affected mRNA's contain premature stop codons (including *PPR1* which contains an upstream ORF in the leader), we propose that premature stop codons can activate an mRNA degradation system that depends on the function of the *UPF1* and *UPF3* gene products. Furthermore, premature stop codons are necessary but not sufficient for activation, since the stability of *GCN4* mRNA containing upstream ORF's is not affected by loss of *UPF1* function.

K 319 **ANALYSIS OF THE DIFFERENT DOMAINS OF THE HIV-1 ENVELOPE SIGNAL PEPTIDE**
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The molecular mechanisms by which signal peptides (SP) direct the nascent polypeptide chains to the ER membrane is still not well understood. Despite a common arrangement in an N-terminal charged region followed by a hydrophobic core and a region defining the cleavage site, SP show little sequence homology.
In addition to this arrangement, the SP of the HIV-1 envelope protein gp160 has unique structural features: an exceptionally highly charged N-terminus, the presence of a tryptophan motif, and the presence of a cysteine in the cleavage site. Because the HIV envelope is a major determinant of viral pathogenesis and because little is known on retroviral envelope SP, we initiated a systematic study of the different regions of the HIV-1 envelope SP by introducing define changes using site-directed mutagenesis. Because of the complex regulatory mechanisms of the HIV-1 Env expression *in vivo*, the effect of the introduced mutations on protein translocation were analyzed in an *in vitro* transcription and translation model.
We made several observations concerning the properties of the HIV-1 Env SP. Thus, the *in vitro* results underlined, as expected, the central role of the hydrophobic core for protein translocation and the importance of a defined sequence of the last three C-terminal amino acids of the SP. However and unexpectedly, no effect on translocation was observed after introduction of either a polar or a charged amino acid in the hydrophobic core. Also, no or limited changes on translocation were observed after either suppression or deletion of N-terminal charges or deletion of the tryptophan motif. Since these features might still be essential for efficient translocation in the presence of other competing mRNAs we are currently studying this possibility.

K 320 FRAMESHIFTING IN TY ELEMENTS OF YEAST: ON THE MECHANISM OF FRAMESHIFTING WITH THE 7 NT MINIMAL SITE. Philip J. Farabaugh, Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21228.

Frameshifting between the TYA andTYB genes of the retrotransposon Ty in yeast occurs within a 7 nucleotide minimal site, promoted by two unusual tRNAs (Belcourt & Farabaugh, Cell **62**:339-352, 1990). One tRNA is an unusual leucyl-tRNA capable of decoding the overlapping CUU and UUA codons within the slip site. The other tRNA is a low abundance tRNA which decodes the AGG "stimulator" codon immediately downstream of the slip site; frameshifting is occurs because the low availability of this tRNA causes a translational pause with the A-site empty. We are pursuing some unusual aspects of this frameshift site principally the fact that frameshifting is inhibited by proximity to the translational initiation codon. We are taking a genetic approach to this problem, isolating suppressors which allow frameshifting.

K 321 **GCD2, A TRANSLATIONAL REPRESSOR OF *GCN4* HAS A GENERAL ROLE IN TRANSLATION INITIATION**, Marco Foiani, Mark Cigan, Christopher Paddon and Alan Hinnebusch, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland, 20892.

Positive (*GCN*) and negative (*GCD*) trans-acting factors control the expression of amino acid biosynthetic enzymes by regulating the synthesis of the transcriptional activator GCN4. Under conditions of amino acid sufficiency, the *GCD2* gene product is required to repress GCN4 synthesis. Because GCD2 regulates *GCN4* at the translational level, and is required for viability under nonstarvation conditions, we conducted a biochemical analysis of *gcd2* mutants to determine whether GCD2 has a general role in translation. The temperature-sensitive lethal mutation *gcd2-503* leads to an increased ratio of 80S monosomes to polysomes only 30' after a shift to the nonpermissive temperature. The 80S particles that accumulate are not bound to mRNA and can be dissociated into 40S and 60S subunits at a high salt concentration. In addition, there is an increased amount of the initiation factor eIF2 associated with 40S subunits at the nonpermissive temperature. Thus, *gcd2-503* may impair translation initiation at the step involving mRNA-binding to 43S preinitiation complexes. The *gcd2-502* mutation leads to the accumulation of "halfmer" polysomes containing an extra 40S subunit bound to mRNA. These halfmers presumably reflect a reduced rate of 40S-60S joining during the initiation process. Halfmers also occur in a strain containing a deletion of the 60S subunit ribosomal protein gene *RPL16B*, probably due to insufficient 60S subunits to drive the 40S-60S joining reaction at the wild type rate. Interestingly, this mutation leads to derepression of *GCN4* expression under nonstarvation conditions similar to *gcd2* mutations. These data indicate an important general role for GCD2 in translation initiation and suggest that a reduction in the efficiency of 40S-60S joining during the initiation process is responsible for increased translation of *GCN4* mRNA in amino acids starved cells.

K 322 PHOSPHORYLATION OF EUKARYOTIC INITIATION FACTOR 4E IS INCREASED IN SRC TRANSFORMED CELL LINES, Robert M. Frederickson and Nahum Sonenberg, Department of Biochemistry, McGill University, Montreal, Canada H3G 1Y6

Eukaryotic initiation factor (eIF-) 4F is a three subunit complex, which binds the 5' cap structure (m^7GpppX, where X is any nucleotide) of eukaryotic mRNAs. This factor facilitates binding of ribosomes by unwinding the secondary structure in the mRNA 5' non-coding region. The limiting component of the 4F complex is believed to be the 24 kDa cap-binding phosphoprotein, eIF-4E. In this report, we describe the phosphorylation of eIF-4E in response to expression of the tyrosine kinase oncoproteins, $pp60^{v\text{-}src}$ or $pp60^{c\text{-}src527F}$. Additionally, we demonstrate that treatment of NIH 3T3 cells with a variety of mitogenic agents, such as PMA, serum, or PDGF, results in a rapid increase in phosphorylated eIF-4E. Taken together, these results suggest eIF-4E functions as a downstream target of the signalling cascade induced by tyrosine-specific protein kinases and various effectors of the mitogenic response.

K 323 CAP IS AN ESSENTIAL REQUIREMENT FOR FUNCTION OF THE POLY(A) TAIL IN HIGHER EUKARYOTES, Daniel R. Gallie, Department of Biochemistry, University of California, Riverside, CA 92521.

Both the cap and the poly(A) tail are known to be essential for efficient translation. The introduction of in vitro-synthesized mRNAs into cells using electroporation has made it possible to dissect both the in vivo functional requirements for the cap and poly(A) tail as well as the temporal dissection of the point during translation in which the poly(A) tail begins to act following ribosomal recruitment. The addition of a poly(A) tail substantially increases translation (>20-fold) but only if the transcript is capped. A poly(A) tail had little effect on the expression from uncapped transcripts. Likewise, a poly(A) tail improved the efficacy of the cap. These data document an entirely new role for the cap whereby the cap plays a vital role in mediating the function of the poly(A) tail and vice versa, suggesting that there exists an interaction between the events occurring at the 5'-terminus (the cap) and the 3'-terminus (the poly(A) tail) that is essential for translation. Similar results were obtained when transcripts terminating in the tobacco mosaic viral 3'-untranslated region (which is functionally equivalent to a poly(A) tail) were tested.

Expression from capped mRNAs with or without a poly(A) tail was observed immediately following electroporation. The presence of a poly(A) tail did not alter the rate of ribosome recruitment until 15 min following electroporation, after which point, ribosome recruitment by polyadenylated mRNAs dramatically outperformed that for non-polyadenylated mRNAs. Although the presence of a cap and poly(A) do increase message stability, their primary effect is increasing translational efficiency.

K 324 A COMPARISON OF THE BINDING OF METHYLATED CAP ANALOGS TO WHEAT GERM PROTEIN SYNTHESIS INITIATION FACTORS 4F AND (ISO)4F, Dixie J. Goss and Susan E. Carberry, Dept. of Chemistry, Hunter College of CUNY, New York, New York 10021

The binding of the mRNA 5' terminal cap analogs m^7GpppG and m^7GTP to wheat germ protein synthesis initiation factors eIF-4F and eIF-(iso)4F as a function of pH, ionic strength and temperature is described. Equilibrium binding data indicate that eIF-4F and eIF-(iso)4F have different mechanisms for interacting with the 5' cap structure, but the complexes formed between m^7GpppG and wheat germ factors eIF-(iso)4F more closely resembel complexes formed between this cap analog and either mammalian eIF-4E or eIF-4F. The binding of these initiation factors to hypermethylated cap analogs has been investigated. The differences in affinity of eIF-4F and eIF-(iso)4F for the hypermethylated 5'terminal cap structures suggest that these factors may have discriminatory activity.

K 325 REGULATION OF EUKARYOTIC INITIATION FACTOR (eIF)-2B ACTIVITY BY SUGAR-PHOSPHATE AND POLYAMINES, Martin Gross, Mark S. Rubino, and Suzanne M. Hessefort, Department of Pathology, The University of Chicago, Chicago, Illinois 60637

We recently reported that sugar-phosphates sustain protein synthesis in gel-filtered rabbit reticulocyte lysate by maintaining the activity of eIF-2B, the factor that catalyzes the recycling of eIF-2·GDP to eIF-2·GTP, via a mechanism that is independent of the phosphorylation of eIF-2α. Furthermore, the stimulation by polyamines (spermidine and spermine) of protein synthesis in gel-filtered lysate is also mediated by an effect on eIF-2B activity that is separate from and in addition to that of sugar-phosphates. We have been able to demonstrate comparable effects of sugar-phosphates and polyamines on relatively crude eIF-2B in an isolated exchange assay that measures the dissociation of eIF-2·[^{3}H]GDP in the presence of GTP and Met-$tRNA_f^{Met}$. Following chromatography on DEAE-cellulose, eIF-2B is no longer dependent upon either sugar-phosphate or polyamines. The dependence upon sugar-phosphate (but not polyamines) can be restored by adding back a protein, removed on DEAE-cellulose, that inhibits exchange by eIF-2B in the absence but not presence of sugar-phosphate. The action of this inhibitor requires ATP (dATP is also effective, ATP(γS) is partly effective, and ITP and GTP are ineffective), Mg^{2+}, and ATP hydrolysis. While a number of sugar-phosphates are effective with crude eIF-2B, only fructose-1,6 (and 2,6)-diphosphate and, to a lesser degree, mannose-6-phosphate restore the activity of crude eIF-2B and eIF-2B (purified through DEAE-cellulose and phosphocellulose) that is incubated with the inhibitor and ATP. It is unclear whether one of these sugar-phosphates or another, that can still be formed from one of these, represents the physiological regulator. (Supported by NIH grant GM 24949).

K 326 CASEIN KINASE I PHOSPHORYLATES THE 25 kDa mRNA CAP-BINDING PROTEIN eIF-4E in vitro, David W. Haas and Curt H. Hagedorn, Departments of Medicine and Cell Biology, Vanderbilt University School of Medicine and VA Medical Center, Nashville, TN 37235

Indirect evidence suggests that phosphorylation of eIF-4E increases the efficiency of the translational apparatus. Among the protein kinases previously studied, only protein kinase C significantly phosphorylated eIF-4E in vitro. While protein kinase C appears to phosphorylate eIF-4E at the constitutive site (serine-53) in intact cells, the kinases that mediate this phosphorylation event under diverse physiologic conditions have not been described. Using eIF-4E as substrate we purified from rabbit reticulocytes a protein kinase that phosphorylated eIF-4E on both serine and threonine residues with an apparent *Km* of 3.7 μM. The molecular mass and specificity for substrates other than eIF-4E suggested that this was a species of casein kinase I. This was confirmed by comparing the phosphopeptide map of the reticulocyte enzyme to that of rabbit skeletal muscle casein kinase I and by comparing phosphopeptide maps of eIF-4E phosphorylated by each enzyme. Reticulocyte casein kinase I incorporated 0.8 pmol of phosphate per pmol of eIF-4E under optimal in vitro conditions. We suggest that casein kinase I may phosphorylate eIF-4E in intact cells under some physiologic conditions.

K 327 USE OF HIGH RESOLUTION DENSITY GRADIENTS TO STUDY TRANSLATION in vivo. J. Hensold. University Ireland Cancer Center, Dept. of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH 44106

Changes in cell translation rate are concurrent with a wide variety of developmental events, including embryogenesis, morphogenesis in Dictylostelium, adipocyte differentiation of 3T3 cells and differentiation of erythroleukemia cells. These changes are likely to affect the interactions of mRNAs with initiation factors and ribosomes and ultimately affect gene expression in the cells. Despite the importance of these changes, there are no well-established techniques for studying these interactions in vivo. Sucrose density gradient fractionation and Northern blotting can be utilized to demonstrate accumulation of mRNA-containing preinitiation complexes in serum-stimulated 3T3 cells and a decrease in these complexes in confluent, serum-starved cells. Transformation of these cells with an activated ras oncogene produces a substantial increase in preinitiation complexes, and in contrast to the nontransformed cells, a high percentage of 40S ribosomes are found in these complexes even with serum-starvation and confluent growth. These results suggest that cell transformation directly affects translation initiation rates and demonstrate the utility of this approach to study translation in vivo. This approach should be compatable with recently described techniques (Wolin and Walter, EMBO J 7:3559, 1988) to identify ribosome-mRNA interactions and experiments are in progress to "footprint" the preinitiation complex in the gradient fractions. The combination of these approaches should provide a means to analyze mRNA-specific context effects on interactions with ribosomes in vivo.

K 328 **IRON-RESPONSIVE ELEMENTS ARE POSITION-DEPENDENT TRANSLATIONAL REGUALTORS OF FERRITIN AND POSSIBLY OTHER mRNAs *in vivo* AND *in vitro*,** **Matthias W. Hentze, Britta Goossen, Anne Constable and Nicola K. Gray, European Molecular Biology Laboratory, Meyerhofstrasse 1, D-6900 Heidelberg, F.R.G., Tel. 06221-387 501**

The translation of the iron storage protein ferritin is regulated in an iron-dependent fashion via a characteristic small RNA element in the 5'UTR of ferritin mRNAs [iron-responsive element, IRE] and a specific cytoplasmic binding protein, the IRE-BP. We have devised a computer search program for the IRE sequence/structure motif which revealed the presence of IRE-like motifs in the 5'UTR of non-ferritin mRNAs and which suggested additional biological roles for these putative IREs.

Functional analyses of the ferritin IRE/IRE-BP interaction in vivo and in vitro demonstrated that an IRE/IRE-BP complex represses mRNA translation. However, the presence of a structurally intact IRE in the 5'UTR of a transcript alone is **not** sufficient for function of the RNA/protein complex as a translational regulator. When the apparent distance between an IRE and the 5' terminus of a transcript is increased by a variety of different "stuffer RNA elements" beyond a relatively well defined threshold, translation of such transcripts remains constitutively de-repressed even under conditions of iron starvation. This position dependence of IRE function gives rise to intriguing models for how the IRE/IRE-BP complex may perturb molecular mechanisms of translation initiation.

K 329 SECONDARY STRUCTURE ANALYSIS CONFIRMS THE PRESENCE OF IRE STEM LOOPS IN TRANSFERRIN RECEPTOR mRNA, Jill A. Horowitz, John L. Casey, Richard D. Klausner and Joe B. Harford, Cell Biology and Metabolism Branch, NICHD, NIH, Bethesda, MD 20892

Ferritin translation and transferrin receptor (TfR) mRNA stability are regulated by iron. Both types of regulation are mediated by similar cis-acting RNA elements termed iron responsive elements (IREs) and by a trans-acting IRE binding protein (IRE-BP). The IREs have potential to form moderately stable stem-loop structures. The 3'UTR of the TfR mRNA contains five IREs all of which have been shown as individual elements to be capable of being recognized by the IRE-BP. A 250nt sequence derived from the TfR 3'UTR has been shown to be sufficient for the regulation of TfR mRNA degradation. This 250nt regulatory sequence contains three IRE sequences, interacts with the IRE-BP, and has been shown to contain a cis-acting rapid turnover determinant. Binding of the IRE-BP to this region appears to regulate the availability of the rapid turnover determinant. Based on computer-assisted folding algorithms, we have postulated a structure for the regulatory region that contains three IRE stem-loop structures although direct structural analysis of the region was lacking until now. We analyzed the secondary structure of a transcript corresponding to the 250nt regulatory region using oligonucleotide-directed RNase H cleavage. The results provide independent confirmation that the secondary structure of the 250nt regulatory sequence contains the three IRE stem loops which we have postulated. Previous data indicate that sequences corresponding to the IREs participate in formation of the rapid turnover determinant. However, despite their similar secondary structures, mutational analysis demonstrates that the ferritin and TfR IREs are not functionally equivalent with respect to formation of the rapid turnover determinant for TfR mRNA degradation. This information appears to reside at least in part in the primary sequence of the TfR IREs.

K 330 **REGULATION OF mRNA STABILITY IN THE YEAST SACCHAROMYCES CEREVISIAE,** Allan Jacobson, Agneta H. Brown, Michael Culbertson*, Janet L. Donahue, David Herrick, Peter Leeds*, Roy Parker, Wendy Sears, and Stuart W. Peltz, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655 and *Laboratory of Molecular Biology, University of Wisconsin, Madison, WI 53706

We have measured the half-lives of over 30 mRNAs encoded by well-characterized yeast genes and found that they ranged from <3' to >45'. To understand the structural features of these mRNAs which dictate their respective decay rates, we have constructed chimeric genes which encode portions of stable and unstable mRNAs and measured the decay rates of the resultant chimeric mRNAs. These experiments identified sequences involved in rapid mRNA decay within the coding regions of the HIS3, MATα1, CDC4, STE2 and STE3 mRNAs. To identify trans-acting factors involved in mRNA decay we have begun to characterize mutants which affect the accumulation of unstable mRNAs. We find that: a) upf1, a mutation originally selected for its ability to suppress HIS4 frameshift mutations, selectively stabilizes mRNAs containing early nonsense mutations without affecting the decay rates of other mRNAs and b) ts352, a mutant with an altered tRNA nucleotidyl-transferase, reduces the decay rates of all classes of mRNA markedly after a shift from 23°C to 36°C. The latter effect is consistent with other experiments implicating a role for translational elongation in mRNA decay.

K 331 **MULTIPLE mRNAS ENCODE THE MURINE TRANSLATION INITIATION FACTOR eIF-4E,** Maria Jaramillo, Jerry Pelletier, Isaac Edery, Peter J. Neilsen* and Nahum Sonenberg, Department of Biochemistry, McGill University, Montreal, Canada, H3G 1Y6 and the *Max-Planck-Institut fur Immunobiologie, Stubewege 51, D-7800, Freiburg, FRG

All eukaryotic cellular mRNAs (except organellar) possess at their 5' end the structure m^7GpppG (where X is any nucleotide) termed the "cap". The cap structure facilitates the melting of mRNA 5' secondary structure through the action of initiation factor-4F (eIF-4F) in conjunction with eIF-4A and eIF-4B. eIF-4F consists of three subunits of which one, eIF-4E contains the cap binding site. Several lines of evidence suggest that eIF-4E regulates the rate of translation initiation and plays an important role in cell growth. Murine eIF-4E cDNAs were isolated which differ in their 3' non-coding region. To investigate the possibility that eIF-4E expression is regulated, we studied the pattern of eIF-4E expression in several cell lines. The existence of multiple mRNAs for eIF-4E that are generated by differential polyadenylation as well as tissue-specific differences in eIF-4E mRNA expression and utilization of polyadenylation sites are shown.

K 332 SELECTIVE TRANSLATIONAL CONTROL OF ELONGATION FACTOR-1α mRNA AFTER MITOGENIC STIMULATION OF SWISS 3T3 CELLS. H.B.J. Jefferies & G. Thomas, Friedrich Miescher-Institut, P.O. Box 2543, 4002 Basel, Switzerland.

Stimulation of quiescent cells by growth factors results in (i) a 2-3-fold increase in the rate of initiation of protein synthesis, (ii) a shift of 80s ribosomes into polysomes accompanied by the recruitment of newly transcribed mRNA and a subset of stored non-polysomal mRNA, (iii) phosphorylation of ribosomal protein S6. Alterations in mRNA expression lead to changes in the pattern of protein synthesis as visualized on 2D NEPHGE. Experiments with actinomycin D suggested that some proteins, including EF-1α (Q49), are controlled at a post-translational level. Cytoplasmic extracts from cells stimulated with serum were fractionated on linear sucrose gradients and analysed by S1-nuclease-protected hybridisation probing for EF-1α mRNA. EF-1α mRNA shifts from mRNP/subpolysome region onto polysomes; this begins within 15 min and ends by ~3 hr. Northern blot analysis and S1-nuclease protected hybridisation confirmed that EF-1α mRNA level remains constant throughout the 3-hr serum stimulation (c.f. β Actin mRNA). Cytoplasmic extracts from quiescent cells run on a linear low-sucrose gradient revealed two populations of EF-1α mRNA, one located around the 80S monosome/disome region and the other in the mRNP region. The implication is that the substantial proportion of total EF-1α mRNA on 80s monosomes/disomes is being translated inefficiently (c.f. β-Actin mRNA). Stimulation of cells with serum initiates rapid changes that select EF-1α mRNA as an inefficiently translated message and allow this message to be utilized efficiently. We aim to determine whether EF-1α mRNA attached to 80s monosomes/disomes in resting cells is being actively translated and if this is a general phenomenon.

K 333 INTRACELLULAR TRAFFICKING OF PROTEINS IN INSECT CELLS AND LARVAE INFECTED WITH RECOMBINANT BACULOVIRUS, P.K. Jha, N.Z. Ehtesham, R.Dhar and S.E. Hasnain, National Institute of Immunology, Shahid Jeet Singh Marg, New Delhi 110067, India

We are interested in understanding the molecular signals and cellular processes involved in intracellular and extracellular targeting of heterologous proteins synthesized in insect cells and larvae using baculovirus vectors. For this , we are using a firefly luciferase (luc) gene as a reporter. The luc cDNA was cloned under the baculovirus polyhedrin promoter and enzymatically active luc was synthesized in insect cells. We previously showed (Hasnain and Nakhai, Gene 91, 135, 1990) that the firefly luc is not secreted into the culture medium of infected cells. By immunofluorescence we showed that this protein is localized within the peroxisomes of infected cells (ms in prep.). Similar results were obtained for recombinant baculovirus expressed in the larvae (Jha et al, FEBS Lett., In Press). Luc protein has a Ser-Lys-Leu motif within the last 20 amino acids at the C-terminal end which acts as a cell sorting domain for intracellular targeting of luc in the peroxisomes (Gould et al, EMBO J. 9,85, 1990). We are asking specific questions about the role of such cell sorting motifs in intracellular targeting and retention, by deleting this domain and/or by replacing it with a heterologous membrane anchor sequence from the rabies virus glycoprotein gene (Rab G). These results have an important bearing on protein trafficking in a heterologous environment.

K 334 CLONING OF THE cDNA ENCODING THE HUMAN IRON-RESPONSIVE ELEMENT BINDING PROTEIN, Stamatina Kaptain,*§ Tracey A. Rouault,* Wilson H. Burgess,† Felipe Samaniego,* O. Wesley McBride,** Joe B. Harford,* and Richard D. Klausner,* *Cell Biology and Metabolism Branch, National Institute of Child Health, Bethesda, MD 20892, †Laboratory of Molecular Biology, Jerome H. Holland Laboratory, American Red Cross, Rockville, MD 20855, Laboratory of Biochemistry, **National Cancer Institute, Bethesda, MD 20892, and §HHMI-NIH Research Scholars Program.

The regulation of cellular iron metabolism in higher eukaryotes is performed by two major proteins: the transferrin receptor (TfR) and ferritin. The molecular basis for their regulation is mediated by stem-loop structures, called iron-responsive elements (IREs), in the 5' untranslated region (UTR) of ferritin mRNA and the 3'UTR of transferrin mRNA. A cytosolic protein, the iron-responsive element binding protein (IRE-BP), binds to the IREs with high affinity under conditions of iron starvation and results in repression of ferritin mRNA translation and increased TfR mRNA stability. The IRE-BP has been identified as a 90 kD cytosolic protein that has been purified to homogeneity using RNA affinity chromatography. The isolated protein was digested with endopeptidases and subsequently sequenced. A degenerate oligonucleotide probe was derived from one peptide sequence and used to secreen a cDNA library. One isolated clone had a DNA sequence with an open reading frame of 790 amino acides, encoding a protein of 87 kD, which included the sequence of 13 other isolated peptides. The cDNA clone hybridized to an mRNA of approximately 4.0 kb by Northern blot analysis and was mapped to chromosome 9 by Southern blot analysis of rodent-human hybrid cell lines. The predicted protein contains a nucleotide binding consensus sequence and regions of cysteine and histidine clusters. It contains no previously defined consensus motifs for DNA or RNA binding. The simultaneous isolation of a highly homologous, but distinct cDNA suggests the existence of a new gene family. We are currently in the process of analyzing genomic clones of the IRE-BP.

K 335 INHIBITION OF eIF-2α PHOSPHORYLATION BY EXPRESSION OF SERINE TO ALANINE 51 MUTANT eIF-2α MITIGATES THE HEAT SHOCK- AND SERUM DEPRIVATION - INDUCED INHIBITION OF GLOBAL PROTEIN SYNTHESIS. Randal J Kaufman, Patricia Murtha, Monique V Davies, and John W B Hershey. Genetics Institute, Cambridge, MA 02140, and Department of Biological Chemistry, School of Medicine, University of California, Davis CA 95616

Phosphorylation of the alpha subunit of eIF-2 correlates with inhibition of translation initiation mediated by heat shock and serum deprivation. We have evaluated the importance of this modification by studying the heat shock response and serum deprivation response in CHO cells which stably overexpress either the wildtype eIF-2α or a serine to alanine mutant at the site of phosphorylation, residue 51. Treatment at 43.5°C for 40 min inhibits protein synthesis 90% in control CHO cells or in CHO cells which overexpress wildtype eIF-2α. In contrast, heat shock treatment of cells that express the 51 alanine mutant, protein synthesis is inhibited 65%. The protein synthesis in these cell lines were compared in response to serum depletion. Wildtype eIF-2α expressing cells exhibit 35% inhibition after 4 days without feeding fresh serum, whereas cells which express 51 alanine eIF-2α do not exhibit reduction in protein synthesis under indentical conditions. These experiments demonstrate that eIF-2α phosphorylation can mediate some degree of protein synthesis inhibition in intact cells. However, under the conditions of analysis, the expression of the 51 alanine mutant eIF-2α did not completely reverse heat shock inhibition of protein synthesis. These results suggest that multiple redundant pathways are likely responsible for control of protein synthesis initiation.

K 336 CHARACTERIZATION OF THE MECHANISM FOR INCREASED SYNTHESIS OF ARTERIAL ELASTIN AND COLLAGEN IN RESPONSE TO ELEVATED WALL STRESS IN VITRO, Fred W. Keeley, L. Bartoscewicz and P. Robson, Division of Cardiovascular Research, Hospital for Sick Children, Toronto, Canada M5G 1X8.

Elevated vascular wall stress appears to be one of the driving forces for accumulation of arterial connective tissue proteins both in normal development and in pathological situations such as hypertension. Our laboratory is investigating the mechanism of transduction of this physical stress into increased synthesis of elastin and collagen, the major connective tissue proteins of the walls of large arteries. By applying a static stress to vessels in *in vitro* organ culture models we can produce an approximately two-fold increase in elastin and collagen synthesis. This response is seen within 2-4 hours, and is not affected by alpha-adrenergic agonists or by inhibitors of angiotensin converting enzyme activity. In contrast, both isoproterenol (10^{-3}) and caffeine (10^{-3}) inhibit the stress-induced response without affecting control synthesis. Selective inhibition of the stress-induced response is also produced by colchicine (10^{-4}), suggesting a role for the cytoskeleton in the transduction process. Verapamil, a calcium channel blocker, shows a similar effect at lower doses (10^{-5}) but at higher doses (10^{-4}) inhibits both control and stress-induced synthesis. Nifedipine has no effect at either dose. On the other hand, heparin (1 mg/ml) partially inhibits control synthesis but does not appear to affect the stress-induced response. Addition of EGTA (1.5mM) to the incubation medium abolishes both control and stress-induced synthesis. The increased synthesis of elastin due to increased wall stress in these models is not accompanied by consistent increases in mRNA for elastin, suggesting a post-transcriptional site of control for this response.

K 337 IDENTIFICATION OF AN INTEGRAL MEMBRANE PROTEIN THAT CAN BE CROSS-LINKED TO NASCENT POLYPEPTIDES UNDERGOING TRANSLOCATION ACROSS THE ENDOPLASMIC RETICULUM. Kennan V. Kellaris, Sharon Bowen and Reid Gilmore, Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester, MA 01655

We have used the chemical cross-linker disuccinimidyl suberate to identify translocation components that are in contact with ^{35}S labelled nascent polypeptides as they are translocated across canine pancreatic microsomal membranes. Two predominant cross-linked products have been obtained. One, with an apparent molecular weight of 62,000, is composed of the nascent polypeptide and the 54 kD subunit of SRP. The second cross-linked product has an apparent molecular weight of 46,000 and is an integral membrane protein, as shown by the continued membrane association of the protein after alkaline extraction. Both SRP and a functional SRP receptor are required to obtain the membrane localized cross-linked product. Nascent secretory chains containing photoactivatable amino acid analogs have been cross-linked to an integral membrane glycoprotein of 35-39 kD known as the signal sequence receptor, or mp39 (Wiedmann et al. *Nature 328* (1987) 830-833; Krieg et al. *J Cell Biol 109* (1989) 2033-2043). Several experiments demonstrated that the 46 kD cross-linked product described here is not identical to SSR. Unlike SSR, the 46kD product does not bind to concanavalin A sepharose, and hence is not a glycoprotein. The 46 kD product is also more resistant to externally added proteinase K than the SSR cross-linked species.

Gene Expression in Eukaryotes B

K 338 INTRACELLULAR DEGRADATION OF APOLIPOPROTEIN B, Janette Le Gros, D. Lesley Graham and James Scott, Division of Molecular Medicine, MRC Clinical Research Centre, Watford Road, Harrow HA1 3UJ, England.

Apolipoprotein (apo) B100 and 48 are synthesised by hepatocytes and/or enterocytes and secreted as lipoprotein particles. These lipoprotein particles are essential for the transport of lipid in the circulation. Previous studies from our lab (Pullinger et al, J. Lipid Res. 30: 1065-1077, 1989) have indicated that apo B expression is regulated post-transcriptionally. One post-transcriptional mechanism known to modulate the quantity of apo B secreted is post-translational degradation (Borchardt and Davis, J. Biol. Chem. 262: 16394-16402, 1987). Approximately 95% of apo B is post-translationally degraded, while albumin, another protein secreted by hepatocytes, is secreted quantitatively. We are characterising both the site(s) of apo B intracellular degradation and signal(s) for degradation. Two cell lines, HepG2 and McArdle 7777, have been used for this purpose. Studies of the general characteristics of apo B degradation in the HepG2 cell line indicate that degradation is temperature-dependent and non-lysosomal. Stably transfected McArdle cell lines expressing various truncated forms of apo B have been used to map degradation signals. Truncated forms of apo B are both degraded and secreted. The efficiency of degradation will be discussed.

K 339 REGULATION OF GRP78/BIP MRNA TRANSLATION BY INTERNAL RIBOSOME BINDING. Dennis Macejak and Peter Sarnow, Biochemistry, Biophysics, & Genetics, Univ. of Colorado Health Sciences Center, Denver, CO 80262

Translation of the cellular mRNA encoding the glucose regulated protein 78/heavy chain binding protein (GRP78/BiP) is enhanced in Poliovirus (PV) infected cells at a time when eIF4F-dependent translation is inhibited (Sarnow, P. 1989. PNAS 86:5795-5799). We have found that the 220 nucleotide (nt) long 5' noncoding region (ncr) of the 2.5 kilobase GRP78/BiP mRNA is sufficient to confer eIF4F-independent translation to a heterologous mRNA containing the firefly luciferase (luc) coding region when transfected into PV-infected cells. Furthermore, dicistronic mRNA molecules containing luc as the second cistron gave rise to 65,000-80,000 light units of luc activity/10^5 cells when either the 220nt 5'ncr of GRP78/BiP mRNA or 700nt of the PV mRNA 5'ncr was inserted between the first and second cistron as compared to 0-5000 light units/10^5 cells with either a 50nt or 400nt insert of control sequences. Thus it appears that GRP78/BiP mRNA can translate by an internal ribosome entry mechanism similar to that of PV mRNA. Recently, we have made stable cell lines expressing dicistronic mRNAs containing GRP78/BiP 5'ncr-luc as the second cistron in order to determine if internal ribosome binding is regulated in a cell cycle dependent manner.

K 340 A CYTOPLASMIC PROTEIN BINDS TO A CONSERVED SEQUENCE IN THE 5' UNTRANSLATED REGION OF ORNITHINE DECARBOXYLASE mRNA, Joyce M. Manzella and Perry J. Blackshear, Howard Hughes Medical Institute Laboratories, Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, NC 27710

An RNA gel retardation assay was used to identify a specific cytosolic protein that bound to the 5'untranslated region (5'UTR) of rat ornithine decarboxylase (ODC) mRNA. Binding was located to within the 3' most 69 bases of the 5'UTR, a region previously suggested to be involved in polyamine feedback inhibition of ODC translation *in vitro*. UV crosslinking demonstrated that the protein:mRNA complexes migrated with an apparent M_r 52,000. Cytoplasmic extracts from human, mouse, rat, *Xenopus*, and *C. elegans* demonstrated similar binding activities. Sequence analysis of the 5'UTR of ODC genes cloned from a variety of animal species suggested the presence of a conserved heptamer. Mutation of this conserved motif abolished protein binding to ODC mRNA. We conclude that the 5'UTR of ODC mRNA contains a conserved sequence responsible for binding a cytoplasmic protein. Current studies are addressing the possible involvement of this protein:mRNA interaction in the translational regulation of ODC expression.

K 341 50 kDa PROTEIN IS RESPONSIBLE FOR REPRESSION OF TRANSLATION OF RABBIT RETICULOCYTE FREE mRNPs, Waldemar B. Minich, Igor P. Maidebura and Lev P. Ovchinnikov, Institute of Protein Research, Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR

mRNA in the cytoplasm of eukaryotic cells is present in the form of free (nontranslatable) and polyribosome-bound (translatable) mRNPs. The nontranslatable mRNPs contain masked mRNA as well as mRNA which is in equilibrium with the mRNA of polyribosomes. We have shown that the state of equilibrium in the distribution of mRNA between free mRNPs and polyribosomes in rabbit reticulocytes is governed by two factors: (1) a translational repressor which is associated with the mRNA within free mRNPs and prevents its translation, and, (2) a translation activator associated with ribosomes, overcoming the effect of the repressor (Minich et al., FEBS Lett., 258, 227-229, 1989). The treatment of free mRNPs by high ionic strength allows to isolate a complex of mRNA with the predominating 50 kDa protein. The mRNA in these particles is still in the repressed state. Highly purified 50 kDa protein inhibits translation of various mRNA in a cell-free system. This effect can be overcome by adding the translational activator. 50% inhibition of globin mRNA translation takes place at a protein/mRNA molar ratio of about 6:1. The 50 kDa protein has a pI of over 9. It interacts with globin 9S mRNA with an association constant ~$4 \cdot 10^5$ l/(M•NMP) (at 150 mM KAc, 4°C). One protein molecule binds ~20 nucleotides. The RNAs and polyribonucleotides have the following relative affinity to 50 kDa protein: poly G > poly U > 9S mRNA ~ rRNA > poly A > poly C. The 50 kDa protein can be phosphorylated both *in vitro* and *in vivo*.

K 342 PHOSPHORYLATION OF eIF-4F STIMULATES PROTEIN SYNTHESIS AT INITIATION, Simon J.Morley[#], Thomas E. Dever*, Diane Etchison** and Jolinda A. Traugh[&], [#]Friedrich Miescher-Institut, PO Box 2543, 4002 Basel, Switzerland, [&]Dept. of Biochemistry, U.C. Riverside, Riverside CA 92521, *Dept. of Biochemistry, School of Medicine, Case Western Reserve Univ., Cleveland OH 44106, **Dept. of Microbiology, Univ. of Kansas Medical Center, Kansas City, KA 66103

eIF-4F is a cap binding protein complex essential for the translation of capped mRNA and composed of 3 subunits; p25 (eIF-4E) which specifically recognises the mRNA cap structure, p46 (eIF-4A) which has an ATP-dependent RNA binding activity and p220, whose function remains elusive, but has been postulated to align p25 and p46 at the 5' end of the mRNA. Phosphorylation of both the p25 and p220 subunits is stimulated in response to tumor promoting phorbol ester and insulin. Previously it was shown that p220 and p25 are phosphorylated *in vitro* by protein kinase C or the multifunctional S6 kinase from liver at the same sites modified *in vivo* in response to PMA or insulin, respectively. Utilising a novel translation system derived from the reticulocyte lysate, we now show that phosphorylation of eIF-4F with protein kinase C or the S6 kinase, results in a 3-5-fold stimulation of protein synthesis. This effect is manifested at the level of initiation complex formation and as shown by chemical cross-linking, by increased interaction of the p25 and p220 subunits of eIF-4F with the 5' end of mRNA. Thus, phosphorylation of an initiation factor enhances binding to mRNA, resulting in a stimulation of translation similar to that observed in response to growth promoting compounds and phorbol esters.

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K 343 INCREASE IN VASOPRESSIN mRNA 3' POLYNUCLEOTIDE TAIL LENGTH FOLLOWING PHYSIOLOGICAL STIMULATION: REGULATION, FUNCTION AND MECHANISM, David Murphy, Karen Pardy, Colin Berry and David Carter, Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511, Republic of Singapore

The gene encoding the neuropeptide vasopressin is expressed in discrete groups of hypothalamic neurones. In response to the physiological stimulus of dehydration the peptide hormone is released into the general circulation from the posterior pituitary. We have shown that the pattern of VP gene expression changes in three ways following dehydration: firstly there is an increase in the transcription of the VP gene as measured by nuclear run-on assays; secondly there is an increase in the steady state levels of the VP mRNA, which correlates with the increase in gene transcription; and thirdly there is an increase in the size of the VP mRNA as a consequence of an increase in the length of the 3' polynucleotide tract (poly(A) tail) which is added post-transcriptionally. Experiments to determine the function of the increased length of the 3' polynucleotide tail have produced no evidence to suggest that it is involved in VP mRNA stability: transcription studies indicate that a stability mechanism need not be invoked to account for changes in steady-state RNA levels and numerous studies have shown that the changes in VP RNA steady state level and polynucleotide tail length are separately regulated. The increase in polynucleotide tail length could influence, presumably by stimulating, the translation of the VP mRNA, but the polysome profile of the VP transcript does not change with the increase in polynucleotide tract size upon dehydration. The mechanisms mediating and controlling the increase in polynucleotide tail length, although shown to be effected within the cell nucleus, remain to be elucidated.

K 344 ISOLATION AND CHARACTERIZATION OF THE *SUA5* GENE, WHICH AFFECTS AUG INITIATION CODON SELECTION IN *SACCHAROMYCES CEREVISIAE*, Jong G. Na, Ines Pinto and Michael Hampsey, Department of Biochemistry and Molecular Biology, LSU Medical Center, Shreveport, LA 71130

We are using genetic reversion analysis to uncover genes involved in selection of the translation initiation codon in yeast. Our approach is to isolate and define suppressors of a mutation that creates an aberrant AUG codon in the transcribed leader region of the *CYC1* gene, which encodes iso-1-cytochrome *c*. This allele, designated *cyc1-362a* (•••AUG CAC ACA CUA AAU UAA UA AUG•••), produces about 1% of the normal amount of iso-1-cytochrome *c*. Five unlinked genes, designated *sua1 - sua5*, have been identified by their ability to suppress the *cyc1-362a* defect. The *sua5-1* allele enhances iso-1-cytochrome *c* expression from *cyc1-362a* to about 60% of normal and also confers a marked slow growth phenotype (Slg^-). Suppression by *sua5-1* does not require the tandem in-frame termination codons in *cyc1-362a*; also, *sua5-1* diminishes iso-1-cytochrome *c* expression from the wild-type *CYC1* gene. These data suggest that the *sua5-1* product promotes ribosomal bypass of the most 5'-proximal AUG codon. *sua5-1* is not complemented by *SUI2* or *SUI3*, which encode the alpha and beta subunits of eIF2, or by *SUI1*. *sua5-1* also affects ß-galactosidase expression from various *gcn4/lacZ* plasmids in a manner different from any of the yeast *gcd* genes. We have cloned *SUA5* from a YCp50 gene bank by complementation of the Slg^- phenotype and have delimited the gene to a 2.7 kb *Nhe*I-*Hind*III DNA fragment. Partial DNA sequence analysis revealed no homology to other yeast genes. We are continuing our analysis of *SUA5* in an effort to define its mechanism of action during translation initiation.

K 345 INTERACTIONS OF THE A1 HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN AND ITS PROTEOLYTIC DERIVATIVE, UP1, WITH RNA AND DNA. Steven G. Nadler, Barbara M. Merrill, William J. Roberts, Kathleen M. Keating, Michael J. Lisbin, Stanley F. Barnett, Samuel H. Wilson*, and Kenneth R. Williams. Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06510 and *National Cancer Institute, NIH, Bethesda, MD.

The 319 residue A1 heterogeneous nuclear ribonucleoprotein is the best studied of the group of major or core mammalian hnRNP proteins that bind pre mRNA immediately following transcription. Fluorescence titrations of A1 and UP1 with poly(A), (U) and d(T) suggest that these two proteins do not bind with significant base specificity. We demonstrate that the cooperativity parameter for A1 binding, which has a value of about 35 for binding to both single stranded RNA and DNA, is insensitive to the NaCl concentration at least up to 0.4M. In contrast to the cooperativity parameter, the occluded site size for A1 binding to RNA is salt dependent and increases from about 14 to 28 upon increasing the NaCl concentration from 25 to 250mM. This variation in site size is best explained by assuming that A1 can interact with nucleic acids via at least two different binding modes. Comparative studies on the binding of A1 versus UP1 to poly r(ϵA) demonstrates that in addition to cooperative protein:protein interactions, the glycine rich COOH-terminal domain of A1 is also directly involved in protein:nucleic acid interactions. These conclusions are directly confirmed by studies on a 48 residue synthetic peptide corresponding to residues 260 to 307 in A1. Our studies demonstrate that the UP1 and glycine rich COOH-terminal domains of A1 make an approximately equal and independent contribution to the overall free energy of binding to single stranded RNA.

K 346 REGULATION OF PROTEIN SYNTHESIS BY CA^{2+}/CALMODULIN-DEPENDENT PHOSPHORYLATION OF ELONGATION FACTOR 2, Angus C. Nairn, Ken-ichi Mitsui, Rockefeller University, New York, NY 10021, Eric A. Jaffe, George Lam, Cornell University Medical College, New York, NY 10021, Matthew Brady, H. Clive Palfrey, University of Chicago, Chicago, Il 60637.

Elongation factor 2 (EF-2), a protein that catalyzes the translocation of peptidyl-tRNA on the ribosome, is phosphorylated in vitro by Ca^{2+}/CaM kinase III with a stoichiometry of ≈1-2 mol/mol on threonyl residue(s) contained within residues 51-61 of EF-2. Phosphorylation of EF-2 inhibits dramatically its activity in vitro, an effect that can be reversed by dephosphorylation of phospho-EF-2 by phosphatase 2A. Ca^{2+}/CaM kinase III has now been purified to near homogeneity from rat pancreas and rabbit reticulocyte cytosol. The enzyme has a native Mr of ≈140,000 and consists of major autophosphorylatable species of Mr 100,000. The physical and enzymological properties of the purified enzyme have been characterized.

In cultured cells Ca^{2+}/CaM kinase III is regulated by a variety of factors including mitogens and growth factors. For example, in endothelial cells, thrombin or histamine causes a transient increase in intracellular Ca^{2+} that activates Ca^{2+}/CaM kinase III resulting in the transient phosphorylation of EF-2. We have now shown that the dephosphorylation of EF-2 is inhibited in endothelial cells by the phosphatase inhibitor, okadaic acid. In addition, histamine in the presence of okadaic acid, caused a large and prolonged inhibition of protein synthesis measured by ^{35}S-methionine incorporation which paralleled the phosphorylation of EF-2. Histamine alone caused a small and transient inhibition of protein synthesis. These results suggest that an initial response of cells to hormones and growth factors is an inhibition of protein synthesis caused by Ca^{2+}/CaM kinase III-catalyzed phosphorylation of EF-2.

K 347 IS PROCESSING OF OVEREXPRESSED PROTEINS IN INSECT CELLS INFECTED WITH RECOMBINANT BACULOVIRUS IMPAIRED DUE TO A "SECRETORY LOAD" ? B.Nakhai,P.Sridhar & S.E.Hasnain.National Institute of Immunology, Shahid Jeet Singh Marg, New Delhi 110 067. India.

The gene coding for the human chorionic gonadotropin hormone (hCG) was cloned under the baculovirus AcNPV polyhedrin gene promoter and used as a reporter to ask fundamental questions on expression, transport & processing of recombinant proteins in insect cells. Native hCG is extensively processed from a precursor to which complex sugars, notably sialic acid is added. Sialic acid is believed to stabilize this hormone in-vivo. Infection of insect cells with a recombinant vAchCG virus resulted in the synthesis of hCG which was immunoreactive and bioactive. PAGE of S^{35}methionine labelled vAchCG-infected cells revealed,besides the presence of an authentic hCG molecule, another protein band corresponding to unprocessed hCG which did not react with the monoclonal antibody. This was perhaps due to a possible impairment in the secretory pathway of these cells as a result of the "secretory load" on the system as a consequence of over expression of the hCG molecule. To investigate further along these lines, the hCG was cloned under a promoter which is active at a stage earlier than polyhedrin promoter in the lytic cycle of the virus and is also not as strong as the latter. These experiments were aimed at understanding the so-called "secretory load" on the system for the processing proteins which are over expressed and require extensive post-translational modification(s).

K 348 MOLECULAR DETERMINANTS OF HEPATITIS B VIRUS NUCLEOCAPSID ASSEMBLY AND CONTROL OF GENOMIC RNA ENCAPSIDATION SELECTIVITY BY TRANSLATION, Michael Nassal, Ralph Bartenschlager, Michael Junker-Niepmann and Heinz Schaller, Zentrum für Molekulare Biologie, University of Heidelberg, D-6900 Heidelberg, Fed. Rep. of Germany

Hepatitis B virus (HBV) is a small DNA virus which replicates by reverse transcription of an RNA pregenome. This reaction takes place inside the viral nucleocapsid. In an attempt to define the molecular determinants governing assembly of replication-competent capsids, we recently characterized a short sequence element near the pregenome's 5'-end as the cis-acting signal ("ε") that mediates RNA encapsidation, most likely through direct interaction with the viral core protein and/or polymerase. That the RNA pregenome serves also as mRNA for these proteins implies the existence of a regulatory switch between the two RNA functions.

Intriguingly, HBV produces a second set of genomic transcripts which, except for some 30 extra nt at their 5'-ends harbouring an upstream initiator codon, are identical to the pregenome, but are excluded from encapsidation. Using a series of mutations we showed that without translation of the 5'-extension the longer transcripts are encapsidated, but that translation from the upstream AUG prevents RNA packaging. Thus, elongating ribosomes encountering ε inactivate the signal, whereas 40S ribosomes scanning through ε on the packaging-competent pregenome do not compete with formation of a preassembly complex and allow its encapsidation.

K 349 **STRUCTURE, FUNCTION AND REGULATION OF THE eEF-2 SPECIFIC Ca^{2+}/CALMODULIN-DEPENDENT KINASE III.**

Odd Nygård, Anders Nilsson, Reinout Amons*, Ulf Carlberg, Kim Palmquist and Lars Nilsson. Dept. Cell Biology, Stockholm University, S-106 91 Stockholm, Sweden and *Dept Medical Biochemistry, Leiden University, 2300 RA Leiden, The Netherlands

Phosphorylation of elongation factor 2 (eEF-2) is catalyzed by the Ca^{2+}/calmodulin-dependent protein kinase III. This kinase has been purified to homogeneity and the kinetics of the phosphorylation reaction have been determined. Phosphorylated eEF-2 has a two orders of magnitude lower affinity for pre-translocation ribosomes compared to the unphosphorylated factor. The regulation of the kinase activity has been studied in detail and the results will be presented. The kinase has been partly sequenced and the data will be discussed in relation to known sequences of other kinases.

K 350 INITIATION OF TRANSLATION BY INTERNAL RIBOSOME BINDING OF THE ANTENNAPEDIA (ANTP) mRNA OF DROSOPHILA MELANOGASTER. Soo-Kyung OH[1], Matthew P. Scott[2] and Peter Sarnow[1]. Department of Biochemistry, Biophysics and Genetics[1], University of Colorado HSC, Denver, CO 80262, and Department of Developmental Biology, Stanford University, Stanford, CA 94305.

The structure of the Antp gene revealed that it has two promoters that direct two transcription units. Both mRNAs contain the same coding but different 5' noncoding regions (NCR); Exons A,B,D,E (1,500 nt) and C,D,E (1,700 nt), respectively. Both 5'NCRs contain many AUG codons that are not used for translational initiation. We tested the translational efficiencies of the Antp 5'NCRs by in vitro transcription of RNAs bearing different parts of the Antp 5'NCR linked to a luciferase coding region. Uncapped hybrid RNAs were transfected into cultured Drosophila (SL2) cells. These RNAs translated effectively in a cap-independent manner. To test whether the Antp 5'NCR can direct internal ribosome binding, we constructed plasmids that can be transcribed to yield dicistronic mRNAs containing chloramphenicol acetyl transferase as a first cistron, 5'NCR of Antp as an intracistronic spacer, and luciferase as a second cistron. Upon transfection into SL2 cells, luciferase could be translated, indicating that the Antp 5'NCR can mediate internal ribosome entry. This hypothesis was confirmed by monitoring the cellular localization of the dicistronic RNA in Cos cells that had been infected with poliovirus. Both intact poliovirus and 5' CAT-5'NCR Antp-LUC 3' RNAs were associated with polysomes but cellular mRNAs were not. Further experiments revealed that the 450 bp exon D/E-containing part of the Antp 5'NCR was sufficient for internal ribosome binding. Thus, these data suggest that translation by internal ribosome binding may be a means of regulating gene expression of eukaryotic cellular mRNAs.

K 351 THE EXPRESSION OF AN *EIMERIA TENELLA* REFRACTILE BODY ANTIGEN DURING SPOROGONY, Helen Profous-Juchelka, Stefan Galuska, Paul A. Liberator, and Mervyn J. Turner, Department of Biochemical Parasitology, Merck, Sharp, and Dohme Research Laboratories, Rahway, N.J. 07065

Eimeria tenella is a protozoan parasite which infects the gut epithelial cells of chickens. It has a complex life cycle with multiple stages of development. The stage which is responsible for infecting the chicken's gut is known as the sporozoite stage. The sporozoite is formed during the process of sporogony or sporulation, which is initiated with the release of an unsporulated oocyst (zygote) into the external environment. During sporogony, eight sporozoites are formed within each oocyst. An *E. tenella* antigen, which appears to be associated with the refractile body found in sporozoites, has been cloned and expressed in *E. coli*. As determined by Northern blot analysis, this antigen appears to be encoded by a single mRNA species. The message encoding this antigen is present in both unsporulated oocysts as well as in oocysts having undergone seven hours of sporulation. In sporozoites, the message is detectable but in relatively low abundance, indicative of turnover. The message, which is present in unsporulated oocysts, is translatable in rabbit reticulocyte lysates; however, it is not being translated *in vivo*. Antiserum directed against the cloned antigen recognizes a predominant 28 kDa polypeptide on Western blots of sporozoite extracts. This 28 kDa polypeptide is not detectable in unsporulated oocysts. It appears to be synthesized during sporulation, within the first 2.5 hours. These observations suggest that the expression of this antigen during sporogony is translationally regulated and that the onset of sporulation may be the triggering event.

K 352 PHOSPHORYLATION AND CONTROL OF INITIATION AND ELONGATION FACTORS, Christopher G. Proud, Nigel T. Price, Nicholas T. Redpath, Harry Mellor and Gavin Welsh, Department of Biochemistry, University of Bristol, Bristol, BS8 1TD, U.K.

Rates of mRNA translation can be regulated by the phosphorylation of initiation or elongation factors.

Initiation factor eIF-2 can be phosphorylated on its α- and β-subunits. Phosphorylation of eIF-2α inhibits its activity. We have shown that inhibition of protein synthesis in reticulocyte lysates by haem-deficiency or double-stranded RNA is associated with the phosphorylation of eIF-2α only at serine-51, and not at serine-48. This is also the only site in eIF-2α phosphorylated in reticulocyte lysates in the presence of the potent protein phosphatase inhibitor microcystin. We have also demonstrated the importance of C-terminal basic residues in the specificity of the eIF-2α kinases regulated by haem and dsRNA, and have assessed the usefulness of a synthetic peptide based on the sequence around Ser^{51}, i.e. ILLSELS^{51}RRRIR, as a substrate for these kinases. We have shown that eIF-2 binds mRNA and that this is regulated allosterically by ATP and GTP, but is unaffected by phosphorylation of eIF-2α.

Elongation factor EF-2 is phosphorylated by a Ca/calmodulin-dependent protein kinase *in vitro* and in reticulocyte lysates on two threonines, Thr^{56} and Thr^{58}. Phosphorylation of both residues completely inactivates EF-2. The effects of phosphorylation at each site EF-2's functional properties will be reported.

Different protein phosphatases (PrP's) act on EF-2 and eIF-2: EF-2 is a good substrate for PrP-2A, but not PrP-1, whereas PrP-1 is the main phosphatase responsible for the dephosphorylation of eIF-2α.

K 353 POST-TRANSCRIPTIONAL REGULATION OF INCREASED EXTRACELLULAR MATRIX PROTEINS CHARACTERIZE THE MIGRATORY VASCULAR SMOOTH MUSCLE CELL PHENOTYPE Marlene Rabinovitch, Nancy Boudreau, Nadine Clausell and Li Zhu. Division of Cardiovascular Research, The Hospital for Sick Children, Toronto, ON., Canada, M 5G 1X 8.

Intimal proliferation is characterized by migration and proliferation of smooth muscle cells in the subendothelial space where the accumulation of extracellular matrix components causes progressive occlusion of the vessel lumen. This is also a feature of diseases such as atherosclerosis, post-transplant coronary arteriopathy and pulmonary hypertension and it also occurs developmentally in a fetal vessel, the ductus arteriosus (DA). In the DA, intimal proliferation in late gestation assures that the vessel will close completely when it constricts in the postnatal period. We compared smooth muscle cells from the fetal lamb DA with those from the aorta (Ao), and have shown that increased migratory behaviour of DA cells in 3-D collagen gels is related to a twofold increase in fibronectin secretion. The increased secretion of fibronectin is not associated with an increase in messenger RNA level, an alteration in mRNA stability or a lack of inclusion of the V 95+ isoform which governs fibronectin secretion from the cell. There is also a threefold increase in tropoelastin secretion in DA compared to Ao cells unaccompanied by an increase in mRNA level. These features suggest, that associated with the migratory DA smooth muscle cell phenotype, increased production of specific extracellular matrix proteins may be related to more efficient translation of mRNA. Similarly, when we compared donor and host smooth muscle cells derived from pig coronary arteries 4 weeks following cardiac transplant, the donor smooth muscle cells were of a migratory phenotype and secreted twofold more fibronectin but no increase in mRNA level was observed. These data suggest that post-transcriptional regulation of extracellular matrix protein synthesis in smooth muscle cells is involved in progressive vascular disease.

K 354 TRANSLATIONAL REGULATION OF HUMAN LOW DENSITY LIPOPROTEIN RECEPTOR mRNA IN FIBROBLASTS BUT NOT HEPATOMA CELLS, Ramharack, R., S-P. Tam, L. Brissette and R.G. Deeley, Cancer Research Laboratories and Department of Biochemistry, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Expression of human low density lipoprotein (LDL) receptor mRNA in primary cultures of fibroblasts is approximately 3 fold lower than in the human hepatoma cell lines HepG2 and Hep3B. When the cells are switched from complete to lipoprotein depleted medium (LPDM) they respond similarly by increasing the level of LDL receptor mRNA 3 fold. Analysis of the LDL receptor protein by Western blotting, pulse and pulse-chase experiments indicated that in hepatomas, the rate of receptor protein synthesis correlated with the change in mRNA concentration and increased 3 fold when the cells are switched to LPDM. However, under these conditions, the rate of synthesis of LDL receptor in fibroblasts increased 30 fold, suggesting that the translational efficiency of this mRNA increased 10 fold. The LDL receptor mRNA polysome profiles in both HepG2 cells and fibroblasts in complete serum were identical. Transfer of the cells to LPDM resulted in no discernible change in HepG2 polysome profile. However, in fibroblasts, LDL receptor mRNA shifted from heavy polysomes to a region of the gradient estimated to contain between 3-6 ribosomes. These observations are consistent with a cell specific increase in elongation or termination rates on LDL receptor mRNA that occurs in fibroblasts but not in hepatoma cells cultured in LPDM.

K 355 STRUCTURAL FEATURES OF mRNA IN ADDITION TO THE 5'-TERMINAL CAP INFLUENCE BINDING TO PROTEIN SYNTHESIS INITIATION FACTOR 4E, Robert E. Rhoads, Susan E. Carberry, Eda Kapsis, and Dixie J. Goss, Department of Biochemistry, University of Kentucky, Lexington, KY 40536 and Department of Chemistry, Hunter College of the City University of New York, New York, NY 10021

A series of capped oligoribonucleotides, analogs of the 5'-terminus of rabbit α-globin mRNA, were used to study the binding of the 25-kDa cap-binding protein eIF-4E. Oligonucleotides were synthesized by T7 RNA polymerase from a synthetic oligodeoxynucleotide template in the presence of m^7GpppG. Equilibrium association constants for the interaction of the oligonucleotides and purified human erythrocyte eIF-4E were obtained from direct fluorescence titration experiments. A capped oligonucleotide which was essentially the same as the 5'-terminal 34 residues of α-globin mRNA, containing the initiator AUG and a stem-loop structure, bound to eIF-4E with nearly the same affinity as α-globin mRNA itself, and with 5-fold higher affinity than m^7GpppG. Binding compared with this oligonucleotide was decreased by *i*) inversion of the AUG, *ii*) deletion of the four nucleotide residues to the 3'-side of the AUG, *iii*) removal of the stem-loop structure or moving it closer to either the 5'- or 3'-terminus, and *iv*) substituting a stem-loop structure with different sequence but equal stability. These results indicate that the high translational efficiency of α-globin mRNA may be due in part to a specific tertiary structure at the 5'-terminus which favors binding to eIF-4E.

K 356 Differntial induction of Ig secretion by phorbor myristate acetate (PMA) and interleukins. Osamu Saiki, and Shungo Abe. Dept. of Microbiology, Ehime University School of Medicine. Shigenobu, Ehime, Japan. 791-02.

Induction of immunoglobulin secretion in EB-transformed human B-lymphoblastoid cell line, SKW 6-4 cells was examined by PMA and several interleukins(IL). IL-2, 3, 4, 5, and 6 alone induced IgM secretion in these cells. Moreover, combination of these ILs generated additional IgM inductions, suggesting these ILs might provide differential signals on induction of Ig secretion. IL-1, however, did not induce any IgM secretion by these cells.

During these interleukins stimulation, Ca-influx did not occur and ionomycin(Ca-ionophore) didn't induce any Ig secretion in SKW6-4 cells. However, PMA (a direct PK-C activator) induced as much IgM secretion as IL-2. Depletion of PK-C activity abrogated IgM induction by IL-2 and PMA, but did not inhibit the induction by other ILs.

These results suggest that the induction by ILs is in a different manner of the Ca-CaM pathway and IgM induction by IL-2 is relatively dependent on a PK-C pathway but the induction by the other ILs is independent of PK-C.(a study at mRNA level was reported by Abe et.al).

K 357 TRANSLATIONAL CONTROL IN SEED GERMINATION, Estela Sánchez-de-Jiménez, Jorge Ibarra, Alma Pérez, Raúl Aguilar. Department of Biochemistry. Facultad de Química. Universidad Nacional Autónoma de México. México, D.F. 04510. MEXICO.

Protein synthesis is one of the earliest events ocurring in seed germination when cells reiniciate active metabolism. Translation in seed embryonic axes starts based on pre-existing stored mRNAs, switching later to newly synthesized messengers.
This research focuses on the translational regulation during seed germination and the role of phytohormones in this process.
Two dimensional analysis of the proteins synthesized in maize axes under conditions of full transcription inhibition indicates that some stored mRNAs are not translated until late in germination. Exposure of the axes to ^{32}P-ortophosphate during germination revealed specific developmental patterns of phosphorylation for ribosomal proteins, susceptible to be modulated by phytohormones only at the end of the process.
The translation efficiency of the phosphorylated ribosomes was tested in an in vitro system using either Poly U or viral mRNAs. Results indicated differential performance of ribosomes of different phosphorylation status. It is concluded that phosphorylation of ribosomal proteins might constitute a means of translational control in seed germination.

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K 358 THE ROLE OF HEMIN-CONTROLLED eIF-2α KINASES FROM MOUSE ERYTHROLEUKEMIA CELLS AS TRANSLATIONAL INHIBITORS, Thomas F. Sarre[1], Richard P. Hummel[1], Nigel T. Price[2], [1)]Institute of Molecular Cell Biology c/o Gödecke AG, University of Freiburg, D-7800 Freiburg, FRG, [2)]Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, U.K.

The reversible phosphorylation of initiation factor 2 (eIF-2) in its α-subunit is an established principle of translational control and two types of eIF-2α kinases - activated by either dsRNA (dsI) or hemin depletion (HCI) - have been characterized so far. We have investigated hemin-controlled eIF-2α kinases from cultured mouse erythroleukemia (MEL) cells, a cell line which can be induced *in situ* to erythroid differentiation marked by the onset of hemoglobin synthesis. From induced MEL cells, a hemin-controlled eIF-2α kinase could be purified, which resembles HCI with respect to its chromatographical characteristics, hemin sensitivity and substrate specificity (phosphorylation of Ser51 of eIF-2α), and in that it is activated *in situ* by iron depletion or heat shock and inhibits protein synthesis in a translation system derived from rabbit reticulocytes. In addition to this type of eIF-2α kinase, both uninduced and induced MEL cells contain a hemin-controlled eIF-2α kinase which is distinct from HCI, though sharing the features of hemin sensitivity, substrate specificity and *in situ* activation by heat shock. This kinase is not activated by *in situ* iron depletion nor does it inhibit *in vitro* protein synthesis.

K 359 TRANSLATIONAL CONTROL AND CYTOPLASMIC POLYADENYLATION OF A DROSOPHILA SPERMATOCYTE MRNA ARE MEDIATED BY THE SAME LEADER ELEMENT, Ulrich Schäfer, Rainer Kuhn, Frank Bosse and Mireille Schäfer, Institut für Genetik, Heinrich-Heine-Universität Düsseldorf, D-4000 Düsseldorf 1, Germany

The Drosophila melanogaster gene Mst87F codes for an mRNA which is exclusively synthesized in the premeiotic spermatocytes. Translation begins at least three days later when the spermatids are fully elongated. Concomitantly, a cytoplasmic polyadenylation of the Mst87F mRNA is observed. A sequence element of twelve nucleotides length within the leader is highly conserved in position and sequence among all seven members of a gene family to which the Mst87F gene belongs. P element-mediated germ line transformations with Mst87F-lacZ fusion genes have shown that both, deletion of this element or alteration of two central nucleotides by in vitro mutagenesis abolish translational control as well as the cytoplasmic polyadenylation. If two peripheral nucleotides are altered the postmeiotic polyadenylation takes place and the translational control is only mildly affected. These experiments demonstrate therefore that in Drosophila spermatogenesis the same sequence element (and most likely the same protein which is postulated to bind there) is responsible for both translational control as well as the concomitant cytoplasmic polyadenylation.

K 360 FERRITIN MRNA/REGULATOR PROTEIN (P-90) INTERACTIONS- Theil, E.C., M°Kenzie, A.R., Harrell, C.M., Patino, M.,M.,and Walden, W., Dept. of Biochemistry, North Carolina State University, Raleigh, NC 27695 and Dept. of Microbiology and Immunology, University of Illinois, Chicago, IL 60680

Ferritin mRNA contains a conserved sequence of 28 nucleotides, called the IRE (iron regulatory element) in the 5'-noncoding region that is required for translational regulation, and is the first identified, conserved mRNA regulatory sequence in eukaryotes. An apparently homologous sequence is repeated in the 3'non-coding region of another protein important in iron metabolism, the transferrin receptor. Iron alters the synthesis of each protein, albeit in opposite directions, via the same structural motif, the IRE; the context (3' or 5') influences IRE function. Trans-acting RNA factor(s) identified in animal cell extracts prevent ferritin mRNA from forming polyribosomes. One factor, a 90 kDa protein (P-90) which inhibits ferritin synthesis in vitro, has been purified. Interactions of ferritin mRNA and P-90 provide a good model for understanding regulation of eukaryotic protein synthesis.

A hairpin loop with several bulges is the computer-predicted structure of the IRE . Recently, using a combination of protein and metal-organic nucleases, we showed that the IRE in native ferritin mRNA was indeed a hairpin loop but Mg-dependent conformational variations occur, e.g. at the base of the hairpin loop [Wang et al., Nucl.Acids. Res(1990)18:4463]. In addition, a base-paired flanking region (FL), which places the entire structure near (10 nucleotides) the cap, was Mg-sensitive. We now report studies of both ferritin m-RNA and a 55-mer containing the IRE and FL with and without P-90. The structure of the 55-mer is much closer to the computer-predicted structure suggesting that the context of the IRE is modulating. Comparative gel-shift assays indicated that the complex of P-90 with mRNA was more stable than with the 55-mer. P-90 contacts detected were exclusively within the IRE, starting at the ends of the conserved sequence. However, binding of P-90 caused changes in the flanking regions. Thus, the structure combining P-90/IRE FL is the likely translational block. [Part support:NIH-DK 20251 (E.C.T.) and NSF-DMB 8818203 (W.W.)]

K 361 INITIATION FACTOR eIF-2 IS ACTIVELY ENGAGED IN mRNA BINDING,

Harry O.Voorma, Gert C.Scheper, Adri Thomas and Han van Heugten, Department of Molecular Cell Biology, University of Utrecht, Transitorium 3, Padualaan 8, 3584 CH Utrecht, The Netherlands.

The mechanism of mRNA recognition by proteins interacting with the cap-structure of messenger RNAs has been investigated by photochemical cross-linking of proteins with ^{32}P-labelled reoviral RNAs. Using eukaryotic initiation factors we identified eIF-4B and eIF-4E. Two pathways for the interaction of eIF-4B with the cap structure were observed, two other initiation factors, eIF-2 and eIF-3, could be cross-linked to the 5′ cap as well; i.e., the β- and γ-subunit of eIF-2 and the 66 kDa subunit of eIF-3. eIF-2 stimulated both pathways of eIF-4B and -4E cross-linking. The affinity of eIF-2 for relatively unstructured mRNA in the proximity of the 5′ cap focuses the attention on a role of eIF-2 in mRNA selection. We suggest that eIF-2 is able to guide the ribosome to a ribosomal binding site even when located internally as is the case with the 5′ UTR of encephalomyocarditis virus RNA. We have obtained strong evidence that the very strong competition of EMVC-RNA towards host mRNAs is due to the very high affinity of eIF-2 for the 5′ UTR of EMCV-RNA. Details of these experiments and the consequences for the initiation model will be discussed.

K 362 REGULATION OF PROTEIN SYNTHESIS IN MAMMALIAN SYSTEMS BY THE GUANINE NUCLEOTIDE EXCHANGE FACTOR. *A. J. Wahba and J.N. Dholakia*

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The guanine nucleotide exchange factor (GEF) is a multisubunit protein which catalyzes the exchange of GDP bound to eIF-2 for GTP and thus facilitates the recycling of eIF-2 during initiation. The activity of GEF may be influenced directly by the phosphorylation of its 82,000 M_r subunit and the redox state of the cell or indirectly by the phosphorylation of the α-subunit of eIF-2 . Recently, we have demonstrated that this reaction follows a sequential mechanism involving the formation of a (GTP)(GEF)(eIF-2·GDP) complex and characterized GEF as a GTP binding protein. In order to identify the nucleotide binding sites, we have covalently modified GEF and eIF-2 with the 8-azido analog of GTP (8-N_3GTP). Autoradiographs of sodium dodecyl sulfate/polyacrylamide gels demonstrate that [γ-^{32}P]8-N_3GTP is crosslinked exclusively to the 40,000 M_r subunit of GEF and the β-subunit of eIF-2. We have used the Friend virus-transformed murine erythroleukemia (MEL) cell system to elucidate the translational regulatory processes that occur during cell differentiation. This system demonstrates both general and specific changes in protein synthesis patterns during the well-characterized differentiation process which is accompanied by morphological and biochemical changes that mimic normal erythroid development. We have standardized the procedure of extraction and the assay of GEF activity in crude MEL cell lysates. GEF activity is decreased by 60% on day 5 of MEL cell differentiation and this parallels the overall decrease in protein synthesis during this period. In order to study the covalent modification of these factors during differentiation we have purified and characterized GEF and eIF-2 from undifferentiated MEL cells and raised antibodies against these factors. MEL GEF contains 5-subunits which appear to be different from the subunits of the rabbit reticulocyte factor. The addition of spermidine stimulates GEF activity in crude and partially purified preparations but has no effect on highly purified GEF from MEL cells. This suggests that the effect of spermidine may be mediated by the stimulation of casein kinase II which in turn may phosphorylate GEF and increase the activity of the partially purified factor. Protein synthesis, GEF activity and the covalent modification of GEF and eIF-2 in MEL cells exposed to heat shock or following treatment with phorbol 12-myristate 13-acetate will be discussed. Grant support: NIH GM 25451.

K 363 ELONGATION AND TERMINATION REACTIONS OF PROTEIN SYNTHESIS ON MAIZE ROOT TIP POLYRIBOSOMES STUDIED IN A HOMOLOGOUS CELL-FREE SYSTEM, Cecelia Webster and Justin K.M. Roberts, Department of Biochemistry, University of California, Riverside, CA 92521

We show that the control of gene expression at the level of elongation and termination of protein synthesis can be observed in vitro. Cytoplasmic polyribosomes isolated from maize root tips were translated in a root tip extract capable of elongation and termination, but not reinitiation. The pH dependence of in vitro elongation\termination reactions was examined. Total protein synthesis exhibited an optimum at pH ~7.5. However, the pH dependence of rates of synthesis of individual proteins was not at all uniform; many polyribosomes stalled when translated at low pH. These data were compared with the elongation\termination capacity of polyribosomes isolated from oxygenated and hypoxic root tips; tissues having, respectively, high and low cytoplasmic pHs. Many of the polyribosomes more severely depleted when root tips become hypoxic were those least sensitive to translational inhibition at low pH in vitro. And some polyribosomes whose translation in vitro is strongly inhibited at low pH are the same polyribosomes that become relatively more abundant when root tips become hypoxic. These results lead to a model in which metabolic reactions in hypoxic root tips communicate with polyribosomes and selectively modulate gene expression.

K 364 ISOLATION OF YEAST GENES WHICH COMPLEMENT EXORIBONUCLEASE MUTATIONS IN *E. COLI*, Ellen M. Welch, Stuart W. Peltz, and Allan Jacobson, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, MA 01655

Our laboratory is interested in delineating the cis- and trans-acting factors which regulate mRNA turnover. As an approach to identifying exoribonucleases involved in mRNA degradation in the yeast *S. cerevisiae* we have used a yeast genomic library to complement a temperature-sensitive *E. coli* strain (SK5003) which is mutant in two exoribonucleases. Previous studies have shown that complementation of either mutation is sufficient to confer growth at the non-permissive temperature (47°C). Yeast DNA fragments which allowed SK5003 transformants to grow at 47°C were isolated and characterized. When those plasmids which alleviated the temperature-sensitive mutation were isolated and transformed again into SK5003, all transformants grew at the non-permissive temperature. Further, transformation of these plasmids into a modified SK5003 strain in which exoribonuclease activity could be monitored demonstrated that some of these plasmids increase the exoribonuclease activity in this strain. Current efforts are focused on defining the yeast gene(s) responsible for the complementing activity.

K 365 REGULATION OF mRNA TRANSLATION BY INSULIN Gavin I Welsh, Nigel T Price and Christopher G Proud, Department of Biochemistry, University of Bristol, Bristol,BS8 1TD,U.K

A major effect of insulin in many cell types is the rapid (15-30 minutes) stimulation of protein synthesis at the translational level, which results from a specific activation of peptide chain initiation. The molecular basis of this action is unknown and at present we are studying two aspects of this:

(1) We have shown that protein synthesis is increased in Swiss 3T3 cells by insulin and that this effect is increased using the diacylglycerol kinase inhibitor R59022. Overall patterns of protein synthesis and protein phosphorylation in response to insulin in this cell type are being studied using two dimensional IEF/SDS gels. The effect of insulin on the phosphorylation of the α- and β-subunits of initiation factor eIF-2 and is being studied using the isoelectric focusing/immunoblotting technique. The results of these experiments will be reported.

(2) Casein kinase 2 (CK-2) has been shown to be stimulated by insulin in cultured cells and in whole tissues. CK-2 phosphorylates eIF-2β at two sites *in vitro*, serine-2 and a site near the C-terminus. The β-subunit is also phosphorylated by cAMP-dependent protein kinase (PKA) and protein kinase C. The position of the sites phosphorylated by CK-2 and PKA and the effects of this phosphorylation on the binding of Met-tRNA $^{met}_i$ and mRNA will be reported.

(Work supported by the British Diabetic Association).

K 366 IDENTIFICATION OF CIS AND TRANS ELEMENTS INVOLVED IN THE TRANSLATIONAL CONTROL OF RIBOSOMAL PROTEIN L32 mRNA, Michael White[2], Roger L. Kaspar[1], Harwood Cranston[2], and David R. Morris[1], [1]Department of Biochemistry, Univ. of Washington, Seattle, WA, 98195. [2]Veterinary Molecular Biology, Montana State Univ., Bozeman, MT, 59717.

Eukaryotic ribosomal protein mRNAs have been shown to be under translational control in a variety of systems. In murine fibroblasts and bovine T-lymphocytes this regulation is manifest in the shift of ribosomal protein mRNAs from translationally inactive mRNP particles to polysomes during the first few hours after mitogenic stimulation. We have utilized the mRNA sequences encoding murine ribosomal protein L32 to identify the cis and trans elements involved in this regulatory phenomenom. Our studies have revealed 1.) The first 11 nucleotides of the 5'-untranslated region of this mRNA are required for translational control in vivo, 2.) Labeled RNA-[^{32}P] containing these sequences form a protein-RNA complex in vitro which can only be competed by unlabeled RNA also containing these sequences and 3.) a pair of ~80K M_r proteins can be specifically crosslinked to RNA containing these sequences.

K 367 INCREASED EXPRESSION OF *Bacillus thuringiensis* INSECTICIDAL PROTEIN GENES IN TRANSGENIC PLANTS, Edith Y. Wong and David A. Fischhoff, Plant Science Technology, Monsanto Co., St. Louis, MO 63198

The development of insect resistant transgenic crop plants has emphasized the use of insecticidal protein genes from *Bacillus thuringiensis* (*B.t.*). Expression of wild type *B.t.* genes in plants is extremely inefficient. Significant increases in *B.t.* protein levels (up to 500-fold) have been obtained through the use of modified coding sequences, and plants expressing these increased levels of *B.t.* protein are significantly protected from insect damage. Experiments were designed to 1) further increase *B.t.* protein levels obtained with modified coding sequences through the use of heterologous 5' untranslated leaders and translational fusions to chloroplast transit peptides, 2) validate the use of a plant cell transient assay for measuring expression changes caused by coding sequence modifications, and 3) analyze the effects of altered leaders, transit peptides or coding sequences both in whole plants and in the transient expression assay. Higher levels (approximately 10-fold) of *B.t.* protein in plants have been obtained through the use of an *Arabidopsis* RUBISCO small subunit promoter with its own 5' untranslated leader and chloroplast transit peptide. The data indicate that the transit peptide and also sequences upstream of the transit peptide are required for the increased expression of *B.t.* protein, which is due to both improved translational efficiency and increased accumulation of mRNA. The transient assay is capable of predicting the expression effects caused by coding sequence modification seen in whole plants.

K 368 PLATELET-DERIVED GROWTH FACTOR STIMULATES PHOSPHORYLATION OF THE 25 kDa mRNA CAP BINDING PROTEIN (eIF-4E) IN HUMAN LUNG FIBROBLASTS, Bu Xin and Curt H. Hagedorn, Departments of Medicine and Cell Biology, Vanderbilt University School of Medicine and the VA Medical Center, Nashville, TN 37232

To explore the possible role of phosphorylation of the 25 kDa mRNA cap a binding protein (eIF-4E) in the regulation of cell growth we have examined the effect that platelet-derived growth factor (PDGF) has on this phosphorylation event. The effect of PDGF on eIF-4E phosphorylation in human lung fibroblasts (WI-38) was studied using both polyclonal antibodies and m^7GTP sepharose affinity purification methods. PDGF produced a rapid stimulation of eIF-4E phosphorylation as early as 15 min. The maximal PDGF effect (400% increase) was observed after 30 - 60 min of incubation. Phosphoaminoacid analysis of eIF-4E isolated from control and PDGF treated cells demonstrated that serine was the sole phosphoamino acid detected. Tryptic phosphopeptide map analysis of eIF-4E isolated from control and PDGF treated cells demonstrated a single phosphopeptide under both conditions. We conclude that PDGF produces a rapid stimulation of eIF-4E in intact WI-38 cells on serine residues. Phosphopeptide map analysis suggests that PDGF does not stimulate phosphorylation of a serine residue that is different from the constitutive phosphorylation site. However, we have not yet excluded the possibility of two or more serine residues being present on the same tryptic phosphopeptide. The specific serine residue(s) phosphorylated in PDGF treated cells, the kinase(s) mediating the PDGF effect and the possible physiologic role of eIF-4E phosphorylation in cell growth control remain to be studied.

K 369 CELL-FREE TRANSLATION SYSTEMS PREPARED FROM STARFISH OOCYTES FAITHFULLY REFLECT *IN VIVO* ACTIVITY; mRNA AND INITIATION FACTORS STIMULATE SUPERNATANTS FROM IMMATURE OOCYTE, Zhe Xu and Merrill B. Hille, Department of Zoology, University of Washington, Seattle, WA 98195.

Meiotic maturation of oocytes stimulates many changes in the translation of their stored mRNAs: mRNAs encoding proteins for oocyte growth are translated before meiotic maturation while those encoding proteins for cleavage are translated after meiotic maturation. Studies of meiotic maturation have been limited by the lack of translationally active cell-free supernatants. We have prepared cell-free translation systems from immature *Pisaster ochraceus* oocytes and oocytes synchronized to mature by the addition of the hormone, 1-methyladenine. These cell-free systems are active in incorporating amino acids with a substantial reinitiation of translation. They mimic *in vivo* changes in the rates of translation and changes in the specificities of proteins synthesized. Cell-free supernatants from mature oocytes have a much higher rate of initiation of translation than those from immature oocytes and use the 43S preinitiation complexes more efficiently in initiation of translation. We have shown that mRNAs and initiation factors are rate-limiting in cell-free systems from immature oocytes.

K 370 SEQUENCE AND SECONDARY STRUCTURE OF A TRANSLATION INHIBITORY RNA OF CHICK EMBRYONIC MUSCLE, Z-C. Zheng, G-J. Cao, Y. Du, P. McCartin and S. Sarkar
Dept. of Anatomy & Cell Biology, Tufts University, Health Sc. Campus, Boston, MA 02111

Previous work by us has shown that the translation inhibitory RNA (iRNA; 60-150 nucleotides size range) isolated from the 12S RNP (iRNP) particles of chick embryonic muscle inhibits mRNA translation in vitro by blocking mRNA binding to the 43S preinitiation complex. The iRNA has no effect on the double-stranded RNA sensitive protein kinase activity. The iRNA has now been resolved into multiple biologically active subspecies which inhibit mRNA translation in a discriminatory manner (muscle poly A$^+$ mRNA being inhibited more strongly than non-muscle mRNA). A cDNA library for iRNA was prepared in pUC18 vector using random priming of total iRNA and the positive clones were identified by screening with radio labeled iRNA. The 114 nucleotide insert of one clone was subcloned in the Bluescript vector. The in vitro transcript of this insert (only the 5'-3' and not the reverse orientation) showed inhibitory activity of TMV RNA translation in vitro. The sequence of the iRNA derived from DNA sequencing of the insert is: 5'AUCGAUUUUCAGGGCUAGUUGAUUCGGCAGGUGAGUUGUUCACACUCCUUAGCGGGUU CCGACUUCCAUGGCCACCGUCCUGCUGUCUAGAUCAACCAGCCCUGAAAAUGGAU 3'. Sequence analysis with the data base indicates that it is a new sequence in which 27 nucleotides underlined at the 3'and 5' ends can form base paired stem, the remaining of the molecule having a loop structure. These results suggest that iRNA(s) are true cellular entities that may play a role in the regulation of translation during myogenesis. Studies on structure-function analysis of cDNA clones for other iRNA subspecis are now in progress.

Phage/Viral Effects on Translation

K 400 *Abstract Withdrawn*

K 401 CELL SPECIFICITY OF TRANSLATIONAL INHIBITION CAUSED BY THE 5'UTR OF HIV-1 mRNA Jian Hong Cao, Ara G. Hovanessian* Hansjörg Hauser; GBF, Department of Cell Biology and Genetics, D-3300 Braunschweig, FRG; *Institut Pasteur, F 75724 Paris, Cedex 15, France

The 5' untranslated region (5'UTR) of HIV-1 provirus-derived mRNAs strongly inhibits translation in vitro and in Xenopus oocytes. The inhibition has been shown to be due to a palindromic region at the extreme 5'end of the mRNAs. It has been reported that the double-stranded region in the HIV-1 5'UTR activates an interferon-induced protein kinase (dsI) (Edery et al., 1989, Cell 56: 303-312). The activation of the dsI leads to an autophosphorylation and a subsequent phosphorylation of the α subunit of eukaryotic translation initiation factor (eIF2α), resulting in an inability to initiate translation. Edery et (Edery et al. have postulated that this is the mechanism through which the 5'UTR from HIV-1 causes inhibition of translation initiation. We have determined the effect of the 5'UTR of HIV-1 on the expression of a reporter gene in heterologous cell lines. In some cell lines like LTk$^-$, BHK and 293 translation of HIV-1 5'UTR-containing mRNAs is inhibited. In contrast, in HeLa and all tested T cell lines the 5'UTR of HIV-1 does not affect translation. To examinate the involvement of dsI in the inhibition we determined the level of dsI in these cell lines by Westernblotting and its response to the dsRNA activation *in vitro*. In both cell groups dsI is present and activatible. It has to be considered that another mechanism is responsible for the HIV-1 5'UTR translational inhibition.

K 402 FROG VIRUS 3 EARLY MESSAGES ARE MORE COMPETITIVE IN VITRO THAN TOBACCO MOSAIC VIRUS RNA. V. G. Chinchar and W. Yu. Dept. of Microbiology, University of Mississippi Medical Center, Jackson, MS 39216.

Frog virus 3 (FV3) messages are translated in vivo in the face of a profound inhibition of host cell protein synthesis. To test whether the selective translation of FV3 mRNA was due to their ability to outcompete cellular messages for the remaining translational capacity of the cell, the translational efficiency of FV3 and a heterologous test message, tobacco mosaic virus (TMV) RNA, was assayed in vitro in the presence of various inhibitors of initiation. In both wheat germ and rabbit reticulocyte lysates, early FV3 mRNAs were more resistant to potassium acetate than TMV RNA. Since hypertonic conditions stabilize secondary structure within the 5' non-translated region (5'NTR) and block ribosomal subunit scanning, these results suggest that the competitiveness of FV3 messages was due to the absence of extensive secondary structure within their leader regions. Interestingly, three other inhibitors of initiation (polyadenylic acid, aurintricarboxylic acid, and 7-methyl-guanosine monophosphate), which block translation by mechanisms distinct from potassium acetate, also selectively inhibited TMV translation. Taken together, these results confirmed the competitiveness of FV3 messages, and suggested that regions other than the 5' NTR control FV3 translational efficiency.

K 403 REGULATION OF EXPRESSION OF EPSTEIN-BARR VIRUS SMALL RNAs EBER-1 AND EBER-2 IN CONTROL AND INTERFERON-TREATED DAUDI CELLS, Paul A. Clarke, Michael J. Luscombe, Nigel Sharp and Michael J.Clemens, Department of Cellular and Molecular Sciences, St.George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, U.K.
Epstein-Barr virus encodes two small RNAs, EBER-1 and -2, that are abundantly expressed at an approximate ratio of 10:1. We have investigated the relative rates of synthesis and turnover of these RNAs, and the effects of interferon treatment, in Daudi cells. Assays using isolated nuclei indicate that EBER-1 and EBER-2 are synthesized in a ratio of approximately 1:1.5. The kinetics of loss of EBER-1 and EBER-2 from cells treated with actinomycin D indicate half-lives of 8-9h and 0.75h respectively, accounting for their different steady state levels. In cells treated with IFN α (in which cell growth is strongly inhibited) the steady state level of EBER-1 increases by up to 2-fold. In contrast, the level of intact EBER-2 declines slightly but this is accompanied by the accumulation of smaller forms of the RNA in two sets of doublets. Nuclear run-on assays show that IFN treatment inhibits both EBER-1 and EBER-2 synthesis by about 65%. Thus the increased level of EBER-1 in IFN-treated cells must be due to stabilization of this RNA. The appearance of the smaller forms of EBER-2 in IFN-treated cells is also a post-transcriptional phenomenon. Nuclease protection analysis indicates that the shortening of the EBER-2 molecule occurs at the 3' end.

K 404 BINDING OF EPSTEIN-BARR VIRUS SMALL RNA EBER-1 TO THE DOUBLE-STRANDED RNA-ACTIVATED PROTEIN KINASE DAI, Michael J. Clemens, Paul A. Clarke, Martin Schwemmle[1], Jürgen Schickinger[1] and Kurt Hilse[1], Department of Cellular and Molecular Sciences, St.George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, U.K. and [1]Institute für Biologie III, Universität Freiburg, D-7800 Freiburg, Federal Republic of Germany.
Epstein-Barr virus encodes two small RNAs, EBER-1 and -2, that are abundantly expressed in latently infected cells. Recent evidence suggests a role for EBER-1 in regulation of translation since this RNA is able to prevent the inhibition of protein synthesis by double-stranded RNA in rabbit reticulocyte lysates. EBER-1 that has been synthesized in vitro forms a complex with the dsRNA-activated inhibitor of protein synthesis DAI, a protein kinase that specifically phosphorylates polypeptide chain initiation factor eIF-2. Gel retardation assays and UV crosslinking experiments indicate that complex formation is specific for EBER-1 and requires the presence of some secondary structure in the molecule. RNA competition studies show that EBER-1-DAI complex formation is not inhibited in the presence of other small RNA species, heparin or the synthetic double-stranded RNA, poly(I).poly(C). SDS gel analysis reveals the existence of two forms of the crosslinked complex, of 64-68kDa and 46-53kDa, both of which are recognized by anti-DAI antibodies in immunoprecipitation experiments. These data suggest that EBER-1 regulates protein synthesis through its ability to interact with DAI.

K 405 The gene 10 frameshift of bacteriophage T7

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Gene 10 codes for the major capsid protein of bacteriophage T7. It has two products which are synthesized at a 10 to 1 ratio. Both are incorporated into the capsid at this proportion. The minor product is produced by a -1 frameshift near the end of the frame that codes for the more abundant product. The frameshift is conserved in gene 10 of bacteriophage T3, a diverged relative of T7. Deletion studies of the cloned gene and subcloning into reporter genes has indicated that sequences necessary for frameshifting have a defined 5' and 3' border. Site directed mutagenesis of the 3' sequences indicate the requirement for a secondary structure over 200 bases downstream from the most likely frameshift site. Complementation of phage mutants with gene 10 clones that fail to frameshift indicate that the reaction is not essential for the phage lifecycle under normal growth conditions.

K 406 REGULATION OF λ N GENE EXPRESSION, Donald Court,[1] Leonor Fernandez,[1] Luis Kameyama[1] and Gabriel Guarneros[2]
[1]ABL-Basic Research Program, NCI-Frederick Cancer Research & Development Center, P.O. Box B, Frederick, Maryland 21702 USA, [2]Department of Genetics & Molecular Biology CINVESTAV-IPN, Mexico City, Mexico.
The N gene is the first gene in the p^L-operon, and its AUG initiation signal is located at 223 nucleotides from the start of transcription. N regulates λ gene expression by transcription antitermination. The long N- mRNA leader contains the nutL region, with which N interacts to cause RNA polymerase to antiterminate. Downstream of nutL, in the N-leader, are RNaseIII processing sites.
To monitor N gene expression, we measured β-galactosidase from N-lacZ fusions. The p^L promoter, the N-leader and the N-lacZ (gene or operon) fusion construct was made and tested in multiple and single copy.
When processed by RNaseIII, N translation is stimulated. No effect was observed by RNaseIII processing upon expression of the operon fusion. When the RNase III processing site was deleted, the presence or absence of RNaseIII in the cell had no effect upon translation.
Expression of the N-lacZ gene fusion but not the operon fusion was inhibited several-fold in the presence of N protein. When the RNaseIII sites of the N-leader were deleted, N still inhibited. However, when part of the nutL site was deleted N inhibition was eliminated. We suggest that N inhibits its own expression at the translational level. This inhibition is dependent upon nutL, but the mechanism by which it occurs is not obvious. We note that N normally interacts with the transcription apparatus via nutL. Could it also interact directly with the translation apparatus, or is the effect on translation indirect?
Research sponsored in part by the National Cancer Institute, DHHS, under contract no. NO1-CO-74101 with ABL.

K 407 STRIKING SIMILARITIES BETWEEN STNV-1 AND STNV-2 RNAs : ROLE OF THEIR PRIMARY AND SECONDARY STRUCTURES DURING TRANSLATION.

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°°Plant Genetic Systems N.V., J. Plateaustraat 22, 9000 Gent, Belgium.

The RNA of the satellite of tobacco necrosis virus (STNV-1) is a highly competitive and very stable messenger, despite the absence of a "cap" structure at the 5'-end and a poly(A) tail at the 3'-end.
We have determined the complete nucleotide sequence of the RNA of STNV-2, a related satellite belonging to the same helper system and characterized by the same properties as STNV-1.
Although they are nearly identical, the leader sequences do not seem to be responsible alone for the translational properties of those RNAs, since they are unable to enhance the expression of heterologous RNAs, as well in vitro as in vivo.
A phylogenetic comparison of the two sequences has revealed the presence of three pseudoknots and a very long hairpin structure in the 3'-untranslated regions. The significance of those secondary structures in the process of translation will be discussed.

K 408 EVIDENCE THAT INTERNAL RIBOSOME INITIATION AT THE EMCV LEADER INVOLVES INTERNAL BINDING AND SCANNING: ITS USE FOR IMPROVED EXPRESSION VECTORS FOR MAMMALIAN CELLS. Monique V. Davies, Louise C. Wasley, Clive R. Wood, Donna N. Michnick, and Randal J. Kaufman. Genetics Institute, Cambridge, MA 02140.
One class of mRNAs for which ribosomes can bind internally to initiate translation belong to the picornaviruses. The 5' untranslated region from poliovirus and encephalomyocarditis virus can promote ribosome binding and translation when introduced internally within an mRNA. We have studied the sequence requirements around the authentic AUG initiation codon for the EMCV polyprotein for translation initiation. The results from analysis of mutations introduced around the authentic initiation codon suggest that the EMCV leader promotes ribosome binding and translation initiation at AUG codons in the vicinity of the authentic AUG. Both sequence context and position influence the choice and frequency of initiation. Based on these observations, a novel set of mammalian expression vectors were derived using the 5' untranslated region from EMCV to promote efficient internal initiation of selectable markers such as dihydrofolate reductase, a methotrexate resistant dihydrofolate reductase, neomycin phosphotransferase, or adenosine deaminase. These improved vectors allow rapid derivation of stable cell lines expressing high levels of the desired protein from a dicistronic mRNA.

K 409 PROTEIN RECOGNITION OF THE HIGHLY CONSERVED GTPase CENTER OF LARGE SUBUNIT RIBOSOMAL RNA, David E. Draper, Patricia C. Ryan, Ming Lu, and Lance G. Laing, Department of Chemistry, Johns Hopkins University, Baltimore, MD 21218.

The L11 protein recognizes bases 1051-1108 of the E. coli 23S rRNA with high specificity; both the protein and the RNA are highly conserved. The same fragment of RNA is also recognized by a family of peptide antibiotics (e.g. thiostrepton); these bind cooperatively with L11 and therefore make a different set of contacts than the protein. We have constructed a large number of mutations in the RNA and measured both L11 and antibiotic affinity for each. Taken together, the set of mutants changes each base in the 1051-1108 region to its Watson-Crick complement; pairwise mutants have been made to preserve the phylogenetically conserved secondary structure. Some of the mutations disrupt the RNA tertiary structure detected in melting experiments. From these measurements we deduce that

1. A tertiary base pair stabilizes the RNA in a "four helix junction" configuration.
2. Several universally conserved nucleotides and base pairs may interact to form the complete RNA tertiary structure.
3. Both L11 and antibiotics require the RNA to fold into a specific tertiary structure; however most of the L11 binding free energy must come from interactions with the RNA backbone, while the antibiotics rely more on contacts with bases.
4. Chemical and nuclease protection experiments ("footprints") are difficult to interpret reliably in terms of specific contacts of a protein with an RNA.

K 410 IN VITRO AND IN VIVO ANALYSIS OF THE SELECTIVE TRANSLATION OF INFLUENZA VIRUS mRNAs, M.S. Garfinkel, M.A. Wambach, and M.G. Katze. University of Washington, Seattle, WA 98195

In cells infected by influenza virus type A, cellular protein synthesis undergoes a rapid and dramatic shut-off. Influenza viral mRNAs are translated preferentially over cellular mRNAs even though the cellular mRNAs are stable and functional when tested *in vitro* . Previous work has suggested that this selective translation may be due, at least in part, to the structure alone of the viral mRNAs. To study the contribution of mRNA structure to the preferential translation of the viral mRNAs, *in vitro* and *in vivo* assays were developed. In the *in vitro* assay, synthetic mRNAs were translated under competitive conditions in two cell-free systems, rabbit reticulocyte lysates and wheat germ extracts. In both systems, a representative viral mRNA, that encoding the influenza viral nucleocapsid protein was preferentially translated over the cellular actin mRNA when both were translated together, even though both were translated with equal efficiency when present alone. In the *in vivo* assay, cellular or viral genes were transfected into COS cells followed by infection with influenza virus. This enabled us to determine whether an exogenously introduced viral gene escapes the virus-induced translational blocks and if so, what structural characteristics impart this resistance. Preliminary results showed that the rate of protein synthesis of the transfected cellular genes decreased after viral infection whereas expression of the transfected viral gene was not compromised. Northern blot analysis is underway to ensure the observed blocks are at the level of mRNA translation. Both assays permit analysis of the structural features of viral mRNAs that contribute to their selective and efficient translation.

K 411 TRANSLATIONAL INHIBITION OF CYTOMEGALOVIRUS GP48 EXPRESSION MEDIATED BY AN UPSTREAM READING FRAME, Adam P. Geballe, Mark R. Schleiss and Catherine R. Degnin, Department of Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Several human cytomegalovirus (CMV) gene transcripts contain AUG codons and associated short reading frames upstream from the protein coding reading frames. Previous studies demonstrated that some, but not all, CMV transcript leaders containing upstream AUG codons inhibited downstream translation. The current studies were designed to assess the translational effects of transcript leader sequences from the structural gene gp48.

The gp48 gene encodes a relatively abundant early transcript (E1) and less abundant early (E2) and late (L) transcripts. The 229 nt leader of the E1 transcript was shown to contain a signal that inhibited translation in transfection assays. Analyses of deletion and point mutation constructs revealed that one of three upstream AUG codons was essential for the inhibitory effect. Polysome analyses confirmed the translational nature of the inhibition. In addition to the upstream AUG codon, sequences 60 nt downstream near the termination codon of the short upstream reading frame were required for inhibition. Thus, studies of the gp48 leader demonstrate that translational inhibition by CMV transcript leaders is dependent on both the upstream AUG codon and additional leader sequences.

K 412 PEPTIDYL-tRNA-HYDROLASE, A TARGET FOR LAMBDA REGULATION. Gabriel Guarneros,[1] Refugio Garcia,[1] Francisco de la Vegal and Richard Buckingham[2]. [1]Department of Genetics and Molecular Biology CINVESTAV-IPN, Mexico City, Mexico, [2]Institut de Biologie Physico-Chimique, Paris-France.

The rap bacterial mutants die upon transcription of certain sites on phage lambda DNA. Another class of mutants, pth, affected in the gene for peptidyl-tRNA-hydrolase, are conditionally lethal because a defect in protein synthesis. We had shown that the rap locus and the gene for peptidyl-tRNA-hydrolase lie in a chromosome segment of 1600 bp. We asked whether rap and pth mutations affected the same gene. When tested rap mutants were found defective in peptidyl-tRNA-hydrolase activity and transcription of the lambda sites mentioned above killed pth mutants . Functional assay of a set of clones containing different segments of the 1600 bp fragment showed that they always co-complemented both, the rap and pth mutations. Sequencing of the 1600 bp segment revealed an ORF encoding a polypeptide of ca. 22 kd. The location of this putative gene agrees with the limits set by the complementation analysis. Analysis of the proteins directed by the different clones, identifyed a polypeptide of ca. 22 kd which correlates with the Rap/Pth functions. This polypeptide migrated in two dimensional gel electrophoresis as predicted from its calculated isoelectric point. The amino-terminal sequence of the protein purified from 2-D gels confirmed that predicted from the nucleotide sequence. Furthermore the sequence of the DNA fragment cloned from the pth mutant differed from the wild type sequence in one base pair which should result in an aminoacid sustitution in the respective protein. We concluded that the rap mutation affects the gene for peptidyl-tRNA-hydrolase.

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K 413 TRANSLATIONAL CONTROL BY HIV-1 TAR RNA

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The 5' LTR of human immunodeficiency virus type-1 (HIV-1) contains a cis-acting element called TAR that confers responsivity to the transacting protein Tat. A transcript of the TAR sequence (TAR RNA) is present in the 5' untranslated region of all HIV-1 mRNAs and adopts a stem-loop structure. It is also present as a free cytoplasmic form of about 60 nts. Extensively purified TAR RNA that was transcribed in vitro, unlike partially purified TAR RNA, did not activate the dsRNA activated inhibitor (DAI or p68 kinase) of protein synthesis which phosphorylates the initiation factor eIF-2. In contrast,like adenovirus VA RNA, purified TAR RNA inhibited the activation of DAI by dsRNA at high concentrations . This was demonstrated both in a kinase assay in vitro, where it inhibited the activation and auto-phosphorylation of DAI, and in a cell-free translation system, where it rescued the dsRNA mediated inhibition of translation. Efforts are underway to demonstrate the effect of TAR RNA on DAI in vivo. The effect of mutations in the TAR sequence on its structure and function will also be discussed.

K 414 TRANSLATIONAL TRANSACTIVATION OF THE POLIOVIRUS 5'NONCODING REGION IN ENTEROVIRUS-INFECTED CELLS, Simon J. Hambidge and Peter Sarnow, Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, CO 80262

We have used an RNA transfection assay to study the translation of the normally uncapped 5' noncoding region (5'NCR) of poliovirus in human cells. Cultured Hela cells were transfected with in vitro-transcribed hybrid RNA molecules containing the 5'NCR of either poliovirus or luciferase linked to the coding region of the firefly luciferase gene. Uncapped hybrid RNAs containing the 5'NCR of poliovirus were translated more efficiently than uncapped or capped hybrid RNAs bearing the cellular 5'NCR. This enhanced translation of the poliovirus 5'NCR-containing RNAs was stimulated five- to tenfold when RNAs were transfected into cells that had previously been infected with either poliovirus type 1 or coxsackievirus B1. This enhancement was observable under conditions in which cellular translation was not inhibited by the viral infection. Thus, the enhanced translation from the poliovirus 5'NCR in infected cells was not due to lack of competition from cellular mRNAs, and we suggest that enteroviruses encode a factor that can act in trans to stimulate cap-independent translation. This may explain the efficient cap-independent translation of enteroviral RNAs in non-dividing target organs such as neurons and myocytes.

K 415 POLYCISTRONIC TRANSLATION FROM CAULIFLOWER MOSAIC VIRUS 35S RNA. Johannes Fütterer, Jean-Mark Bonneville, Marc de Tapia and Thomas Hohn. Friedrich Miescher Institut, CH-4002 Basel.

CaMV 35S RNA has a dual function: template for reverse transcription and mRNA. It has coding potential for all known viral proteins and is in fact the only potential mRNA for ORFs VII,I,II,III and IV. The complexity of the CaMV 35S RNA is further increased by the presence of a 600 nt. long leader sequence containing several short ORFs. By studying the expression of reporter genes fused to the different viral ORFs in plant protoplasts a virus encoded transactivator (tav) was detected that is absolutely required for translation of ORFs I, III and IV and also enhances translation of the ORFs VII, II, V and even VI, which encodes tav and could be translated also from the subgenomic, monocistronic 19S RNA. The mechanism of ORF VII and transactivated ORF I translation was studied in greater detail by deletion mutagenesis and by analysis of the effect of physical hindrance of ribosome migration by antisense RNA or by RNA secondary structure. The results indicate that ribosomes in the absence of viral factors can be transferred directly from the beginning of the leader of the 35S RNA to a region immediately upstream of ORF VII without passing through the intervening sequences. This ribosome-shunt requires the presence of CaMV specific sequences in the 5' part of the leader sequence and close to the beginning of the ORF VII, probably the secondary structure of the bypassed RNA and in addition cellular factor(s). After translation of ORF VII, ribosomes are not able to reinitiate translation at the ORF I start codon in uninfected cells, but this incompetence is alleviated by the viral Tav protein.

K 416 **REGULATION OF eIF-2 ACTIVITY IN VACCINIA VIRUS INFECTED CELLS,** Alyce Emmert[1], Ngoc Thai[1], Bernard Moss[2], and Rosemary Jagus[1], [1]Center of Marine Biotechnology, University of Maryland, Baltimore, MD 21202, and [2]Laboratory of Viral Diseases, NIAD, Bethesda, MD 20205.

A number of animal viruses have evolved mechanisms to inhibit the activation of the interferon-induced eIF-2 alpha kinase, **eIF-2α -PK$_{ds}$,** which forms part of the host antiviral defense mechanisms. The ability of vaccinia to grow in interferon-treated cells stems in part from the production of a vaccinia virus early gene product, termed specific kinase inhibitory factor, **SKIF,** that prevents activation of eIF-2α -PK$_{ds}$. SKIF appears to function as a competitive inhibitor of dsRNA in the activation of eIF-2α -PK$_{ds}$. SKIF has been partially purified from cytosine arabinoside-treated, vaccinia virus infected HeLa cells, making use of the ability of SKIF to prevent dsRNA-dependent inhibition of protein synthesis in a rabbit reticulocyte cell-free translation system. SKIF has an apparent molecular weight of 30,000 and seems to bind dsRNA.

In addition to the induction of SKIF, vaccinia virus appears to encode a gene which has homology to the alpha subunit of eIF-2. This eIF-2 alpha-like gene is also expressed early in infection and encodes a peptide with a lower molecular weight and slightly lower isoelectric point than cellular eIF-2 alpha. A sequence with 30% homology to the human eIF-2 alpha gene has been identified in the Hind III K fragment of vaccinia virus. The accumulated data suggest that the vaccinia virus-specific, eIF-2 alpha-like peptide, in addition to SKIF, may have evolved as part of the virus's strategy to circumvent the antiviral effects of interferon.

K 417 IDENTIFICATION OF A SEQUENCE OF HEPATITIS B VIRUS GENOME WHICH ALLOWS INTERNAL INITIATION OF TRANSLATION. Olivier JEAN-JEAN, Nathalie FOUILLOT and Jean-Michel ROSSIGNOL. UPR 272, Institut de Recherches Scientifiques sur le Cancer; BP n°8, 94802 Villejuif, France.

The HBV P gene is expressed by internal initiation of translation at the first AUG of the P-ORF on the C-gene mRNA. To identify the sequence allowing direct entry of ribosomes, we have designed a bicistronic system in which the *lacZ* and *cat* genes of *E. coli* are placed under the control of an eucaryotic promotor. Defined portions of the HBV C-gene, located upstream of the first ATG of P gene, were inserted in both orientations between *lacZ* and *cat* genes. After transient expression of the plasmids in human cells, ß-galactosidase and CAT assays were performed on cell extracts. The results of repeated experiments show that the introduction of a C-gene sequence of 291 nucleotides leads to a six fold increase of CAT activity when compared to the plasmid containing the same sequence in reverse orientation. Attemps to shorten the HBV intergenic sequence was impeded because the translation of the *cat* gene was increased by the scanning mechanism. The same 291 nucleotides sequence was studied with monocistronic constructions in which the HBV sequence was inserted between the promotor and the *cat* gene. In this case, the CAT activity is 12 fold higher with the sequence in the correct orientation than with the reverse one. Identification of cellular factors involved in HBV P-gene internal initiation of translation is in progress.

K 418 EFFICIENT TRANSLATION OF mRNAs IN CELLS WHICH OVEREXPRESS eIF-4E,
Antonis E Koromilas, Anthoula Lazaris-Karatzas and Nahum Sonenberg
Department of Biochemistry, McGill University, Montreal, Canada

eIF-4E, the cap binding subunit of eIF-4F, is present in cells in limiting amounts relative to other initiation factors. Overexpression of eIF-4E in NIH 3T3 and Rat 2 cells causes tumorigenic transformation. Therefore, eIF-4E has been characterized as a proto-oncogene. One possible mechanism by which eIF-4E transforms NIH 3T3 cells is through the enhancement of translation of mRNAs encoding proteins that control cell growth. This enhancement may be due to increased unwinding of mRNA 5' secondary structure. Since cells overererexpressing eIF-4E contain more eIF-4F complex than normal cells, we want to examine whether overexpression of eIF-4E facilitates the unwinding of the secondary structure at the 5' proximal end of eukaryotic mRNAs. A series of vectors which contain increasing amount of secondary structure at the 5' non-coding region was constructed and transiently expressed in NIH 3T3 cells which overexpress wild type as well as mutant eIF-4E. A relief of translation suppression of mRNA with extensive 5' secondary structure in cells overexpressing wt eIF-4E is being tested.

K 419 Purification and characterization of a cellular inhibitor of the interferon-induced, dsRNA activated protein kinase from influenza virus-infected cells, T.G.Lee(1), J.Tomita(1), A.Hovanessian(2), M.G.Katze(1) 1-Department of Microbiology, University of Washington, Seattle, USA ;Unit of Viral Oncology, 2-Institut Pasteur, Paris, France.
Influenza virus downregulates activity of the interferon-induced protein kinase (referred to as P68) and as a result, the level of eIF-2 α phosphorylation during infection. A repressor of the P68 protein kinase has been purified from virus-infected MDBK cells utilizing in vitro kinase inhibition assays. The inhibitor fractionated at 60% ammonium sulfate and eluted off a FPLC Mono Q column at 280mM KCl. Further purification steps included heparin-agarose and Mono S chromatography and glycerol gradient centrifugation After the final gradient, the kinase inhibitory activity was associated with a single protein with a molecular weight of 58,000 daltons and which sedimented at 3.9-4.0S. . Neither protease nor phosphatase was associated with the purified product. Western blot analysis revealed that the inhibitor was a cellular and not a viral protein. We also have purified an inhibitor with the same chromatographic properties and kinase inhibitory activity from uninfected MDBK cells. Experiments utilizing ammonium sulfate fractions from uninfected cells have suggested a complex regulation of the kinase which may involve not only a repressor of the kinase but also an inhibitor of the repressor itself. A partial amino acid sequence of the 58 Kda protein has been obtained allowing us to prepare peptide antiserum. This antiserum recognized the appropriate protein in infected and uninfected bovine MDBK cells as well as in a variety of human cells. Finally, utilizing this partial sequence and PCR technology, we are attempting to isolate the gene encoding the 58 Kda protein from a MDBK cDNA library. These results and studies elucidating the molecular mechanisms of kinase regulation in influenza virus-infected and uninfected cells, will be presented.

K 420 MOUSE CELL SMALL CYTOPLASMIC B1-Alu RNA; CONSERVATION OF ALU DOMAIN STRUCTURE OF SRP RNA, AND RELEASE FROM POLYSOMES BY HIGH SALT, Richard Maraia, Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, Bethesda, MD, 20892.
SRP RNA and B1-Alu share sequence homology at their 5' and 3' ends. The first 46 bases of SRP RNA fold to form a tRNA-like cruciform structure characteristic of its Alu domain. A cloned mouse B1-Alu gene was previously described whose RNA product underwent processing and cytoplasmic localization as a small RNP in Xenopus oocytes (Nature 1985 317:81-84 and Mol. Cell Biol. 1988 8;4433-4440). In addition, a homologous RNA whose exact sequence was unknown was previously detected in mouse tissue and cell cytoplasm. Secondary structure analyses of the processed RNA from the cloned B1-Alu gene revealed striking conservation of the Alu domain found in SRP RNA despite 20% nucleotide sequence divergence while most B1-Alu sequences in genbank do not form this structure. This conservation involved several base paired nucleotides which co-diverged (from SRP RNA) to maintain base pairing. High stringency B1-specific Northern probing of mouse cells revealed the small cytoplasmic (sc) B1-Alu RNA in the polysomal and cytosolic soluble fractions. Furthermore, high salt treatment stripped the polysomes of scB1-Alu RNA along with SRP RNA which provided an internal control. The scB1-Alu RNA was purified from polyacrylamide gel and used as a substrate for reverse transcription-homopolymeric tailing-PCR amplification. This allowed the characterization of the natural 5' end-most sequences of the RNA. Nucleotide sequence and secondary structural analyses of two different clones revealed conservation of the Alu domain found in SRP RNA. Presumably, these clones reveal the coding sequences of two different genes and begin to define a consensus of expressed B1-Alu sequences. Cumulatively, the results indicate that scB1-Alu RNA may indeed modulate translation or polysome associated processes, and suggest that the previously cloned B1-Alu sequence may be a genuine gene whose RNA product plays a functional role in gene expression.

K 421 MECHANISM OF INTERNAL INITIATION OF TRANSLATION: ROLE FOR p52
Karen Meerovitch, Robert Nicholson and Nahum Sonenberg, Department of Biochemistry, McGill University, Montreal, Quebec, Canada

The mechanism of internal initiation of translation on picornaviral mRNAs is not well understood. In addressing this question we have identified a protein in HeLa cell extracts (p52) that binds specifically to a poliovirus RNA fragment encompassing nucleotides 559-624 of the 5' untranslated region. This 68 nucleotide RNA has two unique features that are highly conserved among poliovirus serotypes and other entero and rhinoviruses (1) a stable stem and loop structure and (2) a stretch of pyrimidine residues. We have created point mutations in this portion of the 5' UTR and examined their effects on internal translation and p52 binding. Our data suggest that nucleotides in the polypyrimidine tract and the AUG in the stem but not the secondary structure are important for translation. The importance of p52 binding and its role in internal initiation of translation is currently under investigation.

K 422 IDENTIFICATION OF AMINO ACID RESIDUES INVOLVED IN BACTERIOPHAGE T4 REGA PROTEIN:RNA INTERACTIONS. Eleanor K. Spicer and Kevin R. Webster, Dept. Molecular Biophysics & Biochemistry, Yale Univ. School of Medicine, New Haven, Ct. 06510
The bacteriophage T4 regA protein (Mr = 14,600) is a translational repressor of a group of T4 early mRNAs. To identify amino acids of regA protein that are involved in RNA binding, [^{32}P]-p(dT)$_{16}$ has been photochemically crosslinked to regA protein. The crosslinked complex was digested with trypsin and peptides were separated by HPLC on a DEAE anion exchange column. This chromatography yielded a single tryptic peptide crosslinked to p(dT)$_{16}$. Gas-phase sequencing of the crosslinked peptide yielded the following sequence: VISXKQKHEWK, which corresponds to residues 103-113 of regA protein. Phe 106 was identified as the site of crosslinking due to the absence of the expected phenylthiohydantoin derivative of Phe in cycle 4 (indicated as X, above). This result indicates that Phe 106 is positioned at the interface of the regA protein:p(dT)$_{16}$ complex. The specificity of the crosslinking was confirmed by the ability of a specific RNA recognition element (from T4 gene 44) to compete efficiently with p(dT)$_{16}$ in the crosslinking reaction. To further probe regA protein functional domains, regA protein fragments, produced by CNBr cleavage, were purified using C_{18} reverse-phase HPLC. CNBr peptide 6, which contains residues 95-122, retains the intrinsic affinity (Ki) of regA protein for poly (rU), as determined by fluorescence quenching assays. However, unlike the intact protein, the 28 residue peptide does not bind to RNA cooperatively. Gel mobility shift assays have demonstrated binding of the peptide to a fragment of gene 44 mRNA, and the specificity of binding is being examined.

K 423 COWPEA MOSAIC VIRUS M-RNA CONTAINS A SEQUENCE THAT ALLOWS INTERNAL BINDING OF RIBOSOMES AND THAT REQUIRES eIF-4F FOR OPTIMAL TRANSLATION, Adri A.M.Thomas, Ernst ter Haar, Joan Wellink [1], and Harry Voorma. Dept of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, and [1]Dept. of Molecular Biology, University of Wageningen, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands.
Cowpea mosaic virus (CPMV) M-RNA translation starts at nt 161 and nt 512, inducing the synthesis of two proteins of 105 and 95 kDa. The similarity of the CPMV-genome to picornaviruses raised the question whether CPMV-RNA, like polio- or EMC-virus, has the ability to direct ribosomes to internal sequences rather than to the 5'-end of its RNA. The 5'UTR of ornithine decarboxylase (ODC) mRNA has a highly stable secondary structure, that prevents efficient in vitro translation of ODC mRNA. Insertion of nt 161-512 of CPMV M-RNA downstream of the 5'UTR of ODC but upstream of the ODC initiation codon resulted in a marked increase of ODC-translation. Translation of this ODC-CPMV construct was independent of the presence of a ^{7}mGppp-structure on the transcripts. The results show that the 161-512 nt CPMV M-RNA sequence comprises an internal ribosomal bindingsite. ODC translation directed by the ODC-CPMV transcript as well as CPMV M-RNA translation was stimulated by addition of eIF-4F, the cap-binding protein complex, showing that eIF-4F exerts a role in internal initiation.

K 424 PREMATURE TRANSLATIONAL TERMINATION OF THE HIV-1 ENVELOPE PROTEIN.

*Catherine Vaquero, Norberto Serpente,*Luc d'Auriol, Marc Sitbon and Heinz Ellerbrok.*
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In infected cells the HIV *env* transcript is initiated in the cytoplasm and the nascent polypeptide chain is translocated into the endoplasmic reticulum. Subsequently, the protein is extensively glycosylated and cleaved into the extra-cellular surface protein SUgp120, and the transmembrane anchor TMgp41. In order to investigate the intermediate steps of translation of the HIV *env*, we derived an in vitro transcriptional and translational model. In a reticulocyte lysate in presence of microsomes the full length *env* transcript coded for two highly glycosylated products of apparent molecular weights of 120 and 160 kD.The 120kD product was produced independently from the cleavage of the gp160 precursor. It was recognized by sera from AIDS patients and by anti-SUgp120 mAb but did not react with anti-TMgp41 mAb. Interestingly, the 120 kD protein appeared slightly larger than the SUgp120 and we will refer to this 120 kD product as to the gp120*.

Using mutant vectors, we showed that the gp120* was not due to internal initiation, but was correctly initiated and produced by a premature translational termination, occurring in a highly structured region, located down-stream from the cleavage site. This translational arrest appeared to be highly dependent on translocation of the protein into the microsomes. The location of this arrest of translation was compatible with a potential role of secondary structures present in the Rev Responsive Element (RRE) located down-stream from the cleavage site. We derived mutants in which various stems of the RRE were eliminated without changing the coding ability of the *env* protein. However all the transcripts obtained so far produced the gp120*. Alternatively, premature translational arrest could also be due to primary structure features of this sequence. Accordingly, we are currently evaluating the role of the primary structure features in ribosomal frame-shifting that would lead to premature termination. Furthermore, we observed that secondary structures of RRE were also involved in premature transcriptional termination.Thus, in addition to the known function of the RRE in RNA processing, it is possible that this structure plays a role in other regulatory events including transcription and translation.

K 425 POLIOVIRUS 2A PROTEASE-INDUCED CLEAVAGE OF THE p220 COMPONENT OF eIF-4F REQUIRES eIF-3

Elizabeth E. Wyckoff and Ellie Ehrenfeld. Department of Cellular, Viral and Molecular Biology, University of Utah School of Medicine, Salt Lake City UT 84132

Eukaryotic translation initiation factor 4F (eIF-4F) is a multiprotein complex which binds the m^7GTP cap on the 5'-end of mRNAs. The p220 subunit of eIF-4F appears to be essential for eIF-4F activity, but its exact contribution to eIF-4F function is not understood. Poliovirus infection of cultured cells induces a rapid inhibition of host protein synthesis, which correlates with cleavage of p220. This is thought to inactivate eIF-4F, so that cellular mRNAs cannot be translated, while the uncapped poliovirus RNA is translated by an eIF-4F-independent mechanism. The poliovirus-encoded 2A protease is required for cleavage of p220, but does not itself cleave p220. The current model is that 2A activates an unidentified, cellular protease which then cleaves p220.

We have developed an in vitro assay to find cellular proteins required for 2A-induced cleavage of p220. Using this assay, we show that an activity present in highly purified eIF-3 is required for efficient, 2A-dependent cleavage of a partially purified p220 substrate. The exact role of eIF-3 in the cleavage reaction and the relationship of eIF-3 to the p220 cleavage activity is currently under investigation.

K 426 TRANSLATIONAL REGULATION AND mRNA STABILIZATION BY THE SINGLE-STRANDED DNA BINDING PROTEIN OF BACTERIOPHAGE M13, Guido J.R. Zaman, John G.G. Schoenmakers and Ruud N.H Konings, Laboratory of Molecular Biology, University of Nijmegen, The Netherlands.

The gene V encoded single-stranded DNA binding protein of bacteriophage M13 has been shown to repress at the translational level the synthesis of its cognate replication proteins encoded by genes II and X [1 - 3]. With the aid of a binary plasmid *in vivo* testsystem we were able to demonstrate that this protein also represses at the level of translation the synthesis of the assembly proteins and the coat proteins encoded by genes I and III, respectively [4, 5]. We furthermore observed that gene V protein functions as a translational autoregulator of its own synthesis. Translational repression of genes I and X is accompanied by stabilization of the respective mRNAs. Mutational analyses of the mRNA leaders of genes II and X indicated that gene V exerts its regulatory effect primarily via a linear consensus sequence that is located just upstream of the Shine-Dalgarno sequences of these mRNAs [5, see also ref. 6].

[1] Model *et al.* (1982) Cell 29, 329-335; [2] Yen and Webster (1982) Cell 29, 337-345; [3] Zaman *et al.* (1990) Eur. J. Biochem. 189, 119-124; [4] Zaman *et al.* (1990) Proc. 5th. Congress on Biotechnology, in press; [5] Zaman *et al.* (1990) submitted for publication; [6] Michel and Zinder (1989) Proc. Natl. Acad. Sci. USA 86,4002-4006.

Late Abstracts

AN INDUCIBLE CYTOPLASMIC FACTOR (AU-B) BINDS SELECTIVELY TO AUUUA-MULTIMERS IN THE 3' UNTRANSLATED REGION OF LYMPHOKINE mRNA. Paul R. Bohjanen[1], Bronislawa Petryniak[2], Carl H. June[6], Craig B. Thompson[1,2,3,5], and Tullia Lindsten[4]. [1]Cellular and Molecular Biology Program, [2]Howard Hughes Medical Institute, [3]Department of Internal Medicine, [4]Department of Pathology, [5]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI 48109. [6]Naval Medical Research Institute, Bethesda, MD 20814.

Recent evidence suggests that the metabolism of lymphokine mRNAs can be selectively regulated within the cytoplasm. However, little is known about the mechanism(s) that cells use to discriminate lymphokine mRNAs from other mRNAs within the cytoplasm. We now report a sequence-specific cytoplasmic factor (AU-B) that binds to AUUUA-multimers present in the 3' untranslated region of lymphokine mRNAs. AU-B does not bind to monomeric AUUUA motifs nor to other AU-rich sequences present in the 3' untranslated region of c-myc mRNA. AU-B RNA-binding activity is not present in quiescent T cells but is rapidly induced by stimulation of the T cell receptor/CD3 complex. Induction of AU-B RNA-binding activity requires new RNA and protein synthesis. Stabilization of lymphokine mRNA induced by co-stimulation with phorbol myristate acetate correlate inversely with binding by AU-B. We also detected an ubiquitous factor (AU-A) that resembles a previously described AU-binding factor, but we found that this factor is localized primarily within the nucleus, is not regulated in an activation-dependent manner, and does not distinguish between lymphokine and c-myc sequences. Together, our data suggest that AU-B is a novel cytoplasmic regulator of lymphokine mRNA metabolism.

MECHANISM OF POST-SEGREGATIONAL KILLING OF PLASMID FREE CELLS BY THE *hok/sok* SYSTEM OF PLASMID R1: A MODEL TO EXPLAIN ACTIVATION OF *hok* mRNA TRANSLATION IN PLASMID FREE SEGREGANTS. Kenn Gerdes, Jan Martinussen, Thomas Thisted, Allan K. Nielsen, and Peter Thorsted. Dept. of Molecular Biology, Odense University, DK-5230 Odense M, Denmark.

The *hok/sok* locus of plasmid R1, which mediates plasmid stabilization by killing of plasmid free segregants, codes for two RNA's, *hok* mRNA, and *sok* antisense RNA. *hok* mRNA encodes Hok killer protein of 52 amino acids. Expression of *hok* is regulated post-transcriptionally by *sok* anti-sense RNA. Killing of plasmid free daughter-cells by the *hok/sok* system is accomplished through differential decay of the *hok* and *sok* RNA's: *hok* mRNA is very stable while *sok* antisense RNA decays rapidly, thus leading to derepression of *hok* mRNA translation in the plasmid free segregants. Gene-control by antisense RNAs usually involves an initial reversible contact ("kissing") between the antisense and the target RNA to which it is complementary, followed by an irreversible inhibition of the target RNA by RNA-RNA duplex formation. *sok* antisense RNA inhibits *hok* mRNA translation by interaction with the *hok* mRNA leader sequence (see Poster by Thisted et al.) From previous experiments we know, that *hok* mRNA translation is activated after decay of *sok* antisense. Therefore this antisense RNA functions in an overall reversible manner. In accordance with recent data, we propose the following model to explain the reversibility accomplished by the *hok/sok* system: The *hok/sok* locus synthesizes a full-length *hok* mRNA, whose 3'-end folds back onto the middle of the molecule. The fold-back structure prevents the translational initiation signals from interaction with the ribosomes and with the *sok* antisense RNA. Thus the full-length hok transcript is translationally inactive without interacting with the antisense RNA. However, the full-length *hok* mRNA is slowly but constitutively processed in the 3'-end to a 70 nucleotides shorter species, which is physically stable. The processed *hok* mRNA lacks the sequences in the 3'-end involved in the fold-back-inhibition. Therefore, the processed mRNA can interact with both the ribosomes and with the *sok* antisense RNA. In plasmid-carrying cells, which obviously contain *sok* antisense RNA, the *sok* RNA forms an inactive duplex with the processed *hok* mRNA. This duplex RNA is rapidly removed by RNase III. In plasmid-free cells, the *sok* RNA decays rapidly. Thus, the slow processing of the full-length transcript allows the formation of the processed, translationally active mRNA in these cells. Hence, we suggest, that killing of plasmid free segregants by the *hok/sok* system is accomplished by a combination of rapid antisense RNA decay and slow mRNA processing.

A NOVEL STRESS - INDUCIBLE GENE, INTERNAL TO THE *dnaN* GENE, IS REGULATED AT THE POST-TRANSCRIPTIONAL LEVEL IN *ESCHERICHIA COLI* , Zvi Livneh, Rami Skaliter, Tamar Elizur, Yaakov Tadmor, Moshe Bergstein, Sara Blumenstein and Rivka Goell. Department of Biochemistry, The Weizmann Institute of Science, Rehovot 76100, Israel

A novel SOS stress-inducible gene, termed *dnaN* *, was discovered in *Escherichia coli* . The unique feature of this gene is that it resides inside the *dnaN* gene, encoding the β subunit of DNA polymerase III holoenzyme, the major replicative polymerase. The *dnaN* * gene is expressed in-frame with *dnaN* , producing a protein of 26kDa which corresponds to the C-terminal portion of the β subunit . Analysis of transcription initiation sites revealed that *dnaN* * mRNA was induced not more than twofold upon treatment with the SOS-inducing agents nalidixic acid or UV light. Under similar conditions translational *dnaN* **-lacZ* gene fusions showed a 10-30 fold induction upon UV irradiation. The induction was not observed in *recA* or *lexA* mutants in which the SOS response could not be induced, however this dependence was not mediated *via* direct repression by LexA. The simplest interpretation of these results is that induction of *dnaN* * is regulated at the post-transcriptional level, by a factor whose expression is regulated by the SOS stress response.

IDENTIFICATION OF CONSERVED AND MUTATIONALLY SENSITIVE AMINO ACIDS IN RegA TRANSLATIONAL REPRESSOR PROTEINS. Eric S. Miller and Catherine E. Jozwik, Department of Microbiology, North Carolina State University, Raleigh, NC 27695

Bacteriophage T4 RegA protein is a translational repressor protein that binds to and affects translational yields of more than a dozen mRNAs. RegA operators are short sequences that include initiator AUGs but do not contain local secondary structure. Progress on defining the RegA mRNA binding sites, by genetic analysis and RNA footprints, contrasts with the limited understanding of functional regions of the RegA protein. We have identified amino acids of the RegA protein that are involved in mRNA site recognition by comparative sequence analysis of *regA* from related phages and by genetic methods. RegA proteins are highly conserved, as amino acid sequences of the T-even and most of the RB phages are identical (Miller, E. S. and C. E. Jozwik. 1990. J. Bacteriol. **172**:5180). RB69 is the exceptional phage. Like the T4 protein, RB69 RegA is comprised of 122 amino acids, with which it shares 78% amino acid sequence identity. Residues in the N-terminal half of the proteins are especially conserved. Amino acid substitutions in T4 *regA* mutants alter RegA mRNA site specificity, autogenous regulation, or interactions with unidentified *E. coli* functions. Mutations in RB69 *regA* frequently affect the same or adjacent amino acids as in the T4 mutants, suggesting an RNA binding function for these residues. The amino-terminal portion of the protein is sensitive to subtitutions, as are some conserved residues throughout the two proteins (e.g., Ala-25, Ser-73, Trp-112). Characterization of the RNA binding and translational repression properties of the various RegA proteins is in progress.

IDENTITY OF A PROKARYOTIC INITIATOR tRNA, Uttam L. RajBhandary, Chan Ping Lee, Umesh Varshney, Nripendranath Mandal, Michael R. Dyson and Baik L. Seong, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Initiator tRNAs function exclusively in the initiation step of protein synthesis and not in the elongation step. To fulfill their special function, initiator tRNAs possess a number of properties which are different from those of all other tRNAs. Through analysis of the properties of mutant initiator tRNAs in vitro and in vivo, we have identified the sequence and/or structural features in E. coli initiator tRNA which account for its distinctive properties. These include sequences necessary for (1) aminoacylation, (2) formylation of the aminoacyl-tRNA, (3) binding to the P site on the ribosome and (4) preventing its binding to the A site on the ribosome. We find that sequences and/or structural elements important for the overall function of E. coli initiator tRNA are clustered mostly in two regions of the tRNA, the anticodon stem and loop and the end of the acceptor stem. In preliminary work, we have shown that introducing these sequences into elongator methionine or glutamine tRNAs allows these tRNAs to act in initiation in E. coli.

TRANSLATIONAL CONTROL OF A RIBOSOMAL PROTEIN mRNA IS MEDIATED BY SEQUENCES PRESENT IN ITS 5'-UNTRANSLATED REGION, Laura F. Steel[1] and Allan Jacobson[2], [1]Fels Institute, Temple Univ. School of Medicine, Philadelphia, PA 19140, [2]Dept. of Molecular Genetics and Microbiology, Univ. of Mass. Medical School, Worcester, MA 01655.

As the rate of ribosome biogenesis decreases during development in *Dictyostelium discoideum*, ribosomal protein (r-protein) synthesis is turned down by controls which act to reduce the level of translation of r-protein mRNAs. In order to localize cis-acting sequences within an r-protein mRNA which play a role in this regulation, we have constructed chimeric genes comprised of portions of the rp1024 gene, which encodes a *Dictyostelium* r-protein mRNA, and portions of the discoidin Ia gene, which encodes an mRNA that is efficiently translated in both growing and early developing cells. The translational activity of the chimeric mRNAs was analysed in transformed cells and we show that sequences present in the 5'-untranslated region of the rp1024 mRNA are responsible for its translational inactivity in developing cells. These sequences apparently lie outside the AUG start codon context since a mutation which greatly improves the poor context of rp1024 mRNA does not increase the efficiency of its translation. We are currently pursuing studies which will identify the cis-acting sequences in the 5'-UTR as well as identify the particular step in translational initiation that is limiting for r-protein mRNAs.

TRANSLATIONAL COUPLING IN THE *hok/sok* SYSTEM OF PLASMID R1: *sok* ANTISENSE RNA REGULATES *hok* via AN OVERLAPPING READING FRAME (*mok*).
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The *hok/sok* locus of plasmid R1, which mediates plasmid stabilization by killing of plasmid free cells, codes for two RNA's, *hok* mRNA, and *sok* antisense RNA. *hok* mRNA encodes Hok killer protein of 52 amino acids. Expression of *hok* is regulated post-transcriptionally by *sok* anti-sense RNA. Killing of plasmid free daughter-cells by the *hok/sok* system is accomplished through differential decay of the *hok* and *sok* RNA's: *hok* mRNA is very stable while *sok* antisense RNA decays rapidly, thus leading to derepression of *hok* mRNA translation in the plasmid free segregants, ensuring a rapid and selective killing of these cells. *sok* antisense RNA is complementary to the leader region of the *hok* mRNA, however, the region of complementarity does not overlap with the *hok* Shine & Dalgarno sequence. Thus, *sok* RNA regulates *hok* translation indirectly and by an unknown mechanism. We show here, that *sok* antisense RNA regulates the translation of another reading-frame located in the *hok/sok* locus. This new reading-frame, which overlaps with almost the entire *hok* gene, was denoted *mok* (*m*ediation of *k*illing). Point-mutations that prevent *mok* translation, abolish *sok* mediated control of *hok* expression. Hence, the antisense mediated regulation of *hok* occurs via translational coupling between the *hok* and *mok* reading-frames. This type of translational control seems to be conserved in 6 out of 7 the members of the *hok* killer gene family, including the plasmid encoded loci *flm*, *srnB*, *pnd* and the *Escherichia coli gef* gene.

MUTATIONAL ANALYSIS OF A DOMAIN OF eIF-2β IN YEAST. Beatriz Castilho-Valavicius, Gloria M. Thompson and Thomas F. Donahue, Disciplina de Microbiologia, Escola Paulista de Medicina, Sao Paulo, Brazil, and Department of Biology, Indiana University, Bloomington, IN.
Mutations in the beta subunit of the translation initiation factor eIF-2, isolated by in vivo selection schemes in S. cerevisiae, have implicated a domain with the structure $C\text{-}X_2\text{-}C\text{-}X_{19}\text{-}C\text{-}X_2\text{-}C$ in providing specificity to the process of initiator codon recognition (Donahue et al.,Cell **54**:621). These mutations, which allow protein synthesis to initiate at UUG codons, alter amino acid positions either in the region between the pairs of cysteines or on the carboxyl side of the domain, immediately following the last cysteine residue. In addition, these mutations cause a transcriptional induction of genes under the general amino acid control, such as HIS4, by directly derepressing translation of GCN4 (Williams et al., PNAS **86**:7515). In order to gain an understanding on the role of this domain of eIF-2β in protein synthesis initiation, we have constructed in vitro mutations in this domain of beta, involving the cysteine residues. Functional analysis of the mutant proteins indicate that alterations of the cysteine residues are incompatible with proper protein function, as the altered proteins are not able to rescue a lethal disruption of the chromosomal gene encoding for beta (SUI3), or to allow initiation at UUG codons. On the other hand, induction of HIS4 is still observed with deletions of the entire domain, and with minor alterations, such as the substitution of serine or histidine for one of the cysteine residues. Simple removal of the carboxyl half of the protein does not lead to an increase in HIS4 expression. Thus, these mutant proteins, although not able to form a functional initiation complex, may impart to the complex alterations that lead to derepression of GCN4 translation.

SEQUENCE AND STRUCTURAL REQUIREMENTS FOR SPECIFIC INTERACTION BETWEEN HIV-1 TAT-DERIVED RNA BINDING PEPTIDES AND TAR RNA, Kevin M. Weeks and Donald M. Crothers, Department of Chemistry, Yale University, New Haven, CT 06511.
HIV-1 Tat is a potent trans-activator of viral long terminal repeat-linked gene expression and is essential for viral replication. The precise mechanism of Tat mediated trans-activation remains unresolved, but involves a novel mechanism requiring specific binding to RNA sequences called TAR. TAR RNA forms a stable hairpin that includes a six residue loop, a trinucleotide pyrimidine bulge and extensive duplex structure. Carboxy terminal fragments of the HIV-1 Tat protein that include a conserved arginine and lysine rich region bind specifically to TAR RNA. A 14 residue peptide spanning the basic subdomain also recognizes TAR, identifying this subdomain as central for RNA interaction. Interference analysis of the RNA demonstrates that the "contact site" spans five base pairs and includes the pyrimidine bulge. Extensive analysis of RNA mutants indicates that within the contact site only a few base pairs are important for specificity and kinetic stability. The sequence and structure of the bulge seem to play a special role in discriminating between specific and non-specific binding modes.