Meeting Report

International symposium on translational/transcriptional regulation of gene expression

Highlights of the Fogarty International Center Meeting held in Bethesda, Maryland on 7–9 April, 1982

B. Safer

National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), Bethesda, MD 20205, USA

M. Grunberg-Manago

Institute de Biologie Physico-Chimique, Paris, France

D. Badman

National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, NIH, Bethesda, MD 20205, USA

F. Bergman

National Institute of General Medical Sciences, NIH, Bethesda, MD 20205, USA

C. Freeman

National Institute of General Medical Sciences, NIH, Bethesda, MD 20205, USA

G. Galasso

National Cancer Institute, NIH, Bethesda, MD 20205, USA

R. Jagus

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20205, USA

B. Williams

National Institute of General Medical Sciences, NIH, Bethesda, MD 20205, USA

Received 20 August 1982

1. INTRODUCTION

This conference focused on the molecular strategies employed by cells to modulate gene expression following the initiation of transcription. Emphasis was placed on recent developments indicating a close interaction of translational components and products with the transcriptional machinery of the cell. Details of molecular structure which specify these complex interactions, as well as their relationship to the overall regulation of cellular structure and function, were emphasized.

Manuscripts of major presentations will be published by Elsevier-North Holland Inc., New York.

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

2. TRANSLATIONAL/TRANSCRIPTIONAL INTERACTIONS IN PROKARYOTES

Three types of post-transcriptional regulation of gene expression (retroregulation, autogenous regulation, and attenuation) were presented in the first session. M. Rosenberg (Bethesda) presented a detailed analysis of the retroregulation of *int* gene expression in bacteriophage λ by the distal *sib* region. The *int* gene can be transcribed from either of 2 sites; one, used early after infection, termed p_L, is positioned 8 kilobases upstream. The large polycistronic mRNA expresses *int* poorly. In contrast, mRNA transcribed from a second promoter, p_I, 137 basepairs upstream of *int* is efficiently translated. The p_L transcription unit, however, in the presence of the phage anti-termination factor N, will read through the termination signals utilized by the p_L transcription unit. This results in p_L transcripts possessing a 3′-region having hyphenated dyad symmetry, which can form a 2 stem, 2 loop structure susceptible to RNase III attack. This appears to predispose the mRNA to subsequent 3′-to-5′ exonucleolytic degradation. In contrast, transcripts originating from p_I terminate within the *sib* region and no RNase III-sensitive structure is generated.

C. Yanofsky (Palo Alto) discussed the attenuation mechanism controlling the expression of several bacterial operons encoding enzymes that participate in amino acid biosynthesis. This regulatory mechanism involves controlled transcription termination at a site, the attenuator, located in the leader region of the operon, in the segment between the transcription start site and the first structural gene. This site consists of an A + T-rich region preceded by a G + C-rich region can form a stable hydrogen-bonded stem and loop structure. This RNA structure, called the terminator, is thought to be the signal that is recognized by the transcribing RNA polymerase. In addition, each leader transcript can potentially form an alternate RNA secondary structure involving an earlier segment of the transcript, and one segment of the G + C-rich stem. This structure is called the 'antiterminator'. The choice between the alternate RNA secondary structures was hypothesized to be regulated by ribosome movement in vivo. To probe the model, Yanofsky and coworkers examined the predictions of the model in vivo and in vitro with studies with the *trp* operons of *Escherichia coli* and *Serratia marcescens*. The studies reported, using different point and deletion mutants, provide strong support for the model of attenuation.

Additional evidence supporting a role of RNA secondary structure in transcription termination was obtained by in vitro studies on the effect of oligonucleotides complementary to an RNA segment. The sequence of oligonucleotide was chosen such that its pairing with the RNA would produce a RNA structure containing the antiterminator. Addition of this oligonucleotide increased read-through transcription as predicted. The studies prove the model of attenuation and reveal new regulatory aspects such as the role of ribosomal pausing and superattenuation that were not previously appreciated.

L. Gold (Boulder) and J. Karam (Charleston) discussed their recent studies on the regulation of early T4 gene expression during infection of *E. coli*. In *regA*$^-$ mutants, early T4 transcripts and their translation products, including *regA* protein, are overproduced. The molecular basis of this regulation appears to be recognition of a common nucleotide sequence near the ribosomal binding site which represses further translation once higher affinity binding sites on phage DNA have been saturated. The sequence in rII*β* mRNA recognized by *regA* is 5′ A U G UACAAU, while that of gene 45 is 5′ AUUACA A U G. An interesting rationale for *regA* function was suggested by Gold based on the observation that most *regA*-sensitive genes are concerned with DNA replication and metabolism. In this model, the primary function of *regA* protein is to bind to the RNA primer used to initiate DNA replication. *regA* would serve as a 'nucleation site' for assembly of other enzymes required both for nucleotide synthesis and DNA replication. Its role in the translational regulation of these proteins would therefore be a secondary one to ensure the balanced biosynthesis of 'replisome' components in analogy to the autogenous feed back repression seen with ribosomal and gene 32 proteins.

B. Weisblum (Madison) presented studies on the acquisition of erythromycin resistance which incorporates many features of the attenuation mechanism. In *Staphylococcus aureus* and *Bacillus subtilis*, induction of erythromycin resistance is achieved by translational activation following a ribosome-dependent alteration of the polycistronic

mRNA encoding a protein methylase. At low levels of erythromycin, ribosomes stall in the leader peptide coding region. This alters the formation of 2 hairpin structures which normally mask its initiation codon. Upon synthesis of the methylase, demethylation of adenine in 23 S rRNA then prevents inhibition of ribosomal function by erythromycin.

3. COORDINATE REGULATION OF GENE EXPRESSION

Aminoacyl-tRNA synthetases are essential enzymes in the cell. Besides being indispensable for protein synthesis, they are also involved in many other cellular processes such as regulation of aminoacyl biosynthetic operons and amino acid transport. Studies on the regulation of the synthetases were previously centered on the isolation and genetic analysis of mutants and the physiological variations of enzyme levels. The latter studies led to the proposal of metabolic control for some of these enzymes. In addition, some of the aminoacyl-tRNA synthetases are depressed under conditions of starvation for their cognate amino acids. Arginyl-tRNA synthetase, isoleucyl-tRNA synthetase and phenylalanyl-tRNA synthetase were shown to exhibit long term depression. The progress in technology due to recombinant DNA and nucleic acid sequence technology has led to a re-examination of these questions.

P. Schimmel (Boston) has cloned and sequenced the structural gene for *E. coli* alanyl-tRNA synthetase, as well as several hundred nucleotides upstream of this region. Using an in vitro protein synthesis system, his group found that alanyl-tRNA synthetase inhibits the transcription of its own gene. This repression is considerably enhanced by the addition of alanine to the system. The protein binds to palindromic sequences on the DNA site which flank the transcription start site. Data such as this may provide a bridge, linking data on genomic structure to physiological findings, such as inhibition of synthetase formation by amino acids. Unfortunately, in vivo data are not available for the effect on the expression of this particular aminoacyl-tRNA synthetase by amino acids. Similar studies are now underway seeking to understand some metabolic regulation of the glycyl-tRNA synthetase in terms of the structure of the gene.

D. Soll (New Haven) reported on work on *E. coli* glutaminyl-tRNA synthetase, a monomeric peptide of 550 amino acids, which again sought to study the interrelationships between the gene for an aminoacyl-tRNA synthetase and its gene product. Unlike the situation with the alanine enzyme, the addition of cognate enzyme, tRNA, and amino acid did not affect the rate of in vitro transcription of the gene. The tentative take-home-lesson is that the regulatory mechanisms controlling expression of this set of genes may be very diverse. Soll feels that each of the systems deserves careful analysis as an individual system, and that broad generalizations about how these enzymes work and how their synthesis might be regulated, may be not only premature, but, in the end, incorrect.

This is well illustrated in the work reported by M. Springer (Paris) on the organization of the *E. coli* phenylalanyl-tRNA synthetase operon and its expression in vivo and in vitro. The genes for the two subunits of this enzyme (*pheS* and *pheT*), as well as those for threonyl-tRNA synthetase, and for initiation factor IF3, cluster in the same region of the *E. coli* chromosome. All were cloned together on a transducing bacteriophage and in a plasmid. In vivo studies indicate that in this system two types of regulation exist:

(1) The two polypeptide chains affect their own expression; mutational alterations in either subunit provokes a decrease in the mRNA levels to about one-third of that of a wild-type plasmid;

(2) It was shown that by fusing *pheS* and *pheT* promoter to the *lac* structural gene in a plasmid, starvation for phenylalanine clearly induces β-galactosidase and that the starvation response is specific for the *pheS* and *pheT* promoter.

This situation is analogous to many amino acid biosynthetic operons which are regulated by attenuation.

The DNA sequence analysis of the *pheS*, *pheT* operon gives strong evidence for an attenuation control mechanism. Specifically, the nucleotide sequences upstream of the *pheS*, *pheT* structural genes indicate a putative phenylalanine rich peptide. This region is followed by a DNA sequence specifying an RNA capable of being folded in several alternative ways, one of which specifies a se-

quence known to result in termination of transcription. This mechanism is also supported by the results of in vitro transcription experiments. It is, as yet, too early to establish if the two mechanisms, autoregulation and attenuation, are related to each other.

M. Nomura (Madison) discussed translational feedback regulation of ribosomal protein synthesis in prokaryotes. The model was originally based on experiments showing that there are no gene dosage effects on the synthesis rate of ribosomal proteins, while the mRNA synthesis rates do have a dependency on gene dosage. He concludes that there is a post-transcriptional feedback regulation. Subsequent experiments identify specific proteins as translational repressor proteins which regulate their own synthesis and the synthesis of some or all of the proteins that are co-transcribed with the repressor. Both in vitro experiments utilizing a DNA-dependent protein synthesizing system and in vivo experiments utilizing gene fusion plasmids were used to identify the translational repressor and their regulatory units. Operons are subdivided into units of translation, regulated by their own unique translation repressors. Multicistronic units are regulated by the action of repressors at a site near the initiation site for the first cistron in the unit. Translation of distal cistrons is dependent on the translation of previous ones. Finally, coordination of regulation is accomplished by competition between ribosomal RNA and mRNA for the repressor ribosomal protein. In addition to the translational feedback regulatory mechanism, there are transcriptional regulatory mechanisms operating in the stringent control of protein synthesis or in conditions of ribosome deficiency. When ribosome assembly is inhibited, a stimulation of transcriptional activities of all ribosomal protein promoters is observed. An hypothesis was also presented that rRNA synthesis is feedback regulated by an excess of ribosomes.

N. Robakis (Nutley) discussed the advantage of a highly defined DNA-directed in vitro protein synthesis system to study gene expression in *E. coli*. A simplified in vitro system developed to study gene expression was based on the formation of the first dipeptide or tripeptide of the gene product. The studies reported used a plasmid containing the L10 operon which contains the genes for ribosomal protein L10 and L12, as well as genes for the β- and β'-subunits of RNA polymerase. They have shown that L10 inhibits the formation of the initiation complex on the L10 mRNA, consistent with the report of Nomura and coworkers. The β-subunit gene is not inhibited by L10 but by the RNA polymerase holoenzyme and is stimulated by a *nusA* gene product (L factor). RNA polymerase holoenzyme inhibits at the level of translation, whereas L factor stimulates transcription of the β-subunit gene probably by altering the extent of attenuation of the L12–β intercistronic region.

L. Lindahl (Rochester) has found that in *E. coli*, the S10 ribosomal protein operon is regulated autogenously by the ribosomal protein L4, which is also encoded by the operon. In addition to the translational regulation of the operon observed, regulation of transcription may also play a role in the autogenous regulation of the S10 operon. The rationale, based on Dr Lindahl's findings, is that regulation of translation, while a rapid means of controlling protein synthesis, might not be efficient for regulating the expression of distant genes in a long operon. Therefore, a second mechanism, operating at the level of transcription, might be beneficial. These results are in apparent contradiction with findings of Nomura et al. It is possible, however, that S10 operon is regulated by a different mechanism.

S. Pederson (Copenhagen) described his studies of two operons, L10 and S1. The molar ratio of L10 and L12 protein in the cell is 1:4. It was found that the synthesis rates of L10 and L12 RNA are about 4-times that of the average ribosomal protein mRNA. By binding to the leader part of the mRNA, the L10 and L10–L12 complex adjusts the synthesis of L10 and L12 to each other and to the amount of rRNA in the cell. Measurements of rate of S1 protein and mRNA synthesis also show the regulation to take place at the translational level. S1 is involved in its own control. However, in contrast to the L10 operon and to other ribosomal operons, S1 regulation does not seem to involve competition between homologous structures on rRNA and S1 leader mRNA. Thus a different mechanism for S1 regulation may be involved.

J. Hershey (Davis) discussed the structure and expression of *E. coli* initiation factor genes. He described 3 methods to determine quantitatively initiation factor (IF-1, IF-2, IF-3) levels: radioim-

munoassay, immunoblotting and two-dimensional gel electrophoresis. The three initiation factors are found in approximately equimolar amounts in cells growing exponentially, although IF-1 levels may be somewhat lower. The molar ratio of factors to ribosomes is in the range 0.15–0.2. Initiation factor levels are coordinately controlled and rise as a function of increasing growth rate. The curves are somewhat similar to that of ribosomal RNA. In addition, it was shown that the level of IF-3 is proportional to the number of its genes.

L. Bosch (Leiden) reported on the expression of *tufA* and *tufB*, the two genes encoding the elongation factor EF-Tu in *E. coli*. EF-Tu is the most abundant protein in the cell. The two EF-Tu encoding genes are distantly located on the *E. coli* chromosome and are positioned in two different transcription units (*tufA* at 73 min is cotranscribed with the S12, S7 EF-G genes, while *tufB* at 88 min is cotranscribed with 4 upstream tRNA genes). Their expression is coordinately regulated and the level of EF-Tu increases proportionally to the growth rate. This coordinate regulation is not affected by a specific point mutation in the *tufB*, but a single site mutation in *tufA* disturbs the coordinate expression of *tufB* and *tufA* and enhances the expression of *tufB*. These data, as well as others, suggest that EF-Tu protein itself is involved in the expression of *tufB*, presumably at a post-transcriptional level. By contrast *tufA* expression is not affected by increasing EF-Tu levels.

J. Rabinowitz (Palo Alto) has attempted to determine the molecular basis for the occurrence of translational specificity in prokaryotes; that is, the basis for response of ribosomal systems from Gram-positive organisms only to mRNA from Gram-positive species, and not to mRNA preparations from Gram-negative or associated phages. The determinants of this specificity are associated with the 30 S ribosomal subunit and the mRNA. Dr Rabinowitz and co-workers have determined a 'Shine-Dalgarno' ribosome binding sequence from Gram-positive organisms. Subsequent studies have led to the proposal that the molecular basis of the 'translational specificity' (in Gram-positive species) is related to the relatively high free energy of base-pairing at the ribosome site as compared to that of mRNAs derived from Gram-negative sources.

The nucleotide environment surrounding codons or anticodons in mRNA or tRNA may affect, in subtle ways, both the rate and fidelity of translation. They are probably responsible for the degeneracy of the initiator codon. UUG has been shown to code for initiator tRNA (J. Rabinowitz), and it was reported by the French group that AUU is the initiator codon in the IF-3 gene. In all of these cases, a strong Shine-Dalgarno sequence is found upstream from the initiation codon. Such local environment effects have been shown also to influence markedly the efficiency of suppression of termination, and may promote or inhibit frameshifts of transcription. In information-dense genomes, such as the small RNA bacteriophages, such frameshifts may, in fact, be essential for proper expression of overlapping genes. All of these modulations are 'context effects' in the sense that the local environment extrinsic to a specific bit of information may affect the manner in which it is expressed or, more anthropomorphically, its 'meaning'.

To discriminate among various regulatory and synthetic functions of tRNA, Yarus (Boulder) varied a specific tRNA gene and its cognate tRNA, one or a few nucleotides at a time. These variants were then used to explore both the gene and its gene product. They illustrate this methodology by showing how they have altered a termination suppressor form of the $tRNA^{Trp}$ (*E. coli*) gene in a way not previously seen: the conversion to a C of the 'universal U', which immediately precedes the anticodon of every known elongation tRNA. Using combinations of oligonucleotide synthesis, nucleic acid enzymology, and DNA cloning methodology, altered tRNA genes are readily introduced into *E. coli* where their expression can be studied. Although replacing the 'universal U' by a C in suppressor RNA does result in somewhat weaker suppression, the effect is not striking. In other words, the 'universal U' may be universal, but it is not irreplaceable.

Bruce and Gesteland (Salt Lake City) use a somewhat similar methodology. tRNA is cleaved by a specific chemical reaction involving the hypermodified Y-base 3′-proximal to the anticodon region. The anticodon is removed by partial digestion with ribonuclease A. A tetramer, including a new anticodon plus a nucleotide to replace the Y-base, is then inserted and sealed with T4 RNA ligase. They discuss the construction of an other-

wise normal phenylalanyl tRNA whose anticodon CpUpAp is complementary to the amber stop codon UAG, which contains an A in lieu of the original Y-base, and which has been shown to function as a phenylalanine-inserting suppressor in an in vitro system. In fact, the artificial tRNA is more effective than a naturally occurring tRNA, which is not too surprising, since highly efficient suppressors would be lethal in vivo.

In this artificial suppressor, the nucleotide adjacent to the 3′-end of the anticodon (and corresponding to the original Y-base) was A. It turns out that tRNAs with the purines A or G in this position are active, while pyrimidines are either inactive, or are extremely weak suppressors. (One possibility that is being considered is that there is some sort of interaction between the 'universal U' and this position.) It is clear that there is much to be learned about context effects in the neighborhood of anticodons.

Buckingham (Paris) studied an in vitro polypeptide synthesis system which includes ribosomes, charged tRNA and various preparations of regularly repeating or random synthetic messages. All contain the UGU codon (coding for cysteine) which is sometimes mistranslated as tryptophan. Their observations point clearly to an effect of codon context on tRNA selection, in that polymers which might, a priori, be expected to result in similar ratios of translation to mistranslation do, in fact, behave in markedly different ways. Certain local environments in which the UGU codon finds itself seem to result in greater fidelity than do others. Such observations may have a bearing on what is possible in evolution without introducing untenable, lethal mayhem on protein synthesis.

Single-stranded RNA bacteriophage such as MS2, f2 and R17, contain information for at least 4 proteins. In bacteriophage MS2, it was recently shown that two such genes are overlapping and must be transcribed in differing reading frames. Specifically, the gene that specifies a lysis, or L protein, overlaps the coat protein gene at its 5′-end, and a synthetase gene at its 3′-end. Van Duin (Amsterdam) has studied this complex system by cloning it as a DNA plasmid under host control. A small genetic region proximal to the region of initiation of the lysis gene translation seems to be essential to expression of this gene. Presumably, this region includes much or all of the region within which a putative frameshift must occur. The Dutch workers conclude that the L gene is not directly accessible to ribosomes, but rather is expressed by a fraction of ribosomes that change their reading frame to reach a stop codon, and then reinitiate out of their original phase. The authors feel that an uncontrolled lysis gene might be particularly dangerous to the host–bacteriophage relationship, and thus envisage the need for such a fail-safe mechanism.

4. STRUCTURE AND EUKARYOTIC TRANSLATION

This session focused on the structural–functional relationships which affect translational efficiency in eukaryotes. S. Penman (Boston) presented a series of whole mount electron micrographs with correlated functional studies of cellular protein and RNA metabolism with alterations of cell architecture. 'Free' polysomes were shown to be associated with the cytoskeleton at the junction of intermediate filaments. Inhibition of initiation (e.g., by drugs, heat shock) results in release of ribosomes, but not mRNA from the cytoskeleton. This may indicate that mRNAs are attached by their mRNP proteins. The progressive loss of translational and transcriptional regulation in cells having an increasingly malignant phenotype could be correlated with alterations of cellular ultrastructure. The basic premise advanced was that complex cytoskeleton and nuclear matrix not only controlled details of translational and transcriptional metabolism, but also was the basis of the mechanism used by the genome to regulate more complex intercellular processes such as tissue organization and developmental regulation. Whether this is cause or effect, however, remains to be demonstrated.

Recent progress in the mechanism of protein translation across the endoplasmic reticulum was presented by G. Blobel (New York). A free signal recognition particle (SRP), an 11 S ribonucleoprotein containing 6 distinct polypeptides and one molecule of 7 S RNA, was shown to be responsible for the transient inhibition of nascent polypeptide elongation during the translation of secretory proteins, if a specific membrane-receptor protein, designated SRP receptor, was unavailable. When SRP

receptor sites become available, 'docking' of the inhibited polysome–SRP complex can occur and the inhibition is relieved. This mechanism may thus assure the correct topological synthesis of secretory proteins and prevent total synthesis of such proteins within the cell cytoplasm. Preliminary sequence data also support the identification of the 7 S SRP RNA as that previously characterized as small cytoplasmic 7 S RNA, which is closely related to the abundant Alu family.

The importance of a second and possibly functionally related class of small RNA has also emerged from the work of T. Shenk (Stoneybrook). The adenovirus VA RNAs I and II, made in large amounts during late infection, exist as RNP particles which are recognized by the anti-La class of lupus sera. A VA I mutant which fails to synthesize VA I RNA appears to have a translational defect which inhibits translation of late adenovirus mRNAs. Normal amounts of functional late mRNAs are present but fail to be translated even though the rate of nascent polypeptide elongation during the translation period is normal. This suggests a role for VA I RNA during initiation of late translation, but at the moment, additional data are required before the mechanism of translational inhibition can be established.

The structural–functional relationship of translational components during formation of the 43 S preinitiation complex were to have been presented by H. Bielka (Berlin). Unfortunately, there were last minute problems which prevented Dr Bielka from arriving; fortunately, his excellent manuscript was received and this is briefly summarized. Studies using crosslinking agents and immunoelectron microscopy show that eIF-2 and Met-tRNA$_i$ interact with a specific subset of 40 S ribosomal proteins, some of which also interact with mRNA. A subset of these interact, in turn, with the 18 S rRNA, and the suggestion is made that part of the 18 S rRNA contributes to functional organization of the binding domains of eIF-2, mRNA and Met-tRNA$_i$ on the 40 S ribosomal subunit. It may be of particular importance that protein S6, whose phosphorylation/dephosphorylation in vivo has recently been correlated with functional changes in the role of translation, is located at the binding site of Met-tRNA$_i$ and eIF-2.

J. Vournakis (Syracuse) summarized his recent work examining the importance of mRNA 2° structure in eukaryotes. By a variety of enzymatic and computer techniques, it was shown that mRNA 2° structure plays a major role in initiation codon selection and ribosomal pausing. Specific 3'-end labeling of globin mRNA was achieved using RNase H after mRNA hybridization to dT$_4$dG. This allowed structural mapping of the 3'-end of globin mRNA by methods previously used for 5'-end analysis. Unlike the prokaryotic system where accessibility of the initiator AUG codon and surrounding regions to the 16 S rRNA (Shine-Dalgarno) appears to be major determinants of ribosomal binding, selection of the eukaryotic initiation codon appears to be located by a ribosomal scanning mechanism, in which both 5'-cap structure and cap-binding proteins may be required to melt the 2° structure of the 5' non-translated sequence. M. Kozak (Pittsburgh) discussed the importance of the surrounding bases in relationship to the ribosome's ability to select correctly the initiator codon. According to the original 'scanning hypothesis' the initiating 43 S preinitiation complex should first bind to the 5'-cap structure and subsequently select the first AUG encountered. Out of 132 eukaryotic 5' non-coding sequences examined, however, 18 exceptions have been found in which the initiator AUG is preceded by an unused upstream AUG. It appears from examination of the flanking sequences that preferential selection of the initiator AUG is favored by the concensus sequences AXX A U G G.

The general theme to emerge from this session appears to be that structure, function and regulation are intimately linked and that whenever possible, all three should be examined and correlated.

5. REGULATION OF INITIATION FACTOR ACTIVITY

Two major topics were covered in this session:

(1) The mechanism of translational inhibition following the activation of eIF-2 kinase;

(2) The identification and role of cap-binding proteins in the regulation of mRNA translation.

There is now major agreement that catalytic function of eIF-2, required for the binding of Met-tRNA$_i$ to initiating 43 S ribosomal subunits, is achieved by an interaction with a large polypeptide complex designated eRF (Voorma, Utrecht),

RF (Safer, Bethesda), SRF (Siekierka, Nutley), and Co-eIF-2C (Gupta, Lincoln). The new factor is a large polypeptide complex of 5 subunits, M_r = 80 000, 67 000, 58 000, 39 000 and 26 000, which can either exist free or as a complex with eIF-2. This factor, which will be referred to as eIF-2B in accordance with the nomenclature officially established in 1977, seems to catalyze the exchange of bound GDP in inactive eIF-2 • GDP, for GTP. Formation of eIF-2 • GTP is required for the subsequent binding of Met-tRNA$_i$. Scatchard analysis of GDP binding data was presented which demonstrated a basis for this exchange activity, since the K_d^{GDP} was 3.1×10^{-8}, while that of eIF-2B • eIF-2 was increased to 2.8×10^{-7} (Safer). A major area that still requires clarification remains, however, and that is how phosphorylation of the eIF-2 α-subunit interferes with this guanine nucleotide exchange function of eIF-2B. Voorma and Siekierka favor a direct inhibition of eIF-2B and eIF-2(αP) association, therefore inhibiting GTP:GDP exchange and eIF-2 recycling. The major problem with such a mechanism, however, is that the majority of eIF-2 (65–75%) is not phosphorylated and would presumably be free to interact normally with eIF-2B. A possible solution is found in the experiments of Safer who reported that there is actually an increased affinity of eIF-2(αP) to eIF-2B, at physiological concentrations of guanine nucleotides, rather than the failure to associate found in the absence of GTP and GDP. If this increased association of eIF-2B and eIF-2 is unaccompanied by guanine nucleotide exchange or inhibits the subsequent release of eIF-2 • GTP, this could explain the almost total inhibition of eIF-2 recycling. That is, partial phosphorylation of the much larger eIF-2 pool (estimates of eIF-2B:eIF-2 approximately 1:5–1:10 were presented) could sequester the smaller eIF-2B pool into an inactive complex. Alternatively, N. Gupta (Lincoln) proposes that the eIF-2B (discussed in terms of two activities, Co-eIF-2B and Co-eIF-2C) reverses protein synthesis inhibition in hemin-deficient lysates by exchanging the phosphorylated α-subunit in eIF-2(αP) with the non-phosphorylated α-subunit of Co-eIF-2C. The relationship of these activities to the highly purified eIF-2B preparations from other laboratories remains uncertain.

Several additional controversies in this field have been clarified:

(1) There has always been disagreement as to whether or not phosphorylation of eIF-2α directly inhibits the Met-tRNA$_i$ binding activity of eIF-2. The general concensus appears to be that it does not, unless sufficient GDP is present to form the inactive complex eIF-2(αP) • GDP. Gupta (Lincoln) proposed that eIF-2(αP) might exist in two conformational states, one active, and one inactive, both derived originally from active eIF-2 • GDP and inactive eIF-2 • GDP complexes, respectively. That is, the continued presence of GDP might not be required to maintain the inactive conformation of eIF-2(αP).

(2) Mg^{2+} inhibition of eIF-2 found by different laboratories and relief of this Mg^{2+} inhibition by eIF-2B now appears comprehensible in terms of the various eIF-2 preparations being contaminated to a variable degree by GDP. Siekierka (Nutley) showed that in the absence of Mg^{2+}, free GDP can readily exchange for bound GDP. In the presence of Mg^{2+}, however, GDP exchange only occurs in the presence of eIF-2B. Their original interpretation, that eIF-2B relieved the Mg^{2+} inhibition of eIF-2 activity but not eIF-2(αP), now becomes understandable in terms of the guanine nucleotide exchange function of eIF-2B and inhibition of this activity by eIF-2α phosphorylation.

The similarity of eIF-2B to EF-Tu was discussed by Dr Y. Kaziro (Tokyo). He also presented arguments supporting the likelihood of a stable eIF-2B • eIF-2 • GTP from which eIF-2 • GTP is displaced by eIF-2 • GDP, rather than a direct guanine nucleotide exchange. Additional discussions identified the major uncertainties remaining in this area. Why is there a requirement for 5 different subunits on eIF-2B? What are their partial functions? Are all necessary for GTP/GDP exchange? What are the energetics of this exchange, since there is an apparent exchange of a high affinity ligand for one of the lower affinity without any apparent input of energy into the system? What are the relative pool sizes of eIF-2B and eIF-2? While clearly there has been remarkable progress and clarification during the past year, much still remains to be done.

S. Tahara (Nutley) described studies on the active form and polypeptide composition of 'cap-binding proteins' (CBP). Two translationally active

forms are present in reticulocyte lysate, designated CBP I and II. CBP I is the original M_r 24 000 cap-binding protein which may be associated loosely with either eIF-3 or eIF-4B. At high [KCl], this association is disrupted, and CBP is only found in a large complex termed CBP II (8–10 S). Since CBP II, but not CBP I, can restore translation of capped mRNA in extracts from polio virus-infected cells, it is felt that CBP II is the active native form of CBP. CBP II contains M_r 50 000 and 80 000 polypeptides previously identified as eIF-4A and eIF-4B, respectively, as well as larger unidentified polypeptides. Interaction of the CBP II polypeptides with the 5′-cap structure occurs in an ATP-dependent process that may be unrelated to a possible role in the hypothetical melting of 5′ mRNA secondary structure during ribosomal mRNA binding. The mechanism of polio virus inactivation is still unknown, but may involve disaggregation of CBP II or modification of its other polypeptide components.

N. Sonnenberg (Montreal) and H. Trachsel (Basel) presented data which examined the structure of CBP II and its role in the mechanism of polio virus translational inhibition. The subunits of CBP II and their possible relationship to eIF-4A and eIF-4B were examined using monoclonal antibodies prepared against CBP. Common antigenic determinants exist among polypeptides of CBP II having M_r-values ($\times 10^{-3}$) of 210, 160, 80, 50, 28 and 24. Since this could result from either similar functional domains (e.g., cap recognition site) of functionally similar proteins, or proteolytic processing of a unique polypeptide during isolation of CBP II, the relationship of CBP subunits to other eIF still remains uncertain. Although the mechanism of polio virus inhibition is still not known, crude initiation factors prepared from polio virus-infected cells were shown to contain an activity which inactivates the cap binding activity of eIF prepared from uninfected cells. The effect of polio virus-infected extracts on mRNA binding to ribosomes, however, does appear to be proportional to the extent of mRNA 2° structure and, similar to uncapped mRNAs, capped mRNAs with little 5′ 2° structure (AMV-4) are still translated efficiently.

The question, 'Does mRNA competition for a rate-limiting mRNA discriminatory factor required for ribosomal binding exist in vivo?' was examined by W. Walden (St Louis). In SC-1 fibroblasts, initiation of 'weak' mRNAs is stimulated by cycloheximide inhibition of the translation of 'stronger' mRNAs, similar to results previously described in reovirus-infected cells. This is the result of a larger pool of the limiting factor now being available for the 'weaker' mRNAs. These results also imply binding of the limiting mRNA discriminatory factor prior to ribosomal binding of mRNA. The identification of the responsible discriminatory factor required for mRNA binding still is unknown, but it is tempting to consider the possible involvement of various cap binding proteins or RNP particles which appear to affect such mRNA binding.

6. TRANSLATIONAL REGULATION IN CELLS

The last session surveyed the mechanisms of translational regulation in intact cells and unfractionated systems. It is evident that considerable progress has been made in several systems asking biochemical questions of complex biological processes. P. Lengyel (New Haven) first reviewed the status of the effects of interferon on translation in virally-infected cells. Activation of the endonuclease RNase L follows the dsRNA-dependent activation of the oligo(A) synthetase system. Activation of RNase L, accompanied by binding of oligo(A) (shown both by filter binding and crosslinking studies) is reversible upon removal of oligo(A). There does not appear to be any change in size (app. M_r = 27 000) or conformation of RNase L. The cellular level of RNase L increases 2–3-fold following treatment with interferon. Interferon also affects translation directly by activation of an eIF-2α kinase distinct from the hemin-regulated kinase. The relative importance of RNase L and eIF-2α kinase to translational regulation in interferon-treated cells is uncertain.

The causes of the apparent decline in the rate of protein synthesis during the transition from exponential to stationary phase growth of CHO cells was addressed by K. Moldave (Irvine). While the amount of mRNA parallels the decline of its translation, its distribution among free mRNP, ribosomal subunits, ribosomes and polysomes appears to be normal. Of the protein translational factors required for protein synthesis, only EF-1 appears

to be significantly affected. The basis for this change is unknown, but the alteration is readily reversible and may be related to a depletion of serum components of the culture medium.

J. Ruderman (Boston) has used the *Spisula* oocyte system to examine the mechanism of translational activation and changes in the specificity of mRNA translation following fertilization. Both a comparison of the in vivo and in vitro polypeptides synthesized and analyses of the polysomal mRNA pool by cDNA hybridization show that large changes in the patterns of proteins synthesized following fertilization result from altered mRNA selection from a relatively fixed maternal mRNA pool, rather than transcription of new mRNA. Such changes are accompanied by large alterations of the sedimentation properties of the processed mRNPs. These major shifts of mRNA translational efficiency can be reproduced by translation of mRNP, but not phenol-extracted mRNA in the heterologous nuclease-treated reticulocyte lysate system. The molecular basis of the translational switch is presently unknown.

Dr C. McLaughlin (Irvine) discussed his studies on the effects of heat shock on transcription and translation in *S. cerevisiae*. In yeast, the enhanced production of specific heat shock proteins is mediated almost exclusively by increased mRNA transcription. Severe heat shock, however, also produces an accompanying inhibition of protein synthesis initiation. The primary emphasis on transcriptional regulation may reflect the short average $t_{1/2}$ of 20 min of mRNA in yeast.

In *Drosophila*, however, S. Lindquist (Chicago) showed that in addition to transcriptional activation of heat shock proteins, there is a preferential translation of heat shock mRNAs over pre-existing mRNAs. The 'normal' complement of mRNAs is only sequestered, however, since a normal pattern of protein synthesis can be immediately resumed upon recovery from heat shock, without any requirement for new transcription. Using a monoclonal antibody, the M_r 70 000 heat shock protein was shown to be rapidly concentrated in the nucleus and was localized to the interband areas of the chromosome. The function of this binding may be to protect 'open' DNA sequences and allow a rapid return to the original pattern of gene expression upon return to normal temperatures. An autogenous feedback mechanism for its synthesis was also suggested, since the incorporation of amino acid analogs into heat shock proteins prevented the shut-off of their synthesis upon return to normal temperatures. This also suggests that heat shock proteins have specific functions that must be fulfilled in order to return to the normal metabolism conditions at standard temperatures.

The meeting was concluded by D. Soll (New Haven) who noted that:

(1) 'Research in prokaryotes is back in vogue', the result of a new appreciation of the variety of their regulatory phenomena, e.g., alteration, transcription, termination and downstream regulation of upstream genes, some or all of which may subsequently be found to operate in eukaryotes;
(2) The importance of RNA structure in specifying its interactions with regulatory and functional proteins;
(3) Biological regulatory mechanisms are very diverse and probably for some time will remain 'a continuous challenge to us to be ever more ingenious...'