Meeting Report

INTERNATIONAL SYMPOSIUM ON PROTEIN SYNTHESIS

Summary of Fogarty Center-NIH Workshop held in Bethesda, Maryland on 18–20 October, 1976

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Received 14 February 1977

1. Nomenclature

A uniform nomenclature for the initiation factors involved in eukaryotic protein synthesis was approved and is listed in table 1. The function(s) of the individual factors is discussed in section 3. An effort to develop a uniform nomenclature for eukaryotic ribosomal proteins is described in section 6.

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2. Initiation in prokaryotic systems

The session devoted to initiation of protein synthesis in prokaryotes centered around two major problems. The most intriguing and so far the most difficult one is that of recognition by the ribosome of initiation signals in the messenger; the other is the assembly of initiation complexes, a process mediated by initiation factors and GTP. L. Bosch (Leiden) reviewed the present status of the field in his opening comments.

The postulate of Shine and Dalgarno (also see

Table 1
Nomenclature for the initiation factors involved in eukaryotic protein synthesis

New	Basel	NIH
eIF-1[a]	IF-E1	–
eIF-2	IF-E2	IF-MP
eIF-3	IF-E3	IF-M5
eIF-4A	IF-E4	IF-M4
eIF-4B	IF-E6	IF-M3
eIF-4C	IF-E7	IF-M2B$_\beta$
eIF-4D	–	IF-M2B$_\alpha$
eIF-5	IF-E5	IF-M2A

[a] eIF Stands for eukaryotic initiation factor – the 'e' can be replaced by: a = animal, p = plant, v = viral, y = yeast, etc.

Special case: M1 (which appears to be an evolutionary remnant): eIF-2A.

section 7), that the 3′-terminus of 16 S rRNA is involved in recognition by complementary base-pairing with base sequences present near the AUG-codon of the initiation region of the messenger, has stimulated a number of workers to study both the secondary structure and the function of the 16 S rRNA 3′-end. Particularly useful proved to be cleavage of 16 S rRNA at a specific site 49 nucleotides from the 3′-terminus by means of bacteriocins like colicin E_3 and cloacin DF 13. Van Knippenberg (Leiden) showed high-resolution nuclear-magnetic-proton-resonance spectra of the 49 nucleotide fragment measured at different temperatures. Individual resonances at low field can be assigned to the imino-protons of particular A · U and G · C base-pairs. From these data, he concluded that the RNA-fragment at 20°C and 5 mM Mg^{2+} exists as a hairpin comprising eight intramolecular base-pairs, the 3′-dodecanucleotide being unpaired. Structures in which the latter dodecanucleotide forms four more base-pairs (closed forms) have a life-time less than a few milliseconds. Evidence for a closed form at lower temperatures (below 20°C) was obtained by J. Steitz (New Haven) who performed *T*-jump experiments and observed two melting transitions (also see section 5). According to her, the closed form is converted to an open form at 21°C. The latter transition disappears in the presence of the ribosomal protein S1, suggesting that S1 stabilizes the open form under these conditions. S1 is required for mRNA binding to the ribosome during the formation of a preinitiation complex, as was shown by J. van Duin (Leiden) and by W. Szer (New York).

Initiation-complex formation with different initiation regions of R17 RNA differ in their dependence on S1 and initiation factors, which, according to Steitz, is related to the degree of complementarity between these regions and the 3′-terminal region of 16 S rRNA. Cleavage of the 16 S rRNA by the bacteriocin causes an interesting defect in the ribosomes: the ability to respond to IF-1 is lost (Bosch). IF-1 displays a number of pleiotropic effects, (H. O. Voorma, Utrecht; M. Grunberg-Manago, Paris; E. Stringer, New York) one of which is the acceleration of ribosome subunit exchange with 70 S ribosome couples. Apparently, the continuity of 16 S rRNA around the 49th nucleotide is a requirement for subunit interaction to be affected by IF-1. Therefore, it was concluded that the 3′-terminal region of 16 S rRNA plays a key role in the interaction between ribosomal subunits. Interesting in this connection was the suggestion by Grunberg-Manago that subunits interact by a base-pairing mechanism between 16 S and 23 S rRNA. This suggestion is based on experiments by van Duin, C. Kurland (Uppsala), Grunberg-Manago, and J. P. Ebel (Strasbourg) that radioactive IF-3 could be cross-linked to the 3′-end of both the 16 S rRNA and the 23 S rRNA following periodate oxidation of the 3′-terminal adenosine. These workers hypothesize that IF-3 exerts its action through the 3′-end of 16 S rRNA, thus preventing the 30 S ribosome from associating with the 50 S ribosome. Concerning the sequence of events in initiation, C. Gualerzi (Berlin) presented a model that reconciles previous controversial data. He concludes, from kinetic data, that the primary binding of mRNA and fMet–tRNA to the 30 S ribosome represent two alternative pathways, and that the sequence of events is not obligatory but depends on the nature of the messenger, the amount of fMet–tRNA, and other experimental conditions.

Stringer presented evidence showing that an important role of IF-1 is to ensure that IF-2 remains bound to 30 S complexes throughout the initiation reaction so that GTP can be hydrolyzed and an active 70 S complex can be formed. Voorma elaborated on the formation of a binary complex between fMet–tRNA and IF-2 (also seen previously by others). He

showed that the binding of this binary complex to the 30 S ribosome is promoted by IF-1. A. J. Wahba (Sherbrooke) described a ribosomal protein very close in size to S1 but differing from the latter in function, N-terminus and immunological behaviour. He identified it as a component of RNA polymerase and showed it to have strong ATPase activity. He also reported an interaction of IF-3 with 30 S, 50 S and 70 S ribosomes and on various ribosomal proteins involved in this interaction. J. A. Lake (Los Angeles) described his model of the 30 S ribosome and the topography of the ribosomal proteins based on electron microscopy, cross-linking data and the binding of antibodies specifically raised against ribosomal proteins (see also section 7 below).

3. Initiation in eukaryotic systems

After a morning meeting on prokaryotic initiation of protein synthesis that was exemplified by painstaking detail, the afternoon session on eukaryotic protein synthesis seemed less exact. As was noted in the opening remarks of W. C. Merrick (Bethesda), one of the current accomplishments is the ability of two laboratories (Bethesda and Basel) to agree on which initiation factors are which. This correlation resulted in the ability of the symposium participants to generate a unified nomenclature (see section 1), a feat accomplished 5 years ago by those working in prokaryotic protein synthesis. The agreement on the identity of the various initiation factors did not, however, imply a single interpretation of the biologic function of each factor.

T. Staehelin (Basel) presented the first paper, which detailed the requirements for the initiation of natural eukaryotic mRNAs. Seven initiation factors, ATP, GTP, Met–$tRNA_f$, 40 S and 60 S subunits and spermidine are required for maximal incorporation of globin mRNA into an 80 S initiation complex. GTP appears to be required in two distinct steps: (1) the formation of a ternary complex of eIF-2 with initiator tRNA and GTP and (2) at subunit joining. The requirement for ATP seems to be associated with binding of the mRNA to the 40 S subunit, but the protein that binds ATP has not yet been identified. Single omission experiments (analyzed by sucrose density-gradients) generally defined points in the 80 S initiation pathway where each initiation factor was required. eIF-2 catalyzes the binding of the initiator tRNA to the 40 S subunit; eIF-3 (which stabilizes the eIF-2·40 S complex) eIF-4A and eIF-4B are subsequently required for the binding of globin mRNA (also see section 5). eIF-5 and eIF-4C catalyze the final step, subunit joining. The pleiotropic effects observed with IF-1 (*E. coli*) seem to be found primarily in eIF-1, although eIF-4C may also have more than one activity.

Data presented by E. C. Henshaw (Rochester) pointed out that, while some reactions can be readily demonstrated by sucrose-gradients, analysis of fixed samples by CsCl centrifugation could also be of considerable importance. By this technique, it is possible to define the characteristics of various 40 S–Met–$tRNA_f$ complexes by their buoyant density. In particular, it will be of importance to define the initiation factors present in complexes of buoyant densities 1.41, 1.49 and 1.51. A point currently in question is: does eIF-2 bind (with Met · $tRNA_f$ and GTP) to the 40 S subunit prior to the binding of eIF-3? The presentation by Staehelin indicated the affirmative; the data presented by R. Benne and J. W. Hershey (Davis) suggested that eIF-3 binds first and eIF-2 second, while eIF-1, eIF-4A and eIF-4B bind subsequently. Henshaw's data seemed to be most consistent with the initial binding of eIF-2, but work is still required to define more clearly differences between kinetic and stabilizing effects of all the components. This technique does have the added advantage of being directly applicable to unfractionated systems, allowing experimentation under a wide variety of conditions, a limitation of the highly fractionated systems.

M. Kozak (Nutley) discussed the partial sequence of two reovirus RNAs: that portion of the 5′-end of the mRNA that can be protected from nuclease digestion by 40 S ribosomal subunits. The sequences are given below:

1 10 20
m^7GpppGmCUAAUCUGCUGACCGUUACUCUGC

30 40 50
AAAG*AUG*GGGAACG(CU,CUUC)CUAUCG(U)

1 10 20
$m^7GpppGmCUAAAGUGACCGUGGUCAUGGCUU$

30 40
CAUUCAAGGGAUUCUCCG(C)

Ribonuclease T1 protected fragments of two medium size class reovirus RNAs (M. Kozak and A. Shatkin, Roche Inst. Molec. Biol.). The entire sequence shown is protected from RNAase T1 by 40 S subunits; the underlined sequence is protected from RNAase T1 by 80 S monosomes. The only obvious similarity between the two sequences (besides the 5′- 'cap') is that the sequence of the five most distal bases is the same (Gm–C–U–A–A). Inasmuch as there seem to be no other areas of homology before the initiating AUG-codon and the distance from the 5′-cap is different (19 vs 30 nucleotides), it is clear that additional sequence data will be required to evaluate possible mRNA · rRNA base pairing (analogous to that observed in prokaryotes). Indeed, data presented by R. Lockard (Cambridge, USA) on the rabbit-globin mRNA sequences show that the alpha- and beta-globin mRNAs have a common 6-base sequence at the 5′-end of the mRNAs, but this sequence (m^6Am–C(m)–A–C–U–U) is different from the common reovirus RNA sequence. The partial sequence of the 5′-end of the alpha- and beta-globin mRNA which was presented is given below:

Alpha-globin mRNA

$m^7Gpppm^6AmC(m)ACUUCUGG$

Beta-globin mRNA

$m^7Gpppm^6AmC(m)ACUUGCUUUUGACACAA$

(R. E. Lockard and U. L. RajBhandary, Mass. Inst. Tech.).

The final two presentations on eukaryotic initiation dealt with an aspect for which there is no known model in prokaryotes; regulation at the translational level. T. Hunt (Cambridge, England) spoke about the molecular mechanism of the shut-off of protein synthesis initiation in a hemin-deficient reticulocyte lysate, a system initially described by M. Rabinovitz and co-workers. During the first 5–10 min incubation of a hemin-deficient lysate, an inhibitor of protein synthesis is activated under conditions otherwise optimal for protein synthesis. This activated inhibitor preparation (which has also been obtained in the laboratories of I. London, B. Hardesty, M. Clemens, and J. Traugh) has protein kinase activity. Based upon the observation of I. London and co-workers that the addition of purified eIF-2 to a hemin-deficient lysate would restore protein synthesis, Hunt and R. J. Jackson examined both crude and purified systems to see if there is a direct correlation between these two observations. The answer was yes. Under conditions where initiation of protein synthesis was inhibited (either by hemin-deficiency, dsRNA, or oxidized glutathione), specific phosphorylation of the low-molecular-weight subunit of eIF-2 appeared to occur (as analyzed by two-dimensional gel electrophoresis). In addition, the same phosphorylation appeared to occur with the partially purified inhibitor and purified eIF-2. The only major reservation was that, in several assay systems, phosphorylated eIF-2 was equally as active as untreated eIF-2. This left the question: 'Is phosphorylation of eIF-2 a necessary and sufficient condition for protein synthesis shut-off, or is it just a necessary condition?'

A second type of regulation of protein synthesis initiation was presented by I. Kerr (London) concerning the effect of interferon treatment on translation. In short, the cell-free protein synthesizing system derived from interferon-treated L-cells is sensitive to (inhibited by) dsRNA while that derived from untreated L-cells is not. The mechanism of initiation shut-off appeared to be similar to that of hemin-deficiency in reticulocytes in that the inhibition showed a 5–10 min lag, was sensitive to ATP-concentration and was more pronounced if the cell-sap was incubated earlier with dsRNA. Comparisons of polyacrylamide gel autoradiograms of cell-sap incubated with [γ-^{32}P]ATP indicated that two polypeptide chains of molecular weight 65 000 and 34 000 were specifically phosphorylated in the interferon-treated cell-extract when compared to control extracts. The 34 000 dalton band could be the low-molecular-weight peptide of eIF-2, but the other band has not been identified. The mechanism of protein synthesis shut-off between the hemin-deficient lysates and extracts from the interferon-treated cells should soon be understood and will add another interesting chapter to the story of eukaryotic protein synthesis.

4. Elongation and termination

Following an overview of prokaryotic and eukaryotic elongation and termination by H. Weissbach (Nutley), the first portion of the session concentrated on the genetics of the bacterial components required for translation. M. Nomura (Madison) described his studies on the mapping of elongation factors Tu, Ts and G and many of the ribosomal proteins. Two transducing phages received special emphasis, namely λRif^{d}18 and λFus3. The former carries the genes for rRNA, RNA polymerase subunits β,β', EF-Tu and several ribosomal proteins from the large ribosome subunit, including L12. The latter phage carries the genetic information for many of the small and large ribosome subunit proteins EF-G and also EF-Tu. Special attention was given to EF-Tu since not only are there two EF-Tu genes, but it is also known that this protein can account for as much as 5% of the soluble protein of *Escherichia coli*. It is apparent from these observations that the regulation of the synthesis of EF-Tu may involve rather unique processes. The availability of in vitro systems directed by phage DNA to study the synthesis of specific proteins provides a powerful tool to investigate the control mechanisms involved. Both Nomura and N. Brot (Nutley) discussed such studies. It is established that many of the gene products carried by the λRif^{d}18 and λFus3 phages are synthesized in vitro. In addition in vivo studies discussed by A. Furano (Bethesda) have shown that the synthesis of EF-Tu parallels the synthesis of tRNA in bacterial cells; this may explain the high level of EF-Tu in *E. coli*. It is also apparent from both in vivo and in vitro studies that EF-Tu synthesis is under stringent control. The in vitro studies have shown that ppGpp inhibits the synthesis of several factors involved in translation such as EF-Tu, EF-G and ribosomal proteins.

A unique observation made several years ago was that EF-Tu and EF-Ts are subunits of Qβ replicase. Recent studies on the relationship between structure and function of EF-Tu in replicase activity were presented by T. Blumenthal (Bloomington). Treatment of EF-Tu with agents that destroy its ability to interact with AA–tRNA did not affect its ability to function in Qβ replicase. Other studies have suggested that the association of EF-Tu with EF-Ts is essential for replicase activity.

The latter portion of the session concerned the role of the eukaryotic factor EF-1 in AA–tRNA binding to ribosomes. The active form of EF-1 is a polypeptide of about 50 000 daltons (EF-1_L). However, this protein is very often present in tissues as high-molecular-weight aggregates (EF-1_H). It appears that disaggregation of EF-1_H must occur during AA–tRNA binding. There is evidence that GTP can accomplish this and general agreement that an AA–tRNA · EF-1_L · GTP complex is an intermediate in the binding reaction. J. Drews (Vienna) discussed studies on Krebs ascites cell EF-1. His studies showed that EF-1_L is the active form of the factor and that it binds tightly to the ribosome during AA–tRNA binding. This bound EF-1 can recycle in the presence of EF-2, presumably due to the ability of EF-2 to free the A-site on the ribosome during polymerization. The discussion on EF-1 was continued by Y. Kaziro (Tokyo). His results suggest that the situation with eukaryotic EF-1 is very similar to what is seen with the prokaryotic factors EF-Tu and EF-Ts. One of his factors (called EF-1_α) appears similar to EF-1_L, i.e., a protein of molecular weight about 50 000. This factor appears to be analogous to EF-Tu. A second factor has been isolated that has two subunits referred to as EF-1_β (molecular weight ~30 000) and EF-1_γ (molecular weight 53 000). EF-1_β appears analogous to EF-Ts in that it catalyzes a nucleotide exchange:

$$\text{EF-}1_\alpha \cdot \text{GDP} + \text{GTP} \overset{\text{EF-}1_\beta}{\rightleftharpoons} \text{EF-}1_\alpha \cdot \text{GTP} + \text{GDP}$$

and also permits EF-1_α to function catalytically. L. Slobin (Leiden) and G. A. Lanzini (Milan) reported that factors similar to those described by Kaziro are present in *A. salina* embryos and wheat-germ embryos.

The final presentation by C. T. Caskey (Houston) concerned recent studies on the role of release factors (RF) in both prokaryotic and eukaryotic systems. In contrast to prokaryotes, in which there are two release factors with different codon specificities, only one release factor has been isolated from rabbit reticulocytes. The active form is a dimer having a subunit of 56 000 daltons. In the prokaryotic systems, antibody studies suggest that there are common antigenic sites on RF-1 and RF-2 and that ribosomal proteins L7/L12 are involved in RF-recognition by

the large subunit. Antibodies to L7/L12 also inhibit eukaryotic termination but, in contrast, antibodies to L40,41 (60 S protein comparable to L7/L12) only inhibit eukaryotic termination.

5. Structure and translation of mRNA

In the initiation of protein synthesis, mRNA interacts with ribosome subunits and several initiation factors. This session, starting with opening comments by H. Lodish (Boston), focused on the features of mRNAs involved in these interactions and also on the reasons why polysomes containing certain mRNAs are bound to membranes. Perhaps the most significant result to emerge was never articulated as such – that both in prokaryotes and eukaryotes, many regions of the mRNA and many types of RNA · protein and RNA · RNA interactions are involved in the initiation event, that certain of these may be more important than others for certain mRNAs or certain cells, and that no universal generalization about the function of any specific nucleotide sequence or interaction is likely to be forthcoming.

There was general agreement that evidence is lacking for the existence of protein factors involved uniquely in translation of only certain mRNAs. R. Thach (St. Louis) summarized his data showing that eIF-4B is required for the translation both of EMC and cellular mRNA, but that lower concentrations are needed for translation of the viral RNA. Hence, at low eIF-4B concentrations, EMC RNA will out-compete cell mRNA in a cell-free system. It is thought that this mechanism might be involved in the shut-off of translation of host mRNA after EMC infection. Experiments using low levels of cycloheximide suggested that, in the infected cell, initiation of translation on EMC–RNA occurs with greater frequency than on host-cell mRNA. D. Kabat (Portland) reported that in vitro translation of rabbit β-globin mRNA requires less eIF-4B than does α-globin mRNA. It is possible that factors previously reported that preferentially stimulate in vitro translation of α-globin mRNA were, in fact, eIF-4B.

P. Hamlyn (Cambridge, England) described rapid techniques for sequencing mRNAs; these involve using specific oligodeoxyribonucleotides (complementary to defined regions of the mRNA) as primer for reverse transcriptase, together with novel labeling and gel techniques to sequence the cDNA. Strikingly, most of the nontranslated regions in ovalbumin, IgG light-chain and globin mRNAs are localized in the 3′-region. At least one sequence in this region – A–A–U–A–A–A – appears common to all mRNAs and comparisons of human and rabbit globin mRNAs show that the sequences of the 3′-nontranslated regions have diverged much less than those in the coding regions. The function of these 3′-sequences (over 200 nucleotides in ovalbumin mRNA, over 150 in IgG light-chain mRNA) are obscure, but obviously important.

Although prokaryotic mRNAs often contain several functional sites for ribosome-attachment and chain-initiation, all eukaryotic mRNAs studied to data contain only one. Nonetheless, several plant and animal virus mRNAs contain masked initiation sites that are not utilized on the full-size RNA but that are utilized by discrete, smaller mRNAs. A. Smith (London) summarized the evidence for this in TMV, BMV and Sindbis viruses. As new examples, he showed that a 19 S mRNA isolated from polyoma-infected cells direct efficient cell-free synthesis of a minor virus protein, VP-2. A smaller 16 S mRNA, which contains nucleotide sequences from the 3′-end of the 19 S RNA, directs synthesis of VP-1, but not of VP-2. Thus, the VP-1 initiation site is masked in the 19 S RNA. Surprisingly, high-molecular-weight nuclear RNA from infected cells also directs synthesis of VP-2, but not of VP-1. Smith suggested that a similar mechanism might exist for translation of the 35 S genome of RSV. Viron 35 S RNA directs cell-free synthesis only of virus *gag*, or internal polypeptides (as had been shown previously by others) while a smaller RNA isolated from infected cells directs cell-free synthesis of a precursor of the virus glycoproteins.

G. Blobel (New York) reviewed the evidence for his well-known 'signal' hypothesis – that the binding to membranes of polysomes containing mRNAs for secretory proteins is due to a certain amino acid sequence on the NH_2-terminus of the nascent chain (also see section 6). This hydrophobic sequence binds to the endoplasmic reticulum (ER) membrane, inducing the formation of a channel for transport of the nascent chain across the membrane, and also causing binding to the membrane of the ribosome–mRNA complex. Lodish showed that a similar situation is

found for certain mRNAs encoding virus glycoproteins; here while most of the protein appears to be transported into the ER lumen, about 30 amino acids, presumably from the COOH-terminus, remain exposed to the cytoplasmic face. He showed that the NH_2-terminus of Sindbis virus glycoproteins determines the specificity of interaction of the ribosomes with the ER-membrane. Here a single mRNA (26 S) directs synthesis of both a soluble capsid protein and two envelope glycoproteins, and the same ribosomes that initiate synthesis of the capsid protein continue on (after proteolytic removal of the C-protein) to synthesize the two Sindbis glycoproteins. Most of the 26 S mRNA is tightly bound to ER-vesicles, and these vesicles direct synthesis of both the C-protein (which remains on the outside of the ER vesicle) and the two glycoproteins that are transported across the ER-membrane.

Turning to the initiation of protein synthesis by prokaryotic mRNAs, J. Steitz (New Haven) showed that all 25 of the ribosome binding sites of viral and bacterial mRNAs so far sequenced contained a region of complementarity (between 3 and 9 bases in length) with a region at the 3′-end of 16 S rRNA (also see sections 2 and 7). In at least one case (the A protein gene of R17) such a hydrogen-bonded complex between these regions of mRNA and rRNA can be isolated from the initiation complex. Steitz presented new physicochemical evidence that the 3′-end of 16 S rRNA has considerable double-stranded regions and that protein S1 can denature part, but not all, of this structure at 37°C. It is proposed that S1 acts by stabilizing single-stranded regions of rRNA so that they can interact with (pair with) ribosome binding sequences in mRNA. Supporting this concept, she showed that both S1 and initiation factors have a greater stimulatory effect on ribosome binding of those initiation sequences on R17 mRNA, which can form fewer potential H-bonds with the 3′-end of 16 S rRNA. mRNA · rRNA Pairing cannot be the only relevant factor; Steitz presented data that indicate that the 3′-end of *E. coli* and *Bacillus stearothermophilus* rRNAs are virtually the same. Thus, the restricted ability of *Bacillus* 30 S ribosome subunits to bind to only some of the initiation sites on R17 RNA cannot be due only to differences in interactions of mRNA to the 3′-end of rRNA; probably ribosomal proteins play crucial roles as well.

6. Structure and function of eukaryotic ribosomes

A great deal is known of the structure and function of *E. coli* ribosomes (see section 7), far less of eukaryotic particles. However, it was clear from the research reported at the eukaryotic ribosome session that the situation is rapidly improving: the gap is now less than a scientific generation. For eukaryotic ribosomes, the future lies ahead.

In his introductory remarks, I. G. Wool (Chicago) pointed out that there are significant structural differences between eukaryotic and prokaryotic ribosomes, which raise potentially important questions. Eukaryotic ribosomes are larger; they contain a greater number of proteins (70 rather than 54), and the individual protein and RNA molecules are, on the average, larger. An explanation of this is lacking. All the evidence supports the contention that eukaryotic ribosomes perform precisely the same function (namely, to assemble polypeptide chains) and they appear to do so by very similar biochemical reactions. It seems important to ask: what was the evolutionary pressure for the accumulation of extra proteins and for an increase in their mass? The very question implies that there may be functions of eukaryotic ribosomes still to be discovered.

Wool then went on to describe research in his laboratory by E. Collatz and K. Tsurgugi that has as its goal the isolation and characterization of the approximately 70 proteins in rat liver ribosomes. Some 52 of the proteins (17 of the 30 in the small subparticle and 35 of the 40 in the large subparticle) have now been purified, and their molecular weights and amino acid compositions determined. The aim has been to obtain the pure proteins in sufficient amounts so as to allow the group to carry out sequence analyses, to produce antibodies, to determine the binding of the proteins to rRNA, etc. Indeed, they are now accumulating large amounts of pure proteins, in some cases as much as 20–40 mg. N-Terminal sequence analyses have been carried out on a number of the proteins by H. G. Wittmann and B. Wittmann-Liebold with material provided by the Chicago group. One purpose is to correlate the eukaryotic sequences with the large number of prokaryotic ribosomal protein sequences accumulated by Wittmann-Liebold. Wool illustrated the potential value of an immunochemical analysis by describing

experiments carried out with Y. Chooi and G. Stöffler. Transcription units of ribosomal DNA from *Drosophila melanogaster* oocytes have been prepared and examined with the electron microscope to localize ferritin-labeled Fabs against ribosomal proteins on nascent rRNA. It is hoped in this way to work out the sequence and mechanism of ribosome assembly.

B. E. H. Maden (Glasgow) described his continuing analysis of the structure of eukaryotic rRNA. The work has concentrated on the localization of methylated nucleotides (of which there are a very large number) and of pseudouridine. This has led to new ideas about their function in the processing of precursor rRNA and in the structure of the ribosome. It is hoped that sequence studies comparable to that being generated by Ebel's group (see section 7) will soon be available for eukaryotic rRNA. Perhaps, the extraordinary new methods used for sequencing DNA (see section 5) that have been used for a determination of the covalent structure of mRNA can be adapted to an analysis of rRNA.

J. Warner (New York) described studies on the regulation of the synthesis of ribosomes in yeast and HeLa cells. He has established, in double-labeling experiments, that yeast ribosomal proteins are not encoded by a single mRNA with the product later cleaved as in polio-virus. It seems, from experiments with HeLa cells (grown in small amounts of actinomycin D to inhibit the synthesis of rRNA), that control is relaxed; i.e., that the synthesis of ribosomal proteins continues even when the synthesis of rRNA ceases. How the two are ordinarily coordinated is still not known. Eukaryotic ribosomes and eukaryotic cells apparently synthesize no ppGpp.

D. Sabatini (New York) described studies on the organization of ribosomes on the ER (also see section 5). His group has established that the rough ER has ribosome receptor proteins (perhaps two in number) that endow the membrane with a fixed number of sites, albeit of different affinities. In turn, the 60 S ribosomal sub-particle has two proteins that can be cross-linked to the ER receptor proteins (free and bound ribosomes have the same proteins; it is the signal peptide on nascent secretory proteins that directs the binding of those ribosomes to the ER). He also reported the preparation of a crude protein fraction from polysomes that prevents binding to the ER.

Experiments aimed at defining the function of some individual proteins were reported by K. Ogata (Niigata). Treatment of rat liver 60 S subparticles with EDTA leads to the removal of a 7 S RNP particle that contains L3 (L5 in the nomenclature of Sherton and Wool) and 5 S RNA. The 7 S particle has GTPase activity, although its relation to elongation factor-dependent hydrolysis of GTP on ribosomes is still not certain. Ogata's group has also succeeded in cross-linking ribosomal proteins S7 and S10 (S6 and S8 in the Sherton and Wool system) to poly(U) by treatment with ultraviolet light and protein S2 to 18 S RNA. These experiments suggest that S2 is a rRNA-binding protein and that S6 and S8 make up a portion of the domain for mRNA binding. The experiments take on even greater significance when coupled with the observation by Gressner and Wool that S6 is the only protein phosphorylated in eukaryotic ribosomes and that the phosphorylation of S6 is altered by hormonal and growth conditions. Perhaps the phosphorylation of S6 conditions the binding of a specific subset of mRNAs.

D. Vasquez (Madrid) described a series of experiments using a variety of techniques designed to identify eukaryotic ribosomal peptidyltransferase. It is likely that proteins involved in peptide bond formation will be found in the group that includes L3, L5, L8, L21, L26, L28, L31, L36.

Finally, the most significant practical consequence of the meeting for eukaryotic ribosomologists was an agreement to adopt a uniform nomenclature for ribosomal proteins based on the coordinates of the proteins on two-dimensional gel electrophoresis. An effort to systematize the nomenclature will be coordinated by Wool and E. McConkey (Boulder).

7. Structure and function of prokaryotic ribosomes

The last session of the symposium dealt with the structure and function of prokaryotic ribosomes. H. G. Wittmann (Berlin) briefly reviewed the various methods being used to elucidate the topography and functions of the numerous *E. coli* ribosomal components, i.e., the 54 proteins and three RNAs. All proteins were isolated and characterized by chemical, physical and immunological methods. More than 5000 of the approximately 8000 amino acids present

in the *E. coli* ribosomes have so far been included in defined sequences, and the primary structures of 30 ribosomal proteins from both subunits have been elucidated in Berlin. From the sequence results there is no indication of strong structural homologies among the *E. coli* proteins, with the exception of the protein pairs L7/L12 and S20/L26. This finding is in excellent agreement with earlier immunochemical studies by G. Stöffler and H. G. Wittmann, which led to the same conclusion.

J. P. Ebel (Strasbourg) presented the present state of the primary structure work on *E. coli* ribosomal RNAs; approximately 90% of the 16 S and 30% of the 23 S RNA have been sequenced so far. Different models of secondary and tertiary structure were discussed. The interactions between the RNAs and ribosomal proteins were studied, using different techniques. A map for the binding sites of numerous proteins on the RNAs was established in collaboration with the groups of R. A. Garrett, in Berlin and R. A. Zimmermann, in Amherst. Additional data on the RNA–protein interactions were obtained using covalent crosslinking between the RNA and the proteins produced by ultraviolet irradiation. The crosslinked peptides contain a large amount of basic amino acids and in some cases also acidic amino acids. Interesting complementarities between the 16 S and 23 S RNAs could be detected, and their possible involvement in the association between the 30 S and 50 S subunits and the initiation process in protein synthesis was discussed in some detail.

R. Brimacombe (Berlin) reported an alternative approach to the study of RNA–protein interactions in the ribosome aimed at investigating the topography of the RNA in the intact particles rather than the RNA binding sites of the proteins. The objective here was to identify points of contact or proximity between regions of RNA and protein. Techniques to identify the oligonucleotides and peptides involved in this type of reaction were developed in a simple ultraviolet-induced crosslinking system where only one protein from each subunit is linked to the RNA. The finding that many proteins have irregular and elongated shapes suggested that such proteins have multiple contacts with widely separated regions of the RNA. Other experiments, using aldehyde-induced crosslinking, showed that this is indeed the case.

L. Dalgarno (Canberra) described studies with J. Shine on the 3′-terminal sequences of prokaryotic and eukaryotic ribosomal RNAs (also see sections 2 and 5). The potential for base-pairing of the 3′-terminus of 16 S RNA with ribosome binding sites was discussed. The existence in 16 S RNAs of 3′-terminal heterogeneity involving a single adenylate residue was discussed in relation to a possible regulatory role. He pointed out that the 3′-octanucleotide of 18 S ribosomal RNA is highly conserved in eukaryotes (the sequence is 5′-C–A–U–C–A–U–U–A_{OH}) and that the last six nucleotides can potentially recognize the three termination triplets (UAA, UAG, UGA) and also AUG. The possible role of this sequence in termination and initiation was discussed, and potential analogies with the 3′-trinucleotide (U–U–A_{OH}) of some bacteria were noted.

C. G. Kurland (Uppsala) spoke about mRNA and factor interactions with the *E. coli* ribosome. Affinity-label experiments by the groups of E. Kuechler in Vienna, O. Pongs in Berlin and H. G. Gassen in Darmstadt show that the 30 S proteins S1, S4, S18 and S21 are in the immediate neighborhood of the codon during translation. The group in Uppsala (C. G. Kurland, A. P. Czernilofsky, J. van Duin and C. San Jose) in collaboration with G. Stöffler (Berlin) found that the 3′-end of 16 S RNA containing the Shine-Dalgarno sequence (for pairing with mRNA) is in the immediate neighborhood of both S1 and S21. In addition, IF-3 is bound to the 30 S subunit in the same domain. This domain contains the 30 S contribution to the A-site. Aminoacyl–tRNA and GTP-dependent binding of EF-Tu takes place at a 50 S subunit domain containing the proteins L1, L5, L7/L12, L15, L20, L30 and L33. This domain makes up the 50 S contribution to the A-site. Localization of these two A-site domains on the topographic models of Stöffler and of Lake give roughly consistent results (see also section 2). These domains are on the closely opposed surfaces of the respective subunits that make up the interface between the subunits. It is easy to see from these data how IF-3 could prevent the association of the subunits.

R. Traut (Davis) and his coworkers used crosslinking with bifunctional reagents in two ways in the study of the protein topography of the *E. coli* ribosome: (1) the identification of specific ribosomal proteins at the binding sites for the three initiation factors IF-1, IF-2 and IF-3 and (2) the mapping of

protein neighborhoods within the 30 S and 50 S ribosomal subunits. The use of the reversible cross-linking reagent, methyl 4-mercaptobutyrimidate, together with diagonal polyacrylamide/dodecylsulfate gel electrophoresis, has led to the identification of 35 protein-pairs in the 30 S ribosomal subunit and 18 pairs in the 50 S subunit. The results permit construction of global maps of the protein topography of the subunits; the protein neighborhoods included by crosslinking are roughly consistent with a variety of genetic, functional and structural studies on the ribosome.

C. R. Cantor (New York) discussed functional sites in *E. coli* ribosomes. Affinity-labeling is a direct method to identify components of functional sites. An analog of aminoacyl–tRNA, ϵBrAc–Lys–tRNA, reacts covalently with protein L27 and with the 23 S rRNA. The latter reaction is abolished when GMP–P[NH]P is substituted for GTP in EF-Tu-dependent binding. Coupling of the covalent reaction to dipeptide formation provides strong proof that the covalent reactions occur directly at the peptidyl transferase center (work of A. Johnson). Singlet–singlet energy transfer allows the distance between functional sites to be measured. FITC-Erythromycylamine, an analog of erythromycin, binds 70 ± 10 Å from the I-AEDANS-reactive site(s) on protein L7 of the 50 S subunit (work of R. Longlois, C. C. Lee, R. Vince and S. Pestka). Yeast $tRNA^{Phe(Pf)}$, in which proflavine has been substituted for the 'Y-base', has been used for two measurements of the distance between the anticodons of tRNAs bound to adjacent sites on the 70 S ribosome (work of J. R. Fairclough, sample provided by W. Wintermeyer and H. G. Zachau). Both measurements show a distance of 20 ± 5 Å. This is quite short and suggests that both tRNAs may interact simultaneously with poly(U). The emission spectra of both copies of $tRNA^{Phe(Pf)}$ bound to 70 S · poly(U) are different from each other and different from $tRNA^{Phe(Pf)}$ bound in the absence of poly(U). This indicates that the mRNA affects the environment of both anticodons in different ways.

8. Final statement

A minute of silence paid tribute to the late Professor Luigi Gorini, who made many scientific and personal contributions to the field of protein synthesis.