Meetings

Translation: Its Mechanism and Control

The purpose of a meeting on Translation, Its Mechanism and Control, held in November 1971 under the auspices of the Fogarty International Center at the National Institutes of Health, was to examine present understanding of protein biosynthesis, to reach a consensus, and to base upon that a uniform system of nomenclature for the variety of factors involved in protein biosynthesis. In this respect, the conference was highly successful, and general agreement was obtained to employ the system of nomenclature for the protein synthetic factors which is indicated in Table 1; references (*1–13*) indicate the correspondence to some earlier terminologies. The nomenclature is more complete for prokaryotic than for eukaryotic systems, and is more certain for factors involved in polypeptide chain elongation than for those required at initiation and termination. The reasons for this can be indicated by a summary of the discussions of protein formation and translational control.

Initiation and the ribosome cycle. In bacterial systems, binding of formylmethionyl-transfer RNA (fMet-tRNA), which is the initiator tRNA, to ribosomes is directed by the initiator codon, AUG (adenosine, uridine, guanosine). This process and subsequent steps up to formation of the first peptide bond require at least three special protein factors [IF-1, IF-2, IF-3 (Table 1)]. The exact roles of these factors are not known, but a number of their activities have been identified.

The IF-1 (molecular weight, 9,000) participates with IF-2 and guanosine triphosphate (GTP) in fMet-tRNA binding to ribosomes; it can bind 30*S* ribosomes but is apparently released when the 50*S* ribosome joins to form the 70*S* initiation complex. The IF-2 has been isolated in two forms (molecular weight, 80,000 and 100,000); both can function with IF-1 and GTP. The IF-2 is bound to an initiation complex formed in presence of [α,β-methylene]-GTP, but not to complexes formed in presence of GTP. This suggests that hydrolysis of GTP results in release of IF-2 from the initiation complex.

Hydrolysis of GTP is also required for formation of the first peptide bond, the reaction that finishes the initiation process. This GTP hydrolysis is not accompanied by advance of the messenger RNA (mRNA) along the ribosome, suggesting that both fMet-tRNA and the initiating AUG codon may be already bound directly to the peptide-holding (P) site on the ribosome.

The role of IF-3 is less clear. At high concentrations of template, IF-3 is not required for AUG-directed fMet-tRNA binding or for polyphenylalanine formation directed by polyuridylic acid [poly-(U)]. However, IF-3 *is* required at limiting concentrations of these templates, and for any translation of phage mRNA. Greater template specificity is suggested for subfractions of IF-3. At least two forms have been separated by DEAE chromatography; one stimulates translation of both MS-2 (a phage) mRNA, the other preferentially stimulates late T4 (a phage) mRNA translation. The two forms also seem to show different preferences for initiation of translation of the MS-2 coat and RNA synthetase cistrons.

The initiation factors also appear to interact with one another. For example, IF-3 stabilizes the complex of IF-2, GTP, and fMet-tRNA. Even more complex are the reactions of the initiation factors with the ribosome. The IF-3—or more precisely, one of its subfractions—dissociates 70*S* ribosomes to 30*S* and 50*S* particles (or, alternatively, holds the subunits apart). It also affects the sedimentation rate of the ribosomes, perhaps because it binds to the 30*S* but not to the 70*S* particle. The IF-1 seems to enhance the dissociating activity of IF-3.

Partially because of the uncertainty of the sequence of reactions involving IF-3, the mechanism and significance of ribosomal dissociation remains incompletely understood. While 70*S* ribosomes in cells seem to exchange 30*S* and 50*S* subunits, the mechanism of exchange is still unclear. In one view, ribosomes are released as 70*S* couples and are then separated by the action of IF-3. In an alternative view, ribosomes are released as subunits and then either join in new 70*S* couples or are held apart by factor IF-3 and reenter polysomes. In the former, free 70*S* ribosomes are an obligate feature of the ribosome cycle; in the latter, they are a side path used in conditions of suboptimal protein synthesis. The results obtained (that is, association or dissociation of ribosomes) depend on the exact environmental conditions and on preparative and analytical methods. Thus, the stability of ribosome couples is affected by the binding of various tRNA derivatives or tRNA itself at different

Table 1. Uniform nomenclature for translation factors.

Factors*	Abbreviation†	Nomenclature replaced
	Prokaryotic factors	
Initiation factor 1	IF-1	F1 (*1*), A (*2*), FI (*3*)
Initiation factor 2	IF-2	F2 (*1*), C (*2*), FIII (*3*)
Initiation factor 3	IF-3	F3 (*1*), B (*2*), FII (*3*)
Elongation factor Tu	EF-Tu	Tu (*4*), FI_u (*5*), S_3 (*6*)
Elongation factor Ts	EF-Ts	Ts (*4*), FI_s (*5*), S_1 (*6*)
Elongation factor G	EF-G	G (*4*), FII (*5*), S_2 (*6*), translocase (*7*)
Release factor 1	RF-1	R (*8*), R1 (*9*)
Release factor 2	RF-2	R2 (*9*)
Release factor 3	RF-3	α (*10*), S (*11*)
	Eukaryotic factors	
Elongation factor 1	EF-1	Transferase I (*12*), TF-I (*13*)
Elongation factor 2	EF-2	Transferase II (*12*), TF-II (*13*)

* Where necessary, species may be indicated in adjacent parentheses, for example, EF-G (*Escherichia coli*). † Abbreviations should be used only where their meaning is clear, otherwise the full term shall be used.

sites on the ribosome. Unanimous agreement with respect to the pathway involved in initiation of the ribosome cycle awaits a more uniform approach to this problem.

Initiation and other possible sites of translational control. Because of convenience and ample physiologic data, translational controls have been studied most intensively in prokaryotic systems with the use of phage mRNA's. One well-established mechanism of translational repression is the repressor-like effect of phage coat protein on f2 and Qβ phage replicase synthesis. In a model that seems to have potentially wider applicability, bacteriophage infection introduces a defect in the ability of the hosts' ribosomes to initiate translation of at least certain mRNA's. Discrimination—for example, initiation of late T4 mRNA but not MS-2 RNA—is suggested by several experiments and may be connected with the existence of the several forms of IF-3; but the results from all laboratories are not in accord. In addition, an antibiotic inhibitor of initiation, kasugamycin, preferentially inhibits the translation in vivo and in vitro of the bacteriophage f2 maturation protein cistron, compared to translation of the coat protein cistron. Again, interaction with initiation factors may be involved. An additional bacterial protein apparently affects initiation specifically by complexing with IF-3; as a result, translation of MS-2 RNA but not of T4 mRNA is inhibited.

With respect to higher organisms, there have been recent successes in the cell-free translation of eukaryotic mRNA's in eukaryotic systems. These systems often cross species barriers successfully. Globin, encephalomyocarditis virus, and lens crystalline protein mRNA's are translated in systems derived from Krebs II ascites cells as well as from rabbit reticulocytes. While these in vitro results suggest an absence of translational discriminatory controls in higher organisms, control of translation in vivo might be expressed quite subtly. For example, there seems to be a different rate of initiation on mRNA's corresponding to the α and β chains of rabbit hemoglobin. A specific factor isolated from avian muscle ribosomes (and again possibly comparable to the prokaryotic initiation factor IF-3) is required for translation of avian myosin mRNA in a cell-free system derived from rabbit reticulocytes. Small molecules also seem to affect the specificity of initiation. For example, altering the Mg^{2+} concentrations in extracts can induce Krebs II tumor cell ribosomes to discriminate between the translation of globin and viral mRNA's; as another example, heme has a stimulatory effect on globin biosynthesis.

Polypeptide chain elongation. In contrast to the initiation step, relatively complete studies with pro- and eukaryotic elongation factors have defined a series of partial reactions involving the required soluble elongation factors EF-Ts, EF-Tu, and EF-G (Table 1). Elongation factor Tu is a protein with a molecular weight of approximately 42,000. This protein binds GTP and aminoacyl-tRNA in a stepwise manner. The ternary complex thus formed reacts with ribosomes, transferring the aminoacyl-tRNA to the binding or A site of the ribosomes and releasing inorganic phosphate (P_i) and the binary complex of EF-Tu·GDP. This is the binding or codon recognition reaction. EF-Tu is inactive while associated with guanosine diphosphate (GDP). The EF-Ts, a second protein, then displaces GDP to form an EF-Ts·Tu complex, and in turn, is displaced by GTP to regenerate EF-Tu·GTP. Thus, EF-Ts catalyzes the exchange of GDP and GTP on EF-Tu, regenerating the active EF-Tu·GTP complex.

The third bacterial elongation factor, EF-G, is also a protein and has a molecular weight of about 73,000. It effects elongation after the ribosomal enzyme peptidyl transferase has catalyzed the formation of a peptide bond between the nascent peptidyl-tRNA and the oncoming aminoacyl-tRNA. Both EF-G and GTP are thought to be required during translocation of the newly formed peptidyl-tRNA and mRNA from A to P sites on the ribosome. In this partial reaction, as in the binding reaction, hydrolysis of GTP occurs.

Both EF-G and GTP also function in the release of deacylated tRNA from the P site of the ribosome. Furthermore, the interaction of EF-G and GTP with the ribosome can be studied in the absence of protein synthesis. When these components are mixed, there is catalytic hydrolysis of GTP. Much of this may result from repeated binding and release of the EF-G from the ribosome. When the antibiotic fusidic acid stabilizes a complex formed between EF-G, ribosome, and GDP, there is hydrolysis of only one molecule of GTP for each ternary complex formed.

The EF-Tu–mediated guanosine triphosphatase activity can also be uncoupled from chain elongation. This occurs, for example, in the presence of the antibiotic sparsomycin. The function of GTP hydrolysis, the mechanism of coupling of the various partial reactions, and the precise mechanism of translocation remain unknown. However, several interesting clues have been obtained. First, thiostrepton and related antibiotics can inhibit both EF-G–mediated and EF-Tu–mediated hydrolysis of GTP. Second, removal of two specific acidic proteins of the 50*S* ribosome—each with a high helical content and molecular weight of approximately about 13,000—suppresses guanosine triphosphatase activities of both EF-Tu and EF-G. Both activities are restored when either of the two proteins is added to the depleted ribosome. These results are consistent with an overlap of sites for function of EF-G and EF-Tu, or a single site for the hydrolysis of GTP by these two factors during protein synthesis. The precise extent of GTP consumption during amino acid incorporation has not yet been determined.

In eukaryotic systems, EF-1 appears to have the properties of the bacterial EF-Tu in binding aminoacyl-tRNA to the ribosome. Whether EF-1 contains a subunit corresponding to EF-Ts, or whether no EF-Ts is required in eukaryotic cells, is unclear. Several chromatographic subfractions of EF-1 that may be relevant were reported. The EF-2 is directly analogous to the prokaryotic EF-G.

Chain termination. With regard to codon-specific release of polypeptides from ribosomes, prokaryotic systems seem to be able to utilize two protein factors, RF-1 or RF-2, with a third, RF-3, acting to stimulate the reaction. The RF-1 mediates release in response to the termination codons UAG and UAA, and RF-2 in response to UAA and UGA. There is some confusion with respect to RF-3, with some evidence relating it to the elongation factor, EF-Tu, and other evidence suggesting that it is distinct.

In contrast, in mammalian cells, a single protein fraction seems to embody all the requisite functions. Furthermore, the mammalian factor shows a specific requirement for GTP, a need not yet demonstrated in bacterial termination. The mechanism in the two systems may yet be similar in both; ribosomal peptidyl transferase can

carry out hydrolysis of the polypeptide chain from tRNA in the absence of any release factor activity. This enzymatic site may therefore be involved in the normal mechanism of chain termination (*14*).

THOMAS CASKEY
Baylor College of Medicine, Texas Medical Center, Houston 77025
PHILIP LEDER
National Institute of Child Health and Human Development, Bethesda, Maryland 20014
KIVIE MOLDAVE
Department of Biochemistry, University of California, School of Medicine, Irvine 92664
DAVID SCHLESSINGER
Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110

References

1. K. Iwasaki, S. Sabol, A. Wahba, S. Ochoa, *Arch. Biochem. Biophys.* **125**, 542 (1968).
2. M. Revel, G. Brawerman, J. C. Lelong, F. Gros, *Nature* **219**, 1061 (1968).
3. S. S. Dubnof and V. Maitra, *Cold Spring Harbor Symp. Quant. Biol.* **34**, 301 (1969).
4. J. Lucas-Lenard and F. Lipmann, *Proc. Nat. Acad. Sci. U.S.* **55**, 1562 (1966).
5. R. L. Shorey, J. M. Ravel, C. W. Garner, W. Shire, *J. Biol. Chem* **244**, 4555 (1969).
6. A. Skoultchi, Y. Ono, H. M. Moon, P. Lengyel, *Proc. Nat. Acad. Sci. U.S.* **60**, 675 (1968).
7. P. Leder, L. S. Skogerson, M. N. Nau, *ibid.* **62**, 454 (1969).
8. M. Capecchi, *ibid.* **58**, 1144 (1967).
9. E. Scolnick, R. Tompkins, T. Caskey, M. Nirenberg, *ibid.* **61**, 768 (1968).
10. M. R. Capecchi and H. A. Klein, *Cold Spring Harbor Symp. Quant. Biol.* **34**, 469 (1969).
11. G. Milman, J. Goldstein, E. Scolnick, T. Caskey, *Proc. Nat. Acad. Sci. U.S.* **63**, 183 (1969).
12. J. M. Fessenden and K. Moldave, *J. Biol. Chem.* **238**, 1479 (1963).
13. B. Hardesty, R. Arlinghaus, J. Shaffer, R. Schweet, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 215 (1963).
14. The participants in the conference were J. Allende, W. Anderson, J. Bodley, L. Bosch, T. Caskey, M. Capecchi, W. Cohn, P. Condliffe, T. Conway, B. Davis, R. Gesteland, J. Gordon, F. Gros, B. Hardesty, M. Harris, S. Heywood, R. Kaempfer, A. Kaji, Y. Kaziro, P. Leder, P. Lengyel, F. Lipmann, H. Lodish, J. Lucas-Lenard, U. Maitra, K. Marcker, M. Mathews, E. Maxwell, K. Moldave, D. Morse, D. Nathans, H. Noll, S. Ochoa, A. Parmeggiani, S. Pestka, A. Peterkofsky, M. Rabinovitz, J. Ravel, M. Revel, D. Schlessinger, T. Staehelin, R. Thach, A. Tissieres, D. Vazquez, H. Voorma, A. Wahba, S. Weiss, and H. Weissbach.

Ethics, Law, and Genetic Counseling

Genetic counseling has moved out of its childhood, into a period of fast-growing adolescence. Advances in genetic knowledge, increased public demand, and refinements in amniocentesis help account for the sudden spurt. But what are its goals, and what ought the means to be?

That was the general theme of an international conference on "Ethical Issues in Genetic Counseling and the Use of Genetic Knowledge," held at Airlie House, Warrenton, Virginia, 12 to 14 October 1971, where 85 participants from six countries met. The interdisciplinary conference, cosponsored by the Institute of Society, Ethics and the Life Sciences and the John E. Fogarty International Center, National Institutes of Health, sought to explore the ethical dilemmas of genetic counseling in their philosophical, scientific, legal, sociological, and political ramifications.

While one must expect arguments in a conference of this kind, the tenor was not that of unbridled disputation. Instead, it was one of serious perplexity and a recognition that the difficulty of the central questions sorely strains the limits of present knowledge, scientific technique, and cultural wisdom. This mood was exemplified in the opening address, given by T. Sonneborn (University of Indiana). He noted that, while the conference brought together scientists and representatives of the humanities and social sciences, no simple polarity can be established between the two groups. The scientists disagree among themselves on the issues no less than the philosophers, lawyers, and social scientists. "Who or what decides what is right or good? By what authority? What do we really mean when we ask about anything: 'Is it ethical?' " On questions of this kind, Sonneborn noted, there is neither certainty nor uniformity of opinion. "Humility and compassion" are imperative in the face of ethical complexity, he said.

After Sonneborn's address, the conference moved systematically through a number of problems, beginning with a series of papers on the present scope of genetic counseling and the variety of dilemmas presented to the counselor. F. C. Fraser (McGill University) presented data to show that, in at least one large and perhaps not untypical counseling center, most of the problems presented (usually on referral) deal with the question "Will it happen again?"—that is, will there be a recurrence of some disease or defect already known to exist in a family? The range of conditions presented is very wide, totaling 349 at the center analyzed by Fraser. Of that number, 61 were either sex-linked or diagnosable in utero, making the possibility of amniocentesis a relevant consideration. Accurate diagnosis, Fraser noted, is the first step in the counseling process, followed by a determination of the probability of recurrence, and concluding with assistance by the counselor in helping the family to reach a decision.

If it is often difficult for patients to know what to do, the pressures on the counselor are often no less. Sometimes the counselor is stymied by an inability legally to get pertinent data from hospital records. At other times, as M. W. Shaw (University of Texas) stressed, de facto and de jure obstacles stand in the way of options available to the counselor or to the family (for example, local antiabortion statutes). "The right of privacy," in particular, poses some acute dilemmas, a theme developed by both Shaw and H. A. Lubs (University of Colorado Medial Center). The law does not require an individual to make known the fact (if he has discovered it) that he harbors a deleterious gene; nor does it require that a physician inform other family members. But should it? Lubs presented a number of case histories to show how painful the dilemmas of privacy can be even at present; and they may increase as nationwide genetic data banks are established.

Another cluster of dilemmas facing the counselor turns on different theories and styles of counseling individual patients. J. Hall (Johns Hopkins Hospital) noted that the traditional role of the counselor has been that of "neutral educator," essentially doing no more than presenting patients the odds and the facts. But this concept faces increasingly heavy weather, particularly because of the rapidly expanding range of options. Not surprisingly, these developments have led to disagreements among counselors about the kind of stance they should take toward their patients. J. R. Sorenson (Princeton University), in a sociological survey of decision-making in counseling practice, noted that, however much the value of neutrality on the part of the counselor may be espoused, counselors do in fact often make decisions for their patients, or at least heavily influence the decisions by the way they present data. J. Fletcher (Ecumenical Training Center) came to a similar conclusion in his findings on