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Ribosome Structure and Function Emergent

Unexpected aspects of ribosome structure and function are revealed by studies of the ribosomal proteins.

C. G. Kurland

The discovery of the genetic code is one of the principal triumphs of molecular biology. Nevertheless, it can be argued that the genetic code will remain just a set of rules until the reasons for that particular kind of solution to the coding problem are forthcoming. This in turn will require a deep understanding of the translation mechanism and of its evolution.

The translation of the coded information from messenger ribonucleic acid (mRNA) into the amino acid sequences of proteins involves the orderly interactions of more than 100 different macromolecules. We have barely identified the contributions that some of these macromolecules make in the translation process, and the physical events that take place in the course of protein synthesis are still quite obscure. To a lesser extent similar remarks could be made about the replication of the genetic material and the transcription of genetic information from deoxyribonucleic acid (DNA) to mRNA molecules. From this point of view, the peroration that Stent (*1*) has recently intoned for molecular biology may seem somewhat premature.

Stent's premise is that at an earlier time the fascination of molecular biology lay in the possibility that new physical laws might be discovered in the domain of molecular genetics. Since such new laws have not been discovered, and since the flow diagram for the transmission of information from DNA to the structure of protein is in hand, there is little to do, Stent thinks, except to fill in the details. Stent's conclusion is based on the assumption that we know enough about the physics of macromolecular replication to decide whether or not anything new and exciting is happening in this domain. I doubt that this assumption is correct, especially since most attempts to describe macromolecular biosynthesis involve a deep faith in the overworked hydrogen bond and a considerable amount of hand waving. Indeed, our knowledge of fundamental aspects of protein synthesis is so bare that we cannot demonstrate at present that this process obeys the first and second laws of thermodynamics (or their statistical analogs). Therefore, it would seem appropriate to postpone any decision concerning the uniqueness of the physical laws governing the domain of molecular genetics until these laws have been discovered.

The analysis of the translation mechanism has been severely limited by the absence of structural information concerning the ribosome, which is the ribonucleoprotein particle that mediates protein synthesis in all organisms of our biosphere. Here I describe current work on the ribosomal proteins, work which may eventually provide the key to the mechanism of protein synthesis. Before doing this, it will be useful to review what is known about protein synthesis.

A View of Protein Synthesis

The process of protein synthesis has been reviewed quite recently by Lipmann (*2*), who focused attention on the role of the protein factors which, together with the ribosome, are responsible for translating the coded information of the mRNA into the amino acid sequences of proteins. In this process each amino acid is brought to its position in the nascent protein by a specific adapter molecule, transfer RNA (tRNA). Each different tRNA molecule can carry one kind of amino acid, and the different aminoacyl-tRNA molecules are selected by specific trinucleotide sequences (codons) in the mRNA. A given aminoacyl-tRNA is transiently bound to the ribosome-mRNA complex until the amino acid is inserted into the nascent protein, and then that tRNA is displaced by the next aminoacyl-tRNA to be inserted into the nascent protein. The nascent protein is at all times coupled to the tRNA that has just carried an amino acid into the assembly line. The stepwise growth of the polypeptide chain is paralleled by a concomitant move-

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ment of the translation apparatus, one codon at a time, along the mRNA.

There are at least two sites on the ribosome for the binding of tRNA molecules. One of these, the A site, is the site at which the specific aminoacyl-tRNA that matches the codon of the mRNA is bound, with the assistance of at least one of the required supernatant proteins. The nascent protein is bound at a second site, the D site, and it is linked to the tRNA molecule that carried the current terminal amino acid of the polypeptide chain to the ribosome-mRNA complex. A peptide bond is formed between the α-amino group of the incoming aminoacyl-tRNA, bound at the A site, and the carboxyl group which links the nascent protein to the tRNA bound at the D site. This transacylation is catalyzed by an active site of the ribosome. Then, the elongated polypeptide chain which is temporarily bound to the tRNA at the A site is translocated to the D site with the assistance of at least one specific supernatant protein. At the same time, or shortly afterward, the mRNA is advanced one codon length relative to the ribosome and the system is readied for the insertion of the next amino acid by the same recursive process. Both the initiation of this process and its termination are mediated by supernatant proteins that are distinguishable from those responsible for polypeptide chain elongation. Similarly, the initiation and termination modes are directed by specific codons in the mRNA.

In effect, the ribosome and its attendant supernatant proteins function as a computer that translates the nucleotide sequences of the mRNA into the amino acid sequences of protein. Although the rules that relate the mRNA input to the protein output can be stated in the form of the genetic code, the internal workings of the translation apparatus are still obscure. One reason for this disparity is the difference between the degree of complexity inherent in the coding problem and the degree of complexity of the translation problem. The elaboration of the genetic code required the solution of a static, one-dimensional problem. In contrast, the description of the translation mechanism must be expressed in three dimensions, along with the appropriate kinetic considerations. Consequently, the technical problems encountered in studying the translation mechanism are quite a bit more complex.

The Ribosome

Until recently there was only a smattering of information concerning the structure and function of the ribosome (*3*). The *Escherichia coli* ribosome, which has been studied more thoroughly than the ribosome from any other organism, is made up by two subunits of unequal size that function together to mediate protein synthesis. Each of these subunits is structurally and functionally unique. The 30*S* subunit has a molecular weight of about 800,000 daltons (*4*). It contains a 16*S* RNA molecule with a molecular weight of approximately 550,000 daltons (*5*) and an aggregate mass of about 250,000 daltons of protein. The 50*S* subunit is roughly twice as large as the 30*S* subunit (*4*). It contains a 23*S* RNA molecule with a molecular weight of approximately 1,100,000 daltons (*5*), a 5*S* RNA molecule with a molecular weight of 40,000 daltons (*6*), and finally an aggregate mass of about 500,000 daltons of protein.

In addition to these gross structural differences, a degree of functional specialization for each of the subunits can be demonstrated by studying the in vitro activities of the isolated subunits. The 30*S* subunit can bind mRNA in the absence of the 50*S* subunits (*7*), and the 30*S*-mRNA complex can in turn bind specific tRNA molecules (*8*). The 50*S* subunit cannot associate with mRNA in the absence of the 30*S* subunit. However, a nonspecific binding site for tRNA is found on the isolated 50*S* subunit (*9*). This site is probably the one responsible for holding the nascent protein attached to tRNA (*10*), and it may therefore be identified as part of the D site discussed above. Finally, the active site responsible for the formation of the peptide bond—the so-called peptidyl transferase—is part of the 50*S* subunit (*11*).

It is evident that these separable functions are distributed between the 30*S* and 50*S* subunits in a nonrandom fashion. The 30*S* subunit seems to be concerned with those operations that relate to mRNA function and the selection of specific aminoacyl-tRNA molecules, while the 50*S* subunit appears to be concerned with the formation of the peptide bond and the processing of the nascent protein. In effect, the two subunits function in a manner which is conjugate with the dual functions of the tRNA molecules: the recognition of codons, on the one hand, and, on the other, the insertion of amino acids into the protein. However, the activities of the two ribosomal subunits must be coordinated. Indeed, there is evidence of a functional interdependence of the two subunits. For example, the binding of aminoacyl-tRNA to the A site requires the presence of a supernatant factor, and this factor-dependent binding of aminoacyl-tRNA to the A site requires the association of both subunits with the mRNA (*12*).

Although it is possible to present such a schematic description of the functions of supernatant factors and ribosomal subunits in protein synthesis, the physical processes that these molecular components initiate are completely mysterious. We might just as well be discussing an IBM typewriter, since in no way do the stated functions of these components relate to their properties as macromolecules. Fortunately, recent work on the ribosomal proteins of *E. coli*, as well as the in vitro assembly of ribosomes from purified molecules, offers an opportunity to study the "black box" responsible for protein synthesis.

Ribosomal Proteins

The structural complexity of the ribosome first became apparent in the work of Waller (*13*), who demonstrated that there are many different proteins in the bacterial ribosome. Waller fractionated the ribosomal proteins by electrophoresis in starch gel as well as by chromatography on carboxymethyl cellulose, and he was able to demonstrate that there are at least 24 separable components that are not artifacts caused by the aggregation of a smaller number of proteins. In addition, Waller showed that the proteins of the 30*S* and 50*S* subunits were characteristic of each subunit. The structural complexity of the ribosome that was established by Waller carried the unwelcome implication that the function of the ribosome would turn out to be complex (*2*). As we shall see, the ultimate complexity of the ribosome was grossly underestimated even after Wallers' conclusions had been accepted.

It gradually became apparent that there would be little progress in analysis of the ribosomes until all of the ribosomal proteins had been isolated and characterized. Then it might be possible to piece together the structure of the ribosomes. Extensive purifications of ribosomal proteins were reported by Wittmann and his co-work-

ers (*14*). Twenty-two ribosomal proteins from *E. coli* were purified by electrophoresis in sheets of polyacrylamide. The amino acid compositions, tryptic peptides, and molecular weights of 16 of these proteins were shown to be unique. Simultaneously, Traut *et al.* (*15*) reported the purification of some of the 30*S* ribosomal proteins. Shortly after this, some of the proteins of the 30*S* ribosomal subunit of *E. coli* were isolated and partially characterized by Fogel and Sypherd (*16*) as well as by Moore *et al.* (*17*). These studies went far toward establishing the minimum number of ribosomal proteins in *E. coli*, but many ribosomal proteins remained to be characterized.

Twenty proteins were purified from the 30*S* ribosomal subunit by my group at the University of Wisconsin, and these were shown to have unique amino acid compositions, tryptic peptides, and molecular weights (*18*). The purity of the isolated proteins was established by comparing the chemical molecular weights, calculated from the amino acid composition and the number of tryptic peptides, with physical molecular weights, obtained from sedimentation equilibrium measurements. Since such molecular weight estimates agree, it is possible to conclude that the protein samples are reasonably pure and, therefore, that there are few if any proteins that had not been resolved. We were also confident that not many of the proteins were contaminants, because our earlier experience had led to a purification scheme which yields ribosomes with less than 1 percent contamination by reversibly bound supernatant proteins (*19*).

Preliminary analysis of the 50*S* ribosomal proteins has not yet been completed. The disk electrophoresis patterns obtained with the 50*S* protein suggest that there are roughly 30 proteins in this subunit (*20, 21*). We have purified and characterized 20 of these; another 10 have been purified, but the chemical and physical analysis of these is not complete.

At present the best estimate is that there are roughly 50 different proteins in the ribosomes of *E. coli*. Several of these are acidic or neutral proteins, but most are basic proteins. Their molecular weights range from 10,000 to 60,000 daltons; the average is in the neighborhood of 20,000. Since the amino acid compositions of some of these are not very different from one another, the possibility existed that some of the proteins are structural homologs (*14*). Indeed, if the bacterial ribosome were a relatively primitive organelle, the evolution of this multiplicity of proteins might be reflected in a repetition of homologous regions in different proteins that might have originated by gene duplication (*14*). However, a comparison of the tryptic peptides of the different proteins reveals very little homology except in a few limited cases that are probably fortuitous (*19, 21*). Furthermore, recent immunochemical studies by Stöffler and Wittmann (*22*) have failed to demonstrate significant cross reactions when the antibodies prepared against individual ribosomal proteins are reacted with heterologous proteins. Therefore, all of the proteins seem to be quite unique. The overwhelming impression is that the ribosome is far from being a primitive organelle. Instead, it seems to be a highly evolved and complicated entity, containing a much larger number of components than can be accounted for by present views of protein synthesis.

In vitro Assembly of Ribosomes

The destructive approach to the elaboration of ribosome structure has limited value by itself. However, when isolated components can be reconstituted to form functional ribosomes, the experimental opportunities become limitless. Happily, we now have procedures for reconstituting ribosomes from purified proteins and RNA, but these did not appear on the scene full-blown. Instead, they were developed over a period of years, largely as a consequence of the perseverance of Nomura and his co-workers.

This methodology had its beginnings in the experiments of Brenner, Jacob, and Meselson (*23*), who discovered anomalous ribonucleoprotein components in the CsCl gradients that they used to demonstrate the conservation of bacterial ribosomes during phage infection. These ribonucleoprotein particles (core particles) were later shown to be degraded ribosomes that lack a class of proteins (the split proteins) which are stripped from the ribosomes during centrifugation in CsCl (*24*). When the appropriate split proteins and core particles are incubated together, fully functional 30*S* and 50*S* subunits are recovered (*25*). The subunits that are assembled in vitro are apparently indistinguishable from the original ribosomal subunits (*26*).

Although this procedure represented only a partial reconstitution of ribosomes, it had important consequences. First, such results indicated that at least part of the ribosomal assembly process was spontaneous. This in turn encouraged the hope that the entire process of ribosome assembly would turn out to be spontaneous and could, therefore, be performed easily in vitro. Finally, it meant that, with some luck, the purification procedures that were being developed for ribosomal proteins could be exploited immediately for functional studies of the individual proteins.

The first such experiments had shown that the core particles and split protein fractions were inactive in protein synthesis by themselves. This might mean that the structure of the active ribosome requires the presence of all the proteins, and that each individual protein contributes an essential, well-defined function. However, subsequent experiments by Traub and Nomura (*27*) indicated that there is, in addition to a class of essential proteins, a group of proteins that stimulate protein synthesis only when they are present in the ribosome. The dispensable proteins were identified by chromatographically fractionating both the 30*S* split proteins and the 50*S* split proteins into acidic (A) and basic (B) subfractions. It was observed that the 30*S* core plus the 30*S* split B proteins are active in protein synthesis, but that the 30*S* core plus the 30*S* split A proteins are inactive. However, when the 30*S* split A proteins are added to the particles formed from cores plus split B proteins, greater activity is recovered than is recovered in the absence of the split A proteins. A parallel subdivision of the 50*S* split proteins leads to similar results, but here the acidic fraction is the essential one, while the basic fraction is dispensable but stimulatory.

The dispensable proteins are something of a puzzle. These proteins could be enhancing the rate of function of active ribosomes or they could be increasing the number of active ribosomes. Initially it was thought that the two alternatives could be distinguished by measuring the number of active ribosomes in the presence and absence of the dispensable proteins (*28*). However, after it was discovered that the number of active ribosomes is enhanced by the dispensable proteins, two contradictory interpretations were possible. First, such results could mean that there is more than one kind of

ribosome and that a subclass of ribosomes requires the so-called dispensable proteins in order to function. According to this interpretation the "essential" proteins are required by all ribosomes, while the "dispensable" proteins are required by some of the ribosomes. Alternatively, the ribosomes lacking the "dispensable" proteins could be structurally unstable and consequently might be distributed among several physically distinct states, only some of which are active. According to this interpretation the role of the "dispensable" proteins would be to trap the ribosomes in an active state. So far, it is not possible to distinguish these alternative hypotheses.

The functional analysis of individual ribosomal proteins was first performed by Traub *et al.* (*29*) with the proteins of the 30*S* split B fraction. These were fractionated by column chromatography. Then 30*S* subunits were reconstituted in the absence of individual proteins so that the separate contribution of each component might be assessed. Here, too, both a class of dispensable proteins and a class of essential proteins were discovered. Unfortunately, the activities of the ribosomes lacking a single protein do not permit unambiguous assignment of specific functions to each individual protein. When the ribosome lacks a single dispensable or essential protein, all of its activities are depressed in a coordinate fashion. For example, if one of the essential proteins is missing, the ribosome cannot bind formyl-methionyl-tNRA to initiate protein synthesis with F2-RNA as messenger; it cannot incorporate valine into an F2-RNA-directed product; it cannot bind phenylalanyl-tRNA with poly U serving as mRNA, and it cannot synthesize polyphenylalanine.

It is difficult to believe that each and every protein of the 30*S* subunit is simultaneously and directly involved in the binding of tRNA, mRNA, and the supernatant factors required for initiation as well as chain propagation. Therefore, the coordinate depression of all functions of the ribosome in the absence of any one protein must reflect a more subtle disruption of cooperative interactions between the proteins that are necessary to maintain the ribosome in a unique optimum configuration. Since the ribosome is a relatively compact object, this aspect of ribosome structure is not surprising. However, the identification of separate roles for each protein becomes more difficult. Indeed, it may be that there is no meaningful distinction between a structural protein of the ribosome and a protein directly involved in some specific function.

A Functional Hierarchy of Ribosomal Proteins

The completely spontaneous nature of the assembly of ribosomes was demonstrated through total reconstitution of 30*S* subunits from purified ribosomal RNA and the separate proteins (*30*). As a consequence, each of the 30*S* proteins was now amenable to functional analysis, and it became possible to identify ribosomal proteins that were altered by mutations leading to streptomycin resistance (*31*), spectinomycin resistance (*32*), and streptomycin dependence (*33*). Although the same coordinate depression of activity is observed when individual core proteins are omitted from the reconstitution mixtures, analysis of such defective ribosomes has led to a preliminary functional classification of the 30*S* ribosomal proteins as well as to a definitive enumeration of the minimum number of different 30*S* proteins (*34*).

Nomura *et al.* (*34*) purified 19 of the 30*S* proteins and used these to reconstruct ribosomes that lacked each of these in turn. In addition to the usual assays for functional activity, an attempt was made to determine the contribution of each of these proteins to the self-assembly process. If the particle assembled in the absence of a given protein migrated on sucrose gradients in a normal manner (28*S* to 30*S*), it was concluded that the omitted protein is not necessary for the self-assembly process. However, when structurally disrupted particles were observed on sucrose gradients, the omitted protein was identified as one essential to the self-assembly of the 30*S* subunit.

In Table 1 I have arranged the 30*S* proteins according to what seems to be a natural, functional hierarchy. The proteins in the first group are those that are required for the physical assembly of the 30*S* subunit; these proteins are necessary for the recovery of functional particles as well. Proteins in the second group are those that are not required for the assembly of the 30*S* subunit but are required for function; when they are omitted, the resulting particles are virtually inactive. Proteins in the third group are not required for assembly or function but stimulate the activity of reconstituted ribosomes; these are the dispensable proteins. Finally, there are two proteins which make no demonstrable contribution to ribosome assembly, the initiation of polypeptide synthesis, or polypeptide chain elongation. Until experiments are made to determine the influence of these proteins on polypeptide chain termination, it will not be possible to decide whether they are ribosomal proteins or contaminants.

A number of important consequences follow from these studies. First, 17 of the 19 proteins that were studied are clearly involved in the assembly and function of the 30*S* particle; therefore, we can be certain that most if not all of the putative 30*S* proteins that were purified and characterized at an earlier time are, indeed, ribosomal proteins. Second, the functional hierarchy that has been established for these proteins serves as a potentially valuable guide to the structure of the ribosome. Finally, the reconstitution method provides, for the first time, an opportunity to study the mechanism of assembly, the genetic organization, and the principles for construction of a complex cellular organelle.

The Structure of the Ribosome

There are two general aspects of ribosome structure which radically influence the kinds of experiments that can be made to study the three-dimensional arrangement of the proteins. First, it is necessary at the outset to know whether or not all of the ribosomes from a single source such as *E. coli* have the same structure. Second, it is helpful to know whether or not there are any repeat-structures in the ribosome. It is unfortunate that the answers to both of these questions are the worst possible ones, from the point of view of simplicity. Thus, recent data indicate that the purified *E. coli* ribosome is heterogeneous and, further, that there is at most one copy of each protein per ribosome.

We first suspected that the *E. coli* ribosome might be heterogeneous after we had isolated 20 different proteins from the 30*S* subunit and realized that this subunit was not sufficiently large to accommodate one copy of each of the proteins (*18*). More specifically, the sum of molecular weights for these proteins is 420,000 daltons, but there is an average of only about 250,000 daltons of protein per 30*S* subunit. If

Table 1. A hierarchy of 30*S* ribosomal proteins. The 30*S* proteins have been arranged in a functional hierarchy on the basis of the data of Nomura *et al.* (*34*), as described in the text. The stoichiometric classification is based on the data of Table 2.

Protein	Nomura's code	Functional class	Stoichiometric class
2a	P4b	Required for assembly	U
6	P9	Required for assembly	U
7	P9	Required for assembly	U
8	P5	Required for assembly	U
10	P4	Required for assembly	U
12	P8	Required for assembly	U
4	P6	Required for function	?
5+9	P3	Required for function	?
11	P7	Required for function	F
12b	P11	Required for function	F
13	P13	Required for function	F
15	P10	Required for function	?
3	P4	Dispensable	?
4a	P2	Dispensable	F
12a	P12	Dispensable	?
14	P10a	Dispensable	?
15a	P15	Dispensable	F
16	P14	Dispensable	F
1	P1	Not known	F
2	P3a	Not known	U

these data are correct, we would expect to find a class of 30*S* proteins that are present in amounts less than one copy per ribosome.

The appropriate stoichiometric data are summarized in Table 2, where each of the 30*S* proteins is classified in one of three categories (*35, 36*). Those proteins that are present in amounts corresponding to between 0.8 copy and 1.2 copies per ribosome are classified as unit proteins. Those that are present in amounts less than 0.65 copy per ribosome are classified as fractional proteins. Finally, the proteins present in amounts corresponding to between 0.65 and 0.80 copy per ribosome are considered unclassifiable because of the uncertainty of our measurements. Although the data have been reproducible to within ±10 percent and the estimates have been obtained by two independent procedures, the absolute uncertainty is probably closer to ±20 percent. Hence, a protein that we estimate to be present on three-fourths of the ribosomes could be a unit protein or a fractional protein.

One unambiguous conclusion that can be drawn from the data of Table 2 is that the purified ribosomes are heterogeneous. This follows from the identification of a substantial group of proteins present in amounts corresponding to much less than one copy per ribosome. Since the conclusion that the ribosomes are heterogeneous is such a startling one, a great deal of caution is required if one is to be certain that this is not an experimental artifact.

It is quite unlikely that errors in our estimates of molecular weight are responsible for artifacts because the molecular weights obtained by equilibrium sedimentation have been verified by chemical molecular weight estimates (*18*). Furthermore, recent molecular weight estimates obtained by a third independent procedure have verified our results (*37*). It is also possible that we have created the heterogeneity by selectively stripping a fraction of some of the proteins from the ribosomes during purification. However, experiments designed to reveal such an artifact have suggested that the proteins that are removed from the ribosome during purification are different from those that remain (*18, 19*). Furthermore, stoichiometric measurements of the proteins from washed and unwashed 30*S* subunits (*35, 36*) indicate that the relative amounts of the proteins are conserved during purification except in the case of two proteins (30*S*-1 and 30*S*-4a). We do not know whether the washing procedure selectively removes a fraction of these two proteins or whether contaminants that migrate with these proteins are lost. As far as we can tell now, the heterogeneity of the purified 30*S* subunits is not an artifact. Although it had been reported previously (*17, 38*) that the proteins of the 30*S* subunit are present in amounts corresponding to one copy per ribosome, the molecular weight data that were used for the first such report (*17*) have since been amended and now are in good agreement with our data (*37*).

One encouraging aspect of the stoichiometric data is the correlation that is found between (i) the identities of the unit proteins and (ii) the identities of those proteins that are required for assembly of the 30*S* subunit (Table 1). This correlation strongly suggests that each of the unit proteins required for assembly is indeed present on every ribosome. In addition, identification of the unit proteins with the proteins required for self-assembly makes it easier to accept the existence of a class of fractional proteins, because none of the fractional proteins would be required for assembly of the 30*S* subunit. Instead, the fractional proteins may differentiate subclasses of ribosomes for special functional roles.

There are at least two extreme interpretations of the heterogeneity of the 30*S* subunits (*35*). One of these, the static model, suggests that the functional specializations of subclasses of ribosomes are permanently fixed by the stable association of specific fractional proteins with a common core of unit proteins. For example, some ribosomes might be competent to initiate protein synthesis at the 5′ end of the mRNA molecule, while others would be able to initiate synthesis only at internal sites of a polycistronic mRNA molecule.

The steady-state model—an alternative interpretation—suggests that the fractional proteins exchange from one ribosome to another in an orderly cycle. Here, each phase of protein synthesis—initiation, propagation, termination, and so on—is mediated by the same 30*S* subunit but each functional mode is associated with a different set of exchangeable fractional proteins. Indeed, we have observed a functional activation of ribosomes in vitro that is caused by an exchange of exogenous and ribosome-bound fractional proteins (*35*). These observations weakly support the steady-state model. However, an unambiguous interpretation of the structural heterogeneity of the 30*S* subunit will require separation and

Table 2. Stoichiometric classification of 30*S* proteins. The molecular weights were measured by the sedimentation equilibrium procedure (*18*), and the number of copies per 30*S* subunit for each protein was calculated from mass fraction data obtained by isotope dilution (*35, 36*). The mass fraction estimate for 30*S*-2 is an indirect one; therefore, it is quite tentative. Proteins 30*S*-5 and 30*S*-9 are grouped as a single protein because their tryptic peptides are almost identical (*18*). The designations U, F, and ? correspond to the classes of proteins which are units, fractionals, or unclassifiable, as described in the text.

Protein	Molecular weight (daltons)	Copies per 30*S* subunit	Class
1	65,000	0.14	F
2	18,000	.81	U
2a	17,600	.90	U
3	24,000	.80	?
4	16,000	.79	?
4a	30,000	.55	F
5+9	32,000	.71	?
6	13,500	.89	U
7	10,700	.83	U
8	21,500	.89	U
10	26,700	.89	U
11	18,300	.40	F
12	21,000	1.06	U
12a	14,600	0.73	?
12b	15,600	.52	F
13	15,000	.60	F
15a	13,000	.34	F
16	14,000	.61	F

analysis of the different kinds of ribosomal subunits.

All of the data suggest that no protein is represented more than once in a single 30*S* subunit. As a consequence, no protein-containing region of the 30*S* subunit would be the same as any other one. This dissymetric arrangement of the proteins suggests that the functions of the ribosome must also be dissymetric. Therefore we can exclude models for protein synthesis that involve multiple, equivalent sites on the ribosome or the movement (rotations) of the ribosomal subunits to multiple, equivalent configurations.

Recognition of Aminoacyl-tRNA

It is generally thought that the stable binding of the aminoacyl-tRNA to the ribosome-mRNA complex is a consequence of the hydrogen-bonded interaction of the messenger codon with an anticodon triplet of the tRNA. The simplicity of this notion, the fact that the "wobble" hypothesis can account for known codon assignments (*39*), and the experimental identification of the predicted anticodon sequences (*40*) have all reinforced this attractive idea. Nevertheless, there are a number of observations which suggest that the recognition and binding of tRNA by the ribosome-mRNA complex may be more complicated.

First, the marked thermal stability of the tRNA-ribosome-mRNA complex, as well as the demonstration that the kinetics of formation of this complex is determined by the source of the ribosome, suggests that the ribosome contributes to the association of tRNA and mRNA (*41*). Indeed, it has been shown that regions of the tRNA other than the anticodon contribute to the stable association of tRNA with the ribosome-mRNA complex (*42*). Therefore, it may be that the ribosome binds the tRNA at sites separate from the nucleotides of the anticodon. In addition, the ribosome seems to influence the specificity of tRNA selection. This follows from the demonstration that the quality and quantity of translation errors can be determined by the structure of the ribosome (*43*).

The most dramatic demonstration of a ribosomal contribution to the fidelity of translation is seen in studies of the activities of ribosomes lacking individual proteins. One protein (30*S*-15) must be present in order to facilitate the induction of error by a variety of pharmacological agents (*31*). Another protein (30*S*-11) must be present in the ribosome to prevent a high frequency of spontaneous errors (*34*). If a simple hydrogen-bonded association of codon and anticodon is the basis of tRNA recognition, how can these two proteins influence the error frequency of this process?

The solution to this problem has several boundary conditions. First, the weak interaction of codon with anticodon must be amplified by a strong ribosomal contribution that does not obliterate the specificity of the weaker codon-anticodon interaction. Second, the ribosomal contribution must be nonspecific, at least to the extent that all tRNA molecules can be bound to the ribosome in equivalent configurations. Finally, the solution should be stated in terms of a single binding site, the A site, rather than in terms of multiplicity of tRNA-specific binding sites.

One way out of this dilemma is to separate the process of tRNA selection into two stages (*44*). Here, the codon and part of the ribosome would provide a stereospecific barrier through which the anticodon loop of the tRNA must pass before the ribosome can bind the tRNA at a site separate from the anticodon. If the anticodon has the correct structure (defined by the "wobble" rules), the tRNA easily passes through the filter formed by the codon and ribosome. If the anticodon is not the correct one, the movement of the tRNA to a stable binding site is retarded. Errors could occasionally occur when incorrect tRNA molecules penetrate the kinetic barrier provided by codon and ribosome. If the structure of the ribosome is altered so that the stereochemical constraints limiting passage through the postulated filter are raised or lowered, there will be a corresponding change in the error frequency. In this way, pharmacological agents as well as alterations in ribosome structure could influence the error frequency. One advantage of this model is the fact that it can explain the contribution of the ribosome to tRNA selection without requiring that the ribosome be privy to the code. Furthermore, kinetic analysis of tRNA binding by the ribosome-mRNA complex could distinguish this model from models in which the codon-anticodon interaction alone provides both the stability and the specificity for the binding of tRNA (*44*).

Conclusions

Although the corresponding data for 50*S* subunits are still being assembled, it is doubtful that these data will significantly alter the general features of ribosome structure that have emerged from analysis of the 30*S* subunits. One overwhelming characteristic of the ribosome is its apparent complexity, which is reflected both in the diversity of ribosomal components and in their dissymmetric arrangement. The absence of repeat-structures implies that the functions of the ribosome are dissymmetric; this should influence the choice of models for protein synthesis.

Since the ribosome is capable of self-assembly, the finding that the functions of the components are highly cooperative is not surprising. However, this means that identification of the specific functional contributions of individual components will be difficult. The analysis of genetically as well as chemically modified proteins should circumvent this technical difficulty. In addition, techniques for identifying the three-dimensional location of each component in the ribosome should have the highest priority for future work.

One step in this direction has recently been taken in Nomura's laboratory (*45*) as well as in my own (*46*). We have begun to study the association of the individual ribosomal proteins with ribosomal RNA. So far, several proteins which can form a stable, specific complex with ribosomal RNA have been identified. It should soon be possible to determine the relative order of these proteins on the ribosomal RNA, as well as to study the binding sites responsible for these protein-nucleic acid interactions.

The most surprising feature of the ribosome to emerge from recent work is the apparent heterogeneity of the 30*S* subunit. The fact that the functional implications of this aspect of ribosome structure cannot yet be assessed is frustrating. It remains to be determined whether the structural heterogeneity of the 30*S* subunit is a trivial complexity or a necessary consequence of ribosome function.

The most challenging problems remain to be tackled. These are elucidation of the detailed mechanisms of tRNA recognition and of the orderly movements of tRNA, mRNA, and nascent protein and supernatant factors during protein synthesis. However, an understanding of these mechanisms will

certainly require much more information about the three-dimensional structure of the ribosome than is now available.

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The "Perfect Contraceptive" Population

The extent and implications of unwanted fertility in the United States are considered.

Larry Bumpass and Charles F. Westoff

Recent discussions of population policy have raised and sharpened the question of unwanted fertility in the United States (*1*). The issue is whether the elimination of unwanted fertility would have a significant effect on our rate of population growth, and the discussion has revolved in part around what might be called the demographic implications of "perfect contraception." We are not suggesting that such a technological development is in sight, or that, if it were, we would not have to be concerned about problems of distribution and use. The "perfect contraceptive" population is simply a model in which couples can avoid having more children than they want and do not have children before they want them. In the broader sense we are visualizing the "complete fertility controlling population" rather than the "perfectly contraceptive population." The achievement of such a state of affairs might well require social policies for the development of more effective contraceptive techniques and more efficient distribution systems as well as the legalization of abortion on request. However, this article is focused on *implications* of the elimination of unwanted fertility rather than on specific policies necessary to realize this goal.

We make no artificial assumptions about fecundity; we assume that the current incidence of subfecundity (less than normal capacity to reproduce) in the United States will continue. Also, we are *not* assuming that every couple will practice contraception or that all couples will begin using contraception at the same stage of their marriage. The system is completely voluntary. The only condition we are imposing is that couples can control their fertility completely in the sense that they can, within the limits of physiological capacity and variability, have the number of children they want, when they want them. If a husband and wife prefer to have chil-

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