# Ribosomes (II): A Complicated Structure Begins to Emerge

The sophistication of biochemical research is increasing rapidly. Fifteen years ago, determining the amino acid sequence of proteins was a tremendous task and the elucidation of their three-dimensional structure had never been accomplished. Today, the former is routine and the latter is becoming so. Then, the sequencing of RNA was an even more difficult task; today, it is much easier. As these techniques have become more usable, biochemists have been able to investigate ever more complex biological structures.

Perhaps the best example of the use of these and other techniques is found in current work on the structure of ribosomes, discrete cellular particles that are the site of protein synthesis. Investigators in many laboratories are studying a variety of structural characteristics of ribosomes and making great progress. It seems likely that the ribosome will, within a few years, be the first such structure for which there is a detailed knowledge of both form and function. This knowledge, in turn, will lead to a better understanding of cellular function.

Ribosomes account for about one-quarter of the dry weight of both prokaryotic cells (those without distinct nuclei) and eukaryotic cells (those with distinct nuclei). Although the two types are structurally and functionally similar, ribosomes from prokaryotic cells are smaller and less complicated. Most work on structure has thus been performed on ribosomes from prokaryotes, particularly those from the bacterial species *Escherichia coli* and *Bacillus stearothermophilus*.

Prokaryotic ribosomes are large particles that have a sedimentation coefficient of 70*S* (the sedimentation coefficient is a convenient way of comparing the molecular weights of large molecules). The particles can be dissociated into two subunits with sedimentation coefficients of 30*S* and 50*S*. The smaller subunit is thought to bind with many of the cellular proteins that are necessary for the initiation of protein synthesis; it also binds the initiator transfer RNA and is the site of interaction of messenger RNA and transfer RNA. The larger subunit is the site of peptidyl transferase activity, peptide bond formation, and hydrolysis of guanosine triphosphate (which is one source of energy for peptide bond formation). Protein synthesis can occur only when the two subunits are combined with messenger RNA.

About two-thirds of the mass of each subunit is RNA and one-third is protein. The RNA of the smaller subunit has a sedimentation coefficient of 16*S* and consists of about 1600 nucleotides. The larger subunit has two pieces of RNA; the sedimentation coefficient of one is 5*S* and that of the other is 23*S*. The 5*S* RNA consists of 120 nucleotides, while the 23*S* consists of about 3200. The complete nucleotide sequence of the 5*S* RNA from *E. coli* has been determined by G. G. Brownlee and Fred Sanger of the Medical Research Council in Cambridge, England.

More than 90 percent of the sequence of 16*S* RNA from the same species has been determined by Chantal Ehresmann and Peter Fellner of the Université Louis Pasteur de Strasbourg and J.-P. Ebel of the Institut de Biologie Moleculaire et Cellulaire in Strasbourg. Only small sections of the sequence of 23*S* RNA have been determined. So far, no regions of identical or very similar sequences have been found in the 16*S* and 23*S* RNA's, but some short identical sequences have been found in the 16*S* and 5*S* RNA's. Corresponding RNA's from other prokaryotes appear to have other nucleotide compositions and base sequences, but there may be short sections that are identical.

The ribosome proteins are rather unusual in comparison to those of most other subcellular structures that have been studied. Most of these, such as the histones associated with chromosomal DNA, have been found to be multiple copies of a few proteins. The number of different ribosomal proteins, in contrast, is large, and most of them occur in only one copy per ribosome. Some proteins have been observed to occur in less than one copy per ribosome, but it now seems likely that these observations are artifacts of the isolation procedure. Some proteins of the larger subunit, however, may occur in two copies per ribosome.

The 30*S* subunits from *E. coli* and *B. stearothermophilus* each contain 21 distinct proteins, which are usually labeled S1 through S21. Functionally corresponding proteins from the two species generally have homologous amino acid sequences, but none have been shown to be identical. The 50*S* subunit from *E. coli* contains 34 distinct proteins, according to Eberhard Kaltschmidt and Heinz-Gunter Wittmann of the Max-Planck-Institut für Molekulare Genetik in West Berlin; these are usually labeled L1 through L34. Immunological and physicochemical studies by the investigators in Berlin have shown that these proteins are different from each other and from proteins of the 30*S* subunit, with only two exceptions. One pair of proteins from the 50*S* subunit differs only in that one of the pair is acetylated at its amino terminus; and one protein from the 50*S* subunit has an amino acid sequence identical to that of a protein from the 30*S* subunit. The intact 70*S* ribosome thus contains 54 proteins.

In contrast, Masayasu Nomura and Jeffrey A. Cohlberg of the University of Wisconsin have shown that the 50*S* subunit from *B. stearothermophilus* contains only 27 distinct proteins. None of these appear to be identical to 30*S* subunit proteins from the same species. It is not yet known whether any are identical to proteins from *E. coli*. The intact 70*S* ribosome from *B. stearothermophilus* thus contains 48 different proteins.

One protein from the 30*S* subunit of both species is much larger than the others, with a mass of about 65,000 daltons. Some investigators now think this protein is cytoplasmic rather than ribosomal. The other proteins from both subunits have masses ranging from 10,000 to 30,000 daltons, with an average of about 17,000 daltons. The proteins contain unusually large amounts of basic amino acids. They also have an unusually low proportion of ordered secondary structure—about 20 to 33 percent, according to Michael Dzionara of the Max-Planck-Institut. The principal exceptions are two 50*S* proteins, 60 percent of which is in alpha-helical form.

Wittmann and his associates have so far determined the complete amino acid sequences of 15 ribosomal proteins and of 26 proteins from mutant strains of *E. coli*. They have also determined partial sequences for another 18 proteins. They have thus sequenced about 3000 (38 percent) of the 8000 amino acid residues in *E. coli* ribosomal proteins. With the exceptions previously discussed, they have so far found no duplicated sequences longer than five residues. It seems likely that the sequences of all the proteins will have been determined within another couple of years.

But knowing the sequences of the RNA and proteins in the ribosome is only a beginning. It is also necessary to know where each component is located within the ribosome, how it interacts with other components, and what its function is. One obvious approach to these problems is x-ray crystallography, but this approach has not yet been successful. For one thing, x-ray crystallography of large structures like the ribosome is very difficult. Furthermore, it has not yet been possible to obtain ribosome crystals that are sufficiently large for high-resolution studies.

A limited amount of progress has been achieved in determining three-dimensional structures from electron micrographs of small ribosome crystals. James A. Lake of the New York University Medical Center and Henry S. Slayter of Harvard Medical School have been able to define the structure of one type of ribosome at a resolution

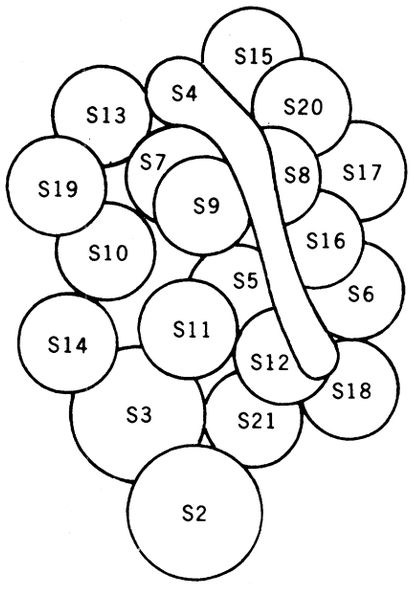


Fig. 1. A schematic diagram showing the spatial relationship of proteins in the 30*S* subunit of *E. coli* ribosomes. The map is based on results obtained from many different experiments. [Source: Robert R. Traut]

of 100 Å. And David Sabatini of the New York University Medical Center and his associates have been able to define the structure of a second type at a resolution of 65 Å. The resolution in both experiments, however, is well above the 10 to 15 Å that is necessary for precise knowledge of structure. Investigators have thus sought other ways to study ribosomal structure. Perhaps the most important step in this direction has been development of the ability to assemble ribosomes in vitro.

Building from their own work and that of other investigators, Nomura, William A. Held, and Shoji Mizushima of the University of Wisconsin 3 years ago showed that protein-free 16*S* RNA could be combined with 21 purified 30*S* subunit proteins under appropriate conditions to produce fully functional *E. coli* subunits. Last year, K. Nierhaus and S. Dohme of the Max-Planck-Institut demonstrated that functional 50*S* subunits from the same species could be assembled from 5*S* RNA, 23*S* RNA, and purified proteins. And earlier this year, Cohlberg and Nomura demonstrated that 50*S* subunits from *B. stearothermophilus* could be reconstituted in the same manner.

These results proved that all the information necessary for assembly of the subunits is contained in their components. They also revealed a great deal about the interactions of proteins and their spatial relationships. Perhaps most important, they opened the door for experiments in which individual components of the subunits can be manipulated to examine their role in the ribosome. Most of this work has been conducted on the 30*S* subunit.

By adding purified proteins to the 16*S* RNA one at a time and in various combinations, Nomura and Mizushima have been able to determine much of the sequence in which the 30*S* subunit is assembled. They and other investigators—Charles G. Kurland of Uppsala Universitet, for example—have found that only six or seven 30*S* subunit proteins bind directly to the 16*S* RNA. A second group of approximately eight or nine, known as "proximal proteins," will bind only after the first group is bound. And the remaining seven, known as "distal proteins," will bind only after the first two groups are bound. Similar, but less complete, results have been obtained with the 50*S* subunit. The proximity of proteins suggested by this type of experiment is one type of evidence that was used in compiling the topographic maps shown in Figs. 1 and 2.

This neat scheme may not be totally complete, however. In as yet unpublished experiments, Gary R. Craven and his associates at the University of Wisconsin have used a different procedure to isolate 16*S* RNA. They found that 12 proteins would independently bind directly to it. Craven's evidence indicates that the RNA they isolated has a more expanded native conformation than that isolated by other investigators, and that this accounts for the difference in binding.

No matter how many proteins are bound directly to the 16*S* RNA, however, Nomura and P. Traub of the University of Wisconsin have shown that the rate-determining step in assembly of the 30*S* subunit is an intramolecular structural rearrangement of a particle containing the RNA and about 12 proteins. This rearrangement is apparently quite extensive, for it changes the sedimentation coefficient of the intermediate particle from about 21*S* to about 25*S*. Similar results have been obtained with the 50*S* subunit.

The availability of such procedures made it possible to conduct a great many experiments in which one or more specific proteins are omitted from the reconstituted ribosome or in which a chemically modified protein is added. In this way, investigators hoped, it might be possible to correlate omissions or modifications with the loss of specific functions of the ribosome. In general, however, the proteins are so interrelated that it has proved quite difficult to assign a particular role to any one protein in this fashion. Omission of a single protein generally affects more than one function, and nearly every function can be shown to require the presence of each of several proteins. A few proteins have been shown to be important for assembly of the ribosome, but not for protein synthesis—although they may participate in as yet undiscerned functions of the ribosome. Others have been shown to participate in specific functions such as initiation of peptide chain synthesis, translation of messenger RNA, binding of transfer RNA, and so forth. But no one has yet been able to show that any protein is an enzyme with a specific function.

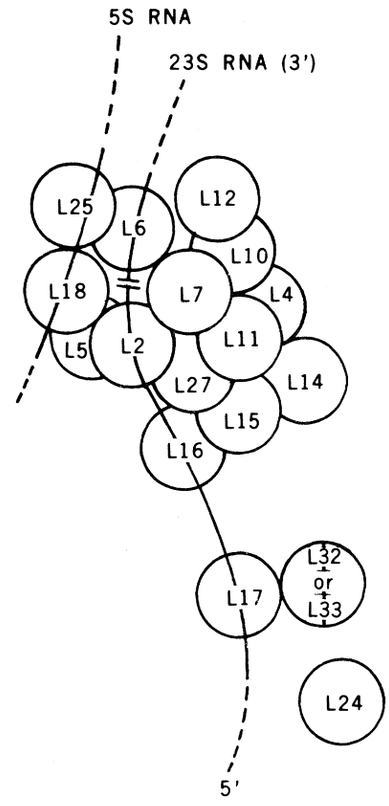


Fig. 2. A schematic diagram showing the spatial relationship of some proteins and part of the RNA in the 50*S* subunit of *E. coli* ribosomes. This map is also based on results from many different experiments. [Source: Robert R. Traut]

The relative ease of disassembly and reconstitution of ribosomes makes possible a great many experiments to determine where proteins are located within the subunits. Specific sites on the ribosome, for example, can be modified with chemical reagents. Purification of the proteins will then reveal which were affected. Individual purified proteins can also be modified and then inserted into the subunits to serve as markers. In this way, maps of the ribosome (Figs. 1 and 2) can slowly be assembled from many different experiments. Results obtained from a variety of experiments have been quite consistent.

The most common technique for this purpose is the reaction of bifunctional reagents with amino acids to link proteins that are adjacent in the intact ribosome. The linked proteins can then be separated from the monomeric proteins and identified. The best reagent for this purpose, developed by Robert R. Traut and his associates at the University of California School of Medicine at Davis, contains a disulfide group that can be cleaved under mild conditions after the dimer has been separated from

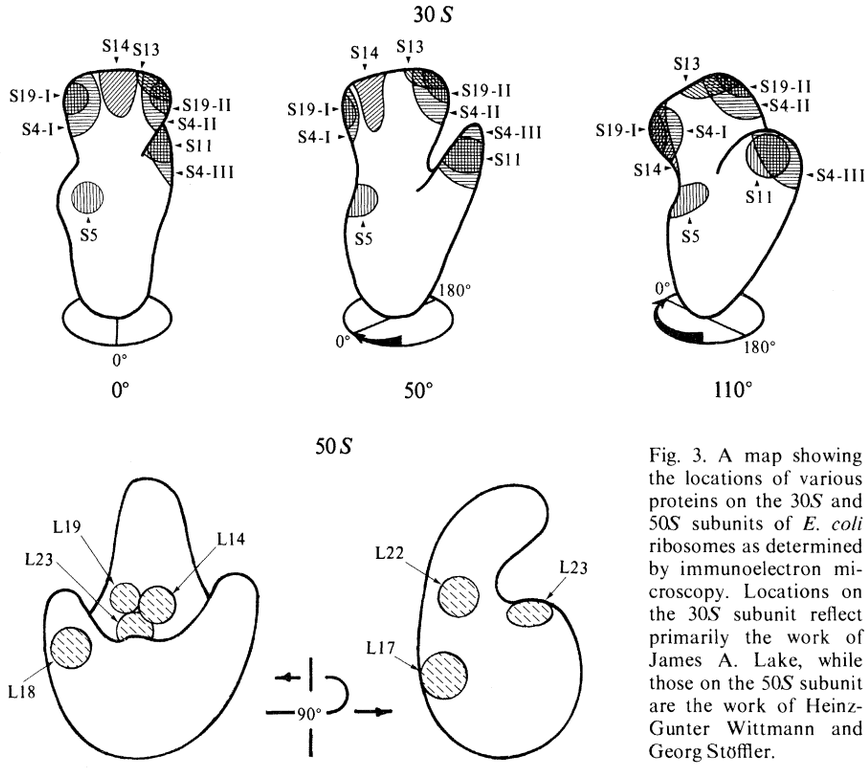


Fig. 3. A map showing the locations of various proteins on the 30*S* and 50*S* subunits of *E. coli* ribosomes as determined by immunoelectron microscopy. Locations on the 30*S* subunit reflect primarily the work of James A. Lake, while those on the 50*S* subunit are the work of Heinz-Gunter Wittmann and Georg Stöffler.

the other proteins. The proteins that were cross-linked can then be identified by conventional chromatographic techniques. So far, about 30 pairs of 30*S* subunit proteins and about 20 pairs of 50*S* subunit proteins have been identified in various laboratories.

Another approach, developed by Craven, involves the reaction of radioactive triiodide ions with proteins in partially assembled ribosomes. As more proteins are added to the ribosomes, he has found, they protect proteins already in the structure from attack by triiodide. Although conformational changes induced by the addition of proteins could provide some protection, Craven's evidence suggests that most of the protection is provided by physical shielding of the proteins. Craven has so far identified about 32 pairs of proteins that exhibit this near-neighbor influence.

A third approach, conceived by Charles R. Cantor of Columbia University, relies on the attachment of two different fluorescent dyes to two different subunit proteins, which are then used in reconstitution of the subunit. By measuring the rate of singlet energy transfer between the two dyes, Cantor has shown, it is possible to measure the distance between the two proteins. The dyes can only be attached to the proteins at random sites, however, so it is possible to measure only the distance between the centers of mass of the two proteins. The technique is relatively new, but Cantor has results for 18 pairs of proteins.

The primary drawback to this technique—and, indeed, to all of the techniques—is that at least some of the proteins in the ribosome are highly elongated. One of the proteins (S4), for example, is thought to be at least six times as long as it is wide. For such a protein, the distance between another protein and its center of mass may be meaningless.

This problem may be partially overcome with a still newer technique developed by Peter B. Moore of Yale University and Benno P. Schoenborn of Brookhaven National Laboratory and by Wittmann and Walter Hoppe of the Max-Planck-Institut in Munich. This technique requires measurement of the scattering of a neutron beam by proteins enriched in deuterium. If two such proteins are incorporated in a ribosomal subunit, the distance between their centers of mass can be obtained from the neutron scattering curve. By appropriate analysis of the data, furthermore, Moore and Schoenborn obtain data about the shapes of individual proteins.

Moore and Schoenborn argue that this technique is the most sophisticated and sensitive technique available for mapping proteins, but it requires extremely careful measurement of the scattering intensities because there is a very low signal-to-noise ratio. The technique has just been shown to work, though, so there are not yet many results obtained with it.

Several investigators, such as Cantor and Olaf Pongs of the Max-Planck-Institut in West Berlin, have used affinity labeling to identify proteins that are at the active sites for selected ribosomal functions. In affinity labeling, a reactive functional group is attached to an otherwise normal substrate in such a fashion that, once the substrate is bound at the active site, it can be irreversibly linked to proteins or RNA in the vicinity. Many of the other techniques can also be adapted to investigate the distance between ribosomal proteins and substrates and other cellular macromolecules that bind to the ribosome.

Perhaps the most useful technique for mapping proteins is immunoelectron microscopy, which has been developed by Wittmann and Georg Stöffler of the Max-Planck-Institut and by Lake, Nomura, and Lawrence Kahan of the University of Wisconsin. The isolation and purification of ribosomal proteins has made it possible to prepare antibodies that are specific for each protein. These antibodies, which are bifunctional, can then be used to link corresponding proteins in two subunits. Electron microscopy of such dimers then reveals where on the distinctively shaped surface of the subunit the antibody is attached, and thus where the protein is located. Ribosome maps constructed by this technique are shown in Fig. 3.

Many variants and combinations of these techniques have also been used. Kurland and R. Brimacombe of the Max-Planck-Institut, for example, have used bifunctional reagents to link proteins to adjacent sites on ribosomal RNA to try to discover what RNA sequences are responsible for protein binding. Other investigators have used flash photolysis with ultraviolet light to create such links. Robert Zimmerman of the University of Massachusetts has used ribonucleases to digest unbound RNA in partially assembled ribosomes in a different type of attempt to determine where proteins bind. Many other investigators have used equally innovative techniques.

Results from all these studies can be combined into various types of topographic maps, such as the three already discussed. These maps are, however, quite obviously incomplete. In particular, they show almost nothing about the conformation of RNA within the ribosome and how it binds with many of the proteins. The maps also provide little information about the binding sites of substrates and cofactors. But they provide much more information about structure than has ever been available, and they are a firm foundation for the research that is likely to be completed within the next few years. And as this research accumulates, investigators will learn much more about the functioning of what might well be the most important organelle within the cell.

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**Additional Reading**

1. M. Nomura, A. T. Tissieres, L. Lengyel, Eds., *The Ribosome* (Cold Spring Harbor Press, New York, 1974).