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Thomas E. Creighton

European Molecular Biology Laboratory
London, England

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RIBOSOMES

A. YONATH
F. FRANCESCHI

Over thirty years ago, the central dogma of molecular biology stated the way genetic information flows: **DNA** is transcribed into **messenger RNA** (mRNA) that, in turn, is translated into **proteins** (1). The *ribosome*, discovered in the mid 1950s, is the universal cellular organelle facilitating the **translation** step, by catalyzing the sequential polymerization of amino acids according to the blueprint encoded in the mRNA. Although the ribosome catalyzes a rather simple chemical reaction, the formation of **peptide bonds**, the process of **protein biosynthesis** is highly complicated and sophisticated, and it depends on a large range of recognitions and interactions. The ribosome guarantees its fidelity and high efficiency by providing highly specific sites with various affinities for a large variety of incoming and outgoing molecules involved in protein synthesis. In **prokaryotes**, during exponential cell growth, the ribosomes may account for up to 50% of the dry cell mass and are distributed in the **cytoplasm**. **Eukaryotic** ribosomes are found also in **mitochondria** and **chloroplasts**. Ribosomes in all organisms are giant **ribonucleoprotein** (RNP) particles consisting of two subunits of unequal size, known as the large and small subunits. Two-thirds of the ribosomal mass is ribosomal RNA (rRNA), the rest is composed of 50 to 82 different ribosomal proteins (r-proteins), depending on the ribosomal source (Table 1). The names of the r-proteins are composed of L or S (depending on whether the protein is from the large or small subunit), and a running number, according to the position of this protein on **two-dimensional electrophoresis** gels.

Owing to the fundamental significance of ribosomes, they have been the target of numerous biochemical, biophysical, and genetic studies (see Appendix 1). These resulted in the elucidation of the gross structure of the ribosome, as well as the approximate locations of several functional sites. Due to recent significant technical advances, current ribosome research is characterized by major conceptual revisions resolving previous ambiguities and introducing substantial spatial rearrangements (2–5).

THE BIOSYNTHETIC PROCESS: AN OVERVIEW

Protein biosynthesis can be divided into three functional steps: initiation, elongation, and termination. The initiation step requires the formation of the initiation complex, which is made of the small ribosomal subunit, **initiation factors** (IF), energy-rich compounds (GTP), initiator formylmethionine-**transfer RNA** (fMet-tRNA), and the mRNA molecule, with the **initiation codon** (usually AUG) in a favorable context. When the large subunit binds to the initiation complex, the elongation cycle can start.

Table 1. Ribosomal Components

Ribosome Source	Avg. Sedimentation Coefficient (range)	rRNA Chains (Large Sub/ Small Sub)	Approx. No. r-Proteins
Bacterial	70S	5S, 23S/16S	50–60
Mitochondria (mammals)	55S	16S/12S	80–90
Chloroplasts	70S	23S, 5S, 4.5S/ 16S	50–60
Mitochondria (fungi, protozoans, mammals)	70S (55S–80S)	21–24S/15–17S	65–90
Archaebacteria	70S	5S, 23S/16S	65–75
Plant mitochondria	75S	5S, 26S/16S	70
Eukaryotes (cytoplasm)	80S	5S, 5.8S, 26–28S/ 17–18S	70–90

The core of translation is the elongation cycle (reviewed in Ref. 6). In this step, one amino acid at a time is integrated into the growing nascent chain. The amino acids are brought to the ribosome in an activated state, bound to their corresponding tRNA through a high-energy phosphodiester bond. To ensure incorporation of the correct amino acid, the **anticodon** of the aminoacyl-tRNA must match the codon in the translated mRNA (see **Aminoacyl tRNA synthetases**). In the early 1960s, Watson (7) and Lipmann (8) suggested a model for the elongation cycle with two tRNA binding sites on the ribosome: the A-site (acceptor site for aminoacyl-tRNA) and P-site (peptidyl-tRNA site). In the beginning of the 1980s, the existence of a third site, E (exit)-site, that binds only deacylated tRNA was proposed (9).

In the first round of the elongation cycle, only one tRNA is bound to the ribosome (the initiator tRNA, which is Met-tRNA in eukaryotes and fMet-tRNA in prokaryotes). During all other elongation rounds, two tRNA molecules are bound to the ribosome. Elongation starts with the f/Met-tRNA at the P-site. Subsequently, the decoding stage takes place, and an aminoacyl-tRNA carrying the amino acid coded by the next mRNA triplet is selected from the tRNA pool and delivered to the A-site by the **elongation factor** (EF) Tu in prokaryotes and EF-l-α in eukaryotes, which is a **GTP-binding protein**, in the form of a ternary complex, aminoacyl-tRNA/EF/GTP. Once the right aminoacyl-tRNA is bound to the A-site, the EF leaves the ribosome as EF-GDP, and the ribosome carries out its intrinsic enzymatic task, the formation of the peptide bond. After peptide bond formation (the pre-translocational stage), the growing peptide is bound to the tRNA (as peptidyl-tRNA) at the A-site. At this point, a second elongation factor (EF-G in bacteria, EF-2 in eukaryotes, both **G proteins**) binds to the pre-translocational ribosome and catalyzes the translocation of the peptidyl-tRNA from the A-site to the P-site. At the same time, the deacylated tRNA from the P-site moves to the E-site (as illustrated in Fig. 1). In this way, the ribosome moves by one codon and reaches the post-translocational state (peptidyl-tRNA at the P-site, deacylated tRNA at the E-site). Now it is ready for the next round of elongation. Once the aminoacyl-tRNA corresponding to the next codon binds to the ribosome at the A-site, the deacylated tRNA bound at the E-site leaves the ribosome and the elongation cycle repeats itself, incorporating a new amino acid to the nascent peptide with each round of elongation. It has recently been suggested that during translocation the ribosome moves like a rigid frame along the mRNA between the three binding sites (A, P, and E), carrying two

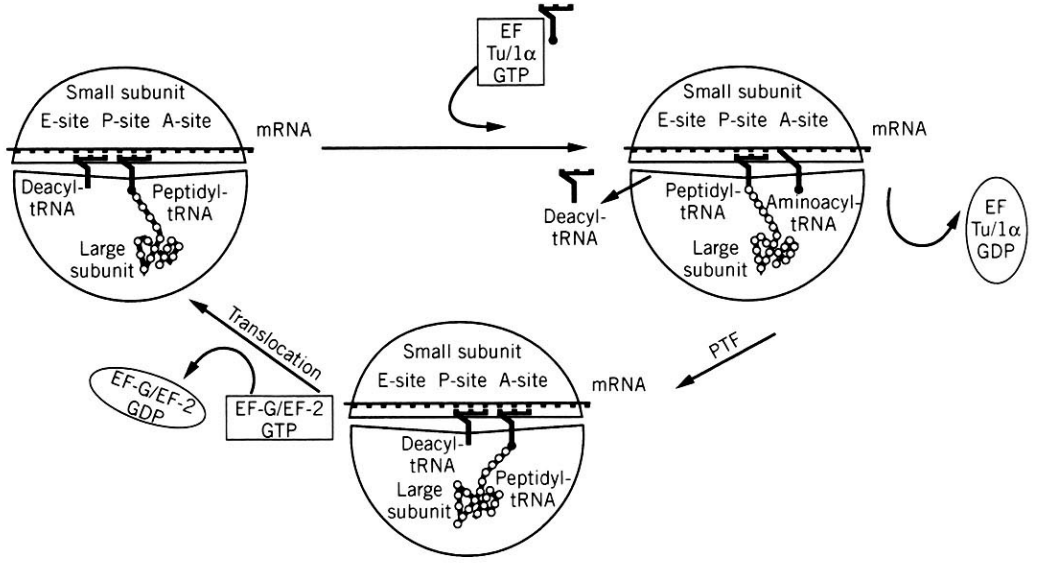


Figure 1. Schematic diagram of the process of the elongation cycle.

tRNA molecules so that the microtopography of the sites adjacent to the decoding region does not change (10).

The elongation process ends when a **stop codon** is present at the A-site. The mechanism of the stop codon recognition by the termination factors and the subsequent release of the nascent protein from the ribosome is still not completely understood. In prokaryotes, it requires two **release factors** and one that stimulates them; in eukaryotes, a **homologous** family has been identified. It is known that the interaction of the release factors with the peptidyl transferase center catalyzes the addition of a **water** molecule instead of an amino acid to the peptidyl tRNA. This reaction frees the carboxyl end of the growing polypeptide chain from its attachment to the tRNA and promotes the release of the mature protein from the ribosome.

Additional compounds involved in this process are the **signal recognition particle** (SRP) in eukaryotes [or the corresponding complex in prokaryotes (11)], which facilitates the targeted translocation of the nascent peptides; **chaperonins**, responsible for the correct folding of the nascent peptide; hydroxylase, acetylase, and aminopeptidase, a series of enzymes catalyzing amino acid modifications that can act cotranslationally; and regulators of ribosomal function under normal or stressful conditions, heat or starvation shock (eg, the relA gene product).

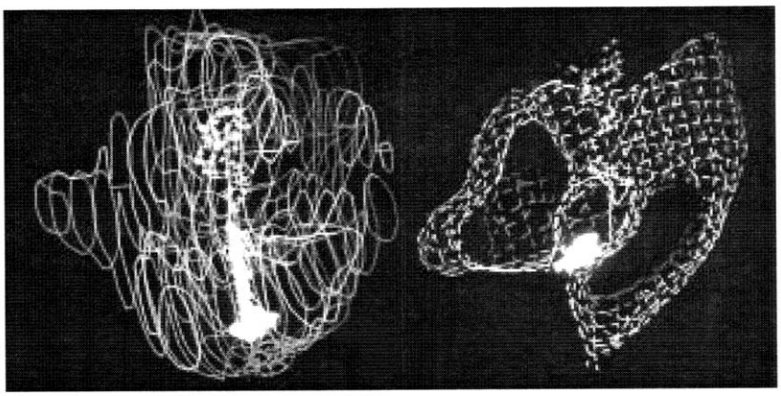
The various enzymatic activities associated with the process of protein biosynthesis take place in an internal ribosomal gap at the interface between the two subunits (12,13). This assignment is based on extensive biochemical investigations that showed that the ribosome masks most of the components participating in the biosynthetic process: a stretch of about 30 nucleotides of the mRNA, the aminoacylated tRNA molecules (14), and a significant part of the nascent polypeptide chain (15–17). Spatial considerations allow the placement of three molecules of tRNA in this void, as well as the factors participating in the elongation cycle (17–22). Two of the tRNA molecules can be positioned so that their anticodons are close to the presumed rRNA-rich path of the bound mRNA, on the surface of the small subunit, and their CCA-termini pointing so that the growing peptide chain may extend into a tunnel spanning the large subunit. This tunnel originates at the subunit interface and terminates on the opposite side, at a location compatible with the exit site of the nascent polypeptide chain identified by **immunoelectron microscopy**, and is thus suggested to be the path for the nascent proteins. Indeed, *in vitro* cotranslational folding was shown for the synthesis of full-length proteins (eg, rhodanese and ricin) while being bound to the ribosome (23). A feasible description of these assignments is given in Figure 2.

The synthesis of a complete protein of average size takes 20 to 60 seconds. Nevertheless, multiple initiations of translation would ensure a faster and efficient translation of mRNA molecules. Accordingly, *polysomes* (or **polyribosomes**) can be formed along a single molecule of mRNA from several ribosomes spaced as close as 25 codons apart.

PROPOSED FUNCTIONAL RELEVANCE OF RIBOSOMAL COMPONENTS

The Ribosomal Components

The natural tendency of the ribosomes to disintegrate led originally to the assumption that they are nonspecific aggregates.



(a) (b)

Figure 2. Structural models of bacterial ribosomes. (**a**) A computer graphics display of the outer contour of the 70S ribosome (13) (in blue). The image was reconstructed from negatively stained crystalline arrays of 70S ribosomes from *B. stearothermophilus* at 47 Å resolution (17,18). A model-built tRNA molecule was placed in the gap at the subunit interface so that its CCA 3′ end points into the tunnel within the 50S subunit. Following an *in vitro* cotranslational experiment (23), the main chain of the MS2 coat protein was placed along the tunnel in a partially unfolded conformation, maintaining the native **beta-strands** and the native (crystallographically determined) conformation of the segment of residues 1 to 47. The C-terminus was placed in the vicinity of the proposed peptidyl transferase center and the N-terminus at the exit domain of the tunnel. The region 1 to 47 includes all atoms and is shown as a space-filling structure. (**b**) A slice of 50Å thickness of the 50S subunit (within the 70S shown on the left), as reconstructed from electron diffraction (11,12) with the same components.

Later on, the contrary was established, and the composition of ribosomal particles from various sources was determined (24). By far the best biochemically characterized bacterial ribosome is that of *Escherichia coli*. It contains about quarter of a million atoms, and has a molecular weight of about 2.3 million daltons and **sediments** with a coefficient of 70S. About two-thirds of the mass of the ribosome is composed of three chains of rRNA (with a total of about 4500 nucleotides). One copy of each of its 58 different proteins is present in the ribosome, with the exception of the tetrameric L12. The ribosomes of **archaebacteria** and eukaryotes are somewhat larger (eg, eukaryotic ribosomes migrate with a sedimentation coefficient of 80S), reflecting the higher complexity of the eukaryotic cellular environment.

Ribosomal RNA Chains

Although it has been suggested that the original, primitive ribosome may have been composed solely of rRNA (see **RNA world**), until recently it was assumed that the catalytic activities of the ribosome are carried out mainly by the r-proteins and that the rRNA molecules have a more passive role in providing the scaffold for the ribosome and in binding the mRNA. Recently, it became clear that the ribosomal functions are no longer due solely to r-proteins, and the ribosomal RNA has been proven to play an active part in the ribosomal functions (25). The prominent catalytic activities of the rRNA are:

1. The **peptidyl transferase** activity (PTF) resides in the large subunit. In some organisms, it was found that a model assay of this activity is relatively resistant to proteolysis (26). The minimal set of components indispensable for peptidyl transferase activity was identified as a stretch of approximately 100 nucleotides, at the central loop of domain V of the 23S RNA, plus a small fraction of the r-proteins (about 5 to 10% of the total mass). The aminoacyl end of the bound tRNA has been shown to make contacts with this rRNA loop, and mutations in this region abolish the PTF reaction completely or make the ribosomes resistant to antibiotics that target the PTF activity.
2. The **GTPase** center is associated with a highly conserved stretch of 23S rRNA that binds the moderately conserved protein L11. Whereas the rRNA is essential for this activity, cells lacking protein L11 are viable.
3. The first step in protein biosynthesis, the formation of the preinitiation complex, depends in prokaryotes on the base-pairing interaction of the small ribosomal subunit with a region of the mRNA, called the **Shine–Dalgarno sequence**, located 3 to 10 bases 5′ of the initiation codon (usually AUG). Moreover, the rRNA from the small subunit provides the decoding site, where the mRNA and anticodon loop of the tRNA interact.

Crystallographic studies led to the determination of the structure of two rRNA domains, both part of the 5S rRNA: a synthetic ribonucleic oligomer of 12 base pairs, imitating Helix A; and stretches of 29 nucleotides containing the sarcin/ricin loop as well as 62 nuclease-resistance nucleotides (helices I and IV, loop E) and a part of it, a dodecamer containing the minimum 11 base pairs required for binding the protein L25. The last, composed of loop E, exhibits an irregular geometry of cross-strand purine stacks.

The r-Proteins

Because of the complexity of the ribosome and its functions, it is still not possible to assign a function to each ribosomal protein. It is assumed that the r-proteins play an important role in the proper folding of the rRNA and enabling it to function efficiently. As mentioned above, some of the r-proteins are essential for the enzymatic properties, such as the PTF (eg, L2), whereas others are involved in the binding of tRNA (S7 and S8). In addition, there is evidence for the bifunctionality of a number of r-proteins (in prokaryotes), which function as regulators of translation by their ability to bind to the polycistronic mRNA coding for them. In some cases, they bind to similar structural motifs in the rRNA and mRNA, suggesting that these proteins interact in a similar fashion with mRNA and rRNA (eg, the interactions of L1 and S8, which possess a stable structure even in isolation); in others, the structural motifs of the rRNA and mRNA target sites do not resemble each other (eg, S15, which exhibits significant flexibility in isolation).

Complete sequences of the r-proteins from *E. coli* and many other bacteria and eukaryotes have been determined (24,26). Some ribosomal proteins undergo **post-translational modifications**. Among others are acylation (eg, L17/L12 in *E. coli*) and phosphorylation (eg, the eukaryotic P1, P2, and P0). Several structural motifs have been suggested for the r-proteins. Among them are clusters of basic/acidic residues; amino acid sequence repeats in shared elements; **zinc finger** domains; basic regions; **leucine zipper** motifs; and carboxyl extensions of **ubiquitin**-like proteins.

For over two decades, the production of crystals of r-proteins useful for **X-ray crystallography** was extremely poor. This, together with the observation that some ribosomal proteins lose their *in situ* conformation upon isolation, led to the assumption that the conformations of almost all r-proteins are dictated by their *in situ* supporting environment. Furthermore, no correlation has been found between the crystallizability of individual r-proteins and the degree of their evolutionary conservation, their localization within the ribosomes, or their involvement in primary contacts with rRNA. An appropriate example of a highly conserved r-protein that is intimately bound to r-RNA in a major functional center (GTPase activity), and undergoes significant conformational changes upon isolation from the ribosome, is protein L11. Interestingly, L11 regains its natural fold and can be reconstituted into core particles lacking it, even when a large chemical moiety with a molecular weight approaching a third of its own is bound to it (17).

The recent increasing sophistication in instrumentation, the implementation of powerful genetic techniques, and the use of ribosomes from **thermophilic** bacteria resulted in major progress in the structure determination of isolated r-proteins. Though it remains to be seen whether the structures of the isolated ribosomal components bear resemblance to their *in situ* conformation, it has been suggested that components possessing an intrinsic characteristic fold may crystallize, provided they are not damaged during their preparation. The structures that have been determined (fully or partly) by either X-ray crystallography or solution heteronuclear NMR are: S4, S5, S6, S7, S8, S17, L1, L6, L9, L7/l12 (the C terminal fragment), L14, L21, L22, L25, L30 (27–28). Most share the split β-α-β fold, called the "common" motif and abbreviated as RRM (RNA recognition motif) (see **RNA-binding pro-**

teins). A few proteins show different folds, called "unique" and "multiple" in which the interacting regions are built mainly of loops. One protein (S8) exhibits versatility in its RNA contact sites, since one interacts with rRNA and the other is involved in binding tRNA. Two novel RNA-binding domains have been recently detected. One can be aligned with **homeodomains** (DNA-binding proteins) consisting predominantly of **alpha-helices** connected by a turn and exhibiting structural flexibility, acquiring stability upon RNA binding (S15 and in the C-terminal of L11). The other is a β-ribbon arm, similar to that found in DNA-bending proteins, observed in S7. This protein acts as the main regulatory element for one of the r-protein operons and is crucial for tRNA binding and assembly of the small subunit.

Assembly and Reconstitution

In prokaryotes, the assembly of ribosomes occurs in the cytoplasm and is coupled to the **transcription** of rRNA molecules. Thus, r-proteins bind to rRNA while being synthesized. In prokaryotes, the *in vivo* ribosome assembly requires between 2 and 3 min. In eukaryotes, the situations is different and more complicated. The r-proteins are synthesized in the cytoplasm and then imported into the **nucleolus** (a substructure of the **nucleus**), where ribosomal assembly takes place. Once the ribosomes are assembled, they are exported back into the cytoplasm. Thus, ribosome assembly and transport take between 30 min (for the small subunit) and 1 hour (for the larger one).

In prokaryotic ribosomes, both subunits can be separated into their components and then reconstituted *in vitro* to fully active articles, even after partial or total unfolding has occurred. Interestingly, the reconstitution process is performed under nonphysiological conditions and takes considerably longer than the *in vivo* assembly (90 min vs 3 min). The ability of the ribosomes to reconstitute *in vitro* shows that the information required to obtain the active **quaternary structure** of the ribosome resides within the ribosomal components. Originally, it was assumed that the r-proteins governed the assembly process and the rRNA chains undergo significant conformational changes throughout the assembly process. However, it has been suggested recently that rRNA may influence the conformations of some r-proteins (eg, S15, L11), as conformational changes in these r-proteins were induced by their interactions with rRNA (29).

Reconstitution experiments led to the construction of ribosome assembly maps, showing the sequential binding of the different r-proteins to the rRNA molecules during assembly. It was found that two ribosomal proteins initiate the assembly of each ribosomal subunit. These proteins are defined as structural inducers, since they bind directly to the respective RNA without cooperativity during the onset of assembly and are assumed to induce the creation of *in situ* microenvironments that serve as assembly nuclei within the ribosome. It has been shown that, in general, the *in vitro* assembly patterns imitate the *in vivo* formation of ribosomes, but recent studies indicate the involvement of nonribosomal cell products in the *in vivo* ribosome assembly. These include the chaperonin DnaK (30) and **RNA helicases** belonging to the **DEAD box** protein family.

Evolution vs Universality

Ribosomes from the three kingdoms (eubacteria, eukaryotes, and archaebacteria) vary considerably in their size and the number of their components. However, they also exhibit a high degree of conservation with respect to their architecture and some r-proteins and rRNA regions (31,32). As the ribosome is basically an RNA enzyme (a **ribozyme**), it can be regarded as ancient on the evolution scale. The existence of r-proteins conserved throughout all three **phylogenetic** kingdoms implies that they appeared in the first stages of evolution. With the progression from primitive to higher organisms, the number of the r-proteins increases, indicating their participation in the more complex functions of the eukaryotic ribosomes.

The ribosomes from archaeabacteria are thought to be vestiges of transition stages in the evolution from prokaryotes to eukaryotes. Thus, it has been frequently remarked that the amino acid sequences of archaebacterial r-proteins are closer to their eukaryotic homologues than their bacterial counterparts, whereas the organization of their genes mimics that of eubacteria. Furthermore, in several *in situ* substructures, the level of conservation is so high that ribosomal proteins from one source can be fully exchanged by their homologues from other ribosomes, even when they belong to two different kingdoms or function under totally different conditions. It is not surprising that protein L11 from *E. coli* binds well to cores of *Bacillus stearothermophilus* lacking it because of the high homology of these ribosomes, but the universality of the internal substructure of L1 and of a segment of the 23S rRNA is rather unpredictable. Despite the evolutionary distance between the eubacteria and archaebacteria, chimeric complexes of L1 were reconstituted between halophilic components and their corresponding mates from *E. coli*.

Inhibitors of Ribosomal Function

Many antibiotics act directly on ribosomes, and most of them interact in one way or another with rRNA. Their binding sites were determined in different ways, including by rRNA mutations leading to drug resistance. The antibiotic functions of the PTF drugs involve interference with the reaction itself (**puromycin** and **chloramphenicol**) or with the movements required to perform the elongation cycle. **Tetracycline** is the classical antibiotic that inhibits the binding of the aminoacyl-tRNA. The aminoglycosides, **streptomycins** and the family of the gentamycins, **kanamycins**, and **neomycins** block A-site occupation and stimulate misreading, resulting in the incorporation of the wrong amino acids and leading to the production of nonfunctional proteins. **Erythromycin** and lincosamide probably stimulate the dissociation of peptidyl-tRNA by blocking the entrance to the tunnel that conveys the exiting nascent peptide. Thiostrepton and spectinomycin prevent translocation, ie, they inhibit the conformational change between the pre- and post-translocational states.

Among the most potent inhibitors of protein synthesis are naturally occurring peptide **toxins** acting mainly as **nucleases** and known as ribosomal-inactivating proteins (RIP). This group includes α-sarcin, **ricin**, arbin, mitogillin, restrictocin, shiga toxin, and vero toxin. Most hydrolyze a single phosphodiester bond in the large subunit rRNA, whereas **colicin** E3 acts on the 16S RNA (Table 1) and pokeweed antiviral protein (PAP) interacts with the EF binding site. α-Sarcin and ricin bind to a loop in the large subunit rRNA that includes a universally conserved dodecamer sequence, which appears even in ribosomes that are not sensitive to these toxins (eg, those from *Haloar-*

cula marismortui). This domain is involved in aminoacyl-tRNA and elongation factor binding, as well as GTPase activity.

STRUCTURAL INFORMATION

Approximate Shapes and Positions

A large number of traditional, as well as specifically designed structural methods (see Appendix 1), have been employed for shedding light on the structural organization of the ribosome and elucidating its **quaternary structure**. The relative positions of the centers of mass of the *E. coli* r-proteins have been determined, and the spatial *in situ* proximities between several ribosomal components could be approximated (2–5). In parallel, attempts to determine accurately the secondary structure of rRNA, pinpoint tertiary structure elements, and assign to them functional relevance, resulted in proposals for fairy detailed topological models for the organization of the rRNA (33). Computational and **phylogenetic** analyses of possible tertiary interactions in the rRNA, combining results of chemical, physical, and functional experiments with energy minimization, were also carried out.

For over three decades, a wide variety of **electron microscopy** techniques have been the methods of choice for viewing ribosomes at various levels of detail. Since the late 1980s, along with the refinement of sophisticated and powerful microscopical and image reconstruction techniques, these studies enabled feasible assignments of functional relevance to a few structural features. The first reconstructed three-dimensional models were obtained from periodically ordered monolayers (ordered arrays) of eukaryotic and prokaryotic ribosomes and their large subunits that occurred naturally (12) or were grown *in vitro* (13). Despite their low resolution, these models revealed several key features, associated mainly with internal vacant spaces, cavities, gaps, tunnels, and partially filled hollows, that had not been detected earlier. Consequently, the ribosome, which was traditionally conceptualized as a compact network, was shown to be rather spongy.

Higher Resolution Studies

The accumulated knowledge about ribosomes has not yet revealed the molecular mechanism of protein biosynthesis. To extend the limits of our understanding of this process, an accurate and reliable model of the ribosome is required. Such a model should be obtained by X-ray crystallography. Two approaches have been taken. One focuses on isolated ribosomal components (r-proteins and rRNA described above); the second aims at the elucidation of the structure of entire ribosomal particles. Being ribonucleoprotein complexes that are notoriously flexible, unstable, and prepared routinely as conformationally mixed populations, ribosomes provide an extremely complicated system for crystallographic studies. On the other hand, the natural periodic organization of ordered helical or two-dimensional arrays of ribosomes has been observed in eukaryotic cells (eg, lizard, chicken, amoebae, and human) exposed to stressful conditions, such as suboptimal temperatures, wrong diet, or lack of oxygen, and it has been hypothesized that these periodically ordered forms are the physiological mechanism for temporary storage of ribosomes, aimed at preserving their integrity and activity for the expected better future. Thus, we have had the crystallization attempts aimed at extending the natural tendency to form periodic arrays into the growth of well-ordered three-dimensional crystals. Perhaps the most striking and unexpected achievement of the last decade is the growth of usable crystals of ribosomal particles from **halophilic** or thermophilic bacteria (17). As a strong correlation was found between the activity of the ribosomes and the quality of their crystals, it has been suggested that these ribosomes are more stable than those from eubacteria and retain their integrity and activity during the isolation and crystallization processes. Furthermore, far beyond the initial expectations, one of these crystal forms (of the 50S subunit from *H. marismortui*) diffracts to almost atomic resolution, 2.7 Å (34,35), the other (of the small subunit from *T. thermophilus*) to 3.0 Å (36). As initial phases could be derived at medium resolution (31), the way to structural analysis of the ribosome has been paved.

APPENDIX 1: Summary of methods used to study ribosomal structure and/or function

- Tests for ribosomal activity: RNA-binding; Poly-U-dependent Poly-Phe synthesis; natural mRNA *in vitro* translation; the use of synthetic tRNA and analogs
- Total ribosomal reconstitution (from isolated r-proteins and r-RNA) for ribosome assembly studies
- Selective removal of r-proteins under mild chemical conditions (salt or organic solvents) followed by partial *in vitro* reconstitution (ribosomal cores + split proteins)
- Heterologous ribosomal reconstitution (using rRNA with r-proteins from a different species and vice versa)
- Reconstitution in the absence of single components, to determine the effect of single r-protein omission in either assembly or ribosomal function
- Chemical **footprinting**, hydroxyl radical cleavage, and protection studies of ribosome–ligand interactions (eg, tRNA, mRNA, and antibiotics)
- Chemical modification probing of selected ribosomal moieties
- Specific cleavages by **nucleases**: α-sarcin, ricin, ribonuclease H, etc.
- rRNA-r-protein, rRNA-rRNA, mRNA-rRNA, and subunit **crosslinking** by mild UV irradiation or chemical agents to map contact topography or functional sites
- Photo-activated reagents in **affinity labeling** of ribosomal ligands or components
- Fluorescence **energy transfer** to follow the binding of ribosomal ligands (eg, tRNA) or the dynamics of the growth of the nascent polypeptide and its folding
- Single-site mutations (in rRNA) leading to antibiotic resistance or ribosome inactivation
- Probing exposed single-stranded rRNA regions with complementary DNA oligonucleotides
- Modeling of rRNA, based on **distance geometry**, energy minimization, or phylogenetic conservation
- Sequence determination and secondary-structure predictions of r-proteins and rRNA
- Comparisons between species and determination of homologous conserved regions
- **Light scattering** and **hydrodynamic** measurements for size/shape estimation

- Triangulation: Reconstitution or binding of one or a few protonated components (r-proteins, tRNAs) into deuterated ribosomes. The locations of the centers of mass are approximated by **neutron scattering**, including proton-spin **contrast variation**.
- **Electron microscopy** (EM), dark field and tunneling, negative and positive staining
- Electron microscopy coupled with three-dimensional image reconstruction using (1) optical diffraction of tilt series of ordered two-dimensional arrays (monolayers) or (2) single particles embedded in vitrified ice and viewed with normal- or low-dose electrons, followed by image processing and angular reconstitution of isolated particles
- **Immunoelectron microscopy**: gross r-protein mapping through electron microscope localization of **antibodies** bound to the r-proteins that were the **immunogen**
- **Neutron diffraction** coupled with contrast variation, investigating the gross localization of the ribosomal components, benefiting from the different scattering properties of protein and RNA
- Heteronuclear three-dimensional **NMR** spectroscopy of isolated small r-proteins or fragments of the larger ones
- X-ray crystallography of ribosomal particles, as well as crystallizable ribosomal components

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