

Review

Toward an understanding of the structural basis of translation

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Abstract

The recently solved X-ray crystal structures of the ribosome have provided opportunities for studying the molecular basis of translation with a variety of methods including cryo-electron microscopy - where maps give the first glimpses of ribosomal evolution - and fluorescence spectroscopy techniques.

The bacterial ribosome, a 2.5 MDa assembly formed by three large RNA molecules and 51 proteins, is the most complex biological structure that is currently known at atomic resolution. It is surpassed by some large viruses in sheer number of atoms (250,000 in the ribosome, if we include the hydrogen atoms), but such viruses are typically formed by symmetrically arranged repeats of a unique structure whose size is easily dwarfed by the ribosome, which has no repeated units. The ribosome's function - to translate the genetic code into protein - is one of the most fundamental processes of life, and intense efforts are going into elucidating the underlying mechanisms. These efforts are reminiscent of the heroic struggle to solve another of life's riddles, the question of how genetic information is stored and replicated, which resulted in the discovery of the structure of DNA 50 years ago. A review of research on the ribosome from its discovery in 1957 until the turn of the century is found in Spirin's comprehensive book [1].

In the ribosome, functional complexity - known from several decades of biochemical studies by thousands of researchers - is matched by structural complexity. Initial visualizations of negatively stained ribosomes by electron microscopy [2] showed little more than a particle that is subdivided into subunits of unequal size. But a first appreciation of the structural complexity came from cryo-electron microscopy (cryo-EM), which in 1995 started to produce density maps revealing an intricate topology [3,4] and allowed mapping of the binding positions of critical ligands such as tRNA [5,6]

and elongation factors Tu (EF-Tu [7]) and G (EF-G [8]). Subsequently, many years of hard work by several X-ray crystallography groups bore fruit in the year 2000, when three groups published X-ray structures of the small [9,10] and large [11] ribosomal subunits of the eubacterium *Thermus thermophilus* and the archeon *Haloarcula marismortui*, respectively. The wealth of data that has emerged from these and ensuing X-ray studies of the complete bacterial ribosome [12], a ribosomal subunit of another species (*Deinococcus radiodurans* [13]), as well as numerous complexes of the subunits with antibiotics, has radically changed the direction and pace of research. Even though it has not yet led to an understanding of ribosome function, it has nevertheless provided the essential structural basis required for the elucidation of the underlying molecular mechanisms.

The ribosome is among the most ancient 'inventions' of evolution, and a detailed comparison of ribosome structures encountered in today's species should shed light on the way new functionality in protein synthesis, and the ancillary functions of protein export and control, have developed over the course of 3.5 billion years. Unfortunately, it has been very difficult to grow crystals suitable for X-ray studies from all but a few 'extremophilic' species of bacteria - those that are adapted to growth under extreme conditions of temperature, salt concentration, radiation exposure, or acidity. This restriction has limited our knowledge in two important ways. Firstly, there is still no X-ray structure for the species for which the largest body of data from biochemical, genetic

and biophysical experiments is available: *Escherichia coli*. Secondly, evolutionary comparisons of structures at atomic resolution are currently possible only for a small number of species that are not widely separated in evolution. As yet there is no X-ray structure available for any eukaryotic ribosome, although an atomic model has been built for the ribosome from yeast on the basis of a cryo-EM map and homology modeling [14]. To date, therefore, the bulk of what we can say about the structures of ribosomes from different species and different kingdoms has come from cryo-EM.

The basic structure and function of the ribosome and its functional centers

Protein synthesis is accomplished by an interaction between the ribosome and amino-acid-bearing tRNAs selected and lined up according to the genetic instructions of the mRNA, in the course of which the amino acids are strung together to form a polypeptide. The basic model of translation, postulated by James Watson [15], remains valid today in its essential features (see, for instance, Wilson and Noller [16] and Ramakrishnan [17]). In this model, there are two sites on the ribosome where tRNAs are bound: the aminoacyl (A) site and the peptidyl (P) site (see Figure 1). Each site is made up of regions on both subunits. Interaction of the tRNA with the mRNA at the A site of the small subunit - the decoding center - determines whether the tRNA is rejected or accepted. The other functional center, the peptidyltransferase center, consists of pocket-like structure on the large subunit. Upon acceptance and accommodation of a cognate tRNA in the A site, we have the following situation: the tRNA residing in the P site is bound through its anticodon to the downstream mRNA codon, while the other end of this tRNA (the CCA end) is bound to the nascent polypeptide; the tRNA residing in the A site is bound to the current codon, while its CCA end carries the new-coming amino acid (Figure 1). This state is short-lived and is immediately followed by the transfer of the peptide to the aminoacyl end of the A-site tRNA, leaving the tRNA that is bound to the P site lacking an amino acid (deacylated). The subsequent step of tRNA translocation, catalyzed by EF-G, moves the entire complex formed by the mRNA and the two tRNAs by one codon relative to the ribosome so that the former P-site tRNA leaves the ribosome; the former A-site tRNA occupies the P site; and the A site is vacated and ready to accept a tRNA cognate to the codon that has just moved in.

The most significant departure from this original model came from Knud Nierhaus' discovery of a separate site into which the deacylated P-site tRNA moves - the exit or E site [18] (Figure 1). In addition, the existence of hybrid states that combine different binding sites on the two subunits, such as 'A/P' and 'P/E' sites, has been inferred from some experiments [19]. Finally, the decoding process is much more complicated than is portrayed in this simple model, as it also involves the participation of EF-Tu and a proofreading step in which near-cognate tRNAs are rejected (see [16]).

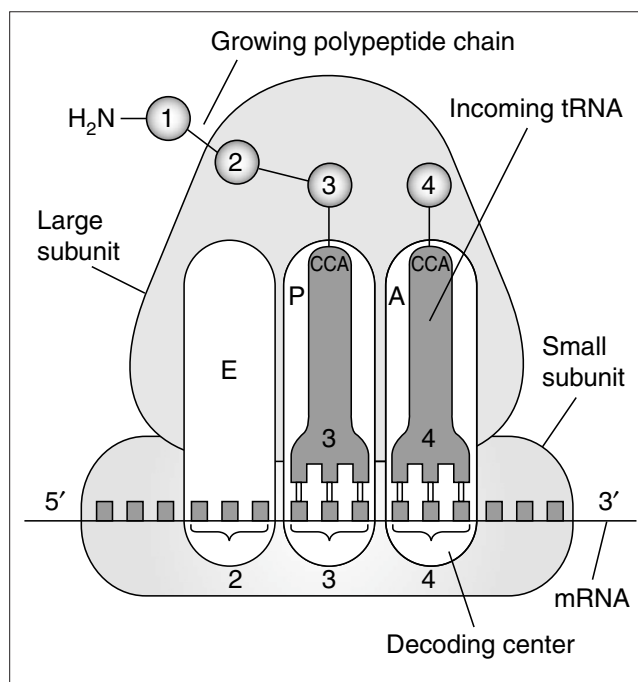
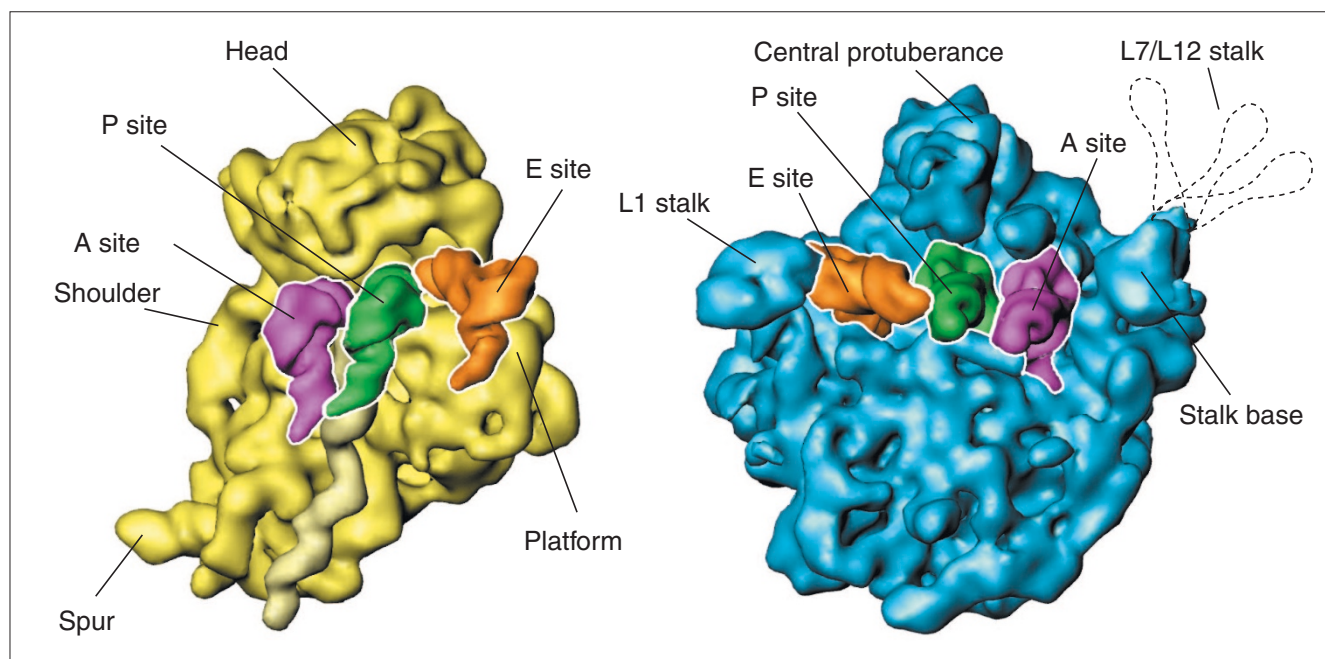


Figure 1

The basic function of the ribosome during the elongation phase of protein synthesis. Amino acids and their corresponding tRNAs and codons are numbered from the amino terminus of the polypeptide. The amino acid is attached to the CCA end of the tRNA and is then transferred to the previous amino acid in the chain. See text for more details. Adapted from [43].

The architecture of the ribosome is determined by the shapes of its two subunits (Figure 2) and their spatial arrangement, which result in a space of complex topology, the inter-subunit cavity. This cavity is tailored for the passage of tRNA, an L-shaped molecule that comes in basically 20 varieties, one for each amino acid. The small subunit has an elongate shape and a pronounced domain structure, with its anthropo-morphically named domains 'head,' 'platform,' 'shoulder,' and 'body' being distinctly recognizable. The large subunit, in contrast, is rather round and monolithic, except for three protrusions that give the subunit the characteristic 'crown' appearance in one of its views. The association of the subunits is mediated by multiple bridges [3,12,20], mostly formed by RNA-RNA contacts, although a few peripheral bridges involve proteins as well. The large and small subunits are made up of the ribosomal proteins L1-L36 and S1-S21, respectively.

The inter-subunit cavity, which is open on both sides, is suitably shaped to act as a conduit for the tRNA's migration through the ribosome from the A to the E site. The 'tRNA entrance' is roughly funnel-shaped and constitutes the binding site for several protein factors: EF-Tu, which delivers the tRNA as part of a ternary complex with GTP to the ribosome; EF-G, which catalyzes the translocation of tRNAs

**Figure 2**

The two ribosomal subunits, as visualized by cryo-EM, with tRNAs attached in the A, P and E sites. The L7/L12 stalk, normally invisible because of its flexibility, is indicated by the dashed contour lines.

and mRNA by one codon; release factors (RF1, RF2, and RF3), which release the polypeptide in response to the appearance of a stop codon at the A site and prepare the ribosome for recycling; and finally, recycling factor (RRF), which in concert with EF-G separates the subunits from each other and liberates the mRNA and the remaining tRNAs. A prominent feature of the ribosome on the tRNA entrance side is a long, flexible stalk formed by proteins L7 and L12, whose function is still unclear; the base of that stalk is formed by ribosomal protein L10 and a 58-nucleotide region of RNA that is tightly associated with the carboxy-terminal domain of protein L11. This component of the ribosome is instrumental in its GTPase activity, which is required both for EF-G-dependent translocation and accommodation of tRNAs.

The 'tRNA exit,' at the opposite end of the inter-subunit space, is gated by a large mushroom-shaped stalk, the L1 stalk, which is formed from an RNA helix and a globular protein, L1. This stalk is mobile and can pivot along an arc (by at least 30 degrees [13,21,22]) around a flex point on the RNA helix. It can apparently alternate between an 'open' position, in which it allows tRNA to exit, and a 'closed' position, in which it blocks the exit (see below).

It has been possible, first by cryo-EM [5,6,23] and then by X-ray crystallography [12,15], to visualize tRNA bound at these sites and to follow the progress of tRNA through the inter-subunit space. As it travels, the tRNA interacts transiently with the two regions of the ribosome called 'func-

tional centers': first the anticodon end interacts with the decoding center, located on the 'neck' region of the small subunit, and second the CCA end interacts with the peptidyl-transferase center, a pocket-like structure on the large subunit made entirely of RNA that also forms the entrance of the polypeptide exit tunnel. The X-ray and cryo-EM maps show many additional contacts of the tRNA with the ribosome in the different stations of its trajectory, suggesting that its movement is tightly controlled for stereochemical precision at the functional binding sites.

After the X-ray structures appeared [9-13], initial attention focused on the molecular processes at the two functional centers. It was suggested that the peptidyl-transferase activity of the ribosome involved a predominantly chemical catalysis of peptide-bond formation [11], but this hypothesis has given way to the view that the proper positioning of the substrates may be all that is required; this issue is still not resolved [24]. Valuable insights into the decoding process have been gained by the group of Ramakrishnan, who have studied the interaction of a tRNA anticodon loop with the decoding center of the isolated 30S subunit in the presence of mRNA at atomic detail [25]. Decoding and accommodation involve a complex sequence of steps that consist of a dynamic interplay between the ribosome, tRNA, and EF-Tu. Many 'snapshots' will need to be analyzed to get the full picture. Toward this end, cryo-EM is starting to provide the first low-resolution (around 10 Å) density maps of the translating ribosome [26,27].

Interaction with elongation factors, and functional dynamics

In vitro translation systems mimic the conditions in the cell by supplying ribosomal subunits and the basic ligands, as well as GTP, in a carefully balanced buffer solution (see [28]). Although not as efficient as in the cell, in which protein synthesis proceeds at 20 amino acids per second, protein synthesis in an *in vitro* system still works at a rate of about 5 amino acids per second, which means that a protein with a length of 200 amino-acid residues can be made in about 40 seconds. Such *in vitro* systems, in which ribosome complexes are free in solution and unimpeded by crystal contacts, can be used to study their dynamic behavior.

The notion that the ribosome changes its conformation during the elongation cycle goes back to early studies [29-31]. Functional dynamics can be studied by various biophysical techniques, such as cryo-EM, hydroxyl radical probing, fluorescence stopped-flow and quench-flow analysis, and single-molecule fluorescent resonance energy transfer (FRET). These techniques complement one another in giving different aspects of the system's time course and in looking at bulk or individual molecules. Significantly, studies using the different approaches all come to the conclusion that the ribosome undergoes periodic changes in confirmation during the elongation cycle ([32,33] and J. Puglisi, personal communication).

So far, the most detailed observations have come from cryo-EM. Here, the dynamic behavior is inferred from a series of three-dimensional 'snapshots' that show the ribosome at different stages of the process. Given that cryo-EM visualization is based on the formation of an average over particles with supposedly identical structures, the taking of a meaningful three-dimensional snapshot requires that a large fraction of the ribosomes is trapped in the same state. This can be accomplished by using either antibiotics or non-hydrolyzable GTP analogs. Antibiotics have been likened to wrenches or 'spanners' that are 'thrown into the works' [34] of the ribosome. They are small molecules that bind at strategic sites and cause the arrest of one or several steps of the dynamic process, mostly by interfering with the required conformational changes. For instance, tetracycline binds to the small subunit at the decoding center and prevents a cognate tRNA from moving into the A site; kirromycin arrests EF-Tu in a conformation that does not allow tRNA accommodation; and fusidic acid prevents a conformational change in EF-G that is required for the factor to leave the ribosome. Nonhydrolyzable GTP analogs, another means of trapping a particular state, bind with high affinity to the GTP-binding site without allowing GTPase activity to take place, resulting in permanent binding of the ligand.

Following such studies, it has been possible, among other results, to observe a large conformational reorganization of the ribosome that occurs in response to the binding of EF-G,

termed the ratchet motion [27,32]. The two subunits rotate relative to each other, and this motion apparently represents the first step in the two-step process of translocation. Early on, Spirin [30] suggested that the very architecture of ribosomes, as a complex formed by two loosely linked massive subunits, implied the existence of a relative motion between these building blocks. Currently, atomic modeling is being used to study the molecular basis of the conformational reorganization [35]. There is the intriguing possibility, brought out by normal-mode analysis of the ribosome structure (an analysis based on classical mechanics, predicting the most important modes of motion), that the observed motions are closely related to the dynamic properties of the ribosome's gross architecture ([36] and Y. Wang, A.J. Rader, I. Bahar and R. Jernigan, personal communication).

Ribosomes from different species and kingdoms

Secondary-structure comparisons indicate that ribosomes from eukaryotes have acquired insertions of additional RNA, the so-called expansion segments (for example, see Figure 3 for the yeast expansion segments). When the locations of the insertion points are traced back to the X-ray structures of the bacterial ribosomes, they prove to be situated exclusively at the periphery of the ribosome, away from the more centrally located functional centers. Other evolutionary developments in the eukaryotes are the addition of new proteins and the expansion and modification of existing ones, again mostly in peripheral locations. Organellar ribosomes, on the other hand, appear to be specialized through the loss of some proteins and substitution of some RNA by new proteins [37].

Although relatively little is known about the structures of the ribosomes of eukaryotes, a cryo-EM look at the ribosome of yeast (Figure 4c) [14] allows us to make some generalizations. The new peripheral acquisitions can be put into three categories: those conveying topological properties that tailor the ribosome for membrane interactions; those involved with protein export; and those required for translational regulation and control. The discovery, mentioned above, that functionally important motions can be reproduced in a normal-mode analysis of the bacterial ribosome structure invites the speculation that some of the added mass might also be required as strategically placed 'counterweights' to other functionally necessary additions, so as to preserve the motions that are required for ribosome function.

With regard to the new additions in eukaryotes with apparent impact on the topology, Dube and coworkers [38] pointed out that for the ribosome of the rabbit reticulocyte, the surface of the 60S subunit interacting with the endoplasmic reticulum membrane (that is, the region surrounding the exit site of the polypeptide) is planar, as though specialized for maximal contact with a planar membrane. This topological trait (see indication in Figure 4c) has been confirmed for all ribosomes of higher eukaryotes investigated so far (yeast

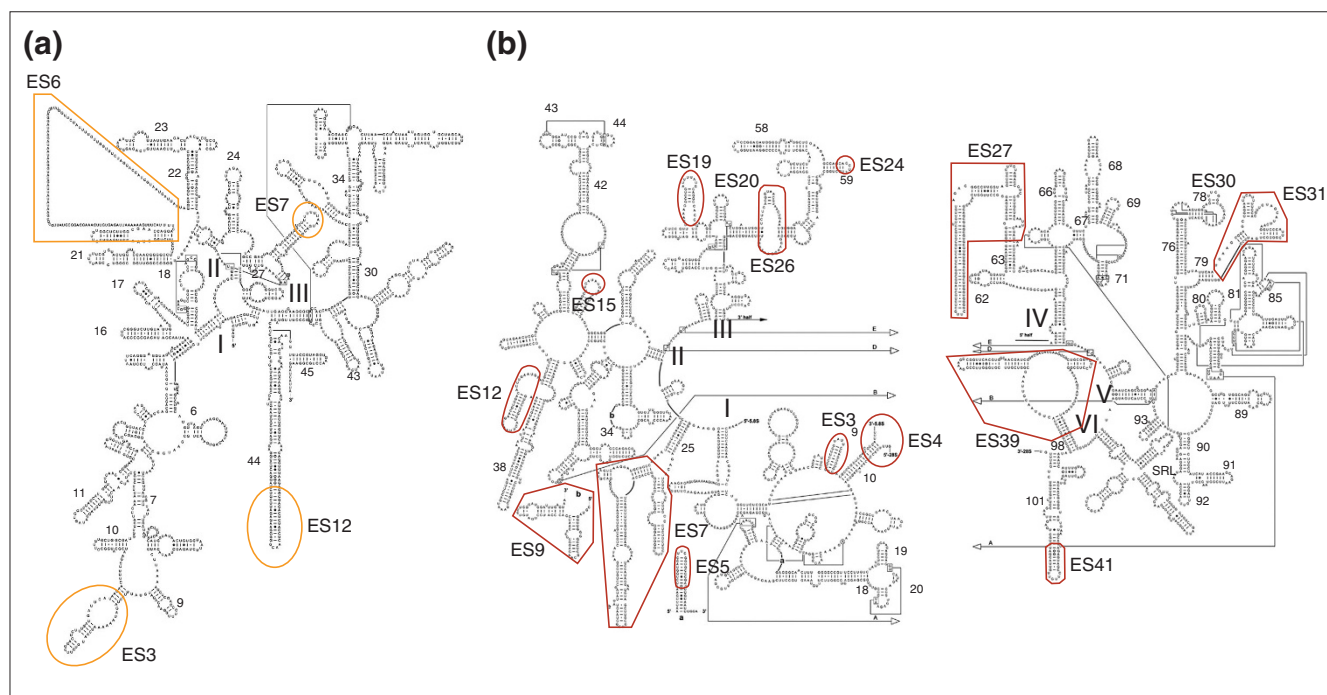


Figure 3 Expansion segments in the secondary structure of yeast ribosomal RNA. **(a)** 18S rRNA; **(b)** 5.8/25S rRNA [44]. Expansion segments (ES) are labeled using Gerbi's nomenclature [45]. Small numbers refer to the helix numbering convention; Roman numerals refer to the RNA domains. Reproduced with permission from [14].

[14,39,40]; rabbit reticulocyte [38,40]; and human (C.M.T. Spahn, E. Jan, A. Mulder, R.A. Grassucci, P. Sarnow and J.F., unpublished data)), and it appears to be a universal feature. Regarding additions affecting protein export, we know through Günther Blobel's seminal work (see [41]) of the existence of a complex apparatus (the 'translocon' for protein insertion into, or export through, the endoplasmic

reticulum membrane. The structural implications of this concept are that some of the new ribosomal proteins and RNA additions near the exit site of the polypeptide tunnel must have a role in interacting with the protein exit channel and the signal recognition particle [39,40,42]. The additional mechanisms for translational regulation and control that have evolved in eukaryotes are least understood and will

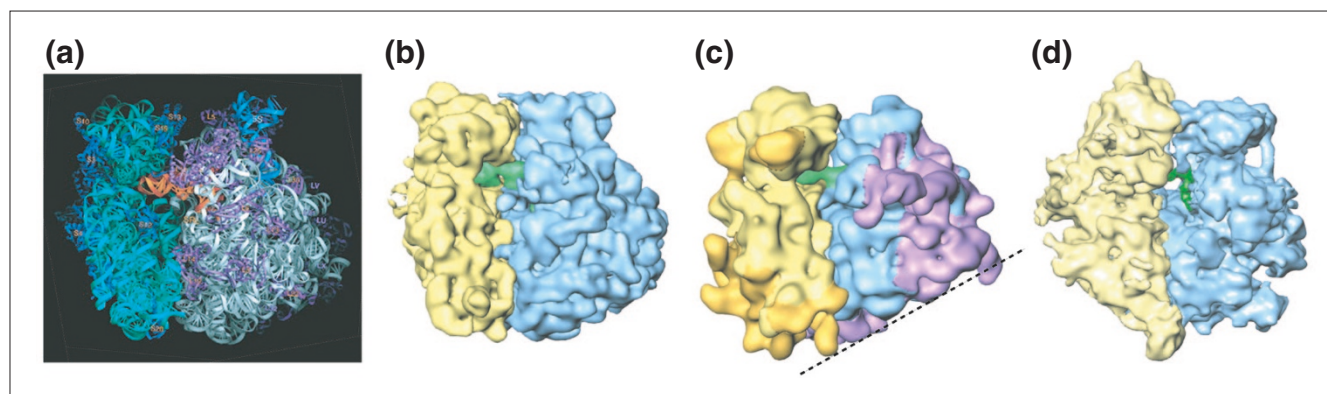


Figure 4 Structures of ribosomes from different species. The small subunit is on the left. **(a)** X-ray structure of the *T. thermophilus* 70S ribosome [12]. **(b)** Cryo-EM map of the *E. coli* 70S ribosome [46]. **(c)** Cryo-EM map of the yeast 80S ribosome [14]. Expansion regions are darker. The dashed line indicates a flat surface that suggests eukaryotic specialization of 60S subunit for association with a planar membrane. **(d)** Cryo-EM map of the mammalian mitochondrial ribosome [37]. Reproduced with permission from (a) [12], (b) [46], (c) [14] and (d) [37].

be a rich area for structural probing by cryo-EM once an X-ray structure of the eukaryotic ribosome is available. Much more work is clearly needed before a full picture of the eukaryotic ribosome can emerge.

In conclusion, thanks to recent advances in X-ray crystallography and cryo-EM of the ribosome, we now have extensive information on its structure, on the makeup of functional centers, and the beginning of an understanding of its functional dynamics. This picture, however, comes mainly from a few bacterial species. Much less is known about the structural basis of translation in eukaryotes, but first insights have come from cryo-EM of the ribosome of yeast and mammals.

Acknowledgements

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