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One core, two shells: bacterial and eukaryotic ribosomes

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Ribosomes are universally conserved enzymes that carry out protein biosynthesis. Bacterial and eukaryotic ribosomes, which share an evolutionarily conserved core, are thought to have evolved from a common ancestor by addition of proteins and RNA that bestow different functionalities to ribosomes from different domains of life. Recently, structures of the eukaryotic ribosome, determined by X-ray crystallography, have allowed us to compare these structures to previously determined structures of bacterial ribosomes. Here we describe selected bacteria- or eukaryote-specific structural features of the ribosome and discuss the functional implications of some of them.

Proteins in a cell are synthesized by ribosomes, which are RNA-based enzymes that use mRNA as a template for protein synthesis in a process called translation (**Fig. 1**). Despite the universal conservation of ribosomes, their composition varies considerably between different domains of life. For example, the ribosomal RNA/protein ratio may vary between 2:1 in bacterial ribosomes to 1:3 in some mitochondrial ribosomes, without substantial differences in molecular weights between the ribosomes. The molecular weight of the ribosomes may vary from 2.3 MDa in bacteria to 4.3 MDa in higher eukaryotes, without substantially changing the RNA/protein ratio. These differences are thought to reflect the functional divergence of ribosomes, the scope of which we are only beginning to uncover. For example, highly specialized ribosomes in mitochondria translate only 13 different mRNAs and are stably associated with membranes[1], whereas ribosomes from higher eukaryotes operate with more than 100,000 different templates and can be transported between different cell types[2].

Although it is known that ribosomes from different domains of life contain many structural and compositional differences, we do not yet understand how these differences correlate to functional divergence. For example, bacterial (such as *Thermus thermophilus*) and eukaryotic (such as *Saccharomyces cerevisiae*) ribosomes contain a total of 65 nonhomologous bacteria- and eukaryote-specific proteins, but only a few of them have well-established species-specific functions.

The first X-ray structures of bacterial and archaeal ribosomal subunits and of the complete 70S ribosome, followed by several higher-resolution structures, revealed the architecture of these complex enzymes, revolutionized our view of the processes carried out by the ribosome and described bacteria-specific features of the ribosome[3–8]. Until recently, the structure of the larger and more complex ribosomes from eukaryotes was studied mainly by cryo-EM. These studies identified the location of most eukaryote-specific expansion segments of ribosomal RNA (rRNA) and of some eukaryote-specific ribosomal proteins[9–16]. However, low-resolution cryo-EM maps precluded complete modeling and, in some cases, correct assignment of eukaryote-specific components of the 80S ribosomes[16].

In 2011, the X-ray structure of the more intricate full 80S eukaryotic ribosome from *Saccharomyces cerevisiae* was determined at 3.0-Å resolution. This structure provides a complete description of the 79 proteins and more than 5,500 RNA bases making up the eukaryotic ribosome, including 32 ribosomal proteins not observed before in X-ray structures of bacterial and archaeal ribosomes and ribosomal subunits. The structure of the 80S ribosome illuminates the precise architecture of eukaryote-specific intersubunit bridges, the conformational changes of rRNA and proteins upon relative movements of ribosomal subunits, and the inhibitory activity of stress-related protein Stm1 in translation[17]. The structures of the 40S and 60S ribosomal subunits from *Tetrahymena thermophila* were determined at 3.9-Å and 3.5-Å resolution, respectively, and provided information on the interaction with initiation factors eIF1 and eIF6 (refs. 18,19).

In this review, we describe bacterial and eukaryotic ribosomes as a conserved core, shared between the 70S and the 80S ribosomes, and two specific shells made of rRNA and protein moieties. The term 'the core' refers to the structurally conserved part of the 70S ribosomes (from *T. thermophilus* and *Escherichia coli*) and the 80S ribosomes (from *S. cerevisiae*), deduced by a standard procedure of structural alignment[20,21]. This approach ignores differences of the sequence in the common core of two ribosomes that may be physiologically important[22] but helps to highlight the major differences between the 70S and the 80S ribosomes.

Historically different names were assigned to several homologous ribosomal proteins in bacteria and eukaryotes. We use the simplified nomenclature based on protein family names (**Supplementary Table 1** and ref. 20). To simplify the discussion, we use terms 'bacteria-specific' and 'eukaryote-specific' to refer to moieties that are present only in 70S and 80S ribosomes, respectively, although some of them may also appear in ribosomes from organelles or Archaea.

Composition of bacterial and eukaryotic ribosomes

Both the 70S and the 80S ribosomes are asymmetric assemblies of more than 50 different proteins and three or four RNA chains. Each ribosomal component is present in the ribosome as a single

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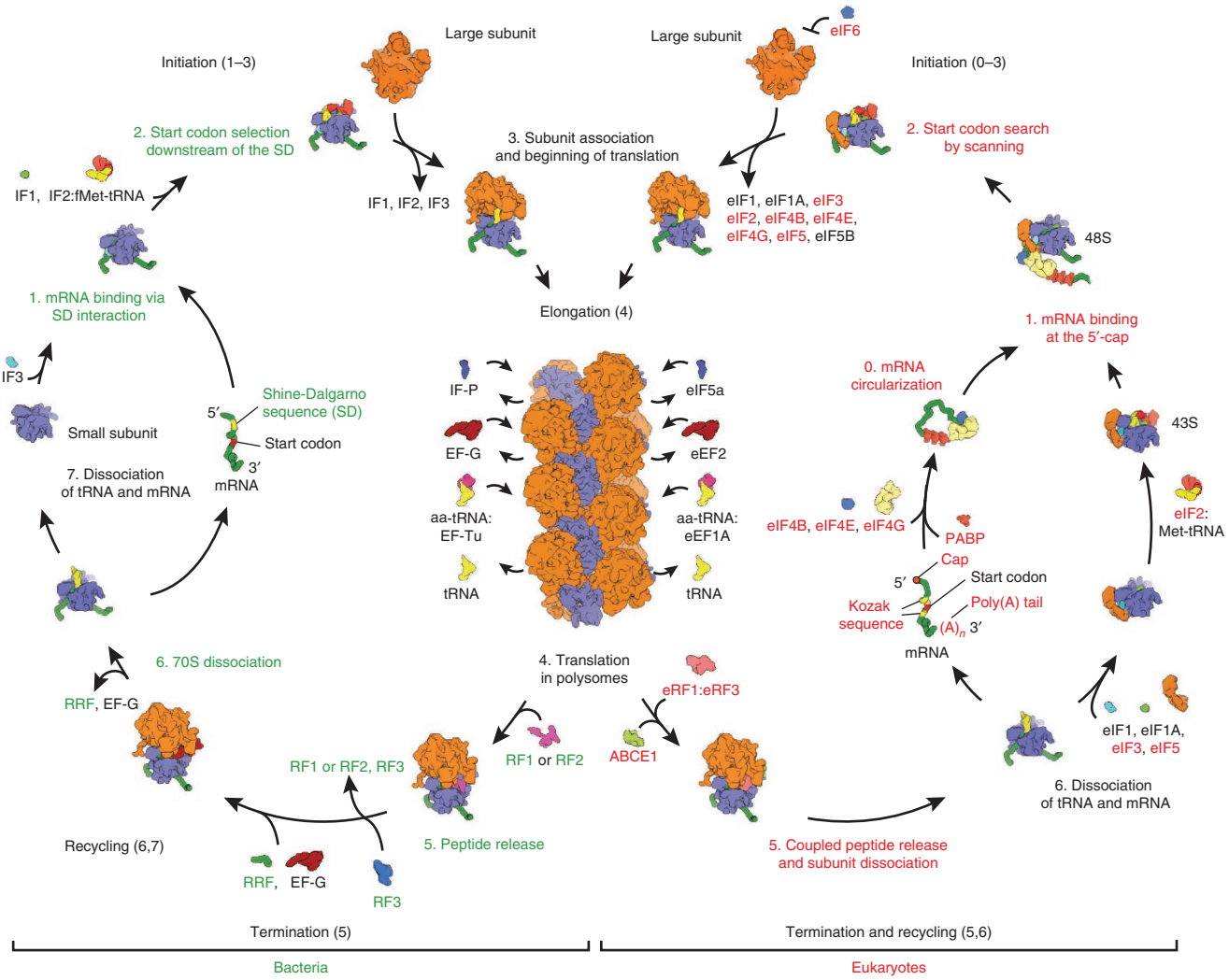


Figure 1 The translation cycle in bacteria and eukaryotes. Translation is a four-stage process that includes the steps of initiation, elongation of the polypeptide chain, termination and recycling of the ribosomes. Each of these steps is assisted by protein factors termed initiation factors (IFs in bacteria or eIFs in eukaryotes), elongation factors (EFs or eEFs), release factors (RFs or eRFs) and recycling factors. The elongation step is the most conserved between bacteria and eukaryotes and is assisted by homologous elongation factors (all homologous factors and common steps of translation are labeled black in the figure). During this step, ribosomes assemble in large helical complexes, termed polysomes, where the inner shell is occupied by the small ribosomal subunit and mRNA and the outer shell is formed by the large ribosomal subunit from which the nascent peptide emerges during translation[79–81] (**Box 1**). The other steps of the translation cycle have diverged and include several stages (indicated by numbers) that differ between bacteria (green) and eukaryotes (red). The initiation, termination and release factors catalyzing these steps include many nonhomologous proteins specific to either bacteria (green) or eukaryotes (red). aa-tRNA, aminoacyl-tRNA.

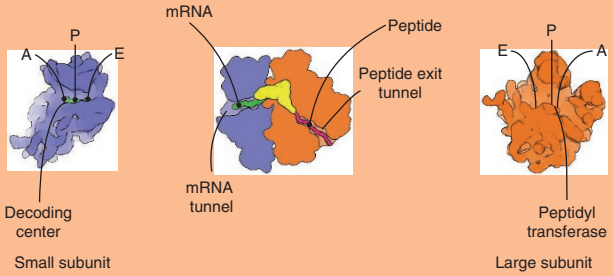
copy except for stalk proteins (L7 and L12 in bacteria, P proteins in eukaryotes) that are present in four or six copies. Early genetic data, corroborated by structural studies, revealed that bacterial and eukaryotic ribosomes share a common structural core, comprising 34 conserved proteins (15 in the small subunit and 19 in the large subunit; see **Supplementary Table 1**) and ~4,400 RNA bases, which harbors the major functional centers of the ribosomes, such as the decoding site, peptidyl transferase center and tRNA-binding sites (**Box 1**)[10,23].

Apart from the core, each of the ribosomes contains its own set of specific moieties: domain-specific proteins, insertions and extensions of conserved proteins and expansion segments of rRNAs[24,25] (**Fig. 2**). The 70S ribosome contains 20 bacteria-specific proteins (6 in the 30S subunit, 14 in the 50S subunit), a few extensions of the conserved proteins (for example, of S2, S3 and S4) and a few extensions of ribosomal RNA (for example, of helices h6, h17 and h33a in 16S rRNA, and helices H1 and H68 in 23S rRNA). The 80S ribosome contains 46 eukaryote-specific proteins (18 in the 40S subunit, 28 in the 60S subunit) and extensions and insertions in most of the proteins of the core, and the rRNA contains several extensions in the conserved rRNA chains, with a total length of nine hundred bases or more[24,25]. Most of these rRNA and protein moieties envelop the core from the solvent side and are thus accessible for potential interactions with molecular partners, such as translation factors and chaperones (see below).

The composition of ribosomes may also vary within bacteria, within eukaryotes and within one species under different conditions of growth and stress, although to a smaller extent. Within each domain of life, the ribosomes usually contain the same set of rRNA and protein chains, and all divergence is achieved via variations of

BOX 1 Core activities of the ribosome

The ribosome core consists of two ribosomal subunits (see illustration) that carry out different roles in translation. The small subunit is responsible for the decoding process where aminoacyl tRNA is selected according to the mRNA sequence. Its major functional sites are the mRNA path used to conduct mRNA during translation, the decoding center responsible for decoding, and the tRNA binding sites (A, P and E). The A site serves to bind aminoacyl-tRNA as it enters the ribosome during protein synthesis, the P site holds tRNA carrying the nascent polypeptide chain (peptidyl-tRNA, in yellow in the illustration), and the E site (exit) is where tRNA dissociates from the ribosome. During translation, tRNAs translocate from the A to the P site and from the P to the E site.



The large subunit catalyzes peptide bond formation. Its major functional sites are the tRNA binding sites (A, P and E), the peptide exit tunnel that extends through the body of the large subunit, and the peptidyl transferase center (PTC). The PTC is responsible for peptide bond formation and is located at the entrance to the peptide tunnel in a conserved region on the interface that is mainly composed of rRNA. As a result of peptide bond formation in the PTC, the nascent polypeptide chain is transferred from the peptidyl-tRNA in the P site to the aa-tRNA in the A site, thus extending the nascent chain by one amino acid.

length and sequence of ribosomal components, mainly rRNA. In some species, the larger size of ribosomal components creates new structural features of the ribosome[26,27]. One example is the 'turret' and the 'spire' formed by rRNA expansions on the solvent side of the 40S ribosomal subunit from *Trypanosoma cruzi*, which are thought to be related to the unusual structure of mRNAs in this species[27]. In eukaryotes, the size of the 80S ribosome varies within an ~1-MDa range, mainly owing to insertions in four RNA expansion segments in 25S–28S rRNA (ES7L, ES15L, ES27L and ES39L)[15]. In a few cases, ribosomes contain one less or additional ribosomal proteins. For example, the thermophilic bacteria *T. thermophilus* contains an additional protein, Thx, that is buried in 16S rRNA and stabilizes its structure[28]. In the budding yeasts, one ribosomal protein is missing (L28e), which is thought to be the result of a gene loss[24]. Under different conditions of growth and stress, the composition of ribosomes may vary within one species[29–36]. The most striking example of such a variation is the ribosome from the human malaria parasite *Plasmodium falciparum*, which expresses different isoforms of rRNA at different stages of the parasite life cycle[36].

The small subunit in bacteria and eukaryotes

The 30S and 40S subunits have similar shapes, including the landmarks known as 'head', 'body', 'platform', 'beak' and 'shoulder' (**Fig. 3a–d**). The mRNA- and the three tRNA-binding sites (A, P and E) are located on the subunit interface. The mRNA enters through a tunnel located between the head and the shoulder and wraps around the neck of the 30S subunit. The mRNA exit site (5′ end of the mRNA) is located between the head and the platform[37,38]. The decoding center of the small subunit, where the codon and anticodon are paired and which convey fidelity to mRNA decoding, is located on the interface surface and is made up of three domains from the head, shoulder and the penultimate stem. When comparing the overall structures, it is evident that there are extensive differences between eukaryotes and bacteria on the small ribosomal subunit solvent side (**Fig. 3c,d**). These differences are directly correlated to the much more complex pathway of translation initiation known to exist in eukaryotic cells.

mRNA exit site and implication for mRNA recruitment. Bacteria and eukaryotes use different strategies to recruit mRNA (**Fig. 1**). In bacteria, the 30S subunit binds mRNA directly in the vicinity of the start codon. This process is mediated by the Shine-Dalgarno sequence, a unique feature of bacterial mRNAs located upstream of the start codon[39]. This sequence interacts with a complementary sequence

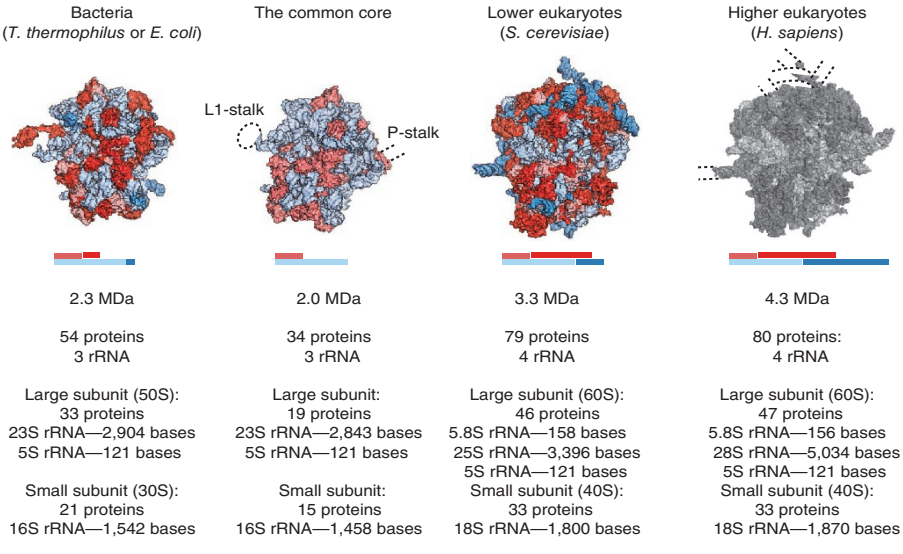


Figure 2 Composition of bacterial and eukaryotic ribosomes and the common core. Bacterial and eukaryotic ribosomes share a conserved core composed of RNA (light blue) and proteins (light red). In addition to the core, ribosomes in each domain of life contain their own set of proteins, extensions and insertions in conserved proteins (both in red), and extension segments in ribosomal RNA (blue). 5.8S and 25S–28S rRNA are both homologous to 23S rRNA in bacteria. Dashed lines around the core indicate positions of flexible stalks of the ribosomes that are usually disordered in X-ray structures. For simplicity these lines are not shown in the other structures. The 80S structure of higher eukaryotes has not yet been determined but is predicted to be highly similar to the structures of yeast and *T. thermophila* ribosomes, based on genetic analysis and cryo-EM studies. Therefore, instead of the human ribosome structure, the yeast 80S structure is shown, in gray with dashed lines indicating the positions of human-specific long rRNA expansion segments, the major distinctive feature of ribosomes from higher eukaryotes. The figure is based on X-ray and cryo-EM structures from refs. 15,20,55,82–84.

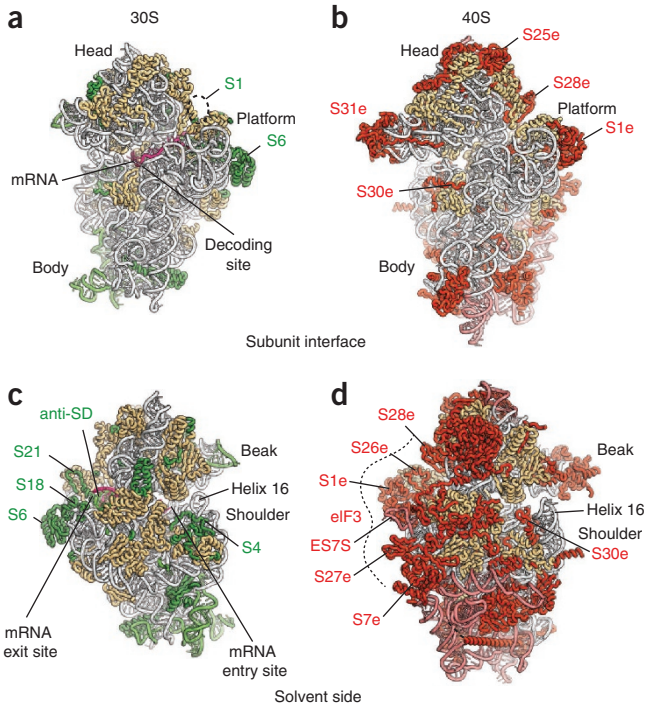


Figure 3 Bacteria- and eukaryote-specific proteins and RNA expansions of the small ribosomal subunit. (**a**–**d**) The common core is shown in white (rRNA) and light orange (proteins); bacteria-specific moieties of the 30S subunit (PDB 3I8H[38]; PDB 3R8N[82]) are shown in green (**a**,**c**), and eukaryote-specific moieties of the 40S subunit (PDB 3U5B and 3U5C[20]) are shown in red (**b**,**d**). For simplicity, extensions and insertions in conserved proteins are not labeled unless they are discussed in the main text. Approximate binding sites for S1 and eIF3 (dashed lines) are shown as in ref. 44 and ref. 46, respectively.

(anti–Shine-Dalgarno or anti-SD) at the 3′ end of the 16S rRNA, which ensures correct placement of the start codon. Crystal structures of ribosomes in complex with mRNAs revealed that Shine-Dalgarno binding results in formation of a helix, which is located in the cleft on the platform[37,38,40–42]. In the 30S subunit, the mRNA exit site is surrounded by four of six bacteria-specific proteins: S1, S6, S18 and S21 (**Fig. 3a**,**c**)[4,8]. Protein S1 (which is not present in all bacteria) participates in mRNA recruitment to the 30S subunit during translation initiation through binding mRNAs 5′ upstream of their start codon[43]. Its location on the solvent side of the small subunit was visualized by cryo-EM studies and correlates with its accessibility for mRNAs[44]. The function of proteins S6, S18 and S21 (all three are located on the platform) is unclear, although proteins S18 and S21 were suggested to modulate interactions between Shine-Dalgarno and anti-SD sequences[37,40,41]. The location of S21 (which is not present in *T. thermophilus*) in crystal structures of vacant *E. coli* ribosomes slightly overlaps with the Shine-Dalgarno duplex position in the ribosome complexes from *T. thermophilus*, suggesting that S21 interacts with the Shine-Dalgarno–anti-SD duplex in *E. coli*[8].

In the eukaryotes, mRNA is recruited through a unique cap feature at the 5′ end of eukaryotic mRNAs to the 43S pre-initiation complex (that is, the 40S subunit in complex with eIF1, eIF1A, eIF2, eIF3, eIF5 and initiator tRNA). This results in formation of a 48S pre-initiation complex. In the 40S subunit, the locations corresponding to the bacterial proteins S6, S18 and S21 are occupied by proteins S1e and S26e. Compared to the 16S rRNA, the 3′ end of the 18S rRNA is shortened and covered by protein S26e[18,20], consistent with the absence of Shine-Dalgarno sequences in eukaryotic mRNAs.

The solvent side around the mRNA exit site of the 40S subunit contains many unique proteins and rRNA expansion segments that have no analogs in the 30S subunit. These include eukaryote-specific proteins and RNA, such as proteins S7e, S17e, S25e, S27e and S28e, RNA expansion segment ES7S and a eukaryote-specific RNA cluster, formed by ES6S. The exact function of these elements is unclear, but they are likely to be associated with eukaryote-specific processes of the translation initiation. There is some evidence from cross-linking and cryo-EM studies of the 40S ribosomal subunit that this area participates in the binding of eIF3, which in turn mediates mRNA recruitment to the ribosome during translation initiation[45–47].

mRNA entry site and implication for scanning. Bacteria and eukaryotes use different strategies to find the start codon within mRNA during translation initiation. In bacteria, start-codon selection is dictated by the Shine-Dalgarno sequence that ensures correct positioning of the start codon on the small subunit. In eukaryotes, the start codon might be located several hundred residues upstream from the point of mRNA attachment, and its location on the ribosome requires 5′–3′ mRNA scanning[48,49].

In bacteria, proteins S3, S4 and S5 form the mRNA entry tunnel[37]. At the mRNA entry site in bacteria, the universally conserved helix 16 (h16) of the small ribosomal subunit is held in a conformation where it is bent toward protein S3 by a bacteria-specific domain of protein S4 that virtually covers a large part of h16 (**Fig. 3c**). However, because this domain does not exist in eukaryotes, h16 is here in a completely different orientation, where it extends away from the ribosome body[20] (**Fig. 3d**). Although protein S30e is located at the base of h16 in eukaryotes, it does not seem to prevent h16 from being able to adopt different orientations in the 80S structure (**Supplementary Fig. 1**). This conformational flexibility of h16 is very relevant for the current model of mRNA scanning. This model proposes that the binding of factors eIF1 and eIF1A to the 40S subunit stimulates scanning by inducing h16 to adopt a closed orientation, which stabilizes opening of the mRNA entry tunnel latch[48,50] and allows scanning to take place.

The large subunit in bacteria and eukaryotes

The 50S and the 60S subunits have similar overall crown-like shapes, which include the 'central protuberance', 'L1-stalk' and the 'L7/L12-stalk' ('P-stalk' in eukaryotes) (**Fig. 4**). On the 60S ribosomal subunit, 27 eukaryote-specific proteins, multiple insertions and extensions of conserved proteins and several rRNA expansion segments are concentrated on the periphery of the subunit forming an almost continuous ring-shaped assembly enveloping the core (**Fig. 4b**,**d**). This ring-shaped assembly comprises two clusters of eukaryote-specific moieties, for which little is known in terms of biological function.

Located on the interface side of the large ribosomal subunit are the three (A, P and E) tRNA-binding sites and the peptidyl transferase center where the peptide bond formation is catalyzed. This peptidyl transferase center is adjacent to the entrance of a tunnel along which nascent proteins progress before they emerge from the ribosome on the solvent side. The overall absence of bacteria- and eukaryote-specific moieties on the central regions of both the solvent and interface sides of the subunit is consistent with the universally conserved functions of these areas. This is seen at the peptidyl transferase center on the intersubunit surface that is relatively devoid of bacteria- and eukaryote-specific moieties as well as around the peptide tunnel on the solvent side, which is used for ribosome association

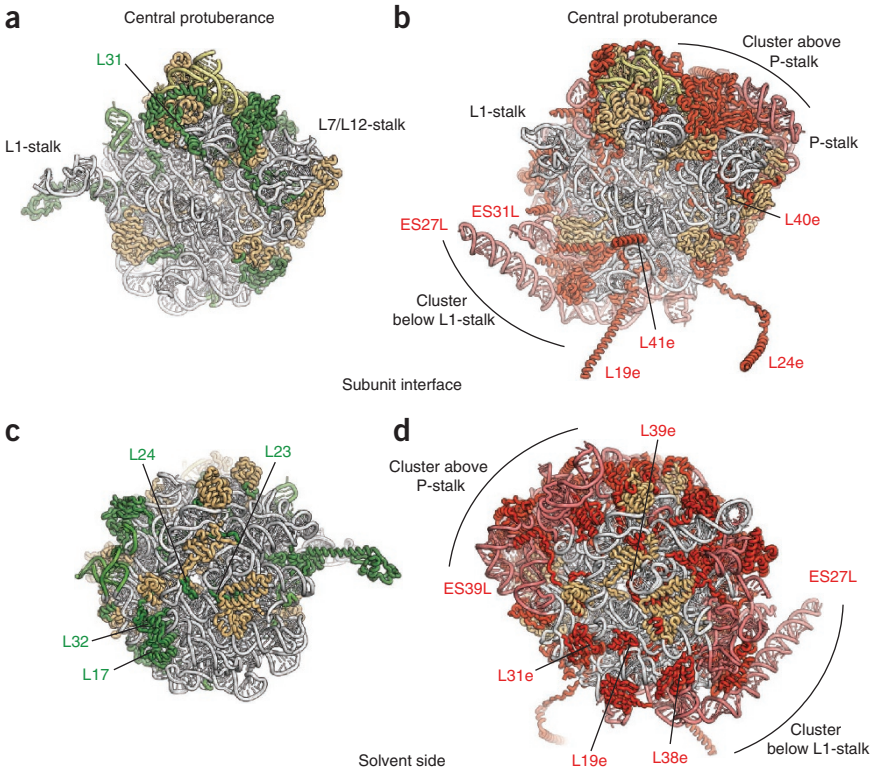


Figure 4 Bacteria- and eukaryote-specific proteins and RNA expansions of the large ribosomal subunit. (**a**–**d**) The common core is shown in white (rRNA) and light orange (proteins); bacteria-specific moieties of the 50S subunit (PDB 3I8I[38], 3R8S[82]) are shown in green (**a**,**c**); eukaryote-specific moieties of the 60S subunit (PDB 3U5D, 3U5E, 3U5H and 3U5I[20] and PDB 3IZD[15]) are shown in red (**b**,**d**). For simplicity, the proteins of flexible stalks are not shown.

with membranes during protein synthesis (**Fig. 4a**–**d**). There are, however, important structural differences between the 50S and the 60S subunits—for example, in the organization of the peptide tunnel and the surrounding area—which can be understood in terms of functional divergence.

The peptide exit tunnel and its surroundings. During translation, the growing peptide chain passes through the peptide exit tunnel to emerge at the solvent side—the place of its processing and folding. In bacteria, the tunnel walls are formed mainly by the conserved parts of the 23S rRNA and contain loops of proteins L4, L22 and a bacteria-specific extension of L23 (refs. 3,6). In eukaryotes, the area corresponding to the bacteria-specific moieties of L23 overlaps with protein L39e[19,20]. In both the 50S and the 60S subunits, proteins L4 and L22 form a constriction of the tunnel, located ~30 Å from the peptidyl transferase center. In eukaryotes, the constriction is narrower because of insertions in protein L4. Although the role of these differences between bacteria and eukaryotes is unclear, it is suggested that the narrower size of the constriction in eukaryotes may block the access of some macrolide antibiotics to the peptidyl transferase center[22,51]. These antibiotics are thought to be delivered to the binding site through the tunnel. Genetic studies have shown that insertion of six amino acids in the loop of L4 in *E. coli* endows bacterial ribosomes with a resistance to larger-size macrolides that is similar to what is found in eukaryotes[51].

On the solvent side, the rim of the polypeptide exit tunnel contains several bacteria- or eukaryote-specific proteins and protein extensions: L17, L32 and an insertion in L24 in bacteria; and proteins L19e and L31e in eukaryotes (**Fig. 4c**,**d**). These differences are, in part, associated with the different processing of N termini of nascent chains in bacteria and eukaryotes. In bacteria, nascent peptides contain a formyl group at the N terminus. This is due to the special modification of aminoacylated initiator tRNA (Met-tRNA^{fMet}), which is formylated to promote its recognition by initiation factor IF2 (**Fig. 1**). During protein synthesis, the formyl group is cleaved by the bacteria-specific enzyme peptide deformylase, which associates with ribosomes through protein L32 (ref. 52). As initiator Met-tRNA^{Met} is not formylated in eukaryotes, the positions corresponding to L17 and L32 on the 60S subunit are occupied by the nonhomologous protein L31e, which is associated with a different activity. In yeasts, L31e interacts with the protein Zuotin, a component of a eukaryote-specific chaperone complex that is involved in co-translational folding of the growing polypeptide[53].

Specific features of the intersubunit surface

Interaction between ribosomal subunits is maintained by several contact points of the interface, called bridges. There are seven bridges in the ribosomal core and a few bacteria- and eukaryote-specific bridges[5,7,20,54]. Recent structural studies of bacterial and eukaryotic ribosomes revealed several unusual new bridges that are markedly distinct from bridges of the core. Each of these unusual bridges are formed by a ribosomal protein of the large subunit, which binds to the small subunit through substantial parts of their structure[20]. In bacteria, one intersubunit bridge of this type is formed by protein L31, which is conserved among bacteria[55] (**Fig. 4a** and **Supplementary Fig. 2**). The N-terminal domain of L31 is located on the central protuberance of the 50S subunit, whereas the C-terminal domain is bound to the labile head domain of the 30S subunit. It was proposed that L31 may modulate the initial swiveling movements of the 30S head domain, which were described as accompanying the ratchet-like movement of the small subunit relative to the large one[55]. In eukaryotes, there are two similar intersubunit bridges, which are formed by the 60S proteins L19e and L24e that are bound to the 40S subunit by two 'arms' on opposite sides (**Fig. 4b** and **Supplementary Fig. 2**)[20,54]. There is evidence suggesting that L19e and L24e may have roles in initiation and reinitiation processes[56–58].

In the 80S ribosome, there is another unusual bridge formed by L41e (**Fig. 4b**), the smallest protein in yeast cells (25 amino acids). L41e is located in the middle of the interface and is almost entirely associated with the 18S rRNA. Although it forms only minor contacts with the 60S, it remains a part of the large subunit upon dissociation.

Other specific features of the intersubunit surface, revealed in the recent structure of the full eukaryotic ribosome, are the proteins S31e (**Fig. 3b**) and L40e (**Fig. 4b**). In eukaryotes, these proteins are produced as fusions with ubiquitin, which is used as a tag to direct other proteins for degradation[59]. Ubiquitin is cleaved from ribosomal proteins, although little is currently known about whether this cleavage occurs before or after S31e and L40e are assembled into the ribosome[59]. The N-terminal tails of S31e and L40e, corresponding to the cleavage site of ubiquitin fusions, are not buried in rRNA, making it possible for ubiquitin

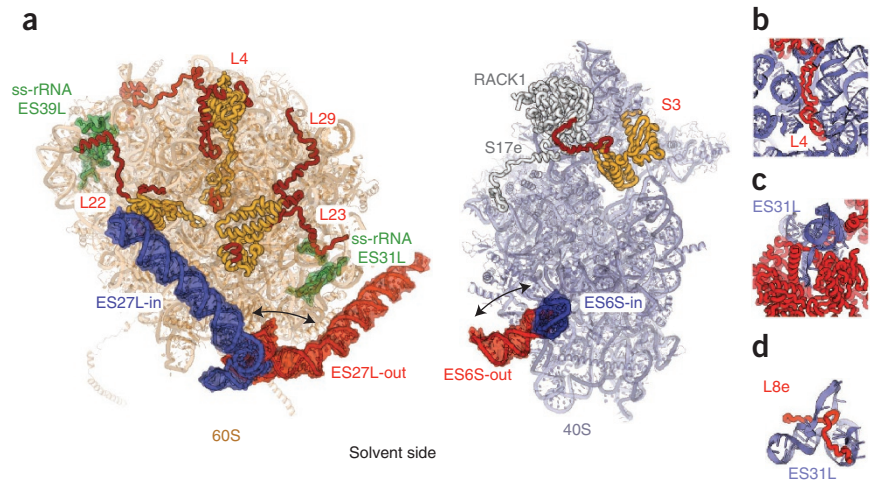


Figure 5 Distinctive features of the 80S ribosome structure. (**a**) Subunits are shown as semitransparent cartoons. Several ribosomal proteins (L4, L22, L23, L29 and S3) are colored as in **Figures 3** and **4**. The proteins RACK1 and S17e are shown in gray to highlight protein-protein interactions of the S3 tail. Single-stranded stretches of ribosomal rRNA (ss-rRNA) are labeled. The suffixes '-in' and '-out' indicate two conformations of RNA expansion segments ES27L and ES6S. (**b**–**d**) rRNA is colored blue, proteins red. (**b**) A loop of L4 (red) in the core stabilizes the three-dimensional fold of rRNA helices (blue). (**c**) The protein cluster around ES31L shows how large assemblies of ribosomal proteins surround a loop of single-stranded rRNA. (**d**) The tail of protein L8e is inserted into an rRNA-helix junction in ES31L. The modeled positions of ES27L were taken from ref. 15.

cleavage from these ribosomal proteins to occur after their incorporation into nascent ribosomal subunits. It was suggested that ubiquitin may prevent subunit association before their complete maturation, which is a common strategy used by factors of ribosome biogenesis[19,60,61].

Novel features of the 80S ribosome

The 80S ribosomes acquired several architecture- and assembly-related features not previously observed in the structures of bacterial ribosomes and the archaeal 50S subunit. These features include long dynamic rRNA helices on the solvent side of both subunits, larger protein clusters assembled around single-stranded rRNA and unusual interactions mediated by protein tails (**Fig. 5a**).

Eukaryote-specific protein tails. Many ribosomal proteins have unusual folds and contain remarkably long tails and loops extending from globular domains[18–20]. Most of them are not buried between rRNA, as in the 70S ribosomes, but are located on the surface of the 80S, where they are mostly associated with other eukaryote-specific moieties (**Fig. 5a**,**b**). It is believed that during the early steps of ribosome biogenesis, ribosomal proteins use globular domains to recognize specific regions of rRNA, whereas their protein tails and loops assist in folding of rRNA by neutralizing the negative charge of RNA phosphates and mediating RNA-RNA interactions[62,63]. With the crystal structure determination of the eukaryotic ribosome, it became clear that these unusual protein folds are even more abundant in eukaryotes (for example, extensions of proteins L4, L22, L23, L29 and S3; **Fig. 5a**) than in bacteria. Most of these insertions and extensions interact with other eukaryote-specific proteins and rRNA expansion segments (for example, L4 with ES7L and ES15L, and L22 with ES39L), suggesting that extensions of the conserved proteins may assist the assembly of eukaryote-specific components of the 80S ribosomes during biogenesis.

RNA expansion segments. The function of these unique features of the 80S ribosome is one of the major unanswered questions of the 80S ribosome biology. Structure determination of the eukaryotic ribosome permitted a detailed analysis of the yeast expansion segments. Structurally, the rRNA expansion segments can be divided into two types[15]. Expansion segments of the first type are grouped in eukaryote-specific RNA-protein clusters and are tightly associated with ribosomal proteins or other rRNA expansion segments. The two most striking examples are ES31L and ES39L, which occupy the central part of two major eukaryote-specific clusters of the 60S subunit (**Fig. 4b,d**). Both ES31L and ES39L contain stretches of a single-stranded rRNA that are surrounded by several ribosomal proteins (**Fig. 5a** and ES31L in **Fig. 5c,d**). The structures of assemblies around ES31L and ES39L were found to be similar to other protein complexes assembled around single-stranded RNA[64].

The second type of RNA expansion comprises long rRNA helices that are attached to the ribosome only at their bases. These helices can adopt different conformations, as described in detail for the flexible helix of ES6S and for ES27L, although their functional role is not fully understood (**Fig. 5a**)[15,20]. However, some data concerning the biological function of these long and exposed helices exist[65–67]. The best studied ES27L was suggested to dock nonribosomal factors, such as chaperones and modifying enzymes, to the nascent chain emerging from the peptide exit tunnel[68]. Another indirect insight into the function of ES27L came from the recent study of protein L38e (**Fig. 4d**)[69], which stabilizes an intermediate conformation of ES27L. It was found that deletion of the gene that encodes L38e in mice selectively affects the 80S ribosome association with a small group of developmentally related mRNAs.

Other examples of the flexibility of expansion segments (ES7L, ES15L, ES27L and ES39L) were shown in low-resolution cryo-EM maps of the mammalian ribosomes (the main difference in higher eukaryotes is that already existing expansion segments have been considerably increased in length; **Fig. 2**). The biological role of these dynamic rRNA expansion segments is largely unknown, but their accessibility on the 60S subunit surface suggests a possible involvement in recruitment of unknown ribosomal factors and partners[15].

Perspectives

The crystal structures of eukaryotic ribosomes showed that the 80S ribosomes are not merely larger than their bacterial counterparts but also contain unique structural features, many of which have still not been studied functionally. We expect that the successful structure determination of the yeast 80S ribosomes will facilitate biochemical, genetic and cryo-EM studies of the initiation and regulation of translation, ribosome biogenesis, ribosome-associated processes of mRNA decay and protein degradation, and other translation research that exploits X-ray structures[70–76].

Further developments in the isolation, purification and crystallization methods for the 80S ribosome and ribosomal subunits from higher eukaryotes will allow for structural studies of functional complexes. These complexes could be formed with functional ligands such as transfer RNAs and different types of mRNA—for example, with internal ribosome entry sites (IRES) and translation factors.

Structures of functional complexes will give snapshots of the ribosome in action, in order to increase our understanding of the fundamental aspects of eukaryotic protein synthesis.

One of the unexploited directions of research is the elucidation of the function of the long dynamic rRNA expansion segments. These features are exposed on the ribosome surface for possible interactions with various partners. Because of the high abundance of ribosomes in eukaryotic cells, these features correspond to several percent of the total cellular RNA and constitute the major part of the weight difference between the yeast and human ribosomes[15,77]. Surprisingly, to date not a single protein has been found to interact with these features of eukaryotic ribosomes.

The availability of highly detailed structures of eukaryotic ribosomes allows us to sort through differences in the interpretation of already existing molecular and biochemical data. The crystal structures of the eukaryotic ribosomes will provide a tool for studying the details of, for example, attachment of mRNA on the 43S pre-initiation complex, where there are currently experimental discrepancies.

Additionally, the functions of many eukaryote-specific proteins are largely unknown. Some have been shown to be related to the intricate pathways of ribosome biogenesis. Ribosome biogenesis is currently one of the least studied processes in ribosome biology, even though it is the most energy-demanding process in the cell and requires more than 200 assembly cofactors in eukaryotes[78]. Crystal structures of the eukaryotic small subunit have already been used to interpret cryo-EM maps of biogenesis intermediates, but additional studies are still needed to complete our understanding of the order and dynamics of ribosome biogenesis[61].

Yeast has for many years been a model organism for studying genetics. With the availability of the structure of the 80S eukaryotic ribosome from this particular organism, it is now possible to combine yeast genetics with structural studies.

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