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Structure and Function of the Eukaryotic Ribosome: The Next Frontier

Minireview

Jennifer A. Doudna[1,3] and Virginia L. Rath[2,3]
[1]Department of Molecular Biophysics
and Biochemistry and
Howard Hughes Medical Institute
Yale University
New Haven, Connecticut 06520
[2]Department of Molecular and Cell Biology
University of California, Berkeley
Berkeley, California 94720

As the catalytic and regulatory centers of protein synthesis in cells, ribosomes are central to many aspects of cell and structural biology. Recent work highlights the unique properties and complexity of eukaryotic ribosomes and their component rRNAs and proteins.

Ribosomal RNAs, the most abundant cellular RNA species, have evolved as the catalytic, organizational, and regulatory hub of protein biosynthesis in all cells. These RNAs form the bulk of the ribosome, a large RNA-protein particle whose two subunits together decode messenger RNAs and synthesize the corresponding polypeptides. The structure and function of ribosomes have been studied extensively over the course of the last half-century, culminating in the recent publication of crystal structures of the prokaryotic 50S and 30S ribosomal subunits and the intact 70S ribosome. These crystallographic results revealed unequivocally that the ribosome is a ribozyme, confirming a starring role for ribosomal RNA as the catalyst of peptide bond formation. In addition to spurring enormous progress in understanding the translational mechanism and the mode of antibiotic inhibitors of ribosome function (Ramakrishnan, 2002), they have stimulated new interest in extending our understanding to the more complicated eukaryotic ribosome. How does this complex machine assemble inside cells? What unique activities are conferred by the additional rRNA and proteins that increase its mass by ~30% relative to bacterial ribosomes? And how does the eukaryotic ribosome interact with cellular and viral factors that regulate its function? These questions are beginning to be addressed using a combination of genetic, biochemical, and structural approaches.

In the mature 80S eukaryotic ribosome, the 60S subunit and the smaller 40S subunit together contain about 80 proteins articulated around highly modified ribosomal RNAs (rRNAs). While early in vitro experiments to reconstitute prokaryotic ribosomes suggested that all necessary information was contained in the purified rRNA and protein components, it is now clear that approximately some 100 accessory proteins and a similar number of small nucleolar RNAs are involved in ribosome assembly in yeast (Warner, 2001). These assembly factors participate in a series of complicated pathways throughout the cell that mediate all aspects of ribosome biogenesis.

[3]Correspondence: jennifer.doudna@yale.edu (J.A.D.), vlrath@lbl.gov (V.L.R.)

All three RNA polymerases are required: RNA polymerase I (Pol 1) makes the 28S, 18S, and 5.8S rRNAs, Pol II produces the messenger RNAs encoding ribosomal proteins and Pol III synthesizes the remaining 5S rRNA. These building blocks come together in the nucleolus as preribosomal particles, cross the nucleoplasm, exit through nuclear pores, and mature into functional ribosomes in the cytoplasm, requiring coordination in several cellular compartments. This assembly pathway is likely to be closely coupled to ribosome function, producing ribosomes at the rates and cellular locations required for various activities. For example, the ribosome participates directly in polypeptide transport across membranes and the correct insertion of polytypic membrane proteins into specific bilayers. Furthermore, eukaryotic ribosomes are highly regulated by an array of initiation and elongation factors, many of which differ from those associated with prokaryotic ribosomes. Here, we focus on recent structural work on the eukaryotic ribosome, highlighting both the exceptional progress in this field and the exciting questions that remain.

Structural Studies of the Eukaryotic Ribosome

The eukaryotic ribosome contains an additional rRNA molecule and 20–30 more proteins compared to prokaryotic ribosomes, yet similarities between many components suggested that the ribosome "core" is conserved in all organisms. This fundamental conservation, as well as some fascinating differences, have been revealed recently in a reconstruction of the yeast 80S ribosome at 15 Å resolution by cryo-electron microscopy (Spahn et al., 2001a). Cryo-electron microscopy (cryo-EM) is an attractive approach to the study of ribosome structures because the requirements for sample amounts and purity are considerably lower than typically necessary for obtaining crystals. Furthermore, conformational dynamics of ribosomes and their complexes are readily visualized.

The small subunit of the ribosome is responsible for binding and decoding messenger RNAs by ensuring correct base pairing between codons and aminoacylated tRNAs. In the recent work, the 40S subunit of *S. cerevisiae* shows the classical division into head, body, and platform comprising the 1798 nucleotide long 18S rRNA and 32 ribosomal proteins (Figure 1). The 18S rRNA is 256 nucleotides longer than the 16S rRNA of *E. coli* and the yeast 40S subunit contains 11 more proteins. Outside of the conserved rRNA core, intriguing conformational changes between the yeast 40S and bacterial 30S subunits are observed that may be functionally important. For example, helix 16 within the shoulder of the 18S rRNA in the yeast 40S subunit has the same conformation as observed in a mammalian 40S subunit, perhaps reflecting features of eukaryotic-specific translation initiation mechanisms (discussed below). Furthermore, differences in positioning of the helix 44 segment of the 18S rRNA in the lower part of the body may correlate with association of the large and small subunits into the complete ribosome (Gabashvili et al., 2000). Other differences between the 40S and 30S subunits that are not yet associated with function include changes

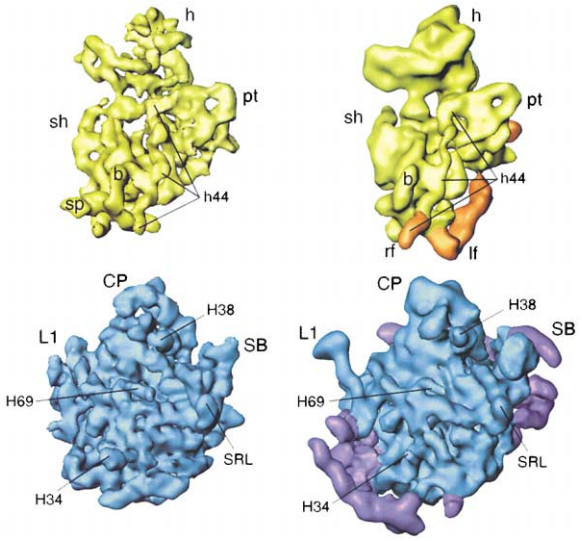

Figure 1. Comparison of EM Density for rRNA from *E. coli* and *S. cerevisiae* Ribosomes

Upper panels, rRNA density computationally identified within the small ribosomal subunit of *E. coli* (left) or *S. cerevisiae* (right), respectively. The head (h), shoulder (sh), platform (pt), spur (sp), body (b), and helix 44 (h44) are indicated; in orange, expansion segments in the *S. cerevisiae* rRNA. Lower panels, rRNA density computationally identified within the large ribosomal subunit of *E. coli* (left) or *S. cerevisiae* (right), respectively. The central protuberance (CP), stalk base (SB), sarcin-ricin loop (SRL) and several helices are indicated; in purple, expansion segments in the *S. cerevisiae* rRNA. Note that in the *E. coli* density, parts of the L1-bearing protuberance and the stalk base are missing, while in *S. cerevisiae*, these features are visible in the map.

in the shape and position of the beak (see Figure 1), a result of differences in the length of a region known as helix 33 and the presence of additional protein density. Segments of the rRNA that contain additional sequences, or insertions, in the yeast ribosome can be identified in some cases based on the EM density and sequence comparisons. A series of these expansion segments (ES) (ES12, ES6, ES3, see Figure 1) that connect the platform of the 40S with the lower part of the body form a structure of unknown function not found in the prokaryotic ribosome.

The large ribosomal subunit contains the peptidyl transferase active site responsible for catalyzing peptide bond formation during protein synthesis. In yeast, this 60S subunit includes 25S rRNA (3392 nucleotides), 5.8S rRNA (158 nucleotides), 5S rRNA (121 nucleotides), and 45 proteins. This makes the yeast 5.8S/25S rRNA 646 nucleotides longer than the corresponding bacterial (*E. coli*) rRNA and 505 nucleotides longer than the archaeal *(H. marismortui)* rRNA. The yeast large subunit has 12 and 14 proteins more, respectively, than its bacterial or archaeal counterparts. Despite these additional components, its core is structurally similar to that of the *H. marismortui* 23S rRNA. Segments of the rRNA that contact ribosomal proteins L1 and L11 were taken from the crystal structures of the bacterial *T. thermophilus* (Yusupov et al., 2001) and *T. maritima* (Wimberly et al., 1999) rRNAs, respectively, because these regions are disordered in the *H. marismortui* coordinates. Adjustments in position were required to fit these segments into the EM map, revealing large differences in the positions of the rRNA segments that contact L1 and L11. For example, helices 43 and 44 shift by some 15 Å and helix 78 moves by 30 Å. Movements of the part of the ribosome bearing L1 and L11 have also been observed in the yeast ribosome on binding to the translocation factor EF2 (Gomez-Lorenzo et al., 2000). These two regions are important in tRNA binding and release, and their mobility may be required to accomplish both tasks.

One of the more intriguing aspects of the yeast 80S ribosome images is the presence of expansion segments in the large subunit rRNA, visible as extra density in the separated RNA map that is not accounted for by the X-ray model of the archaeal large ribosomal subunit 23S rRNA. These expansion segments can be found in all domains of the 5.8S/25S rRNA, and are concentrated on approximately opposite sides of the 60S subunit. Many participate in tertiary and quaternary contacts, including two additional bridges to the 40S subunit not seen in bacteria. Ribosomal proteins not found in bacterial or archaeal large subunits were also identified, using homology modeling, but some twelve regions of unmodeled protein density are present on the solvent side of the large subunit. Interestingly, much of this density forms multiple contacts to other parts of the ribosome, implicating these additional yeast proteins in stabilizing the extra rRNA or interacting with eukaryotic-specific translation factors or regulators.

Interaction between the large and the small subunit of the ribosome is a fundamental property of translation. The small subunit binds mRNA and the anticodon portion of the tRNA and is responsible for translational fidelity by ensuring base pairing between the codon and anticodon during the decoding process. The large subunit binds the acceptor ends of the tRNAs and catalyzes peptide bond formation between the nascent polypeptide chain and the incoming aminoacylated tRNA. Both subunits are involved in translocating the mRNA by one trinucleotide codon each cycle. Previous EM work on the *E. coli* 70S ribosome combined with the X-ray crystallographic map of the *T. thermophilus* 70S ribosome identified 7 bridges between the two subunits, all of which occur in the yeast ribosome. In addition, four new bridges, which may be eukaryotic specific, can be identified from the present work. Most of the subunit interactions involve direct RNA-RNA contacts, consistent with the idea that an ancestral form of the ribosome might have been composed entirely of RNA.

A glimpse of the mechanism of tRNA recognition by a eukaryotic ribosome comes from the tRNA observed in the peptidyl or P site of the yeast 80S structure. Based on the docked models of the rRNAs, ribosomal proteins and the tRNA, Spahn et al. propose that the P site codon may interact with helix 44 of the 18S rRNA, and the tRNA with helices 24, 30, 31, and 43 of 18S rRNA as well as ribosomal protein S16. These interactions in yeast appear similar to those in bacteria, supporting the idea that the most fundamental, and ancient, activities of the ribosome are the same in all kingdoms of life (Carter et al., 2000; Yusupov et al., 2001).

Targeting Ribosomes to Membranes

In addition to synthesizing proteins, ribosomes are responsible for directing polypeptides to their correct cel-

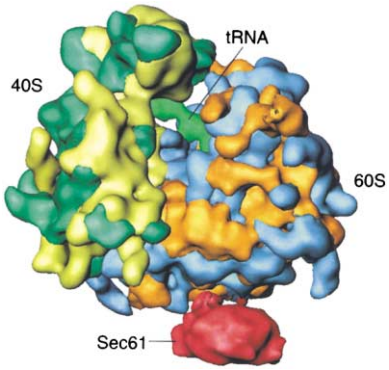

Figure 2. Yeast 80S Ribosome Bound to the Sec61 Protein Complex
Density corresponding to Sec61, red; P-site-bound peptidyl tRNA, green; 40S proteins, aqua; small subunit (18S) rRNA, yellow; 60S proteins, orange; large subunit (25S/5.8S/5S) rRNAs, blue.

lular location. Many of the approximately 30% of proteins that are inserted into or transported across cellular membranes contain an N-terminal signal sequence that is recognized during translation by the highly conserved signal recognition particle (SRP). As the signal sequence emerges from the ribosome, SRP binds and targets this ribosome-nascent chain complex (RNC) to the ER membrane. Hand-off of the nascent chain from the SRP to the translocation channel, a heterotrimeric membrane protein called, in yeast, the Sec61p complex, creates a leak-proof seal that enables cotranslational export of the polypeptide (Figure 2).

Direct association of the ribosome with the Sec61p complex in the absence of SRP or its receptor has been demonstrated in several ways, and this interaction has been visualized at a new level of detail using cryo-EM (Beckmann et al., 2001). Preparation of an appropriate sample required the clever design of an mRNA encoding a yeast membrane protein, dipeptidylaminopeptidase B (DAP2), known to be translocated cotranslationally. Yeast ribosomes were programmed in a cell-free translation system with a truncated form of this mRNA containing an N-terminal affinity tag and the first 120 amino acids of DAP2 including the signal sequence. Following translation termination by the addition of cyclohexamide, active translocating ribosomes were assembled using immunoprecipitated RNCs and a purified sample of the heterotrimeric Sec61 in a membrane free system. A control sample contained Sec61p complexed with ribosomes isolated from a translation reaction with no added mRNA.

Both samples revealed the expected overall appearance of the 80S ribosome, with additional density for the Sec61p complex visible at the exit site of the large ribosomal subunit tunnel. In both the translating and inactive ribosomes, a gap of at least 15 Å between the channel surface and the ribosome leaves the channel unsealed. This gap, observed in similar mammalian and yeast ribosomal complexes at lower resolution, leaves open the question of channel function without concomitant membrane leakage. The overall shape of the Sec61p complex in the presence or absence of mRNA is the same, appearing as a compact disc with a central indentation rather than the discrete pore previously observed for an empty ribosome channel complex in detergent (Beckmann et al., 1997). This suggests that the signal sequence itself doesn't alter the channel shape or fill the gap.

The four attachment sites observed at the outer surface of the polypeptide exit tunnel in the 60S subunit and on the Sec61p complex apparently include both ribosomal protein and rRNA components, a result which contradicts an experiment showing that the isolated large subunit rRNA alone bound to the channel with high affinity (Prinz et al., 2000). It may be that ribosomal proteins do not contribute energetically to channel docking, though this has not yet been investigated. In any case, the EM structure provides a set of predicted contacts that can now be explored genetically and biochemically, an important step toward understanding ribosome-channel communication.

Interestingly, rRNA may play an important role in regulating access to the emerging polypeptide during protein synthesis. Comparison of the translating complex with previous cryo-EM studies of yeast ribosomes reveals a significant rRNA conformational change that occurs near the polypeptide exit site. The location of a large segment of density identified as the main helix of expansion segment 27 (ES27) in 25S rRNA, one of the rRNA insertions characteristic of 80S ribosomes, adopts one of two preferred positions. In one conformation, the helix is found close to the polypeptide exit site on the ribosome, while in the other it rotates 90 degrees away from the exit tunnel and binds near the arm of ribosomal protein L1. Reconstructions of different samples reveal both conformations, and in some cases, evidence for partial occupancy of both positions in the same EM map. However, in the complex with Sec61p, ES27 is found only near L1, implying that Sec61p binding stabilizes ES27 to a locus where it cannot interfere with nascent chain translocation. This highly dynamic rRNA structure may control access of nonribosomal factors such as chaperones, modifying enzymes, or the Sec61p complex to the tunnel exit site and thereby to the emerging nascent chain.

Viral Recruitment of Ribosomes

The unique features of the eukaryotic ribosome, located primarily on its outer solvent-exposed surfaces, are prime targets for interaction with regulatory factors. These surfaces can also be exploited by viruses for ribosome recruitment during host cell infection. Many viruses circumvent cellular controls on 5′-end-dependent translation initiation by utilizing internal ribosome entry site (IRES) RNAs located within viral mRNAs upstream of coding sequences. In hepatitis C virus (HCV), the IRES binds the 40S subunit in the absence of any other factors to form a high-affinity complex that precedes translation initiation. A 20 Å resolution cryo-EM reconstruction of the mammalian 40S subunit complexed with functional and disabled forms of the HCV IRES provides clues to the mechanisms of ribosome recruitment and translation initiation (Spahn et al., 2001b).

The overall structure of the mammalian 40S subunit compares well with the 40S portion of the yeast 80S ribosome EM map described above. The IRES RNA binds on the solvent side of the 40S subunit and involves

recognition of structural elements unique to eukaryotic ribosomes. Since the HCV IRES is functional only with mammalian ribosomes, its mode of recognition may in fact be highly specific for particular rRNA, proteins, or structures. An ~100 nucleotide region at the 5′ end of the IRES, domain II, is particularly interesting because its location on the ribosome partially overlaps with the exit (E) site that houses the deacylated tRNA prior to its release after peptide bond formation. Although domain II alone does not bind the 40S subunit or contribute to IRES affinity for the 40S subunit, translation initiation is impaired without it. Domain II also appears responsible for a pronounced 40S subunit conformational change induced by IRES binding that may correlate with IRES translational efficiency. The 40S subunit head position relative to the body is altered near the beak, clamping down on the mRNA on one side while simultaneously opening up the helix 18–34 region to allow the translational start site in the mRNA to insinuate into the entry channel. This conformational change, not observed in a complex between the 40S subunit and an IRES RNA lacking domain II, suggests a key role for domain II in the correct positioning of viral mRNA. The IRES may directly place the message in the decoding center of the ribosome or stabilize the 40S subunit in a conformation necessary for mRNA binding in the absence of canonical initiation factors. Many intriguing questions remain: what is the functional relevance of the conformational change observed with the HCV IRES? Do other IRES RNAs bind and influence ribosome conformation in a similar way? Is this conformation a common feature of ribosomes primed for translation by cellular translation initiation factors? Future structural and biochemical experiments will be required to address these and other aspects of the complicated process of translation initiation.

Conclusion

As some of the most ancient cellular components, ribosomal RNAs have been crafted over evolutionary eons for efficient catalysis and regulation of all aspects of protein synthesis. Much attention has lately been focused on the conserved "core" of the ribosome, which appears to be structurally similar in all kingdoms of life. But it may well be that investigation of its less conserved rRNA and protein elements will uncover properties and activities of the eukaryotic ribosome that are unique in higher organisms. It also seems likely that understanding eukaryotic ribosomal RNA processing, chemical modification, folding, structure, and activities will illuminate fundamental aspects of cellular RNA metabolism in general. The eukaryotic ribosome represents the next frontier in cell and structural biology, whose mysteries now seem much more tractable, and more interesting, the more we know.

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