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Making ends meet: a universal driver of large ribosomal subunit biogenesis

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A common aspect of ribosome assembly, conserved across all domains of life, is the establishment of connections between the 5′ and 3′ ends of the large subunit (LSU) ribosomal RNA (rRNA) to initiate rRNA domain compaction and subunit assembly. We discuss the diverse mechanisms employed in different organisms to accomplish this important event.

Ribosomes are the macromolecular machines driving cellular protein synthesis. Correct assembly of these large ribonucleoprotein (RNP) complexes involves coordinating rRNA processing, modification, and folding with hierarchical ribosomal protein (RP) recruitment (Box 1). As well as fulfilling the catalytic function, the rRNA serves as an architectural scaffold, and a major challenge of ribosome assembly is achieving faithful construction on this highly dynamic and flexible framework. Structural and biochemical studies of ribosome biogenesis in diverse species have generated a wealth of insights revealing common features and unique characteristics of the assembly process. A key principle of eukaryotic LSU assembly is the 'ends-to-middle' order of rRNA folding and structural domain stabilization, where the domains transcribed first and last are the first to compact, followed by the internal ones [1]. Across all domains of life, LSU biogenesis is initiated by bringing together the LSU rRNA ends. While the concept of making the LSU rRNA ends meet has been discussed recurrently (e.g., [2,3]), new evidence illuminating how this is accomplished in different species reveals a surprising diversity of mechanisms involving RNA base-pairing, RNA circularization, RNA-mediated chaperoning, and RP/assembly factor bridging. Here, we contrast the various ways in which LSU rRNA end proximity is achieved in different organisms and compare LSU rRNA circularization with other examples of RNAs that make ends meet to facilitate RNP biogenesis.

Bacterial ribosomes have long served as prototypes for elucidating principles of ribosome assembly and, more than two decades ago, it was discovered that a key step in *Escherichia coli* LSU (bLSU) assembly is the establishment of base-pairing between the 5′ and 3′ ends of the bLSU rRNA (23S) [Figure 1A; Protein Data Bank (PDB) ID: 6PJ6] [4]. Complementarity between the first and last nucleotides of the bLSU rRNA, as well as the immediately flanking precursor sequences, leads to formation of a thermodynamically stable stem structure, part of which is present in mature complexes. Deletion of either end of the 23S rRNA or introduction of mutations that disrupt base-pairing dramatically impair bLSU formation, underlining the fundamental importance of this structure. Formation of the stem is necessary for both processing of the pre-bLSU transcript and binding of the key RP uL3. During biogenesis, uL3 acts as a universally conserved 'initiator' RP, the recruitment of which further stabilizes early pre-bLSU particles, facilitating downstream maturation steps.

Mitochondrial ribosomes are of bacterial ancestry but, while the ends of the human mitochondrial LSU rRNA (mLSU; PDB ID: 3J9M) are held in close proximity, they do not base-pair (Figure 1B). In this case, uL3m contacts the 3′ end of the mLSU rRNA, while another mitochondrial RP (mRP), uL22m, binds the 5′ end and bL32m bridges interactions between these two RPs. All three mRPs are recruited to very early mLSU particles [5], suggesting that bridging the mLSU rRNA ends is also important for initiating mLSU assembly. The establishment of a proteinaceous bridge between the mLSU ends is in line with the higher protein content of mitoribosomes compared to their bacterial or eukaryotic cytosolic counterparts.

A long-known general scheme for pre-rRNA processing in archaea involves base-pairing of sequences flanking the archaeal small subunit (SSU) and LSU rRNAs (aSSU and aLSU, respectively), to form a bulge-helix-bulge motif. This is then processed by a tRNA splicing-like mechanism, which is followed by less well understood endoribonucleolytic cleavage and exoribonucleolytic trimming to produce the mature rRNAs [6]. Intriguingly, in some archaea, the splicing step

Box 1. Overview of the process of ribosomal subunit assembly

Across all species and organelles, assembly of rRNAs and RPs to form functional ribosomal subunits is facilitated by assembly factors that bind transiently to pre-ribosomal particles but are not present in mature complexes. The number of such assembly factors dramatically increases with organismal complexity with ~20, 30, 80, 200, and potentially upwards of 400 being used in bacterial, mitochondrial, archaeal, yeast cytosolic, and human cytosolic ribosome assembly, respectively. Only bacterial ribosomes can self-assemble *in vitro* and, in all contexts, assembly factors facilitate efficient and high-fidelity construction as well as providing a means for regulation. A characteristic feature of archaeal and eukaryotic ribosome assembly, not used in bacteria or mitochondria, is the involvement of RNA-based assembly factors. These include both RNA modification enzymes targeted to specific rRNA nucleotides by RNA guides, and chaperone RNAs that exploit their base-pairing abilities to regulate rRNA folding and subunit assembly by tethering particular rRNA regions and/or blocking formation of aberrant rRNA–rRNA interactions. Alongside the assembly factors, the hierarchical recruitment of the ribosomal proteins also contributes to the step-wise assembly of the subunit domains, with domains I and V/VI compacting first, followed by domains II, III, and IV.

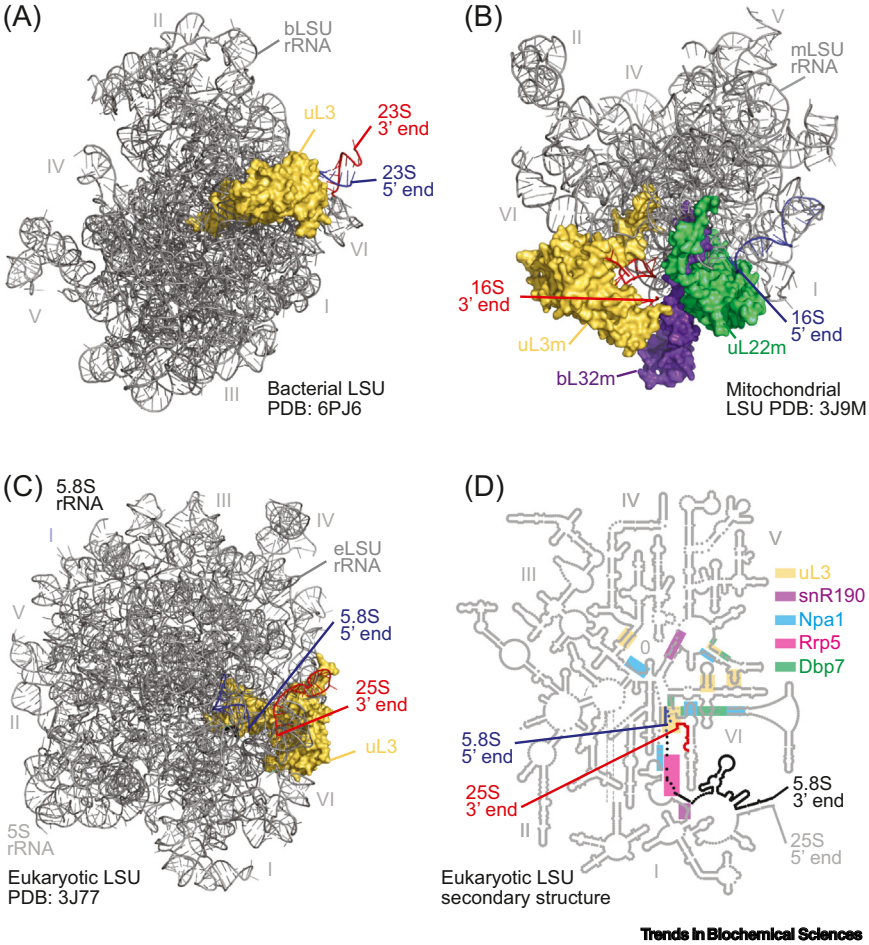

Figure 1. Structures of bacterial, and eukaryotic cytosolic and mitochondrial large ribosomal subunits (LSUs). (A) Tertiary structure of the *Escherichia coli* (b)LSU rRNA (23S) with domain numbers indicated, the nucleotides forming the terminal stem highlighted, and uL3 shown in surface view. Protein Data Bank (PBD) ID: 6PJ6. (B) Tertiary structure of the human mitochondrial (m)LSU) rRNA (16S) with domain numbers indicated, the ten 5′ and 3′ nucleotides highlighted in blue and red, respectively, and uL3, bL32m, and uL22m shown in surface view. PBD ID: 3J9M. (C) Tertiary structure of the *Saccharomyces cerevisiae* (e) LSU rRNAs with domain numbers indicated, the 5′ end of the 5.8S rRNA and the 3′ end of the 25S rRNA marked in blue and red, respectively, and uL3 shown in surface view. (D) Secondary structure of the *S. cerevisiae* eLSU rRNAs with the 5′ end of the 5.8S rRNA and the 3′ end of the 25S rRNA marked in blue and red, respectively. The base-pairing sites of the snR190 snoRNA and selected crosslinking sites of several proteins involved in rRNA end-tethering are indicated by colored boxes.

joins the mature ends, resulting in a mature circular aLSU rRNA. In *Pyrococcus furiosus*, an otherwise highly conserved helix (H98) is then excised leaving a circularly permuted aLSU rRNA [7]. It is tempting to speculate that the strategy of covalent rRNA end joining in some hyperthermophilic archaea is preferable to base-pairing due to its greater stability at higher temperatures.

During evolution, the 5′ end of the LSU rRNA was partitioned by an internal transcribed spacer in eukaryotes to form separate 5.8S and 25S/28S species. In eukaryotic pre- and mature ribosomes, the 3′ end of the 5.8S rRNA and the 5′ end of the 25S rRNA form a strong stem and the 5′ end of the 5.8S rRNA represents the 5′ end of the eukaryotic LSU (eLSU) rRNA. Structural snapshots of intermediate, late, and mature eukaryotic (pre-)LSU also reveal the close proximity of the 5′ end of the 5.8S rRNA and the 3′ end of the 25S rRNA (Figure 1C; PDB ID: 3J77) (reviewed in [8]). Notably, the pre-rRNA processing events generating these rRNA ends have been suggested to be coupled [9]. While uL3, which binds domain VI close to the 3′ end of the 25S rRNA and proximal to the 5′ end of the 5.8S rRNA (Figure 1C; PDB ID: 3J77), is recruited to early pre-eLSU particles within primordial yeast pre-LSU complexes, it recently emerged that a step-wise pathway involving several *trans*-acting assembly factors is responsible for tethering the eLSU rRNA ends. In the earliest pre-60S particles, the large assembly factors Npa1 and Rrp5 directly contact the eLSU rRNA at both the 5′ end (5.8S rRNA and 25S domain I) and the 3′ end (25S domain V/VI), thus capturing and tethering the two ends of the transcript (Figure 1D) [9,10]. Similar to Npa1, the small nucleolar RNA (snoRNA) snR190, which is highly abundant in early pre-60S particles [11], has the potential to base-pair with the root helices of both domains I and V of the 25S rRNA, likely consolidating bridging between the two terminal regions of the eLSU rRNA [12]. Both snR190 and the Npa1 complex accumulate on pre-ribosomal particles in the absence of the RNA helicase Dbp7. In contrast, recruitment of uL3 is impeded. This implies that the remodeling activity of the helicase triggers exchange of the initial tethering factors for uL3, which then helps maintain the two rRNA ends in their final positions, enabling the remaining architecture of the LSU to be correctly established [13]. Much less is known about ribosome assembly in human cells, but homologs of Rrp5 and Npa1 exist. Human SNORD12 is most similar to snR190 and base-pairs to equivalent rRNA target sequences, although it guides 2′-*O*-methylation in contrast to snR190. Perhaps another snoRNA, such as U8 (SNORD118), may contribute RNA-mediated bridging to the

rRNA end association during human eLSU biogenesis.

The importance of bringing the rRNA ends together is emphasized by the turnover of pre-ribosomal particles in which rRNA end-bridging is impaired [4,13]. Establishing contact between the rRNA ends during initial particle assembly appears to be an essential pre-requisite, licensing subsequent maturation steps. Across different species, bringing the rRNA ends together is important for rRNA maturation as well as folding. Beyond coupling pre-rRNA processing to rRNA folding, there can be several reasons why bringing the rRNA ends together is favorable for subunit assembly. Tethering the rRNA ends will significantly reduce the degrees of freedom of the rRNA scaffold, thus promoting step-wise domain compaction and facilitating recruitment of RPs. In addition, this approach has the advantage that only fully transcribed rRNAs can assemble into subunits, reducing the need for energetically costly degradation of aberrant pre-ribosomal particles.

In conclusion, the evolutionarily conserved strategy of tethering the two ends of the long LSU rRNAs to nucleate subunit assembly emerges as a common pivotal step but is accomplished by remarkably varied mechanisms in different gene expression systems. Notably, the mechanisms of protein bridging, base-pairing, and RNA-mediated chaperoning utilized to bring together LSU rRNA ends are also used to promote RNA looping in various other contexts, such as mRNA circularization to promote transcript stability and efficient translation, as well as during removal of both group I and II introns. The 'circularized' approach to initiating folding is in contrast to linear RNA folding occurring sequentially from the 5′ end, which has been described for other RNAs in the context of co-transcriptional assembly. The establishment of stable base-pairing between the 5′ and 3′ ends of tRNAs and other small noncoding RNAs, such as snoRNAs and RNase P, is known to be important for efficient RNP assembly/RNA maturation. However, the transcriptional distance between the rRNAs ends is much greater, potentially rationalizing the more complex mechanisms utilized to tether the RNA ends. Whether making the ends meet is also a conserved principle of SSU assembly remains unclear. While the bacterial SSU domains can assemble individually *in vitro*, suggesting minimal interdependence in terms of folding (reviewed in [14]), in eukaryotes, the 5′ and 3′ ends of the SSU rRNA are bound by specific subsets of initiator RPs, implying that stabilization and positioning of the ends is likely an important early maturation event [15].

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Declaration of interests

No interests are declared.

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