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Structural aspects of mitochondrial translational apparatus

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Abstract

During the last decade groundbreaking progress has been made towards the understanding of structure and function of cell's translational machinery. Cryo-electron microscopic (cryo-EM) and X-ray crystallographic structures of cytoplasmic ribosomes from several bacterial and eukaryotic species are now available in various ligand-bound states. Significant advances have also been made in structural studies on ribosomes of the cellular organelles, such as those present in the chloroplasts and mitochondria, using cryo-EM techniques. Here we review the progress made in structure determination of the mitochondrial ribosomes, with an emphasis on the mammalian mitochondrial ribosome and one of its translation initiation factors, and discuss challenges that lie ahead in obtaining their high-resolution structures.

Certain organelles of the cell, such as mitochondria and chloroplasts, possess their own translational machineries, including ribosomes [1,2]. These ribosomes synthesize proteins with very specialized and important functions, including the proteins that are involved in oxidative phosphorylation (ATP generation) and photosynthesis. Since mitochondria and chloroplasts are thought to have evolved from endosymbiotic primitive bacteria in pre-eukaryotic host cells [3,4], structures of their ribosome are expected to be more similar to that of bacterial ribosomes [5,6] rather than to that of cytoplasmic ribosomes from eukaryotes [7•,8•]. Like all ribosomes, organellar ribosomes are made up of two unequally sized subunits, a small subunit (SSU) and a large subunit (LSU), each composed of at least one ribosomal RNA (rRNA) molecule and up to several dozens of ribosomal proteins (r-proteins). The primary functions of SSU and LSU are to facilitate the processes of mRNA decoding and peptide-bond formation, respectively.

The overall biochemical composition of the chloroplast ribosomes is very similar to that of a bacterial ribosome [9,10], whereas the composition of mitochondrial ribosomes (mitoribosome) is dramatically different (Table 1). Unlike the situation with cytoplasmic ribosomes, for which several high-resolution X-ray crystallographic structures are available [5,6,7•,8•, for recent reviews see 11,12•,13•], only a few cryo-electron microscopic (cryo-EM) structures are available for the organellar ribosomes, including two mitoribosomes, one each from mammalian tissues [14] and a protistan cell [15], and two chloroplast ribosomes [9,10]. As expected, structures of the chloroplast ribosome show greater resemblance to that

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of the bacterial ribosome, while a number of distinct structural features have been identified in the structures of both mitoribosomes [for a recent review see 16•]. Unfortunately there is no structure available for the mitoribosome from yeast, where the mitochondrial translation system is best understood biochemically and genetically [for a recent review see 17•]. In this article, we first summarize the information available on overall structural organization of the mammalian and protistan mitoribosomes and then describe the recent progress in structural characterization of one of the mammalian mitochondrial translation initiation factors. Since all of the polypeptides synthesized on the mammalian mitoribosome are inserted into the mitochondrial inner membrane (mtIM) [18,19], we discuss the structural relationship between the mitoribosome and mtIM in context of the recent biochemical findings.

Structures of Mitochondrial Ribosomes

The cryo-EM structures of two mitoribosomes (Fig. 1), studied so far, have revealed several of their unique features. Both mitoribosomes are found to be highly porous structures [14,15], as compared to their bacterial counterpart despite similar molecular masses (Table 1). The porosity in structure is primarily due to the absence of several bacterial rRNA segments in those mitoribosomes [see ref 16•, for comparative secondary structures of rRNA]. Even though the proportion of r-proteins is significantly increased as compared to that in the bacterial ribosome [20,21], many of new protein masses occupy new spatial positions in the mitoribosome structures. Thus, there is limited structural compensation for missing rRNA segments by new, or enlarged homologs of bacterial, r-proteins. For example, in the mammalian (*Bos taurus*) mitoribosome, only ~20% of missing rRNA segments is structurally replaced by mitochondrial r-proteins (MRPs) [14], while such a compensation is much higher (>50%) in the protistan (*Leishmania tarantolae*) mitoribosome [15].

Due to significant reduction in the size of their rRNAs, both subunits of the mitoribosome possess several tunnel-like features that appear to connect the inter-ribosomal subunit space with the bulk solvent. However, the functional core of both mitoribosomes, i.e., the regions involved in mRNA decoding on the SSU [22] and peptide-bond formation on the LSU [23], are predominantly made up of conserved rRNA segments, even though there are greater occurrences of protein masses on the interface side of both their subunits as compared to that in the subunits of the cytoplasmic ribosomes. Many of these protein masses participate in the formation of protein-protein inter-subunit bridges, and paths of mRNA and tRNA interactions [14,15]. The rRNA scaffolds within the mitoribosome structures are highly shielded by MRPs, such that the solvent-facing surfaces of the mitoribosomal subunits are predominantly made up of MRPs. This situation is in sharp contrast to the pattern of distribution of rRNA and r-protein masses within the structures of bacterial or chloroplast ribosomes, where proteins are present in relatively small patches on the solvent-side surfaces (Fig. 1) [16•]. Interestingly, most (11 out of 12) bacterial 23S rRNA segments that form the tRNA-exit site (E site) [24] are absent in the mito-LSU rRNAs [14,15,25,26], implying that the E site is either absent or very weak in both these mitoribosomes and that the deacylated mitochondrial tRNA (tRNA_{mt}) may not stay on either of these two mitoribosomes after its release from the ribosomal peptidyl site (P site) during the translation elongation cycle [27]. These unique features of the mitoribosome structures suggest that the *modus operandi* of these ribosomes has diverged significantly from their cytoplasmic or chloroplast counterparts.

Structure of the Mammalian Mitoribosome

In addition to the common features of the mitoribosome structures described in the previous section, here we highlight some of the unique features of the mammalian mitoribosome structure. Due to the absence of bacterial SSU rRNA helices 16 and 17 that are not

compensated by MRPs, the mammalian mitoribosomal SSU has a narrower shoulder and body regions and a more exposed translation factor-binding region, as compared to its bacterial counterpart. However, the body of the mammalian mitoribosomal SSU is significantly elongated due to the addition of MRPs to than in its lower body region. In addition, a triangular gate-like structure, made entirely of MRPs, partially covers the mRNA entry site on the solvent side of the mitoribosomal SSU [14] (Fig. 1B). More recent 7.0 Å resolution cryo-EM maps of the mammalian mitoribosome (MR Sharma, TM Booth, ME Haque, LL Spremulli, RK Agrawal, in preparation) suggest that this gate-like feature is a highly dynamic structure, and may be involved in recruiting the generally leaderless mitochondrial mRNAs [28] to the mitoribosomal SSU.

On the mammalian mitoribosomal LSU, the central protuberance is much larger in size as compared to that in a bacterial ribosome, even though density for the 5S rRNA has not been detected within the cryo-EM map of the mitoribosomal LSU. [It should be noted that only two rRNAs, 12S and 16S, corresponding to the mitoribosomal SSU and LSU, respectively, are encoded by the mammalian mitochondrial genome.] However, a recent biochemical study suggests that the 5S rRNA is transported into the mitochondria along with MRP L18 [29]. Thus, the possibility that the 5S rRNA is present only in a very small subset of isolated mitoribosomal population, and therefore goes undetected in the averaged cryo-EM maps, cannot be ruled out. In all of the cryo-EM maps, a mito-specific MRP emerges from the inter-subunit face of the central protuberance of the LSU and contacts the P-site tRNA_{mt} at its T-loop side [14]. This extended structure, referred to as the P-site finger, is a dynamic feature that may stabilize and regulate the binding and movements of generally short mammalian tRNA_{mt}s [30]. Another important structural aspect of the mammalian mitoribosomal LSU is its nascent polypeptide exit tunnel, which has been separately described later in this article.

Structure of the Mammalian Mitochondrial Translation Initiation Factor 2

Steps in the mechanism of mammalian mitochondrial translation initiation are somewhat similar to those in a bacterial cell. However, of the three bacterial initiation factors (IF1, IF2, and IF3), homologs of only two, IF2_{mt} and IF3_{mt}, have been identified in the mammalian mitochondria [31]. Both these factors show significant alterations in their structural organization as compared to their bacterial counterparts [32,33]. Interestingly, IF2_{mt}, which stimulates the binding of fMet-tRNA to the SSU of the mitoribosome, lacks domains I and II of *E. coli* IF2 [34] but contains a functionally important 37 amino-acid insertion domain (Fig. 2A).

Cryo-EM maps of the IF2_{mt}•GMPPNP•fMet-tRNA in complex with both the 70S bacterial ribosome [35] and the 55S mitoribosome (MR Sharma, AS Yassin, ME Haque, LL Spremulli, RK Agrawal, in preparation) have been determined. The overall binding positions of conserved domains of IF2_{mt} to the ribosome are similar to those of the bacterial IF2 [36]. However, the density corresponding to the insertion domain protrudes out from the rest of the IF2_{mt} density (Fig. 2B) to partially occupy the ribosomal aminoacyl-tRNA binding site (A-site) on the SSU, overlapping with the binding site of bacterial IF1 [37]. Thus, this structural study [35] shows that IF2_{mt} possesses dual functions corresponding to bacterial IF1 and IF2, corroborating a previous genetic study [38]. Despite the fact that there are significant size mismatch and no sequence homology or structural similarity between the insertion domain (37 amino acid) of IF2_{mt} and the bacterial IF1 (71 amino acids) (Fig. 2C,D), both structures bear similar surface charge distributions, which might play an important role in recognition of the same ribosomal binding pocket [35,39]. The presence of long linkers between the insertion domain and the rest of the IF2_{mt} molecule [35,39] suggests that the relative position of the insertion domain might vary between its native and

ribosome-bound states, as also observed for the bacterial release factors [40]. An integration of a relatively small IF1-mimicking feature onto the IF2_{mt} might have occurred during the course of evolution to serve a dual purpose: (i) one less protein had to be transported into the mitochondrial matrix, and (ii) to ensure an efficient transport of the IF1 feature to the mitochondrial protein synthesis site within highly dense mitochondrial matrix [41].

The Polypeptide-Exit Tunnel and Its Interaction with mtIM

In contrast to the variety of possible destinations for the nascent polypeptide chain synthesized by the cytoplasmic and chloroplast ribosomes, all of the nascent chains synthesized by the mammalian mitoribosome are inserted into the mtIM, and it is most likely that these polypeptides are inserted co-translationally into mtIM [18,19]. The nascent chain exit tunnel in the mitoribosomes appears to be tailor made for this purpose [16•]. The exit tunnel in the mitoribosome LSU has two openings on the solvent side, one corresponding to the conventional polypeptide exit site (PES) [42], and the second site at a distance of ~25 Å from the PES. This site of premature exposure of the nascent chain is located closer to the peptidyl-transferase center and allows free access to the solvent, and is designated as the polypeptide-accessible site [PAS, 14,15]. PAS is formed because the majority of the rRNA domain I and a significant portion of domain III of LSU rRNA, which together form the inner lining of the lower portion of the exit tunnel in bacterial and archaeal ribosomes [42], are absent in the mitoribosome and are not compensated by MRPs. The solvent-side openings of both PES and PAS are predominantly encircled by mito-specific MRPs [16•], which may be involved in the adherence of the mitoribosome to the mtIM [43,44] through either PES (Fig. 3A) or PAS, such that the lower portion of the mitoribosome is embedded into the mtIM with PAS and PES exposed at opposite sides of mtIM (Fig. 3B).

In yeast, Oxa1p, an integral membrane protein [45] and a mitochondrial homolog of bacterial YidC [46], interacts with the nascent chain while it is still on the ribosome. The human homolog of Oxa1p is known as Oxa1L [47]. The C-terminal tail of Oxa1L (Oxa1L-CTT) is exposed to the mammalian mitochondrial matrix and has been cross-linked to the mitoribosomal LSU [48]. Interestingly, Oxa1L-CTT does not interact with the conserved components (homologs of bacterial r-proteins L22, L23, L24, and L29) of conventional PES. Instead, it crosslinks to other homologs of bacterial r-proteins (L13, L20, and L28) and several mito-specific MRPs (MRPL48, MRPL49, and MRPL51), implying that these MRPs are situated close to the PAS, which would then be close to the matrix side of the mtIM (as depicted in Fig. 3B). A recent study showed that a mitochondrial protein ATAD3 can interact with both the mitochondrial DNA nucleoid and mitoribosome, as well as with mtIM [49•]. Another study showed an interaction between MRPL7 and the mitochondrial RNA polymerase [50•]. Both these studies suggest a coupling between mitochondrial transcription and translation machineries, and strengthen the view that mitoribosomes are either situated very close to, or partially embedded into, the mtIM. Based on these findings it is tempting to suggest that each mitoribosome could be associated with its own mitochondrial DNA (as there are more than 1000 copies of DNA in a given mitochondrion) to facilitate the synthesis and mtIM insertion of a defined set of polypeptide(s) and to ensure the high level of accuracy and efficiency needed to assemble the complexes of oxidative phosphorylation.

Concluding Remarks

From the cryo-EM structures of mitoribosomes and their comparison with the atomic structures of the cytoplasmic ribosomes, some novel aspects of mitochondrial protein synthesis have begun to emerge, suggesting that mitoribosomes have diverged significantly to adopt different host cell types during the course of evolution. However, higher resolution

structures are necessary to unravel details of the mechanisms of three very fundamental processes associated with the mammalian mitoribosome: (i) recruitment of leaderless mRNAs, (ii) decoding of atypical codons by unusual tRNA_{mt}, and (iii) the release of nascent chains through the dramatically remodeled exit tunnels. Structural characterization of the protein components present near key functional sites, such as at the mRNA entrance, and PAS and PES of the nascent chain exit tunnel, will be critical in establishing detailed molecular mechanism of these processes. While the tracing of the rRNA scaffolds can be achieved with high confidence in current 7 Å resolution maps, identification and modeling of mito-specific MRPs, especially those with undefined secondary structure motifs, into the cryo-EM map is still challenging. In the absence of any atomic structures of MRPs or mitochondrial translational factors, molecular interpretation of structures of all mitoribosomes and their functional complexes has to be based currently on docking of homology models into the cryo-EM maps. Furthermore, mitoribosomes seem to be inherently heterogeneous in composition due to (i) dramatic reduction in size of their rRNAs and significant increase in the number of MRPs; consequently, some of the mito-specific MRPs may be less firmly attached to the rest of the mitoribosome in the absence of their direct interaction with the main rRNA scaffolds, and (ii) presence of multiple isoforms of MRPs (e.g., MRPS18) in a given mitoribosome population. Thus, the progress in determining their higher resolution structure is hindered mainly due to challenges in obtaining highly homogeneous preparations. Nevertheless, because of improved algorithms to classify particle images, cryo-EM has been very effective in providing the overall structures of the mitoribosome and associated translation factors at reasonable resolutions.

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Abbreviations

SSU	Small subunit
LSU	Large subunit
rRNA	ribosomal RNA
r-protein	ribosomal protein
mitoribosome	mitochondrial ribosome
mtIM	mitochondrial inner membrane
MRP	mitochondrial r-protein
IF	translation initiation factor
PES	polypeptide exit site
PAS	polypeptide accessible site

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Highlights

1. We reviewed structures of mitochondrial ribosomes, which are significantly different from their cytoplasmic counterparts.
2. Functional rRNA core of the mitochondrial ribosomes is conserved, but their surfaces were remodeled during evolution.
3. Cryo-EM structures suggest that the protistan and mammalian mitochondrial ribosomes must have evolved differently.
4. The translation initiation factor 2 of the mammalian mitochondria also possess the features of bacterial initiation factor 1.
5. The nascent polypeptide exit tunnel seems to be tailor made to meet the specialized needs of the mitochondrial translation.

Fig. 1. Comparison between structures of bacterial and mitochondrial ribosomes

RNA-protein segmented structures of ribosomes from (A) bacteria (70S, *E. coli*) (B) mammalian mitochondria (55S, *Bos taurus*), and (C) protistan mitochondria (50S, *Leishmania tarentolae*). In panel A, atomic structure [5] has been low-pass filtered to match the resolution of the cryo-EM map of mammalian mitoribosome shown in panel B, whereas the cryo-EM map of the protistan mitoribosome is shown at ~ 14 Å [15]. Mito-specific MRPs of SSU and LSU are colored yellow and blue, respectively; conserved MRPs of SSU and LSU are colored green and aquamarine, respectively; and rRNAs of SSU and LSU are colored orange and magenta, respectively. Landmarks of the small subunit: h, head; mgt, mRNA gate; sh, shoulder; sp, spur. Landmarks of the large subunit: CP, central protuberance; Sb, Stalk base or protein L11 region.

Fig. 2. Structure of the mammalian IF2_{mt} as derived by cryo-EM and location of its insertion domain relative to the binding site of bacterial IF1

(A) Domain alignment of *E. coli* IF2 and bovine IF2_{mt}. Note that the mature IF2_{mt} (i.e., after deletion of the import sequence) starts from aa residue 78. The 37-aa insertion domain in IF2_{mt} is highlighted in red. (B) Fitting of atomic models of the IF2_{mt} and initiator tRNA (P/I stands for P-site tRNA at initiator position) into the corresponding cryo-EM densities (meshwork) extracted from the map of the 70S•IF2_{mt}•GMPPNP•fMet-tRNA complex [35••]. The color codes used for various domains of IF2_{mt} are the same as in panel A. Asterisk (*) point to the region that would correspond to domain III of IF2_{mt} but was not modeled. (C) Binding positions of the insertion domain (red), and (D) IF1 [37] (green), into a common binding pocket on the SSU of the ribosome. Landmarks of the SSU: h44 and h18, 16S rRNA helices; and S12, r-protein S12 (adopted from ref. [35••]).

Fig. 3. Hypothetical models of the mammalian mitoribosome interaction with the mtIM
 (A) The conventional polypeptide-exit site (PES) of the ribosome interacts with the matrix side of the mtIM, which is depicted as a generic lipid bilayer (semitransparent grey). (B) The lower portion of the mitoribosome is partially embedded into the mtIM, such that the conventional PES is exposed in the inter-membrane space side and the polypeptide accessible site (PAS) of the mitoribosome remains exposed on the matrix side of the mtIM. Other labels: NPC, modeled nascent polypeptide chain (red); 28S, SSU (yellow); and 39S, LSU (blue).

Table 1

Comparison of composition and physical properties of bacterial, mammalian mitochondrial, and protistan mitochondrial ribosomes ^{*}.

Ribosome source → Properties ↓	Bacteria <i>E. coli</i>	Mammalian mitochondria <i>B. taurus</i>	Protistan mitochondria <i>L. tarentolae</i>
Molecular mass	2.3 MDa	2.7 MDa	2.2 MDa
Sedimentation coefficient	70S	55S	50S
RNA : protein ratio	~2:1	~1:2	~1:3
Subunits	30S + 50S	28S + 39S	(28S – 30S) + 40S
Small subunit composition	16S rRNA (1542 nt) + 21 proteins	12S rRNA (950 nt) + 29 proteins (15) ^{**}	9S rRNA (610 nt) + 56 proteins [†] (46)
Large subunit composition	23S rRNA and 5S rRNA (total 3024 nt) + 34 proteins	16S rRNA (1560 nt) + 50 proteins (20)	12S rRNA (1173 nt) + 77 proteins [†] (66)
Diameter	~260 Å	~320 Å	~245 Å

^{*} Modified from [16•]

^{**} The number of mitochondrial proteins that do not have bacterial homologs are shown in brackets.

[†] The estimated number of proteins in the *L. tarentolae* mitoribosomal subunits has been derived from *trypanosoma brucei* [21], the closest known relative of *Leishmania*.

nt, nucleotide(s)