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Hierarchical folding of the catalytic core during mitochondrial ribosome biogenesis

Elena Lavdovskaia,[1,2] Hauke S. Hillen,[1,2,3,*] and Ricarda Richter-Dennerlein[1,2,*]

Final maturation steps during ribosome biogenesis require the assistance of assembly and quality control factors to ensure the folding of rRNA and proteins into a functional translation machinery. Here we integrate several recent structural snapshots of native large ribosomal subunit intermediates into the complex pathway of mitochondrial ribosome assembly.

Late assembly steps of the large ribosomal subunit (LSU) require the action of RNA helicases, RNA-modifying enzymes, chaperones, and GTPases. These mediate key quality control steps and facilitate final maturation of the peptidyl transferase center (PTC), the catalytic core of the ribosome. Folding of the PTC, which is mostly composed of rRNA, is the last and most critical step during LSU biogenesis and relies on a combination of conserved and species- or organelle-specific biogenesis factors. Recently, several structural snapshots of intermediate states of LSU assembly isolated from human and protist mitochondria as well as from bacteria have been reported. This was achieved by applying genetic perturbation or affinity purification of ribosome biogenesis factors followed by cryo-electron microscopy (cryo-EM). These structures reveal the molecular function and hierarchical order of action for several essential assembly factors. Here, we integrate these recent studies into a stepwise model of mitochondrial LSU (mtLSU) biogenesis, which highlights similarities to its bacterial counterpart and organelle-specific features.

The supposedly earliest reported human mtLSU intermediate lacks the ribosomal proteins bL33m, bL35m, and bL36m and displays an immature central protuberance (CP) and delocalized domains IV and V (state A) (Figure 1) [1]. However, it has the mitochondrial assembly of ribosomal large subunit protein 1 (MALSU1) module, the RNA helicase DDX28, the GTPase GTPBP10 and a dimer of the 2′-*O*-methyltransferase MRM3 bound. Acronyms of discussed biogenesis factors are defined in Table 1 including the respective functions and corresponding PDB codes. During ribosome maturation, dedicated factors prevent premature subunit joining. In mitochondria, MALSU1 appears to act as an anti-association factor. Its bacterial homolog RsfS interferes with intersubunit bridge B8 formation by preventing uL14 from associating with h14 of the small ribosomal subunit (SSU) rRNA. MALSU1 associates with L0R8F8 and mitochondrial acyl carrier protein (mtACP) to form an antiassociation module that interacts with uL14m, bL19m, and the sarcin-ricin loop (SRL, h95) to obstruct subunit joining [2]. This may be explained by evolutionary changes in the mitochondrial ribosome, which lacks h14. Interestingly, the MALSU1 module was also present in mtLSU particles derived from recycled stalled complexes, indicating that it also acts as an antiassociation factor upon subunit dissociation, reminiscent of the role of mitochondrial initiation factor 3 bound to the mitochondrial small subunit (mtSSU) [3]. In *Trypanosoma brucei*, the MALSU1 module was identified in an even earlier intermediate lacking the CP and L12 stalk and with an immature L1 stalk [4,5]. Thus, the MALSU1 module binds early during the final mtLSU maturation steps and remains associated until a translationally competent subunit is formed. DDX28 binds to h88 and stabilizes the CP in a premature conformation in state A. GTPBP10 interacts with the SRL and the L12 stalk base, similarly to its bacterial homolog ObgE in the 50S LSU assembly intermediate [6]. The Obg domain of GTPBP10 stacks against h89, which is shifted compared with mature mtLSU particles. The abstraction of h89 enables MRM3 to reach h92 of domain V to methylate G3040 within the A loop (h92) [1]. Comparable observations were made in *T. brucei*, where the MRM3 homolog mt-LAF5 adopts a similar conformation, although mt-LAF5 is enzymatically inactive and differences in the overall folding of domain V indicate species-specific differences [4].

On the basis of biochemical data, the next intermediate should contain all ribosomal proteins except bL36m as well as GTPBP10, GTPBP7, and MTERF4-NSUN4 (state B) [7]. MTERF4 and NSUN4 act as another antiassociation module, as they interact with h68-h71 and prevent the folding of structural elements involved in intersubunit bridging [1,8–11]. GTPBP7, the homolog of bacterial RbgA, binds early, but it is not clear when GTPBP7 joins the assembling human mtLSU. The *T. brucei* homolog was solved in assembly intermediates lacking the CP, L12, and L1 stalk, indicating that GTPBP7 might also bind upstream of state A [4,5]. Nevertheless, state B has not been structurally captured, and instead a state lacking all assembly factors except MALSU1 was reported (B′) [2].

The recruitment of the last ribosomal protein, bL36m, to the maturing particle results in state C, to which MTERF4-NSUN4 remains bound. Independent studies suggest that GTPBP7 binding may be transient and that this state can exist with (C) or without it (C′). Despite copurifying this mtLSU intermediate via a tagged variant of GTPBP7, a density for GTPBP7 in reconstructions could not be observed [10]. Similarly, other isolated state C complexes do not reveal density for GTPBP7, although detectable by mass spectrometry [8]. However, in the

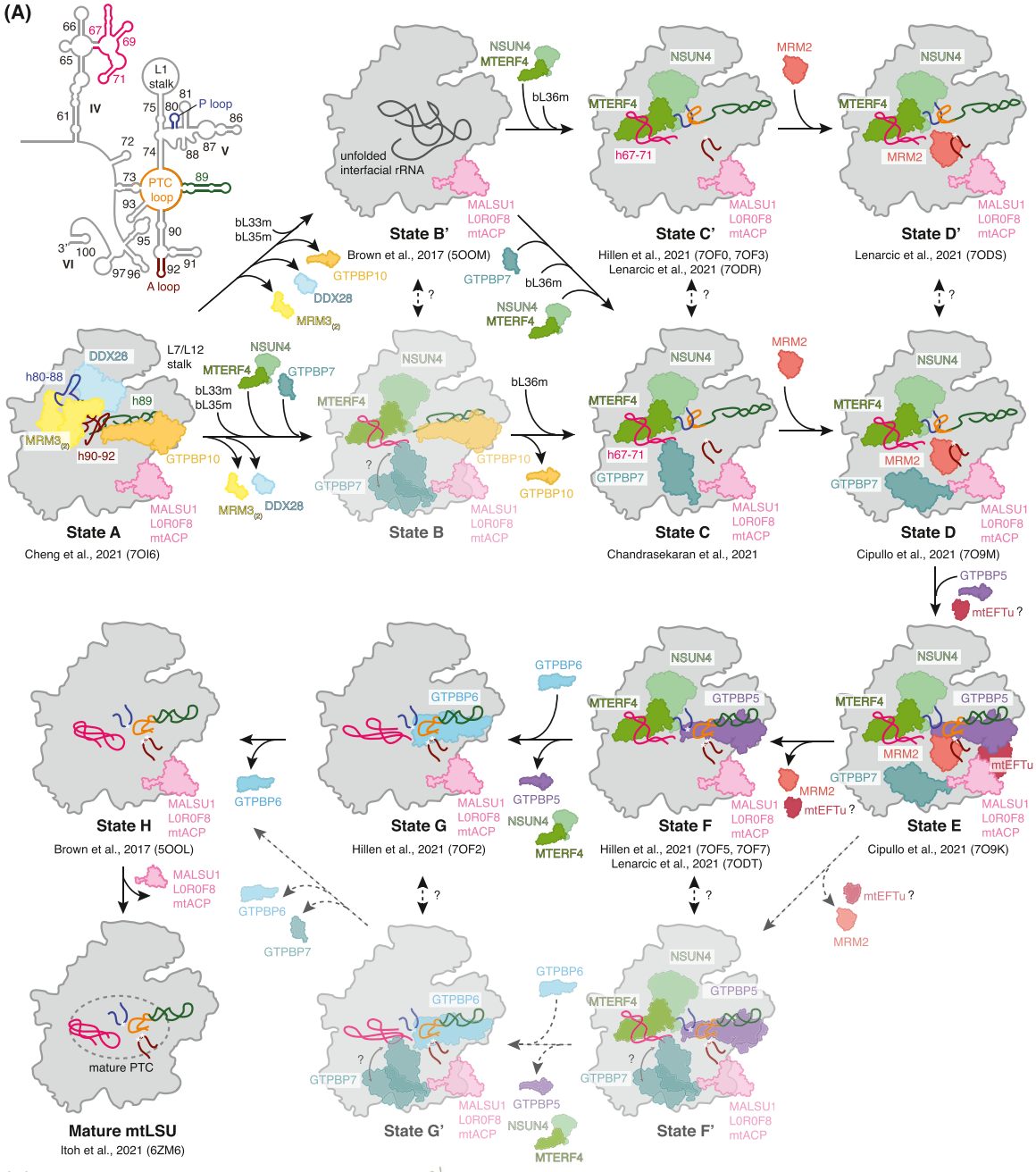

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Table 1. Functions of mitoribosome biogenesis factors during mtLSU maturation

Biogenesis factor	Bacterial homolog	Function	State	Protein Data Bank code
MALSU1 (mitochondrial assembly of ribosomal large subunit protein 1)	RsfS (ribosomal silencing factor)	uL14m chaperone; forms a module with L0R0F8 and mtACP; prevents premature subunit association	A, B/B′, C/C′, D/D′, E, F/F′, G/G′, H	7OI6, 5OOM, 7OF0, 7OF3, 7ODR, 7ODS, 7O9M, 7O9K, 7OF5, 7OF7, 7ODT, 7OF2, 5OOL
L0R0F8 (MIEF1 upstream open reading frame protein)	–	Prevents premature subunit association together with MALSU1 and mtACP	A, B/B′, C/C′, D/D′, E, F/F′, G/G′, H	7OI6, 5OOM, 7OF0, 7OF3, 7ODR, 7ODS, 7O9M, 7O9K, 7OF5, 7OF7, 7ODT, 7OF2, 5OOL
mtACP (acyl carrier protein, mitochondrial, NDUFAB1)	–	Prevents premature subunit association together with MALSU1 and L0R0F8	A, B/B′, C/C′, D/D′, E, F/F′, G/G′, H	7OI6, 5OOM, 7OF0, 7OF3, 7ODR, 7ODS, 7O9M, 7O9K, 7OF5, 7OF7, 7ODT, 7OF2, 5OOL
DDX28 (DEAD box RNA helicase 28)	–	Keeps h88 of the CP immature	A	7OI6
MRM3 (rRNA methyltransferase 3)	–	Methylates G3040 (h92, A loop)	A	7OI6
GTPBP10 (GTP-binding protein 10)	ObgE (GTPase ObgE/CgtA)	Abstracts h89 from its mature position in the PTC; assists MRM3; antiassociation function	A, B	7OI6
GTPBP7 (GTP-binding protein 7; or mitochondrial ribosome-associated GTPase 1, MTG1)	RbgA (ribosome biogenesis GTPase A)	Verifies 2′-*O*-methylation of U3039 (A loop); promotes h89-h93 folding	B, C, D, E, F′, G′	7O9M, 7O9K
MTERF4 (mitochondrial transcription termination factor 4)	–	Acts as an antiassociation factor by preventing folding of h68-h71	B, C/C′, D/D′, E, F/F′	7OF0, 7OF3, 7ODR, 7ODS, 7O9M, 7O9K, 7OF5, 7OF7, 7ODT
NSUN4 (5-methylcytosine rRNA methyltransferase)	RsmB (ribosomal RNA small subunit methyltransferase B)	N-terminal tail facilitates PTC loop folding together with GTPBP5	B, C/C′, D/D′, E, F/F′	7OF0, 7OF3, 7ODR, 7ODS, 7O9M, 7O9K, 7OF5, 7OF7, 7ODT
MRM2 (rRNA methyltransferase 2)	RlmE/RrmJ/FtsJ (ribosomal RNA large subunit methyltransferase E)	Methylates U3039 (h92, A loop); might facilitate PTC folding	D/D′, E	7ODS, 7O9M, 7O9K
GTPBP5 (GTP-binding protein 5; or mitochondrial ribosome-associated GTPase 2, MTG2)	ObgE	Facilitates PTC loop maturation together with NSUN4; assists MRM2; antiassociation function	E, F/F′	7O9K, 7OF5, 7OF7, 7ODT
GTPBP6 (GTP-binding protein 6)	HflX (GTPase HflX, ribosome splitting factor)	Triggers the release of GTPBP5 and MTERF4-NSUN4; promotes P loop and PTC loop maturation; antiassociation function	G	7OF2

presence of GMPPCP (nonhydrolyzable GTP analog), state C intermediates have been isolated with corresponding density for both MTERF4-NSUN4 and GTPBP7, which is locked in a prehydrolysis conformation, similar to its bacterial counterpart [11]. This structure shows that GTPBP7 interacts with h92 and directly contacts U3039 to monitor its methylation status. This base in the A loop is methylated during mtLSU biogenesis by MRM2, another 2′-*O*-methyltransferase, the recruitment of which

Figure 1. Late maturation steps during mitochondrial large ribosomal subunit (mtLSU) biogenesis. (A) Top left: A schematic depiction of the 16S rRNA secondary structure with domains IV–VI and highlighted regions, which adopt different folding states during the maturation of the peptidyl transferase center (PTC): h67-71 (pink), h80/P loop (dark blue), PTC loop (orange), h89 (dark green), and h92/A loop (dark red). Biogenesis factors discussed in this review are highlighted as followed: rRNA methyltransferase 3, MRM3 (yellow); DEAD box RNA helicase 28, DDX28 (light blue); GTP-binding protein 10, GTPBP10 (orange); mitochondrial assembly of ribosomal large subunit protein 1, MALSU1 module (pink); transcription termination factor 4, MTERF4 (green); 5-methylcytosine rRNA methyltransferase, NSUN4 (mint); GTP-binding protein 7 GTPBP7 (turquoise); GTP-binding protein GTPBP5 (purple); rRNA methyltransferase 2, MRM2 (salmon); mitochondrial elongation factor Tu, mtEFTu (red); GTP-binding protein GTPBP6 (blue). 2′-O-methylations are indicated with a white asterisk. In states F and G, the resolution of the P and A loop allowed modeling for methylation of G2815, U3039, and G3040 [8]. In states D and E, G3040 was shown to be methylated [9]. As the rRNA was not as well resolved in state C/C′, the methylation of G3040 is indicated on the basis of the assumption that it represents a state downstream of an MRM3-bound state. It is currently unclear in which state G2815 undergoes methylation. Potential alternative assembly states and lines are indicated in transparent with broken arrows and a question mark. These states were not structurally resolved, but biochemical data indicate that they might exist. (B) Close-up view of PTC maturation states corresponding to selected assembly states depicted in (A) [8,9,13]. Biogenesis factors are highlighted as in (A).

leads to state D. As previously, this intermediate was shown to exist with (D) and without (D') GTPBP7 bound. Although MRM2 is essential for mtLSU biogenesis, its methyltransferase activity is dispensable, suggesting another function during PTC maturation besides U3039 methylation [12]. Interestingly, GTPBP7 adopts a different conformation in state D than in state C, as it would otherwise sterically clash with MRM2. Thus, GTPBP7 may bind to different locations on the mtLSU in different states, but the functional relevance of this is not clear [9]. In contrast, RbgA from *Bacillus subtilis* and GTPBP7 from *T. brucei* were only observed in the conformation corresponding to state C, although the latter also undergoes some conformational changes during mtLSU assembly [4,5]. Recruitment of GTPBP5, a second human homolog of ObgE, leads to state E, in which the A loop is displaced from the active site of MRM2 [9]. State E contains GTPBP7, GTPBP5, and MRM2, suggesting that these factors can act simultaneously, which is in contrast to their bacterial homologs [6]. Strikingly, mtEFTu was suggested to accommodate GTPBP5 similarly as it delivers aminoacyl tRNAs to the A site, an intriguing idea that requires further validation [9]. After methylation of U3039, MRM2 dissociates, whereas GTPBP5 and MTERF4-NSUN4 remain bound (state F). NSUN4 and GTPBP5 cooperate to facilitate folding of the PTC [8,10]. The N-terminus of NSUN4, which is not conserved in its bacterial homolog RsmB, is stabilized by GTPBP5 and protrudes into the PTC. While NSUN4 breaks the interaction between the PTC loop and the P loop (h80), GTPBP5 stabilizes a refolded conformation of the PTC loop. Although the structures of state F did not contain GTPBP7, biochemical data suggest that an equivalent state with GTPBP7 bound may exist (state F'). The next step in mtLSU biogenesis entails an exchange of the maturation factors MTERF4-NSUN4 and GTPBP5 for GTPBP6 (state G) [8]. GTPBP6 is homologous to the bacterial ribosome splitting factor HflX and binds to the same position on the mtLSU as GTPBP5. Thus, GTPBP6 binding may trigger a molecular switch by releasing GTPBP5 and the MTERF4-NSUN4 module to mediate further progression of PTC folding. In the GTPBP6-bound state, the PTC adopts a near-mature conformational and modification state, and it thus likely represents one of the last mtLSU maturation steps.

In conclusion, single-particle cryo-EM has proved to be a powerful tool to reveal the molecular function of ribosome assembly factors and the progression of the catalytic core during distinct assembly steps. The dissection of these structural snapshots allows one to draw a comprehensive assembly scheme of late mtLSU maturation steps and to assign the hierarchical order of action of essential assembly factors. Future aspects that need to be addressed include the earlier assembly steps, which may also be coupled to mitochondrial transcription or RNA processing, and whether mitoribosome biogenesis occurs in close proximity to defined membrane foci. In addition, the process of mtSSU maturation is thus far poorly understood. The combination of biochemical approaches with cryo-EM and cryo-electron tomography holds promise to provide answers to these open questions.

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Declaration of interests

The authors have no interests to declare.

[1]Department of Cellular Biochemistry, University Medical Center Goettingen, D-37073 Goettingen, Germany
[2]Cluster of Excellence 'Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells' (MBExC), University of Goettingen, D-37075 Goettingen, Germany
[3]Research Group Structure and Function of Molecular Machines, Max Planck Institute for Biophysical Chemistry, D-37077 Goettingen, Germany

*Correspondence:
hauke.hillen@med.uni-goettingen.de (H.S. Hillen) and
ricarda.richter@med.uni-goettingen.de (R. Richter-Dennerlein).

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