Divergent functions of a ribosome maturation factor

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The ribosome, the large macromolecular complex responsible for the translation of messenger RNA (mRNA) into proteins, must be matured and assembled correctly for effective function. Generally, ribosome maturation and assembly involves (I) translation of ribosomal proteins, and transcription and processing of ribosomal RNA (rRNA); (II) modification of ribosomal proteins and rRNA; (III) shaping and remodeling of rRNA in parallel with ribosomal protein recruitment (1). These processes are reliant on assembly factors that include nucleases, rRNA-modifying enzymes, GTPases, RNA helicases and chaperones (1,2). Dysregulation of this process has been implicated in a wide variety of human diseases.

In general, protein translation and ribosomal assembly is less well studied for mitochondrial ribosomes (mitoribosomes) than bacterial and eukaryotic ribosomes. Recent high-resolution electron cryomicroscopy (cryo-EM) structures have revealed considerable evolutionary divergences of mitoribosomes from their bacterial ancestors both in structure and composition (3-7). For example, the mammalian mitoribosome, which consists of the 39S large subunit (mt-LSU) and 28S small subunit (mt-SSU), has 36 additional mitoribosome specific proteins compared to the bacterial ribosome and considerable shortening of rRNA (6,8). However, the core scaffold of rRNA retains essential features such as the peptidyl transferase centre (PTC) and the messenger RNA decoding site. The assembly of human mitoribosomes involves the coordination of 82 nuclear-encoded mitochondrial proteins with mitochondrially encoded RNA (1 mt-tRNA and 2 mt-rRNA; 12S of the mt-SSU and 16S of the mt-LSU), and is dependent on nuclear-encoded assembly factors. Many mitoribosomal maturation and assembly factors have bacterial homologs, but given the increased complexity of mitoribosomal assembly, additional factors unique to the mitoribosome have been found (9-18).

In a recent Biochemical Journal article by Rozanska et al. entitled “The human RNA-binding protein RBFA promotes the maturation of the mitochondrial ribosome”, the authors examined the role that RBFA plays in the assembly of human mitoribosomes (19). RBFA is homologous to bacterial RbfA, and both contain an RNA-binding ‘KH’ domain. However, the puzzle is that while bacterial RbfA is involved in the processing of the 5’ region of 17S rRNA precursor to form mature 16S rRNA (20), human mt-rRNA is excised from polycistronic transcripts without the need for further processing. Thereby alternative roles are likely to have evolved for these structurally related proteins. Consistent with this, RBFA has acquired considerable N- and C-terminal extensions and is over twice the size of its bacterial counterpart.

To study the cellular role of RBFA the authors first demonstrated that RBFA associates preferentially with the mt-SSU. By sequencing RNA isolated by crosslinking immunoprecipitation (CLIP-seq) they found that RBFA associated largely with helices 44 and 45 at the 3’ end of 12S rRNA. Helix 44 is an integral part of the decoding centres and forms several inter-subunit bridges. Helix 45 harbours two highly conserved dimethylated adenines in a tetraloop that tucks into a groove of h44 close to the decoding centre (Figure 1). These are two of only ten nucleotides of
mt-rRNA that have been found to be post-transcriptionally modified in mammals (21). This is modest compared to eukaryotic cytoplasmic and bacterial ribosomes which have in excess of 200 and 30 modifications, respectively (21). In bacteria, loss of helix 45 dimethylation has been shown to decrease translational fidelity (22). Knockout of the methyltransferase enzyme responsible for these modifications, TFB1M, is embryonically lethal in mice and heterozygosity showed impaired pancreatic islet cell mitochondrial function contributing to the pathogenesis of type II diabetes (23,24).

ERAL1, the GTPase and 12S mt-rRNA chaperone, also binds to this site (12), but is not functionally interchangeable with RBFA in human cell lines. The authors were therefore interested to see the effect of RBFA and ERAL1 on the methylation status of h45. To achieve this, they performed parallel immunoprecipitations of RBFA and ERAL1-FLAG, and determined the methylation status of the extracted rRNA. The results showed that RBFA was mostly bound to dimethylated h45 while ERAL1 was largely bound to unmethylated h45. It is likely that ERAL1 and RBFA bind 12S rRNA sequentially, as mass spectrometry data did not detect a significant proportion of ERAL1 co-immunoprecipitating with RBFA, and the CLIP-protected RNA fragments were found to be similar for the two proteins.

The next step was to look for newly synthesized 12S rRNA to ascertain the effect of RBFA on promoting modification and maturation. To do this the authors first decreased the level of mature modified mt-SSU by depletion of ERAL1. Loss of ERAL1 has been shown to lead to depletion of nascent 12S mt-rRNA (12). This was followed by repletion of ERAL1 with either concomitant RBFA depletion or a non-depleted control. The modification status of the rRNA was then evaluated. They found that when 12S rRNA was depleted together with RBFA the number of unmodified nucleotides was substantially increased compared to the non-depleted control, indicative of RBFA playing a role in promoting 12S rRNA methylation. The authors propose that RBFA functions by pushing h45 out to expose the adjacent adenines to its methyltransferase, TFB1M. This is consistent with a low-resolution cryo-EM structure (25) that shows RbfA displaces h44 and the adjacent area of h45 by ~25 Å when bound to the bacterial SSU (25). However, the exact molecular mechanism by which RBFA influences the modification process remains speculative.

The authors then demonstrated that unmodified 12S rRNA could be incorporated into mt-SSU, but only mt-SSU with the correctly modified and matured 12S rRNA forms monosomes. Thus, this paper shows that RBFA plays a role in promoting dimethylation of the 12S rRNA of the mt-SSU, and this is an important quality control step in monosome formation. Binding of RBFA to h44/45 may promote dimethylation of highly conserved consecutive adenines by increasing accessibility of these residues to their methyltransferase TFB1M. It does not appear that the dimethylation is a prerequisite for assembly of the mt-SSU.

**Figure 1** Interaction between the dimethylated adenines of helix 45 with helix 44 in the human mitoribosome (protein data bank entry 3J9M) (6).
Instead the dimethylation appears instrumental in mt-rRNA maturation and functional monosome formation.

In summary, the authors have shown an example of a known ribosomal maturation factor acquiring alternative functions to support ribosome maturation as an adaptive strategy, most likely resultant of the evolutionary divergence of the mitoribosome.

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