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Review

Supernumerary proteins of mitochondrial ribosomes ☆

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ABSTRACT

Background: Messenger RNAs encoded by mitochondrial genomes are translated on mitochondrial ribosomes that have unique structure and protein composition compared to prokaryotic and cytoplasmic ribosomes. Mitochondrial ribosomes are a patchwork of core proteins that share homology with prokaryotic ribosomal proteins and new, supernumerary proteins that can be unique to different organisms. In mammals, there are specific supernumerary ribosomal proteins that are not present in other eukaryotes.
Scope of review: Here we discuss the roles of supernumerary proteins in the regulation of mitochondrial gene expression and compare them among different eukaryotic systems. Furthermore, we consider if differences in the structure and organization of mitochondrial genomes may have contributed to the acquisition of mitochondrial ribosomal proteins with new functions.
Major conclusions: The distinct and diverse compositions of mitochondrial ribosomes illustrate the high evolutionary divergence found between mitochondrial genetic systems.
General significance: Elucidating the role of the organism-specific supernumerary proteins may provide a window into the regulation of mitochondrial gene expression through evolution in response to distinct evolutionary paths taken by mitochondria in different organisms. This article is part of a Special Issue entitled Frontiers of Mitochondrial Research.

1. Introduction

Mitochondrial genomes encode a limited set of mRNAs that are translated on mitochondrial ribosomes to produce specific protein subunits of the oxidative phosphorylation (OXPHOS) complexes. Mitochondrial ribosomes are composed of rRNAs that are encoded by the mitochondrial genome and ribosomal proteins that are typically encoded by the nuclear genome and imported post-translationally into mitochondria. Mitochondrial genomes in some fungi, plants and protists also encode several ribosomal proteins. Mitochondria encode either a complete set of tRNAs within their genomes or import some of their tRNAs from the cytoplasm that are required for translation by mitochondrial ribosomes. The composition of mitochondrial ribosomes can vary between different organisms, and it is likely that the size and organization of mitochondrial genomes have necessitated the acquisition of new domains and proteins within the ribosome. Here we briefly summarize the knowledge about mitochondrial ribosomes, since a detailed description of the composition and structure of mitochondrial ribosomes has been covered recently [1–4]. Instead we focus on the known and suspected roles of the supernumerary and mammalian specific ribosomal proteins identified to date. Finally, we present the knowledge gained about the role of the mammalian specific ribosomal proteins from human diseases caused by mutations in the genes encoding these proteins.

2. Mitochondrial ribosomes

Mitochondrial ribosomes are located inside the matrix, the site of the transcriptome, and they closely associate with the mitochondrial inner membrane to allow co-translational insertion of the hydrophobic proteins into the inner membrane followed by their assembly into the OXPHOS complexes [5]. The mitochondrial ribosome is composed of over 80 proteins and two rRNAs that make up the small and large ribosomal subunits [1,3,6–10]. Mitochondrial ribosomal RNA has reduced in size considerably during evolution and has been replaced by additional proteins. These proteins share homology with cytoplasmic ribosomal proteins from prokaryotes and yeast and some of them are unique to mitochondrial ribosomes. Mitochondrial ribosomal proteins that have conserved rRNA-binding sites have been observed to be similar in size to those in *Escherichia coli* ribosomes, and ribosomal proteins whose binding sites on rRNA are shortened or lost, carry N- or C-terminal extensions [1]. Although it has been suggested that an increased ribosomal protein content may compensate for the loss of rRNA, it has been shown that many of these additional ribosomal proteins do not replace the missing RNA helices but instead have unique positions, decorating the exterior of the mitochondrial ribosome [1,11]. One example is the loss of the 3′ end of the mitochondrial small subunit rRNA, which contains

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the message-binding site (MBS) in bacterial rRNA and recruits mRNAs for translation initiation. This is perhaps not surprising, given that mitochondrial mRNAs do not have the characteristic ribosome-binding sequences (RBS) that directly base pair with the MBS, however how ribosomal proteins can compensate for this loss and ensure accurate start codon recognition is not currently understood. About 20% of mitochondrial ribosomal proteins replace the rRNA helices that are lacking in mitochondrial ribosomes, however in the mitochondrial protist *Leichmania* ribosomes more than 50% of the missing rRNA helices are replaced by proteins [9,12]. The mRNA decoding and peptide-bond forming active sites in the small and large ribosomal subunits are composed of conserved rRNA helices, although it has been observed that there is higher protein abundance around these sites compared to cytoplasmic or prokaryotic ribosomes [1]. The significance of the supernumerary proteins and the extended domains of the conserved proteins is unclear. It is suggested that the organization of the mitochondrial genomes in different organisms has driven the evolution of the diversity in mitochondrial ribosomal proteins.

A heterogeneous complement of proteins has been observed in prokaryotic as well as cytoplasmic ribosomes of eukaryotes [13–15], however whether this is the case for mitochondrial ribosomes remains to be determined. There is some notion that there may be heterogeneous ribosomes through the discovery of three different isoforms of MRPS18, however this remains to be determined experimentally. Changes in the expression of ribosomal proteins, including mitochondrial ribosomal proteins, have been observed between different organisms, developmental stages and growth conditions, possibly reflecting specific requirements for translation [16–22].

3. Supernumerary ribosomal proteins in mitochondria

The additional proteins found in mitochondrial ribosomes do not share homology with bacterial and cytoplasmic ribosomal proteins, although some have homology between yeast and animal mitochondria and some are unique to specific organisms such as in *Leishmania* and yeast mitochondria [8,23]. Recently, 95 additional supernumerary proteins have been identified in mitochondrial ribosomes from *Trypanosoma brucei* that are unique to kinetoplastids [24], although their functions are not known. In contrast, there are fewer yeast specific supernumerary proteins compared to those found in humans, and some of these are transcript specific regulators of translation (Tables 1 and 2). Clearly organism specific supernumerary proteins have unique roles within their mitochondrial ribosomes. In archea, ribosomes have lost proteins in contrast to eukaryotic mitochondrial ribosomes that have acquired additional proteins [23]. It has been suggested that the composition of mitochondrial ribosomes in different organisms is still rapidly evolving, unlike that of cytoplasmic and bacterial ribosomes [8,23,25].

The mammalian mitochondrial ribosome is composed of large 39S and small 28S subunits that associate and sediment as 55S particles [26,27]. Mammalian mitochondrial ribosomes have only two rRNAs encoded by the mitochondrial genome, the small subunit 12S rRNA and the large subunit 16S rRNA [28]. In addition to the core ribosomal proteins that share homology with prokaryotic ribosomes, human mitochondrial ribosomes have 35 supernumerary proteins, some of which may have new functions in mitochondrial translation and recognition of mitochondrial mRNAs within the small and large ribosomal subunits [1].

Table 1
Supernumerary ribosomal proteins of the small mitochondrial subunit.

Human MRP	Yeast MRP	Present in last common eukaryotic ancestor?[a]	Homologous domains	Role in mitochondria
MRPS22				Genetic defects result in combined oxidative phosphorylation deficiency [61] and Cornelia de Lange-like dysmorphic features [64].
MRPS23	Rsm25			
MRPS25	Mrp49		NDUFB8	Not essential for translation in yeast [65].
MRPS26			Coiled coil	
MRPS27			Pentatricopeptide repeats (PPRs)	Required for translation [36].
MRPS28				
MRPS29	Rsm23		Death associated protein 3 (DAP3)	Promotes apoptosis [31,34,66], Binds GTP and is phosphorylated [67]. Cross-links to mitochondrial translational initiation factor 3 (IF3(mt)) [2]. Associates with NOA1 and Complex I [30].
MRPS30			PDCD9/MRPL37	Promotes apoptosis [32,34,68].
MRPS31				Autoantigen in type 1 diabetes [69].
MRPS32				Identical to MRPL42. Cross-links to mitochondrial translational initiation factor 3 (IF3(mt)) [2].
MRPS33	Rsm27			Heterozygous deletion causes cardiomyopathy in flies [41].
MRPS34				
MRPS35	Rsm24			
MRPS36	Ymr31			Cross-links to mitochondrial translational initiation factor 3 (IF3(mt)) [2].
PTCD3			PPRs	Required for translation [37]. Cross-links to mitochondrial translational initiation factor 3 (IF3(mt)) [2]. Associates with TEFM [40].
	Rsm22		rRNA methyltransferase	
	Ppe1			
	Mrps35			
	Mrp51			Genetic interactions with mutations in the COX2 and COX3 mRNA 5′-UTRs in yeast [45].
	Mrp13			
	Mrp1			Genetic interaction with PET122, a COX3-specific translational activator, in yeast [46].
	Rsm26		Superoxide dismutase (SOD)	
	Pet123		Coiled coil	Genetic interaction with PET122, a COX3-specific translational activator, in yeast [46].
	Mrp10		COX19, NDUFA8	
	Rsm28		Kinase	Genetic interactions with mitochondrial translation initiation factor 2 (IF2) and the methionyl-tRNA-formyltransferase (FMT1) suggest a role in translation initiation in yeast [47].
	Mrp8			

[a]The supernumerary proteins present in the last common eukaryotic ancestor are shaded in gray [64,65,66,67,68,69].

3.1. Supernumerary proteins of the small ribosomal subunit

The mitochondrial small ribosomal subunit is responsible for mRNA recruitment, association with initiation factors and mRNA decoding [15]. The small subunit of mammalian ribosomes is composed of 29 proteins, of these 14 are core ribosomal proteins that share homology with prokaryotic ribosomal proteins and 15 are unique proteins (Table 1). Some progress has been made to elucidate the role of supernumerary proteins, however a confounding factor to the identification of their functions within the mitochondrial ribosome is the lack of a robust *in vitro* mitochondrial translation system. One of the unusual features of mitochondrial ribosomes is the presence of a GTP binding protein in the small ribosomal subunit. This is the mammalian supernumerary protein MRPS29 that is located adjacent to the interface between ribosomal subunits, hypothesized to be the binding site of mitochondrial initiation factor 3 (IF3mt) [2]. MRPS29 has been found to associate