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# Striking Diversity of Mitochondria-Specific Translation Processes across Eukaryotes

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1 Review

## 2 **Striking diversity of mitochondrial specific translation processes** 3 **across eukaryotes**

4  
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6  
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### 13 **Keywords**

14 Mitochondria, gene expression, translation, mitoribosome, mRNA, pentatricopeptide repeat proteins

15

16 **Mitochondria are essential organelles acting as energy conversion powerhouses and metabolic**  
17 **hubs. Their gene expression machineries combine traits inherited from prokaryote ancestors and**  
18 **specific features acquired during eukaryote evolution. Mitochondrial research has wide**  
19 **implications going from human health to agronomy. Here, we highlight recent advances on**  
20 **mitochondrial translation. Functional, biochemical, and structural data have revealed the**  
21 **unexpected diversity of mitochondrial translation systems, in particular of their key players, the**  
22 **mitoribosomes. Ribosome assembly and translation mechanisms, such as initiation, are discussed**  
23 **and put in perspective with the prevalence of eukaryote specific families of factors, such as**  
24 **pentatricopeptide repeat proteins, for mitochondrial translation.**

25

### 26 **Mitochondria are semi-autonomous organelles holding complete gene expression machineries**

27 Within eukaryote cells, **mitochondria** (see Glossary) perform in fundamental energy conversion and  
28 metabolic pathways. Their main purpose is to perform metabolic respiration. They are also involved  
29 in amino acid and nucleotide metabolism, lipid, quinone, and steroid biosynthesis, and iron-sulphur  
30 (Fe/S) cluster biogenesis [1–4]. They have an exogenous origin and were acquired during the  
31 eukaryogenesis [5] through the **endosymbiosis** of a bacteria from a sister group of  $\alpha$ -proteobacteria,  
32 as recently proposed [6]. Mitochondria are non-autonomous **organelles**, as the large majority of  
33 genes originally encoded in the endosymbiont were either lost or transferred to the nucleus of the  
34 host cell [7,8]. However, in most eukaryotes, mitochondria still possess their own genome, vestige of  
35 their once free-living bacterium ancestor. This genome is rather small comparatively to the original  
36 endosymbiont genome and only a few genes are still encoded in mitochondria. Thus the majority of  
37 mitochondrial proteins are nuclear encoded, their messenger RNAs (mRNAs) translated in the  
38 cytosol, and imported in mitochondria [9,10]. This is also the case for transfer RNAs (tRNAs), where a  
39 complete set of tRNA genes encoded by **mitochondrial DNA (mtDNA)** is found in some eukaryotes,  
40 such as Human or *Saccharomyces cerevisiae*, but is absent from many other eukaryote groups, i.e.  
41 from most plants and protists [11].

42

43 Moreover, as mitochondria were acquired before the evolutive radiation of eukaryotes, major  
44 differences in term of structure, size, gene content, and expression mechanisms are observed  
45 between the different groups of eukaryotes. The size and structure of mitochondrial genomes greatly  
46 varies between organisms, ranging from 6 kb in *Plasmodium falciparum* to an 11 Mb  
47 multichromosomal genome in *Silene conica* [12,13]. The number of protein coding genes is also  
48 variable, e.g. 3 in *P. falciparum* and 69 in *Reclinomonas americana* [14,15]. These proteins are  
49 functionally conserved, being very often core components of the respiratory chain complexes, which  
50 are mostly hydrophobic. Nonetheless, constituents of the mitochondrial ribosomes (**mitoribosomes**)  
51 and additional proteins can also be commonly found (i.e. cytochrome c maturation and RNA

polymerase subunits), especially in plants and jakobids [16]. For the expression of their genomes, mitochondria use processes relying on a degenerate bacterial scaffold completed by nuclear-encoded factors acquired during eukaryote evolution. Translation, the final step of gene expression, remained completely elusive until recent years.

Translation is performed by ribosomes, the ubiquitous molecular machines that read mRNAs and catalyze the assembly of amino acids to form proteins [17]. They are composed of a small subunit (SSU) that binds mRNA and decodes the genetic information and a large subunit (LSU) that actually catalyzes the synthesis of polypeptide chains. Even if ribosomes are ubiquitous to all living organisms, their structures and compositions, along with the factors that contribute to translation, have diverged. Ribosomes are not only different between prokaryotes and eukaryotes, but also between bacteria and archaea [7,18]. Eukaryotes are unique in the sense that different types of ribosomes co-exist in the same cells. Beyond the cytosolic ribosomes, ribosomes are also present in organelles containing a genome and a gene expression machinery (i.e. in mitochondria and chloroplasts in the plant lineage). It is now clear that organellar ribosomes have a prokaryote origin [19]. In contrast with chloroplast ribosomes that strongly resemble bacterial ribosomes [20,21], mitoribosomes greatly diverged from their bacterial counterparts [22]. A high number of studies have been published very recently describing mitochondrial translation systems, in particular mitoribosomes, in a wide diversity of eukaryotes and will be discussed below. They revealed that mitoribosomes diverged in the different eukaryote lineages, both in terms of global architecture and of ribosomal RNA (rRNA) and ribosomal protein (r-protein) components. This review focuses on specific features of mitochondrial translation, including the composition and structures of mitoribosomes, their assembly, translation initiation, and their specialization for membrane proteins synthesis.

#### **Mitoribosomes compositions and structures**

Mitoribosomes perform the translation of the few mRNAs encoded by mitochondrial genomes. While the exact nature of mitoribosomes had remained elusive for many years, early biochemical studies suggested that mitoribosomes were protein-rich as compared with bacterial ribosomes [23–26]. In the past few years, biochemical and structural data have been obtained for mitoribosomes from diverse eukaryotes, i.e. mammalian, ascomycetes, trypanosomes, and plants [27–32]. High-resolution structures obtained by **cryo-electron microscopy (cryoEM)** (Box 1) confirmed that mitoribosomes are indeed protein-rich and revealed an unexpected diversity of architectures (Figure 1). Mitoribosomes are larger than their bacterial counterpart, with diameters of 320, 330, 360, and 385Å for mammalian, yeast, plant, and trypanosome mitoribosomes, as compared to 260Å for *Escherichia coli* ribosome [27,28,30,31,33]. This is mostly due to extended small subunits, particularly in plants and trypanosomes where the SSU is actually more extended than the LSU [27,30].

#### *Diversity of mitoribosome rRNAs across eukaryotes*

These studies revealed that mitoribosomes are very diverse in structure; however, their catalytic core composed of rRNAs resembles that of bacterial ribosomes. mt-rRNAs are encoded in mitochondrial genomes [16], though their lengths are variable across eukaryotes [22]. The length of rRNAs are much shorter in metazoans, kinetoplastids, and apicomplexans due to large sequence deletions [12,34,35], while in fungi and particularly in plants, the length of rRNAs are significantly longer than bacterial ones because of large sequence insertions [36–38]. In particular, in most plants (i.e. in Angiosperms), a large expansion of the 18S rRNA makes the core of an elongated additional domain on the head of the SSU [27]. Beyond insertions and deletions, the organization of rRNAs in mitoribosomes is sometimes unusual. For example, in *Chlamydomonas* and *Plasmodium*, both LSU and SSU rRNAs are fragmented into several small RNAs encoded by gene pieces in the mtDNA [12,39].

Mitoribosomes are also defined by specific and variable central protuberances (CP). The core of this LSU domain contains the 5S rRNA in both bacterial and cytosolic ribosomes and mediates inter-

104 subunit contacts with the SSU head [40]. Conversely, in mitochondria, the 5S rRNA is often absent. In  
105 mammals, it is replaced by a mt-tRNA (tRNA<sup>Val</sup> in Human and tRNA<sup>Phe</sup> in *Sus scrofa*) [41,42]. The  
106 incorporation of a tRNA in the mitoribosome of both species appears to result from its position in the  
107 mitochondrial genome, where both mt-tRNAs flank the SSU rRNA with which they are co-transcribed  
108 [41–43]. In yeast, the 5S rRNA is also absent, but its loss is compensated by an expansion of the 21S  
109 rRNA [28]. In contrast to these examples, in plants and jakobids, 5S rRNAs are present similar to  
110 bacteria [27], and in Trypanosoma, the CP is devoid of RNA, being entirely made of proteins [30].

111  
112 Finally, another difference that has been observed in mitoribosomes but not in bacterial or  
113 cytoplasmic ribosomes is that the mt-rRNAs from investigated systems (e.g. animals, fungi, plants) do  
114 not possess anti-Shine-Dalgarno sequences at the 3' end of SSU rRNAs. This implies that their  
115 translation initiation systems have significantly diverged from the bacterial Shine-Dalgarno  
116 translation initiation [44].

#### 117 118 *Mitoribosome specific sets of r-proteins*

119 In contrast with mt-rRNAs, whose sizes were shortened or lengthened during eukaryote evolution,  
120 the number of r-proteins associated to mitoribosomes increased considerably in all eukaryote  
121 lineages compared to bacteria [27–30]. High resolution cryoEM structures of mitoribosomes  
122 identified proteomes ranging from 80 in yeast to 127 proteins in trypanosome as compared to the 54  
123 proteins of *E. coli* ribosome (Table 1). Still, some proteins of the bacterial core were lost from  
124 mitoribosomes [45]. The loss of these proteins from the mitoribosome is often linked with the  
125 deletion of their corresponding rRNA binding sites [29–31]. Beyond bacterial type r-proteins,  
126 additional proteins have been specifically recruited to mitoribosomes. Some of them are conserved  
127 in all investigated eukaryotes, while other novel mitochondrial r-proteins only occur in specific  
128 eukaryote lineages [27,30,46,47]. For example, mL41 and mL46 are part of the central protuberance  
129 and are present in all eukaryotes [22].

130  
131 All these proteins form an extensive interaction network on the surface of the rRNA core and might  
132 have contributed to the specialization of mitoribosomes [19], as species-specific proteins appear to  
133 give mitoribosomes distinctive properties. For instance, mL57 and mL58 only found in yeast appear  
134 to stabilize rRNA expansions [28]. Interestingly, the majority of these additional r-proteins, as well as  
135 specific insertions in canonical r-proteins, are characterized by  $\alpha$ -helical secondary structure  
136 elements [30]. Several of these proteins belong to the family of **pentatricopeptide repeat** (PPR)  
137 proteins (Figure 2 and Box 2). These proteins are found in the mitoribosomes of animals,  
138 trypanosoma, and plants (with 2, 6, and 10 PPRs respectively). The canonical mode of action of PPR  
139 proteins involves the binding to single stranded RNA. While this seems to be the case for mS39 in  
140 animals, which appears to make a platform to stabilize mRNAs during translation [48], all the other  
141 ribosomal PPR proteins make contacts with rRNAs or bind other r-proteins and act as structural  
142 components [29–31].

#### 143 144 **Evolution of mitoribosomes**

145 As described above, the composition and structure of mitoribosomes essentially rely on a bacterial  
146 scaffold that has differently diverged in the various eukaryote lineages. The first major steps of  
147 mitoribosome evolution seemingly occurred during eukaryogenesis, soon after the endosymbiosis  
148 event [22]. Indeed, given the composition of mitoribosomes, it is clear that several mitochondria  
149 specific proteins were acquired, and other proteins lost, during the early evolution of eukaryotes,  
150 before their radiation into the groups of modern eukaryotes. For instance, bS20 is missing in every  
151 mitochondrial ribosome investigated to date, indicating an early loss of the protein [49]. In contrast,  
152 other novel proteins (i.e. mS29, mL41, and mL46) are present in all mitoribosomes investigated to  
153 date and might constitute a common core of proteins that defined ancestral mitochondrial  
154 ribosomes (Table 1) [22,49].

155

156 The main reason towards the rapid evolution of the mitoribosomes might be explained by the  
157 erosion of mt-genomes. Genome reduction is thought to have played a crucial role toward energy  
158 production optimization, allowing faster replication, but resulted in the loss of mitoribosomal  
159 proteins, as well as in the reduction of mt-rRNAs in some cases [16,22]. In this line, the condensation  
160 of mtDNA also resulted in specific losses of mRNA needing to be translated [22]. This might have led  
161 to further specialization of mitoribosomes in specific eukaryote groups. For instance, in mammals,  
162 the mitoribosome always translates mRNAs encoding respiratory chain membrane proteins [50]. This  
163 might have led to the specific recruitment of mL45 to ease the interactions between the ribosome  
164 exit tunnel and the mitochondrial inner membrane [29,31]. In other cases, the specialization of  
165 mitoribosomes might have resulted from neutral evolution where structural features were fixed into  
166 mitoribosomes following codependent mutations without any obvious functional reason [16,51,52].  
167 It was also proposed that this rapid evolution of mitoribosome structures was facilitated by  
168 “structural patching” where preexisting RNAs or proteins were recruited to compensate for losses of  
169 ribosome structural elements [22]. In particular, throughout evolution, this might have resulted in  
170 the successive accretion of novel proteins, such as PPR proteins (but also other alpha helical proteins  
171 like Tetratricopeptide repeat proteins (TPR), Huntingtin, elongation factor 3 (EF3) (HEAT), protein  
172 phosphatase 2A (PP2A), the yeast kinase TOR1, and Armadillo (ARM) motif proteins) [27,28,30,31] on  
173 the existing core, in some cases to replace reduced rRNA elements and in other cases to gain new  
174 functions and/or to adapt to specialized translation processes in the respective eukaryote niches.

175

#### 176 **Mitochondrial translation machinery biogenesis and assembly**

177 Mitoribosome biogenesis occurs in the mitochondrial matrix [53]. It is a complex process which  
178 requires the coordinated synthesis of mitochondrial r-proteins, most of whose mRNAs are encoded  
179 in the nucleus, translated in the cytosol, and imported in mitochondria [46,47], with the synthesis of  
180 the components encoded by the mt-genome (i.e. rRNAs and r-proteins, in some cases) [16]. The  
181 latter are not encoded in animal mt-genomes, but are present in other organisms, in particular in  
182 jakobids where 27 r-proteins are still encoded by the mt-genome [14]. Thus, mitoribosome  
183 biogenesis heavily relies on cytosolic translation. Few studies have investigated the assembly of  
184 mitoribosomes [54–57]. Still, evidences suggest that in human and yeast, this process is very similar  
185 to what is observed in bacteria; in prokaryotes, the rRNAs serve as scaffolds that are stabilized and  
186 protected by r-proteins. These proteins are sequentially recruited, with the help of assembly factors,  
187 and stabilize the folding of the rRNAs [58].

188

189 In human, the structures of two assembly intermediates of the LSU were resolved by cryoEM [54].  
190 These revealed, similar to bacteria, that a late intermediate structure where the mt-rRNA  
191 constituting the peptidyl transferase center (PTC) is unfolded and bL36m is missing. In addition, a  
192 complex of three proteins (MALSUI1 an RsfS family protein, LOR8F8, and mt-ACP) interacting with  
193 uL14m and the sarcin–ricin stem–loop (SRL; helix H95) of mt-LSU rRNA was also described, and its  
194 function is proposed to prevent premature subunit association [54]. It is also noteworthy that in  
195 human, similar to bacteria, rRNA modifications, i.e. methylations and pseudouridylations, are  
196 required for the biogenesis of the mitoribosome LSU [59–61]. Finally, a SILAC pulse labeling approach  
197 was used to determine the order and kinetics of mitoribosomal r-proteins recruitment [57]. This  
198 showed that full mitoribosome assembly occurs in about 2–3 hours and revealed that most  
199 mitochondrial r-proteins are synthesized and imported to mitochondria in considerable excess.

200

201 Likewise, in yeast, a genetic approach showed that LSU assembly is rather similar to the biogenesis of  
202 *E. coli* LSU. For this, the 44 genes coding for mtLSU r-proteins were systematically deleted and the  
203 effects on the sequence of events leading to ribosome biogenesis were observed [56]. Nonetheless,  
204 yeast LSU assembly also involves the incorporation of additional mitochondria-specific r-proteins and  
205 uses novel biogenesis factors [62]. This genetic study also showed that many r-proteins (conserved in  
206 ribosomes or specific to mitochondria) are not essential. Finally, it revealed that yeast LSU assembly  
207 is localized at the mitochondrial inner membrane.

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In contrast, in trypanosoma, mitoribosome assembly is likely to be very different from bacteria. It was proposed that mitoribosome assembly is driven by proteins and not by rRNAs because of the extreme reduction of rRNA sizes. It appears unlikely that the reduced rRNAs can coalesce into rRNA tertiary structures that would then be completed by proteins. It is more likely that unfolded rRNA bind to and are shaped on preformed protein sub-complexes to build the trypanosoma mitoribosome SSU and LSU [30]. Recently, this hypothesis was reinforced with the structures resolved by cryoEM of three different assembly intermediates of the *T.brucei* mitoribosome SSU [55]. The three different intermediates allowed to determine a partial order of r-proteins integration in the mitoribosome SSU. The most immature intermediate, termed assemblosome, is also the largest of the three intermediates forming a 4-MDa complex. Although presenting an incomplete set of r-proteins, it is shaped by 34 maturation factors that interact with the partially immature 9S rRNA.

### **Recruitment of mRNA to mitoribosomes and translation initiation**

While mitochondrial translation initiation shares some similar features with bacterial translation initiation, it mainly involves specific processes that differ in distinct eukaryote lineages. For example, in mitochondria, similar to bacteria, translation initiation uses a formylated fMet-tRNA<sup>Met</sup>, even though in yeast it has been suggested that formylation is not required [63,64]. However, the factors involved are different. In bacteria, translation initiation involves three essential initiation factors, IF1, IF2, and IF3 [65]. In brief, IF1 associates with the SSU in the A site and prevents an aminoacyl-tRNA from entering. IF2 binds to the initiator tRNA and controls its entry in the ribosome. Finally, IF3 is required for SSU binding to the initiation site in mRNA. IF1 and IF2 are called “universal” translation initiation factors because they have conserved functional and structural homologs in both eukaryotes and Archaea [66]. However, the sets of IF proteins used for mitochondrial translation initiation diverged considerably as compared to bacteria. While mitochondrial IF2 (mt-IF2) has been observed in all mitochondria studied thus far, mt-IF3 does not always occur (in yeast Aim23p was proposed to be a mt-IF3 orthologue [67] and IF1 appears to have been lost during mitochondrial evolution as it has not been characterized in any mitochondria studied yet [67]). The observation that bovine mt-IF2 is able to complement the essential functions of both bacterial IF1 and IF2 suggests that mt-IF2 might have acquired the function of IF1 [68]. This appears to indeed be the case as a 37 amino acids insertion conserved in vertebrate mitochondrial IF2 proteins appears to have taken over IF1 function [68,69]. This was further confirmed with the recent determination of human mitoribosome structures in complex with (i) mtIF2 and fMet-tRNA<sup>Met</sup> and (ii) with mtIF3 alone [48,70].

Beyond initiation factors, a main difference between mitochondrial and bacterial translation initiation processes is that mitochondrial mRNAs and SSU rRNAs lost their respective Shine Dalgarno (SD) and anti-SD sequences [46]. Hence, the mechanisms for mRNA recruitment to the small subunit, as well as for the correct positioning of start codons, was unclear [69]. In animals, this process was even more elusive as some mt-mRNAs do not have 5' untranslated regions (UTR) [71]. Possible cis-elements involved in ribosome binding thus appeared to be absent. However, the recent determination of yeast and mammalian mitoribosome high-resolution structures provided the first evidences to understand mRNA recruitment in fungi and animals mitochondria [28,48].

In mammalian mitoribosomes, translation initiation uses a PPR protein (Box 2) [48]. This protein, mS39, is located at the SSU head near the mRNA entry channel. It was proposed that mS39 could be involved in the binding of the leaderless mRNAs to aid their threading through the remodeled mRNA channel entrance toward the decoding center [29,31]. The recent structure of the mammalian translation initiation complex reconstituted *in vitro* confirmed this hypothesis. The PPR motifs of mS39 bind the mRNA downstream of its AUG initiation codon in a U-rich region (Figure 2) [48]. Such U-rich sequences are conserved (downstream of codon 7) in the 11 mRNAs of mammalian mitochondria and might all be specifically bound by mS39 for mRNA recruitment to mitoribosomes [19,31,48].

260  
261 Yeast mitochondria use a different translation initiation system. mRNAs have long 5' UTRs bound by  
262 translation activators. These factors are usually specific to a particular transcript and are essential for  
263 the translation of the respective transcripts [72]. Moreover, the mRNA exit channel of yeast  
264 mitoribosome is highly remodeled, composed of yeast specific mitoribosomal r-proteins, forming a V-  
265 shaped canyon at the channel exit. The cryoEM map established in 2017 showed that additional  
266 electron densities are detected on the V-shaped canyon, suggesting that this canyon might act both  
267 as an extended mRNA channel and a binding platform for translation activators [28].  
268

269 Similar to yeast, plant mt-mRNAs have long 5' UTRs, and several PPR proteins occur as core  
270 mitoribosome proteins [27]. Some of them might be involved in translation initiation, similar to mS39  
271 in animals, but this has yet to be studied in depth.  
272

### 273 **Specialization for membrane proteins translation and interaction with insertase systems**

274 The few protein-coding genes retained in mt-genomes are relatively conserved across eukaryotes.  
275 Most of them are components of the respiratory chain [16]. The two proteins universally conserved  
276 in all known mt-genomes are *cob* and *cox1*, which encode proteins of the oxidative phosphorylation  
277 (OXPHOS) complexes III and IV respectively [5,16]. Respiratory chain components are often highly  
278 hydrophobic membrane proteins [73]. Mitoribosomes therefore appear to have evolved to  
279 synthesize a number of hydrophobic respiratory proteins.  
280

281 Recent studies have shown that mitoribosomes are often localized and bound to the mitochondrial  
282 inner membrane (IMM) [74,75]. However, different membrane anchors are used in different systems  
283 (Figure 3). *In situ cryo-electron tomography* studies performed with purified mitochondria have  
284 shown how yeast and human mitoribosomes are arranged and organized at the IMM [74,75]. It  
285 suggested the presence of polysome-like structures. It also revealed additional densities on  
286 ribosomal particles that were not observed with isolated mitoribosomes in cryoEM studies. The  
287 additional density observed for the human mitoribosome is illustrated in Figure 3. In both yeast and  
288 human, it was shown that some mitochondrial encoded membrane proteins are co-translationally  
289 inserted into the IMM and that at least a sub-population of mitoribosomes are bound to the IMM  
290 [74,75]. In the case of mammalian mitoribosomes, this attachment is most likely mediated by the  
291 mitochondria-specific protein mL45, located next to the ribosomal exit tunnel [48]. In contrast, in  
292 yeast, the mitoribosome attachment to the IMM is mediated by the protein Mba1, as well as by the  
293 rRNA expansion segment of helix 96 [75,76]. In both mammals and yeast, the nascent protein is  
294 thought to be co-translationally inserted into the IMM by the insertase Oxa1 [19,77,78] (Figure 3).  
295 Moreover, yeast, mammals, and trypanosoma systems diverge significantly as their polypeptide exit  
296 tunnels have been extensively remodeled with several lineage-specific mitoribosomal proteins, most  
297 likely to facilitate membrane association and co-translational insertion of proteins in the IMM [28–  
298 31]. However, while the attachment of mitoribosomes to the IMM has been described for fungi and  
299 mammalian, it might not be ubiquitous across eukaryotes. Mitochondria might have different sets of  
300 ribosomes bound or not to the IMM to accommodate the synthesis of both hydrophobic and  
301 hydrophilic proteins.  
302

### 303 **Interplay between mitochondrial translation and RNA processing pathways**

304 While the composition and architecture of mitoribosomes is beginning to be unravelled in an  
305 increasing number of representative eukaryotes, little is known on translation regulation. Recent  
306 studies have shown that translational activators play key roles in this regulation and that  
307 mitochondrial translation is tightly coupled with other RNA maturation, processing, and degradation  
308 pathways [79].

309 While PPR proteins have been identified as core ribosomal proteins, several other PPR proteins have  
310 also been recognized as translation activators in many different eukaryotes in the recent years. For  
311 example, in human, the PPR protein LRPPRC acts together with SLIRP to resolve mRNA secondary

312 structures, to enable translation initiation, and to promote RNA stabilization and **polyadenylation**  
313 [80]. Similarly, in yeast, PPR proteins, such as Pet111, Atp22, or Aep1 bind mRNAs and act as  
314 translation activators [81], although their mode of action remains elusive. Pet111 was recently  
315 proposed to resolve mRNA secondary structures to allow translation initiation, similar to LRPPRC  
316 [82]. Likewise, in *T. brucei*, non-mitoribosomal PPR proteins were identified during affinity  
317 purification of both mitoribosomes and the polyadenylation complex [83]. Functional analysis of two  
318 of these PPR proteins suggest that they make an interface between polyadenylation and translation  
319 machinery, and appear to be essential to stimulate mRNA translation [84]. In the same line,  
320 translation is a dynamic and regulated process in plant mitochondria [85] and plant PPR proteins  
321 were identified as translation regulators [86,87]. The immunoprecipitation of complexes with a core  
322 mitoribosome protein used as a bait identified gene expression regulators associated to the  
323 ribosome, including several PPR proteins and other RNA binding proteins [27]. Altogether, studies  
324 performed in different eukaryotes all suggest a close interaction between RNA processing and  
325 translation in mitochondria.

326

### 327 **Mitochondrial translation dysfunctions in diseases and drug design**

328 Mitochondrial translation and its regulations are essential processes in eukaryote cells. Dysfunctions  
329 of these processes lead to severe disorders in very diverse eukaryotes. In particular, in human,  
330 several inherited disorders are caused by mitochondrial translation dysfunctions. In the case of  
331 mitoribosome dysfunctions, diseases have been biochemically characterized by alterations in  
332 OXPHOS complexes containing mitochondrial encoded subunits [88]. Translation deficiencies are  
333 caused by either mitochondrial or nuclear mutations affecting genes encoding r-proteins, rRNAs, or  
334 tRNAs [89–93]. Additionally, mutations in initiation factors, elongation, and termination factors, or in  
335 aminoacyl tRNA synthetases, also lead to severe clinical cases [94–97]. These mutations generally  
336 lead to severe growth retardation, neurological disorders, myopathies, and/or other characteristic  
337 symptoms (e.g. earing-loss) [98]. Beyond genetic inherited diseases, it was shown that many cancer  
338 cells have altered OXPHOS capacity and that elevated mitochondrial translation stimulates cell  
339 proliferation [99]. Mitochondrial translation thus constitutes an important target for cancer therapy  
340 [100,101].

341

342 In plants as well, mitochondrial translation defects lead to disorders. A widely expanded trait called  
343 cytoplasmic male sterility (CMS), characterized by an inability of plants to produce functional pollen  
344 is caused by mitochondrial dysfunctions [102]. The CMS phenotype can be suppressed by nuclear  
345 “restorers of fertility” (Rf) genes. The phenomenon of CMS and nuclear fertility restoration are of  
346 great commercial interest and are extensively used for the production of higher yielding hybrid crop  
347 varieties world-wide. Interestingly, most Rf genes encode PPR proteins and some of them were  
348 experimentally shown to be regulators of translation [103,104].

349

350 In a wider context, the evolutionary drift of mitochondria in the different eukaryote groups has  
351 allowed the appearance of highly divergent mitoribosomes that nevertheless all perform the same  
352 core function. This diversity implies that many components, either r-proteins or rRNAs, can be  
353 specifically targeted for drug development. In particular, mitoribosomes constitute a great target for  
354 species-specific targets to tackle non-vaccine-preventable diseases such as malaria, sleeping sickness,  
355 and Chagas disease, which are mainly caused by protozoan parasites [105].

356

### 357 **Concluding Remarks and Future Perspectives**

358 The recent analysis of compositions and structures of mitoribosomes from diverse eukaryotes  
359 (animal, fungi, plants, and trypanosomes) has shown that mitoribosomes are incredibly diverse,  
360 while cytosolic ribosomes are comparatively much more conserved across eukaryotes [19].  
361 Nonetheless, a lot of information is still missing to fully apprehend the diversity of mitochondrial  
362 translation systems across eukaryotes (see Outstanding Questions). For instance, no data is available  
363 for the eukaryote supergroup containing Stramenopiles, Alveolata and Rhizaria (Figure 1). Among

364 them, it should be very informative to determine mitoribosome structures for prevalent eukaryotes  
365 such as diatoms or parasites responsible for diseases such as *P. falciparum*. Similarly, in other super-  
366 groups such as Archaeplastida (the plant group), no data is available for unicellular green algae or  
367 brown and red algae (Chlorophyta such as *Chlamydomonas reinhardtii*, Glaucophyta and  
368 Rhodophyceae). In some species such as *C. reinhardtii* and *P. falciparum* where rRNAs genes have  
369 been split into several sub-fragments, it will be instrumental to determine how mitoribosome  
370 architecture is reconstituted from these sub-fragments [12,106]. Moreover, in the plant group, some  
371 specific features (i.e. 18S rRNA additional domains) are only predicted to occur in flowering plants.  
372 Therefore mitoribosomes should also be very different in other major groups of the green lineage  
373 such as ferns, mosses or gymnosperms [27]. Finally, among Excavata, while mitoribosome structure  
374 was determined for *T. brucei*, it will be essential to understand mitoribosome evolution to study  
375 jakobids such as *R.americana* whose mitochondria seemingly closely resemble the original  
376 endosymbiont, therefore their mitoribosomes are predicted to be more bacterial-like as compared to  
377 the other eukaryotes [107]. By comparing all these systems, we should obtain clues to understand  
378 why mitoribosomes are so diverse as compared to their cytosolic counterparts across eukaryotes.  
379

380 Other major challenges will be to understand the exact functions of all the novel r-proteins identified  
381 in different eukaryote groups. It will also be essential to understand the diversity of mitochondrial  
382 translation initiation mechanisms, to capture ribosome assembly intermediates and to understand  
383 how mitoribosomes are integrated within the global contexts of (i) mRNA biogenesis and turnover  
384 processes and (ii) machineries responsible for the coordinated assembly of mitochondrial and  
385 nuclear encoded proteins into OXPHOS complexes. Overall, understanding the diversity and the  
386 evolution of mitochondrial translation systems in eukaryotes will reveal common features and  
387 specificities that will possibly be used in the longer term for socio-economical applications going from  
388 agriculture to human health.  
389

390

391

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401 **References**

- 402 1 Spinelli, J.B. and Haigis, M.C. (2018) The multifaceted contributions of mitochondria to cellular  
403 metabolism. *Nat. Cell Biol.* 20, 745–754
- 404 2 Mitchell, P. (1961) Coupling of Phosphorylation to Electron and Hydrogen Transfer by a  
405 Chemi-Osmotic type of Mechanism. *Nature* 191, 144–148
- 406 3 Lill, R. *et al.* (1999) The Essential Role of Mitochondria in the Biogenesis of Cellular Iron-Sulfur  
407 Proteins. *Biol. Chem.* 380, 1157–66
- 408 4 Krebs, H.A. and Johnson, W.A. (1937) Metabolism of ketonic acids in animal tissues. *Biochem.*  
409 *J.* 31, 645–660
- 410 5 Gray, M.W. (2015) Mosaic nature of the mitochondrial proteome: Implications for the origin  
411 and evolution of mitochondria. *Proc. Natl. Acad. Sci.* 112, 10133–10138
- 412 6 Martijn, J. *et al.* (2018) Deep mitochondrial origin outside the sampled alphaproteobacteria.  
413 *Nature* 557, 101–105
- 414 7 Eme, L. *et al.* (2017) Archaea and the origin of eukaryotes. *Nat. Rev. Microbiol.* 15, 711–723
- 415 8 Yang, D. *et al.* (1985) Mitochondrial origins. *Proc. Natl. Acad. Sci. U. S. A.* 82, 4443–7
- 416 9 Roise, D. *et al.* (1986) A chemically synthesized pre-sequence of an imported mitochondrial  
417 protein can form an amphiphilic helix and perturb natural and artificial phospholipid bilayers.  
418 *EMBO J.* 5, 1327–1334
- 419 10 Abe, Y. *et al.* (2000) Structural Basis of Presequence Recognition by the Mitochondrial Protein  
420 Import Receptor Tom20. *Cell* 100, 551–560
- 421 11 Salinas-Giegé, T. *et al.* (2015) tRNA Biology in Mitochondria. *Int. J. Mol. Sci.* 16, 4518–4559
- 422 12 Feagin, J.E. *et al.* (2012) The Fragmented Mitochondrial Ribosomal RNAs of *Plasmodium*  
423 *falciparum*. *PLoS One* 7, e38320
- 424 13 Sloan, D.B. *et al.* (2012) Rapid Evolution of Enormous, Multichromosomal Genomes in  
425 Flowering Plant Mitochondria with Exceptionally High Mutation Rates. *PLoS Biol.* 10,  
426 e1001241
- 427 14 Burger, G. *et al.* (2013) Strikingly Bacteria-Like and Gene-Rich Mitochondrial Genomes  
428 throughout Jakobid Protists. *Genome Biol. Evol.* 5, 418–438
- 429 15 Tyagi, S. *et al.* (2014) Whole mitochondrial genome sequence of an Indian *Plasmodium*  
430 *falciparum* field isolate. *Korean J. Parasitol.* 52, 99–103
- 431 16 Sloan, D.B. *et al.* (2018) Cytonuclear integration and co-evolution. *Nat. Rev. Genet.* 19, 635–  
432 648
- 433 17 Petrov, A.S. *et al.* (2015) History of the ribosome and the origin of translation. *Proc. Natl.*  
434 *Acad. Sci.* 112, 15396–15401
- 435 18 Greber, B.J. *et al.* (2012) Cryo-EM Structure of the Archaeal 50S Ribosomal Subunit in Complex  
436 with Initiation Factor 6 and Implications for Ribosome Evolution. *J. Mol. Biol.* 418, 145–160
- 437 19 Bieri, P. *et al.* (2018) High-resolution structures of mitochondrial ribosomes and their  
438 functional implications. *Curr. Opin. Struct. Biol.* 49, 44–53
- 439 20 Boerema, A.P. *et al.* (2018) Structure of the chloroplast ribosome with chl-RRF and  
440 hibernation-promoting factor. *Nat. plants* 4, 212–217
- 441 21 Bieri, P. *et al.* (2017) The complete structure of the chloroplast 70S ribosome in complex with  
442 translation factor pY. *EMBO J.* 36, 475–486
- 443 22 Petrov, A.S. *et al.* (2019) Structural Patching Fosters Divergence of Mitochondrial Ribosomes.  
444 *Mol. Biol. Evol.* 36, 207–219
- 445 23 Goldschmidt-Reisin, S. *et al.* (1998) Mammalian Mitochondrial Ribosomal Proteins. *J. Biol.*  
446 *Chem.* 273, 34828–34836
- 447 24 Kitakawa, M. *et al.* (1997) Identification and Characterization of the Genes for Mitochondrial  
448 Ribosomal Proteins of *Saccharomyces Cerevisiae*. *Eur. J. Biochem.* 245, 449–456
- 449 25 Cavdar Koc, E. *et al.* (2001) The Small Subunit of the Mammalian Mitochondrial Ribosome. *J.*  
450 *Biol. Chem.* 276, 19363–19374
- 451 26 Koc, E.C. *et al.* (2001) The Large Subunit of the Mammalian Mitochondrial Ribosome. *J. Biol.*  
452 *Chem.* 276, 43958–43969

453 27 Waltz, F. *et al.* (2019) Small is big in Arabidopsis mitochondrial ribosome. *Nat. Plants* 5, 106–  
454 117

455 28 Desai, N. *et al.* (2017) The structure of the yeast mitochondrial ribosome. *Science (80-. )*. 355,  
456 528–531

457 29 Greber, B.J. *et al.* (2015) The complete structure of the 55S mammalian mitochondrial  
458 ribosome. *Science (80-. )*. 348, 303–308

459 30 Ramrath, D.J.F. *et al.* (2018) Evolutionary shift toward protein-based architecture in  
460 trypanosomal mitochondrial ribosomes. *Science (80-. )*. 362, eaau7735

461 31 Amunts, A. *et al.* (2015) The structure of the human mitochondrial ribosome. *Science (80-. )*.  
462 348, 95–98

463 32 van der Sluis, E.O. *et al.* (2015) Parallel Structural Evolution of Mitochondrial Ribosomes and  
464 OXPHOS Complexes. *Genome Biol. Evol.* 7, 1235–1251

465 33 Noeske, J. *et al.* (2015) High-resolution structure of the Escherichia coli ribosome. *Nat. Struct.*  
466 *Mol. Biol.* 22, 336–341

467 34 Kirby, L.E. and Koslowsky, D. (2017) Mitochondrial dual-coding genes in Trypanosoma brucei.  
468 *PLoS Negl. Trop. Dis.* 11, e0005989

469 35 Taanman, J.-W. (1999) The mitochondrial genome: structure, transcription, translation and  
470 replication. *Biochim. Biophys. Acta - Bioenerg.* 1410, 103–123

471 36 Marienfeld, J. *et al.* (1999) The mitochondrial genome of Arabidopsis is composed of both  
472 native and immigrant information. *Trends Plant Sci.* 4, 495–502

473 37 Sloan, D.B. *et al.* (2018) Correction of Persistent Errors in Arabidopsis Reference  
474 Mitochondrial Genomes. *Plant Cell* 30, 525–527

475 38 Wolters, J.F. *et al.* (2015) Population structure of mitochondrial genomes in Saccharomyces  
476 cerevisiae. *BMC Genomics* 16, 451

477 39 Salinas-Giegé, T. *et al.* (2017) Polycytidylation of mitochondrial mRNAs in Chlamydomonas  
478 reinhardtii. *Nucleic Acids Res.* 45, 12963–12973

479 40 Ban, N. *et al.* (2000) The complete atomic structure of the large ribosomal subunit at 2.4 Å  
480 resolution. *Science (80-. )*. 289, 905–920

481 41 Brown, A. *et al.* (2014) Structure of the large ribosomal subunit from human mitochondria.  
482 *Science* 346, 718–722

483 42 Greber, B.J. *et al.* (2013) Architecture of the large subunit of the mammalian mitochondrial  
484 ribosome. *Nature* 505, 515–519

485 43 Chrzanowska-Lightowlers, Z. *et al.* (2017) Human mitochondrial ribosomes can switch  
486 structural tRNAs – but when and why? *RNA Biol.* 14, 1668–1671

487 44 Yusupova, G.Z. *et al.* (2001) The Path of Messenger RNA through the Ribosome. *Cell* 106, 233–  
488 241

489 45 Smits, P. *et al.* (2007) Reconstructing the evolution of the mitochondrial ribosomal proteome.  
490 *Nucleic Acids Res.* 35, 4686–4703

491 46 Ott, M. *et al.* (2016) Organization and Regulation of Mitochondrial Protein Synthesis. *Annu.*  
492 *Rev. Biochem.* 85, 77–101

493 47 Greber, B.J. and Ban, N. (2016) Structure and Function of the Mitochondrial Ribosome. *Annu.*  
494 *Rev. Biochem.* 85, 103–132

495 48 Kummer, E. *et al.* (2018) Unique features of mammalian mitochondrial translation initiation  
496 revealed by cryo-EM. *Nature* 560, 263–267

497 49 Desmond, E. *et al.* (2011) On the last common ancestor and early evolution of eukaryotes:  
498 reconstructing the history of mitochondrial ribosomes. *Res. Microbiol.* 162, 53–70

499 50 Anderson, S. *et al.* (1981) Sequence and organization of the human mitochondrial genome.  
500 *Nature* 290, 457–465

501 51 Finnigan, G.C. *et al.* (2012) Evolution of increased complexity in a molecular machine. *Nature*  
502 481, 360–364

503 52 Stoltzfus, A. (1999) On the possibility of constructive neutral evolution. *J. Mol. Evol.* 49, 169–  
504 181

505 53 De Silva, D. *et al.* (2015) Mitochondrial ribosome assembly in health and disease. *Cell Cycle* 14,  
506 2226–2250

507 54 Brown, A. *et al.* (2017) Structures of the human mitochondrial ribosome in native states of  
508 assembly. *Nat. Struct. Mol. Biol.* 24, 866–869

509 55 Saurer, M. *et al.* (2019) Mitoribosomal small subunit biogenesis in trypanosomes involves an  
510 extensive assembly machinery. *Science* 365, 1144–1149

511 56 Zeng, R. *et al.* (2018) Yeast Mitoribosome Large Subunit Assembly Proceeds by Hierarchical  
512 Incorporation of Protein Clusters and Modules on the Inner Membrane. *Cell Metab.* 27, 645-  
513 656.e7

514 57 Bogenhagen, D.F. *et al.* (2018) Kinetics and Mechanism of Mammalian Mitochondrial  
515 Ribosome Assembly. *Cell Rep.* 22, 1935–1944

516 58 Davis, J.H. and Williamson, J.R. (2017) Structure and dynamics of bacterial ribosome  
517 biogenesis. *Philos. Trans. R. Soc. B Biol. Sci.* 372, 20160181

518 59 Rorbach, J. *et al.* (2014) MRM2 and MRM3 are involved in biogenesis of the large subunit of  
519 the mitochondrial ribosome. *Mol. Biol. Cell* 25, 2542–2555

520 60 Bar-Yaacov, D. *et al.* (2016) Mitochondrial 16S rRNA Is Methylated by tRNA Methyltransferase  
521 TRMT61B in All Vertebrates. *PLOS Biol.* 14, e1002557

522 61 Antonicka, H. *et al.* (2017) A pseudouridine synthase module is essential for mitochondrial  
523 protein synthesis and cell viability. *EMBO Rep.* 18, 28–38

524 62 Chen, S.S. and Williamson, J.R. (2013) Characterization of the Ribosome Biogenesis Landscape  
525 in *E. coli* Using Quantitative Mass Spectrometry. *J. Mol. Biol.* 425, 767–779

526 63 Spencer, A.C. and Spremulli, L.L. (2004) Interaction of mitochondrial initiation factor 2 with  
527 mitochondrial fMet-tRNA. *Nucleic Acids Res.* 32, 5464–70

528 64 Li, Y. *et al.* (2000) Initiation of protein synthesis in *Saccharomyces cerevisiae* mitochondria  
529 without formylation of the initiator tRNA. *J. Bacteriol.* 182, 2886–92

530 65 Gualerzi, C.O. and Pon, C.L. (1990) Initiation of mRNA translation in prokaryotes. *Biochemistry*  
531 29, 5881–5889

532 66 Roll-Mecak, A. *et al.* (2001) Engaging the ribosome: universal IFs of translation. *Trends*  
533 *Biochem. Sci.* 26, 705–709

534 67 Atkinson, G.C. *et al.* (2012) Evolutionary and genetic analyses of mitochondrial translation  
535 initiation factors identify the missing mitochondrial IF3 in *S. cerevisiae*. *Nucleic Acids Res.* 40,  
536 6122–6134

537 68 Gaur, R. *et al.* (2008) A Single Mammalian Mitochondrial Translation Initiation Factor  
538 Functionally Replaces Two Bacterial Factors. *Mol. Cell* 29, 180–190

539 69 Kuzmenko, A. *et al.* (2014) Mitochondrial translation initiation machinery: Conservation and  
540 diversification. *Biochimie* 100, 132–140

541 70 Koripella, R.K. *et al.* (2019) Structural insights into unique features of the human  
542 mitochondrial ribosome recycling. *Proc. Natl. Acad. Sci.* 116, 8283–8288

543 71 Montoya, J. *et al.* (1981) Distinctive features of the 5'-terminal sequences of the human  
544 mitochondrial mRNAs. *Nature* 290, 465–470

545 72 Derbikova, K.S. *et al.* (2018) Activation of Yeast Mitochondrial Translation: Who Is in Charge?  
546 *Biochem.* 83, 87–97

547 73 Sousa, J.S. *et al.* (2018) Mitochondrial Respiratory Chain Complexes. pp. 167–227

548 74 Englmeier, R. *et al.* (2017) Structure of the Human Mitochondrial Ribosome Studied In Situ by  
549 Cryoelectron Tomography. *Structure* 25, 1574-1581.e2

550 75 Pfeiffer, S. *et al.* (2015) Organization of the mitochondrial translation machinery studied in situ  
551 by cryoelectron tomography. *Nat. Commun.* 6, 6019

552 76 Ott, M. *et al.* (2006) Mba1, a membrane-associated ribosome receptor in mitochondria.  
553 *EMBO J.* 25, 1603–1610

554 77 Haque, M.E. *et al.* (2010) Properties of the C-terminal Tail of Human Mitochondrial Inner  
555 Membrane Protein Oxa1L and Its Interactions with Mammalian Mitochondrial Ribosomes. *J.*  
556 *Biol. Chem.* 285, 28353–28362

557 78 Szyrach, G. (2003) Ribosome binding to the Oxa1 complex facilitates co-translational protein  
558 insertion in mitochondria. *EMBO J.* 22, 6448–6457

559 79 Kehrein, K. *et al.* (2015) Organization of Mitochondrial Gene Expression in Two Distinct  
560 Ribosome-Containing Assemblies. *Cell Rep.* 10, 843–853

561 80 Siira, S.J. *et al.* (2017) LRPPRC-mediated folding of the mitochondrial transcriptome. *Nat.*  
562 *Commun.* 8, 1532

563 81 Herbert, C.J. *et al.* (2013) Yeast PPR proteins, watchdogs of mitochondrial gene expression.  
564 *RNA Biol.* 10, 1477–1494

565 82 Jones, J.L. *et al.* (2019) Yeast mitochondrial protein Pet111p binds directly to two distinct  
566 targets in COX2 mRNA, suggesting a mechanism of translational activation. *J. Biol. Chem.* 294,  
567 7528–7536

568 83 Aphasizheva, I. *et al.* (2011) Pentatricopeptide Repeat Proteins Stimulate mRNA  
569 Adenylation/Uridylation to Activate Mitochondrial Translation in Trypanosomes. *Mol. Cell* 42,  
570 106–117

571 84 Aphasizheva, I. *et al.* (2016) Ribosome-associated pentatricopeptide repeat proteins function  
572 as translational activators in mitochondria of trypanosomes. *Mol. Microbiol.* 99, 1043–1058

573 85 Planchard, N. *et al.* (2018) The translational landscape of Arabidopsis mitochondria. *Nucleic*  
574 *Acids Res.* 46, 6218–6228

575 86 Uyttewaal, M. *et al.* (2008) PPR336 is Associated with Polysomes in Plant Mitochondria. *J.*  
576 *Mol. Biol.* 375, 626–636

577 87 Haïli, N. *et al.* (2016) The MTL1 Pentatricopeptide Repeat Protein Is Required for Both  
578 Translation and Splicing of the Mitochondrial NADH DEHYDROGENASE SUBUNIT7 mRNA in  
579 Arabidopsis. *Plant Physiol.* 170, 354–366

580 88 Fiedorczuk, K. and Sazanov, L.A. (2018) Mammalian Mitochondrial Complex I Structure and  
581 Disease-Causing Mutations. *Trends Cell Biol.* 28, 835–867

582 89 Liu, Z. *et al.* (2014) The novel mitochondrial 16S rRNA 2336T>C mutation is associated with  
583 hypertrophic cardiomyopathy. *J. Med. Genet.* 51, 176–184

584 90 Menezes, M.J. *et al.* (2015) Mutation in mitochondrial ribosomal protein S7 (MRPS7) causes  
585 congenital sensorineural deafness, progressive hepatic and renal failure and lactic acidemia.  
586 *Hum. Mol. Genet.* 24, 2297–2307

587 91 Lake, N.J. *et al.* (2017) Biallelic Mutations in MRPS34 Lead to Instability of the Small  
588 Mitoribosomal Subunit and Leigh Syndrome. *Am. J. Hum. Genet.* 101, 239–254

589 92 Carroll, C.J. *et al.* (2013) Whole-exome sequencing identifies a mutation in the mitochondrial  
590 ribosome protein MRPL44 to underlie mitochondrial infantile cardiomyopathy. *J. Med. Genet.*  
591 50, 151–159

592 93 Distelmaier, F. *et al.* (2015) MRPL44 mutations cause a slowly progressive multisystem disease  
593 with childhood-onset hypertrophic cardiomyopathy. *Neurogenetics* 16, 319–323

594 94 Scheper, G.C. *et al.* (2007) Mitochondrial aspartyl-tRNA synthetase deficiency causes  
595 leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation. *Nat.*  
596 *Genet.* 39, 534–539

597 95 Pierce, S.B. *et al.* (2011) Mutations in mitochondrial histidyl tRNA synthetase HARS2 cause  
598 ovarian dysgenesis and sensorineural hearing loss of Perrault syndrome. *Proc. Natl. Acad. Sci.*  
599 108, 6543–6548

600 96 Valente, L. *et al.* (2007) Infantile Encephalopathy and Defective Mitochondrial DNA  
601 Translation in Patients with Mutations of Mitochondrial Elongation Factors EFG1 and EFTu.  
602 *Am. J. Hum. Genet.* 80, 44–58

603 97 Mootha, V.K. *et al.* (2003) Identification of a gene causing human cytochrome c oxidase  
604 deficiency by integrative genomics. *Proc. Natl. Acad. Sci.* 100, 605–610

605 98 Boczonadi, V. *et al.* (2018) Mitochondrial DNA transcription and translation: clinical  
606 syndromes. *Essays Biochem.* 62, 321–340

607 99 Ashton, T.M. *et al.* (2018) Oxidative Phosphorylation as an Emerging Target in Cancer Therapy.  
608 *Clin. Cancer Res.* 24, 2482–2490

609 100 Škrtić, M. *et al.* (2011) Inhibition of Mitochondrial Translation as a Therapeutic Strategy for  
610 Human Acute Myeloid Leukemia. *Cancer Cell* 20, 674–688  
611 101 Sotgia, F. *et al.* (2012) Caveolin-1 and Cancer Metabolism in the Tumor Microenvironment:  
612 Markers, Models, and Mechanisms. *Annu. Rev. Pathol. Mech. Dis.* 7, 423–467  
613 102 Gualberto, J.M. and Newton, K.J. (2017) Plant Mitochondrial Genomes: Dynamics and  
614 Mechanisms of Mutation. *Annu. Rev. Plant Biol.* 68, 225–252  
615 103 Uyttewaal, M. *et al.* (2008) Characterization of *Raphanus sativus* Pentatricopeptide Repeat  
616 Proteins Encoded by the Fertility Restorer Locus for *Ogura* Cytoplasmic Male Sterility. *Plant*  
617 *Cell* 20, 3331–3345  
618 104 Dahan, J. and Mireau, H. (2013) The Rf and Rf-like PPR in higher plants, a fast-evolving  
619 subclass of PPR genes. *RNA Biol.* 10, 1469–1476  
620 105 Andrews, K.T. *et al.* (2014) Drug repurposing and human parasitic protozoan diseases. *Int. J.*  
621 *Parasitol. Drugs Drug Resist.* 4, 95–111  
622 106 Denovan-Wright, E.M. and Lee, R.W. (1995) Evidence that the fragmented ribosomal RNAs of  
623 *Chlamydomonas* mitochondria are associated with ribosomes. *FEBS Lett.* 370, 222–226  
624 107 Lang, B.F. *et al.* (1997) An ancestral mitochondrial DNA resembling a eubacterial genome in  
625 miniature. *Nature* 387, 493–497  
626 108 Hammani, K. *et al.* (2014) Helical repeats modular proteins are major players for organelle  
627 gene expression. *Biochimie* 100, 141–150  
628 109 Aubourg, S. *et al.* (2000) In *Arabidopsis thaliana*, 1% of the genome codes for a novel protein  
629 family unique to plants. *Plant Mol. Biol.* 42, 603–613  
630 110 Lurin, C. *et al.* (2004) Genome-Wide Analysis of *Arabidopsis* Pentatricopeptide Repeat  
631 Proteins Reveals Their Essential Role in Organelle Biogenesis. *Plant Cell* 16, 2089–2103  
632 111 Barkan, A. *et al.* (2012) A Combinatorial Amino Acid Code for RNA Recognition by  
633 Pentatricopeptide Repeat Proteins. *PLoS Genet.* 8, 4–11  
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637 **Glossary**

638

639 **Cryo-electron microscopy (cryoEM):** cryoEM is an electron microscopy technique applied on  
640 biological objects cooled to cryogenic temperatures and embedded in vitreous water. Near atomic  
641 resolution structures of biological complexes can be obtained with image processing and averaging  
642 of multiple images of the complexes.

643 **Cryo-electron tomography:** cryoET is a technique by which complexes can be studied *in situ*.  
644 Complexes are maintained by cryo-fixation, visualized with an electron microscope and structures  
645 can be derived by subtomogram averaging.

646 **Endosymbiosis:** It is an evolutionary process during which a living organism (the endosymbiont) was  
647 engulfed by another organism, both organisms maintaining a mutualistic relationship. In the case of  
648 mitochondria, the endosymbiont, related to an  $\alpha$ -proteobacteria was engulfed by the ancestor of  
649 eukaryotes, an archeal-like organism.

650 **Mitochondria:** they are the two membrane endosymbiotic organelles primarily responsible for ATP  
651 production in eukaryote cells through processes such as  $\beta$ -oxidation, the citric acid (or Krebs) cycle,  
652 and the respiratory chain.

653 **Mitoribosome:** the mitochondrial ribosomes are of prokaryote origin but have significantly diverged  
654 during eukaryote evolution with the recruitment of many mitochondria specific protein subunits and  
655 the gain or loss of specific RNA domains. Mitoribosomes have evolved for the specialized translation  
656 of specific mRNAs encoded in mitochondrial genomes.

657 **Mitochondrial DNA (mtDNA):** it makes the compact mitochondrial genomes, ranging from a few kbp  
658 in many eukaryotes (16 kbp in Human) to ca. 300-500 kbp in most land plants. It encodes a few  
659 essential proteins, i.e. essential hydrophobic respiratory chain proteins, as well as components of the  
660 translation machinery, i.e. rRNA, tRNA and ribosomal proteins in some eukaryotes.

661 **Organelle:** subcellular specialized structures delimited by membranes. Here, it refers to  
662 endosymbiotic compartments such as mitochondria and chloroplasts containing a genome and a  
663 complete gene expression machinery.

664 **OXPHOS:** oxidative phosphorylation is the metabolic pathway, localized in mitochondria, that  
665 oxidizes nutrients, thereby releasing energy which is used to produce ATP. It involves respiratory  
666 complexes I to IV and the ATP synthase complex.

667 **Pentatricopeptide repeat proteins:** PPR proteins are a family of eukaryote specific RNA binding  
668 proteins folded in  $\alpha$ -helical repeated modules, involved in all steps of organellar gene expression  
669 processes.

670 **Polyadenylation:** addition of a polyA tail to the 3' end of mRNAs. In mitochondria, similar to bacteria,  
671 polyA tails serve as a signal for 3' to 5' exonucleolytic RNA degradation.

672

673

674 **Box 1. Challenges and technologies in mitochondrial translation research**

675 Tremendous progress has been achieved in the field of mitochondrial translation in the last five  
676 years, largely thanks to cryoEM analyses. This technology, in particular, has revealed high resolution  
677 structures of a diversity of mitoribosomes in various eukaryote groups [28–31]. Challenges now  
678 remain to capture and characterize less abundant and more transient complexes, such as  
679 mitochondrial translation initiation complexes or mitoribosome assembly intermediates in most  
680 eukaryotes. In the same line, the characterisation of super-complexes involving mitoribosome and  
681 insertases or OXPHOS subcomplexes will reveal how mitochondrial translation is coupled with post-  
682 translational processes, such as co-translational insertion into the IMM. While cryoEM continues to  
683 improve rapidly, both in terms of hardware and data analysis software, other approaches such as  
684 electron cryo-tomography will surely be instrumental to apprehend the integration of mitochondrial  
685 translation machineries with other cellular processes. Other technologies, for instance ribosome  
686 profiling coupled with next generation sequencing, will be instrumental to understand the functions  
687 of the mitoribosome specific r-proteins that have been identified in recent years [27]. It will e.g.  
688 reveal if these proteins are specific for the translation of particular mRNAs or generic translation  
689 factors.

690

691 **Box 2. Prevalence of PPR proteins as mitochondria specific r-proteins**

692 Pentatricopeptide repeat (PPR) proteins belong to the wide family of helical repeats modular  
693 proteins [108]. They are composed of repeated modules of ca. 35 amino acids folded into helix-turn-  
694 helix structures, with the succession of PPR motifs making an  $\alpha$  super-helix. PPR proteins compose a  
695 eukaryote specific family of proteins, present in various numbers across eukaryote groups, e.g. 7 in  
696 human, 15 in *Saccharomyces cerevisiae*, 28 in *Trypanosoma brucei*, and 400-600 in most land plants,  
697 thus making one of the largest gene family in plants [109]. PPR proteins are mostly localized in  
698 mitochondria (and/or chloroplasts in photosynthetic organisms) [110], and their functions appear to  
699 be related to all steps of organellar gene expression, i.e. transcription, the maturation of RNA ends,  
700 splicing, RNA editing, and translation. The canonical mode of action of PPR proteins involves the  
701 specific recognition of an RNA sequence, with each PPR motif recognizing a specific nucleotide [111],  
702 similar to other helical repeat proteins such as Pumilio-related proteins [108]. The identification of  
703 PPR proteins as core ribosomal proteins has revealed that PPR proteins mode of action is more  
704 diverse than previously anticipated, with some PPR proteins indeed connecting single stranded  
705 mRNA, while other bind the convex surface of double stranded rRNA helices and others are present  
706 as ribosome structural components and do not interact with RNA at all [30,48,55].

707 **Table 1. Mitoribosome proteins and rRNA contents across eukaryotes compared with *E. coli***  
 708 **ribosome<sup>a</sup>.**

**A**

Bact.	Mamm.	Yeast	Trypa.	Ara.	Bact.	Mamm.	Yeast	Trypa.	Ara.
<b>Small Subunit</b>					<b>Large Subunit</b>				
<b>Ribosomal RNAs</b>									
<b>16S</b> (1534 nt)	<b>12S</b> (962 nt)	<b>15S</b> (1649 nt)	<b>9S</b> (620 nt)	<b>18S</b> (1935 nt)	<b>23S</b> (2904 nt) 5S	<b>16S</b> 1569 nt) CP tRNA	<b>21S</b> (3296 nt) –	<b>12S</b> (1176 nt) –	<b>26S</b> (3169 nt) 5S
<b>Ribosomal proteins</b>									
<b>bS1</b>	Q9Y2Q9 <sup>b</sup>	Q02950	–	–	<b>uL1</b>	Q9BYD6	Q04599	–	Q8RWT4
<b>uS2</b>	Q9Y399	P32902 P02381	–	Q9GCB9	<b>uL2</b>	Q5T653	P32611	–	Q8VZU4 <i>m</i>
<b>uS3</b>	Q96EL2	<i>m</i>	MURF-5 <i>m</i>	Q95749 <i>m</i>	<b>uL3</b>	P09001	P31334	Q580R4	Q9LRN8
<b>uS4</b>	–	P27929	–	Q31708 <i>m</i>	<b>uL4</b>	Q9BYD3	P51998	A0A1G4HYD1*	Q8VY61
<b>uS5</b>	P82675	P33759	Q38AX6	Q6GKU7	<b>uL5</b>	–	P36519	–	P42793 <i>m</i>
<b>bS6</b>	P82932	P28778	Q38BW5	Q9LSA1	<b>uL6</b>	–	P32904	–	Q9ZPX2
<b>uS7</b>	Q9Y2R9	P47150	–	P92557 <i>m</i>	<b>bl9</b>	Q9BYD2	P53724	Q57UC5	Q9LVU5
<b>uS8</b>	–	Q03799	Q388R7	Q9M0E0	<b>uL10</b>	Q7Z7H8	P36521	Q57ZV7	Q9LHH1
<b>uS9</b>	P82933	P38120	Q57W62	Q8L6Z4	<b>uL11</b>	Q9Y3B7	P53875	Q586R9	Q9SVW7
<b>uS10</b>	P82664	Q03201	Q57Z45	P42797	<b>bl12</b>	P52815	P53163	Q584I9	Q9M7T1
<b>uS11</b>	P82912	P42847	Q389T7	Q8VZT8	<b>uL13</b>	Q9BYD1	Q12487	Q580D5	Q7XA68
<b>uS12</b>	Q15235	P53732	RPS12 <i>m</i>	P92532 <i>m</i>	<b>uL14</b>	Q6P1L8	P35996	–	Q93Z17
<b>uS13</b>	–	P53937	–	Q9CA19	<b>uL15</b>	Q9P015	P36520	Q57U68	Q9FLF3
<b>uS14</b>	Q60783	P10663	Q580I0	Q9SMX4	<b>uL16</b>	Q9NX20	P38064	Q57UM6	Q95747 <i>m</i>
<b>uS15</b>	P82914	P21771	Q4GZ99	Q9LMQ3	<b>bl17</b>	Q9NRX2	P22353	Q57YI7	Q8LD39
<b>bS16</b>	Q9Y3D3	Q02608	Q384N9	Q9LTS6	<b>uL18</b>	Q9H0U6	–	–	Q8LDK5
<b>uS17</b>	Q9Y2R5	Q03246	Q38DP8	Q9XIA6	<b>bl19</b>	P49406	P25626	Q4GZ98	Q48691
<b>bS18</b>	Q9Y3D5	P40033	Q38AS2	Q9LML3	<b>bl20</b>	Q9BYC9	–	Q383R2	Q8LCN1
<b>uS19</b>	–	P53733	Q584T8	P39697	<b>bl21</b>	Q7Z2W9	P40858	Q57UP4	Q8L9A0
<b>bS20</b>	–	–	–	–	<b>uL22</b>	Q9NWU5	P53881	Q57Y86	Q56W16
<b>bS21</b>	P82921	P38175	Q580M9	F4JCI2	<b>uL23</b>	Q16540	P32387	Q387G3	Q9SMR5
<b>bTHX</b>	–	–	–	Q9SIU8	<b>uL24</b>	Q96A35	P36534	Q57ZE0	Q9LT09
					<b>bl25</b>	–	–	–	F4JPA3
					<b>bl27</b>	Q9P0M9	P12687	A0A1G4I8T3*	Q9ZVX0
					<b>bl28</b>	Q13084	P36525	Q586A2	Q9SV23
					<b>uL29</b>	Q9HD33	P36517	Q38EM7	Q94JQ7
					<b>uL30</b>	Q8TCC3	P20084	C9ZY77*	Q8L908
					<b>bl31</b>	Q7Z7F7	P36531	Q38AB0	Q1G3G5
					<b>bl32</b>	Q9BYC8	P25348	Q584F4	–
					<b>bl33</b>	O75394	P36533	Q581Q0	Q9SQT5
					<b>bl34</b>	Q9BQ48	Q04598	–	–
					<b>bl35</b>	Q9NZE8	P53921	D0A1K1*	–
					<b>bl36</b>	Q9P0J6	O14464	Q583E9	Q8W464

709 <sup>a</sup> Composition of the mammalian (Mamm.), yeast, trypanosome (Trypa.) and Arabidopsis (Ara.) mitoribosomes  
 710 compared with the bacterial 70S ribosome.  
 711

712 <sup>b</sup> For each protein, the UNIPROT identification is indicated. Proteins highlighted in light blue are bacterial type  
 713 (Bact.) ribosomal proteins universal to prokaryotes and eukaryotes.  
 714

**B**

Mito <sup>a</sup>	Mamm.	Yeast	Trypa.	Ara	Mito	Mamm.	Yeast	Trypa.	Ara
mS22 <sup>b,d</sup>	P82650	–	Q38DR3	Q8GW63	mL37	Q9BZE1	–	–	–
mS23	Q9Y3D9	P40496	Q57VB2	F4HPB7	mL38	Q96DV4	Q06678	Q381T7	–
mS25 <sup>c</sup>	P82663	–	–	–	mL39	Q9NYK5	–	–	–
mS26	Q9BYN8	P17558	Q38DK6	Q945P2	mL40	Q9NQ50	P36527	D0A511 <sup>*f</sup>	Q9M0V5
mS27 <i>p</i> <sup>e</sup>	Q92552	–	–	–	mL41	Q8IXM3	P36526	D0A8I6 <sup>*</sup>	Q9LUJ9
mS29	P51398	Q01163	Q585C2	Q8W4K2	mL42	Q9Y6G3	–	Q383S1	–
mS31	Q92665	–	–	–	mL43	Q8N983	Q06090	Q583E5	F4J9E4
mS33	Q9Y291	P53305	Q57WW0	Q8GXH5	mL44	Q9H9J2	P36516	–	–
mS34	P82930	–	Q57UK0	Q9FHC3	mL45	Q9BRJ2	–	–	–
mS35	P82673	Q03976	Q387C7	Q9LJQ6	mL46	Q9H2W6	P36528	Q57X87	Q8L7U3
mS37	Q96BP2	O75012	Q38C96	Q1G3Z3	mL48	Q96GC5	–	–	–
mS38	Q9NWT8	P32344	Q57VQ9	–	mL49	Q13405	P25642	Q57Z82	–
mS39 <i>p</i>	Q96EY7	–	–	–	mL50	Q8N5N7	Q02204	–	–
mS40	Q9Y676	–	–	–	mL51	Q4U2R6	–	–	–
mS41	–	P38783	Q389L3	Q6IDB8	mL52	Q86TS9	–	Q385V2	–
mS42	–	P47141	Q586A1	–	mL53	Q96EL3	P19956	Q57YA9	Q940G3
mS43	–	P10662	Q585I1	–	mL54	Q6P161	P36532	–	Q9S799
mS44	–	P12686	–	–	mL57	–	P36523	–	–
mS45	–	P53292	–	Q9LVA9	mL58	–	P22354	–	–
mS46	–	Q03430	–	–	mL59/mL64	Q8TAE8	P23369	Q383B7	O65448
mS47	–	P28817	Q383R4	Q8RXN4	mL60	–	P14063	–	–
mS48	–	–	Q57UJ2	–	mL61	–	P32388	–	–
mS49	–	–	Q586P5	–	mL62	Q14197	–	–	–
mS50	–	–	Q57YB5	–	mL63	Q9BQC6	–	Q57XS1	–
mS51 <i>p</i>	–	–	Q385L8	–	mL65	Q9NP92	–	–	–
mS52	–	–	Q386Q7	–	mL66	Q9NVS2	–	–	–
mS53	–	–	Q38ET1	–	MHR1	–	Q06630	–	–
mS54 <i>p</i>	–	–	Q57ZP8	–	mL67	–	–	D0A5V6 <sup>*</sup>	–
mS55 <i>p</i>	–	–	Q580V1	–	mL68	–	–	Q383B7 <sup>*</sup>	–
mS56	–	–	Q587C2	–	mL69	–	–	Q584V5	–
mS57	–	–	Q584U8	–	mL70	–	–	Q586Y7	–
mS58	–	–	Q38BP1	–	mL71	–	–	Q57WG1	–
mS59	–	–	Q38BS2	–	mL72	–	–	Q585Z1	–
mS60	–	–	Q57XL2	–	mL73	–	–	Q57Y49	–
mS61	–	–	Q38D60	–	mL74	–	–	Q38AM5	–
mS62 <i>p</i>	–	–	Q383D1	–	mL75 <i>p</i>	–	–	Q38CK0	–
mS63 <i>p</i>	–	–	Q38F25	–	mL76	–	–	Q383M2	–
mS64	–	–	Q382E8	–	mL77	–	–	Q584Q8	–
mS65	–	–	Q57UA2	–	mL78	–	–	D0A4P5 <sup>*</sup>	–
mS66	–	–	Q388L7	–	mL79	–	–	Q381N5	–
mS67	–	–	Q586G5	–	mL80	–	–	Q585A3	Q9C9B5
mS68	–	–	Q582T9	–	mL81	–	–	D0A755 <sup>*</sup>	–
mS69	–	–	Q57U26	–	mL82	–	–	D0A0S6 <sup>*</sup>	–
mS70	–	–	D0A8P6 <sup>*</sup>	–	mL83	–	–	Q57WF8	–
mS71	–	–	Q383G5	–	mL84	–	–	Q586A6	–
mS72	–	–	Q57YD4	–	mL85	–	–	Q38FG8	–
mS73	–	–	Q587C4	–	mL86	–	–	Q57Z37	–
mS74	–	–	D0A3P2 <sup>*</sup>	–	mL87	–	–	D0A7Z9 <sup>*</sup>	–
mS76 (rPPR1)	–	–	–	Q8LE47	mL88	–	–	C9ZUW3 <sup>*</sup>	–
mS77 (rPPR2)	–	–	–	Q8LEZ4	mL89	–	–	Q57WW5	–
mS78 (rPPR3a)	–	–	–	Q9LG23	mL90	–	–	Q585P1	–
mS79 (rPPR3b)	–	–	–	Q9LK57	mL91	–	–	Q580U5	–
mS80 (rPPR6)	–	–	–	POC896	mL92	–	–	Q383Y4	–
mS81 (rPPR8)	–	–	–	Q8LPF1	mL93	–	–	D0A4T0 <sup>*</sup>	–
mS83	–	–	–	Q1JPP0	mL94	–	–	Q38EP7	–
mS84	–	–	–	Q9C8L9	mL95	–	–	Q389K3	–
mS85	–	–	–	Q9FZ84	mL96	–	–	Q381W9	–

mL97	-	-	Q388L8	-
mL98	-	-	Q388M2	-
mL99	-	-	Q587H8	-
mL100	-	-	A0A1G4HYZ0*	-
mL101 (rPPR4)	-	-	-	Q22714
mL102 (rPPR5)	-	-	-	Q9ZUU3
mL103 (rPPR7)	-	-	-	Q9M065
mL104 (rPPR9)	-	-	-	Q9FME4
mL105	-	-	-	Q9SD44

<sup>a</sup> Mitoribosome proteins specific to eukaryotes (Mito).

<sup>b</sup> The proteins in yellow are specific to mitochondria and shared by different organisms

<sup>c</sup> The ones in orange are specific to a respective organism.

<sup>d</sup> *m* specifies mitochondria encoded genes

<sup>e</sup> *p* PPR proteins

<sup>f</sup> In the case of *Trypanosoma*, due to poor annotations, the proteins correspond to *Trypanosoma brucei brucei* (strain 927/4 GUTat10.1), except for the proteins with a \* that correspond to other strains.

## Figure legends

**Figure 1. Structural divergence of mitoribosomes across eukaryotes.** Mitoribosome structures, at high resolution, for representatives from Excavata (*Trypanosoma*) (PDB ID: 6HIV), Fungi (budding yeast) (PDB ID: 5MRC) and Animal (human) (PDB ID: 6GAW) and a low resolution for *Arabidopsis* (EMDB ID:4408), are represented at the same scale. Structures are overlaid on a schematic representation of the major eukaryote lineages and compared with *E. coli* ribosome (PDB ID:5KCR) that resembles the ancestral  $\alpha$ -proteobacteria like ribosome that are hypothesised to give rise to mitoribosomes after divergence from the last eukaryote common ancestor (LECA). Ribosomes are shown oriented with the SSU at the bottom and the LSU at the top. It shows the global increase in mitoribosome sizes as compared to the bacterial ribosome and illustrates the diversity of mitoribosome architectures, in particular with very large SSU in *Arabidopsis* (Viridiplantae, Green plants) and *Trypanosomes* (Excavata). Ribosomal RNAs are shown in grey. Proteins of bacterial origin are shown in dark blue. Proteins specific to mitochondria and found in all analyzed eukaryotes are shown in yellow. Finally, proteins specific for the respective eukaryote groups are shown in red. For plants, SSU expansions specific to angiosperms are represented in red. The composition and architecture of mitoribosomes is unknown for many eukaryotes, in particular for the super group of Stramenopiles, Alveolates, Rhizaria (SAR).

**Figure 2. Functional diversity of PPR proteins in mitoribosomes.** (A) High resolution structures of Human (PDB ID: 6GAW) and *Trypanosoma* (PDB ID: 6HIV) mitoribosomes, indicating PPR proteins in blue. (B) and (C) Mammalian PPR proteins mS39 and mS27. mS39 appears to mediate the stabilization of mRNA (depicted as a green line) on the SSU head. In contrast, mS27 located in the SSU body has contact, although limited, with rRNA. (D) *Trypanosoma* PPR protein mS51 binds r-proteins and does not make any contact with RNA. r-proteins are shown in gray, rRNAs in red-orange, and proteins containing PPR motifs in blue.

**Figure 3. Mitoribosomes membrane attachment.** Membrane attachment models for the yeast mitoribosome (A) and the mammalian mitoribosome (B). LSU are shown in blue shades and SSU in yellow shades. Polypeptide tunnels are highlighted in yellow. (A) The yeast mitoribosome (PDB ID: 5MRC) is bound to the inner membrane (IM) due to the expansion segment of H96 and a non-ribosomal protein Mba1, which contact the insertase Oxa1 [65]. In yeast, the classical bacterial exit tunnel is blocked (red star) and replaced by another exit [28]. The new polypeptide path is shown by a red dashed line. (B) The human mitoribosome (PDB ID: 6GAW) is bound to the IM by the intrinsic r-protein mL45 (shown in red), directly interacting with Oxa1. It has been shown that the N-terminal part of mL45 probes the polypeptide channel when the mitoribosome is not bound to the IM of mitochondria, thus acting almost as a maturation factor preventing protein synthesis when the mitoribosome is not bound to the IM [45]. Moreover cryo-ET revealed the presence of an additional density bound to the PPR protein mS39 in native conditions [66]. OM, outer membrane.





