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REVIEW ARTICLE

The post-transcriptional life of mammalian mitochondrial RNA

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Mammalian mitochondria contain their own genome that encodes mRNAs for thirteen essential subunits of the complexes performing oxidative phosporylation as well as the RNA components (two rRNAs and 22 tRNAs) needed for their translation in mitochondria. All RNA species are produced from single polycistronic precursor RNAs, yet the relative concentrations of various RNAs differ significantly. This underscores the essential role of post-transcriptional mechanisms that control the maturation, stability and translation of mitochondrial RNAs. The present review provides a detailed summary on the role of RNA maturation in the regulation of mitochondrial gene expression, focusing mainly on messenger RNA polyadenylation and stability control. Furthermore, the role of mitochondrial ribosomal RNA stability, processing and modifications in the biogenesis of the mitochondrial ribosome is discussed.

Key words: mitochondrion, ribosome biogenesis, RNA degradation, RNA polyadenylation, RNA turnover.

INTRODUCTION

According to the endosymbiotic theory, mitochondria originated from proteobacteria that were taken inside another cell (either by a primitive 'eukaryotic cell ancestor' or anaerobic archeobacteria) and have since almost completely lost their autonomy [1]. As a result, present-day eukaryotes possess nDNA (nuclear DNA) and mtDNA (mitochondrial DNA) genomes.

Human mtDNA is a double-stranded closed circular molecule of 16.6 kb [2]. The two strands of mtDNA have been designated as the light inner strand (L-strand) and the heavy outer strand (H-strand). Most of the information is encoded on the heavy strand: 14 mt-tRNAs (mitochondrial tRNA), two mt-rRNAs (mitochondrial rRNA) and 12 polypeptides; whereas the light strand encodes the remaining eight mt-tRNAs and one polypeptide, the ND (NADH dehydrogenase) 6 subunit. Human mtDNA has a very compact organization. The genes lack introns and the coding sequences are separated by only a few bases [2]. In two cases, the protein genes are overlapping: genes for ATPase 6 and 8 as well as those for ND4 and ND4L have overlapping regions of 46 bp and 7 bp respectively [2,3]. All thirteen of the protein products of the mitochondrial genome are indispensable parts of multi-enzymatic complexes in the oxidative phosphorylation system. To translate the thirteen protein-coding genes mitochondria have a specific protein synthesis machinery, in which all factors apart from mt-tRNAs and mt-rRNAs are encoded by the nucleus.

The long polycistronic precursor RNAs of the H-strand and L-strand are transcribed from the H-strand promoters [HSP (major H-strand promoter) 1 and HSP2] and L-strand promoter [LSP (major L-strand promoter)] [4,5] respectively by mitochondrial transcription machinery that includes a monomeric RNA polymerase {POLRMT [polymerase (RNA) mitochondrial (DNA directed)]}, TFAM (mitochondrial transcription factor A) and TFB2M (mitochondrial transcription factor B2) and several regulatory factors [TEFM (mitochondrial transcription elongation factor), mTERFs (mitochondrial transcription termination factors) and MRP (mitochondrial ribosomal protein) L12] [6–11]. The polycistronic transcripts generated by both strands are processed and subsequently matured to generate mt-rRNA, mt-tRNA and mt-mRNA (mitochondrial mRNA) molecules. According to the 'tRNA punctuation model', mt-tRNA sequences located between most of the rRNA and mRNA genes form cloverleaf structures on the nascent transcripts, acting as signals for the processing machinery [2,12]. It has been postulated that at the sites lacking the 'punctuating' tRNA genes, other tRNA-like secondary structures may be formed; nevertheless, no sequence similarity has been found for these cleavage sites that might be important for the recognition by the processing machinery [13]. Several observations of mitochondrial transcription products suggest that a number of transcript cleavages take place in the mitochondria that cannot be routinely explained by the 'punctuating' model [14–16]. The 5′ endonucleolytic cleavage of tRNAs from polycistronic transcripts is performed by the mitochondrial RNase P (as suggested by [17]). The main mitochondrial RNase P, a multimeric enzyme consisting of three different proteins and (in stark contrast with earlier assumptions)

Abbreviations used: CO, mitochondrially encoded cytochrome *c* oxidase; Dis3L, DIS3 mitotic control homologue-like; Elac2, elaC homologue 2; EndoG, endonuclease G; ERAL1, Era G-protein-like 1; ERI, exoribonuclease; ExoG, exonuclease G; GFP, green fluorescent protein; HRSP12, heat-responsive protein 12; HSP, mitochondrially encoded major H-strand promoter; LRPPRC, leucine-rich pentatricopeptide-repeat-containing; LSFC, Leigh syndrome French Canadian variant; m-AAA, mitochondrial AAA; m7G, 7-methylguanylate; MRP, mitochondrial ribosomal protein; MTase, methyltransferase; mtDNA, mitochondrial DNA; mTERF, mitochondrial transcription termination factor; hMTERF4, human mTERF4; mtEXO, mitochondrial degradosome; mt-LSU, mitochondrial large 39S subunit; mt-mRNA, mitochondrial mRNA; mt-SSU, mitochondrial small 28S subunit; mt-rRNA, mitochondrial rRNA; MTS, mitochondrial targeting signal; mt-tRNA, mitochondrial tRNA; ND, mitochondrially encoded NADH dehydrogenase; NOA1, nitric-oxide-associated 1; NSUN4, NOP2/Sun domain family 4; PABP, poly(A)-binding protein; PAP, poly(A) polymerase; mtPAP, mitochondrial PAP; hmtPAP, human mtPAP; PARN, poly(A)-specific exoribonuclease; PDE12, phosphodiesterase 12; PNPase, polynucleotide phosphorylase; POLG, polymerase (DNA directed) γ; POLRMT, polymerase (RNA) mitochondrial (DNA directed); PPR, pentatricopeptide repeat; PTCD, pentatricopeptide repeat domain; REXO2, RNA exonuclease 2 homologue; RNAi, RNA interference; RT, reverse transcription; siRNA, short interfering RNA; SLIRP, steroid receptor RNA activator stem-loop-interacting RNA-binding protein; snoRNA, small nucleolar RNA; SUV3, suppressor of var1 3-like protein 1; hSUV3, human SUV3; TACO1, translational activator of mitochondrially encoded cytochrome *c* oxidase I; TEFM, mitochondrial transcription elongation factor; TFB1M, mitochondrial transcription factor B1; TFB2M, mitochondrial transcription factor B2; UTR, untranslated region.

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lacking the RNA component, has been fully characterized by Rossmanith and colleagues [18]. Nevertheless, the presence of a second RNA-containing mitochondrial RNase P with differing substrate specificity (such as cleavage of adjacent tRNAs) has not been completely ruled out [19]. The 3′-ends of mt-tRNAs have been proposed to be cleaved by the RNase Z endonuclease [20]. ELAC2 (elaC homologue 2) has been demonstrated to localize to the mitochondria and affect the 3′-end processing of tRNAs, suggesting that it functions as an RNase Z in human mitochondria [21–24]. Released tRNA molecules are matured by the addition of the CCA trinucleotide to their 3′-end by ATP(CTP):tRNA nucleotidyltransferase [25]. Mammalian mitochondrial genes do not contain introns, thus maturation of nascent mRNAs is limited to constitutive polyadenylation of the 3′-ends of these molecules [12]. Further details regarding this process and its role in mitochondria are provided in the sections below.

THE POST-TRANSCRIPTIONAL REGULATION OF mt-mRNAS

In mammalian mitochondria all RNA species are produced from single polycistronic precursor RNAs, yet significant differences in the relative concentrations of various mt-mRNAs have been reported [16,26,27]. Concentrations of particular transcripts might be, in theory, regulated by transcription processivity during elongation so that the transcripts located in the proximity of the promoters would be more abundant as compared with the promoter-distal ones. We have recently identified TEFM as an interactor of POLRMT and showed that it is required for synthesizing promoter-distal transcripts from both strands [7]. The up-regulation of TEFM may have an opposite effect and increase the concentration of the promoter-proximal transcripts. Therefore levels of TEFM expression, and possibly other factors, could be a means of controlling mtRNA abundance. Apart from co-transcriptional regulation, active post-transcriptional mechanisms must exist to determine maturation, translation and degradation of mtRNA. This section outlines the current knowledge on human mt-mRNA metabolism and discusses progress in the identification of proteins that are involved in these processes. Figure 1 accompanies the text to provide a graphical representation of the key pathways and factors.

Polyadenylation of mt-mRNA: tricky tale

One of the common features of mammalian mt-mRNAs is stable polyadenylation of their 3′-termini upon processing. The essential purpose of polyadenylation is to create a functional stop codon for seven of the mitochondrial mRNAs excised from polycystronic precursors with U or UA at their 3′-termini, thus requiring one or two adenosine residues to complete the codon [2] (Table 1). However, despite many years of research the broader role of poly(A) tails in human mitochondria remains unclear.

Polyadenylation appears to play contrasting roles in the gene expression of different systems. In the eukaryotic cytosol, poly(A) tails confer stability on the transcript, playing a role in its exit from the nucleus and promoting initiation of translation. In bacteria and chloroplasts, polyadenylation stimulates RNA degradation [28]. Interestingly, despite their monophyletic origin, mitochondria from different organisms display varied polyadenylation patterns. In plants, similarly to the bacterial system, addition of 3′-adenylates is a signal for RNA degradation. In contrast, yeast transcripts do not display any polyadenylation, instead containing an AU-rich dodecamer sequence at the 3′-end, required for stability and translatability [29]. The situation is even more complicated in protist mitochondria, where the pre-editing

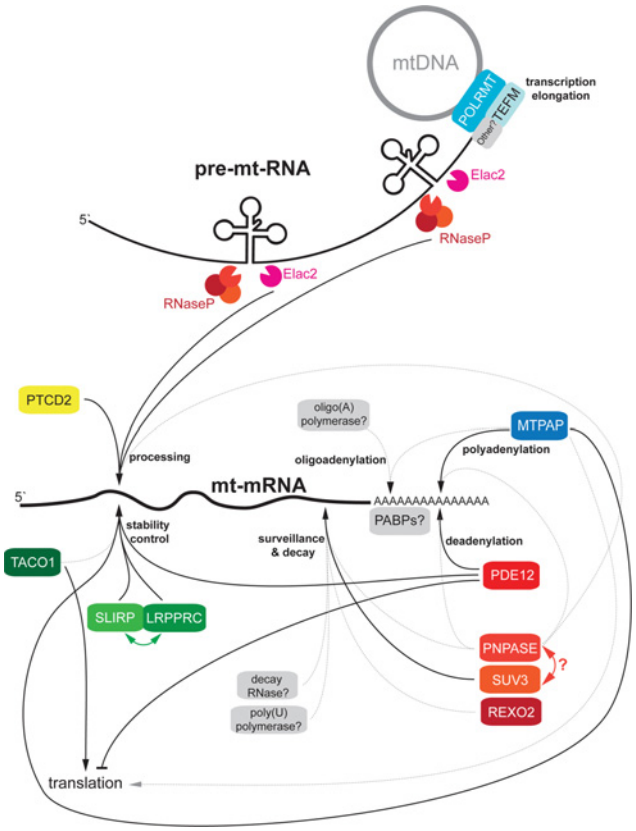

Figure 1 Factors involved in mitochondrial mRNA metabolism

Well-established interactions are represented by solid lines and broken lines indicate hypothetical connections.

addition of short 3′ adenosine tails stabilizes transcripts during and after the editing, and subsequent extension of short poly(A) tails is critical for protein synthesis [30,31].

Human mitochondria do not seem to follow any of the paradigms described above. Transcripts generated from the mtDNA heavy strand acquire a poly(A) extension of approximately 45 nt; however, there are marked differences in the length of the poly(A) tail of the same transcript between different cell types, as well as between different transcripts within a cell type [13]. Notably, the ND6 transcript, the only light strand transcript, is not polyadenylated at all [32]. Recently, the 3′-end of the ND6 transcript has been mapped, revealing a 33/34 nt UTR (untranslated region) following the stop codon and confirming the lack of a poly(A) tail [27]. Strikingly, this same study suggests that other mitochondrial mRNAs can be present in both polyadenylated and non-polyadenylated forms at variable frequencies; however, this intriguing observation requires further investigation.

The enzyme responsible for the addition of poly(A) tails in human mitochondria, hmtPAP {human mitochondrial PAP [poly(A) polymerase]}, was identified by two research groups [33,34]. Unexpectedly, hmtPAP is not a bacterial-type PAP, but is homologous with the family of regulatory cytoplasmic PAPs that are employed in polyadenylation-regulated translation in a broad range of biological processes such as gametogenesis, cell-cycle progression and synaptic activity [35–37]. Down-regulation of the hmtPAP protein by siRNA (short interfering RNA) results in profoundly shortened poly(A) tails of mt-mRNAs, consistent

Table 1 Effects of mitochondrial protein manipulations on transcript stability, polyadenylation profile and processing

A/AA, 'A' additions required for stop codon; A, <10; AA, 20–50; AAA, >50; ↓↑, changes in the steady-state levels of mature transcripts; ∼, no changes in the steady-state levels of mature transcripts; p+, precursors and/or truncated mt-RNA species observed; [2], inconsistent data on poly(A) between siRNA oligonucleotides; [3], the length of the poly(A) tail deduced from reduced size on Northern blot; [4], except for ND1 and CO2 the length of the poly(A) tail deduced from a reduced size on Northern blot; [5], taken from [27]; KO, precursor RNAs analysed in mouse knockout (KO)-derived cells [19]; O-E, overexpression.

Mitochondrial transcript	Stop codon	3′ UTR (nt)	Knockdown of PNPase			Inactivation of SUV3	Knockdown of mtPAP				O-E of PARN[3]	O-E of PDE12[4]
CO1	UAG	72	∼/AAA	∼/A/p+	↓↓/p+ KO				↓/A	↓↓	↓↓/A	↓↓/A
CO2	UAG	24	∼/AAA		↓↓/p+ KO	∼/p+			↓↓/A	↓↓	↓↓/A	↓↓/A
CO3	UAA	0	∼/AAA	∼/AA	∼/p+ KO		∼/A	↓	↓↓/A	↓↓		∼/A
ND1	UAA	0				↑↑/p+		↑↑		∼	↑↑/A	↑↑/A
ND2	UAA	0				↑↑/p+		↑↑		∼	↑↑/A	↑↑/A
ND3	UAA	0	∼/AAA	∼[2]		↑↑/AAA/p+	↑/A	↑/A	∼/A	∼	∼/A	∼/A
ND4/4L	UAA	0				∼/p+		↑			∼/A	∼/A
ND5	UAA	568	∼/AAA	∼/AAA	∼					∼	↑/A	∼/A
ND6	UAG	33/34[5]										∼
CytB	UAA	0								∼		↑/A
ATP6/8	UAA	0			↓/p+ KO	AAA/p+	↑/A	↓	↓/A	↓↓	↓/A	∼/A
12S					↓	↓↓/AAA/p+					∼	∼
16S				∼/A/p−	↓↓			∼	∼		∼	∼
Reference		[13]	[34]	[43]	[19]	[144]	[33]	[48]	[34]	[47]	[49]	[50]

with the enzyme being responsible for polyadenylation within the organelle [33,34]. Recently, a mutation in hmtPAP associated with autosomal recessive spastic ataxia present in an Ohio Amish family has been described [38]. The identified N478D substitution occurs in a highly conserved region of PAP, and leads to a severe truncation of mitochondrial poly(A) tails, resulting in oligoadenylated species that are less than 10 nt long.

It is still unknown if polyadenylation is a two-step process with oligoadenylation at the 3′-termini preceeding addition of the long poly(A) tails by hmtPAP. It is important to note that no mRNA species, completely devoid of 3′-added adenosine residues have been identified in either hmtPAP siRNA-knockdown experiments or in cells from patients with hmtPAP mutations [33,38]. This may suggest that another as yet undiscovered enzyme is responsible for the oligoadenylation of mitochondrial transcripts.

One of the protein candidates that may be involved in poly(A) metabolism and oligo(A) synthesis in the absence of PAP activity is PNPase (polynucleotide phosphorylase). PNPases exhibit both PAP and phosphorolytic 3′→5′ exoribonuclease activities. In an *Escherichia coli* mutant lacking PAP activity, PNPase is capable of synthesis of poly(A) tails that sporadically contain non-adenine ribonucleotides [39]. A homologue of bacterial PNPase has been shown to be present in human mitochondria [40,41] and was demonstrated to possess PAP activity *in vitro* [34,42]. Down-regulation of the human PNPase led to extended poly(A) tails of all mt-RNAs analysed [CO (mitochondrially encoded cytochrome *c* oxidase) 1, CO2, CO3, ND3 and ND5)] [34] (Table 1), consistent with the role of PNPase in poly(A) degradation. However, when the involvement of PNPase in mt-RNA metabolism was further addressed by stable silencing of the gene in human cells, a varied effect on 3′-ends of mitochondrial transcripts was reported [43]: (i) the stable poly(A) tails of CO2 were abolished; (ii) CO3 remained unaffected; and (iii) ND5 poly(A) tails extended (Table 1). Despite the lack of polyadenylation, normal steady-state levels of CO1 transcript and protein were observed. Additionally, it was reported that ATP levels were depleted upon PNPase down-regulation and ATP depletion alone can result in the shortening of poly(A) tails, suggesting an indirect path by which PNPase could influence mt-mRNA polyadenylation [43]. Indeed, other studies have demonstrated that the majority of human PNPase pool localizes to the intermembrane space [44] separate from the mt-mRNA transcription and processing machineries of the mitochondrial matrix. This again suggests that PNPase may play an indirect role in mitochondrial polyadenylation (and/or stability) [44]. In agreement with this, a recent study by Wang et al. [19] proposed a novel, and rather unexpected, role for PNPase in the import of nuclear-encoded RNAs, including RNase P, 5S RNA and MRP RNAs into the mitochondrial matrix, implying more a general role of the enzyme in the maintenance of mitochondrial homoeostasis [19], reviewed in [45]. The latter is further discussed in the following section. Further research will be necessary to answer whether or not the mitochondrially localized PNPase is directly involved in the poly(A) tail metabolism.

How does mitochondrial polyadenylation influence the stability of transcripts?

The correlation between shortening of poly(A) tails and decreased transcript stability was first reported by Temperley et al. [32] in a patient-derived cell line bearing a micro-deletion in the ATP8/6 bicistronic mRNA, resulting in the loss of the two terminal nucleotides. This loss removes the terminal uridine, which serves as the first letter of the UAA stop codon, and would be completed in the healthy mitochondria by polyadenylation. In this cell line, processing and polyadenylation still occurs and the resulting transcripts undergo translation, but later are rapidly degraded in a deadenylation-dependent manner. These data were interpreted as consistent with the role of polyadenylation in promoting stability of the non-stop transcript, thus allowing translation and generation of stable functional polypeptides [46]. Moreover, the study suggested the potential existence of a putative PABP [poly(A)-binding protein] in mitochondria that could be involved in the protection of transcripts, similarly to cytosolic PABPs.

Conflicting results have been obtained from studies of the effect of mtPAP silencing on transcript stability. Work by Nagaike et al. [34] suggested that the poly(A) tail is important for mRNA stability, as shortening of the poly(A) tails resulted in a decreased steady-state level of several mRNAs including CO1, CO2, CO3 and ATP8/6, but not the ND3 mRNA. Similar conclusions were drawn from a further study by Nagao et al. [47] (Table 1). In contrast, Tomecki and colleagues [33] postulated that long

poly(A) tails are not required for transcript stability as hmtPAP depletion did not alter the stability of ND3 or ATP8/6 [33]. An additional report by the same group extended this analysis [48] to several further transcripts, indicating that levels of transcripts coding for subunits of complex I are generally elevated, whereas CO3 and ATP8/6 levels are decreased upon mtPAP silencing. The variation in steady-state levels of the same transcripts between independent experiments most probably reflects the variability in hmtPAP silencing.

Recently, another study by Wydro et al. [49] showed a variable effect of polyadenylation on transcript stability. When the native poly(A) tails were interfered with by binding of mitochondrially targeted cytosolic PABP1 or deadenylated with the cytosolic PARN [poly(A)-specific exoribonuclease] targeted to the mitochondria, a severe mitochondrial phenotype was observed. Mitochondrial targeting of PARN completely removed the 3′ adenylate extensions of the transcripts and resulted in increased steady-state levels of ND1, 2 and 5 mRNAs, with a concomitant decrease in expression of the other mRNAs (CO1, CO2 and ATP8/6). Strikingly similar effects on the steady-state levels of the transcripts have been observed when human 2′-PDE12 (phosphodiesterase 12), was overexpressed in cultured human cells. PDE12 is the first poly(A)-specific exoribonuclease that has been shown to exist in human mitochondria [50,51], specifically removing poly(A) tails of mitochondrial transcripts both *in vitro* and *in vivo*. In both aforementioned studies, removing poly(A) tails from mt-mRNAs destabilized CO1 and CO2, whereas the steady-state levels of ND1 and ND2 mRNAs increased, suggesting that there is no universal role of mitochondrial poly(A) tails in the regulation of RNA stability.

Finally, the co-existence of two types of polyadenylation (stabilizing and destabilizing) has been suggested by Slomovic et al. [16]. In addition to the regular polyadenylation at the 3′-end, mt-RNAs, including mt-tRNAs and mt-rRNAs, can be internally polyadenylated, albeit at very low levels. Using RT (reverse transcription)–PCR-based techniques in conjunction with bioinformatic analysis, non-abundant, internally polyadenylated RNAs were detected in mitochondria, suggesting that polyadenylation-dependent degradation, similar to the prokaryotic mechanism, may co-exist with stabilizing polyadenylation. This necessitates the existence of mechanisms that can distinguish between these two forms and probably depends on additional poly(A)-binding factors or sequence-specific proteins. It cannot be excluded, however, that the appearance of a small population of internally polyadenylated RNA species may represent aberrantly processed intermediates that are exposed to the activity of the polyadenylating enzyme. Indeed, when mRNA is endonucleotically cleaved, the truncated species are immediately readenylated [52]. High rates of readenylation in truncated RNAs have also been observed in other studies [32,50].

To summarize, there is no generic effect of 3′ polyadenylation on the stability of mitochondrial RNAs (Table 1), and polyadenylation is not sufficient to direct mt-RNA for degradation. The mechanism of degradation is probably dependent on additional as yet unidentified factors. In yeast mitochondria, all mRNAs lack poly(A) tails and are stabilized by mRNA-specific factors {e.g. CBP1 (cap-binding protein 1), [53], Aep3 (ATPase expression protein 3) [54] or Pet309 (PETite colonies 309) [55]}. Although no homologues have been identified, it could be speculated that specific factors are involved in the stabilization of individual transcripts in human mitochondria. Intriguingly, several reports suggest the existence of stable antisense transcripts in mitochondria [27,56,57], although their role in the regulation of transcript stability and metabolism remains to be addressed.

Mitochondrial poly(A) tails and protein synthesis: is there a link?

In the cytosol, poly(A) tails play an important function during the initiation of translation. EIF4F (eukaryotic cytoplasmic initiation factor 4F) interacts with the 5′ mRNA m7G (7-methylguanylate) cap and 3′ poly(A) via PABP [58]. This interaction effectively circularizes the mRNA, and recruits the small ribosomal subunit to promote translation initiation. In mitochondrial transcripts the translational start codon is typically located within the three nucleotides of the 5′-end of the mRNA [2]. Also, mt-mRNAs are not capped by m7G at their 5′-end and lack the traditional Shine–Dalgarno sequence, which facilitates ribosome binding in bacteria, hence neither the cytosolic or prokaryotic mechanism for translational initiation could operate in this system.

Very limited progress has been made towards understanding the role of polyadenylation in mitochondrial translation in human cells. Recently, some light has been shed on this problem by inducing the trimming of 3′ poly(A) extensions, either by targeting of cytosolic PARN into mitochondria or by overexpression of mitochondrial PDE12 deadenylase [49,50]. In both cases de-adenylation led to severe inhibition of protein synthesis. It cannot be ruled out, however, that the activity of PDE12 and PARN causes a functional removal of stop codons, leading to the loss of the capacity of an mRNA to be translated. Interestingly, targeting of cytosolic PABP1 to mitochondria did not lead to shortening or decay of transcripts, but caused an inhibition of mitochondrial translation, suggesting that poly(A) tails are indeed necessary for regulation of protein synthesis in mitochondria.

It is tempting to speculate that deadenylation-dependent inhibition of protein synthesis could play a regulatory role in gene expression in response to different stimuli. Mitochondrial protein synthesis is decreased under conditions of low pH and anoxia [59]. Both oxygen deprivation and acidic pH (pH 6.4) lead to an increase in the half-life of selected mitochondrial transcripts, indicating that protein synthesis alteration is not the result of transcripts limitation [60]. Interestingly, in the same conditions, a significant deadenylation of stabilized transcripts is observed [61]. This implies that modulation of poly(A) tail length may indeed play a regulatory role in protein synthesis; however, this intriguing hypothesis needs to be supported by further studies.

Polyadenylation has a profound effect on mitochondrial function, as evidenced by the severe neurodegeneration disorder presented in patients with mtPAP mutation; however, until now there has been no consensus regarding the precise role of poly(A) tails in transcript stability and translation. Addressing the questions of poly(A) function and regulation, as well as identification of the mitochondrial PABP, constitutes a challenge for further investigation.

Mitochondrial mRNA regulatory proteins and translational activators

In yeast mitochondria a group of regulatory proteins exist to optimize translation of individual mRNAs. The regulatory role of these proteins can be realized via regulation of the stability of specific transcripts or through promoting translation by binding to the 5′-UTRs of the mRNA (e.g. [62–64], reviewed in [65]). As mammalian mitochondrial mRNAs do not contain significant 5′-UTRs, alternative mechanisms must exist to promote stabilization or translation of individual transcripts.

Previously, the first human translational activator specific for CO1, TACO1 [translational activator of mitochondrially encoded cytochrome *c* oxidase I; CCDC44 (coiled-coil domain containing 44)] has been identified [66]. Mutations in TACO1 result in cytochrome *c* oxidase deficiency and late-onset Leigh

syndrome. A significant reduction in synthesis of the CO1 protein was observed in patient fibroblasts, despite a normal steady-state level of CO1 mRNA. It is possible that the protein interacts with the long 3′-UTR of CO1 to promote translation; however, this has not yet been investigated. Understanding the mechanism of TACO1 may provide an insight into the general method of how mRNAs are recognized for translation in human mitochondria.

Several proteins of the PPR (pentatricopeptide repeat) family have been proposed to play a regulatory role in mitochondrial translation. The PPR family is characterized by the presence of a 35-amino-acid structural motif that is tandemly repeated 2–26 times per protein. Most members of the PPR family are plant proteins and nearly all localize to chloroplasts or mitochondria. The PPR domain appears to be involved in RNA–protein interactions, and PPR proteins are known to play various roles in RNA editing, stability and translation activation [67]. In mammals, only seven PPR proteins have been characterized thus far (reviewed in [68]), all of them localized predominantly to mitochondria: POLRMT, PTCD (pentatricopeptide repeat domain) 1, PTCD2, PTCD3, MRPS27 (mitochondrial ribosomal protein S27), LRPPRC (leucine-rich pentatricopeptide-repeat-containing) and MRPP3 (mitochondrial RNase P protein 3). The PPR proteins with a documented role in post-transcriptional regulation of mt-mRNAs, LRPPRC and PTCD2, are described below. The remaining proteins with a role in the regulation of non-messenger mt-rRNAs, PTCD1 and PTCD3, will be discussed in the 'Role of mitochondrial rRNA stability, processing and modifications in mitoribosome biogenesis' section.

LRPPRC (or LRP130), has been implicated in mitochondrial disease; however, its precise role is far from clear. Mutations in LRPPRC cause LSFC (Leigh syndrome French Canadian variant), characterized by a tissue-specific decrease in cytochrome *c* oxidase activity [69]. LRPPRC has been proposed to act as a specific regulator of the expression of mitochondrially encoded subunits of cytochrome *c* oxidase [70]. However, further studies in cells depleted of LRPPRC by siRNA [71] or in LSFC patient-derived fibroblasts [72] showed that a decreased LRPPRC level results in a general reduction of steady-state levels of most mitochondrial mRNAs. Nevertheless, the steady-state levels of cytochrome *c* oxidase mRNAs were the most sensitive to LRPPRC inactivation and, importantly, their translation was disproportionately decreased, supporting the notion that LRPPRC is involved in translational control of these mRNAs. A recent mouse model study has confirmed the role of LRPPRC in the production of a functional cytochrome *c* oxidase and identified the short fragment of CO1 to which the protein specifically binds [73]. It is still unclear how LRPPRC regulates the metabolism of transcripts. It has been shown that LRPPRC may play a role in mitochondrial transcription [74] and a recent study suggested an involvement in transcript polyadenylation [75]. Notably, in the latter study, loss of LRPPRC in a conditional mouse knockout led not only to decreased transcript stability and loss of poly(A) tails, but also aberrant translation with excessive translation of some transcripts and inhibition of others [75]. This suggests, in contrast with other studies [49,50], that translation, although heavily misregulated, may occur in the absence of mRNA polyadenylation.

In addition, several reports have shown the presence of LRPPRC in the nucleus, where it seems to play a role in the regulation of mitochondrial biogenesis and energy homoeostasis [71,76], thus suggesting an involvement in the co-ordination of interactions between the nucleus and mitochondria. In cultured cell lines only a mitochondrial form of LRPPRC was detected [77], therefore dual localization of LRPPRC might be a tissue-specific phenomenon, or may depend on specific cell conditions.

LRPPRC has been found to be associated with another mitochondrial RNA-binding protein, SLIRP (steroid receptor RNA activator stem-loop-interacting RNA-binding protein) [72,75]. SLIRP was first identified as a nuclear protein interacting with steroid receptor RNA activator, although the majority of the protein was shown to reside in mitochondria [78], implying mitochondrial function. Supporting this conclusion, SLIRP was identified in a screen for regulators of oxidative phosphorylation as an essential factor in the maintenance of mitochondrial transcripts [79]. LRPPRC and SLIRP are postulated to be part of the high-molecular-mass ribonucleoprotein complex that regulates metabolism of mitochondrial transcripts [72]. Expression of this complex correlates with total mt-mRNA levels, as in rho0 cells (cells devoid of mtDNA) the levels of both proteins were significantly reduced, indicating mutual partnership [72].

Recent years have seen the identification of several RNA-binding proteins in mitochondria, providing a greater understanding of various aspects of mitochondrial gene regulation; however, the list is probably far from complete, and other factors remain to be identified.

THE POST-TRANSCRIPTIONAL REGULATION OF NON-MESSENGER RNAs IN MAMMALIAN MITOCHONDRIA

In addition to a differential stability of mRNAs, maintaining a correct concentration of tRNAs and rRNAs constitutes an additional regulatory mechanism for the proper function of these molecules and consequently gene expression. Accordingly, in mammalian mitochondria a variation in steady-state levels of mature mt-rRNAs and mt-tRNAs have been reported [27]. Since mitochondrial rRNA and tRNA molecules, similarly to mt-mRNAs, are generated from a common precursor transcript, it is likely that post-transcriptionally acting factors are involved in the regulation of their abundance. However, the details of how these mechanisms operate in mammalian mitochondria are lacking.

Post-transcriptional nucleotide modifications of non-messenger RNAs are often required for their correct function [80]. Many nucleotides in mitochondrial rRNAs and tRNAs undergo modifications, although our knowledge of the mechanisms and enzymes that are involved in these processes in mammalian mitochondria is, to say the least, limited. To put this into context, over two dozen nucleotides in various mitochondrial tRNAs are subjected to post-transcriptional modifications, but only five enzymes responsible for these changes have been identified [81] (Figure 2). Astonishingly, although there are at least nine modifications in both mitochondrial rRNAs, only one enzyme (that performs two of these modifications) has been identified thus far.

This section is devoted to recent advances in analysing the post-transcriptional mechanisms responsible for the stability and modifications of mitochondrial non-messenger RNAs in mammalian cells. Recent and comprehensive reviews by experts in the field deal with biogenesis, function and structural aspects of mammalian mt-tRNAs and hence they will not be described in detail [81,82]. Instead, we will focus on the current knowledge on stability and modifications of mitochondrial rRNA highlighting their importance in the biogenesis of the mitochondrial ribosome. In contrast with the relatively well-studied role of rRNA transacting factors in the biogenesis of the ribosome in bacteria and cytoplasm of eukaryotes, thus far such factors have not received a great deal of attention in mammalian mitochondria. Again, a graphical abstract of this section is provided (Figure 2).

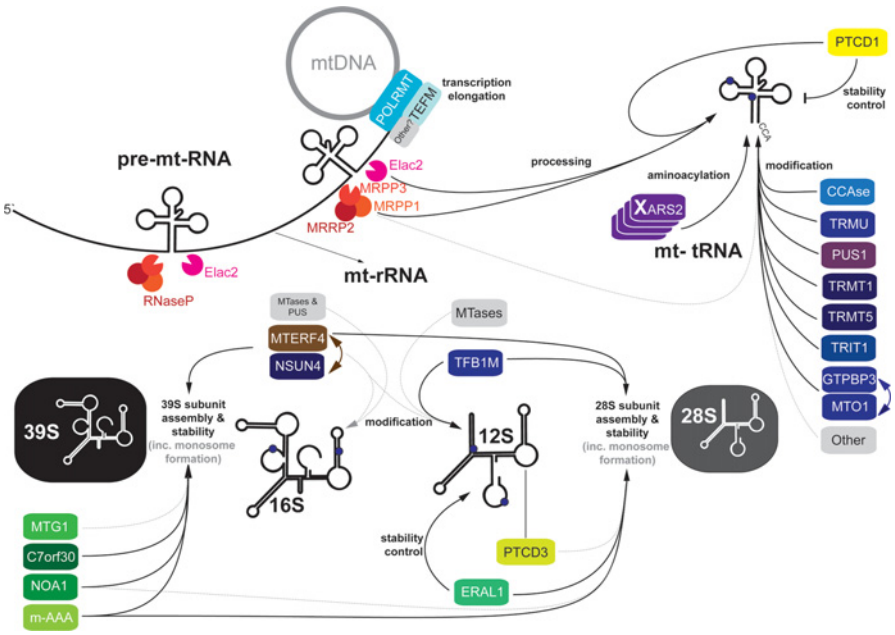

Figure 2 Post-transcriptional metabolism of non-messenger RNAs and protein factors engaged in these processes

Processing of mitochondrial tRNAs, characterization of aminoacyl-tRNA synthetases (XARS2) and mt-tRNA modification enzymes are reviewed in [81]. Solid lines and broken lines represent well-characterized and hypothetical interactions respectively. GTPBP3, GTP-binding protein 3; MTO1, mitochondrial translation optimization 1 homologue; PUS1, pseudouridylate synthase 1; TRIT1, tRNA isopentenyltransferase 1; TRMT, TRM1 tRNA methyltransferase 1 homologue; TRMU, tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase.

Role of mitochondrial rRNA stability, processing and modifications in mitoribosome biogenesis

The human mitochondrial ribosome consists of mt-SSU (mitochondrial small 28S subunit) and mt-LSU (mitochondrial large 39S subunit), containing 12S and 16S rRNAs respectively. The 3′-termini of mt-rRNAs normally do not have more than one or two adenine nucleotides [27,33]. Whether or not a small rRNA, analogous to the 5S RNA in the *E. coli* ribosome, is present in the mitoribosome is still under debate [83]. Similarly to the majority of mt-mRNAs, mt-rRNAs are generated by the processing of mt-tRNAs located on either ends. mt-rRNAs exhibit 10–30-fold higher steady-state expression levels compared with the most abundant mt-mRNAs such as CO1 or CO2 [5,27,84]. The overabundance of mt-RNA is believed to result primarily from a higher transcription rate from the HSP1 promoter. There are also marked differences in the concentrations within rRNA species, and usually 16S rRNA are 2-fold more abundant than 12S [27]. Therefore post-transcriptional mechanisms regulating transcript stability are also likely to contribute to this effect.

Ribosome production entails a complex multi-step pathway in all organisms studied thus far and rRNA plays a vital role in this process. In the yeast cytoplasm, approximately 200 proteins and 70 snoRNAs (small nucleolar RNAs) have been reported to participate in ribosome biosynthesis. Also, biosynthesis of the bacterial ribosome requires auxiliary factors; however, owing to the lack of compartmentalization of the ribosome synthesis steps, a much smaller number of proteins are involved in this process. Many of the non-ribosomal factors involved in the ribosome biogenesis directly regulate rRNA stability and processing (e.g. sequence-specific helicases that aid folding and RNA chaperones) [85].

Maturation of rRNA requires a number of post-transcriptional nucleotide modifications, some of which are co-transcriptional, whereas others occur once the rRNA has been assembled into pre-ribosomes [86]. These modifications consist mostly of base methylation, 2′-*O*-ribose methylation and pseudouridylation and are conserved in Prokaryota and Eukaryota. Base methylation is performed by MTases (methyltransferases) that directly recognize specific targets [87]. However, the methylation of ribose and conversion into pseudouridine, although common to both Prokaryota and Eukaryota, involves a different mechanism in these two groups. Cytoplasmic eukaryotic rRNAs require a number of snoRNAs in order to mark the target nucleotide by forming a transient duplex with the precursor, whereas prokaryotic rRNA modification is performed by several site-specific enzymes that recognize their target without a guide RNA [87]. The modifications made to rRNAs have been mapped for yeast and mammalian mitochondrial ribosomes (Table 2). In contrast with eukaryotic cytoplasmic and bacterial rRNAs that contain a large number of modified nucleotides [88], only a handful of rRNA nucleotides are modified in mitochondria. Similarly to bacteria, mitochondrial rRNA modifications depend rather on site-specific enzymes that are not guided by small RNAs [89].

12S rRNA and mt-SSU biogenesis

A number of protein factors contributing to the assembly and function of mt-SSU via interaction with 12S rRNA have been reported in mammalian mitochondria.

A homologue of the bacterial Era protein, ERAL1 (Era G-protein-like 1), which is a member of a conserved family of GTP-binding proteins with RNA-binding activity, has been identified in human mitochondria [90,91]. It has been demonstrated that ERAL1 is important for the formation of mt-SSU and down-regulation of its expression by siRNA results in decreased mitochondrial protein synthesis and a decay of nascent 12S mt-rRNA [90,91]. It has been suggested that ERAL1 functions as a mitochondrial RNA chaperone to protect the 12S mt-rRNA on mt-SSU during the assembly [90].

Table 2 Ribosomal RNA modifications in bacteria and mitochondria of yeast and mammals

N/A, not applicable.

E. coli			Yeast mitochondria			Mammalian mitochondria					
Position	Modification	Gene	Position	Modification	Gene	Hamster position	Hamster modification	Human position	Human modification	Gene	References
(a) Large rRNA											
2251	Gm	*RlmB*	2270	Gm	*Pet56p*	1144	Gm	1145†	To be confirmed	Not identified	[98,99,101, 122,123]
2552	Um	*RrmJ*	2791	Um	*MRM2*	1370	Um	1369†	To be confirmed	Not identified	[98,99,101,124,125]
2553	Not modified	N/A	2792	Not modified	N/A	1371	Gm	1370†	To be confirmed	Not identified	[99,101]
2580	Psi	*RluC*	2819	Psi	*PUS5*	1398†	Not detected	1397	Psi	Not identified	[130,131]
(b) Small rRNA											
788	Not modified	N/A	1243	Not modified	N/A	426	m^5U	429 †	To be confirmed	Not identified	[98,99,101]
1402	m^4Cm	*RsmH*, *RsmI*	1862	Not modified	N/A	847	m^4C	846 †	To be confirmed	Not identified	[99–101, 113]
1403	Not modified	N/A	1863	Not modified	N/A	848	m^5C	847†	To be confirmed	Not identified	[98,99–101]
1518	$m^6{}_2A$	*KsgA*	2002	Not modified	N/A	939	$m^6{}_2A$	936	$m^6{}_2A$	TFB1M (TFB2M)	[98,100–102, 110]
1519	$m^6{}_2A$	*KsgA*	2003	Not modified	N/A	940	$m^6{}_2A$	937	$m^6{}_2A$	TFB1M (TFB2M)	[98,100–102, 110]

†Predicted from a sequence alignment.

As mentioned above, PPR proteins play important roles in RNA metabolism in organelles. PTCD3 is involved in the regulation of protein synthesis in mitochondria [92]. PTCD3 is tightly associated with the ribosome and has been classified as a mt-SSU protein [93,94]. PTCD3 is also associated with 12S and knockdown of the protein levels significantly decreased protein synthesis without affecting the abundance or processing of mitochondrial transcripts. Whether or not ribosome integrity is compromised in PTCD3-depleted cells remains an open question. It has been shown that the abundance of two marker proteins for mt-SSU and mt-LSU (MRPS15 and MRPL11) are not affected in PTCD3-depleted cells [92]; however, the integrity of the subunit has not been studied by other methods such as sucrose-gradient sedimentation. Other studies have shown that mitoribosome assembly can be compromised without affecting the steady-state levels of individual components [50,95]. The exact role of PTCD3 in the mt-SSU function is yet to be elucidated, but given its properties it may be involved in the assembly or the recognition/decoding of mRNA transcripts by the mitoribosome.

Although mitochondrial tRNA metabolism is beyond the scope of the present review, it is worth briefly mentioning another PPR protein, PTCD1, involved in the regulation of post-transcriptional mitochondrial gene expression. PTCD1 is a low-abundance protein found mostly in the heart, testes and muscles. Overexpression of PTCD1 reduces the levels of leucine tRNAs in mitochondria, whereas its knockdown by siRNA increases the levels of these tRNAs [96]. Down-regulation of PTCD1 did not alter mitochondrial mRNA levels, but increased the protein levels of several mitochondrially encoded subunits of Complexes I and IV, suggesting that leucine tRNAs may be a rate-limiting factor for mitochondrial protein synthesis. This led to the conclusion that PTCD1 acts as a negative regulator of translation at the tRNA level [96]. A more recent study, however, suggested a further function of the protein in 3′ processing of mt-tRNAs from their precursors [97]; although the relationship between these two roles needs to be addressed in the future.

Post-transcriptional nucleotide modifications of 12S rRNA

Detailed studies of hamster cells the allowed identification of five modified nucleotides in the small 12S rRNA [98–101] (Table 2). All positions that are modified in 12S rRNA in rodent mitochondria are absolutely conserved in the human sequence and, although not experimentally confirmed, are likely to be present in human rRNA. Surprisingly, in yeast mitochondria the small 15S rRNA does not to harbour any modifications (Table 2). In the present review we briefly characterize the rRNA modifications detected in mammalian 12S rRNA (using human rRNA position numbering) discussing their documented or predicted role in mitoribosome assembly and function.

$m^6{}_2A^{936}$ and $m^6{}_2A^{937}$: critical modification performed by protein(s) related to mitochondrial transcription factor

It has been shown that two very highly conserved adenines in a stem-loop near the 3′-end of 12S rRNA are modified by N6-dimethylation in hamster and human mitochondria [101,102]. TFB1M (mitochondrial transcription factor B1) and its paralogue TFB2M are capable of dimethylation of these residues and are the only rRNA-modifying enzymes identified in mammalian mitochondria thus far. TFB1M and TFB2M were initially discovered as human mitochondrial transcription factors by homology with the only known mitochondrial transcription factor in yeast [103]. Both proteins are able to stimulate transcription initiation *in vitro*, although TFB2M is significantly more efficient as compared with TFB1M [103,104]. Sequence homology analysis revealed that both TFB1M and TFB2M have an *S*-adenosylmethionine-dependent MTase domain homologous with *E. coli* KsgA that dimethylates the corresponding adenines in 16S [103,104]. Furthermore, it has been shown that TFB1M, and to a lesser extend TFB2M, are not only capable of stimulating transcription, but also can dimethylate the adenines (A^{1518} and A^{1519}) in the conserved stem-loop in bacterial 16S rRNA in KsgA-deficient *E. coli* strains [102,105]. Therefore each of the factors is formally capable of contributing to both rRNA methylation and transcription *in vivo*, albeit TFB1M is mainly the 12S rRNA MTase, whereas TFB2M is primarily a transcription factor (discussed further in [106]).

More recently, studies in a mouse model have shown that ablation of TFB1M in the heart leads to the loss of adenine dimethylation in 12S rRNA, a reduction in the steady-state levels of 12S rRNA and a dramatic impairment in the stability/assembly of mt-SSU, with a consequent loss of mitochondrial translation [107]. Consistent with this data are the studies of Matsushima et al. [108], who found that RNAi (RNA interference) knockdown of TFB1M in insect cells also impaires mitochondrial

translation. Why is dimethylation of a stem-loop structure in 12S rRNA of critical importance for the integrity of the mammalian mitoribosome? One of the possibilities is that rRNA dimethylation plays a direct and crucial role mt-SSU assembly. However, this would be in disagreement with results from many studies of bacteria and yeast cytoplasmic ribosomes showing that fully assembled small subunits are produced in the absence of dimethylated adenines [109]. An alternative explanation could be that this modification constitutes an obligatory late step in the assembly of the complete mitoribosome, consequently mt-SSU subunits with unmethylated 12S rRNA fail to interact with mt-LSU and become unstable. This hypothesis would be consistent with some earlier data showing that extracts from KsgA-deficient *E. coli* cells have reduced affinity between the 30S and 50S ribosomal subunits [110]. This is also in agreement with a more recent functional model for bacterial KsgA proposing that dimethylation of adenines is a part of the universal checkpoint mechanism during ribosome assembly. This model proposes that KsgA plays a role in the final stages of ribosome assembly by establishing an optimal subunit conformation and preventing not fully assembled small ribosomal subunits from entering the translational cycle prematurely [111,112]. However, it is unclear why such a dramatic effect on the stability of the unmethylated mt-SSU would be observed in mammalian mitochondria, whereas in bacteria and the cytoplasm of yeast, rRNA adenine dimethylation only fine-tunes ribosomal function [109]. This issue certainly needs more attention.

12S m^4C^{846}: enzyme awaiting identification

The biogenesis and function of the methylation of m^4C^{846} remains unclear and no enzyme catalysing this modification has been identified. In *E. coli* 16S rRNA the base corresponding to C^{846} (C^{1402}) is also N^4-methylated, however, in contrast with mammalian 12S rRNA, *E. coli* C^{1402} is additionally ribose methylated (N^4, 2′-*O*-dimethylcytidine-m^4Cm; Table 2). It has been shown that a lack of N^4-methylation of C^{1402} (but not 2′-*O*-ribose methylation) increases the efficiency of non-AUG initiation and decreases the rate of UGA stop codon read-through. This led to a model whereby m^4Cm^{1402} in the 16S rRNA plays a role in fine-tuning the structure and function of the P-site and regulates decoding fidelity [113]. It would be very interesting to see if this function is evolutionarily conserved and if m^4C^{846} has the same function in mammalian mitochondria.

12S m^5C^{847}: a target for the mTERF4–NSUN4 (NOP2/Sun domain family 4) complex?

In contrast with C^{846}, the base corresponding to C^{847} is not modified either in 16S rRNA of *E.coli* (C^{1403}) or other small rRNAs analysed [88] (Table 2). No m^5C MTase has been assigned to perform this modification in mammalian mitochondria. However, recently a putative RNA m^5C MTase NSUN4 has been identified in mammalian mitochondria that forms a complex with hMTERF4 (human mTERF4), a member of a family of mitochondrial proteins involved in transcription regulation [114]. The hMTERF4–NSUN4 complex co-migrates with mt-LSU and primarily binds the large 16S mitochondrial rRNA, suggesting a role in the biosynthesis of mt-LSU. However, in the absence of hMTERF4 both subunits of the mitoribosome are present at increased levels, but do not interact to form a functional assembled ribosome indicative for the hMTERF4–NSUN4 complex being a part of the late stages of mitoribosome assembly [114]. Importantly, no m^5C methylation has been detected in the mammalian mitochondrial 16S rRNA thus far (Table 2). Is it, therefore, possible that NSUN4 modifies the small 12S rRNA while being localized on the mt-LSU, with this modification being necessary for the completion of mitoribosome assembly? Or, is there an m^5C methylation on 16S rRNA that has not so far been detected? Further studies of the mTERF4–NSUN4 complex are necessary in order to answer these questions. Interestingly, the closest bacterial homologue of NSUN4, YebU, is responsible for the methylation of a cytosine in the rRNA of the bacterial small 28S subunit (C^{1407}), and can only perform the reaction when rRNA is present in the assembled small ribosomal subunit (free rRNA or 70S ribosomes cannot be methylated by YebU) [115].

12S m^5U^{429}: enzyme awaiting identification

Methylation of m^5U^{429} occurs in the central domain of 12S rRNA as a part of the conserved sequence GGAm^5UUAGA. The human mitochondrial enzyme responsible for this modification has not been identified, so nothing is known about the function of U^{429} in ribosome biogenesis. A corresponding uracil residue in *E. coli* 16S rRNA (U^{788}) is located within the 790-loop of helix 24 [88]. Although U^{788} is not methylated in bacteria, it has been shown that mutants of the 790-loop yielded no fully formed 30S subunits, indicating that this loop is critical to the 30S ribosomal biogenesis and assembly pathways [116]. Interestingly, it is has been observed that the 790-loop plays an important role in catalysis by the N^6-dimethyltransferase KsgA, the bacterial homologue of TFB1M (see above) [116]. Again, it would be very interesting to see if these features are conserved in mammalian mitochondria and how U^{429} methylation contributes to it.

16S rRNA and mt-LSU biogenesis

Not much is known about the role of 16S rRNA in biogenesis of the human mitoribosome. mTERF4 is the only recognized protein that binds mitochondrial 16S rRNA and participates in the biogenesis of the mitoribosome [114]. However, as already discussed (see the '12S m^5C^{847}: a target for the mTERF4–NSUN4 complex?' section), whether or not mTERF4 plays a direct role in production of the mature mt-LSU remains to be elucidated. Of note, a number *trans*-acting factors that play a role in the regulation of the integrity of mt-LSU have been reported recently and we would like to briefly characterize them in the present review.

NOA1 (nitric-oxide-associated 1; C4orf14, mAtNOS1) is a member of the cpGTPase (circularly permuted GTPase) family that play various roles in ribosome function [117]. Knockout of NOA1 in mouse cells led to impaired protein synthesis without loss of mtDNA and/or transcription. The analysis of mitochondrial ribosomal subunits from Noa1-null cells by sucrose-gradient centrifugation showed anomalous sedimentation of mt-LSU, consistent with a defect in assembly. However, a role of Noa1 in assembly of mt-SSU cannot be formally ruled out [117].

The yeast Mtg1 protein, a homologue of bacterial YlqF GTPase (also known as RbgA) [118], has been suggested to function in the assembly of mt-LSU in yeast mitochondria without a role in transcription or processing of mt-rRNAs [119]. The human orthologue of Mtg1 is capable of partial rescue of respiratory deficiency in a yeast mtg1-null mutant and has been localized to mitochondria in human cells [119]. Therefore it is likely that the human Mtg1 protein also participates in the mitoribosome assembly.

Another non-ribosomal protein involved in the late stages of mitoribosomal biogenesis is m-AAA (mitochondrial AAA) protease. Studies in yeast mitochondria revealed that m-AAA protease is involved in the processing of the mitochondrial ribosomal protein MRPL32 allowing for its association with pre-ribosomal particles and hence completion of mitoribosome assembly. In mammals the m-AAA protease constitutes hetero-oligomeric complex composed of Afg3l2 (AFG3 ATPase family gene 3-like 2) and paraplegin, and its function is conserved as maturation of MrpL32 is impaired in a mouse model lacking the paraplegin subunit [120].

Finally, very recently we [95] and others [121] have characterized a novel human protein, C7orf30, that specifically associates with mt-LSU. Inactivation of C7orf30 in human cells by RNAi-impaired mitochondrial translation without any appreciable effects on the steady-state levels of mitochondrial mRNAs and rRNAs. The translation defect in C7orf30-depleted cells results from aberrant assembly of mt-LSU and consequently reduced formation of the monosome. We proposed that C7orf30 is a human assembly and/or stability factor involved in the biogenesis of mt-LSU [95].

Post-transcriptional nucleotide modifications of 16S rRNA

In parallel with the studies of 12S rRNA in hamster cells, detailed analysis of the modifications in the large 16S rRNA identified four modified nucleotides (Table 2). Perfect sequence conservation between rodent and human mitochondrial rRNA sequences at the modified residues suggests that the modifications will also be conserved, subject to experimental verification. In vertebrates, three out of four modifications present are also present in the large 21S rRNA in yeast mitochondria (Table 2). In the present study, we briefly characterize the rRNA modifications detected in mammalian 16S rRNA (using human rRNA position numbering) considering their predicted role in mitoribosome assembly and function.

16S Gm^{1141}: enzyme awaiting identification

The predicted 2′-*O*-methylguanosine at human position 1141 occurs in a universally conserved nucleotide in the peptidyl transferase centre of the large rRNA. The enzyme responsible for this modification in human mitochondria has not been identified. In the yeast mitochondrial large rRNA (21S) the corresponding position (Gm^{2270}) is ribose-methylated by the Pet56 protein [122]. A Pet56-deletion mutant lacks MTase activity and, importantly, is deficient in the formation of a functional yeast mt-LSU, indicating a vital role in the assembly *in vivo* [122]. It is therefore possible that the human orthologue might be essential for the biogenenesis of the mitochochondrial ribosome. However, any speculation on the function of a putative human mitochondrial 2′-*O*-methylguanosine MTase is difficult, in the view that RlmB, the *E. coli* enzyme responsible for the formation of Gm in the corresponding position (2251) of the bacterial large rRNA (23S), has no important role in ribosome maturation and function [123].

16S Um^{1369} and Gm^{1370}: enzymes awaiting identification

The predicted human 2′-*O*-methyluracil and 2′-*O*-methylguanosine at positions 1369 and 1370 respectively, occur in the large rRNA A-loop. The A-loop is an essential component of the peptidyl transferase centre that is implicated in the interaction with aminoacyl (A)-site tRNA. The human enzymes responsible for the modification of these sites have not thus far been identified (Table 2).

The base equivalent of Um^{1369} has been shown to be methylated in the majority of organisms investigated thus far [89]. In yeast mitochondria Mrm2p is required for methylating the corresponding position (Um^{2791}) in the large 21S rRNA. Mrm2p co-sediments with the large rRNA on sucrose gradients and methylates U^{2791} only when assembled into the large 39S subunit suggesting that the Mrm2p-mediated methylation occurs late in the maturation process of the ribosome. Deletion of the *MRM2* gene causes a thermosensitive respiratory growth phenotype accompanied with a rapid loss of mitochondrial DNA [124]. The analysis of RrmJ, a bacterial homologue that is responsible for methylation of the corresponding position (Um^{2552}) in the large 23S rRNA, revealed that RrmJ-deficient strains exhibit growth defects and reduced translation rates. RrmJ is active against ribosomes and the free large 39S subunit, but not on free rRNA, and the Um^{2552} methylation is important for the stability of the 70S ribosome [125,126]. More recent studies have shown that U^{2552} methylation negatively regulates translational accuracy. The lack of this modification in bacterial 23S rRNA causes a decrease in programmed $+1$ and -1 translational frameshifting and a reduction in read-through of UAA and UGA stop codons [127]. It would be interesting to see if the predicted Um^{1369} in human 16S rRNA fulfils a similar function, especially in the context of the recently discovered programmed -1 mitoribosome frameshifting during translation termination in mammalian mitochondria [52].

Nothing is known about the predicted Gm^{1370} modification in human 16S rRNA. The corresponding position is not modified in yeast mitochondrial 21S rRNA (G^{2792}) nor in *E. coli* 23S rRNA (G^{2553}). However, it has been reported that the loop containing G^{2553} (and the preceding Um^{2552}; see above) in bacterial 23S rRNA plays an essential role in translation by base pairing with the tRNA 3′-terminal region. Mutation of G^{2553} abolishes the interaction with tRNA and thus the peptidyl transferase activity [128]. Interestingly, the analogous 2′-*O*-methylguanosine is present in the cytoplasmic yeast 25S rRNA (Gm^{2922}). The formation of Gm^{2922} is catalysed by Spb1p, occurs late in the processing stage, and is essential for normal ribosome production and translation [129]. Collectively, the data obtained for *E. coli* and yeast mitochondria and cytoplasm suggest that modifications of the nucleotides of the A-loop are indispensible for ribosome function, hence there is a pressing need for study of the predicted Um^{1369} and Gm^{1370} in human mitochondrial 16S rRNA.

16S Psi^{1397}: enzyme awaiting identification

So far, the pseudouridine conversion at position 1397 is the only confirmed modification of the human 16S rRNA [130]. However, the enzyme catalysing this modification remains to be discovered. The corresponding position in yeast mitochondrial 21S rRNA (Psi^{2819}) is modified by Pus5p, is not essential for cell viability and its function is not clear [131]. Curiously, the analogous modification has not been detected in hamster mitochondrial rRNA, implying that some modifications might not be conserved within mammals. This signifies the need to experimentally confirm all of our predictions for the modifications to the human mitochondrial rRNAs described above

TURNOVER OF MITOCHONDRIAL RNA: WHICH ENZYMES FOR THE JOB?

In contrast to the relatively well-characterized mechanisms of mt-RNA degradation in yeast or plant mitochondria, there are many key outstanding questions on the RNA decay machinery

in mammalian mitochondria, the most important being: which enzyme is responsible for the ribonuclolytic activity of the mt-RNA-degrading apparatus? This section reviews the existing knowledge on the factors with confirmed functions in RNA decay in mammalian mitochondria and discusses other proteins that are potentially involved in this process.

SUV3 (suppressor of var1 3-like protein 1): an mt-RNA decay and surveillance helicase

Large multiprotein complexes that degrade RNA, such as the eukaryotic exosome complex [132] or the bacterial degradosome [133], often contain RNA helicases in addition to ribonucleases. The activity of these helicases increases the efficiency of exoribonuclease-dependent decay by unwinding RNA secondary structures in an ATP-dependent manner. In line with this, in yeast mitochondria the nuclear-encoded RNA helicase SUV3 is essential for the proper function of the mtEXO (mitochondrial degradosome) complex. In mtEXO the exoribonucleolytic activity is provided by DSS1 [134] and the function of this enzyme in mitochondrial RNA decay will be discussed in the sections below.

Extensive yeast genetics and biochemical studies have established yeast SUV3 as one of the major players in the degradation of mitochondrial transcripts and mt-RNA surveillance. As a member of the Ski2 family of DExH-box RNA helicases, Suv3 has an ATP-dependent RNA helicase activity [135,136]. Inactivation of SUV3 in yeast cells leads to severe perturbations in mt-RNA metabolism such as the accumulation of misprocessed transcripts and introns [135,137,138] and consequently translation inhibition and respiratory incompetence. There is a consensus in the field that yeast Suv3, as an essential component of mtEXO, functions as a regulator of mature mt-RNA half-life and as a surveillance factor that controls the degradation of unnecessary or aberrant mt-RNA molecules [135].

The SUV3 gene is well evolutionarily conserved among Prokaryota and Eukaryota. A high degree of sequence homology with the yeast SUV3 gene has allowed identification of the human orthologue: hSUV3 (or SUPV3L1) [139]. The hSuv3 protein is localized predominately in mitochondria and is a part of the mitochondrial nucleoid [140,141]. The recombinant enzyme exhibits an ATP-dependent helicase activity *in vitro* with a broad substrate specificity that includes dsDNA, dsRNA and DNA/RNA hybrids [140,142]. The involvement of hSuv3 in mt-RNA degradation and surveillance has been relatively well documented. Initially, Khidr et al. [143] have shown that the RNAi-mediated down-regulation of hSuv3 results in the accumulation of shortened mt-RNA species (of note, only the ND2 mRNA was analysed) that were interpreted to be a consequence of impaired mt-RNA degradation. The analysis of the hSuv3 function in mitochondrial RNA metabolism has been extended recently by inactivation of the protein through overexpression of a catalytically inert mutant causing a dominant-negative effect [144]. This resulted in the accumulation of processing and decay intermediates in all mt-RNAs analysed, including: (i) non-coding antisense species transcribed from both the H- and L-strands; (ii) aberrant mt-mRNA species with misprocessed mt-tRNAs; and (iii) degradation intermediates truncated predominantly at 3′-ends (Table 1).

Apart from the role of SUV3 in mt-RNA turnover and surveillance, some data have implicated this protein in the maintenance of mtDNA in yeast [145] and humans [143]. In yeast, inactivation of SUV3, in addition to perturbing mt-RNA metabolism, also leads to loss of mtDNA. However, yeast SUV3 mutants defective in RNA unwinding and degradation can rescue mtDNA-loss phenotypes, implying that the role of the yeast Suv3 in mtDNA maintenance is uncoupled from its function in RNA stability and turnover [145]. Furthermore, down-regulation of human SUV3 by RNAi leads to reduced mtDNA copy number [143]. These results are compatible with the biochemical properties of the enzyme, as it has been shown that hSuv3, in addition to dsRNA, also efficiently unwinds dsDNA *in vitro* [140,142]. Further support for a potential role of mammalian Suv3 in maintaining the mitochondrial genome integrity comes from the studies of the gene in mouse models. Although homozygous knockout of SUV3 leads to early embryonic lethality [146], conditional ablation of SUV3 leads to delayed growth and aging-like phenotypes, such as sarcopenia, kyphosis, cachexia and premature death [147]. Very similar phenotypes were observed in transgenic mice with dysfunctional mitochondrial genomes owing to high levels of somatic point mutations in mtDNA introduced by the expression of the proofreading-deficient mitochondrial replicase POLG [polymerase (DNA directed) γ; POLG mitochondrial mutator mice] [148,149]. It is possible that these aging-like phenotypes of the SUV3-knockout mouse result from problems in mtDNA maintenance. However, the interpretation of these data is complex as, in addition to the mitochondrially localized pool of human SUV3, the protein can be detected in the cell nucleus [150]. Extramitochondrially localized SUV3 interacts with WRN (Werner syndrome, RecQ helicase-like) and BLM (Bloom syndrome, RecQ helicase-like), which are the RecQ helicases involved in nuclear genome maintenance [146], as well as RPA (replication protein A), FEN1 (flap endonuclease 1) [151] and co-factor of survivin HBXIP (hepatitis B virus x-interacting protein) [152]. Therefore an equally probable explanation for the aging-like phenotypes in SUV3-deficient animals is that the protein is involved in protecting nuclear genome integrity.

In summary, SUV3, despite diverse intracellular localization, plays a vital role in mitochondrial RNA metabolism, participating in the regulation of mt-RNA surveillance and decay. Further studies of mt-RNA-related processes in the already existing *SUV3*-knockout-mouse model could be very helpful in order to gain a better understanding of the mitochondrial function of this gene. Also, the development of new cellular models where the mitochondrial form of SUV3 is specifically inactivated would enable additional studies of the gene in the context of mt-RNA metabolism. However, this is currently hampered by the lack of sufficient data on the localization signals within the mammalian SUV3 protein.

A hunt for a turnover RNase in mammalian mitochondria

In yeast mtEXO, in addition to the helicase activity provided by SUV3, the exoribonucleolytic activity is provided by DSS1 (MSU1, YMR287C) [134]. DSS1 contains the RNB catalytic domain typical for proteins related to bacterial 3′→5′ exoribonuclease II (RNase II) and ribonuclease R [153]. The two-subunit helicase–exoribonuclease organization has been conserved through evolution and orthologous mtEXO-like complexes are found in the mitochondria of *Trypanosoma brucei* [154] and *Schizosaccharomyces pombe* [155]. It is also important to stress that, apart from mtEXO, other as yet unidentified ribonucleases have to be involved in RNA decay in yeast mitochondria. This is based on the observation that in mtEXO-deficient strains, some RNA species, such as transcribed intergenic regions, are not accumulated, hence other mechanisms must be responsible for their decay.

The identification and characterization of the human orthologue of SUV3 has been a very promising start towards describing the mammalian mitochondrial mechanisms involved in RNA

degradation (see above). However, identification of the mammalian orthologue of DSS1 has proven unachievable thus far. Therefore, it is conceivable that mammalian mitochondrial RNA catabolic processes are governed by a different type of molecular machinery, in which the role of DSS1 is fulfilled by a distinct ribonuclease. Below we discuss the current status of knowledge regarding the candidates for mt-RNA decay ribonucleases in mammalian mitochondria. We also include selected data on our attempts to identify novel human mitochondrial ribonucleases using a gene candidate approach.

Does PNPase have a direct role in mt-RNA degradation?

As mentioned in the preceding section, several years ago PNPase was identified in human mitochondria [40]. As PNPase is capable of degrading RNA in a processive 3′→5′ phosphorolytic reaction, it instantly become a plausible candidate for the human mitochondrial RNA-decay enzyme [40]. This concept was supported by the occurrence of PNPase in a bacterial RNA degradosome and its participation in the degradation, processing and polyadenylation of RNA in plant mitochondria and chloroplasts [156]. The bacterial degradosome is a multiprotein complex involved in the decay of mRNA, conserved throughout the Proteobacteria, that in addition to PNPase consists of a helicase (RhlB), endonuclease (RNase E) and enolase [157]. In plants mitochondrial and chloroplast RNA is degraded by PNPase and this process is stimulated by RNA polyadenylation [156].

The role of PNPase in RNA metabolism in mammalian mitochondria has been addressed by gene inactivation; however, a consensus on whether or not the enzyme is involved directly in RNA degradation has not been reached. As has been reported in many systems, inactivation of the RNA decay machinery results in accumulation of RNA classes not detected normally owing to their short half-life, including for example misprocessed precursors or intergenic regions, which may or may not be accompanied by a perturbation in the mature RNA species. In a study by Nagaike et al. [34], down-regulation of PNPase by transient delivery of siRNA did not produce a significant effect on the steady-state levels of the mature human mt-mRNAs analysed, but the data presented do not allow any conclusions to be drawn as to whether or not RNA precursors were accumulated. On the other hand, in another study stable down-regulation of PNPase by siRNA resulted in an increased abundance of aberrant CO1 mRNA as established by circularization of RNA and RT–PCR followed by sequencing [43]. The nature of the aberrantly processed transcripts resembled those observed upon silencing of hSUV3, i.e. RNA molecules retained fragments of misprocessed mt-tRNAs and intergenic regions, and there were also molecules truncated at their 3′-ends. A similar procedure has been applied in order to analyse the 16S rRNA, but no adverse effects of stable PNPase silencing have been detected [43]. No other mt-mRNA species have been analysed in detail upon stable PNPase silencing that would allow verification of whether or not precursors were accumulated. Furthermore, in a recent study by Wang et al. [19] using mouse liver cells from liver-specific PNPase-knockout animals, in addition to the mature CO2 and CO3 transcripts, a range of larger and smaller RNA species were present in Northern blot analysis. Also, the same cells accumulated unprocessed RNA containing the CO1, mt-tRNA-SerUCN, mt-tRNA-Asp, CO2 genes and RNA precursors containing CO2, mt-tRNA-Lys, ATP6/8 genes. Collectively, the aberrant RNA species observed upon PNPase inactivation in both of these studies could either reflect problems with transcript processing or a deficiency in RNA decay, at least for the CO1, CO2, CO3 and ATP6/8 transcripts. The recent discovery of the binding of PNPase to hSuv3p *in vitro* [158], and co-purification of these two proteins from human mitochondria [144], points towards a role for PNPase in mt-RNA degradation. According to this, the human mtEXO would consist of only two proteins: hSuv3p, providing a helicase activity, and PNPase, acting as a ribonuclease [159]. This design of the human mtEXO, however, would be incompatible with a recently postulated model, whereby mitochondrial intermembrane space-localized PNPase mediates the translocation of various RNAs into mitochondria (see above, [19], comprehensively reviewed in [45]). More research is needed to resolve this issue.

Poly(U) tail: scavenger for aberrant mt-RNA?

An interesting feature of the misprocessed mt-RNA species that accumulate upon inactivation of either hSUV3 or PNPase has been observed [43,144]: a significant proportion of these molecules contained a poly(U) tail. Polyuridinylated aberrantly processed RNA molecules have been reported also in mouse mitochondria and this modification was not present in correctly matured transcripts [160]. It is, therefore, conceivable that polyuridynylation is used in mammalian mitochondria to mark improperly formed RNAs for degradation. Such a decay process would require a mitochondrially localized enzyme responsible for catalysing the addition of poly(U) tails, but no poly(U) polymerase has been reported in mitochondria thus far. Interestingly, the recently discovered mitochondrial deadenylase PDE12 degraded poly(A) and poly(U) RNA substrates *in vitro* with comparable efficacy, suggesting that this enzyme might contribute to a potential poly(U)-dependent RNA turnover.

Other mitochondrial RNases

Apart from PNPase, other RNases with a documented or predicted mitochondrial localization have been reported thus far. In the present review, we summarize the information available on these RNases and discuss their potential roles in mt-RNA degradation.

REXO2 (RNA exonuclease 2 homologue)

REXO2 [also known as oligoribonuclease, ORN or SFN (small fragment nuclease)] is a human orthologue of the bacterial oligoribonuclease [161] and yeast mitochondrial Ynt20 [162]. A knockout of the *E. coli* oligoribonuclease is lethal [161] and a temperature-sensitive mutant accumulates small oligoribonucleotides [163], consistent with an essential role of this protein in the removal of short RNAs resulting from RNA degradation processes [164]. Inactivation of yeast mitochondrial *Ynt20* results only in a temperature-sensitive respiratory growth defect suggesting that its function is redundant, but the exact mitochondrial role of the gene is still unknown [162]. Human nuclear-encoded REXO2 is a 3′→5′ exoribonuclease specific for small ($\leqslant$ 5 nt) oligonucleotides. Although it is active on both RNA and DNA oligomers, it degrades RNA more efficiently [165]. One of the alternatively spliced isoforms of REXO2 encodes an N-terminally extended protein that contains a predicted MTS (mitochondrial targeting signal) [165]. Therefore REXO2 appears to be a very plausible candidate for the human mitochondrial oligoribonuclease, but its cellular localization and function needs to be confirmed experimentally.

EndoG (endonuclease G) and ExoG (exonuclease G)

Mitochondria of many species contain at least one highly conserved sugar-non-specific nuclease. Yeast mitochondria

possess the endo-/exo-nuclease Nuc1p (also known as Endo G or EndoG), which is one of the best studied enzymes of this mitochondrial family. Aside from endonucleolytic activity on ss- and ds-DNA and exonucleolytic activity on dsDNA, yeast Nuc1p has a strong RNase activity on ssDNA, but not dsRNA, suggesting a role in mt-RNA degradation [166]. However, Nuc1p is not essential for mitochondrial gene expression and its role in mt-RNA degradation may only be limited to very specific species, such as transcribed intergenic regions that do not accumulate in mtEXO mutants (see above) [138]. Other roles for Nuc1p have been proposed including DNA recombination [167] and regulation of apoptosis and necrosis [168].

In contrast with the yeast homologue, mammalian EndoG exhibits exclusive endonucleolytic activity and cleaves ss- and ds-DNA, ss- and ds-RNA, and RNA/DNA hybrids with almost identical rates [169]. Mammalian EndoG has been reported mainly to play a role in programmed cell death, as it translocates from mitochondria to the nucleus and induces nucleosomal DNA fragmentation during caspase-independent apoptosis [170]. The involvement of mammalian EndoG in mt-RNA degradation has not thus far been studied, however, this potential function is not supported by the cellular localization of the protein mainly to the mitochondrial intermembrane space, but not in the matrix where mt-RNA is located. However, recently a mitochondrial paralogue of EndoG, named ExoG, has been identified in higher eukaryotes. ExoG is localized in the inner mitochondrial membrane and primarily exerts 5′→3′ exonucleolytic activity towards ssDNA and ssRNA [169]. So far the only mitochondrial role reported for ExoG is participation in the repair of DNA single-strand breaks in the mitochondrial genome [171]. The cellular localization and RNase activity of ExoG make this protein a possible candidate for an mt-RNA degradation enzyme, but its involvement in RNA decay needs to be addressed experimentally.

RNase L

RNase L is a part of the interferon-inducible innate immunity system protecting the cell from infections by some types of viruses. This is achieved through cleavage of viral and cellular ssRNA, hence inhibiting of both viral replication and cellular proliferation [172]. It has been shown that a subpopulation of cellular RNase L is located in mitochondria and regulates mt-mRNA stability in lymphocytes [173] mouse embryonic fibroblasts [174] upon interferon activation. More recently it has been proposed that the interferon-dependent regulation of mitochondrial mRNA stability by RNase L occurs through its interaction with the mitochondrial translation initiation factor (IF2mt). In this model, the IF2mt–RNase L interaction brings RNase L into close association with the mt-mRNA, where it can act as an endoribonuclease before the translation initiation complex is assembled [175]. Taken together, RNase L might be an important regulator of mt-RNA stability, but most probably this function is only activated as part of a general anti-proliferative response and the enzyme might not participate in mt-RNA turnover as a housekeeping enzyme.

Gene candidate approach to identify novel mitochondrial RNases

With the aim of identifying the main turnover RNase in mammalian mitochondria we have analysed the proteins with a known RNase domain present in the human genome for the presence of an N-terminally located MTS. Below we briefly summarize the results of our, as yet unsuccessful, experimentation towards identifying novel human mitochondrial RNases.

Dis3L (DIS3 mitotic control homologue-like) 1

Yeast Dis3p is one of the catalytic subunits of the cytoplasmic exosome that is involved in RNA processing and turnover [132]. The human genome contains three homologues of Dis3p (Dis3, Dis3L1 and Dis3L2). We have analysed the protein sequences of all human homologues of Dis3p for the presence of a putative MTS using several computer programmes. The analysis revealed a high probability of a MTS in Dis3L1 (Figure 3A). In order to experimentally test these predictions, we fused the predicted MTS (the first 84 amino acids) to GFP (green fluorescent protein) and expressed this construct in human osteosarcoma cells. The N-terminal part of Dis3L1 can indeed serve as an MTS since the recombinant MTS–GFP fusion co-localized with mitochondria, suggesting a mitochondrial localization for Dis3L1 (Figure 3B). However, experiments with the full-length protein did not confirm this prediction and recently Dis3L1 has been reported to reside exclusively in the cytoplasm of human cells where it interacts with the human core exosome [176,177]. Hence, Dis3L1 is unlikely to be involved in mt-RNA metabolism.

ERI (exoribonuclease) 2

The human genome contains three homologues of ERI-1 (named ERI1, 2 and 3), which is a DEDDh-type 3′→5′ exonuclease initially identified in *Caenorhabditis elegans* as a negative regulator of RNAi [178]. Later it was shown that an isoform of ERI-1 also plays a role in 3′-end processing of the 5.8S ribosomal RNA [179] and interacts with a conserved stem-loop of histone mRNA [180]. Here again, we have scanned the protein sequences of all human homologues of ERI-1 for the presence of a MTS and found a putative MTS in ERI2 (Figure 3A). The predicted MTS (the first 35 amino acids) was fused to GFP and expressed in human osteosarcoma cells. Also, in order to study the cellular localization of the full-length protein we transiently expressed the corresponding cDNA fused to a FLAG tag in osteosarcoma cells. Immunofluorescence analysis revealed that none of the recombinant protein co-localizes with mitochondria (Figure 3B). We concluded that ERI2 is not a mitochondrial protein.

HRSP12 (heat-responsive protein 12)

The human HRSP12 protein (also known as UK114, L-PSP or P14.5) belongs to the evolutionarily well-conserved YER057c/Yjgf/Uk114 family of proteins. A number of functions have been proposed for the proteins of this family including, for example, isoleucine biosynthesis [181] and inhibition of phosphoribosylamine synthesis [182]. Importantly, it has also been reported that human HRSP12 is an RNase responsible for translation inhibition via endonucleolytic degradation of mRNA [183,184]. Published proteomics data [185] and our *in silico* analysis predicted a mitochondrial localization for HRSP12 and so the protein appeared to be an attractive candidate for an RNase involved in mt-RNA metabolism. In agreement with this prediction are data relating to the yeast homologue of HRSP12, MMF1, that has been shown to localize to mitochondria, to be indispensible for respiration and to genetically interact with the mitochondrial ribosome [186,187]. Moreover, human HRSP12 can be imported to yeast mitochondria and rescue the MMF1-null phenotypes. The same study also reported a fractionation experiment of rat liver mitochondria indicating that rat HRSP12, like Mmf1p, is a soluble protein of the matrix [186]. In order to study the cellular localization of HRSP12 in more detail, the full-length, C-terminally FLAG-tagged protein was transiently expressed in HeLa cells and detected by immunofluorescence.

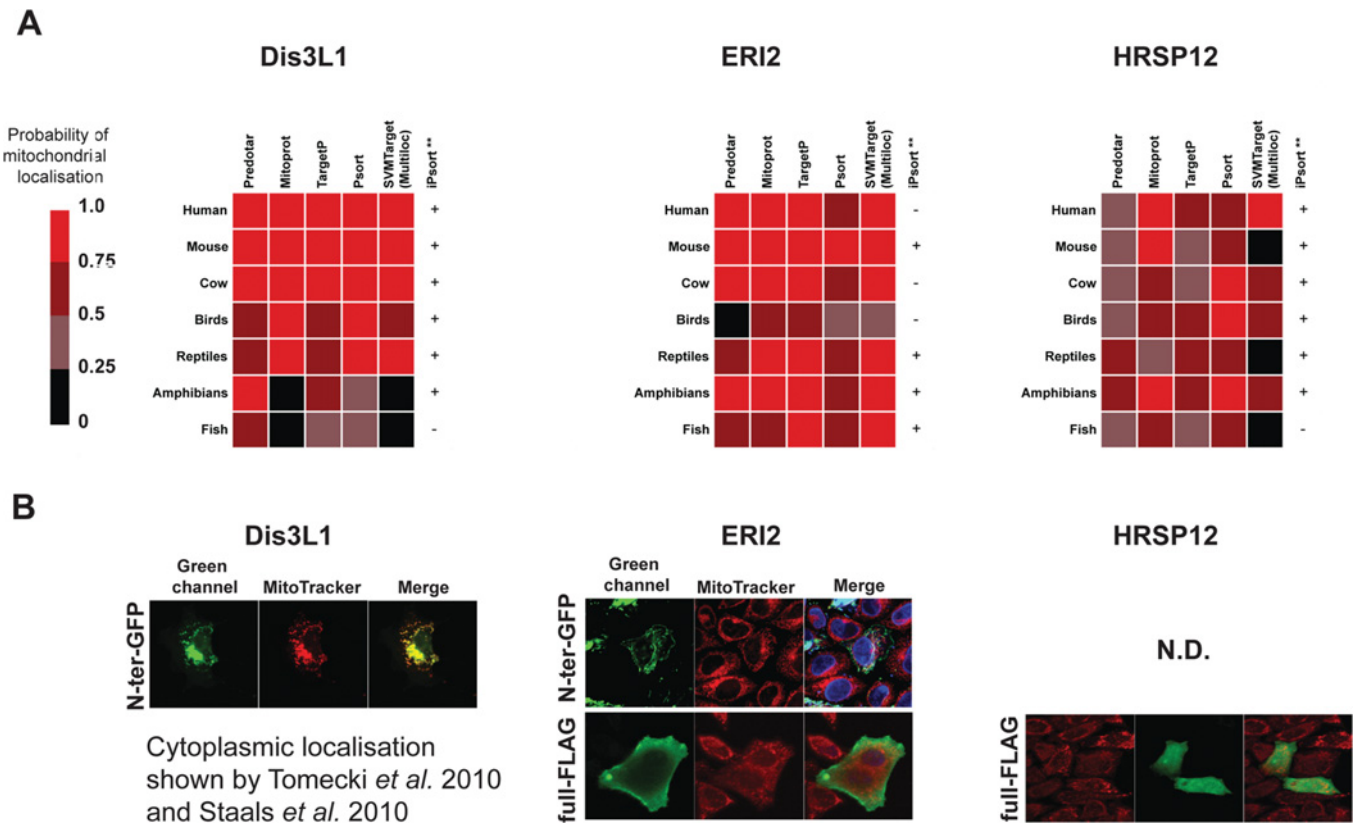

Figure 3 Investigations of the subcellular localization of candidate mitochondrial RNases

(**A**) Bioinformatic prediction tools were used to investigate the mitochondrial localization of human candidate proteins and their homologues from different vertebrates. Red colour indicates a strong probability of mitochondrial localization. (**B**) Microscopic analysis of the subcellular localization of candidate proteins. HeLa cells (HSRP12) or human osteosarcoma cells (ERI2, Dis3L1) were transiently transfected with a plasmid encoding the N-terminal part of the candidate protein fused to GFP or full-length proteins tagged with the FLAG tag at their C-terminus. The FLAG-tagged proteins were visualized by immunocytochemistry using anti-FLAG antibodies and Alexa Fluor® 488-conjugated secondary antibodies (green). Mitochondria (red) were stained with Mitotracker CM-H2XRos and nuclei (blue) were stained with DAPI (4′,6-diamidino-2-phenylindole). Co-localization of protein and mitochondria appears yellow in digitally merged images. The cytoplasmic localization of Dis3L1 was shown in other studies [176,177]. The GenBank® accession numbers for protein sequences used in the present review are: NP_001137160 (Dis3L1), NP_001136197 (ERI2) and AAH93059 (HSRP12). N.D. not determined.

Surprisingly, this analysis revealed that the recombinant HRSP12 protein does not co-localize with mitochondria (Figure 3B). Further research in necessary to resolve whether or not HRSP12 resides in mitochondria.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Regulation of mitochondrial gene expression is essential for adapting oxidative phosphorylation in response to physiological demands in health and disease. Recent years have witnessed significant progress towards understanding the role of RNA metabolism in the regulation of human mitochondrial gene expression. Several new factors have been characterized that play important roles in these processes and some, such as hmtPAP and LRPPRC, have already been linked to human disease. The latest advances in compiling full lists of mammalian mitochondrial proteins using high-throughput methods of tandem mass spectrometry [185,188,189] and comparative genomics [190] should aid in the discovery of missing factors such as decay RNases, poly(U) polymerase or rRNA-modifying enzymes. Also, recent rapid progress in next generation sequencing technology will be helpful in the characterization of mitochondrial RNA abundance, processing and maturation events in the context of up- or down-regulation of specific RNA-transacting factors. These methods will also be useful in establishing a potential role for mitochondrial antisense, small RNA or long non-coding RNAs that are reported more commonly in recent studies [27,191].

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REFERENCES

1 Gray, M. W., Burger, G. and Lang, B. F. (1999) Mitochondrial evolution. Science **283**, 1476–1481
2 Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F. et al. (1981) Sequence and organization of the human mitochondrial genome. Nature **290**, 457–465
3 Fearnley, I. M. and Walker, J. E. (1986) Two overlapping genes in bovine mitochondrial DNA encode membrane components of ATP synthase. EMBO J. **5**, 2003–2008
4 Montoya, J., Christianson, T., Levens, D., Rabinowitz, M. and Attardi, G. (1982) Identification of initiation sites for heavy-strand and light-strand transcription in human mitochondrial DNA. Proc. Natl. Acad. Sci. U.S.A. **79**, 7195–7199

5 Montoya, J., Gaines, G. L. and Attardi, G. (1983) The pattern of transcription of the human mitochondrial rRNA genes reveals two overlapping transcription units. Cell **34**, 151–159
6 Falkenberg, M., Larsson, N. G. and Gustafsson, C. M. (2007) DNA replication and transcription in mammalian mitochondria. Annu. Rev. Biochem. **76**, 679–699
7 Minczuk, M., He, J., Duch, A. M., Ettema, T. J., Chlebowski, A., Dzionek, K., Nijtmans, L. G., Huynen, M. A. and Holt, I. J. (2011) TEFM (c17orf42) is necessary for transcription of human mtDNA. Nucleic Acids Res. **39**, 4284–4299
8 Kruse, B., Narasimhan, N. and Attardi, G. (1989) Termination of transcription in human mitochondria: identification and purification of a DNA binding protein factor that promotes termination. Cell **58**, 391–397
9 Wenz, T., Luca, C., Torraco, A. and Moraes, C. T. (2009) mTERF2 regulates oxidative phosphorylation by modulating mtDNA transcription. Cell Metab. **9**, 499–511
10 Park, C. B., Asin-Cayuela, J., Camara, Y., Shi, Y., Pellegrini, M., Gaspari, M., Wibom, R., Hultenby, K., Erdjument-Bromage, H., Tempst, P. et al. (2007) MTERF3 is a negative regulator of mammalian mtDNA transcription. Cell **130**, 273–285
11 Wang, Z., Cotney, J. and Shadel, G. S. (2007) Human mitochondrial ribosomal protein MRPL12 interacts directly with mitochondrial RNA polymerase to modulate mitochondrial gene expression. J. Biol. Chem. **282**, 12610–12618
12 Ojala, D., Montoya, J. and Attardi, G. (1981) tRNA punctuation model of RNA processing in human mitochondria. Nature **290**, 470–474
13 Temperley, R. J., Wydro, M., Lightowlers, R. N. and Chrzanowska-Lightowlers, Z. M. (2010) Human mitochondrial mRNAs-like members of all families, similar but different. Biochim. Biophys. Acta **1797**, 1081–1085
14 Guan, M. X., Enriquez, J. A., Fischel-Ghodsian, N., Puranam, R. S., Lin, C. P., Maw, M. A. and Attardi, G. (1998) The deafness-associated mitochondrial DNA mutation at position 7445, which affects tRNASer(UCN) precursor processing, has long-range effects on NADH dehydrogenase subunit ND6 gene expression. Mol. Cell. Biol. **18**, 5868–5879
15 Sbisa, E., Tullo, A., Nardelli, M., Tanzariello, F. and Saccone, C. (1992) Transcription mapping of the Ori L region reveals novel precursors of mature RNA species and antisense RNAs in rat mitochondrial genome. FEBS Lett. **296**, 311–316
16 Slomovic, S., Laufer, D., Geiger, D. and Schuster, G. (2005) Polyadenylation and degradation of human mitochondrial RNA: the prokaryotic past leaves its mark. Mol. Cell. Biol. **25**, 6427–6435
17 Doersen, C. J., Guerrier-Takada, C., Altman, S. and Attardi, G. (1985) Characterization of an RNase P activity from HeLa cell mitochondria. Comparison with the cytosol RNase P activity. J. Biol. Chem. **260**, 5942–5949
18 Holzmann, J., Frank, P., Loffler, E., Bennett, K. L., Gerner, C. and Rossmanith, W. (2008) RNase P without RNA: identification and functional reconstitution of the human mitochondrial tRNA processing enzyme. Cell **135**, 462–474
19 Wang, G., Chen, H. W., Oktay, Y., Zhang, J., Allen, E. L., Smith, G. M., Fan, K. C., Hong, J. S., French, S. W., McCaffery, J. M. et al. (2010) PNPASE regulates RNA import into mitochondria. Cell **142**, 456–467
20 Dubrovsky, E. B., Dubrovskaya, V. A., Levinger, L., Schiffer, S. and Marchfelder, A. (2004) *Drosophila* RNase Z processes mitochondrial and nuclear pre-tRNA 3′ ends *in vivo*. Nucleic Acids Res. **32**, 255–262
21 Brzezniak, L. K., Bijata, M., Szczesny, R. J. and Stepien, P. P. (2011) Involvement of human *ELAC2* gene product in 3′ end processing of mitochondrial tRNAs. RNA Biol. **8**, 616–26
22 Rossmanith, W. (2011) Localization of human RNase Z isoforms: dual nuclear/mitochondrial targeting of the *ELAC2* gene product by alternative translation initiation. PLoS ONE **6**, e19152
23 Sanchez, M. I., Mercer, T. R., Davies, S. M., Shearwood, A. M., Nygard, K. K., Richman, T. R., Mattick, J. S., Rackham, O. and Filipovska, A. (2011) RNA processing in human mitochondria. Cell Cycle **10**, 2904–2916
24 Mineri, R., Pavelka, N., Fernandez-Vizarra, E., Ricciardi-Castagnoli, P., Zeviani, M. and Tiranti, V. (2009) How do human cells react to the absence of mitochondrial DNA? PLoS ONE **4**, e5713
25 Nagaike, T., Suzuki, T., Tomari, Y., Takemoto-Hori, C., Negayama, F., Watanabe, K. and Ueda, T. (2001) Identification and characterization of mammalian mitochondrial tRNA nucleotidyltransferases. J. Biol. Chem. **276**, 40041–40049
26 Duborjal, H., Beugnot, R., Mousson de Camaret, B. and Issartel, J. P. (2002) Large functional range of steady-state levels of nuclear and mitochondrial transcripts coding for the subunits of the human mitochondrial OXPHOS system. Genome Res. **12**, 1901–1909
27 Mercer, T. R., Neph, S., Dinger, M. E., Crawford, J., Smith, M. A., Shearwood, A. M., Haugen, E., Bracken, C. P., Rackham, O., Stamatoyannopoulos, J. A. et al. (2011) The human mitochondrial transcriptome. Cell **146**, 645–658
28 Slomovic, S. and Schuster, G. (2011) Exonucleases and endonucleases involved in polyadenylation-assisted RNA decay. Wiley Interdiscip. Rev. RNA **2**, 106–123
29 Butow, R. A., Zhu, H., Perlman, P. and Conrad-Webb, H. (1989) The role of a conserved dodecamer sequence in yeast mitochondrial gene expression. Genome **31**, 757–760
30 Aphasizheva, I., Maslov, D., Wang, X., Huang, L. and Aphasizhev, R. (2011) Pentatricopeptide repeat proteins stimulate mRNA adenylation/uridylation to activate mitochondrial translation in trypanosomes. Mol. Cell **42**, 106–117
31 Etheridge, R. D., Aphasizheva, I., Gershon, P. D. and Aphasizhev, R. (2008) 3′ Adenylation determines mRNA abundance and monitors completion of RNA editing in *T. brucei* mitochondria. EMBO J. **27**, 1596–1608
32 Temperley, R. J., Seneca, S. H., Tonska, K., Bartnik, E., Bindoff, L. A., Lightowlers, R. N. and Chrzanowska-Lightowlers, Z. M. (2003) Investigation of a pathogenic mtDNA microdeletion reveals a translation-dependent deadenylation decay pathway in human mitochondria. Hum. Mol. Genet. **12**, 2341–2348
33 Tomecki, R., Dmochowska, A., Gewartowski, K., Dziembowski, A. and Stepien, P. P. (2004) Identification of a novel human nuclear-encoded mitochondrial poly(A) polymerase. Nucleic Acids Res. **32**, 6001–6014
34 Nagaike, T., Suzuki, T., Katoh, T. and Ueda, T. (2005) Human mitochondrial mRNAs are stabilized with polyadenylation regulated by mitochondria-specific poly(A) polymerase and polynucleotide phosphorylase. J. Biol. Chem. **280**, 19721–19727
35 Read, R. L., Martinho, R. G., Wang, S. W., Carr, A. M. and Norbury, C. J. (2002) Cytoplasmic poly(A) polymerases mediate cellular responses to S phase arrest. Proc. Natl. Acad. Sci. U.S.A. **99**, 12079–12084
36 Kwak, J. E., Wang, L., Ballantyne, S., Kimble, J. and Wickens, M. (2004) Mammalian GLD-2 homologs are poly(A) polymerases. Proc. Natl. Acad. Sci. U.S.A. **101**, 4407–4412
37 Saitoh, S., Chabes, A., McDonald, W. H., Thelander, L., Yates, J. R. and Russell, P. (2002) Cid13 is a cytoplasmic poly(A) polymerase that regulates ribonucleotide reductase mRNA. Cell **109**, 563–573
38 Crosby, A. H., Patel, H., Chioza, B. A., Proukakis, C., Gurtz, K., Patton, M. A., Sharifi, R., Harlalka, G., Simpson, M. A., Dick, K. et al. (2010) Defective mitochondrial mRNA maturation is associated with spastic ataxia. Am. J. Hum. Genet. **87**, 655–660
39 Mohanty, B. K. and Kushner, S. R. (2000) Polynucleotide phosphorylase, RNase II and RNase E play different roles in the *in vivo* modulation of polyadenylation in *Escherichia coli*. Mol. Microbiol. **36**, 982–994
40 Piwowarski, J., Grzechnik, P., Dziembowski, A., Dmochowska, A., Minczuk, M. and Stepien, P. P. (2003) Human polynucleotide phosphorylase, hPNPase, is localized in mitochondria. J. Mol. Biol. **329**, 853–857
41 Sarkar, D., Park, E. S., Emdad, L., Randolph, A., Valerie, K. and Fisher, P. B. (2005) Defining the domains of human polynucleotide phosphorylase (hPNPaseOLD-35) mediating cellular senescence. Mol. Cell. Biol. **25**, 7333–7343
42 Portnoy, V., Palnizky, G., Yehudai-Resheff, S., Glaser, F. and Schuster, G. (2008) Analysis of the human polynucleotide phosphorylase (PNPase) reveals differences in RNA binding and response to phosphate compared to its bacterial and chloroplast counterparts. RNA **14**, 297–309
43 Slomovic, S. and Schuster, G. (2008) Stable PNPase RNAi silencing: its effect on the processing and adenylation of human mitochondrial RNA. RNA **14**, 310–323
44 Chen, H. W., Rainey, R. N., Balatoni, C. E., Dawson, D. W., Troke, J. J., Wasiak, S., Hong, J. S., McBride, H. M., Koehler, C. M., Teitell, M. A. and French, S. W. (2006) Mammalian polynucleotide phosphorylase is an intermembrane space RNase that maintains mitochondrial homeostasis. Mol. Cell. Biol. **26**, 8475–8487
45 Wang, G., Shimada, E., Koehler, C. M. and Teitell, M. A. (2011) PNPASE and RNA trafficking into mitochondria. Biochim. Biophys. Acta, doi:10.1016/j.bbagrm.2011.10.001
46 Chrzanowska-Lightowlers, Z. M., Temperley, R. J., Smith, P. M., Seneca, S. H. and Lightowlers, R. N. (2004) Functional polypeptides can be synthesized from human mitochondrial transcripts lacking termination codons. Biochem. J. **377**, 725–731
47 Nagao, A., Hino-Shigi, N. and Suzuki, T. (2008) Measuring mRNA decay in human mitochondria. Methods Enzymol. **447**, 489–99
48 Piechota, J., Tomecki, R., Gewartowski, K., Szczesny, R., Dmochowska, A., Kudla, M., Dybczynska, L., Stepien, P. P. and Bartnik, E. (2006) Differential stability of mitochondrial mRNA in HeLa cells. Acta Biochim. Pol. **53**, 157–168
49 Wydro, M., Bobrowicz, A., Temperley, R. J., Lightowlers, R. N. and Chrzanowska-Lightowlers, Z. M. (2010) Targeting of the cytosolic poly(A) binding protein PABPC1 to mitochondria causes mitochondrial translation inhibition. Nucleic Acids Res. **38**, 3732–3742
50 Rorbach, J., Nicholls, T. J. and Minczuk, M. (2011) PDE12 removes mitochondrial RNA poly(A) tails and controls translation in human mitochondria. Nucleic Acids Res. **39**, 7750–7763
51 Poulsen, J. B., Andersen, K. R., Kjaer, K. H., Durand, F., Faou, P., Vestergaard, A. L., Talbo, G. H., Hoogenraad, N., Brodersen, D. E., Justesen, J. and Martensen, P. M. (2011) Human 2′-phosphodiesterase localizes to the mitochondrial matrix with a putative function in mitochondrial RNA turnover. Nucleic Acids Res. **39**, 3754–3770
52 Temperley, R., Richter, R., Dennerlein, S., Lightowlers, R. N. and Chrzanowska-Lightowlers, Z. M. (2010) Hungry codons promote frameshifting in human mitochondrial ribosomes. Science **327**, 301

53 Chen, W. and Dieckmann, C. L. (1994) Cbp1p is required for message stability following 5′-processing of COB mRNA. J. Biol. Chem. **269**, 16574–16578
54 Ellis, T. P., Helfenbein, K. G., Tzagoloff, A. and Dieckmann, C. L. (2004) Aep3p stabilizes the mitochondrial bicistronic mRNA encoding subunits 6 and 8 of the H^+-translocating ATP synthase of *Saccharomyces cerevisiae*. J. Biol. Chem. **279**, 15728–15733
55 Manthey, G. M. and McEwen, J. E. (1995) The product of the nuclear gene *PET309* is required for translation of mature mRNA and stability or production of intron-containing RNAs derived from the mitochondrial COX1 locus of *Saccharomyces cerevisiae*. EMBO J. **14**, 4031–4043
56 Tullo, A., Tanzariello, F., D'Erchia, A. M., Nardelli, M., Papeo, P. A., Sbisa, E. and Saccone, C. (1994) Transcription of rat mitochondrial NADH-dehydrogenase subunits. Presence of antisense and precursor RNA species. FEBS Lett. **354**, 30–36
57 Lung, B., Zemann, A., Madej, M. J., Schuelke, M., Techritz, S., Ruf, S., Bock, R. and Huttenhofer, A. (2006) Identification of small non-coding RNAs from mitochondria and chloroplasts. Nucleic Acids Res. **34**, 3842–3852
58 Kapp, L. D. and Lorsch, J. R. (2004) The molecular mechanics of eukaryotic translation. Annu. Rev. Biochem. **73**, 657–704
59 Kwast, K. E. and Hand, S. C. (1996) Acute depression of mitochondrial protein synthesis during anoxia: contributions of oxygen sensing, matrix acidification, and redox state. J. Biol. Chem. **271**, 7313–7319
60 Hardewig, I., Anchordoguy, T. J., Crawford, D. L. and Hand, S. C. (1996) Profiles of nuclear and mitochondrial encoded mRNAs in developing and quiescent embryos of *Artemia franciscana*. Mol. Cell. Biochem. **158**, 139–47
61 Eads, B. D. and Hand, S. C. (2003) Mitochondrial mRNA stability and polyadenylation during anoxia-induced quiescence in the brine shrimp *Artemia franciscana*. J. Exp. Biol. **206**, 3681–3692
62 Costanzo, M. C. and Fox, T. D. (1988) Specific translational activation by nuclear gene products occurs in the 5′ untranslated leader of a yeast mitochondrial mRNA. Proc. Natl. Acad. Sci. U.S.A. **85**, 2677–2681
63 Mulero, J. J. and Fox, T. D. (1993) PET111 acts in the 5′-leader of the *Saccharomyces cerevisiae* mitochondrial COX2 mRNA to promote its translation. Genetics **133**, 509–516
64 Rodel, G. and Fox, T. D. (1987) The yeast nuclear gene *CBS1* is required for translation of mitochondrial mRNAs bearing the cob 5′ untranslated leader. Mol. Gen. Genet. **206**, 45–50
65 Chacinska, A. and Boguta, M. (2000) Coupling of mitochondrial translation with the formation of respiratory complexes in yeast mitochondria. Acta Biochim. Pol. **47**, 973–991
66 Weraarpachai, W., Antonicka, H., Sasarman, F., Seeger, J., Schrank, B., Kolesar, J. E., Lochmuller, H., Chevrette, M., Kaufman, B. A., Horvath, R. and Shoubridge, E. A. (2009) Mutation in TACO1, encoding a translational activator of COX I, results in cytochrome *c* oxidase deficiency and late-onset Leigh syndrome. Nat. Genet. **41**, 833–837
67 Schmitz-Linneweber, C. and Small, I. (2008) Pentatricopeptide repeat proteins: a socket set for organelle gene expression. Trends Plant Sci. **13**, 663–670
68 Rackham, O. and Filipovska, A. (2011) The role of mammalian PPR domain proteins in the regulation of mitochondrial gene expression. Biochim. Biophys. Acta, doi: 10.1016/j.bbagrm.2011.10.007
69 Mootha, V. K., Lepage, P., Miller, K., Bunkenborg, J., Reich, M., Hjerrild, M., Delmonte, T., Villeneuve, A., Sladek, R., Xu, F., Mitchell, G. A. et al. (2003) Identification of a gene causing human cytochrome *c* oxidase deficiency by integrative genomics. Proc. Natl. Acad. Sci. U.S.A. **100**, 605–610
70 Xu, F., Morin, C., Mitchell, G., Ackerley, C. and Robinson, B. H. (2004) The role of the LRPPRC (leucine-rich pentatricopeptide repeat cassette) gene in cytochrome oxidase assembly: mutation causes lowered levels of COX (cytochrome *c* oxidase) I and COX III mRNA. Biochem. J. **382**, 331–336
71 Cooper, M. P., Qu, L., Rohas, L. M., Lin, J., Yang, W., Erdjument-Bromage, H., Tempst, P. and Spiegelman, B. M. (2006) Defects in energy homeostasis in Leigh syndrome French Canadian variant through PGC-1α/LRP130 complex. Genes Dev. **20**, 2996–3009
72 Sasarman, F., Brunel-Guitton, C., Antonicka, H., Wai, T. and Shoubridge, E. A. (2010) LRPPRC and SLIRP interact in a ribonucleoprotein complex that regulates posttranscriptional gene expression in mitochondria. Mol. Biol. Cell **21**, 1315–1323
73 Xu, F., Addis, J. B., Cameron, J. M. and Robinson, B. H. (2012) LRPPRC mutation suppresses cytochrome oxidase activity by altering mitochondrial RNA transcript stability in a mouse model. Biochem. J. **441**, 275–283
74 Sondheimer, N., Fang, J. K., Polyak, E., Falk, M. J. and Avadhani, N. G. (2010) Leucine-rich pentatricopeptide-repeat containing protein regulates mitochondrial transcription. Biochemistry **49**, 7467–7473
75 Ruzzenente, B., Metodiev, M. D., Wredenberg, A., Bratic, A., Park, C. B., Camara, Y., Milenkovic, D., Zickermann, V., Wibom, R., Hultenby, K. et al. (2011) LRPPRC is necessary for polyadenylation and coordination of translation of mitochondrial mRNAs. EMBO J. **31**, 443–456
76 Cooper, M. P., Uldry, M., Kajimura, S., Arany, Z. and Spiegelman, B. M. (2008) Modulation of PGC-1 coactivator pathways in brown fat differentiation through LRP130. J. Biol. Chem. **283**, 31960–31967
77 Sterky, F. H., Ruzzenente, B., Gustafsson, C. M., Samuelsson, T. and Larsson, N. G. (2010) LRPPRC is a mitochondrial matrix protein that is conserved in metazoans. Biochem. Biophys. Res. Commun. **398**, 759–764
78 Hatchell, E. C., Colley, S. M., Beveridge, D. J., Epis, M. R., Stuart, L. M., Giles, K. M., Redfern, A. D., Miles, L. E., Barker, A., MacDonald, L. M. et al. (2006) SLIRP, a small SRA binding protein, is a nuclear receptor corepressor. Mol. Cell **22**, 657–668
79 Baughman, J. M., Nilsson, R., Gohil, V. M., Arlow, D. H., Gauhar, Z. and Mootha, V. K. (2009) A computational screen for regulators of oxidative phosphorylation implicates SLIRP in mitochondrial RNA homeostasis. PLoS Genet. **5**, e1000590
80 Decatur, W. A. and Fournier, M. J. (2002) rRNA modifications and ribosome function. Trends Biochem Sci. **27**, 344–351
81 Suzuki, T. and Nagao, A. (2011) Human mitochondrial tRNAs: biogenesis, function, structural aspects, and diseases. Annu. Rev. Genet. **45**, 299–329
82 Watanabe, K. (2010) Unique features of animal mitochondrial translation systems. The non-universal genetic code, unusual features of the translational apparatus and their relevance to human mitochondrial diseases. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. **86**, 11–39
83 Smirnov, A. V., Entelis, N. S., Krasheninnikov, I. A., Martin, R. and Tarassov, I. A. (2008) Specific features of 5S rRNA structure: its interactions with macromolecules and possible functions. Biochemistry **73**, 1418–1437
84 Gelfand, R. and Attardi, G. (1981) Synthesis and turnover of mitochondrial ribonucleic acid in HeLa cells: the mature ribosomal and messenger ribonucleic acid species are metabolically unstable. Mol. Cell. Biol. **1**, 497–511
85 Wilson, D. N. and Nierhaus, K. H. (2007) The weird and wonderful world of bacterial ribosome regulation. Crit. Rev. Biochem. Mol. Biol. **42**, 187–219
86 Venema, J. and Tollervey, D. (1999) Ribosome synthesis in *Saccharomyces cerevisiae*. Annu. Rev. Genet. **33**, 261–311
87 Motorin, Y. and Helm, M. (2011) RNA nucleotide methylation. Wiley Interdiscip. Rev. RNA **2**, 611–631
88 Piekna-Przybylska, D., Decatur, W. A. and Fournier, M. J. (2008) The 3D rRNA modification maps database: with interactive tools for ribosome analysis. Nucleic Acids Res. **36**, D178–D83
89 Sirum-Connolly, K., Peltier, J. M., Crain, P. F., McCloskey, J. A. and Mason, T. L. (1995) Implications of a functional large ribosomal RNA with only three modified nucleotides. Biochimie **77**, 30–39
90 Dennerlein, S., Rozanska, A., Wydro, M., Chrzanowska-Lightowlers, Z. M. and Lightowlers, R. N. (2010) Human ERAL1 is a mitochondrial RNA chaperone involved in the assembly of the 28S small mitochondrial ribosomal subunit. Biochem. J. **430**, 551–558
91 Uchiumi, T., Ohgaki, K., Yagi, M., Aoki, Y., Sakai, A., Matsumoto, S. and Kang, D. (2010) ERAL1 is associated with mitochondrial ribosome and elimination of ERAL1 leads to mitochondrial dysfunction and growth retardation. Nucleic Acids Res. **38**, 5554–5568
92 Davies, S. M., Rackham, O., Shearwood, A. M., Hamilton, K. L., Narsai, R., Whelan, J. and Filipovska, A. (2009) Pentatricopeptide repeat domain protein 3 associates with the mitochondrial small ribosomal subunit and regulates translation. FEBS Lett. **583**, 1853–1858
93 Koc, E. C. and Spremulli, L. L. (2003) RNA-binding proteins of mammalian mitochondria. Mitochondrion **2**, 277–291
94 Haque, M. E., Koc, H., Cimen, H., Koc, E. C. and Spremulli, L. L. (2011) Contacts between mammalian mitochondrial translational initiation factor 3 and ribosomal proteins in the small subunit. Biochim. Biophys. Acta **1814**, 1779–1784
95 Rorbach, J., Gammage, P. A. and Minczuk, M. (2012) C7orf30 is necessary for biogenesis of the large subunit of the mitochondrial ribosome. Nucleic Acids Res., doi:10.1093/nar/gkr1282
96 Rackham, O., Davies, S. M., Shearwood, A. M., Hamilton, K. L., Whelan, J. and Filipovska, A. (2009) Pentatricopeptide repeat domain protein 1 lowers the levels of mitochondrial leucine tRNAs in cells. Nucleic Acids Res. **37**, 5859–5867
97 Lopez Sanchez, M. I., Mercer, T. R., Davies, S. M., Shearwood, A. M., Nygard, K. K., Richman, T. R., Mattick, J. S., Rackham, O. and Filipovska, A. (2011) RNA processing in human mitochondria. Cell Cycle **10**, 2904–2916
98 Dubin, D. T. (1974) Methylated nucleotide content of mitochondrial ribosomal RNA from hamster cells. J. Mol. Biol. **84**, 257–273
99 Dubin, D. T. and Taylor, R. H. (1978) Modification of mitochondrial ribosomal RNA from hamster cells: the presence of GmG and late-methylated UmGmU in the large subunit (17S) RNA. J. Mol. Biol. **121**, 523–540
100 Baer, R. and Dubin, D. T. (1980) The 3′-terminal sequence of the small subunit ribosomal RNA from hamster mitochondria. Nucleic Acids Res. **8**, 4927–4941
101 Baer, R. J. and Dubin, D. T. (1981) Methylated regions of hamster mitochondrial ribosomal RNA: structural and functional correlates. Nucleic Acids Res. **9**, 323–337

102 Seidel-Rogol, B. L., McCulloch, V. and Shadel, G. S. (2003) Human mitochondrial transcription factor B1 methylates ribosomal RNA at a conserved stem-loop. Nat. Genet. **33**, 23–24

103 Falkenberg, M., Gaspari, M., Rantanen, A., Trifunovic, A., Larsson, N. G. and Gustafsson, C. M. (2002) Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. Nat. Genet. **31**, 289–294

104 McCulloch, V., Seidel-Rogol, B. L. and Shadel, G. S. (2002) A human mitochondrial transcription factor is related to RNA adenine methyltransferases and binds *S*-adenosylmethionine. Mol. Cell. Biol. **22**, 1116–1125

105 Cotney, J. and Shadel, G. S. (2006) Evidence for an early gene duplication event in the evolution of the mitochondrial transcription factor B family and maintenance of rRNA methyltransferase activity in human mtTFB1 and mtTFB2. J. Mol. Evol. **63**, 707–717

106 Shutt, T. E. and Shadel, G. S. (2010) A compendium of human mitochondrial gene expression machinery with links to disease. Environ. Mol. Mutagen. **51**, 360–379

107 Metodiev, M. D., Lesko, N., Park, C. B., Camara, Y., Shi, Y., Wibom, R., Hultenby, K., Gustafsson, C. M. and Larsson, N. G. (2009) Methylation of 12S rRNA is necessary for *in vivo* stability of the small subunit of the mammalian mitochondrial ribosome. Cell Metab. **9**, 386–397

108 Matsushima, Y., Adan, C., Garesse, R. and Kaguni, L. S. (2005) *Drosophila* mitochondrial transcription factor B1 modulates mitochondrial translation but not transcription or DNA copy number in Schneider cells. J. Biol. Chem. **280**, 16815–16820

109 Lafontaine, D. L., Preiss, T. and Tollervey, D. (1998) Yeast 18S rRNA dimethylase Dim1p: a quality control mechanism in ribosome synthesis? Mol. Cell. Biol. **18**, 2360–2370

110 Poldermans, B., Bakker, H. and Van Knippenberg, P. H. (1980) Studies on the function of two adjacent N6,N6-dimethyladenosines near the 3′ end of 16S ribosomal RNA of *Escherichia coli*. IV. The effect of the methylgroups on ribosomal subunit interaction. Nucleic Acids Res. **8**, 143–151

111 Xu, Z., O'Farrell, H. C., Rife, J. P. and Culver, G. M. (2008) A conserved rRNA methyltransferase regulates ribosome biogenesis. Nat. Struct. Mol. Biol. **15**, 534–536

112 Demirci, H., Murphy, F. T., Belardinelli, R., Kelley, A. C., Ramakrishnan, V., Gregory, S. T., Dahlberg, A. E. and Jogl, G. (2010) Modification of 16S ribosomal RNA by the KsgA methyltransferase restructures the 30S subunit to optimize ribosome function. RNA **16**, 2319–2324

113 Kimura, S. and Suzuki, T. (2010) Fine-tuning of the ribosomal decoding center by conserved methyl-modifications in the *Escherichia coli* 16S rRNA. Nucleic Acids Res. **38**, 1341–1352

114 Camara, Y., Asin-Cayuela, J., Park, C. B., Metodiev, M. D., Shi, Y., Ruzzenente, B., Kukat, C., Habermann, B., Wibom, R., Hultenby, K. et al. (2011) MTERF4 regulates translation by targeting the methyltransferase NSUN4 to the mammalian mitochondrial ribosome. Cell Metab. **13**, 527–39

115 Andersen, N. M. and Douthwaite, S. (2006) YebU is a m5C methyltransferase specific for 16 S rRNA nucleotide 1407. J. Mol. Biol. **359**, 777–786

116 Desai, P. M., Culver, G. M. and Rife, J. P. (2011) Site-directed mutants of 16S rRNA reveal important RNA domains for KsgA function and 30S subunit assembly. Biochemistry **50**, 854–863

117 Kolanczyk, M., Pech, M., Zemojtel, T., Yamamoto, H., Mikula, I., Calvaruso, M. A., van den Brand, M., Richter, R., Fischer, B., Ritz, A. et al. (2011) NOA1 is an essential GTPase required for mitochondrial protein synthesis. Mol. Biol. Cell **22**, 1–11

118 Uicker, W. C., Schaefer, L. and Britton, R. A. (2006) The essential GTPase RbgA (YlqF) is required for 50S ribosome assembly in *Bacillus subtilis*. Mol. Microbiol. **59**, 528–540

119 Barrientos, A., Korr, D., Barwell, K. J., Sjulsen, C., Gajewski, C. D., Manfredi, G., Ackerman, S. and Tzagoloff, A. (2003) MTG1 codes for a conserved protein required for mitochondrial translation. Mol. Biol. Cell **14**, 2292–2302

120 Nolden, M., Ehses, S., Koppen, M., Bernacchia, A., Rugarli, E. I. and Langer, T. (2005) The m-AAA protease defective in hereditary spastic paraplegia controls ribosome assembly in mitochondria. Cell **123**, 277–289

121 Wanschers, B.F.J., Szklarczyk, R., Pajak, A., van den Brand, M.A.M., Gloerich, J., Rodenburg, R.J.T., Lightowlers, R. N., Nijtmans., L. G. and Huynen, M. A. (2012) C7orf30 specifically associates with the large subunit of the mitochondrial ribosome and is involved in translation. Nucleic Acids Res., doi: 10.1093/nar/gkr1271

122 Sirum-Connolly, K. and Mason, T. L. (1993) Functional requirement of a site-specific ribose methylation in ribosomal RNA. Science **262**, 1886–1889

123 Lovgren, J. M. and Wikstrom, P. M. (2001) The *rlmB* gene is essential for formation of Gm2251 in 23S rRNA but not for ribosome maturation in *Escherichia coli*. J. Bacteriol. **183**, 6957–6960

124 Pintard, L., Bujnicki, J. M., Lapeyre, B. and Bonnerot, C. (2002) *MRM2* encodes a novel yeast mitochondrial 21S rRNA methyltransferase. EMBO J. **21**, 1139–1147

125 Caldas, T., Binet, E., Bouloc, P. and Richarme, G. (2000) Translational defects of *Escherichia coli* mutants deficient in the Um2552 23S ribosomal RNA methyltransferase RrmJ/FTSJ. Biochem. Biophys. Res. Commun. **271**, 714–718

126 Caldas, T., Binet, E., Bouloc, P., Costa, A., Desgres, J. and Richarme, G. (2000) The FtsJ/RrmJ heat shock protein of *Escherichia coli* is a 23 S ribosomal RNA methyltransferase. J. Biol. Chem. **275**, 16414–16419

127 Widerak, M., Kern, R., Malki, A. and Richarme, G. (2005) U2552 methylation at the ribosomal A-site is a negative modulator of translational accuracy. Gene **347**, 109–114

128 Kim, D. F. and Green, R. (1999) Base-pairing between 23S rRNA and tRNA in the ribosomal A site. Mol. Cell **4**, 859–864

129 Lapeyre, B. and Purushothaman, S. K. (2004) Spb1p-directed formation of Gm2922 in the ribosome catalytic center occurs at a late processing stage. Mol. Cell **16**, 663–669

130 Ofengand, J. and Bakin, A. (1997) Mapping to nucleotide resolution of pseudouridine residues in large subunit ribosomal RNAs from representative eukaryotes, prokaryotes, archaebacteria, mitochondria and chloroplasts. J. Mol. Biol. **266**, 246–268

131 Ansmant, I., Massenet, S., Grosjean, H., Motorin, Y. and Branlant, C. (2000) Identification of the *Saccharomyces cerevisiae* RNA:pseudouridine synthase responsible for formation of ψ2819 in 21S mitochondrial ribosomal RNA. Nucleic Acids Res. **28**, 1941–1946

132 Lykke-Andersen, S., Tomecki, R., Jensen, T. H. and Dziembowski, A. (2011) The eukaryotic RNA exosome: same scaffold but variable catalytic subunits. RNA Biol. **8**, 61–66

133 Carpousis, A. J. (2002) The *Escherichia coli* RNA degradosome: structure, function and relationship in other ribonucleolytic multienzyme complexes. Biochem. Soc. Trans. **30**, 150–155

134 Dmochowska, A., Golik, P. and Stepien, P. P. (1995) The novel nuclear gene *DSS-1* of *Saccharomyces cerevisiae* is necessary for mitochondrial biogenesis. Curr. Genet. **28**, 108–112

135 Dziembowski, A., Piwowarski, J., Hoser, R., Minczuk, M., Dmochowska, A., Siep, M., van der Spek, H., Grivell, L. and Stepien, P. P. (2003) The yeast mitochondrial degradosome. Its composition, interplay between RNA helicase and RNase activities and the role in mitochondrial RNA metabolism. J. Biol. Chem. **278**, 1603–1611

136 Malecki, M., Jedrzejczak, R., Stepien, P. P. and Golik, P. (2007) *In vitro* reconstitution and characterization of the yeast mitochondrial degradosome complex unravels tight functional interdependence. J. Mol. Biol. **372**, 23–36

137 Margossian, S. P., Li, H., Zassenhaus, H. P. and Butow, R. A. (1996) The DExH box protein Suv3p is a component of a yeast mitochondrial 3′-to-5′ exoribonuclease that suppresses group I intron toxicity. Cell **84**, 199–209

138 Dziembowski, A., Malewicz, M., Minczuk, M., Golik, P., Dmochowska, A. and Stepien, P. P. (1998) The yeast nuclear gene *DSS1*, which codes for a putative RNase II, is necessary for the function of the mitochondrial degradosome in processing and turnover of RNA. Mol. Gen. Genet. **260**, 108–114

139 Dmochowska, A., Kalita, K., Krawczyk, M., Golik, P., Mroczek, K., Lazowska, J., Stepien, P. P. and Bartnik, E. (1999) A human putative Suv3-like RNA helicase is conserved between *Rhodobacter* and all eukaryotes. Acta Biochim. Pol. **46**, 155–162

140 Minczuk, M., Piwowarski, J., Papworth, M. A., Awiszus, K., Schalinski, S., Dziembowski, A., Dmochowska, A., Bartnik, E., Tokatlidis, K., Stepien, P. P. and Borowski, P. (2002) Localisation of the human hSuv3p helicase in the mitochondrial matrix and its preferential unwinding of dsDNA. Nucleic Acids Res. **30**, 5074–5086

141 Wang, Y. and Bogenhagen, D. F. (2006) Human mitochondrial DNA nucleoids are linked to protein folding machinery and metabolic enzymes at the mitochondrial inner membrane. J. Biol. Chem. **281**, 25791–25802

142 Shu, Z., Vijayakumar, S., Chen, C. F., Chen, P. L. and Lee, W. H. (2004) Purified human SUV3p exhibits multiple-substrate unwinding activity upon conformational change. Biochemistry **43**, 4781–4790

143 Khidr, L., Wu, G., Davila, A., Procaccio, V., Wallace, D. and Lee, W. H. (2008) Role of SUV3 helicase in maintaining mitochondrial homeostasis in human cells. J. Biol. Chem. **283**, 27064–27073

144 Szczesny, R. J., Borowski, L. S., Brzezniak, L. K., Dmochowska, A., Gewartowski, K., Bartnik, E. and Stepien, P. P. (2010) Human mitochondrial RNA turnover caught in flagranti: involvement of hSuv3p helicase in RNA surveillance. Nucleic Acids Res. **38**, 279–298

145 Guo, X. E., Chen, C. F., Wang, D. D., Modrek, A. S., Phan, V. H., Lee, W. H. and Chen, P. L. (2011) Uncoupling the roles of the SUV3 helicase in maintenance of mitochondrial genome stability and RNA degradation. J. Biol. Chem. **286**, 38783–38794

146 Pereira, M., Mason, P., Szczesny, R. J., Maddukuri, L., Dziwura, S., Jedrzejczak, R., Paul, E., Wojcik, A., Dybczynska, L., Tudek, B. et al. (2007) Interaction of human SUV3 RNA/DNA helicase with BLM helicase; loss of the *SUV3* gene results in mouse embryonic lethality. Mech. Ageing Dev. **128**, 609–617

147 Paul, E., Cronan, R., Weston, P. J., Boekelheide, K., Sedivy, J. M., Lee, S. Y., Wiest, D. L., Resnick, M. B. and Klysik, J. E. (2009) Disruption of Supv3L1 damages the skin and causes sarcopenia, loss of fat, and death. Mamm. Genome **20**, 92–108

148 Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J. N., Rovio, A. T., Bruder, C. E., Bohlooly, Y. M., Gidlof, S., Oldfors, A., Wibom, R. et al. (2004) Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature **429**, 417–423

149 Kujoth, G. C., Hiona, A., Pugh, T. D., Someya, S., Panzer, K., Wohlgemuth, S. E., Hofer, T., Seo, A. Y., Sullivan, R., Jobling, W. A. et al. (2005) Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science **309**, 481–484

150 Szczesny, R. J., Obriot, H., Paczkowska, A., Jedrzejczak, R., Dmochowska, A., Bartnik, E., Formstecher, P., Polakowska, R. and Stepien, P. P. (2007) Down-regulation of human RNA/DNA helicase SUV3 induces apoptosis by a caspase- and AIF-dependent pathway. Biol. Cell **99**, 323–332

151 Veno, S. T., Kulikowicz, T., Pestana, C., Stepien, P. P., Stevnsner, T. and Bohr, V. A. (2011) The human Suv3 helicase interacts with replication protein A and flap endonuclease 1 in the nucleus. Biochem. J. **440**, 293–300

152 Minczuk, M., Mroczek, S., Pawlak, S. D. and Stepien, P. P. (2005) Human ATP-dependent RNA/DNA helicase hSuv3p interacts with the cofactor of survivin HBXIP. FEBS J. **272**, 5008–5019

153 Amblar, M., Barbas, A., Fialho, A. M. and Arraiano, C. M. (2006) Characterization of the functional domains of *Escherichia coli* RNase II. J. Mol. Biol. **360**, 921–933

154 Mattiacio, J. L. and Read, L. K. (2009) Evidence for a degradosome-like complex in the mitochondria of T*rypanosoma brucei*. FEBS Lett. **583**, 2333–2338

155 Hoffmann, B., Nickel, J., Speer, F. and Schafer, B. (2008) The 3′ ends of mature transcripts are generated by a processosome complex in fission yeast mitochondria. J. Mol. Biol. **377**, 1024–1037

156 Lange, H., Sement, F. M., Canaday, J. and Gagliardi, D. (2009) Polyadenylation-assisted RNA degradation processes in plants. Trends Plant Sci. **14**, 497–504

157 Carpousis, A. J. (2007) The RNA degradosome of *Escherichia coli*: an mRNA-degrading machine assembled on RNase E. Annu. Rev. Microbiol. **61**, 71–87

158 Wang, D. D., Shu, Z., Lieser, S. A., Chen, P. L. and Lee, W. H. (2009) Human mitochondrial SUV3 and polynucleotide phosphorylase form a 330-kDa heteropentamer to cooperatively degrade double-stranded RNA with a 3′-to-5′ directionality. J. Biol. Chem. **284**, 20812–20821

159 Borowski, L. S., Szczesny, R. J., Brzezniak, L. K. and Stepien, P. P. (2010) RNA turnover in human mitochondria: more questions than answers? Biochim. Biophys. Acta **1797**, 1066–1070

160 Brown, T. A., Tkachuk, A. N. and Clayton, D. A. (2008) Native R-loops persist throughout the mouse mitochondrial DNA genome. J. Biol. Chem. **283**, 36743–36751

161 Deutscher, M. P. (1993) Promiscuous exoribonucleases of *Escherichia coli*. J. Bacteriol. **175**, 4577–4583

162 Hanekamp, T. and Thorsness, P. E. (1999) YNT20, a bypass suppressor of yme1 yme2, encodes a putative 3′→5′ exonuclease localized in mitochondria of *Saccharomyces cerevisiae*. Curr. Genet. **34**, 438–448

163 Ghosh, S. and Deutscher, M. P. (1999) Oligoribonuclease is an essential component of the mRNA decay pathway. Proc. Natl. Acad. Sci. U.S.A. **96**, 4372–4377

164 Morrissey, J. P. and Tollervey, D. (1995) Birth of the snoRNPs: the evolution of RNase MRP and the eukaryotic pre-rRNA-processing system. Trends Biochem. Sci. **20**, 78–82

165 Nguyen, L. H., Erzberger, J. P., Root, J. and Wilson, III, D. M. (2000) The human homolog of *Escherichia coli* Orn degrades small single-stranded RNA and DNA oligomers. J. Biol. Chem. **275**, 25900–25906

166 Dake, E., Hofmann, T. J., McIntire, S., Hudson, A. and Zassenhaus, H. P. (1988) Purification and properties of the major nuclease from mitochondria of *Saccharomyces cerevisiae*. J. Biol. Chem. **263**, 7691–702

167 Zassenhaus, H. P. and Denniger, G. (1994) Analysis of the role of the NUC1 endo/exonuclease in yeast mitochondrial DNA recombination. Curr. Genet. **25**, 142–149

168 Buttner, S., Eisenberg, T., Carmona-Gutierrez, D., Ruli, D., Knauer, H., Ruckenstuhl, C., Sigrist, C., Wissing, S., Kollroser, M., Frohlich, K. U. et al. (2007) Endonuclease G regulates budding yeast life and death. Mol. Cell **25**, 233–246

169 Cymerman, I. A., Chung, I., Beckmann, B. M., Bujnicki, J. M. and Meiss, G. (2008) EXOG, a novel paralog of endonuclease G in higher eukaryotes. Nucleic Acids Res. **36**, 1369–1379

170 Li, L. Y., Luo, X. and Wang, X. (2001) Endonuclease G is an apoptotic DNase when released from mitochondria. Nature **412**, 95–99

171 Tann, A. W., Boldogh, I., Meiss, G., Qian, W., Van Houten, B., Mitra, S. and Szczesny, B. (2011) Apoptosis induced by persistent single-strand breaks in mitochondrial genome: critical role of EXOG (5′-EXO/endonuclease) in their repair. J. Biol. Chem. **286**, 31975–31983

172 Liang, S. L., Quirk, D. and Zhou, A. (2006) RNase L: its biological roles and regulation. IUBMB Life **58**, 508–514

173 Le Roy, F., Bisbal, C., Silhol, M., Martinand, C., Lebleu, B. and Salehzada, T. (2001) The 2-5A/RNase L/RNase L inhibitor (RLI) [correction of (RNI)] pathway regulates mitochondrial mRNAs stability in interferon α-treated H9 cells. J. Biol. Chem. **276**, 48473–48482

174 Chandrasekaran, K., Mehrabian, Z., Li, X. L. and Hassel, B. (2004) RNase-L regulates the stability of mitochondrial DNA-encoded mRNAs in mouse embryo fibroblasts. Biochem. Biophys. Res. Commun. **325**, 18–23

175 Le Roy, F., Silhol, M., Salehzada, T. and Bisbal, C. (2007) Regulation of mitochondrial mRNA stability by RNase L is translation-dependent and controls IFNα-induced apoptosis. Cell Death Differ. **14**, 1406–1413

176 Tomecki, R., Kristiansen, M. S., Lykke-Andersen, S., Chlebowski, A., Larsen, K. M., Szczesny, R. J., Drazkowska, K., Pastula, A., Andersen, J. S., Stepien, P. P. et al. (2010) The human core exosome interacts with differentially localized processive RNases: hDIS3 and hDIS3L. EMBO J. **29**, 2342–2357

177 Staals, R. H., Bronkhorst, A. W., Schilders, G., Slomovic, S., Schuster, G., Heck, A. J., Raijmakers, R. and Pruijn, G. J. (2010) Dis3-like 1: a novel exoribonuclease associated with the human exosome. EMBO J. **29**, 2358–2367

178 Kennedy, S., Wang, D. and Ruvkun, G. (2004) A conserved siRNA-degrading RNase negatively regulates RNA interference in *C. elegans*. Nature **427**, 645–649

179 Gabel, H. W. and Ruvkun, G. (2008) The exonuclease ERI-1 has a conserved dual role in 5.8S rRNA processing and RNAi. Nat. Struct. Mol. Biol. **15**, 531–533

180 Yang, X. C., Purdy, M., Marzluff, W. F. and Dominski, Z. (2006) Characterization of 3′hExo, a 3′ exonuclease specifically interacting with the 3′ end of histone mRNA. J. Biol. Chem. **281**, 30447–30454

181 Kim, J. M., Yoshikawa, H. and Shirahige, K. (2001) A member of the YER057c/yjgf/Uk114 family links isoleucine biosynthesis and intact mitochondria maintenance in *Saccharomyces cerevisiae*. Genes Cells **6**, 507–517

182 Lambrecht, J. A., Browne, B. A. and Downs, D. M. (2010) Members of the YjgF/YER057c/UK114 family of proteins inhibit phosphoribosylamine synthesis *in vitro*. J. Biol. Chem. **285**, 34401–34407

183 Oka, T., Tsuji, H., Noda, C., Sakai, K., Hong, Y. M., Suzuki, I., Munoz, S. and Natori, Y. (1995) Isolation and characterization of a novel perchloric acid-soluble protein inhibiting cell-free protein synthesis. J. Biol. Chem. **270**, 30060–30067

184 Morishita, R., Kawagoshi, A., Sawasaki, T., Madin, K., Ogasawara, T., Oka, T. and Endo, Y. (1999) Ribonuclease activity of rat liver perchloric acid-soluble protein, a potent inhibitor of protein synthesis. J. Biol. Chem. **274**, 20688–20692

185 Pagliarini, D. J., Calvo, S. E., Chang, B., Sheth, S. A., Vafai, S. B., Ong, S. E., Walford, G. A., Sugiana, C., Boneh, A., Chen, W. K. et al. (2008) A mitochondrial protein compendium elucidates complex I disease biology. Cell **134**, 112–123

186 Oxelmark, E., Marchini, A., Malanchi, I., Magherini, F., Jaquet, L., Hajibagheri, M. A., Blight, K. J., Jauniaux, J. C. and Tommasino, M. (2000) Mmf1p, a novel yeast mitochondrial protein conserved throughout evolution and involved in maintenance of the mitochondrial genome. Mol. Cell. Biol. **20**, 7784–7797

187 Accardi, R., Oxelmark, E., Jauniaux, N., de Pinto, V., Marchini, A. and Tommasino, M. (2004) High levels of the mitochondrial large ribosomal subunit protein 40 prevent loss of mitochondrial DNA in null mmf1 *Saccharomyces cerevisiae* cells. Yeast **21**, 539–548

188 Forner, F., Foster, L. J., Campanaro, S., Valle, G. and Mann, M. (2006) Quantitative proteomic comparison of rat mitochondria from muscle, heart, and liver. Mol. Cell. Proteomics **5**, 608–619

189 Taylor, S. W., Fahy, E., Zhang, B., Glenn, G. M., Warnock, D. E., Wiley, S., Murphy, A. N., Gaucher, S. P., Capaldi, R. A., Gibson, B. W. and Ghosh, S. S. (2003) Characterization of the human heart mitochondrial proteome. Nat. Biotechnol. **21**, 281–286

190 Calvo, S., Jain, M., Xie, X., Sheth, S. A., Chang, B., Goldberger, O. A., Spinazzola, A., Zeviani, M., Carr, S. A. and Mootha, V. K. (2006) Systematic identification of human mitochondrial disease genes through integrative genomics. Nat. Genet. **38**, 576–582

191 Rackham, O., Shearwood, A. M., Mercer, T. R., Davies, S. M., Mattick, J. S. and Filipovska, A. (2011) Long noncoding RNAs are generated from the mitochondrial genome and regulated by nuclear-encoded proteins. RNA **17**, 2085–2093

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