Biochemical Society Transactions (2016) **44** 1475–1482
DOI: 10.1042/BST20160126

Polyadenylation and degradation of RNA in the mitochondria

Shiri Levy* and Gadi Schuster

Faculty of Biology, Technion — Israel Institute of Technology, Haifa 32000, Israel

Correspondence: Gadi Schuster (gadis@tx.technion.ac.il)

Mitochondria have their own gene expression machinery and the relative abundance of RNA products in these organelles in animals is mostly dictated by their rate of degradation. The molecular mechanisms regulating the differential accumulation of the transcripts in this organelle remain largely elusive. Here, we summarize the present knowledge of how RNA is degraded in human mitochondria and describe the coexistence of stable poly(A) tails and the nonabundant tails, which have been suggested to play a role in the RNA degradation process.

Gene expression in human mitochondria

Mitochondria are critical for many metabolic pathways, including in the production of ATP via oxidative phosphorylation. The organelle is an evolutionary remnant of an endosymbiotic event that occurred between an α-proteobacterium and an ancient host cell 1.5 billion years ago, after which, most of the bacterial genes were transferred to the nuclear genome of the ancient host [1,2]. The mammalian mitochondrial genome preserved a total of 37 genes, encoding 2 ribosomal RNAs, 22 tRNA, and 13 proteins, all of which are oxidative phosphorylation component subunits, essential in several critical metabolic pathways and in maintaining cell viability [3,4]. Mitochondrial RNAs are transcribed from the mitochondrial DNA, as polycistronic molecules, in a process in which the mRNAs and rRNAs are mostly punctuated by tRNAs [3–5]. Endonucleolytic cleavage of tRNAs, at both 5′- and 3′-ends, is driven by RNase P and RNase Z, respectively, producing, in addition to processed tRNAs, the rRNA and mRNA transcripts (Figure 1) [5–9]. The released mRNA species are then decorated with stable poly(A) tails and translated by mitochondrial ribosomes. Owing to mitochondrial genome reduction that evolved with time, 7 of the 13 mRNA molecules contain truncated translational stop codons, composed of only U or UA instead of UAA; therefore, posttranscriptional addition of a stable poly(A) tail at the 3′-end of the molecule is required for the production of a functional stop codon [10] (Figure 1). Additional functions of the stable poly(A) tails were also proposed [11–14]. Aside from the addition of a stable poly(A) tail at the 3′-end, the addition of transient and unstable poly(A) tails at the 3′-end of truncated transcripts has also been observed [15]. These tails may indicate the polyadenylation-assisted degradation pathway of RNA described in bacteria, archaea, organelles, as well as in the nucleus and cytosol [10,16–18]. Although produced from only a few polycistronic transcripts, the rRNA, tRNA, and mRNA transcripts accumulate in the mitochondria to varying concentrations, indicating the importance of a modulated and well-controlled RNA degradation mechanism [19]. The presence of RNA granules, associated with RNA-binding proteins and enzymes that are functionally linked to mitochondrial transcript processing and degradation, has been recently described [20–23].

Mitochondrial ribonucleases

To better understand defects in mitochondrial RNA turnover and consequential mitochondrial disorders, extensive investigations to identify the ribonucleases responsible for mitochondrial transcript processing and degradation are underway [3,4]. The mitochondrial RNase P and RNase Z (ELAC2), which process the tRNAs, have been characterized [6–8]. Owing to the established role of polynucleotide phosphorylase

*Present address: Department of Biochemistry, University of Washington, Seattle, WA, USA.

Received: 25 May 2016
Revised: 24 June 2016
Accepted: 28 June 2016

Version of Record published: 19 October 2016

Biochemical Society Transactions (2016) **44** 1475–1482
DOI: 10.1042/BST20160126

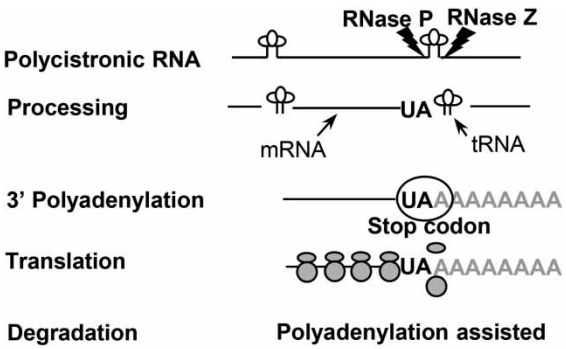

Figure 1. RNA processing in animal mitochondria.

The genome is transcribed into polycistronic RNAs, and mRNAs are punctuated by tRNAs. The tRNAs are then cleaved at the 5′- and 3′-ends by the endoribonucleases RNase P and RNase Z, respectively. Several mRNAs contain incomplete translational stop codons composed of only U or UA instead of UAA. The addition of a stable poly(A) tail at the 3′-end by mtPAP creates a complete stop codon. The mRNA is then translated, and eventually degraded by the polyadenylation-assisted degradation pathway.

(PNPase) in 3′–5′ exoribonuclease activity on RNA and polyadenylation in prokaryotes, chloroplasts, and plant mitochondria [22,24–26], its human counterpart has been designated as the most suitable candidate to execute identical activities in human mitochondria. However, this assumption was questioned when PNPase was found to be primarily located in the mitochondrial intermembrane space of human cells [27]. A recent work has shown that a significant amount of PNPase complexes with the hSuv3p helicase to form the mitochondrial exosome (also known as the mitochondrial degradosome), in RNA granules [22], and degrades 'mirror' RNAs that are complementary to mitochondrial genes within defined regions, termed D-foci. These mitochondrial defined regions could be the same RNA granules described before for RNA-binding proteins [21]. An additional RNA exonuclease, termed REXO2, is located both in the mitochondrial intermembrane space and in the matrix, and has been proposed to degrade oligoribonucleotides, byproducts of the activities of PNPase and other ribonucleases [28]. PDE12, a mitochondrial 2′- and 3′-phosphodiesterase, has been shown to remove stable poly(A) tails [29,30]. Endonuclease G is a powerful, nonspecific DNA/RNA endonuclease that is located in the intermembrane space of the mitochondria and functions during apoptosis [31,32]. Several RNA-binding proteins that are essential for the correct processing and stability of mitochondrial transcripts, but that do not possess ribonucleolytic activity, have also been described [6,12,20,21,23,33,34].

In a search for ribonucleases that are responsible for RNA degradation in human mitochondria, an uncharacterized protein from the metallo-β-lactamase (MBL) superfamily was identified. This previously unannotated, soluble, monomeric, human mitochondrial matrix protein, LACTB2, has been recently shown to display endoribonucleolytic activity [35]. Unlike other mitochondrial proteins, such as GRSF1 [23] and RNase P [6] or PNPase and hSuv3 [22], that form protein complexes, LACTB2 is not engaged in a higher-order complex of proteins. Recombinant and purified LACTB2 cleaves single-stranded RNA molecules and knocking down its expression by RNAi results in a modest elevation of the levels of several mitochondrial mRNAs, in mitochondrial dysfunction, and in rapid cell death [35]. However, its specific biological function remains to be discovered. Interestingly, RNase J, another MBL protein, is a pivotal ribonuclease in the RNA degradation process in bacteria, archaea, and the chloroplast [36]. Accordingly, LACTB2 could be a remnant of the evolutionary prokaryotic ancestor of the mitochondria, which has remained intimately involved in RNA degradation.

Degradation and polyadenylation of RNA in different mitochondria

The mechanism of mitochondrial RNA degradation varies between organisms. Despite the addition of a stable poly(A) tail at the 3′-end of the RNA molecules in trypanosomes and mammals [10] (Table 1), and evolutionary adaptions in the enzymatic players driving RNA degradation in different organisms, in general, mitochondria have

preserved the prokaryotic RNA decay system [37–40]. Organisms representing the different forms of polyadenylation in mitochondria are plants, yeast, trypanosomes, and mammals [10] (Table 1). RNA in plant mitochondria is polyadenylated with unstable poly(A) tails, synthesized by the activity of a poorly characterized poly(A) polymerase (PAP). This PAP is responsible for the catalysis of homopolymeric extensions at the 3′-ends of truncated molecules (perhaps cleavage products), as well as the 3′-end [41,42]. The two plant proteins responsible for 3′–5′ exoribonucleolytic activity are RNase II/R [43], and PNPase, which is also known for its dual phosphorylase activity, as a polymerase and as a ribonuclease, in bacteria and organelles [15,25]. A poly(A)-specific ribonuclease has also been characterized in plant mitochondria [42]. However, to date, no endonuclease has been linked to the initial endonucleolytic cleavage, which is believed to initiate the degradation pathway in plant mitochondria [10].

In yeast mitochondria, where no polyadenylation and no *PNP* genes are found, a protein complex, defined as the yeast mtExo or the yeast mitochondrial degradosome, consisting of an RNase R exoribonuclease and an RNA helicase, is responsible for RNA degradation [44,45]. Mitochondrial/chloroplast RNase R exonucleases are inhibited by a secondary stem-loop structure at the 3′-end of the RNA molecules and, therefore, require endonucleolytic cleavage and polyadenylation, or the recruitment of an RNA helicase, before they can act on the RNA. In yeast mitochondria, the RNase R homolog, Dss1, digests RNA secondary structures in complex with the helicase SUV3 [22,38,44].

The trypanosomes constitute another organismal group that express RNase R exoribonucleases. Trypanosomal mitochondrial transcripts, like mammals, contain both stable and unstable tails, composed of both short oligo(A) and long poly(A) sequences that in this group is dependent on the editing stage of the particular transcript [46,47]. In at least one case, the long tail is composed of a significant number of uridines, and is therefore termed an A/U extension. In addition, a mitochondrial PAP (mtPAP) was characterized and found to be essential for the parasite's viability [46,48].

Since human metabolism of mitochondrial RNA resembles, in some aspects, that of its prokaryotic ancestor, it was questioned whether in the background of the stable poly(A) tails, unstable poly(A) tails are present and play a role in the polyadenylation-assisted degradation pathway. Slomovic et al. [15] discovered polyadenylation on the immature, truncated ends of mitochondrial mRNA, rRNA, and tRNA transcripts in human cells, most likely a remnant of the poly(A)-assisted RNA degradation pathway in prokaryotes/organelles [10].

As described above, genes encoding both mtPAP and PNPase exist in the mammalian genome, and their products are targeted to the mitochondria. To date, PNPase polymerization activity in prokaryotes and the chloroplast has been associated with the formation of heteropolymeric tails only, thus suggesting that the homogeneic poly(A) tails, characterized thus far in human mitochondria, are formed by mtPAP, and not by PNPase [14,49,50]. Knocking down the expression of both mtPAP and PNPase resulted in shortening of the stable poly(A) tails of ND3 and ATP6/8 from about 50 nt to 8 nt, suggesting that there is an additional, yet uncharacterized PAP in the mitochondria [12,14,26,51]. However, a recent mtPAP knockout study in *Drosophila*, demonstrating the almost complete depletion of oligo(A) tails, suggested that at least in this organism, a single mtPAP is responsible for the polyadenylation in the mitochondria [52].

The coexistence of stable and unstable poly(A) tails was also observed in the cytosol and nuclei [15,53–55], and has been shown to play a crucial role in determining RNA fate [10]. This dual function of polyadenylation in marking mtRNA for exonucleolytic degradation or translation resembles the role of the stable versus

Table 1 Characteristics of mitochondrial polyadenylation and degradation in different organisms

No mtPAP or PNPase is found in yeast mitochondria and therefore no poly(A)-tail is created. However, the RNase R exoribonucleases are found in complex with RNA helicase. Plants and mammals express PNPase and at least one mtPAP. Tails produced by mtPAP are homopolymeric, with the exception of poly(A/U) extensions found in Trypanosoma brucei. Tails are both stabilizing and destabilizing in trypanosomes and mammals, but only destabilizing in plants, where the 3′-ends of the transcripts are not decorated with a stable poly(A) tail.

Organism	Polymerizing enzyme of the tails	Poly(A) tails	Stability of poly(A) tails	Degradation enzymes
Plants	PAP (uncharacterized)	Homopolymeric	Unstable	PNPase and RNR
Yeast	–	No tails	–	RNase R/helicase
Trypanosomes	mtPAP	Poly(A/U)	Unstable + Stable	RNase R
Human	mtPAP	Homopolymeric	Unstable + Stable	(PNPase/LACTB2?/Dec12?)

Biochemical Society Transactions (2016) **44** 1475–1482
DOI: 10.1042/BST20160126

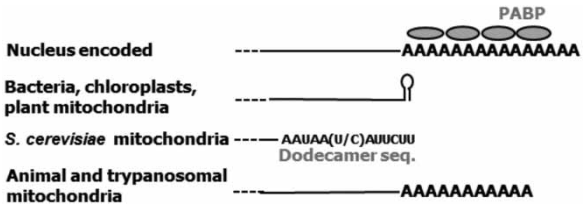

Figure 2. The 3′-ends of mature mRNAs in various systems.

The details of each are discussed in the text. 'Dodecamer seq.' is an encoded tag found at the end of yeast mitochondrial transcripts.

transient poly(A) tails in marking the mature versus degradation products of nuclear mRNA. It is believed that the poly(A)-binding protein binds the stable poly(A)-tail of nuclear and cytosolic mRNAs, differentiating it from the transient and nonabundant tails of the degradation products (Figure 2) [56]. However, as of yet, no such protein has been identified in the mitochondria. Moreover, introducing the cytosolic poly(A)-binding protein, PABPC1, into the mitochondria resulted with translation inhibition [57].

The poly(A)-assisted RNA degradation pathway

Poly(A)-assisted RNA degradation was initially described in *Escherichia coli* and its molecular mechanism has been extensively studied in this bacterium. Later, similar pathways were discovered in other systems, such as other bacteria, chloroplasts, plants, trypanosomes, human mitochondria, certain archaea, and the nucleus and cytosol of yeast and human cells [58–60]. The poly(A)-assisted degradation pathways are generally similar across species, with slight variations between organisms. The 'classic' sequence of events commences (in prokaryotes and organelles) with the endonucleolytic cleavage of the transcript, followed by the addition of poly (A) or poly(A)-rich 3′-extension, and then with exonucleolytic 3′–5′ degradation of the tagged RNA fragment [61,62] (Figure 3). Transient poly(A) tails can be homopolymeric (composed of adenosine residues only) or heteropolymeric/poly(A)-rich (mostly, but not exclusively, adenosines) [50]. In *E. coli*, the endonuclease RNase E is believed to carry out the initial transcript cleavage, while polyadenylation is primarily executed by a nucleotidyl transferase-type PAP, which produces homopolymeric poly(A) tails. PNPase has also been implicated in the latter activity, but to a lesser extent, and produces heteropolymeric extensions [63]. The role of exonucleolytic degradation is shared by PNPase, RNase II, and RNase R [61,62,64,65].

The existence of the poly(A)-assisted degradation pathway was first discovered upon the detection of nonabundant, truncated, adenylated RNA fragments. The adenylated degradation intermediates serve as a tell-tale sign of the presence of poly(A)-assisted RNA decay, where the tail nucleotide composition provides an initial hint as to the identity of the polymerizing enzyme [50]. As described above, mRNAs of animal mitochondria are decorated with a stable poly(A) tail at the 3′-end, resembling those of nucleus-encoded mRNAs (Figure 2). In addition, low-abundance poly(A) tails have been identified on the 3′-ends of truncated transcripts of all types of mitochondrial transcripts, as well as on transcripts derived from the intergenic regions in this organelle [15]. These intermediate transcripts may serve as a marker for the presence of the poly(A)-assisted degradation pathway. However, obtaining the proof that this is indeed the case in human mitochondria, which is to show that under inhibition of the polyadenylation, or the various steps of the exonucleolytic degradation, the polyadenylated intermediates accumulate, was not yet achieved in this system. Therefore, at present, other suggestions like that the truncated low-abundance poly(A) tails and polyadenylated transcripts represent 'leakage' products of the polyadenylation pathway, or the removal of early terminated truncated transcription products, cannot be ruled out. Nevertheless, in all systems analyzed thus far, inhibition of exonucleolytic degradation, or of various steps in the polyadenylation process, resulted in the accumulation of the degradation products [55,59,60,66,67].

Assuming that the poly(A)-assisted degradation pathway is the mechanism of RNA degradation in human mitochondria, the mechanisms regulating mitochondrial transcript levels remain to be identified. The mitochondrial transcripts are processed from only a few initial, long primary transcripts, and then accumulate to different levels, which are largely determined by the modulation of their stability. Their stability is therefore primarily determined by the specific degradation of each transcript. The rate-limiting step of the degradation pathway is believed to be the initial endonucleolytic cleavage. In *E. coli*, this step is triggered by the removal of

Biochemical Society Transactions (2016) **44** 1475–1482
DOI: 10.1042/BST20160126

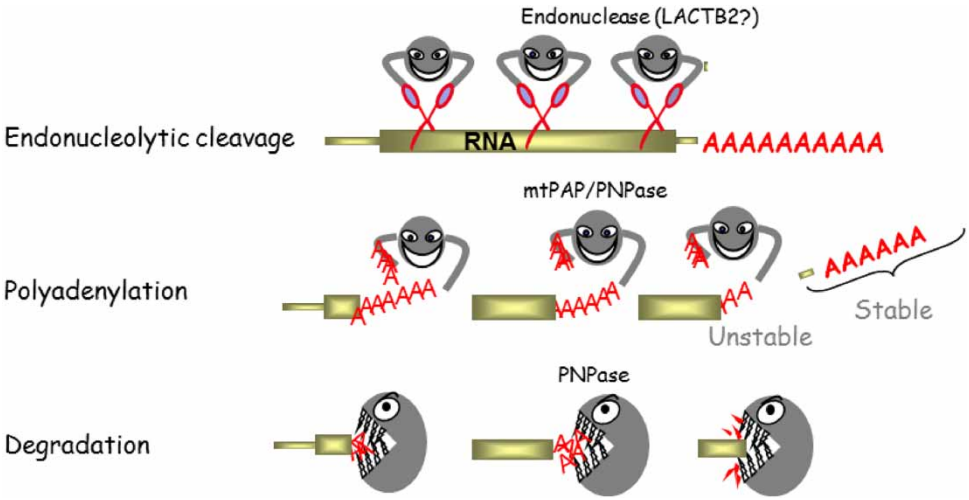

Figure 3. The polyadenylation-assisted degradation pathway.

The stages of polyadenylation-assisted RNA degradation are: (1) endonucleolytic cleavage, (2) polyadenylation, and (3) exonucleolytic digestion. Polyadenylation is executed by mtPAP, producing homopolymeric poly(A) tails, or by PNPase, producing heteropolymeric poly(A)-rich tails. The 3′–5′ exonucleolytic degradation step is carried out in bacteria and organelles by PNPase and/or RNase II/R. The first endonucleolytic cleavage step was not yet defined in human mitochondria.

the pyrophosphate at the 5′-end, resembles the decapping step in nucleus-encoded mRNAs of eukaryotes [68,69]. However, in human mitochondria, whose RNA transcripts do not contain three phosphates at the 5′-end, and which do not express the responsible endoribonuclease, no such step has been identified.

In addition, the enzymes that are responsible for each step of the poly(A)-assisted degradation pathway in human mitochondria remain to be characterized. While mtPAP and PNPase may be responsible for the second and third steps, respectively, the initial endonucleolytic cleavage step, if exists, is yet to be shown. As described above, it could be performed by LACTB2 [35]. The other known human mitochondrial endoribonucleases, RNase P and RNase Z, may be specifically responsible for tRNA processing [6–8].

Polyadenylation of RNA is a posttranscriptional modification that plays an important role in gene expression. Unlike other modifications, RNA polyadenylation plays a dual role in regulating both RNA function and stability. The addition of a stable poly(A) tail at the 3′-end of transcripts contributes to RNA functionality and stability, whereas unstable polyadenylation targets RNA to poly(A)-assisted degradation. RNA polyadenylation in human mitochondria, where its encoded transcripts are long known to harbor stable poly(A) tails at their mature 3′-ends, akin to nucleus-encoded mRNA, can undergo transient, internal polyadenylation as well. Indeed, the presence of unstable polyadenylation in the mitochondrion is consistent with its evolutionary prokaryote origin and elucidates the mechanism of RNA degradation in this organelle. Yet the underlying molecular mechanism and the enzymes involved remain to be deciphered. The recent findings presented here have directed mitochondrial RNA metabolism research to now focus on characterizing the mechanisms that differentiate between these opposing forms of RNA polyadenylation.

Abbreviations

MBL, metallo-β-lactamase; mtPAP, mitochondrial poly(A)-polymerase; PAP, poly(A)-polymerase; PNPase, polynucleotide phosphorylase.

Funding

Work in the authors' laboratory was supported by a grant from the Israel Science Foundation.

Acknowledgments

The authors thank the anonymous reviewers for making constructive comments and suggestions that have helped us improve the revised manuscript.

Biochemical Society Transactions (2016) **44** 1475–1482
DOI: 10.1042/BST20160126

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Gray, M.W., Burger, G. and Lang, B.F. (1999) Mitochondrial evolution. *Science* **283**, 1476–1481 doi:10.1126/science.283.5407.1476
2 Lane, N. and Martin, W. (2010) The energetics of genome complexity. *Nature* **467**, 929–934 doi:10.1038/nature09486
3 Rackham, O., Mercer, T.R. and Filipovska, A. (2012) The human mitochondrial transcriptome and the RNA-binding proteins that regulate its expression. *Wiley Interdiscip Rev. RNA* **3**, 675–695 doi:10.1002/wrna.1128
4 Van Haute, L., Pearce, S.F., Powell, C.A., D'Souza, A.R., Nicholls, T.J. and Minczuk, M. (2015) Mitochondrial transcript maturation and its disorders. *J. Inherited Metab. Dis.* **38**, 655–680 doi:10.1007/s10545-015-9859-z
5 Ojala, D., Montoya, J. and Attardi, G. (1981) tRNA punctuation model of RNA processing in human mitochondria. *Nature* **290**, 470–474 doi:10.1038/290470a0
6 Holzmann, J., Frank, P., Löffler, 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 doi:10.1016/j.cell.2008.09.013
7 Lopez Sanchez, M.I.G, Mercer, T.R., Davies, S.M.K., Shearwood, A.-M.J., Nygard, K.K.A., Richman, T.R. et al. (2011) RNA processing in human mitochondria. *Cell Cycle* **10**, 2904–2916 doi:10.4161/cc.10.17.17060
8 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**, 616–626 doi:10.4161/rna.8.4.15393
9 Levinger, L., Morl, M. and Florentz, C. (2004) Mitochondrial tRNA 3′-end metabolism and human disease. *Nucleic Acids Res.* **32**, 5430–5441 doi:10.1093/nar/gkh884
10 Schuster, G. and Stern, D. (2009) RNA polyadenylation and decay in mitochondria and chloroplasts. *Prog. Mol. Biol. Transl. Sci.* **85**, 393–422 doi:10.1016/S0079-6603(08)00810-6
11 Temperley, R.J., Seneca, S.H., Tonska, K., Bartnik, E., Bindoff, L.A., Lightowlers, R.N. et al. (2003) Investigation of a pathogenic mtDNA microdeletion reveals a translation-dependent deadenylation decay pathway in human mitochondria. *Hum. Mol. Genet.* **12**, 2341–2348 doi:10.1093/hmg/ddg238
12 Wilson, W.C., Hornig-Do, H.-T., Bruni, F., Chang, J.H., Jourdain, A.A., Martinou, J.-C. et al. (2014) A human mitochondrial poly(A) polymerase mutation reveals the complexities of post-transcriptional mitochondrial gene expression. *Hum. Mol. Genet.* **23**, 6345–6355 doi:10.1093/hmg/ddu352
13 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 doi:10.1074/jbc.M500804200
14 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 doi:10.1093/nar/gkh923
15 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 doi:10.1128/MCB.25.15.6427-6435.2005
16 Slomovic, S., Portnoy, V., Liveanu, V. and Schuster, G. (2006) RNA polyadenylation in prokaryotes and organelles; different tails tell different tales. *Crit. Rev. Plant Sci.* **25**, 65–77 doi:10.1080/07352680500391337
17 Lange, H., Sement, F.M., Canaday, J. and Gagliardi, D. (2009) Polyadenylation-assisted RNA degradation processes in plants. *Trends Plant Sci.* **14**, 497–504 doi:10.1016/j.tplants.2009.06.007
18 Wang, S.-W., Stevenson, A.L., Kearsey, S.E., Watt, S. and Bahler, J. (2008) Global role for polyadenylation-assisted nuclear RNA degradation in posttranscriptional gene silencing. *Mol. Cell. Biol.* **28**, 656–665 doi:10.1128/MCB.01531-07
19 Wolf, A.R. and Mootha, V.K. (2014) Functional genomic analysis of human mitochondrial RNA processing. *Cell Rep.* **7**, 918–931 doi:10.1016/j.celrep.2014.03.035
20 Antonicka, H., Sasarman, F., Nishimura, T., Paupe, V. and Shoubridge, E.A. (2013) The mitochondrial RNA-binding protein GRSF1 localizes to RNA granules and is required for posttranscriptional mitochondrial gene expression. *Cell Metab.* **17**, 386–398 doi:10.1016/j.cmet.2013.02.006
21 Jourdain, A.A., Koppen, M., Rodley, C.D., Maundrell, K., Gueguen, N., Reynier, P. et al. (2015) A mitochondria-specific isoform of FASTK is present in mitochondrial RNA granules and regulates gene expression and function. *Cell Rep.* **10**, 1110–1121 doi:10.1016/j.celrep.2015.01.063
22 Borowski, L.S., Dziembowski, A., Hejnowicz, M.S., Stepien, P.P. and Szczesny, R.J. (2013) Human mitochondrial RNA decay mediated by PNPase-hSuv3 complex takes place in distinct foci. *Nucleic Acids Res.* **41**, 1223–1240 doi:10.1093/nar/gks1130
23 Jourdain, A.A., Koppen, M., Wydro, M., Rodley, C.D., Lightowlers, R.N., Chrzanowska-Lightowlers, Z.M. et al. (2013) GRSF1 regulates RNA processing in mitochondrial RNA granules. *Cell Metab.* **17**, 399–410 doi:10.1016/j.cmet.2013.02.005
24 Lisitsky, I., Klaff, P. and Schuster, G. (1996) Addition of destabilizing poly(A)-rich sequences to endonuclease cleavage sites during the degradation of chloroplast mRNA. *Proc. Natl Acad. Sci. USA* **93**, 13398–13403 doi:10.1073/pnas.93.23.13398
25 Yehudai-Resheff, S., Hirsh, M. and Schuster, G. (2001) Polynucleotide phosphorylase functions as both an exonuclease and a poly(A) polymerase in spinach chloroplasts. *Mol. Cell. Biol.* **21**, 5408–5416 doi:10.1128/MCB.21.16.5408-5416.2001
26 Chujo, T., Ohira, T., Sakaguchi, Y., Goshima, N., Nomura, N., Nagao, A. et al. (2012) LRPPRC/SLIRP suppresses PNPase-mediated mRNA decay and promotes polyadenylation in human mitochondria. *Nucleic Acids Res.* **40**, 8033–8047 doi:10.1093/nar/gks506
27 Wang, G., Chen, H.-W., Oktay, Y., Zhang, J., Allen, E.L., Smith, G.M. et al. (2010) PNPASE regulates RNA import into mitochondria. *Cell* **142**, 456–467 doi:10.1016/j.cell.2010.06.035
28 Bruni, F., Gramegna, P., Oliveira, J.M.A., Lightowlers, R.N. and Chrzanowska-Lightowlers, Z.M.A. (2013) REXO2 is an oligoribonuclease active in human mitochondria. *PLoS ONE* **8**, e64670 doi:10.1371/journal.pone.0064670
29 Rorbach, J., Nicholls, T.J.J. and Minczuk, M. (2011) PDE12 removes mitochondrial RNA poly(A) tails and controls translation in human mitochondria. *Nucleic Acids Res.* **39**, 7750–7763 doi:10.1093/nar/gkr470
30 Fiedler, M., Rossmanith, W., Wahle, E. and Rammelt, C. (2015) Mitochondrial poly(A) polymerase is involved in tRNA repair. *Nucleic Acids Res.* **43**, 9937–9949 doi:10.1093/nar/gkv891

31 Bruni, F., Gramegna, P., Lightowlers, R.N. and Chrzanowska-Lightowlers, Z.M.A. (2012) The mystery of mitochondrial RNases. *Biochem. Soc. Trans.* **40**, 865–869 doi:10.1042/BST20120022
32 Low, R.L. (2003) Mitochondrial endonuclease G function in apoptosis and mtDNA metabolism: a historical perspective. *Mitochondrion* **2**, 225–236 doi:10.1016/S1567-7249(02)00104-6
33 Ruzzenente, B., Metodiev, M.D., Wredenberg, A., Bratic, A., Park, C.B., Cámara, Y. et al. (2012) LRPPRC is necessary for polyadenylation and coordination of translation of mitochondrial mRNAs. *EMBO J.* **31**, 443–456 doi:10.1038/emboj.2011.392
34 Baggio, F., Bratic, A., Mourier, A., Kauppila, T.E.S., Tain, L.S., Kukat, C. et al. (2014) *Drosophila melanogaster* LRPPRC2 is involved in coordination of mitochondrial translation. *Nucleic Acids Res.* **42**, 13920–13938 doi:10.1093/nar/gku1132
35 Levy, S., Allerston, C.K., Liveanu, V., Habib, M.R., Gileadi, O. and Schuster, G. (2016) Identification of LACTB2, a metallo-β-lactamase protein, as a human mitochondrial endoribonuclease. *Nucleic Acids Res.* **44**, 1813–1832 doi:10.1093/nar/gkw050
36 Dominski, Z., Carpousis, A.J. and Clouet-d'Orval, B. (2013) Emergence of the β-CASP ribonucleases: highly conserved and ubiquitous metallo-enzymes involved in messenger RNA maturation and degradation. *Biochim. Biophys. Acta, Gene Regul. Mech.* **1829**, 532–551 doi:10.1016/j.bbagrm.2013.01.010
37 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, Bioenerg.* **1797**, 1066–1070 doi:10.1016/j.bbabio.2010.01.028
38 Szczesny, R.J., Borowski, L.S., Malecki, M., Wojcik, M.A., Stepien, P.P. and Golik, P. (2012) RNA degradation in yeast and human mitochondria. *Biochim. Biophys. Acta, Gene Regul. Mech.* **1819**, 1027–1034 doi:10.1016/j.bbagrm.2011.11.010
39 Gagliardi, D., Stepien, P.P., Temperley, R.J., Lightowlers, R.N. and Chrzanowska-Lightowlers, Z.M.A. (2004) Messenger RNA stability in mitochondria: different means to an end. *Trends Genet.* **20**, 260–267 doi:10.1016/j.tig.2004.04.006
40 Bobrowicz, A.J., Lightowlers, R.N. and Chrzanowska-Lightowlers, Z. (2008) Polyadenylation and degradation of mRNA in mammalian mitochondria: a missing link? *Biochem. Soc. Trans.* **36**, 517–519 doi:10.1042/BST0360517
41 Zimmer, S.L., Schein, A., Zipor, G., Stern, D.B. and Schuster, G. (2009) Polyadenylation in Arabidopsis and *Chlamydomonas* organelles: the input of nucleotidyltransferases, poly(A) polymerases and polynucleotide phosphorylase. *Plant J.* **59**, 88–99 doi:10.1111/j.1365-313X.2009.03853.x
42 Hirayama, T., Matsuura, T., Ushiyama, S., Narusaka, M., Kurihara, Y., Yasuda, M. et al. (2013) A poly(A)-specific ribonuclease directly regulates the poly (A) status of mitochondrial mRNA in *Arabidopsis*. *Nat. Commun.* **4**, 2247 doi:10.1038/ncomms3247
43 Gagliardi, D., Perrin, R., Marechal-Drouard, L., Grienenberger, J.-M. and Leaver, C.J. (2001) Plant mitochondrial polyadenylated mRNAs are degraded by a 3′- to 5′-exoribonuclease activity, which proceeds unimpeded by stable secondary structures. *J. Biol. Chem.* **276**, 43541–43547 doi:10.1074/jbc.M106601200
44 Dziembowski, A., Piwowarski, J., Hoser, R., Minczuk, M., Dmochowska, A., Siep, M. et al. (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 doi:10.1074/jbc.M208287200
45 Piwowarski, J., Dziembowski, A., Dmochowska, A., Minczuk, M., Tomecki, R., Gewartowski, K. et al. (2004) RNA degradation in yeast and human mitochondria. *Toxicol. Mech. Methods* **14**, 53–57 doi:10.1080/15376520490257473
46 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 doi:10.1038/emboj.2008.87
47 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 doi:10.1016/j.molcel.2011.02.021
48 Etheridge, R.D., Clemens, D.M., Gershon, P.D. and Aphasizhev, R. (2009) Identification and characterization of nuclear non-canonical poly(A) polymerases from *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* **164**, 66–73 doi:10.1016/j.molbiopara.2008.11.004
49 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 doi:10.1016/S0022-2836(03)00528-X
50 Slomovic, S., Portnoy, V., Yehudai-Resheff, S., Bronshtein, E. and Schuster, G. (2008) Polynucleotide phosphorylase and the archaeal exosome as poly (A)-polymerases. *Biochim. Biophys. Acta, Gene Regul. Mech.* **1779**, 247–255 doi:10.1016/j.bbagrm.2007.12.004
51 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 doi:10.1261/rna.697308
52 Bratic, A., Clemente, P., Calvo-Garrido, J., Maffezzini, C., Felser, A., Wibom, R. et al. (2016) Mitochondrial polyadenylation is a one-step process required for mRNA integrity and tRNA maturation. *PLoS Genet.* **12**, e1006028 doi:10.1371/journal.pgen.1006028
53 Wyers, F., Rougemaille, M., Badis, G., Rousselle, J.-C., Dufour, M.-E., Boulay, J. et al. (2005) Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. *Cell* **121**, 725–737 doi:10.1016/j.cell.2005.04.030
54 Vaňáčová, Š., Wolf, J., Martin, G., Blank, D., Dettwiler, S., Friedlein, A. et al. (2005) A new yeast poly(A) polymerase complex involved in RNA quality control. *PLoS Biol.* **3**, e189 doi:10.1371/journal.pbio.0030189
55 Slomovic, S., Fremder, E., Staals, R.H., Pruijn, G.J. and Schuster, G. (2010) Addition of poly(A) and poly(A)-rich tails during RNA degradation in the cytoplasm of human cells. *Proc. Natl Acad. Sci. USA* **107**, 7407–7412 doi:10.1073/pnas.0910621107
56 Wigington, C.P., Williams, K.R., Meers, M.P., Bassell, G.J. and Corbett, A.H. (2014) Poly(A) RNA-binding proteins and polyadenosine RNA: new members and novel functions. *Wiley Interdiscip. Rev. RNA* **5**, 601–622 doi:10.1002/wrna.1233
57 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 doi:10.1093/nar/gkq068
58 Slomovic, S., Portnoy, V. and Schuster, G. (2008) Detection and characterization of polyadenylated RNA in eukarya, bacteria, archaea, and organelles. *Methods Enzymol.* **447**, 501–520 doi:10.1016/S0076-6879(08)02224-6
59 Mühlemann, O. and Lykke-Andersen, J. (2010) How and where are nonsense mRNAs degraded in mammalian cells? *RNA Biol.* **7**, 28–32 doi:10.4161/rna.7.1.10578
60 Houseley, J. and Tollervey, D. (2009) The many pathways of RNA degradation. *Cell* **136**, 763–776 doi:10.1016/j.cell.2009.01.019
61 Kushner, S.R. (2004) mRNA decay in prokaryotes and eukaryotes: different approaches to a similar problem. *IUBMB Life* **56**, 585–594 doi:10.1080/15216540400022441

Biochemical Society Transactions (2016) **44** 1475–1482
DOI: 10.1042/BST20160126

62 Régnier, P. and Hajnsdorf, E. (2009) Poly(A)-assisted RNA decay and modulators of RNA stability. *Prog. Mol. Biol. Transl. Sci.* **85**, 137–185 doi:10.1016/S0079-6603(08)00804-0
63 Mohanty, B.K. and Kushner, S.R. (2000) Polynucleotide phosphorylase functions both as a 3′-right-arrow 5′-exonuclease and a poly(A) polymerase in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **97**, 11966–11971 doi:10.1073/pnas.220295997
64 Arraiano, C.M., Andrade, J.M., Domingues, S., Guinote, I.B., Malecki, M., Matos, R.G. et al. (2010) The critical role of RNA processing and degradation in the control of gene expression. *FEMS Microbiol. Rev.* **34**, 883–923 doi:10.1111/j.1574-6976.2010.00242.x
65 Reis, F.P., Pobre, V., Silva, I.J., Malecki, M. and Arraiano, C.M. (2013) The RNase II/RNB family of exoribonucleases: putting the 'Dis' in disease. *Wiley Interdiscip. Rev. RNA* **4**, 607–615 doi:10.1002/wrna.1180
66 Kushner, S.R. (2015) Polyadenylation in *E. coli*: a 20 year odyssey. *RNA* **21**, 673–674 doi:10.1261/rna.049700.115
67 Schuster, G., Lisitsky, I. and Klaff, P. (1999) Polyadenylation and degradation of mRNA in the chloroplast. *Plant Physiol.* **120**, 937–944 doi:10.1104/pp.120.4.937
68 Hui, M.P., Foley, P.L. and Belasco, J.G. (2014) Messenger RNA degradation in bacterial cells. *Annu. Rev. Genet.* **48**, 537–559 doi:10.1146/annurev-genet-120213-092340
69 Deana, A., Celesnik, H. and Belasco, J.G. (2008) The bacterial enzyme RppH triggers messenger RNA degradation by 5′-pyrophosphate removal. *Nature* **451**, 355–358 doi:10.1038/nature06475