Fidelity and coordination of mitochondrial protein synthesis in health and disease

Danielle L. Rudler[1,2,3*], Laetitia A. Hughes[1,2,3*], Helena M. Viola[4], Livia C. Hool[4,5], Oliver Rackham[1,2,6,7,8] and Aleksandra Filipovska[1,2,3,8,9+]

[1]Harry Perkins Institute of Medical Research and [2]ARC Centre of Excellence in Synthetic Biology, QEII Medical Centre, Nedlands, Western Australia 6009, Australia

[3]Centre for Medical Research, The University of Western Australia, QEII Medical Centre, Nedlands, Western Australia 6009, Australia

[4]School of Human Sciences, The University of Western Australia, 35 Stirling Highway, Nedlands, Western Australia 6009, Australia

[5]Victor Chang Cardiac Research Institute, Sydney, NSW, Australia

[6]School of Pharmacy and Biomedical Sciences, Curtin University, Bentley, Western Australia 6102, Australia

[7]Curtin Health Innovation Research Institute, Curtin University, Bentley, Western Australia 6102, Australia

[8]Telethon Kids Institute, Northern Entrance, Perth Children's Hospital, 15 Hospital Avenue, Nedlands, Western Australia, Australia

[9]School of Molecular Sciences, The University of Western Australia, Crawley, Western Australia 6009, Australia

*co-first authors

[+]Corresponding author: aleksandra.filipovska@uwa.edu.au

Keywords: mitochondria, ribosomes, protein synthesis

Running title: Fidelity and coordination of mitochondrial protein synthesis

This is an Accepted Article that has been peer-reviewed and approved for publication in The Journal of Physiology, but has yet to undergo copy-editing and proof correction. Please cite this article as an 'Accepted Article'; doi: 10.1113/JP280359.

Abstract

The evolutionary acquisition of mitochondria has given rise to the diversity of eukaryotic life. Mitochondria have retained their ancestral α-proteobacterial traits through the maintenance of double membranes and their own circular genome that varies in size, ranging from very large in plants to the smallest in animals and their parasites. The mitochondrial genome encodes essential genes for protein synthesis and has to coordinate its expression with the nuclear genome from which it sources most of the proteins required for mitochondrial biogenesis and function. The mitochondrial protein synthesis machinery is unique because it is encoded by both the nuclear and mitochondrial genome thereby requiring tight regulation to produce the respiratory complexes that drive oxidative phosphorylation for energy production. The fidelity and coordination of mitochondrial protein synthesis are essential for ATP production. Here we compare and contrast the mitochondrial translation mechanisms in mammals and fungi to bacteria and reveal that their diverse regulation can have unusual impacts on the health and disease of these organisms. We highlight that in mammals the rate of protein synthesis is more important than the fidelity of translation, enabling coordinated biogenesis of the mitochondrial respiratory chain with respiratory chain proteins synthesised by cytoplasmic ribosomes. Changes in mitochondrial protein fidelity can trigger the activation of the diverse cellular signalling networks in fungi and mammals to combat dysfunction in energy conservation. The physiological consequences of altered fidelity of protein synthesis can range from liver regeneration to the onset and development of cardiomyopathy.

Introduction

Sustained and regulated energy conservation is essential for cell health and survival. Mitochondria supply ATP, the energy currency of cells, through oxidative phosphorylation (OXPHOS) by the electron transport chain (ETC) and the ATP synthase. Mitochondria also play essential roles in cell metabolism and signalling, cell death, reactive oxygen species (ROS) production, antioxidant and redox regulation, ion homeostasis and stress responses (Nunnari & Suomalainen, 2012). Mitochondria have retained a circular genome that is a remnant of the endosymbiotic union of an α-proteobacterium and an ancestor of the modern eukaryotic cell (Margulis, 1981; Martin *et al.*, 2015). The mammalian mitochondrial genome is compact, encoding 13 protein subunits of the OXPHOS system, 22 tRNAs and 2 rRNAs (Rackham *et al.*, 2012), produced by excision of the genome length polycistronic RNAs (Rackham *et al.*, 2016; Siira *et al.*, 2018). In yeast, despite considerable variation in genome size, all species have retained a core set of 7 genes, which encode proteins of the OXPHOS complexes and ribosomal proteins (Freel *et al.*, 2015), in addition to ~26 tRNAs and 2 rRNAs.

Mitochondrial ATP production relies on the coordinated expression of genes from both the mitochondrial and nuclear genomes. Synthesis of proteins from both genomes must adapt synergistically to meet cellular energy demands (Lee *et al.*, 2018). The stages of mitochondrial translation, from initiation through to elongation, termination, and ribosome recycling are all regulated by nuclear-encoded translation factors. By analogy to bacterial and cytoplasmic translation, elongation is considered to proceed at maximal rates, while initiation is the rate limiting and most highly regulated phase of protein synthesis (Hershey *et al.*, 2012).

Species-specific selective pressures have resulted in altered regulation of translation initiation in mitochondria. Advances in cryo-EM reconstructions of mitoribosomes, the use of new genetic models and RNA sequencing technologies have promoted a renaissance of the mitochondrial translation field that has important implications in health and disease. Mutations in genes encoding components of the translation machinery are one of the most common causes of mitochondrial diseases (Boczonadi & Horvath, 2014). Furthermore, a large proportion of clinically useful antibiotics function by interfering with the bacterial translation machinery (McCoy *et al.*, 2011), and off-target effects of these drugs can have negative side effects in mitochondria due to similarities between these systems (Singh *et al.*,

2014). Disruptions in coordination of nuclear and mitochondrial translation have been implicated in aging and age-related disease (Finley & Haigis, 2009), while mistranslated proteins also result in OXPHOS defects and cardiovascular dysfunction (Suhm *et al.*, 2018; Ferreira *et al.*, 2019). Here we contrast and compare mitochondrial translation initiation across species, highlighting the importance of coordination and fidelity in mitochondrial protein synthesis.

Mitochondrial ribosomes are molecular machines that recognize unique mRNAs

Mitochondria have diverged significantly from bacteria, which is reflected in mitoribosomes with variable RNA content and additional mitochondria-specific proteins (Suzuki *et al.*, 2001) (Figure 1). The mammalian and yeast mitoribosomes have lost the 5S rRNA found in bacterial ribosomes; instead in mammalian mitochondria this is replaced with a tRNA, for example in humans this is mt-tRNA^{Val} and mt-tRNA^{Phe} in pigs (Brown *et al.*, 2014; Greber *et al.*, 2014). Interestingly, if the levels of mt-tRNA^{Val} are reduced, the large ribosomal subunit can accommodate mt-tRNA^{Phe} as a replacement structural element (Rorbach *et al.*, 2016). Mammalian mitoribosomes have reduced rRNA content but have acquired additional proteins (Suzuki *et al.*, 2001; Amunts *et al.*, 2015; Greber *et al.*, 2015). It is not clear if these mitochondria-specific ribosomal proteins replace structural rRNAs that were lost during evolution since the yeast mitoribosome also has gained numerous mitochondria-specific proteins without reducing its rRNA content (Amunts *et al.*, 2014; De Silva *et al.*, 2015). It is possible that mitochondria-specific ribosomal proteins have been acquired in response to changes in the mitochondrial genome and the unique requirements for its expression in different organisms (Hill, 2020).

Mitochondrial mRNAs lack untranslated regions and significant secondary structures required by bacterial or cytosolic ribosomes for ribosome binding and start codon scanning (Jones *et al.*, 2008; Liu *et al.*, 2013). Consequently, mitoribosome recognition of the three different start codons of the leaderless mammalian mRNAs is significantly different to that of yeast mRNAs that have 5′ sequence elements for translational activator binding of each specific mRNA (Herrmann *et al.*, 2013). While yeast have dedicated translational activators that assist in mRNA docking and interaction with the mitoribosome (Jones *et al.*, 2019; Salvatori *et al.*, 2020), mammalian mitochondria only possess one known translational activator. The translational activator of cytochrome c oxidase subunit 1 (TACO1) binds the *mt-Co1* mRNA to facilitate its translation (Richman *et al.*, 2016), and mutations in the

TACO1 gene cause late onset Leigh Syndrome (Weraarpachai *et al.*, 2009; Seeger *et al.*, 2010). The paucity of translational activators in mammalian mitochondria indicates that mitoribosomes may require additional proteins to fulfil this regulatory capacity. Interestingly evolutionarily new mitochondria-specific ribosomal proteins, such as PTCD3 (mS39 or MRPS39), decorate the periphery of the ribosomal subunits, specifically around the ribosomal mRNA entrance site and can associate with mRNAs and facilitate mRNA recognition and docking (Davies *et al.*, 2009; Kummer *et al.*, 2018). Understanding the roles of the unique proteins that make up the mitoribosome in addition to the factors that interact with the initiation complex will be important in understanding how individual mRNAs are translated in both mammals and yeast.

Evolutionary differences in translation initiation factors between bacteria and eukaryotic mitochondria

Translation initiation is composed of a series of reactions that position the start codons of mRNAs in the peptidyl decoding site of the ribosome, paired to the anticodon of the aminoacylated and formylated initiation methionyl transfer RNA ($tRNA^{fMet}$) (Figure 2). Coordination of initiation factor binding is likely essential for initiation complex formation, and the timing of this binding is key in bacteria (Milón *et al.*, 2012). In bacteria, initiation is tightly controlled by three factors, IF1, IF2, and IF3 (Iwasaki *et al.*, 1968), while only two homologous initiation factors have been identified in mitochondria, MTIF2 and MTIF3 (Liao & Spremulli, 1990; Koc & Spremulli, 2002). Bacterial IF1 is the smallest initiation factor and binds to the A-site of the 30S subunit, enhancing the function of IF2 and IF3 by providing key anchoring points for binding (Carter *et al.*, 2001; Hussain *et al.*, 2016). IF1 blocks premature binding of elongator tRNAs during initiation of translation by occupying the same place on the 16S rRNA that could be bound by an elongator tRNA (Moazed *et al.*, 1995). IF1 can have cooperative functions with the other initiation factors, for example, IF1 and IF2 stabilise the 30S initiation complex, and IF1 is also involved in the proof-reading of initiation complexes with IF3 (Yassin *et al.*, 2011). There is no protein equivalent of IF1 in mitochondria and it is widely accepted that mammalian MTIF2 has compensated for the loss of IF1 via a 37 amino acid insertion (Gaur *et al.*, 2008), which forms an α-helix and blocks the ribosomal A site during initiation, functionally replacing IF1 (Yassin *et al.*, 2011; Kummer *et al.*, 2018). As IF1 is an essential protein in bacteria (Cummings & Hershey,

1994), its loss in mitochondria indicated that the interactions of the remaining initiation factors with ribosomes would have divergent roles in translation initiation.

In bacteria IF2 functions as a GTPase involved in the recruitment of $tRNA^{fMet}$ to the P site of the 30S subunit, and in regulating the formation of the 70S initiation complex (Gualerzi & Pon, 1990). Bacteria possess two forms of $tRNA^{Met}$, one for initiation, which is accepted directly into the P site, and one for elongation, which as accepted into the A site and translocated into the P site. IF2 mediates the selection of the initiator $tRNA^{fMet}$ recognising its 3′ single stranded acceptor stem and formylated methionine (Hartz *et al.*, 1989; Guenneugues *et al.*, 2000; Milón *et al.*, 2010). Mitochondrial MTIF2 is the only factor that is universal throughout mitochondria in eukaryotes (Liao & Spremulli, 1990). The function of the factor in mammalian mitochondria is unique as they only encode one form of $tRNA^{Met}$, and therefore rely exclusively on the formylation of the methionine by the mitochondrial methionyl-tRNA formyltransferase (MTFMT) (Takeuchi *et al.*, 1998) to allow the mitoribosome to distinguish the initiator $tRNA^{fMet}$ from the elongator $tRNA^{Met}$.

MTIF2 has maintained its native bacterial function in enhancing binding of $tRNA^{fMet}$ to the 28S subunit in the presence of a canonical start codon (Ma & Spremulli, 1996; Spencer & Spremulli, 2005), because it has a 50-fold preference for this tRNA (Spencer & Spremulli, 2004). Interestingly, this is not the case for yeast, which have retained similarities to bacteria, such as separate initiator and elongator $tRNA^{Met}$ species and in *Saccharomyces cerevisiae* mIF2 has a greater affinity for $tRNA^{fMet}$ (Martin *et al.*, 1977; Garofalo *et al.*, 2003). In bacteria IF2 promotes the association of the 50S subunit with the 30S initiation complex, in a GTP dependent reaction, to form the elongation competent 70S initiation complex (Antoun *et al.*, 2003; Grigoriadou *et al.*, 2007*a*). It is unclear whether mammalian and yeast MTIF2 have retained this function. The structure of the interaction between MTIF2 and the mitoribosome, identified that the five major domains of MTIF2 can interact with the decoding centre of the ribosome in the A-site, the sarcin-ricin loop, and the 3′ CCA end of the $tRNA^{fMet}$ (Kummer *et al.*, 2018). Recently the *in vivo* association of MTIF2 with the mitoribosome was shown to be stable, and loss of MTIF3 reduced the stability of MTIF2, indicating a cooperative role for these two proteins during translation initiation (Rudler *et al.*, 2019).

Bacterial IF3 binds to dissociated small ribosomal subunits to prevent reassembly of the monosome, thereby increasing the availability of free 30S subunits to proceed with translation initiation (Dottavio-Martin *et al.*, 1979). The ability of IF3 to enhance subunit

dissociation is attributed to the specific sites this factor occupies on the 16S rRNA, which are essential binding sites for the 50S subunit (Moazed *et al.*, 1995). IF3 is also essential in proofreading the codon-anticodon interaction between the mRNA and initiator tRNA at the P site (Meinnel *et al.*, 1999). In this way IF3 plays an important role in the fidelity of initiation by promoting the conversion of the 30S initiation complex to the 70S initiation complex only in complexes containing the canonical initiation codons, and by enhancing the dissociation of initiator tRNAs from 30S initiation complexes containing non-canonical initiation codons (Grigoriadou *et al.*, 2007*b*; Milón *et al.*, 2008).

Mammalian MTIF3 has evolved from its bacterial ancestor, and has acquired extensions on both its N- and C-termini of approximately 30 amino acids (Koc & Spremulli, 2002), which are important for its function (Haque *et al.*, 2008; Koripella *et al.*, 2019). *In vitro* studies suggested that these extensions assisted in actively disassociating the subunits at the end of translation, and prevented monosome reassembly (Koc & Spremulli, 2002; Derbikova *et al.*, 2018). *In vivo* findings identified that MTIF3 is instead required for the correct positioning of the mRNA within the preinitiation complex, and the removal of prematurely bound initiator tRNA from the ribosome (Rudler *et al.*, 2019). MTIF3, unlike MTIF2, is transiently bound to the ribosome (Haque *et al.*, 2011; Koripella *et al.*, 2019; Rudler *et al.*, 2019). Further, MTIF3 was found to regulate the rate of translation, such that loss of MTIF3 caused increased translation at the expense of fidelity and ribosome stalling (Figure 3). Loss of MITF3 caused embryonic lethality indicating that its function is non-redundant and essential for life (Rudler *et al.*, 2019). The altered fidelity of translation caused by heart and skeletal muscle-specific loss of MTIF3 impaired coordination with cytosolic translation and consequently caused reduction in the *de novo* biogenesis of the OXPHOS system resulting in reduced ATP levels (Rudler *et al.*, 2019).

Unlike the bacterial system, which has been extensively investigated, the formation of the mammalian mitochondrial initiation complex has recently been revealed, while in yeast the initiation of translation is still poorly understood. There is a divergence in the requirement of initiation factors found in bacteria and mammals, instead an assortment of other factors may substitute for their functions. In some species of yeast, such as *S. cerevisiae,* a homolog of IF3 was not identified and Aim23 was suggested as a potential candidate capable of fulfilling the function of the third initiation factor (Atkinson *et al.*, 2012). Interestingly, while MTIF3 is an essential factor for mammalian mitochondria (Rudler *et al.*, 2019), loss of Aim23 in *S. cerevisiae* did not completely arrest translation and instead only caused an

imbalance in protein production (Kuzmenko *et al.*, 2016), supporting the idea that there are likely additional factors involved in translation initiation in yeast.

Balancing efficiency and fidelity in mitochondrial translation

Changes in developmental or environmental conditions, as well as diverse and fluctuating cellular signals impose different energy demands on cells. Efficiency of translation is important to ensure mitochondria can meet these demands, whereas fidelity of protein synthesis, stress signalling, and protein homeostasis need to be coordinated and can influence cellular lifespan (Suhm *et al.*, 2018; Ferreira *et al.*, 2019). Translational efficiency is a fine balance between fidelity and speed, where an increase in one is often at the expense of the other. A reduction in fidelity can lead to an accumulation of misfolded proteins, which are toxic for the cell, while a reduction in speed can reduce the biogenesis of the OXPHOS system thereby compromising energy requirements. The rate of translation also impacts the correct folding of *de novo* synthesized proteins (Cabrita et al. 2010). In general, the speed of translation is determined by the rates of the three main stages of translation: initiation, elongation, and termination. Correct initiation and elongation are key for maintaining translational fidelity, and disruptions to these processes can occur in several ways.

Recognition of mitochondrial mRNA start codons is unique as they contain several non-standard sequences. The ability for mitochondrial systems to recognise alternative start codons suggests that there are already fidelity differences between mitoribosomes. For example, yeast Aim23 affects the stoichiometry of certain, but not all, mRNAs, a mechanism that is possibly involved in regulating mitochondrial subunit levels (Kuzmenko *et al.*, 2016). Human MTIF3 expressed in *Escherichia coli* permits a third position mismatch on mRNAs, unlike bacterial IF3 that only allows mismatching at the first position (Ayyub *et al.*, 2018). While the steady state levels of certain mitochondrial peptides are unaffected by loss of MTIF3, it does not discriminate between canonical and non-canonical start codons (Rudler *et al.*, 2019). The loss of MTIF3's proof-reading of correct initiation complex formation, where misregulated protein synthesis leads to increased translation at the expense of fidelity (Rudler *et al.*, 2019), is a response that appears unique to mammalian mitochondria. Unhindered mitochondrial translation in the absence of MTIF3, produces a subset of proteins at an increased rate which disrupts the balance of nuclear and mitochondrial subunits (Figure 3).

Other ways translational errors can occur is via incorrect aminoacylation of the tRNAs or errors in tRNA selection during mRNA decoding by the ribosome. One of the first

major steps in elongation that is crucial for translation fidelity is the aminoacylation of tRNAs by the correct aminoacyl-tRNA synthetases (aaRS). AaRSs can specifically differentiate between tRNA species via identity elements in the anticodon loop or amino acid accepting stem (Suzuki *et al.*, 2011). In mitochondria, elongation factor EF-Tu performs an additional proof-reading step to reduce errors in the aminoacylation of tRNAs (Nagao *et al.*, 2007). Defects in mitochondrial aaRSs have been observed to lead to diseases caused by mitochondrial dysfunction, indicating that proofreading by aaRSs is essential for mitochondrial function (Riley *et al.*, 2010; Diodato *et al.*, 2014; Dogan *et al.*, 2014). Despite this, aminoacylation of tRNAs by aaRSs is very accurate, and it is generally accepted that errors in translation are most often caused by errors in ribosomal mRNA recognition.

The question of ribosome fidelity was raised over 60 years ago, following findings that codon-anticodon interactions in solution were weak and required ribosomes (Lipsett *et al.*, 1960). Treatment of translating ribosomes with streptomycin identified that the ribosome and its structure play essential roles in translation and fidelity (Davies *et al.*, 1964). Investigations into the interactions of antibiotics with the ribosome with streptomycin-resistant *E. coli* mutants (displaying streptomycin-dependent phenotypes) identified mutations in the *rpsL* gene corresponding to the ribosomal subunit protein 12 (S12) that restricted the capacity for streptomycin to induce translation misreading (Nomura *et al.*, 1969). Further studies identified mutations in S12 that conferred error-prone and error-restrictive (hyper-accurate) activity (Agarwal *et al.*, 2011). This meant that mutations in a single ribosomal protein could be used to investigate the role of the ribosome in fidelity. Analysis of the structure of the ribosome found that S12 was positioned in the A site of the bacterial ribosome (Yusupov *et al.*, 2001), where it makes contact along the acceptor arm of tRNA (Valle *et al.*, 2002). In the case of the error-prone systems, this positioning allows S12 to accelerate domain closure of the SSU, resulting in incorrect acceptance of near-cognate tRNAs (Gromadski & Rodnina, 2004). Further, this region was identified to be a conserved element of the decoding centre between species (Greber *et al.*, 2015). Error-prone ribosomes result in increased ROS production and activate a general stress response in yeast (Suhm et al. 2018), a response similar to that in bacteria (Agarwal *et al.*, 2011). In mammals, mitochondrial mistranslation reduced translation and respiratory function, which elicited a stress signalling response that enabled the recovery of mitochondrial translation via mitochondrial biogenesis, telomerase expression, and cell proliferation (Ferreira *et al.*, 2019). In contrast, increased fidelity of mitochondrial translation reduced the rate of protein

synthesis without eliciting a mitochondrial stress response, where an inability to upregulate biogenesis and recover ATP levels resulted in cardiomyopathy. Error-prone mitochondrial translation showed more deleterious consequences in yeast (Suhm *et al.*, 2018), while hyper-accurate mitochondrial translation had a greater physiological impact in mice (Ferreira *et al.*, 2019). This suggests that in mammals there is a threshold of tolerance for mistranslation of proteins in mitochondria, where the rate of translation is more important than accuracy for mitochondrial function. The differences in responses between the error-prone and hyper-accurate mutations in yeast and mammals highlight the mammalian-specific signalling pathways that respond to changes in the fidelity of mitochondrial protein synthesis. This is supported by studies investigating the relationship between translational speed and protein folding efficiency, where Siller et al. (2010) found that mutant bacteria expressing eukaryotic proteins could not effectively fold these proteins until the speed of translation was reduced, as eukaryotic cytosolic translation proceeds at a slower rate than that of bacteria. Mice carrying a V338Y mutation in MRPS5, which is another mitoribosomal protein required for translational fidelity, have increased mitochondrial mistranslation and these mice have enhanced anxiety and were more susceptible to noise-induced hearing loss (Akbergenov *et al.*, 2018). The effects of altered translation fidelity differ between organisms and highlight the distinct responses and mechanisms that have developed to deal with changes in organelle translation between yeast and mammals. It would be interesting to investigate other key ribosomal proteins and their roles in fidelity to further elucidate the extent of control the ribosome has over the correct recognition of mRNA codons.

Coordination of mitochondrial and nuclear translation in health and disease

Translation is an energetically costly process, so tailoring protein expression to demand is critical. Protein production in bacteria is tightly coordinated, and the subunits of multi-protein complexes are produced according to their stoichiometries (Li *et al.*, 2014). In mammalian mitochondria, the coordinated expression of proteins from both the nuclear and mitochondrial genomes is essential for OXPHOS function (Rudler *et al.*, 2019). Controlled protein production is particularly important in mitochondria because subunits produced in excess have the potential to form toxic intermediates, leading to damaging ROS production (Khalimonchuk *et al.*, 2007). In *S. cerevisiae* cytosolic and mitochondrial translation are synchronously regulated by the nuclear genome in response to mitochondrial biogenesis (Couvillion *et al.*, 2016). When cells were shifted from a fermentable to a non-fermentable carbon source to induce respiratory metabolism, translation of cytosolic and mitochondrial

OXPHOS subunits increased simultaneously, in a response coordinated unidirectionally by the nuclear genome. While the pathways exerting this control are not fully understood, it is likely that in yeast translational activators play an essential part in this control. Translational activators operate within feedback loops, and some are present in rate limiting amounts, and therefore have the capacity to match mitochondrial protein expression to nuclear protein availability (Ott *et al.*, 2016). In mammals, assembly factors are emerging as key players in the nuclear control of mitochondrial protein expression (Richter-Dennerlein *et al.*, 2016). It is possible that during active mitoribosome translation these factors are sequestered to facilitate OXPHOS complex assembly in the inner membrane (Wang *et al.*, 2020).

Perturbations to the coordination of mitochondrial and cytoplasmic protein synthesis are managed by downstream pathways operating to restore the stoichiometric balance of OXPHOS subunits and function. Mitochondrial proteins are highly hydrophobic and must be co-translationally inserted into the inner membrane. Following insertion into the inner membrane, mitochondrial OXPHOS subunits must assemble with the nuclear encoded subunits to form functional OXPHOS complexes. In yeast, Oxa1 and Mba1 are both essential proteins required for the co-translational insertion of hydrophobic mitochondrial proteins (Preuss *et al.*, 2001). In mammals, the MRPL45 protein anchors the translating ribosome to the mitochondrial inner membrane, facilitating the insertion of proteins likely via OXA1L (Greber *et al.*, 2014; Kummer *et al.*, 2018) using the unique mitochondrial membrane lipid cardiolipin as a platform (Lee et al. 2020). Loss of cardiolipin disrupts protein synthesis in mitochondria by destabilising the mitoribosome-OXA1L interaction, causing mitochondrial fragmentation that produces ROS and diminishes ATP production, which has also been observed in Barth Syndrome patients (Lee et al. 2020).

The mitochondrial unfolded protein response (UPR^{mt}) is another mechanism that has been shown to restore homeostasis during mitochondrial stress, including that induced by imbalanced mitochondrial and cytosolic protein levels. When an accumulation of unfolded proteins is detected in mitochondria, the UPR^{mt} activates the transcription of mitochondrial proteases and chaperones capable of restoring homeostatic conditions (Zhao *et al.*, 2002). In *Caenorhabditis elegans*, inducing protein imbalance by blocking mitoribosome formation resulted in increased lifespan, despite the resulting decrease in mitochondrial respiration (Houtkooper *et al.*, 2013). Interestingly, this increase in longevity was only observed if the disruption to translation coordination and the resulting UPR^{mt} was induced during development. It would be important to gain detailed mechanistic understanding of

evolutionary different responses to mitochondrial stress induced by altered translation to help guide treatments for diseases associated with aging.

Despite previous evidence for unidirectional control of mitochondrial and cytosolic translation coordination, new evidence has suggested that bidirectional control is more likely. Recently, it was shown that inhibiting mitochondrial translation by knocking out MRPS5 in *C. elegans* led to increased ATF4 and ATF5 levels and induced coordinated changes in cytosolic translation (Molenaars *et al.*, 2020). The reduction in mitochondrial translation resulted in a balanced decrease in nuclear encoded mRNAs and proteins involved in cytosolic translation, which was independent of oxidative stress or the UPR^{mt}. Interestingly, in other mouse models of impaired mitochondrial protein synthesis, a significant increase in *Atf4* and *Atf5* mRNAs has been observed following inhibition of mitochondrial translation, however, in these models mRNAs involved in cytosolic translation were increased (Rackham *et al.*, 2016; Perks *et al.*, 2018; Siira *et al.*, 2018). This is a different response to that observed by Molenaars *et al.* (2020) where the mRNA levels of the transcription factors *Atf5* and *Atf4* were not changed, but their protein levels were increased. This highlights the complexity of responses to reduced mitochondrial protein synthesis, and that upregulation of *Atf5* and *Atf4* does not always have the same effect on cytosolic translation. It is likely that there are many pathways relaying changes in mitochondrial translation to the cytosol to maintain coordinated protein synthesis, and further insight into these processes is necessary. Nonetheless, these findings indicate that coordinated mitochondrial and nuclear protein synthesis is controlled bidirectionally, as the status of the mitochondrial ribosomes has a direct impact on the status of cytosolic translation.

The mechanisms operating to coordinate mitochondrial and cytosolic translation and subunit availability, as well as the systems involved in restoring proteostasis following perturbations to this balance are essential to mitochondrial and cellular fitness. Active research in this area will unravel the complex nature of these pathways, and how disruption to this coordination can result in disease and contribute to ageing.

Acknowledgements

We thank members of the Filipovska and Rackham laboratories for their scientific contributions and constructive advice.

Funding

This project was supported by fellowships and project grants from the National Health and Medical Research Council (APP1058442, APP1045677, APP1041582, APP1023460, APP1005030, APP1043978 to AF and OR and APP1117366 to LH), the Australian Research Council (DP180101656 to AF and OR), the Cancer Council of Western Australia (to OR and AF). HV is supported by a Heart Foundation Future Leader Fellowship, DLR and LAH are supported by UWA Postgraduate Scholarships.

Conflict of Interest: None of the authors has any conflicts of interests.

References

Agarwal D, Gregory ST & O'Connor M (2011). Error-Prone and Error-Restrictive Mutations Affecting Ribosomal Protein S12. *J Mol Biol* **410,** 1–9.

Akbergenov R et al. (2018). Mutant MRPS5 affects mitoribosomal accuracy and confers stress-related behavioral alterations. *EMBO Rep* **19,** e46193.

Amunts A, Brown A, Bai X, Llácer JL, Hussain T, Emsley P, Long F, Murshudov G, Scheres SHW & Ramakrishnan V (2014). Structure of the Yeast Mitochondrial Large Ribosomal Subunit. *Science (80-)* **343,** 1485–1489.

Amunts A, Brown A, Toots J, Scheres SHW & Ramakrishnan V (2015). The structure of the human mitochondrial ribosome. *Science (80-)* **348,** 95–98.

Antoun A, Pavlov MY, Andersson K, Tenson T & Ehrenberg M (2003). The roles of initiation factor 2 and guanosine triphosphate in initiation of protein synthesis. *EMBO J* **22,** 5593–5601.

Antoun A, Pavlov MY, Lovmar M & Ehrenberg M (2006). How initiation factors tune the rate of initiation of protein synthesis in bacteria. *EMBO J* **25,** 2539–2550.

Atkinson GC, Kuzmenko A, Kamenski P, Vysokikh MY, Lakunina V, Tankov S, Smirnova E, Soosaar A, Tenson T & Hauryliuk V (2012). Evolutionary and genetic analyses of mitochondrial translation initiation factors identify the missing mitochondrial IF3 in S. cerevisiae. *Nucleic Acids Res* **40,** 6122–6134.

Ayyub SA, Aswathy SL, Dobriyal D, Aluri S, Spremulli LL & Varshney U (2018). Fidelity of translation in the presence of mammalian mitochondrial initiation factor 3. *Mitochondrion* **39,** 1–8.

Bhargava K & Spremulli LL (2005). Role of the N- and C-terminal extensions on the activity of mammalian mitochondrial translational initiation factor 3. *Nucleic Acids Res* **33,** 7011–7018.

Boczonadi V & Horvath R (2014). Mitochondria: Impaired mitochondrial translation in human disease. *Int J Biochem Cell Biol* **48,** 77–84.

Brown A, Amunts A, Bai X, Sugimoto Y, Edwards PC, Murshudov G, Scheres SHW &

Ramakrishnan V (2014). Structure of the large ribosomal subunit from human mitochondria. *Science (80-)* **346,** 718–722.

Cabrita LD, Dobson CM & Christodoulou J (2010). Protein folding on the ribosome. *Curr Opin Struct Biol* **20,** 33–45.

Carter AP, Clemons Jr. WM, Brodersen DE, Morgan-Warren RJ, Hartsch T, Wimberly BT & Ramakrishnan V (2001). Crystal Structure of an Initiation Factor Bound to the 30S Ribosomal Subunit. *Science (80-)* **291,** 498–501.

Chicherin I V, Zinina V V, Levitskiy SA, Serebryakova M V & Kamenski PA (2019). Aim23p Interacts with the Yeast Mitochondrial Ribosomal Small Subunit. *Biochem* **84,** 40–46.

Couvillion MT, Soto IC, Shipkovenska G & Churchman LS (2016). Synchronized mitochondrial and cytosolic translation programs. *Nature* **533,** 499–503.

Cummings HS & Hershey JW (1994). Translation initiation factor IF1 is essential for cell viability in Escherichia coli. *J Bacteriol* **176,** 198–205.

Davies J, Gilbert W & Gorini L (1964). Streptomycin, Suppression, and the Code. *Proc Natl Acad Sci U S A* **51,** 883–890.

Davies SMK, Rackham O, Shearwood A-MJ, Hamilton KL, Narsai R, Whelan J & Filipovska A (2009). Pentatricopeptide repeat domain protein 3 associates with the mitochondrial small ribosomal subunit and regulates translation. *FEBS Lett* **583,** 1853–1858.

Derbikova K, Kuzmenko A, Levitskii S, Klimontova M, Chicherin I, Baleva M, Krasheninnikov I & Kamenski P (2018). Biological and Evolutionary Significance of Terminal Extensions of Mitochondrial Translation Initiation Factor 3. *Int J Mol Sci* **19,** 3861.

Desai N, Brown A, Amunts A & Ramakrishnan V (2017). The structure of the yeast mitochondrial ribosome. *Science (80-)* **355,** 528–531.

Diodato D, Ghezzi D & Tiranti V (2014). The Mitochondrial Aminoacyl tRNA Synthetases: Genes and Syndromes. *Int J Cell Biol* **2014,** 2014:787956.

Dogan SA, Pujol C, Maiti P, Kukat A, Wang S, Hermans S, Senft K, Wibom R, Rugarli EI & Trifunovic A (2014). Tissue-Specific Loss of DARS2 Activates Stress Responses Independently of Respiratory Chain Deficiency in the Heart. *Cell Metab* **19,** 458–469.

Dottavio-Martin D, Suttle DP & Ravel JM (1979). The effects of initiation factors IF-1 and IF-3 on the dissociation of escherichia coli 70 S ribosomes. *FEBS Lett* **97,** 105–110.

Ferreira N, Perks KL, Rossetti G, Rudler DL, Hughes LA, Ermer JA, Scott LH, Kuznetsova I, Richman TR, Narayana VK, Abudulai LN, Shearwood AJ, Cserne Szappanos H, Tull D, Yeoh GC, Hool LC, Filipovska A & Rackham O (2019). Stress signaling and cellular proliferation reverse the effects of mitochondrial mistranslation. *EMBO J* **38,** e102155.

Finley LWS & Haigis MC (2009). The coordination of nuclear and mitochondrial communication during aging and calorie restriction. *Ageing Res Rev* **8,** 173–188.

Freel KC, Friedrich A & Schacherer J (2015). Mitochondrial genome evolution in yeasts: an all-encompassing view. *FEMS Yeast Res* **15,** fov023.

Gabashvili IS, Agrawal RK, Spahn CMT, Grassucci RA, Svergun DI, Frank J & Penczek P (2000). Solution Structure of the E. coli 70S Ribosome at 11.5 Å Resolution. *Cell* **100,** 537–549.

Garofalo C, Trinko R, Kramer G, Appling DR & Hardesty B (2003). Purification and characterization of yeast mitochondrial initiation factor 2. *Arch Biochem Biophys* **413,** 243–252.

Gaur R, Grasso D, Datta PP, Krishna PDV, Das G, Spencer A, Agrawal RK, Spremulli L & Varshney U (2008). A Single Mammalian Mitochondrial Translation Initiation Factor Functionally Replaces Two Bacterial Factors. *Mol Cell* **29,** 180–190.

Greber BJ, Bieri P, Leibundgut M, Leitner A, Aebersold R, Boehringer D & Ban N (2015). The complete structure of the 55S mammalian mitochondrial ribosome. *Science (80-)* **348,** 303–308.

Greber BJ, Boehringer D, Leibundgut M, Bieri P, Leitner A, Schmitz N, Aebersold R & Ban N (2014). The complete structure of the large subunit of the mammalian mitochondrial ribosome. *Nature* **515,** 283–286.

Grigoriadou C, Marzi S, Kirillov S, Gualerzi CO & Cooperman BS (2007*a*). A quantitative

kinetic scheme for 70 S translation initiation complex formation. *J Mol Biol* **373,** 562–572.

Grigoriadou C, Marzi S, Pan D, Gualerzi CO & Cooperman BS (2007*b*). The Translational Fidelity Function of IF3 During Transition from the 30S Initiation Complex to the 70S Initiation Complex. *J Mol Biol* **373,** 551–561.

Gromadski KB & Rodnina M V (2004). Streptomycin interferes with conformational coupling between codon recognition and GTPase activation on the ribosome. *Nat Struct Mol Biol* **11,** 316–322.

Gualerzi CO & Pon CL (1990). Initiation of mRNA translation in prokaryotes. *Biochemistry* **29,** 5881–5889.

Guenneugues M, Caserta E, Brandi L, Spurio R, Meunier S, Pon CL, Boelens R & Gualerzi CO (2000). Mapping the fMet-tRNA(f)(Met) binding site of initiation factor IF2. *EMBO J* **19,** 5233–5240.

Haque ME, Grasso D & Spremulli LL (2008). The interaction of mammalian mitochondrial translational initiation factor 3 with ribosomes: Evolution of terminal extensions in IF3mt. *Nucleic Acids Res* **36,** 589–597.

Haque ME, Koc H, Cimen H, Koc EC & Spremulli LL (2011). Contacts between mammalian mitochondrial translational initiation factor 3 and ribosomal proteins in the small subunit. *Biochim Biophys Acta* **1814,** 1779–1784.

Hartz D, McPheeters DS & Gold L (1989). Selection of the initiator tRNA by Escherichia coli initiation factors. *Genes Dev* **3,** 1899–1912.

Herrmann JM, Woellhaf MW & Bonnefoy N (2013). Control of protein synthesis in yeast mitochondria: The concept of translational activators. *Biochim Biophys Acta - Mol Cell Res* **1833,** 286–294.

Hershey JWB, Sonenberg N & Mathews MB (2012). Principles of translational control: an overview. *Cold Spring Harb Perspect Biol* **4,** a011528.

Hill GE (2020). Mitonuclear Compensatory Coevolution. *Trends Genet* **36,** 403–414.

Houtkooper RH, Mouchiroud L, Ryu D, Moullan N, Katsyuba E, Knott G, Williams RW &

Auwerx J (2013). Mitonuclear protein imbalance as a conserved longevity mechanism. *Nature* **497,** 451–459.

Hussain T, Llácer JL, Wimberly BT, Kieft JS & Ramakrishnan V (2016). Large-Scale Movements of IF3 and tRNA during Bacterial Translation Initiation. *Cell* **167,** 133-144.e13.

Iwasaki K, Sabol S, Wahba AJ & Ochoa S (1968). Translation of the genetic message: VII. Role of initiation factors in formation of the chain initiation complex with Escherichia coli ribosomes. *Arch Biochem Biophys* **125,** 542–547.

Jones CN, Wilkinson KA, Hung KT, Weeks KM & Spremulli LL (2008). Lack of secondary structure characterizes the 5' ends of mammalian mitochondrial mRNAs. *RNA* **14,** 862–871.

Jones JL, Hofmann KB, Cowan AT, Temiakov D, Cramer P & Anikin M (2019). Yeast mitochondrial protein Pet111p binds directly to two distinct targets in COX2 mRNA, suggesting a mechanism of translational activation. *J Biol Chem* **294,** 7528–7536.

Khalimonchuk O, Bird A & Winge DR (2007). Evidence for a Pro-oxidant Intermediate in the Assembly of Cytochrome Oxidase. *J Biol Chem* **282,** 17442–17449.

Koc EC & Spremulli LL (2002). Identification of mammalian mitochondrial translational initiation factor 3 and examination of its role in initiation complex formation with natural mRNAs. *J Biol Chem* **277,** 35541–35549.

Koripella RK, Sharma MR, Haque ME, Risteff P, Spremulli LL & Agrawal RK (2019). Structure of human mitochondrial translation initiation factor 3 bound to the small ribosomal subunit. *iScience* **12,** 76–86.

Kummer E, Leibundgut M, Rackham O, Lee RG, Boehringer D, Filipovska A & Ban N (2018). Unique features of mammalian mitochondrial translation initiation revealed by cryo-EM. *Nature* **560,** 263–267.

Kuzmenko A, Atkinson GC, Levitskii S, Zenkin N, Tenson T, Hauryliuk V & Kamenski P (2014). Mitochondrial translation initiation machinery: Conservation and diversification. *Biochimie* **100,** 132–140.

Kuzmenko A, Derbikova K, Salvatori R, Tankov S, Atkinson GC, Tenson T, Ott M,

Kamenski P & Hauryliuk V (2016). Aim-less translation: loss of Saccharomyces cerevisiae mitochondrial translation initiation factor mIF3/Aim23 leads to unbalanced protein synthesis. *Sci Rep* **6,** 1–9.

Lee RG, Rudler DL, Rackham O & Filipovska A (2018). Is mitochondrial gene expression coordinated or stochastic? *Biochem Soc Trans* **46,** 1239–1246.

Lee, RG, Gao, J, Siira, SJ, Shearwood, A-MJ, Ermer, JA, Hofferek, V, Mathews, JC, Zheng, M, Reid, GE, Rackham O & Filipovska A (2020) Cardiolipin is required for mitoribosomal membrane docking and protein synthesis. *J Cell Sci*. In Press.

Li G-W, Burkhardt D, Gross C & Weissman JS (2014). Quantifying Absolute Protein Synthesis Rates Reveals Principles Underlying Allocation of Cellular Resources. *Cell* **157,** 624–635.

Liao HX & Spremulli LL (1990). Identification and initial characterization of translational initiation factor 2 from bovine mitochondria. *J Biol Chem* **265,** 13618–13622.

Lipsett MN, Heppel LA & Bradley DF (1960). Complex formation between adenine oligonucleotides and polyuridylic acid. *BBA - Biochim Biophys Acta* **41,** 175–177.

Liu G, Mercer TR, Shearwood A-MJ, Siira SJ, Hibbs ME, Mattick JS, Rackham O & Filipovska A (2013). Mapping of Mitochondrial RNA-Protein Interactions by Digital RNase Footprinting. *Cell Rep* **5,** 839–848.

Ma J & Spremulli LL (1996). Expression, Purification, and Mechanistic Studies of Bovine Mitochondrial Translational Initiation Factor 2. *J Biol Chem* **271,** 5805–5811.

Margulis L (1981). *Symbiosis in cell evolution : life and its environment on the early Earth* . W. H. Freeman, San Francisco.

Martin RP, Schneller JM, Stahl AJ & Dirheimer G (1977). Study of yeast mitochondrial tRNAs by two-dimensional polyacrylamide gel electrophoresis: characterization of isoaccepting species and search for imported cytoplasmic tRNAs. *Nucleic Acids Res* **4,** 3497–3510.

Martin WF, Garg S & Zimorski V (2015). Endosymbiotic theories for eukaryote origin. *Philos Trans R Soc B Biol Sci* **370,** 20140330.

McCoy LS, Xie Y & Tor Y (2011). Antibiotics that target protein synthesis. *Wiley Interdiscip Rev RNA* **2,** 209–232.

Meinnel T, Sacerdot C, Graffe M, Blanquet S & Springer M (1999). Discrimination by Escherichia coli initiation factor IF3 against initiation on non-canonical codons relies on complementarity rules. *J Mol Biol* **290,** 825–837.

Milón P, Carotti M, Konevega AL, Wintermeyer W, Rodnina M V & Gualerzi CO (2010). The ribosome-bound initiation factor 2 recruits initiator tRNA to the 30S initiation complex. *EMBO Rep* **11,** 312–316.

Milón P, Konevega AL, Gualerzi CO & Rodnina M V (2008). Kinetic Checkpoint at a Late Step in Translation Initiation. *Mol Cell* **30,** 712–720.

Milón P, Maracci C, Filonava L, Gualerzi CO & Rodnina M V. (2012). Real-time assembly landscape of bacterial 30S translation initiation complex. *Nat Struct Mol Biol* **19,** 609–615.

Moazed D, Samaha RR, Gualerzi C & Noller HF (1995). Specific protection of 16 S rRNA by translational initiation factors. *J Mol Biol* **248,** 207–210.

Molenaars M, Janssens GE, Williams EG, Jongejan A, Lan J, Rabot S, Joly F, Moerland PD, Schomakers B V, Lezzerini M, Liu YJ, McCormick MA, Kennedy BK, van Weeghel M, van Kampen AHC, Aebersold R, MacInnes AW & Houtkooper RH (2020). A Conserved Mito-Cytosolic Translational Balance Links Two Longevity Pathways. *Cell Metab* **31,** 549–563.

Nagao A, Suzuki T & Suzuki T (2007). Aminoacyl-tRNA surveillance by EF-Tu in mammalian mitochondria. *Nucleic Acids Symp Ser* **51,** 41–42.

Nomura M, Ozaki M & Mizushima S (1969). Identification and Functional Characterization of the Protein controlled by the Streptomycin-resistant Locus in E. coli. *Nature* **222,** 333–339.

Nouet C, Bourens M, Hlavacek O, Marsy S, Lemaire C & Dujardin G (2007). Rmd9p Controls the Processing/Stability of Mitochondrial mRNAs and Its Overexpression Compensates for a Partial Deficiency of Oxa1p in Saccharomyces cerevisiae. *Genetics* **175,** 1105–1115.

Nunnari J & Suomalainen A (2012). Mitochondria: in sickness and in health. *Cell* **148,** 1145–1159.

Ott M, Amunts A & Brown A (2016). Organization and Regulation of Mitochondrial Protein Synthesis. *Annu Rev Biochem* **85,** 77–101.

Perks KL, Rossetti G, Kuznetsova I, Hughes LA, Ermer JA, Ferreira N, Busch JD, Rudler DL, Spahr H, Schöndorf T, Shearwood AMJ, Viola HM, Siira SJ, Hool LC, Milenkovic D, Larsson NG, Rackham O & Filipovska A (2018). PTCD1 Is Required for 16S rRNA Maturation Complex Stability and Mitochondrial Ribosome Assembly. *Cell Rep* **23,** 127–142.

Preuss M, Leonhard K, Hell K, Stuart RA, Neupert W & Herrmann JM (2001). Mba1, a Novel Component of the Mitochondrial Protein Export Machinery of the Yeast Saccharomyces cerevisiae. *J Cell Biol* **153,** 1085–1095.

Rackham O, Busch JD, Matic S, Siira SJ, Kuznetsova I, Atanassov I, Ermer JA, Shearwood AMJ, Richman TR, Stewart JB, Mourier A, Milenkovic D, Larsson NG & Filipovska A (2016). Hierarchical RNA processing is required for mitochondrial ribosome assembly. *Cell Rep* **16,** 1874–1890.

Rackham O, Mercer TR & Filipovska A (2012). The human mitochondrial transcriptome and the RNA-binding proteins that regulate its expression. *Wiley Interdiscip Rev RNA* **3,** 675–695.

Richman TR et al. (2016). Loss of the RNA-binding protein TACO1 causes late-onset mitochondrial dysfunction in mice. *Nat Commun* **7,** 1–14.

Richter-Dennerlein R, Oeljeklaus S, Lorenzi I, Ronsör C, Bareth B, Schendzielorz AB, Wang C, Warscheid B, Rehling P & Dennerlein S (2016). Mitochondrial Protein Synthesis Adapts to Influx of Nuclear-Encoded Protein. *Cell* **167,** 471–483.

Riley LG, Cooper S, Hickey P, Rudinger-Thirion J, McKenzie M, Compton A, Lim SC, Thorburn D, Ryan MT, Giegé R, Bahlo M & Christodoulou J (2010). Mutation of the mitochondrial tyrosyl-tRNA synthetase gene, YARS2, causes myopathy, lactic acidosis, and sideroblastic anemia--MLASA syndrome. *Am J Hum Genet* **87,** 52–59.

Rorbach J, Gao F, Powell C a, D'Souza A, Lightowlers RN, Minczuk M & Chrzanowska-

Lightowlers ZM (2016). Human mitochondrial ribosomes can switch their structural RNA composition. *Proc Natl Acad Sci U S A* **113,** 12198–12201.

Rudler DL, Hughes LA, Perks KL, Richman TR, Kuznetsova I, Ermer JA, Abudulai LN, Shearwood A-MJ, Viola HM, Hool LC, Siira SJ, Rackham O & Filipovska A (2019). Fidelity of translation initiation is required for coordinated respiratory complex assembly. *Sci Adv* **5,** eaay2118.

Salvatori R, Kehrein K, Singh AP, Aftab W, Möller-Hergt BV, Forne I, Imhof A & Ott M (2020). Molecular Wiring of a Mitochondrial Translational Feedback Loop. *Mol Cell* **77,** 887-900.e5.

Seeger J, Schrank B, Pyle A, Stucka R, Lörcher U, Müller-Ziermann S, Abicht A, Czermin B, Holinski-Feder E, Lochmüller H & Horvath R (2010). Clinical and neuropathological findings in patients with TACO1 mutations. *Neuromuscul Disord* **20,** 720–724.

Siira SJ, Rossetti G, Richman TR, Perks K, Ermer JA, Kuznetsova I, Hughes L, Shearwood AJ, Viola HM, Hool LC, Rackham O & Filipovska A (2018). Concerted regulation of mitochondrial and nuclear non-coding RNAs by a dual-targeted RNase Z. *EMBO Rep* **19,** e46198.

Siller E, DeZwaan DC, Anderson JF, Freeman BC & Barral JM (2010). Slowing Bacterial Translation Speed Enhances Eukaryotic Protein Folding Efficiency. *J Mol Biol* **396,** 1310–1318.

De Silva D, Tu YT, Amunts A, Fontanesi F & Barrientos A (2015). Mitochondrial ribosome assembly in health and disease. *Cell Cycle* **14,** 2226–2250.

Singh R, Sripada L & Singh R (2014). Side effects of antibiotics during bacterial infection: Mitochondria, the main target in host cell. *Mitochondrion* **16,** 50–54.

Spencer AC & Spremulli LL (2004). Interaction of mitochondrial initiation factor 2 with mitochondrial fMet-tRNA. *Nucleic Acids Res* **32,** 5464–5470.

Spencer AC & Spremulli LL (2005). The interaction of mitochondrial translational initiation factor 2 with the small ribosomal subunit. *BBA - Proteins Proteomics* **1750,** 69–81.

Suhm T, Kaimal JM, Dawitz H, Peselj C, Masser AE, Hanzén S, Ambrožič M, Smialowska A, Björck ML, Brzezinski P, Nyström T, Büttner S, Andréasson C & Ott M (2018).

Mitochondrial Translation Efficiency Controls Cytoplasmic Protein Homeostasis. *Cell Metab* **27,** 1309-1322.e6.

Suzuki T, Nagao A & Suzuki T (2011). Human Mitochondrial tRNAs: Biogenesis, Function, Structural Aspects, and Diseases. *Annu Rev Genet* **45,** 299–329.

Suzuki T, Terasaki M, Takemoto-Hori C, Hanada T, Ueda T, Wada A & Watanabe K (2001). Structural compensation for the deficit of rRNA with proteins in the mammalian mitochondrial ribosome. Systematic analysis of protein components of the large ribosomal subunit from mammalian mitochondria. *J Biol Chem* **276,** 21724–21736.

Takeuchi N, Kawakami M, Omori A, Ueda T, Spremulli LL & Watanabe K (1998). Mammalian mitochondrial methionyl-tRNA transformylase from bovine liver. Purification, characterization, and gene structure. *J Biol Chem* **273,** 15085–15090.

Valle M, Grassucci RA, Burkhardt N, Agrawal RK, Frank J, Swami NK, Sengupta J & Nierhaus KH (2002). Cryo-EM reveals an active role for aminoacyl-tRNA in the accommodation process. *EMBO J* **21,** 3557–3567.

Wang C, Richter-Dennerlein R, Pacheu-Grau D, Liu F, Zhu Y, Dennerlein S & Rehling P (2020). MITRAC15/COA1 promotes mitochondrial translation in a ND2 ribosome–nascent chain complex. *EMBO Rep* **21,** e48833.

Weraarpachai W, Antonicka H, Sasarman F, Seeger J, Schrank B, Kolesar JE, Lochmüller H, Chevrette M, Kaufman BA, Horvath R & Shoubridge EA (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.

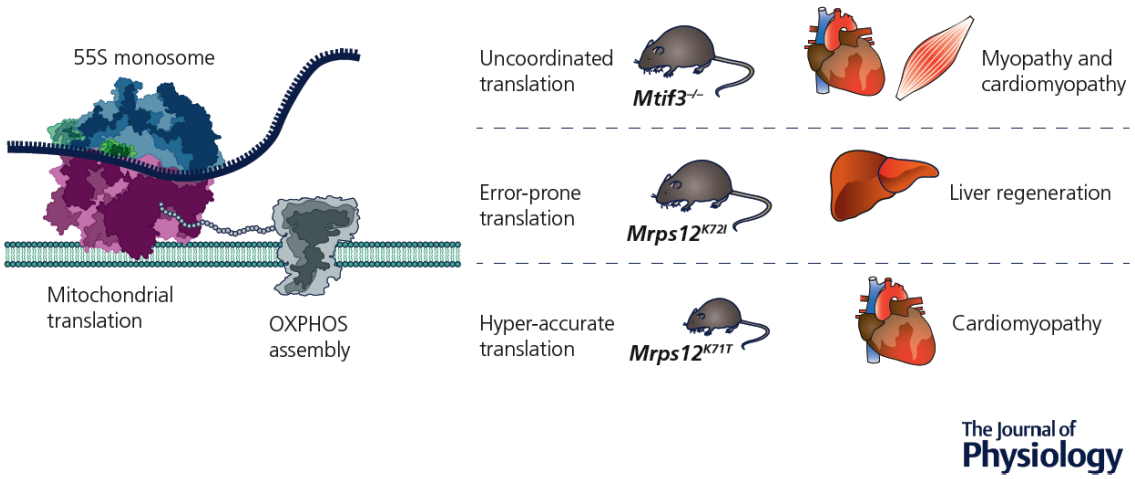
Williams EH, Butler CA, Bonnefoy N & Fox TD (2007). Translation initiation in Saccharomyces cerevisiae mitochondria: Functional interactions among mitochondrial ribosomal protein Rsm28p, initiation factor 2, methionyl-tRNA-formyltransferase and novel protein Rmd9p. *Genetics* **175,** 1117–1126.

Yassin AS, Haque ME, Datta PP, Elmore K, Banavali NK, Spremulli LL & Agrawal RK (2011). Insertion domain within mammalian mitochondrial translation initiation factor 2 serves the role of eubacterial initiation factor 1. *Proc Natl Acad Sci U S A* **108,** 3918–3923.

Yusupov MM, Yusupova GZ, Baucom A, Lieberman K, Earnest TN, Cate JH & Noller HF (2001). Crystal structure of the ribosome at 5.5 A resolution. *Science* **292,** 883–896.

Zhao Q, Wang J, Levichkin I V, Stasinopoulos S, Ryan MT & Hoogenraad NJ (2002). A mitochondrial specific stress response in mammalian cells. *EMBO J* **21,** 4411–4419.

Abstract Figure Legend



The rate and fidelity of mitochondrial protein synthesis impacts the coordinated biogenesis of the oxidative phosphorylation system. Evolutionary divergence in mitochondrial genomes and translational machineries have given rise to different modes for protein synthesis regulation. Altered protein synthesis can elicit a variety of cellular responses to cope with compromised biogenesis and function of the oxidative phosphorylation system.

Figure Legends

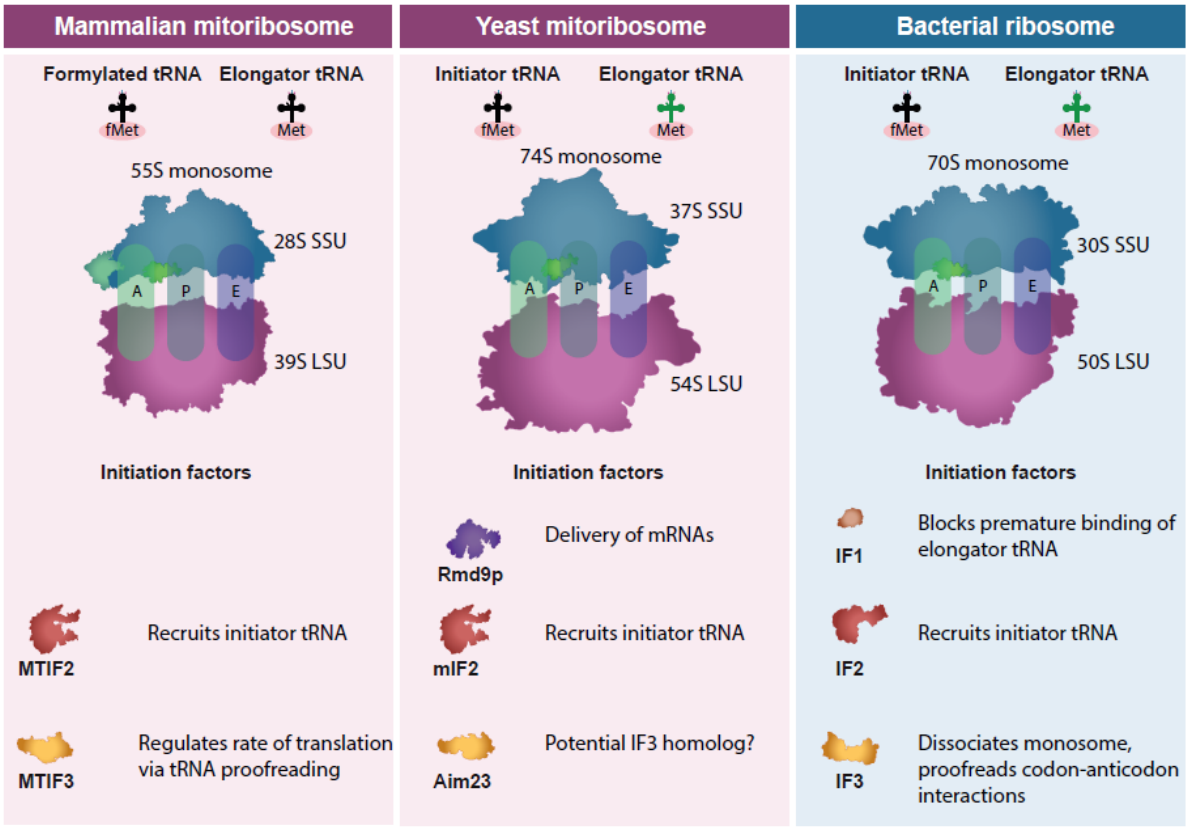


Figure 1. Evolutionary divergence of the mitochondrial ribosome from its ancestral bacterial ribosome. Mitoribosomes are evolutionally derived from the bacterial ribosome but exhibit vast structural and functional variation. The cryo-EM structures of the bacterial ribosome (Gabashvili *et al.*, 2000), mammalian (Amunts *et al.*, 2015; Greber *et al.*, 2015) and yeast (Desai *et al.*, 2017) mitoribosomes reveal variation between species. In bacteria, the 70S ribosome is composed of a 30S small subunit (blue) containing the 16S rRNA and a 50S large subunit (purple) containing the 23S and 5S rRNA. Bacteria have three translation initiation factors: IF1, IF2 and IF3, and each of them have specific roles shown in the schematic. *S. cerevisiae* yeast mitoribosomes have a sedimentation coefficient of 74S and are composed of a 37S small subunit with a 15S rRNA and 34 proteins (Desai *et al.*, 2017) and a 54S large subunit with a 21S rRNA and 39 proteins (Amunts *et al.*, 2014). Initiation of translation in yeast mitochondria is poorly understood and the factors involved may differ between different species; the current players are shown in the schematic. Mammalian mitoribosomes sediment as 55S particles, with a 28S small subunit composed of the 12S rRNA and 30 proteins, while the 39S large subunit contains the 16S rRNA and 52 proteins (Greber *et al.*, 2014, 2015). There are two initiation factors in mammalian

mitochondria, MTIF2 and MTIF3, both essential for translation initiation and cell survival. The bacterial and mitochondrial ribosomes have three main sites where factors involved in the translation cycle bind, the aminoacyl (A) site, peptidyl (P) site, and the exit (E) site. The A-site binds an aminoacyl-tRNA or termination release factors, the P-site binds a peptidyl-tRNA (a tRNA bound to the poly-peptide chain) or initiator tRNAs; and the E-site (exit) binds a free tRNA. The small subunit (SSU) contains the decoding site where the codon-anticodon interactions between the aminoacyl-tRNA and mRNA take place, while the LSU contains the active site where peptidyl transfer and hydrolysis reactions occur.

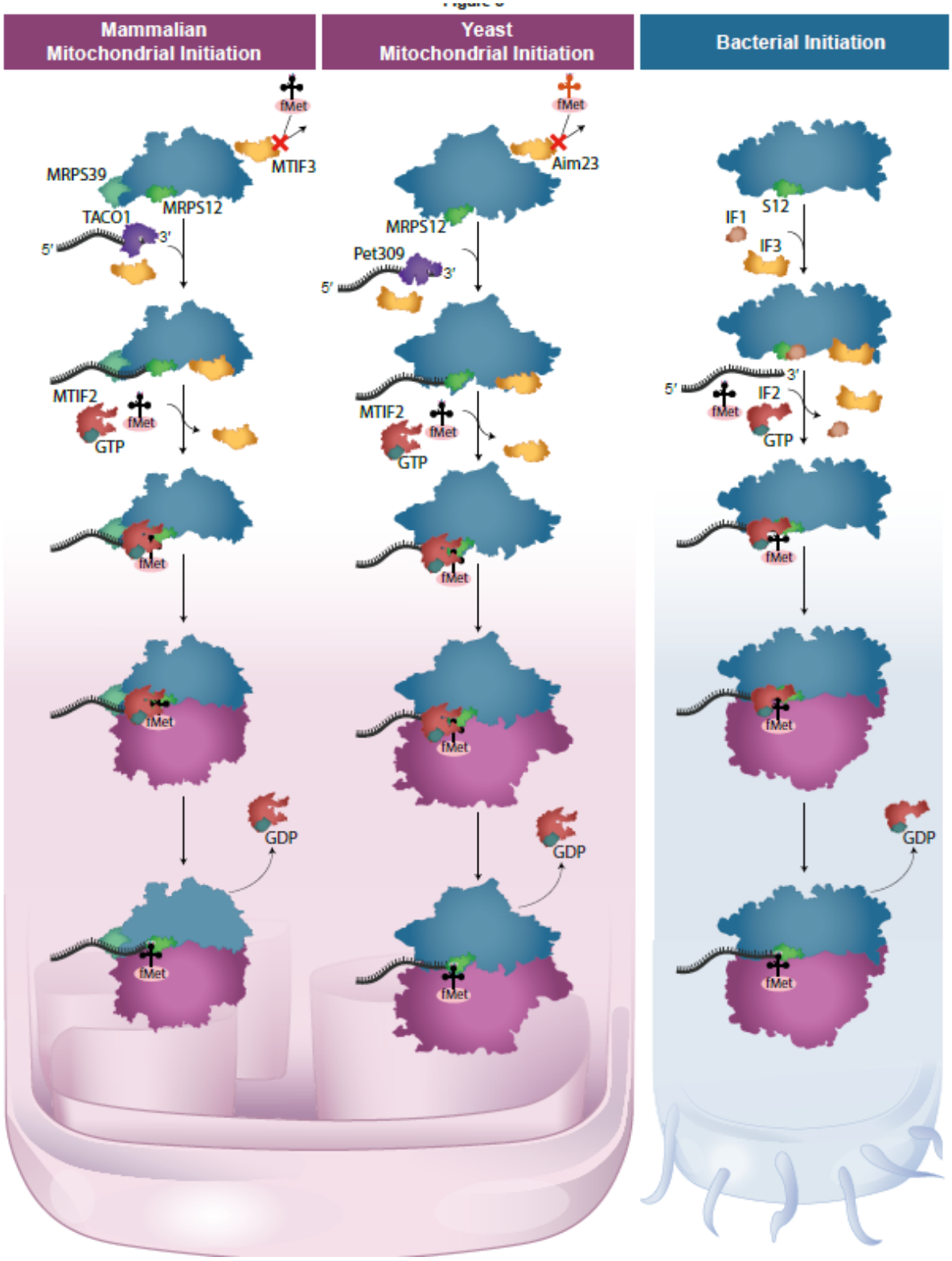


Figure 2. Initiation of mitochondrial protein synthesis in mammals and yeast compared to bacteria. Dissociation of the small and large ribosomal subunits gives prepares the ribosome for a new round of translation initiation. This process starts with the formation of a preinitiation complex. Following correct pre-initiation complex formation, the initiation

factors disassociate and the large subunit (LSU) binds to complete the monosome, allowing the elongation phase to begin.

In bacteria the pre-initiation complex is composed of an mRNA bound by the SSU, initiator $tRNA^{fMet}$, and all three initiation factors. Following ribosome dissociation and IF3 binding to the SSU, canonical mRNA start codons are positioned at the P-site of the SSU, often relying on cognate pairing of the Shine-Dalgarno sequences to the 16S rRNA, with the assistance of IF3. Subsequently, GTP-bound IF2 binds to the pre-initiation complex. The last initiation factor to bind is IF1, which stabilises the pre-initiation complex and enhances the function of IF2 and IF3. Once all the initiation factors are bound, IF2 recruits the initiator $tRNA^{fMet}$. Dissociation of IF3 is required for the binding of the 50S subunit to the 30S preinitiation complex, to form the 70S initiation complex. The rate of IF3 dissociation and 50S docking is increased on canonically assembled pre-initiation complexes, indicating the importance of the coordinated assembly of all the initiation components (Antoun *et al.*, 2006). A series of reactions, including GTP hydrolysis on IF2 and the conformational rotation of the subunits, completes the initiation and allows the elongation phase of translation to begin.

Translation initiation in mammalian mitochondria requires mRNA bound SSU, initiator $tRNA^{fMet}$, MTIF2 and MTIF3. After disassociation of the ribosomal subunits, the SSU is free for preinitiation-complex formation. The SSU is bound by mRNA and MTIF3, where MTIF3 destabilises the initiation complex if $tRNA^{fMet}$ binds without an mRNA (Rudler *et al.*, 2019). Subsequently, the MTIF2-GTP complex is recruited and MTIF2 recognises the correct initiator $tRNA^{fMet}$ and facilitates its joining. MTIF3 then disassociates in a way that is currently not known but may involve the unique features of its terminal extensions (Haque *et al.*, 2008). Once MTIF3 dissociates, the LSU can bind, completing the initiation complex. GTP hydrolysis to GDP releases MTIF2 and allows the complex to progress to the elongation phase.

Experimental insight into translation initiation complex formation in yeast mitochondria is still lacking, although it is possible that this system relies on a combination of mechanisms operating in the bacterial and mammalian mitochondrial translation systems. Although Aim23 is not essential in yeast, since its loss only causes imbalanced protein synthesis (Kuzmenko *et al.*, 2016), it was found to bind to the SSU (Chicherin *et al.*, 2019), although its mechanistic role is unknown. Subsequent to the recruitment of mRNA to the SSU, mIF2 functions to recognise the correct tRNA (Kuzmenko *et al.*, 2014), possibly with the help of

Rsm28p (Williams *et al.*, 2007). Several other factors may then assist in delivery of mRNAs such as Rmd9p (Nouet *et al.*, 2007), in addition to translational activators assisting in recruiting specific mRNAs to the ribosomes for translation to proceed.

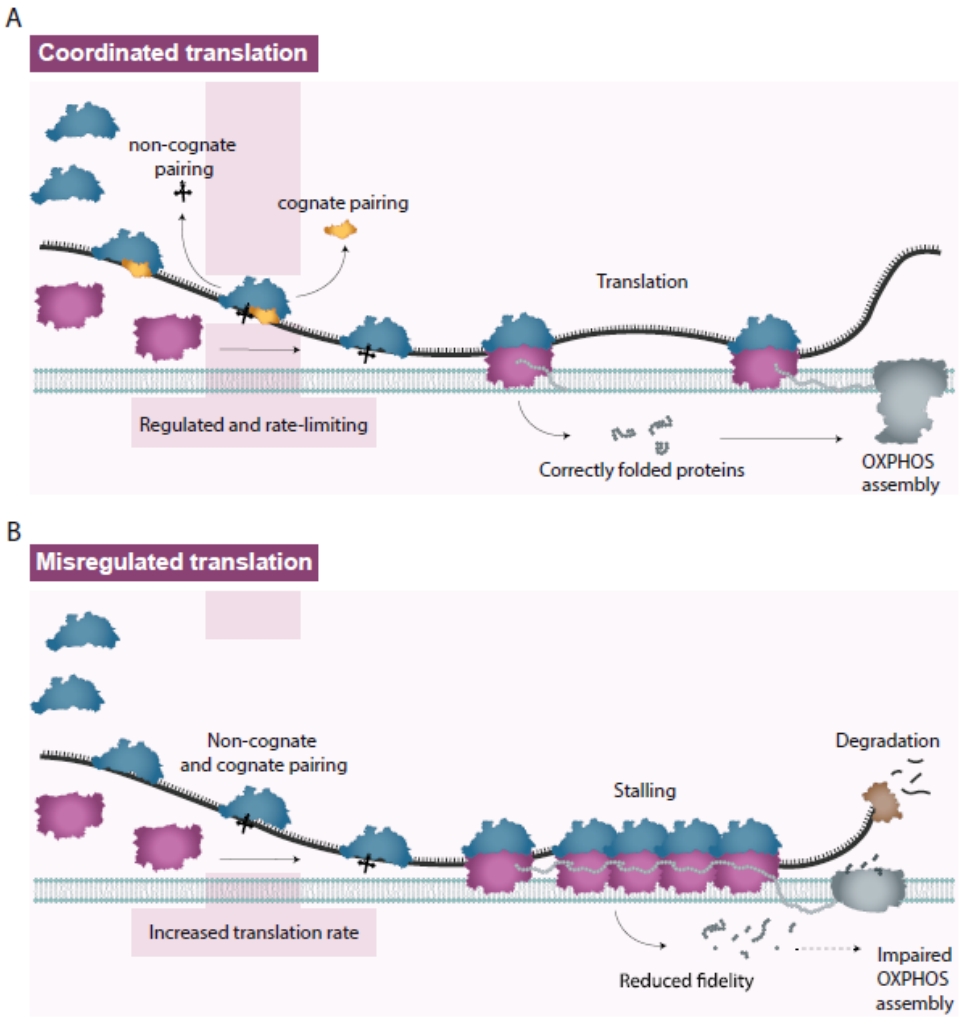


Figure 3. Coordinated regulation of mitochondrial protein synthesis is required for the assembly of the energy producing molecular machines. (A) MTIF3 prevents the translation initiation complex formation if it is bound by a tRNA in the absence of mRNA. Only small ribosomal subunits that have bound mRNA before the recruitment of tRNA and MTIF2 are able to proceed from translation initiation to elongation. This proofreading by MTIF3 coordinates the fidelity of protein synthesis of OXPHOS assembly required for ATP production. **(B)** MTIF3 performs molecular proofreading and in its absence translation initiation proceeds at an accelerated rate but at the expense of fidelity. When fidelity of initiation is compromised initiation complexes can stall at the start of mRNAs, leaving the remainder of the mRNA prone to degradation. This results in reduced stability of mitochondrially encoded OXPHOS subunits and impaired assembly of the respiratory chain compromising ATP production.

Biography

Aleksandra Filipovska is a Professor and NHMRC Senior Research Fellow at the University of Western Australia, the Harry Perkins Institute of Medical Research and Telethon Kids Institute in Perth, Australia. She received her PhD from the University of Otago, New Zealand and she was a NZ Foundation for Research, Science and Technology Fellow at the MRC Mitochondrial Biology Unit in Cambridge, the United Kingdom. She established her group in 2006 focusing on the regulation of gene expression by RNA-binding proteins and the use of animal models and multi-omic technologies to elucidate their molecular functions in health and disease. Her research group uses genomic technologies to design new models of metabolic and cardiovascular diseases and develops treatments for these disorders.