Review

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Translation termination in human mitochondria – substrate specificity of mitochondrial release factors

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Abstract: Mitochondria are the essential players in eukaryotic ATP production by oxidative phosphorylation, which relies on the maintenance and accurate expression of the mitochondrial genome. Even though the basic principles of translation are conserved due to the descent from a bacterial ancestor, some deviations regarding translation factors as well as mRNA characteristics and the applied genetic code are present in human mitochondria. Together, these features are certain challenges during translation the mitochondrion has to handle. Here, we discuss the current knowledge regarding mitochondrial translation focusing on the termination process and the associated quality control mechanisms. We describe how mtRF1a resembles bacterial RF1 mechanistically and summarize in vitro and recent in vivo data leading to the conclusion of mtRF1a being the major mitochondrial release factor. On the other hand, we discuss the ongoing debate about the function of the second codon-dependent mitochondrial release factor mtRF1 regarding its role as a specialized termination factor. Finally, we link defects in mitochondrial translation termination to the activation of mitochondrial rescue mechanisms highlighting the importance of ribosome-associated quality control for sufficient respiratory function and therefore for human health.

Keywords: COX1 translation; mitoribosome-associated quality control; mitoribosome rescue; mtRF1; mtRF1a; non-canonical stop codons

1 Introduction

Mitochondria are essential organelles of eukaryotic cells well-known for their function in energy production via oxidative phosphorylation (OXPHOS). Deriving from an endosymbiotic event with a proteobacterial ancestor and an eukaryotic host cell (Martijn et al. 2018; Roger et al. 2017), mitochondria retained only a fraction of mitochondrial DNA (mtDNA) while most of the genetic information was transferred into the nucleus (Petrov et al. 2018). All proteinaceous components of the mitochondrial ribosome (mitoribosome) and assisting translational factors are transcribed in the nucleus, translated by cytosolic ribosomes and need to be transferred into mitochondria via dedicated import machineries (Pfanner et al. 2019). Additionally, also the majority of OXPHOS subunits must be imported. Hence all of these multi-subunit complexes, except complex II which is completely nuclear-encoded, are of dual genetic origin. OXPHOS components can further assemble into macromolecular supercomplexes (respiratory chain supercomplexes, RSC), which appear to confer functional advantages to the OXPHOS machinery. The extent of their formation can be adapted to the energy demand of the cell (Cogliati et al. 2021; Milenkovic et al. 2017). Almost one fifth of the mitochondrial proteome is dedicated for proper expression of the mtDNA and its maintenance (Morgenstern et al. 2021). Malfunction of the mitochondrial translation machinery leads to a heterogeneous group of mitochondrial diseases characterized, inter alia, by a dysfunctional OXPHOS system (Gorman et al. 2016). Therefore, understanding the architecture and function of the mitoribosome itself and the distinct steps of protein synthesis aided by mitochondria-specific translation factors is of high scientific interest and clinical relevance.
2 Maturation of mitochondrial transcripts and characteristic features of mitochondrial mRNAs

Even though the basic mechanistic steps of translation are conserved from bacteria, there are certain mitochondrial-specific features of the translation factors that have been adapted to the needs of the evolved mitoribosomes. This can either be the acquisition of mitochondria-specific insertions; the loss of certain factors accompanied by transfer of its function to another; or gene duplications of factors which function then even further specialized. All these alterations aim to ensure proper function of this high-fidelity process (Kummer and Ban 2021; Nadler et al. 2022b).

The highly condensed, intron-less mitochondrial genome is transcribed as two long, polycistrionic transcripts and encodes for two mitochondrial ribosomal RNAs (12S and 16S mt-rRNA) and 22 transfer RNAs (mt-tRNA) besides the 13 mitochondrial transcripts with the exception of \( MT-ND6 \) is transcribed as two long, polycistronic transcripts and encodes for two mitochondrial ribosomal RNAs (12S and 16S mt-rRNA) and 22 transfer RNAs (mt-tRNA) besides the 13 encoded for two mitochondrial ribosomal RNAs (12S and 16S mt-rRNA) and 22 transfer RNAs (mt-tRNA) besides the 13 members of the FASTK protein family (Antonicka and Shoubridge 2015; Jourdain et al. 2015; Ohkubo et al. 2021). Further posttranscriptional maturation like methylation and pseudouridylation of certain mt-tRNA and mt-rRNA bases is required to ensure their correct folding and stability (Bohnsack and Sloan 2018). The addition of the conserved 3′ CCA tail by the nucleotidyltransferase TRNT1 is required for attaching the respective amino acid as well as for the correct positioning within the mitoribosome and is essential to complete the maturation of the tRNAs (Nagaike et al. 2001; Sasarman et al. 2015).

Importantly, mt-mRNAs harbor some characteristic features and also the codon usage differs in mitochondria (Rackham et al. 2012; Temperley et al. 2010b). The majority of mitochondrial transcripts with the exception of \( MT-ND6 \) is getting polyadenylated at the 3′ terminus by the mitochondrial poly(A) polymerase (mtPAP). In contrast to bacteria, polyadenylation does not mark mitochondrial transcripts for degradation, however the role of this 3′ modification in human mitochondria is not completely understood. Nevertheless, it is requisite to complete the stop codon UAA of seven mt-mRNAs, thus creating a functional mRNA, and it appears to be required for the maturation of certain mt-tRNAs (Bratic et al. 2016; Chang and Tong 2012; Levy and Schuster 2016; Pearce et al. 2017; Temperley et al. 2010b). The stability of mt-mRNA is enhanced by the interaction with the RNA-binding protein (RBP) LRPPRC, which in turn is stabilized by its co-factor SLIRP (Sasarman et al. 2010; Siira et al. 2017). In this way, this complex not only provides further access for mtPAP, but also prevents cleavage of the mt-mRNA by the mitochondrial degradosome, consisting of the phosphorolytic exonuclease PNPase (PNPT1) and the ATP-dependent helicase SUV3. By controlling RNA abundance and removing antisense and potentially toxic RNA fragments like double-stranded mtDNA, whose release into the cytosol would otherwise unintentionally activate the innate immune system, the degradosome is implicated in gene expression and regular RNA turnover (Borowsk i et al. 2013; Dhir et al. 2018; Wang et al. 2009; Wang et al. 2014).

Mitochondrial mRNAs lack significant 5′ and 3′ untranslated regions (UTR). Therefore, elements like a Shine-Dalgarno sequence or a Kozak sequence and a 5′ 7-methylguanosine cap, which are crucial for translation initiation in prokaryotes or the eukaryotic cytosol, respectively, are missing in mitochondria (Montoya et al. 1981). Instead, LRPPRC together with its co-factor SLIRP is acting as a mRNA-chaperone. By binding mt-mRNAs and interacting with the mitoribosome-specific constituent mS39 and mS31, LRPPRC-SLIRP enables the engagement of the mt-mRNA with the initiation-competent mtSSU and the assembled 55S mitoribosome at the site of the mRNA channel (Aibara et al. 2020; Siira et al. 2017; Singh et al. 2022). Electrostatic interactions with the positively-charged residues of uS5m, another mitochondrion-specific protein and part of the mRNA channel, further drags the mRNA into the mitoribosome until the start codon is positioned in the mitoribosomal P-site. Complementary pairing with the anticodon of the initiator tRNA sets the mitoribosome into the correct reading frame (Aibara et al. 2020; Kummer et al. 2018). To date only one auxiliary factor enhancing the initiation of a mt-mRNA is identified: TACO1 is a specific activator for translation of the core component of complex IV, COXI (Weraarpachai et al. 2009). TACO1 interacts with \( MT-COI \) (mt-mRNA encoding for COXI) and associates with the mitoribosome, thereby enhancing the translation of COXI by a yet unknown mechanism (Richman et al. 2016; Weraarpachai et al. 2009).

Remarkably, the otherwise conserved universal code differs in mitochondria and mt-mRNAs apply a slightly altered codon usage (Figure 1b and d). Whereas the majority of mt-mRNA uses the common start codon AUG, also two alternative start codons AUU and AUA are present in human
mitochondria (Montoya et al. 1981). Another alteration of the mitochondrial codon usage is the reassignment of the universal stop codon UGA to encode for tryptophan (Barrell et al. 1979). While UAG and UAA still serve as conventional stop codons, two other codons, namely AGA and AGG, have no cognate tRNA and are therefore implemented as

Figure 1: The role of human mtRF1a and mtRF1 during translation termination. (a) Overview of mitochondrial translation – from mtDNA to OXPHOS proteins. The mitochondrial DNA (mtDNA, left) encodes for 2 mitochondrial rRNAs (12S and 16S, shown in light blue), 22 tRNAs (dark grey) and 13 subunits of the OXPHOS machinery, which also form macromolecular respiratory chain supercomplexes (RSC) composed of complex I, III and IV (right). The respective genes, mRNAs and corresponding complexes are colored as followed: green for complex I, orange for complex III, blue for complex IV and pink for complex V. The nuclear-encoded complex II is depicted in yellow. Non-coding region is shown in light grey. The UTRs of mRNAs are depicted in medium grey and the poly A tails in dark red. Mitochondrial translation is facilitated by mitoribosomes, which co-translationally insert the nascent polypeptides into the inner mitochondrial membrane. Depicted is a termination complex with a stop codon in the mitoribosomal A-site. There are four mitochondrial stop codons: two canonical stop codons (UAA, UAG) and two unconventional stop codons (AGA, AGG). Panels (b) and (d) show the different specificities of the two codon-dependent mitochondrial release factors regarding stop codon recognition. While mtRF1a is the major mitochondrial release factor by being able to recognize stop codons of all mitochondrial mRNAs except of MT-CO1, mtRF1 is specifically required to terminate translation of COX1. The termination of MT-ND6 at AGG codon by mtRF1 requires further investigation. Panels (c) and (e) illustrate the consequences of the loss of the respective release factor. Ablation of mtRF1a leads to combined OXPHOS deficiency and to the accumulation of COX1-containing MITRAC complexes as complex IV assembly is stalled due to the lack of additional complex IV subunits like COX2 or COX3. Loss of mtRF1 reduces the storage of newly-synthesized COX1 in MITRAC, but ensures the formation of respiratory supercomplexes by activating C12ORF65-based mtRQC.
alternative stop codons at the end of the open reading frame of MT-CO1 and MT-ND6, respectively (Anderson et al. 1981). A controversial debate about the purpose of these two non-standard codons ensued. It was suggested that a dedicated release factor might recognize them and thus is responsible for the release of the two affected peptides (Soleimanpour-Lichaei et al. 2007; Young et al. 2010). Another possible hypothesis is a shift of the mitoribosome in the reading frame. Since both unconventional stop codons are preceded by a uridine, a −1 frameshift would lead to termination in a conventional UAG stop codon (Temperley et al. 2010a).

3 Mitochondrial translation initiation and elongation

As this review mainly focuses on translation termination, we refer for the process of translation initiation and elongation to other recent comprehensive reviews (Kummer and Ban 2021; Nadler et al. 2022b). Briefly, translation starts with the step-wise assembly of the mitoribosomal subunits together with the mRNA and the initiator fMet-tRNA^Met to mature the initiation complex (IC). Unlike bacteria, mitochondria only have two initiation factors: mtIF2 and mtIF3. The role of mtIF3 appears to differ from its bacterial homolog: mtIF3 acts as a recycling factor as well as anti-association and assembly factor (Itoh et al. 2022; Khawaja et al. 2020; Kummer et al. 2018; Remes et al. 2023). Once a translation-competent IC has formed, polypeptide elongation can take place via a cycle of three distinct, highly conserved steps: decoding of the mRNA base triplet by delivery of a cognate acylated (aa-) tRNA, peptide bond formation, and translocation of the mRNA-tRNA complex. It requires three mitochondrial elongation factors: mtEFTu, mtEFTs and mtEFG1 (Desai et al. 2020; Koripella et al. 2020; Kummer and Ban 2020).

4 Mitochondrial translation termination

To terminate translation a release factor (RF) has to recognize sequence-specifically the stop codon, which is lacking a cognate tRNA, followed by the release of the nascent polypeptide. Although the basic principles of translation termination are conserved in human mitochondria, some unique aspects of mt-mRNAs and mitochondrial RFs challenge our understanding. Four RFs that harbor the highly conserved GGQ motif, which is crucial to facilitate peptide hydrolysis (Frolova et al. 1999; Mora et al. 2003), have been identified in human mitochondria: mtRF1, mtRF1a, ICT1 (mL62) and C12ORF65 (mtRF-R) (Antoniccka et al. 2010; Duarte et al. 2012; Handa et al. 2010; Richter et al. 2010; Soleimanpour-Lichaei et al. 2007; Zhang and Spremulli 1998). However, only mtRF1 and mtRF1a have a so-called decoding domain and are supposed to cognon-dependent recognize and bind stop signals. As all translation factors passively test whether their specific action is required, highly accurate discrimination between stop and sense codons is essential to consequently only release mature polypeptides. If a stop codon is sensed correctly, the RF binds to it with high affinity (Freistroffer et al. 2000). Like their bacterial counterpart RF1, which senses UAG and UAA stop codons, mtRF1 and mtRF1a share the codon recognition domain consisting of the PxT motif and the tip of the α5 helix (Ito et al. 2000; Laurberg et al. 2008; Petry et al. 2005). However, whereas mtRF1a shows a great sequence similarity compared to RF1 regarding the codon recognition domain, namely a PKT motif and comparable amino acids within the α5 helix, two insertions in the respective domains of mtRF1 raised doubts about the function of mtRF1 as a canonical RF (Lind et al. 2013; Soleimanpour-Lichaei et al. 2007). In vitro termination assays demonstrated that mtRF1a is able to terminate translation on UAG and UAA stop codons supporting its role as the major mitochondrial RF (Nozaki et al. 2008; Soleimanpour-Lichaei et al. 2007). Contemplating the potential −1 frameshift, this factor would be able to terminate all 13 open reading frames (Temperley et al. 2010a). Yet, definite evidence was lacking as no in vivo results could confirm this assumption (Soleimanpour-Lichaei et al. 2007). Only quite recently, the long-postulated role of mtRF1a as canonical mitochondrial RF has structurally and biochemically been verified (Figure 1b) (Kummer et al. 2021; Nadler et al. 2022a). The decoding and peptide release mechanism is highly similar to the one of bacterial RF1 reading UAG and UAA stop codons (Laurberg et al. 2008; Petry et al. 2005). In vitro reconstitution of the termination complex and subsequent structural analysis via cryo-electron microscopy (cryo-EM) showed that contacts of critical residues of the decoding motifs of mtRF1a with the stop codon are only possible with a uridine at the first and an adenine in the second position. Stacking interactions with the essential G256 residue of the 12S rRNA and further residues of mtRF1a with the third stop codon position allow the presence of either another adenine or guanosine (Kummer et al. 2021). Binding to a stop codon at the DC of the mtSSU causes conformational changes within the so-called switch loop of the RF, which liberates the GGQ-containing domain III, usually tethered at the decoding domain. The rearrangement causes elongation of the factor so that the GGQ motif can reach into the PTC of the mtLSU. Here, it binds close to the 3′ CCA end of the P-site tRNA in a way that a single water molecule...
can nucleophilically attack the ester bond between the tRNA and the nascent polypeptide chain subsequently releasing the protein (Korostelev 2021; Kummer et al. 2021). Ablation of mtRF1a in HEK293 cells confirmed in vivo that this factor is critical for translation termination in human mitochondria and thus ensures correct respiratory function (Nadler et al. 2022a). Loss of mtRF1a leads to highly disturbed de novo translation of almost all mitochondrial-encoded proteins and consequently completely prevents assembly of OXPHOS complexes I, III, IV and V. Lack of aerobic respiration forces the cells to produce energy via glycolysis only, hence explaining the poor growth phenotype (Figure 1c) (Nadler et al. 2022a).

Surprisingly, mtRF1a is not required to terminate translation of MT-COI. Instead, it was recently demonstrated that mtRF1 is the RF responsible to terminate translation of this key component of complex IV, the terminal electron acceptor of the respiratory chain (Figure 1d) (Krüger et al. 2023; Nadler et al. 2022a). However, the function of mtRF1 is still greatly discussed. It was questioned for a long time whether this factor is either involved in (in-)directly terminating the non-standard stop codons AGA and AGG or acts as a rescue factor on stalled ribosomes with an empty A-site (Duarte et al. 2012; Huynen et al. 2012; Soleimanpour-Lichaei et al. 2007; Young et al. 2010). Two recent publications demonstrated that indeed, mtRF1 is a genuine mitochondrial RF, but came to different conclusions regarding the specificity of the factor (Krüger et al. 2023; Nadler et al. 2022a). Either mtRF1 can directly detect the non-standard stop codons AGA and AGG, and with that can facilitate the release of COX1 and ND6 as suggested by ribosome profiling and an in vitro termination assay (Krüger et al. 2023). Alternatively, mtRF1 is a dedicated RF specialized only for the release of COX1 as demonstrated by in vivo metabolic labeling (Nadler et al. 2022a). In this case, it is possible that either mtRF1 detects the non-canonical stop codon of MT-COI by an unconventional decoding mechanism or a −1 ribosomal frameshift would allow the termination by mtRF1 in the standard stop codon UAG. The latter is actually supported by a former bioinformatic model that suggested that mtRF1 might co-evolved to adapt the changes of the also altered mt-rRNA core of the mitoribosome and is able to act as RF reading the conventional stop codons UAA and UAG, but not AGA or AGG (Lind et al. 2013; Nadler et al. 2022a). However, a very recently published structure of mtRF1 bound to a termination complex demonstrated an unconventional codon recognition mechanism: a mtRF1-specific N-terminal extension forms unique contacts with the mtLSU and its mt-rRNA, thereby stabilizing the binding of the RF (Saurer et al. 2023). The bespoken two extensions in the decoding domain of mtRF1 then alter the structure of the mt-mRNA in a way that the unconventional stop codons, both starting with an adenosine and guanosine base, can be accommodated and recognized by the RF as well as the bases of the mtSSU-rRNA and surrounding mt-mRNA residues. Recognition of the last stop codon bases resembles the commonly observed mode of third base recognition and appears to be less restricted, as both adenosine or guanosine can be tolerated (Saurer et al. 2023). Nevertheless, it is still questionable, whether mtRF1 is required for the termination of both of the transcripts with unconventional stop codons. There is agreement regarding the involvement of mtRF1 in terminating MT-COI translation (Krüger et al. 2023; Nadler et al. 2022a; Saurer et al. 2023). Yet, there are still uncertainties regarding termination of MT-ND6. Whereas decrease of ND6 in newly synthesized and steady state protein levels can be assigned to the loss of mtRF1a (Nadler et al. 2022a), there is no unambiguous evidence for the involvement of mtRF1 in ND6 translation termination. Even though ribosome profiling shows a certain occupancy at the end of the ORF of MT-ND6 (Krüger et al. 2023; Saurer et al. 2023), intensities vary to an extent where the mitoribosomal occupancy at the end of MT-ND6, reflecting translational stalling due to the loss of mtRF1, is almost the same as for a standard stop codon (Saurer et al. 2023). Furthermore, neither an effect on the protein level of ND6 nor a complex I deficiency could be observed upon mtRF1 ablation (Krüger et al. 2023; Nadler et al. 2022a). The fact that MT-COI as well as MT-ND6 terminate in classical stop codons in other vertebrate species, which also express mtRF1, indicate that the function of mtRF1 is not completely dependent on and/or restricted to non-standard stop codons (Nadler et al. 2022a).

5 COX1 – a special case in mitochondrial translation

Translation of COX1 appears to be special in several aspects. Not only is MT-COI the only transcript having a dedicated translational activator, namely TACO1, but apparently also a dedicated RF: mtRF1 (Nadler et al. 2022a; Weraarpachai et al. 2009). What the specificity-conferring element(s) might be, is the object of future research. COX1 is the core subunit of the cytochrome c oxidase of the respiratory chain and with that highly essential for the regulatory role of complex IV for the energy production via OXPHOS (Kadenbach 2021). This also explains the various cellular mechanisms to guarantee sufficient amounts of COX1 above the ‘respiratory threshold’ to keep the electron transport and with that ATP production going by rescuing COX1 translation when mtRF1 is lost (Figure 1e). The required level of COX1 is about 40–50 % of
wildtype control (D’Aurelio et al. 2006), which ensures its incorporation into the functional active RSC and thus proper respiration (D’Aurelio et al. 2006; Lobo-Jarne et al. 2020). Preferential incorporation of complex IV subunits into RSC in cases of isolated complex IV deficiency is a mechanism of the cell to stabilize existing subunits from general turnover and to keep COX1 levels above the respiratory threshold (D’Aurelio et al. 2006; Lazarou et al. 2009). This was also recently demonstrated in mtRF1-deficient cells, in which the mitoribosome-associated quality control (mtRQC; further discussed below) becomes activated (Nadler et al. 2022a) to ensure the critical COX1 threshold level. Additionally, loss of mtRF1 is accompanied by a reduction of the MITRAC12 (mitochondrial translation regulation assembly intermediate of cytochrome c oxidase) complex as a consequence of the stalled COX1 translation (Figure 1e). CI2ORF62 and MITRAC12, the two MITRAC components primary interacting with nascent COX1 and coordinating further cytochrome c complex assembly, are decreased upon loss of mtRF1, while elevated in mtRF1a-deficient cells (Mick et al. 2012; Nadler et al. 2022a; Richter-Dennerlein et al. 2016). As COX2 and COX3 are strongly diminished in mtRF1a-ablated cells, COX1 is getting stalled within MITRAC (Figure 1c). This underlines again the importance of proper COX1 translation and the role of MITRAC in coordinating the synthesis and subsequent assembly of newly synthesized COX1. A diminished level of a nuclear-encoded complex IV subunit as well as decreased levels and activity of free complex IV reflects the isolated complex IV deficiency in mtRF1-deficient cells while all other OXPHOS complexes remain unchanged, as demonstrated for complex I (Nadler et al. 2022a). This again highlights that mtRF1 is specifically required for COX1 translation and consequently for complex IV assembly, but not for the essential structural component of complex I, ND6.

6 Mitochondrial ribosome recycling

Recycling of mitoribosomes is the last step of a translation cycle, which is required to be able to engage in another round of translation and to prevent energy-consuming de novo assembly of mitoribosomal subunits (Maiti et al. 2021). For disassembly and ejection of the mRNA and deacylated tRNAs, a recycling factor (mtRF) binds to the vacant A-site, preferentially in a state where the mitoribosomal subunits are rotated against each other and the P-site tRNA is in a hybrid P/E-state, ensuring that only posttermination complexes are recycled (Koripella et al. 2019; Rorbach et al. 2008). Binding of mtRF disturbs intersubunit bridge formation and blocks further tRNA binding. Only then, mtEFG2, another paralog of bacterial elongation factor EF-G, can bind and further drive the separation of the two mitoribosomal subunits (Tsuboi et al. 2009). Interestingly, mtEFG2 sterically and electrostatically diverged from mtEFG1 in a way that it necessarily requires mtRRF to bind to the mitoribosome and with that ensures that not actively elongating but only splitting-competent mitoribosomes can be disassembled (Koripella et al. 2021; Kummer et al. 2021). Recently, an alternative ribosome recycling factor, GTPBP6, has been identified in human mitochondria. GTPBP6 is a homolog of bacterial ribosome splitting factor HfIx and actively dissociates vacant ribosomes or posttermination complexes (Hillen et al. 2021; Lavdovskaia et al. 2020). Further research is required to unveil the physiological conditions under which GTPBP6-mediated ribosome recycling is required.

7 Mitochondrial ribosome rescue mechanisms

In cases where translation cannot resume due to various reasons like aa-tRNA starvation, truncated mRNAs or problematic secondary structures and/or sequences, the mitoribosome pauses or is getting stalled, respectively. In the latter case, the ribosome needs to be rescued and the aberrant protein and potentially truncated mRNA need to be eliminated. These quality control mechanisms are essential for proper cellular function as accumulation of malfunctioning peptides and mRNAs is detrimental for the cell. Bacteria and eukaryotes developed several rescue systems to be able to react to different scenarios (Keiler 2015; Nürenberg-Goloub and Tampé 2019). Trans-translation based on tmRNA and SmpB, which acts in non-stop situations where translation is arrested during elongation or termination due to the lack of a codon in the A-site, is the main rescue pathway in bacteria and is present in most bacteria (Karzai et al. 1999; Keiler et al. 1996; Keiler 2015). Ribosome rescue mediated by the alternative rescue factor A (Arfa) or B (ArfB) as well as ribosome-associated quality control (RQC), long-known in eukaryotes but also relatively recently discovered in prokaryotes, serve as backup mechanisms or under certain stress conditions and are partially redundant (Crowe-McAuliffe et al. 2021; Filbeck et al. 2022; Joazeiro 2019; Ltvynenko et al. 2019).

In mitochondria, there are two mitoribosome rescue pathways driven by the two mitochondrial RFs without codon recognition motifs: ICT1 and CI2ORF65. Being a homolog of ArfB, ICT1 with its long, positively-charged C-terminal extension can detect non-stop mitoribosomes with an empty mRNA.
channel and vacant A-site. When bound to the mitoribosomal A-site, ICT1 facilitates the release of the premature polypeptide from the peptidyl tRNA by placing its GGQ motif into the PTC (Feaga et al. 2016; Handa et al. 2010; Kogure et al. 2014; Kummer et al. 2021; Richter et al. 2010). The absence of mRNA in the decoding site is mandatory for the binding of ICT1 and therefore a function of ICT1 as alternative release factor for AGA and AGG stop codons, as it was initially postulated (Akabane et al. 2014), can be excluded. Furthermore, ICT1 (here referred to as mL62) is also an integral part of the mitoribosome (Brown et al. 2014; Greber et al. 2014; Richter et al. 2010) located in the central protuberance. However, only an extraribosomal copy can enter the ribosomal A-site and thus alleviate stalled translation. Whether ICT1-mediated ribosome rescue is required under physiological or specific stress conditions remains to be addressed.

Mitoribosome-associated quality control (mtRQC) is reminiscent of cytosolic or prokaryotic RQC and is required when ribosomes are trapped in no-go complexes with intact mRNA and peptidyl tRNA in the P-site, for example if aa-tRNAs are lacking or if elongation or termination is inhibited. In this case, C12orf65, together with its co-factor MTRES1, can rescue stalled protein synthesis via binding at the A-site close to the P-site tRNA on the split mtLSU (Figure 1e), which is prevented from re-association by an anti-association module consisting of MALSU1-mtACP-L0RF8 (Desai et al. 2020). However, the GGQ motif, which is required for C12orf65 function and highly conserved in all release factors (Ng et al. 2022; Richter et al. 2015), was quite far from the PTC in the pre-hydrolysis complex suggesting that further conformational changes are actually required to facilitate the release of the nascent peptide chain. Nevertheless, using a hybrid in vitro system to test C12orf65 and MTRES1 on stalled 70S monosomes or split 50S LSU with a peptidyl-tRNA clearly demonstrated that C12orf65 together with MTRES1 can facilitate peptide hydrolysis on split LSU, but not on 70S ribosomes (Desai et al. 2020). Hence, dissociation of the no-go mitoribosome complex is a necessary but mechanistically still unknown prerequisite. It is tempting to speculate that either the canonical recycling system composed of mtRF1 and mtEF2 or the alternative recycling factor GTBP6 are needed in this step. However, as both systems are shown to only act on mitoribosomes having a deacylated tRNA in the P-site, additional alternative factors might be involved (Hillen et al. 2021; Kummer et al. 2021; Lavdovskaia et al. 2020). As it was recently demonstrated, C12orf65-mediated mtRQC appears to be the mechanism responsible for rescuing COX1 translation in mtRF1-ablated cells. In absence of mtRF1, C12orf65 is elevated and apparently activated. The depletion of C12orf65 in mtRF1-deficient cells reduces the levels of newly synthesized COX1 further indicating that mtRQC ensures respiratory competent levels of COX1 upon loss of mtRF1 (Nadler et al. 2022a). Depletion of mt-mRNAs of the respective transcripts is most likely a precaution to minimize stalling events and hence to avoid an overload of the rescue system (Nadler et al. 2022a). Whether C12orf65, next to alleviating no-go complexes, might also be involved in rescuing no-stop mitoribosomal complexes stalled on aberrant and/or truncated mRNA, as recently suggested by deep-sequencing and metabolic labeling, is currently under debate (Ng et al. 2022). However, since this mode of action is mutually exclusive with acting on the split mtLSU originating from stalled 55S with intact mRNA, further supporting evidence such as structural analysis of C12orf65 bound to no-stop 55S is required. Although, recent attempts to reconstitute such a scenario failed under chosen conditions (Kummer et al. 2021).

Nevertheless, the clinical relevance of mtRQC is demonstrated by an increasing number of patients with mutations in the gene encoding for C12orf65 presented with OXPHOS deficiency-related symptoms (Antonicka et al. 2010; Perrone et al. 2020; Wesolowska et al. 2015), highlighting the importance of C12orf65 as mitochondrial rescue factor per se and the need for mitochondrial quality control for human health.

8 Concluding remarks

During the last years, structural studies and CRISPR/Cas9-mediated knockout models contributed tremendously to the progress in understanding the distinct steps of mitochondrial translation, especially the hitherto relatively ill-defined termination step. Although many aspects are reminiscent of the bacterial system, many factors evolved from their bacterial ancestors to adapt to the changes in the mitochondrial translation apparatus and to ensure accurate translational fidelity. Mitochondrial translation concerns one critical process in the eukaryotic cell: energy production via oxidative phosphorylation. Particularly mitochondrial translation termination was for more than two decades a black box regarding possible redundancy of two similar RFs and their mode of action. It is now proofed structurally and in vivo that mtRF1a is the canonical mitochondrial RF responsible for termination of the majority of mitochondrial transcripts as it was suggested by bioinformatic analysis and in vitro release assays (Kummer et al. 2021; Lind et al. 2013; Nadler et al. 2022a; Soleimanpour-Lichaei et al. 2007). Recent publications also shed light on the role of mtRF1 as proper mitochondrial RF (Krüger et al. 2023; Nadler et al. 2022a; Saurer et al. 2023). However, there is still some disagreement regarding the codon-reading qualities and the substrate specificity of mtRF1. High-resolution structures demonstrate
the capability of mtRF1 to recognize non-canonical stop codons AGA and AGG, respectively (Saurer et al. 2023). However, isolated complex IV deficiency as a result of a specific COX1 translation defect and unaltered ND6 synthesis with stable complex I in mtRF1-deficient cells, raises the question whether mtRF1 is really responsible for MT-ND6 termination, reading AGG codon, particularly if mtRF1a loss results in severe ND6 reduction (Krüger et al. 2023; Nadler et al. 2022a). Here, also knockouts in different species can provide insights into the real purpose of mtRF1. As this factor is evolutionary conserved in vertebrates but apparently the postulated unconventional stop codons AGA and AGG are not, assessing the results obtained from human knockout experiments in other species can be a valuable finding for further understanding of the function of mtRF1.

Additionally, our knowledge regarding the precise steps of the mtRQC mechanism(s) is far from complete. How are the complexes initially ‘prepared’ so that an available substrate for the mtRQC is generated? Which factors are responsible for the degradation of mitochondrial mRNAs? How are aborted translation products labelled for degradation and which machinery is responsible for the elimination of these polypeptides? It is tempting to speculate that a tagging mechanism as seen for cytosolic or prokaryotic RQC, where C-terminal alanine and threonine or alanine tails, respectively, are added to the aberrant polypeptide to mark them for degradation, is taking place in human mitochondria as well. Further investigations are required to provide a complete picture concerning the molecular basis of translation termination and associated quality control mechanisms within these essential organelles, which would also help to understand the complex nature of associated diseases.

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F. Nadler and R. Richter-Dennerlein: Translation termination in human mitochondria


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