MINIREVIEW

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Termination of Protein Synthesis in Mammalian Mitochondria*

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All mechanisms of protein synthesis can be considered in four stages: initiation, elongation, termination, and ribosome recycling. Remarkable progress has been made in understanding how these processes are mediated in the cytosol of many species; however, details of organellar protein synthesis remain sketchy. This is an important omission, as defects in human mitochondrial translation are known to cause disease and may contribute to the aging process itself. In this minireview, we focus on the recent advances that have been made in understanding how one of these processes, translation termination, occurs in the human mitochondrion.

The synthesis of proteins is a fundamental mechanism of life. It is clearly essential that the process of mRNA translation, from which proteins are generated, is accurate and well controlled. Translation consists of four stages. The first is initiation, in which a repertoire of initiation factors coordinates the association of mRNA with the ribosomal subunits, recognition of the start codon, and its alignment with an fMet-tRNA^{Met} in the ribosomal P-site. The second stage is elongation, which is facilitated by the action of elongation factors. These act in concert, causing the mRNA to move through the ribosome in steps corresponding to three nucleotides commonly referred to as a codon (1). This stepwise progression allows each codon to be decoded by the cognate aminoacylated tRNA, with the consequent addition of the corresponding amino acid to the nascent polypeptide (2). The third stage is termination, which occurs when a stop codon arrives in the ribosomal A-site and is recognized by a *trans*-acting protein termed a translation release (RF)[2] or termination factor. This acts in a ribosome-dependent manner, resulting in the nascent polypeptide being separated from the P-site tRNA, allowing the newly synthesized protein to be released from the ribosome. The final stage is that of ribosome recycling, in which the large (LSU) and small (SSU) ribosomal subunits are separated, and the mRNA is released so that the components can be used in a fresh cycle of translation.

Extensive research on these individual steps has shown that, across the three domains of life, there are differences in both the *cis*- and *trans*-acting factors involved in carrying out these processes, resulting in mechanistic variations. Moreover, investigations into intraorganellar protein synthesis suggest that there are further subtleties to this process, with differences even between organelles from different organisms. To illustrate how the systems can differ as well as retain similarities, this minireview will concentrate on a single step in this process, namely translation termination, and focus on how mitochondria have organized their machinery to accomplish this process.

Termination

Termination of translation occurs when a stop codon becomes positioned in the ribosomal A-site and is decoded by a protein moiety. This *trans*-acting factor (an RF) acts in an analogous fashion to the tRNAs, as it shows sequence-specific recognition (Fig. 1*A*). The crucial difference from the mechanism of elongation is that the codon is recognized solely by a proteinaceous factor rather than a tRNA. Discrimination of the A-site codon is accomplished by the interactions of two domains within the RF that are brought into apposition in the tertiary structure, entering the decoding center (DC) of the ribosome at the interface of the SSU and LSU. On recognition and interaction between the RF and the A-site stop codon, the third RF domain containing the GGQ motif swivels into the peptidyl transferase center (PTC) (Fig. 1*B*) (3). This arrangement facilitates the hydrolysis of the ester bond between the last decoding tRNA that is still in the P-site and the completed polypeptide. This cleavage allows the nascent protein to complete its migration out of the exit tunnel, leaving a ribosome with a deacylated tRNA in the P/E-site (Fig. 1*C*) (2).

Mitochondrially Encoded Translation Termination Codons Vary between Species: Who Uses What?

With the extensive availability of genomic and transcriptomic sequences, the gene organization of many mitochondrial genomes has now been analyzed and compared with the standard genetic codon usage. Among the identified variations are changes to or from the three canonical stop codons (UAA, UAG, and UGA) that are used in eu/archaebacteria, plant plastids, and the eukaryotic cytosol (4–6). Some examples of these variants are given below.

In addition to the three standard termination codons, *Thraustochytrium* (marine protist) mitochondrial DNA is predicted to utilize UUA as a fourth termination codon (Table 1) (The Organelle Genome Megasequencing Program (OGMP) and Ref. 8). The unicellular green alga *Scenedesmus obliquus* has retained a three-stop codon system, but only two of the standard stop codons are used, UGA/UAA, with UCA converted to a novel stop codon and UAG recoded as a leucine (9). In contrast, analysis of ORFs in mitochondrial DNA from the vast majority of organisms indicates a reduction from the standard three to two stop codons, retaining UAA/UAG but disposing of UGA, which is decoded by a tRNA^{Trp} (TriTrypDB

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[2] The abbreviations used are: RF, release factor; LSU, large ribosomal subunit; SSU, small ribosomal subunit; DC, decoding center; PTC, peptidyl transferase center; mt, mitochondrial; aRF, archaebacterial RF; eRF, eukaryotic RF.

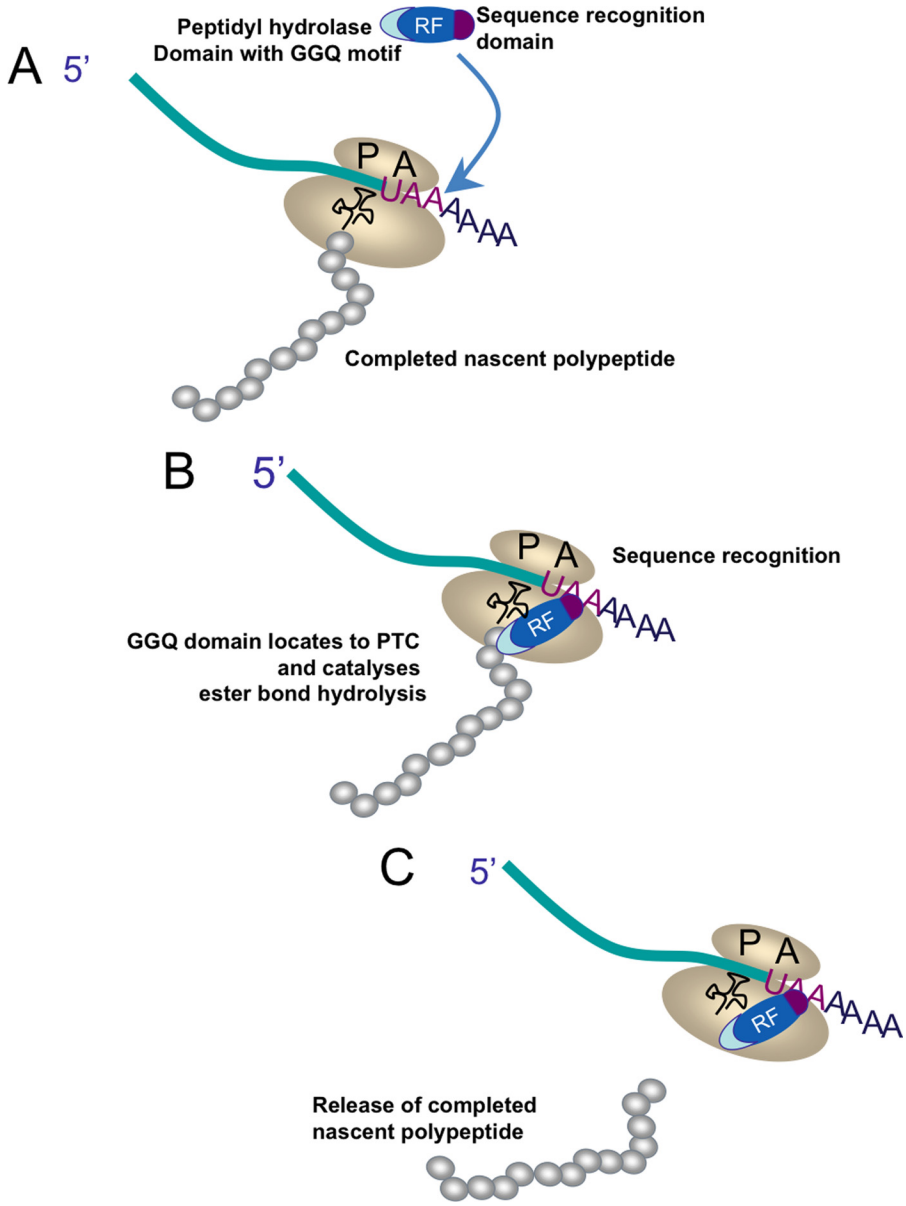

FIGURE 1. **Schematic illustrating the action of an RF during translation termination.** *A*, a ribosome that has completed protein synthesis and translocated the stop codon to the A-site. The RF has two critical functional domains; one is required to recognize the stop triplet, and the other, which on correct positioning in the PTC, facilitates the hydrolysis of the ester bond between the terminal P-site tRNA and the nascent polypeptide. *B*, the correct insertion and spanning of these two RF functional domains that are ~70 Å apart when in the ribosome. *C*, the completed protein has dissociated from the tRNA and is released from the ribosome, and in all probability, this is accompanied by specific chaperones to ensure correct folding and association with partner proteins (not depicted here).

Database and Refs. 10–12). Chlorophycean (freshwater green algae) mitochondrial DNA also uses only two stop codons, retaining, in this instance, UAA and UGA, with UAG being recognized by $tRNA^{Leu}$ (14, 15). Although there are conflicting opinions, it has been suggested that flatworms and roundworms have diminished their mitochondrial stop codon usage to a single triplet, retaining UAG, with UAA and UGA recoded as tyrosine and tryptophan, respectively (16, 17). Transcriptomic data from the nematode *Radopholus similis* suggest that the system has been minimized further, with 7 of the 12 ORFs lacking any canonical stop codon. Moreover, the lack of transcript polyadenylation implies that these could not be generated post-transcriptionally (18).

This very brief summary illustrates how the *cis*-acting elements determining translation termination have diverged from the bacterial and eukaryotic cytosol but also quite considerably between mitochondria from different organisms (Table 1). It is not simply in the number of stop codons, from an increased quota of four down to one single stop codon or possibly no stop codon at all, but also in the choice of nucleotide triplets that can act as stop codons and to which amino acid the redundant stop will be recoded (6, 11). The majority of these mtDNA stop codons have been determined by analysis of DNA sequences and predicted ORFs. Analysis of the DNA sequence alone can, however, be misleading. The mtDNA of Atlantic codfish has been analyzed, and the length of the ORFs inferred, accepting AGA as a stop codon. Later analysis of experimentally determined mitochondrial (mt) mRNA sequences from these gadid species, together with the poly/oligo(A) status, revealed that the transcripts were shorter than had been predicted and that only

TABLE 1
Variations in stop codon triplets
Sources are given in text.

Organism/compartment	Stop codon usage					
	UAA	UAG	UGA	UCA	UUA	Other
Eubacteria	TER[a]	TER	TER			
Archaebacteria	TER	TER	TER			
Eukaryotic cytosol	TER	TER	TER			
Yeast mitochondria	TER	TER	**Trp**[b]			
Human mitochondria	TER	TER	**Trp**			**AGA/AGG** (unassigned)
Drosophila mitochondria	TER	TER				
Trypanosome mitochondria	TER	TER	**Trp**			
Platyhelminth/nematode mitochondria	**Tyr**	TER	**Trp**			7/12 non-canonical stop codons
Chlorophycean mitochondria	TER	**Leu**	TER			
S. obliquus mitochondria	TER	**Leu**	TER	**TER**		
Thraustochytrium mitochondria	TER	TER	TER		**TER**	

[a] TER, termination codon.
[b] Boldface type indicates deviation from standard genetic code.

TABLE 2
Alignment of GGQ motifs from RFs from selected species

Human mtRF1a	RIDTKRASGA**GGQ**HVNTTDSAVRIVHLP
Human mtRF1	RIDTFRAKGA**GGQ**HVNKTDSAVRLVHIP
Mouse mtRF1	RVDTFRARGA**GGQ**HVNTTDSAVRLVHIP
Candida glabrata MRF1	RIDVKRASGK**GGQ**HVNTTESAVRLTHIP
S. pombe MRF1	KIEVMRSRGA**GGQ**HVNRTESAVRLTHIP
Caenorhabditis elegans MRF1	KIEAMRASGP**GGQ**NVNKRSTAVRMTHKE
Drosophila melanogaster MRF1	KIETKRASGA**GGQ**HVNTTDSAVRIVHLP
Anopheles gambiae MRF1	EMQTSRSGGA**GGQ**NVNKVETKVQLTHKP
Trypanosoma brucei mtRF1	NIEFVRGSGP**GGQ**GMQSSSNAVCLTHKP
E. coli RF1	RIDTFRSSGA**GGQ**HVNTTDSAIRITHLP
Rickettsia prowazekii RF1	RIDTYRASGA**GGQ**HVNTTDSAVRITHIP
R prowazekii RF2	RIDTFRSSGA**GGQ**HVNTTDSAVRITHIP
Human ICT1	TISYCRSSGP**GGQ**NVNKVNSKAEVRFHL
Human C12orf65	EEQFVKGHGP**GGQ**ATNKTSNCVVLKHIP
Human eRF1	TVDLPKKHGR**GGQ**SALRFARLRMEKRHN
Methanococcus maripaludis aRF1	TSGVPGKFKA**GGQ**SARRLERLIDDAAHQ

canonical termination signals were used (19). It was just such visual inspection of the human mtDNA that, taken together with evolutionary comparisons, led to the assumption that the AGA/AGG triplets that are decoded as arginine in the cytosol had been reassigned as termination triplets (20, 21). This original prediction proposed that human mitochondria use an extended repertoire of four stop codons, an assumption that was adopted into the literature (16, 22–25). Recent experimental evidence has revealed, however, that human mitochondria use only UAA/UAG as stop codons (26). Taken together with the recoding of UGA to tryptophan, human mitochondria have therefore evolved the most common deviation from the standard genetic code.

As mentioned above, termination codons are not unassigned. Instead of recognition being mediated by an RNA *trans*-acting factor in the well characterized mRNA/tRNA codon/anticodon interaction, these triplets are recognized by proteins that demonstrate sequence specificity, namely class I RFs. The distinct variation in the codons that are or are not used in mitochondria from different organelles suggests that elements of the *trans*-acting RFs will need to have evolved to be able to discriminate accordingly. The features responsible for this are described below.

What Are the Protein Factors That Recognize the Stop Codons?

RFs are divided into two groups, those that demonstrate sequence-specific recognition of the mRNA (class I) and those that do not (class II). A brief description of the critical features of only class I RFs will be given here. Extensive research has been performed on RFs from eu/archaebacteria and the eukaryotic cytosol. These structural, genetic, and biochemical analyses have allowed accurate definition of the regions that are important for function and a deeper understanding of the molecular mechanisms in which they take part (1, 3, 27–31). RFs essentially have two main aspects to their activity. First is the discrimination of A-site triplets, which requires sequence-specific recognition of an appropriate stop codon in the A-site. Following such recognition, the RF remains associated with the ribosome and adopts a rigid open conformation (3, 27, 28, 32). The second RF activity is catalysis of the hydrolysis of the ester bond that links the P-site tRNA and the terminal amino acid, which effectively anchors the nascent peptide to the ribosome (33). This second activity is dependent on the first, and both are dependent on the context of the ribosome for the RF to promote peptidyl-tRNA hydrolysis.

A single RF is present in archaebacteria (aRF1) and the eukaryotic cytosol (eRF1), and each recognizes all three of the canonical stop codons used in these compartments (34, 35). Eubacteria also use the three canonical stop codons but have two RFs; both recognize UAA, but RF1 alone has specificity for UAG, and only RF2 has specificity for UGA (29, 36). Although the tertiary structure of most RFs has been described as resembling that of a folded tRNA (32, 37–39), as might be expected, the differences in codon recognition requirement are reflected in the structure and sequence of the domains responsible for this activity. Two regions are separated in the linear amino acid sequence but become apposed in the DC at the interface of the SSU/LSU and the A-site mRNA (3, 28). These two regions are the tip of the α5 helix and a tripeptide motif in domain 2 (3, 28). The latter has a consensus sequence of P*X*T for RF1 (where *X* is variable) and SPF for RF2 proteins. For eRF1, it is the conserved NIKS and Y*X*C*XXX*F motifs in the N-terminal domain that are implicated in stop codon recognition (31, 35, 40–43). Although there are differences in the amino acid sequences between eRFs and RF1/RF2 proteins, the GGQ motif that mediates peptidyl-tRNA hydrolysis is conserved throughout all the ribosome-dependent RFs (44). This motif is within a relatively conserved stretch of amino acids in domain 3 (Table 2), ensuring that once the RF is in the open conformation, the GGQ motif will be positioned in the PTC to promote peptidyl-tRNA hydrolysis by the ribosome.

How Do These Features Relate to the Requirements in the Numerous Mitochondrial Systems with Their Unusual Termination Codon Usage?

In comparison with the research on aRF1/eRF1 and the eubacterial RF1/RF2 proteins, little is known about the mitochondrial counterparts. However, alignment of amino acid sequences against non-mitochondrial RFs indicates proteins with which there is the highest identity/similarity. Thus far, the mitochondrial factors most closely resemble the eubacterial RF1-type proteins (45, 46).

Which of These Factors Actually Function to Terminate Protein Synthesis in Human Mitochondria?

In 1998, analysis of expressed sequence tags revealed a candidate for the human mitochondrial RF that appeared to correspond to a full-length RF with similarity to eubacterial RF1 (23). As a consequence, this protein was assimilated into the literature as mtRF1 and reported as the single mitochondrial RF (29, 42, 47). On alignment with RF1 and RF2, there was more similarity to RF1, but there were still clear differences in the regions now known to be responsible for sequence recognition. This appeared to be fully compatible with the perceived change in stop codon usage, with AGA/AGG being taken as termination codons in addition to the standard UAA/UAG codons. The alteration of the P*X*T tripeptide motif to a PEVGLS hexapeptide was assumed to account for the need to recognize this expanded and varied repertoire of termination triplets. Indeed, the tip of the α5 helix also differs compared with the majority of eubacterial RFs as shown (see supplemental Fig. S2 in Ref. 45). Further substantiating evidence was the presence of an almost identical protein in other mammals. Although mtRF1 appeared to be a credible candidate and indeed was later determined to be mitochondrial, functional *in vitro* assays were unable to confirm any peptidyl-tRNA hydrolase activity for this protein using the *Escherichia coli* ribosome (45). Moreover, human mtRF1 was unable to rescue the loss of either fission or fusion yeast mitochondrial RFs *in vivo* (45). Chimeric forms of bacterial RF1 were generated that substituted particular amino acids corresponding to the putative sequence recognition domains from human mtRF1. Under particular conditions in an *in vitro* assay with standard stop codons, these chimeras lost normal peptidyl-tRNA hydrolysis activity, but with codons beginning with A (particularly AAG), some activity was detected (48). It has now been demonstrated that it is a second mitochondrial RF family member, mtRF1a (also known as mtRF1-L (49)), that is wholly responsible for translation termination of all ORFs in human mitochondria (26).

Does mtRF1a Retain All the Characteristic Features of an RF?

Structural data for a number of RFs are available, and overall, mtRF1a appears to conform to the general pattern of RF1-type proteins. The protein database was primed with the amino acid sequence of human mtRF1a to generate a model (Fig. 2*A*) based on RF1 from *Thermus thermophilus* and a superimposition of the two structures in given in Fig. 2*B*. This indicates that, in mtRF1a, the important domains and motifs that are characteristic of class I RFs are correctly positioned to generate a functional RF. Although RFs have been reported to adopt either an open or closed structure, it is the open form with domain 3 almost perpendicular to domain 2 (3, 30) that is functional when found resident in the ribosome. This open form "locks" into position only when a termination codon has been recognized as being in the A-site. The specificity for the A-site stop codon is determined by the tripeptide motif together with the tip of the α5 helix. In human mtRF1a, the former is present as PKT, with high levels of identity in the flanking region. The latter, unlike mtRF1, conforms to the length and amino acid content of a number of RF1 proteins. When extended, domain 3 with the GGQ motif at the distal tip positions this motif at the PTC (reviewed in Ref. 50); again, mtRF1a not only retains this conserved motif but has high levels of identity in the flanking amino acids (45). Thus, mtRF1a appears to have retained the crucial features and structures expected in a class I RF1-type protein.

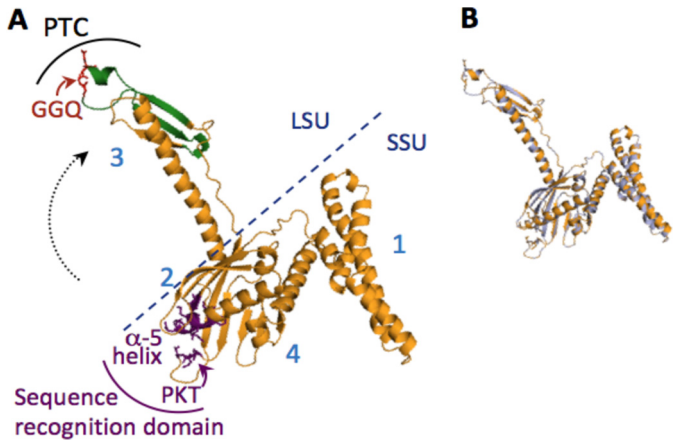

FIGURE 2. **Model of the human mitochondrial RF (mtRF1a).** *A*, mtRF1a is modeled on *T. thermophilus* RF1 (Protein Data Bank code 2B64 (28)), and the structural domains are numbered according to the bacterial notation (domains 1–4, *blue*). After entering the ribosome, the RF first has to distinguish whether a stop codon is present in the A-site. If termination is to proceed, domain 3 then swings away from domain 2 (*dotted arrow* showing direction of movement) and locks into the fully extended conformation. This positions the distal tip of domain 3 containing the conserved GGQ motif (*brown*) at the PTC. The PTC is embedded in the LSU of the mitoribosome, and this orientation of the RF and ribosome allows peptidyl-tRNA hydrolysis to occur. The GGQ motif, which is characteristic of ribosome-dependent peptidyl-tRNA hydrolases (*PTK*), is flanked by a region that is also relatively well conserved (*green*). A second critical feature of RFs determines stop codon selectivity. This is the sequence recognition domain (*purple*) and includes the PKT motif (within domain 2) and the tip of the α5 helix (within domain 4). These align with the mRNA in the A-site at the interface between the ribosomal subunits. It is this open conformation that is represented here, and it results in the RF spanning the two ribosomal subunits, with the approximate position of the LSU and SSU interface illustrated by the *dashed blue line*. *B*, the Protein Data Bank-generated superimposition of human mtRF1a (*orange*) and *T. thermophilus* RF1 (*gray*) indicates the level of similarity in structure as predicted from the primary amino acid sequence.

Co-crystallization and structural analyses have given us details of specific interactions between the nucleotides of the stop codon with the ribosome and the amino acid residues of eubacterial RFs. Although a great deal can be inferred from this with respect to the mitochondrial system, mitoribosomes (mitochondrial ribosomes) can be quite different, as described below, and thus far, no structure has been generated of mitoribosomes with RFs physiologically positioned in the A-site. Moreover, because human mitochondria have now been shown to frameshift at the end of the *MTCO1* and *MTND6* ORFs, it is

probable that the interactions at the DC behave differently. This −1 frameshift functions to position a standard UAG stop codon in the A-site rather than either the AGA or AGG codon described above. To allow any frameshift to occur, there are interactions between the mRNA, rRNA, and tRNAs that need to be broken and reformed. Differences in the structure of the mammalian mitoribosome compared with standard bacterial 70 S and eukaryotic 80 S particles are discussed below; however, particular features of the mammalian mitoribosome, including the loss of mt-rRNA domains, the increased porosity, and the apparent loss of an exit site tRNA, are likely to facilitate the −1 shift that repositions the mt-mRNA codon in the A-site so that UAG rather than AGA or AGG triggers RF activity and release of the nascent polypeptide from the mitoribosome.

What About the GGQ Motif?

Here, there is very high identity of both mtRF1a and mtRF1 compared with other RF proteins. Mitochondrial RF sequences have also been identified in a number of mammals, yeasts (including but not restricted to *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, and *Kluyveromyces lactis*) nematodes, *Drosophila*, *Anopheles*, and trypanosomes. In these, as with the human mitochondrial RFs, the GGQ motif has been conserved, with high levels of similarity in the flanking sequence (Table 2) to the eubacterial counterparts. This is not surprising because this motif has been demonstrated to be critical in catalyzing peptidyl-tRNA hydrolysis in the PTC (44).

Interestingly, bioinformatics has identified two additional members of the mitochondrial RF family in humans, namely ICT1 and C12orf65. Each of these has been reported to play a crucial role in mitochondrial protein synthesis, as depletion or expression of mutant forms results in mitochondrial dysfunction in cultured cell lines and also in patients for C12orf65 (46, 51). Both of these proteins have retained the GGQ motif, and in both cases, the flanking regions show similarity to other RFs (Table 2). Although *in vitro* release activity assays demonstrated ribosome-dependent peptidyl-tRNA hydrolase activity for ICT1, no such activity was detected for C12orf65 (46, 51). Moreover, for ICT1, this activity was independent of the A-site codon sequence, consistent with alignments indicating that these two proteins lack both of the domains (tripeptide and tip of the α5 helix) important in decoding the mRNA and so are unlikely to act as conventional RFs (46).

Mitoribosomes: Importance of Context for Ester Bond Hydrolysis

Recognition of the stop codon by the RF takes place within the ribosome. Moreover, the essential RF activity in promoting peptidyl-tRNA hydrolysis is ribosome-dependent. Therefore, it is critical to consider the contribution made to this process by the ribosomes to appreciate how the ribosomes in various systems can differ and to assess the potential impact this may have on the polypeptide release mechanism.

Ribosomes are megadalton molecular machines, with both RNA and protein components independently assembled into two subunits that trap mRNA between them. The ribosomes of the eukaryotic cytosol are 80 S particles made up of a 40 S SSU and a 60 S LSU, whereas bacterial 70 S monosomes comprise a 30 S SSU and a 50 S LSU. It was expected that mitochondria would follow their ancestral origins and harbor 70 S ribosomes, but this does not appear to be universally true. Although the mitoribosomes from the yeast *S. cerevisiae* do have a 70 S monosome, this is where the similarity to characterized eubacterial ribosomes ends. Work in the 1970s from a number of different groups established that *Candida utilis* has a 72 S particle made up of 50 S and 36 S subunits (52), *Neurospora* mitochondria contain 73 S monosomes (51 S LSU and 30 S SSU) (53), *Tetrahymena* monosomes are 80 S (54), and *Xenopus* mitoribosomes are 60 S (55).

By comparison, mammalian mitoribosomes appear to have one of the lowest sedimentation coefficients at only 55 S, consisting of large and small subunits of 39 S and 28 S, respectively (56). Despite this difference in sedimentation, the monosome particles have a similar mass (2.71 MDa) to the *E. coli* ribosome.

Another contrasting feature is the antibiotic sensitivity, which is consistent with a "loss or modification of components" of the 28 S and 39 S subunits (57). This means that although mammalian mitoribosomes are susceptible to peptidyl transferase inhibition by chloramphenicol and a subset of aminoglycosides that also target eubacterial ribosomes, they are resistant to fusidic acid, kirromycin, and amikacin (described in Refs. 57 58). This phenomenon has indeed now been shown to be a consequence of both the "loss" and "modification" of ribosomal elements. These losses and modifications have had a profound effect on the RNA/protein ratio. Where the 80 S and 70 S particles comprise ~67% RNA and only 33% protein, this ratio is almost exactly reversed in the mammalian mitoribosome. Here, the rRNA species contribute only 31% of the mass, with the remaining 69% being made up of mitoribosomal proteins (described in Ref. 59).

How and Why Is This Dramatic Reversal Achieved, and What, if Any, Are the Functional Differences?

Analysis of the rRNA encoded by the mammalian mitochondrial genome immediately explains one reason for this change in the RNA/protein ratio. It is currently accepted that there are only two (12 S and 16 S), not three, rRNA species and that these are truncated compared with their eubacterial and eukaryotic counterparts, although recent publications implicate the 5 S species as an rRNA component imported into mitochondria (7, 13). By overlaying the predicted secondary structures of these rRNAs, the loss of sequence from the mt-rRNAs is clearly not randomly dispersed across the sequence but can be seen to lie in selected areas. This has ensured the preservation of conserved functional residues, critical helices, and structural domains (depicted in Ref. 59) while dispensing with regions that appear to be surplus to requirements. One such example relates to the Shine-Dalgarno sequences that promote initiation of translation. The majority of human mt-mRNA species do not contain 5′-untranslated regions, and so upstream Shine-Dalgarno-like sequences are absent. For this reason, there has been no requirement to maintain the anti-Shine-Dalgarno-like sequences in the mt-rRNA with the consequent loss of this region. However, dispensing with such domains from the mt-rRNA alone would not be sufficient to cause this dramatic change in the RNA/protein ratio. To effect this, mammalian

mitoribosomes have acquired a number of additional proteins that do not have eubacterial ribosomal orthologs (58).

What Effect Does This Have on the Overall Structure of the 55 S Mitoribosome?

Although the mammalian mitoribosomes have maintained a similar molecular mass (2.7 MDa) as bacterial ribosomes, the change in RNA/protein composition has had an effect on the structure. The relative loss of mt-rRNA coupled with the increased protein content has resulted in a reduced sedimentation coefficient, generating a 55 S particle. This information, combined with the cryo-EM data, indicates that the mitoribosome has a more porous and open structure than its bacterial counterpart. The mt-SSU comprises ~30 polypeptides, 15 of which are regarded as mitochondrion-specific with no bacterial orthologs. The mt-LSU has ~50 polypeptides, again with almost half constituting a new class of ribosomal proteins (58). Hence, it is a combination of the overall protein content and the acquisition of new polypeptides that contributes to a change in structure.

This increased protein composition has another important structural consequence. In several instances, these newly acquired polypeptides are positioned to generate protein-dense regions. One example is in the polypeptide exit tunnel that is now composed of mitochondrion-specific proteins that line the last two-thirds of the tunnel (59). Furthermore, at the surface beyond the tunnel, the exit site is also flanked by mitochondrion-specific proteins (59). This mitochondrion-specific, protein-rich environment may have been specifically adapted to cope with the highly hydrophobic nature of the mtDNA-encoded proteins that are being synthesized (for a comprehensive recent review, see Ref. 58). Because of their hydrophobicity, these nascent polypeptides are likely to need either a direct interaction with the inner mitochondrial membrane or an indirect interaction via particular chaperones. It may be that the mitochondrion-specific components surrounding the exit site facilitate just such interactions, ensuring accurate insertion into the membrane and potentially also association with the correct complement of respiratory complex proteins.

In summary, we are now aware that only a single polypeptide, mtRF1a (mtRF1-L), is required for terminating the translation of all 13 human mitochondrial polypeptides. It has also become apparent that there are three other members of the mitochondrial translation RF family, all three of which are essential for cell viability. The exact function of these factors remains elusive, but it is striking that RF paralogs in the yeast cytosol perform crucial functions in mRNA surveillance systems such as non-stop and no-go decay mechanisms, which necessitate the restarting of stalled ribosomes.

Three major goals need to be achieved if we are to further our understanding of human mitochondrial gene expression. First, we must reconstitute a faithful *in vitro* mitochondrial translation system, without which we can never be certain what constitutes a true mitoribosome. Second, we need a high resolution structure of this complex. Third, we need to determine the methods of quality control in mitochondrial protein synthesis. It is undoubtedly going to be a very busy period for mitochondrial biology for the foreseeable future.

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