Translation termination in human mitochondrial ribosomes

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Abstract

Mitochondria are ubiquitous and essential organelles for all nucleated cells of higher eukaryotes. They contain their own genome (mtDNA), and this autosomally replicating extranuclear DNA encodes a complement of genes whose products are required to couple oxidative phosphorylation. Sequencing of this human mtDNA more than 20 years ago revealed unusual features that included a modified codon usage. Specific deviations from the standard genetic code include recoding of the conventional UGA stop to tryptophan, and, strikingly, the apparent recoding of two arginine triplets (AGA and AGG) to termination signals. This latter reassignment was made because of the absence of cognate mtDNA-encoded tRNAs, and a lack of tRNAs imported from the cytosol. Each of these codons only occurs once and, in both cases, at the very end of an open reading frame. The presence of both AGA and AGG is rarely found in other mammals, and the molecular mechanism that has driven the change from encoding arginine to dictating a translational stop has posed a challenging conundrum. Mitochondria from the majority of other organisms studied use only UAA and UAG, leaving the intriguing question of why human organelles appear to have added the complication of a further two stop codons, AGA and AGG, or have they? In the present review, we report recent data to show that mammalian mitochondria can utilize a −1 frameshift such that only the standard UAA and UAG stop codons are required to terminate the synthesis of all 13 polypeptides.

Introduction

Mitochondria are vital organelles that are present in all nucleated cells of higher eukaryotes. They play critical roles in many processes, including calcium homeostasis, apoptosis, Fe–S cluster formation and oxidative phosphorylation. Mitochondria contain their own genome (mtDNA, where mt is mitochondrial) encoding mt-mRNAs that are translated within the organelle [1]. In humans, these transcripts encode 13 proteins that are all components of the oxidative phosphorylation machinery, in addition to the two mt-rRNAs and 22 mt-tRNAs, which are required for the intramitochondrial translation of the mt-mRNAs. The remaining protein components required for intra-organelar translation and mitochondrial biogenesis are nuclear-encoded and need to be imported from the cytosol. Human mtDNA is a relatively small genome (16569 bp) and is found in many copies per mononucleate cell. A minimal non-coding sequence is present, which contains regions that control the initiation of mtDNA replication and transcription. From these regions, this compact genome is almost fully transcribed from both strands [2]. As a consequence, long polycistronic transcriptional units are generated, which are subsequently processed to separate the mt-rRNA, mt-tRNAs and mt-mRNAs, and are then matured [2,3]. For the mt-tRNAs, this involves the addition of -CCA to the 3′-teminus of the precursor by the tRNA-nucleotidyltransferase, a protein encoded by TRNT1 [4]. The 3′-termini of the mt-mRNAs are also matured, but by the addition of a poly(A) tail. This modification appears to be constitutive and is effected by mtPAP, a nuclear-encoded poly(A) polymerase that is specific to mitochondria [5]. In contrast with cytosolic mRNAs, their mitochondrial counterparts lack any modification to the 5′-termini and remain as a simple 5′-PO₄. Another contrasting feature is the relative lack of UTRs (untranslated regions), leaving most ORFs (open reading frames) unflanked. Thus initiation commences at or within three nucleotides of the extreme 5′-teminus for all except two of the ORFs, whereas completion of the UAA stop codon is facilitated by the addition of ‘A’ residues as a consequence of polyadenylation of seven ORFs.

Protein synthesis in the human mitochondrion

Initiation of protein synthesis occurs mainly at AUG codons, but AUU and AUA can also be decoded as initiating methionines [1]. Compared with translation in the eukaryotic cytosol, where there are many IFs (initiation factors) that come together to form the initiation complex, there appears to be a much reduced system in mammalian mitochondria. In the latter, the initiation complex appears to have retained only two homologues: IF2 and IF3 [6,7]. It does appear, however, that mtIF2 may have taken on the functions of...
bacterial IF1 [8]. Elongation proceeds along the mt-mRNA facilitated by mtEF-Tu, mtEF-Ts and mtEF-G1 [9–12]. Another major difference lies in the general structure of the mammalian mitochondrial ribosome when compared with the more familiar bacterial 70S and eukaryotic cytosolic 80S counterparts [13]. Rather than a predominance of rRNA, mammalian mitochondrial ribosomes have reduced the RNA component to two shorter rRNA species, 12S and 16S, while concomitantly increasing the number of protein components, thus reversing the conventional ratio to ~70% protein and only ~30% rRNA. As a consequence, the structure is more open and porous with no conventional E-site and with altered sedimentation values of 28S and 39S for the small and large subunits respectively and 55S for the complete monosome [13].

**Termination of protein synthesis in human mitochondria**

The 55S particle continues protein synthesis until a stop codon is reached and positioned within the A-site. Sequencing of the human mtDNA almost 30 years ago [1] revealed the features mentioned above. This included the reassignment of the standard stop codon UGA as a tryptophan, a not uncommon reassignment in mitochondrial genetic codes. Strikingly, it also indicated the apparent recoding of AGA and AGG as stop signals since each of these triplets is found only once and, in both cases, only at the very end of the ORFs of mitochondrial transcripts MTCO1 or MTND6 respectively. The conclusion that these were now stop signals was driven by the fact that the mtDNA does not code for any tRNA that could decode either AGA/AGG triplets and no tRNA has been shown to be imported physiologically into the human organelle [14]. The remaining 11 mitochondrial ORFs terminate in either of the two standard stops, UAA or UAG. The original dilemma was therefore what form of the class I RF (release factor) would be required to promote peptidyl-tRNA hydrolysis at these stop codons? Do human mitochondria follow the bacterial paradigm where two RFs are required to decode the three stop codons (RF1 recognizing UAA/UAG, and RF2 showing specificity for UGA/UAA [15,16]), or do they utilize a single RF more akin to eRF1 (eukaryotic RF1)/aRF1 (archaeal RF1) in the eukaryotic or archaeal cytosol respectively [17,18]? A single mitochondrial RF, however, would have to recognize an unusual and expanded repertoire of four triplets.

Over 12 years ago, bioinformatic mining identified an encouraging candidate for the role of human mtRF1. This protein contained a predicted decoding tripeptide motif (comprising proline and threonine with a variable amino acid between the two and thus designated the ‘PXT’ motif) which, although divergent, aligned more closely with that of bacterial RF1 than RF2 [19]. The PXT motif in mtRF1 is made up of six amino acids, PEVGLS, rather than just the three, thus differing from the accepted RF1 type PXT consensus in both length and sequence. It was considered that these differences, when taken with a second sequence variation at the tip of the α-5 helix, could have evolved to allow recognition of this extended repertoire of stop triplets. Subsequent biochemical analysis with mtRF1, however, failed to identify any release activity with any codons. A more recent search for a further candidate identified a protein, mtRF1a, with high overall identity with mtRF1 [20]. Analysis of the codon recognition domains revealed greater similarity to that of the bacterial RF1 homologue and consensus sequences, with PKT as the sequence constituting the tripeptide motif. Activity assays demonstrated that mtRF1a has a specificity for the standard UAA and UAG codons, but fails to recognize AGA/AGG or any other codon tested [20]. Since the *in vitro* assays are performed with 70S bacterial ribosomes, the lack of recognition of AGA/AGG by mtRF1 or mtRF1a could have been the consequence of using a heterologous system, especially in the light of the significant differences between 55S and 70S particles described above. Further bioinformatic searches have now revealed a family of four predicted mitochondrial RFs, with ICT1 and C12orf65 adding to the previously described mtRF1 and mtRF1a. Intriguingly, these two new members, ICT1 and C12orf65, both lack the two regions involved in codon recognition and are therefore unlikely candidates for AGA/AGG recognition [21].

Reanalysis of the two transcripts containing the AGA or AGG codons indicated that each of these triplets is directly preceded by a ‘U’ nucleotide, which, following procession of the mitochondrial ribosome to the end of the reading frame, would be placed immediately adjacent, in the P-site [1]. Thus, by invoking a single nucleotide shift, a conventional UAG stop signal would be positioned in the A-site. In support of this hypothesis, although 3′-UTRs are unusual in human mt-mRNAs, these are present in both of the transcripts containing in-frame AGA or AGG, and are predicted to form stable secondary structures [22]. Frameshifting on mitochondrial mRNAs is not common and no examples have been identified previously in mammalian mitochondria. Indeed there are only very few examples in mitochondria, all of which thus far are in the +1 direction and occur within the mt-ORFs [23–25]. The frameshifting proposed in human mitochondria would contrast not only with these other mitochondrial examples (as it is in the −1 direction), but also with all standard frameshifts, as it would occur after protein synthesis has been completed. Furthermore, many frameshifts offer an inefficient alternative to readthrough [26], which can be regulated by changes in physiological conditions [27]. In the instance of this human mitochondrial example, there would be no possibility for readthrough, as there is no cognate mt-tRNA for the AGA/AGG triplets. We have been able to test this hypothesis by combining the use of a mitochondrially targeted bacterial A-site-specific endonuclease, mtRelE, together with sequence analysis of the resultant cleaved RNA [22]. These fine mapping data revealed the A-site codon at termination and confirmed that a −1 frameshift does indeed occur. Thus the human mitochondrial translation system only requires the more standard UAG and UAA codons for termination. Moreover, as a consequence,
only a single RF is required, mtRF1a, which has already been characterized to show selectivity and specificity for these two termination signals [20].

Recycling of the human mitochondrial ribosome following translation termination

No orthologue of a class II RF has been identified in human mitochondria, leaving the question of how mtRF1a is removed from the mitoribosome after translation. Recycling of the post-termination complex to release the decylated mt-tRNA, mt-mRNA and separation of the monosome into the two subunits is effected by the mitochondrial ribosome recycling factor, mtRRF, in conjunction with mt-EF-G2 and mt-1F3 [28–31]. This second elongation factor mt-EF-G2 was characterized recently and appears to play no role in elongation, but co-operates exclusively with mtRRF in the recycling process [29]. The eukaryotic 80S and bacterial 70S ribosomes were believed to employ only a single EF-G for both processes. However, bioinformatic, in vitro and in vivo data now show that in fact it is not uncommon for bacteria to have separated the elongation and recycling activities and have two EF-G paralogues, as is the case in human mitochondria [32]. Human mtRRF has an N-terminal presequence that targets the protein to the mitochondrion, but this is not cleaved after successful import into the matrix of the organelle, as is the case for many of the nuclear-encoded but mitochondrially destined proteins [28]. Alignment with numerous RRF sequences suggests that the human mtRRF has a 79-amino-acid N-terminal extension that lacks homology with other proteins [28]. Since this is retained in the mature protein, it is tempting to speculate that it may contain as yet uncharacterized functional domains. Investigations are ongoing in our group to determine whether such domains are present and, if so, what their contribution might be to the process of mitoribosomal recycling.

In conclusion, mitochondrial translation and termination, particularly in humans, shares a number of similarities, but also differs in many ways from these processes in bacteria and the eukaryotic cytosol. This is perhaps not surprising, as it has become clear over the last 30 years that the mitochondrial protein synthesis machinery has an essential association with the membrane, reflecting the highly hydrophobic nature of its translation products, exclusively so in mammals. A greater understanding of this process is hampered by a lack of two important factors. First, our inability to manipulate the mitochondrial genome, which means that it is currently not possible to investigate the role of any cis-acting elements in mitochondrial translation. Second, although impressive reconstituted protein synthesis systems have been reported, these are essentially hybrid systems and we therefore lack a faithful reconstituted mitochondrial translation system. If either of these two issues can be resolved in the near future, our in-depth understanding of these processes will increase rapidly.

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