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Review

Mitochondrial protein synthesis: Figuring the fundamentals, complexities and complications, of mammalian mitochondrial translation

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Mitochondrial protein synthesis is essential for all mammals, being responsible for providing key components of the oxidative phosphorylation complexes. Although only thirteen different polypeptides are made, the molecular details of this deceptively simple process remain incomplete. Central to this process is a non-canonical ribosome, the mitoribosome, which has evolved to address its unique mandate. In this review, we integrate the current understanding of the molecular aspects of mitochondrial translation with recent advances in structural biology. We identify numerous key questions that we will need to answer if we are to increase our knowledge of the molecular mechanisms underlying mitochondrial protein synthesis.

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1. Introduction

It has been known for over 50 years that isolated rat liver mitochondria can incorporate radiolabelled amino acids into nascent polypeptides [1–4]. Although these first reports were challenged by researchers claiming that this incorporation was due to bacterial contamination of isolated mitochondrial preparations [5], by the late 1960s it had become well accepted that mammalian mitochondria were capable of intraorganellar synthesis of proteins [6,7]. We now know that the mitochondrial genome (mtDNA), which is housed in the mitochondrial matrix, contains the blueprint for thirteen polypeptides and all the RNA molecules believed to be necessary and sufficient for intramitochondrial protein synthesis [8]. All the other required components are imported from the cytosol. During this 50-years period, many factors have been identified that are critical for mitochondrial translation, but despite this we are still surprisingly unsure about many details underlying this process. This is due in major part to the lack of a faithful *in vitro* reconstituted system. Progress is further impeded by our inability to use standard molecular genetic manipulations, as there is no robust process for transfecting mammalian mitochondria [9,10]. In this short review, we mention important contributions to this field, but highlight fundamental questions that still remain.

2. What is so unusual about the mammalian mitochondrial ribosome?

Central to the process of mitochondrial protein synthesis is the mitochondrial ribosome, or mitoribosome. Pioneering work from O'Brien, Spremulli and others, showed that the bovine mitoribosome comprises 2 subunits of unequal size, a 28S small subunit (mt-SSU) and 39S large subunit (mt-LSU) [11]. Only one molecule of relatively short mtDNA-encoded ribosomal RNA could be identified in each subunit of the human mitoribosome – 12S rRNA in the small subunit (954 nt) and 16S rRNA in the large subunit (1559 nt) (however, see recent observations below) [8]. Intact mitoribosomes from a variety of mammalian sources were shown to be less dense (55S) than either their cytosolic (80S) or eubacterial (70S) counterparts and even differed from other organellar sources, such as *Saccharomyces*, *Neurospora*, *Tetrahymena* or *Xenopus* mitochon-

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dria [11–15] reviewed in [16]. This is largely due to a reversal in their protein to RNA ratio, changing from approximately 1:2 protein:RNA for eubacterial/eukaryotic cytosolic ribosomes to approximately 2:1 for the mammalian mitoribosome. The reduced ribosomal RNA species have not become shortened through stochastic loss of nucleotides but by selective excision of regions, including the anti-Shine–Dalgarno region, consistent with a corresponding lack of S–D sequence in mammalian mt-mRNAs. Although conservation of certain domains is clear, such as the sarcin–ricin loop and helix 45 of the SSU [17], there is little overall preservation of actual nucleotide sequence or even base composition [18]. Loss of part of the rRNA species would have been expected to reveal a number of spatial domains in a standard ribosomal structure. Intriguingly, some but not all of these domains have become occupied by a series of 'newly acquired' mitoribosome-specific proteins that have no apparent orthologues [17,19,20]. One consequence of these changes is a more porous structure, which is consistent with the original data indicating that a mitochondrial monosome had a low sedimentation coefficient of 55S [11,17].

Analysis of the many polypeptide constituents from a variety of mitoribosomal sources has been an iterative enterprise reflecting the constant technological improvement in detection methods [19,21–26]. Mass spectrometry of peptide fragments from the 39S large subunits of isolated bovine mitochondria identified 48 independent gene products. Many of these proteins could be assigned positions in an early and seminal cryo-EM structure from Agrawal and colleagues of the entire 55S mitoribosome at 13.5 A resolution [17,27]. Crystals of the mitochondrial ribosome have been elusive, but in their absence cryo-EM has continued to provide vital structural information, with improvements increasing the resolution to 12.1 Å of the bovine mt-LSU [27] and to 7 Å for the mt-SSU [28]. Recently, however, Greber and colleagues, using a combination of techniques, have produced a structure of the mt-LSU at 4.9 Å that is approaching the resolution achieved with crystallography. By subjecting porcine 39S mt-LSU preparations to chemical cross-linking followed by controlled proteolysis and MS analysis, contacts between numerous polypeptides have been unequivocally established. This information combined with the near-atomic resolution of the cryo-EM has both increased the number of mt-LSU assigned proteins to at least 51 members and identified the positions within the porcine mt-LSU of a number of these recently identified polypeptides [20]. Intriguingly, the structural analysis has also identified an RNA component that does not correspond to the 16S rRNA (see below). Currently there is no parallel study on the 28S mt-SSU, although previous mass spectrometry and increasingly sensitive analyses have revealed that it comprises at least 30 individual mitoribosomal proteins (MRPs) [18,23,24,29,30]. Cryo-EM has also very recently been used to generate high definition structural information on the yeast mitochondrial ribosome, but again it is the large subunit rather than the small that has been investigated. Single particle cryo-EM, using high-speed direct electron detectors, has been used to produce an almost complete model of this mt-LSU [31]. In this case there is no evidence of a 5S rRNA particle, consistent with the lack of the 5S RNA binding proteins L18 and L25 [31]. As a consequence of the loss of these elements, the central protuberance is significantly remodelled, with mitochondrial specific proteins occupying the vacated space. The yeast mt-LSU has 8 mitochondrial specific proteins that are common to both yeast and mammals, but a further 5 that so far are believed to be specific to yeast [31]. The accumulated data raises the question – *are these really yeast specific or are there still more components to the mammalian mitoribosomes that, as yet, have escaped detection?*

3. New versus old ribosomal proteins

As mentioned above mitoribosomes have acquired a number of new protein components. This means that the MRPs can be divided into two groups, new and old and these are roughly equal in number The old group includes those with clear eubacterial orthologues, evidencing the bacterial origin of the mitochondria, which therefore follow a similar nomenclature (e.g. MRPL1 is the orthologue of RPL1). The second group of 'new' mitochondrial specific MRPs (reviewed in [18,31,32]) appears to be evolving more rapidly than cytosolic ribosomal proteins and have adopted functions that suggest they do not merely act as fillers to occupy the space generated by the reduced rRNAs [33–36]. Acquisition of these novel mitoribosomal proteins appears to be through gene duplication or through the requisition of non-ribosomal proteins that have become targeted to mitochondria, often bearing post-translational modifications (discussed in [37]). One clear example of such gene duplication in mammals results in the presence of MRPS18A, B and C [24]. The difference in function of these distinct isoforms has not yet been elucidated, but tissue specificity, or the formation of specialised ribosomes dedicated to the translation of subsets of mt-mRNAs, are potential explanations. The acquisition and adaptation of pre-existing proteins is a fascinating phenomenon. A case in point is that of MRPL39, originally termed MRPL5 [21,26]. A heart specific variant of this protein was identified, which displayed sequence similarity to the N-terminal domain of cytosolic threonyl-tRNA synthetase that had maintained its tRNA binding site [38]. Adaptive evolution presumably dispensed with the mid and C-terminal regions, leaving a mitoribosomal protein with a currently undefined function. *Has this substantial increase in the relative amount of protein only evolved to shield the rRNA from damaging reactive oxygen species as speculated by a number of groups, or are there other novel functions still waiting to be disclosed?*

In contrast to those novel proteins of unknown function that have been acquired by the mitoribosome, a number of other new MRPs have brought defined but unexpected functions to their new home. An example of such a protein is ICT1 (redefined as MRPL58 in [18]). The transcript encoding this protein was first reported in a cell culture model of colon carcinoma, where levels varied between differentiated and undifferentiated HT29-D4 cells [39]. Consequently, it became known as immature colon carcinoma transcript 1 (ICT1). This deceptive nomenclature delayed its recognition as an MRP. Characterisation of ICT1 later revealed that this protein exhibited peptidyl-tRNA hydrolase activity. Any uncontrolled ability to cleave the elongating peptide from the P-site tRNA is potentially lethal to the cell. It is therefore a somewhat surprising function to incorporate into the mature mitoribosome [40]. How this activity is restricted is part of an ongoing investigation in our laboratory. Other bifunctional MRPs with deceptive nomenclature exist, including the mt-SSU associated Programmed Cell Death Protein 9, PDCD9 (or MRPS30), a protein involved in apoptosis [24,41] and another mt-SSU component, Death Associated Protein 3, DAP3 (or MRPS29), also reported to be an apoptotic factor [42,43]. This mitochondrial specific protein brings a novel GTP-binding activity to the ribosome [35]. *Is it possible that further bifunctional proteins will be identified as important in the assembly, or as structural components of mammalian mitoribosomes?*

4. Are all mitoribosomes born equal?

It is often assumed that all ribosomes are constitutively active and are identical, irrespective of the different tissues, environmental cues or species of transcript to be translated. However, this is in contrast to the established concept of a ribosome filter, where translational control is exerted at the level of ribosome selection

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