2014-08-04

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Cell Metab. 2014 Aug 5;20(2):226-40.
Cell Press
http://doi.org/10.1016/j.cmet.2014.07.001
http://hdl.handle.net/10616/42130

Making proteins in the powerhouse

B. Martin Hällberg[1,2,3*] and Nils-Göran Larsson[4,5*]

[1]Department of Cell and Molecular Biology, Karolinska Institutet, 171 77 Stockholm, Sweden.

[2]Centre for Structural Systems Biology, DESY-Campus, 22603 Hamburg, Germany

[3]European Molecular Biology Laboratory, Hamburg Unit, 22603 Hamburg, Germany

[4]Department of Mitochondrial Biology, Max Planck Institute for Biology of Ageing, 509 31 Cologne, Germany

[5]Department of Laboratory Medicine, Karolinska Institutet, 171 77 Stockholm, Sweden.

[*]Correspondence: martin.hallberg@ki.se or larsson@age.mpg.de

Abstract

Understanding regulation of mitochondrial DNA (mtDNA) expression is of considerable interest as mitochondrial dysfunction is important in human pathology and ageing. Similar to the situation in bacteria, there is no compartmentalization between transcription and translation in mitochondria; hence, both processes are likely to have a direct molecular crosstalk. Accumulating evidence suggests that there are important mechanisms for regulation of mammalian mtDNA expression at the posttranscriptional level. Regulation of mRNA maturation, mRNA stability, translational coordination, ribosomal biogenesis and translation itself, all form the basis for controlling oxidative phosphorylation capacity. Consequently, a wide variety of inherited human mitochondrial diseases are caused by mutations of nuclear genes regulating various aspects of mitochondrial translation. Furthermore, mutations of mtDNA, associated with human disease and ageing, often affect tRNA genes critical for mitochondrial translation. Recent advances in molecular understanding of mitochondrial translation regulation will likely provide novel avenues for modulating mitochondrial function to treat human disease.

Introduction

Expression of mtDNA is essential for biogenesis of the oxidative phosphorylation system (Larsson et al., 1998) and has a complex regulation at various levels, such as genome maintenance, transcription initiation, mRNA stability and translation (Falkenberg et al., 2007). It is therefore not surprising that mutations of mtDNA are major causes of human disease, and are also heavily implicated in common forms of age-associated disease and the ageing process (Larsson, 2010). Human pathogenic mtDNA mutations were first described more than 25 years ago (Holt et al., 1988; Wallace et al., 1988) and it soon became apparent that many of them affect tRNA genes and thereby impair mitochondrial translation (Larsson and Clayton, 1995). Two mutations (m.1555A>G and m.1494C>T) in the 12S ribosomal RNA (rRNA) gene of mtDNA predispose to maternally inherited aminoglycoside-induced deafness (Prezant et al., 1993; Vandebona et al., 2009; Zhao et al., 2004). This shows that also side effects, of commonly used antibiotics, can originate in the mitochondrial translation system. During the last decade, it has become increasingly clear that also nuclear gene mutations can impair mitochondrial translation to cause human mitochondrial disease (Boczonadi and Horvath, 2014; Rötig, 2011). Furthermore, at least a subset of the many different types of mtDNA mutations found in ageing impair mitochondrial translation and these mutations undergo clonal expansion to create a mosaic pattern of respiratory chain deficiency in many different ageing mammalian tissues (Larsson, 2010).

In this review, we will briefly describe the principles for transmission, maintenance and expression of mtDNA with special emphasis on mitochondrial translation. We will summarize the current knowledge of the regulation of mitochondrial translation, including novel insights into the biogenesis, structure and function of the mitoribosome.

Transmission of mtDNA

It is estimated that the human mtDNA mutation rate is at least an order of magnitude higher than the corresponding nuclear rate (Pakendorf and Stoneking, 2005). The transmission of mammalian mtDNA is asexual as it is strictly maternally inherited (Kaneda et al., 1995) and

does not undergo germ line recombination (Hagström et al., 2014). It has been predicted that any genome with these features would undergo what is known as the Muller's ratchet effect, that is the accumulation of deleterious mutations over time that thereby causes a mutational meltdown (Muller, 1964). Mammals have several poorly understood mechanisms that counteract the Muller's ratchet effect by decreasing the inheritance of mtDNA mutations between generations: (i) The bottleneck phenomenon is a mechanism that ensures that only a subset of the molecules in the mother's mtDNA pool is transmitted to the offspring, which may lead to an mtDNA genotype shift in just a few generations (Hauswirth and Laipis, 1982). (ii) There is a purifying selection mechanism in the germ line that selectively removes the mtDNA mutations that cause amino acid substitutions (Stewart et al., 2008a; Stewart et al., 2008b). (iii) There is a mechanism in developing embryos that selects against fetuses with high levels of pathogenic tRNA mutations (Freyer et al., 2012). (iv) High mutation levels in the female germ line decrease fertility (Ross et al., 2013). Accumulation of mtDNA mutations is heavily implicated in human ageing (Larsson, 2010) and recent experimental data suggest that low levels of maternally transmitted mtDNA mutations act synergistically with somatic mutagenesis to increase the total mtDNA mutation load and thus accelerate mammalian ageing (Ross et al., 2013).

Maintenance and transcription of mtDNA

The cellular mtDNA copy number varies by several orders of magnitude between tissues and is ~1 x 10^5 in human oocytes (Chen et al., 1995) and ~3 x 10^3 in human fibroblasts (Kukat et al., 2011). Recent work using super-resolution microscopy has shown that mammalian mtDNA is packaged into compacted nucleoid structures with a mean size of ~100 nm (Brown et al., 2011; Kukat et al., 2011). Most nucleoids seem to contain just a single copy of mtDNA and the main packaging factor is the high mobility group box domain protein called mitochondrial transcription factor A (TFAM) (Kukat et al., 2011). TFAM binds mtDNA cooperatively and can increase the flexibility of mtDNA and bend it to facilitate compaction (Fisher et al., 1992; Hallberg and Larsson, 2011; Ngo et al., 2011; 2014; Rubio-Cosials et al.,

2011; Farge et al., 2012). Although TFAM is the main factor in the packaging of mtDNA, it is possible that other proteins also make significant contributions to the structure of the nucleoid (Kukat and Larsson, 2013).

The two strands of mtDNA, which can be separated by buoyant-density ultracentrifugation, are, for historical reasons, called the heavy (H) and light (L) strands (Figure 1) (Battey and Clayton, 1978). Replication of mtDNA is often abortive and leads to the formation of a triple-stranded structure called the displacement (D-) loop, which contains a prematurely terminated nascent H strand. The D-loop region contains the origin for initiation of H (leading) strand mtDNA replication (O_H), which requires an RNA primer formed by transcription at the light strand promoter (LSP). After mtDNA replication has been initiated, a poorly understood mechanism operating at the end of the D-loop will determine whether it will be abortive and lead to the formation of a D-loop structure or if it will proceed and result in the replication of the whole mtDNA molecule (Brown and Clayton, 2002). The replication of the lagging strand is initiated when leading-strand mtDNA replication is two-thirds complete and the light-strand replication origin (O_L) is activated (Clayton, 1982). Once it has been exposed in its single-stranded form, O_L forms a stem-loop structure to which mitochondrial RNA polymerase (POLRMT) binds to produce the RNA primer necessary for initiation of lagging-strand mtDNA replication (Wanrooij et al., 2008; Wanrooij et al., 2012). There is currently no scientific consensus about the mode of mtDNA replication and in this regard several other models have been proposed (Lightowlers and Chrzanowska-Lightowlers, 2012).

The mammalian mtDNA contains a dedicated main promoter for the transcription of each strand called the LSP and the H-strand promoter (HSP). The basal transcription initiation machinery consists of three proteins: POLRMT, TFAM and the mitochondrial transcription factor B2 (TFB2M) (Falkenberg et al., 2002; Shi et al., 2012). It is important to note that TFAM has dual roles because it is required for both transcription initiation (Shi et al., 2012) and the packaging of mtDNA into nucleoids (Bogenhagen et al., 2008; Ekstrand et al., 2004). There has been a long-standing hypothesis that the genes encoding rRNAs and mRNAs on the

H strand are transcribed from two distinct promoters called HSP1 and HSP2, respectively (Kruse et al., 1989). According to this model transcription initiated at HSP1 is terminated immediately downstream of the rRNA genes as the mitochondrial transcription termination factor 1 (MTERF1) binds its target sequence in the $tRNA^{L(UUR)}$ gene (Figure 1) (Kruse et al., 1989; Martin et al., 2005). However, a recent knockout of the mouse *Mterf1* gene has shown that the loss of MTERF1 does not affect rRNA gene expression, whereas the amount of antisense RNAs increases (Terzioglu et al., 2013). This result suggests that there is only one transcription unit for expression of H-strand genes that is regulated by one single HSP (Terzioglu et al., 2013). Contrary to previous assumptions, the main function of MTERF1 seems to be to prevent transcription initiated at LSP from proceeding over the rRNA gene region to produce antisense transcripts. It is possible that such antisense transcripts may be disadvantageous as they may prevent proper folding of the 12S and 16S rRNAs. In addition, antisense transcription has been shown to interfere with LSP activity, possibly by transcriptional collision preventing re-initiation of transcription (Terzioglu et al., 2013).

Mitochondrial RNA processing

Transcription by POLRMT yields two long transcripts, one originating from LSP and the other from HSP. These two long transcripts are processed by the mitochondrial RNA-processing machinery to release the individual tRNAs, rRNAs, and mRNAs (Figure 2). The processing is initiated on the tRNA sequences to release, according to the generally accepted tRNA punctuation model, the mRNAs and rRNAs that they intersperse (Figures 1 and 2) (Ojala et al., 1981). However, not all mRNAs are flanked by tRNA genes, as exemplified by ATP6-COIII, and ND5-Cyt b (Figure 1). The pentatricopeptide repeat domain 2 (PTCD2) protein has been reported to be involved in processing of ND5–Cyt b (Xu et al., 2008), but it is otherwise unclear how mRNAs not flanked by tRNAs are processed.

Evidence is accumulating to indicate that the early transcript processing takes place co-transcriptionally in small distinct speckles tentatively named mitochondrial RNA granules (MRGs) (Antonicka et al., 2013; Jourdain et al., 2013; Lee et al., 2013). The mitochondrial

5´-processing machinery in the form of the mitochondrial protein-only version of RNase P, consisting of three subunits (MRPP1-3), is found in these granules. In contrast, RNase Z, which constitutes the 3´-processing machinery of tRNAs, is not present in the MRGs (Jourdain et al., 2013). This fact, in combination with the primary structure of the intermediates that are formed upon knockdown of the 5´- and 3´- tRNA-processing machinery and the known inhibition of RNase Z by long 5´ leaders (Rossmanith et al., 1995; Levinger et al., 2001; Nashimoto et al., 1999) argues that the 5´ end of the tRNAs are processed first and the 3´ ends in a subsequent step (Figure 2). RNase Z in mammalian mitochondria consists of ELAC2 (Brzezniak et al., 2011; Rossmanith, 2011) possibly in complex with PTCD1 (Sanchez et al., 2011). According to this model, the primary transcripts undergo an initial and partly co-transcriptional processing in the MRGs followed by a second round of processing outside the MRGs.

Two of the three subunits of mitochondrial RNase P, MRPP1 and MRPP2, form a strong complex whereas MRPP3 binds the complex weakly and only under low-salt conditions *in vitro* (Holzmann et al., 2008). MRPP1 shows strong sequence homology to a family of tRNA N1-methyltransferases that methylate on position 9 in tRNAs and this m1A9 or m1G9 modification is critical for obtaining a cloverleaf fold of mitochondrial $tRNA^K$ (Helm et al., 1998; Helm et al., 1999). Elegant single-molecule FRET experiments have shown an equilibrium between an extended non-physiological conformation and a cloverleaf conformation of mitochondrial $tRNA^K$ (Voigts-Hoffmann et al., 2007). The reason for the extended non-physiological conformation is the possibility of a Watson-Crick base pairing between A9 and U64 in mitochondrial $tRNA^K$. However, methylation of A9 renders the A9-U64 Watson-Crick base pairing sterically impossible hence shifting the balance to a cloverleaf conformation. Interestingly, it was recently shown that the RNase P subcomplex of MRPP1-2 was necessary and sufficient for N1-methylation of both A and G (m1R9) of mitochondrial tRNAs (Vilardo et al., 2012). The methylation was shown to be rate limiting, so one can envision that the pre-tRNA structure of the primary transcript is bound by the MRPP1-2 subcomplex and that MRPP3 then comes in and trims the 5´ end while the tRNA is

being methylated on R9. Since the m1R9-methylation is likely to be crucial for cloverleaf formation for a majority of mitochondrial tRNAs, it is likely important that the tRNA gets this modification before it is released by the tRNA 5´- and 3´-processing machinery.

The CCA in the acceptor stem of tRNAs is not encoded by mtDNA. It is instead added by the mitochondrial CCA-adding enzyme TRNT1 (Nagaike et al., 2001). Once the CCA sequence has been added to the 3´ end of the tRNA, it is no longer a substrate for RNAse Z because the CCA addition is an anti-determinant for RNase Z (Mohan et al., 1999). This arrangement makes unproductive cycling between TRNT1 and RNase Z impossible.

Mitochondrial mRNA stability and polyadenylation

The release of the 11 mtDNA-encoded mRNAs (Figure 1) occurs by RNA-processing events, as discussed above. Remarkably, the ND6 mRNA seems to be immediately competent for translation once liberated from the primary transcript and does not need any further maturation steps. The ND6 mRNA contains no 5´ untranslated region (UTR) and has a relatively short 3´ UTR that is not polyadenylated in humans (Temperley et al., 2010a) and mice (Ruzzenente et al., 2012). Also the other 10 mRNAs have no or strikingly short 5´ UTRs and often completely lack a 3´ UTR (Figure 1) (Montoya et al., 1981; Temperley et al., 2010a)

Two mRNAs, ND4L/ND4 and ATP8/ATP6, are bicistronic and each of them contains two open reading frames (ORFs) that are partly overlapping (Figure 1). It is possible that both ORFs are translated by a single interaction event between the mRNA and the ribosome, e.g. by first translating the 5´ ORF followed by backtracking and repositioning to translate the 3´ ORF. Alternatively, the mRNA may be released after translation of the 5´ ORF and a second interaction event facilitated by one as-yet-undefined internal ribosome entry site of the mRNA may allow translation of the 3´ ORF. In this latter case, the mRNA would contain a long 5´ or 3´ UTR depending on which ORF is subject to translation.

A 7-methylguanosine cap at the 5´-end of nucleus-encoded mRNAs is essential for translation in unicellular eukaryotes and metazoans (Cowling, 2010), but this modification is

lacking in the mtDNA-encoded mRNAs (Grohmann et al., 1978). All mRNAs except ND6 contain short polyadenylation tails of ~45–50 nucleotides in length (Figure 1) (Ojala et al., 1981b). A mitochondrial polyA polymerase (mtPAP) is required for polyadenylation of the mitochondrial mRNAs (Nagaike et al., 2005; Tomecki et al., 2004). Decreased polyadenylation affects the stability of a subset of mRNAs and leads to impaired mitochondrial translation and mitochondrial dysfunction (Nagaike et al., 2005; Tomecki et al., 2004). The importance of mtPAP is further underscored by the finding that an autosomal recessive mutation in its gene leads to defective mRNA polyadenylation and clinical manifestations such as spastic ataxia and optic atrophy (Crosby et al., 2010). The 2′-phosphodiesterase (PDE12) has been reported to remove polyA tails from mitochondrial mRNAs (Rorbach et al., 2011).

The leucine-rich pentatricopeptide repeat (PPR)-containing (LRPPRC) protein belongs to the large family of PPR proteins that bind RNA and members of which are predominantly found in organelles (Small and Peeters, 2000; Barkan et al., 2012). LRPPRC is mainly present in the mitochondrial matrix (Sterky et al., 2010), where it controls mRNA stability (Sasarman et al., 2010). Mice with knockout of *Lrpprc* and fruit flies with decreased expression of the fly ortholog of LRPPRC, called Bicoid stability factor (BSF or dmLRPPRC), have drastically reduced steady-state levels of mRNAs and drastically decreased polyadenylation of mRNAs and transcript processing defects (Bratic et al., 2011; Harmel et al., 2013; Ruzzenente et al., 2012). Interestingly, LRPPRC seems to stabilize a pool of translationally inactive transcripts and mitochondrial translation becomes chaotic and uncoordinated in the absence of LRPPRC in mammals and flies (Bratic et al., 2011; Ruzzenente et al., 2012). Consistent with the genetic studies, *in vitro* biochemistry has shown that LRPPRC suppresses RNA degradation by blocking the action of polynucleotide phosphorylase (PNPase) and promotes polyadenylation by stimulating the activity of mtPAP (Chujo et al., 2012). LRPPRC and the stem loop-interacting RNA-binding protein (SLIRP) form a stable complex (Ruzzenente et al., 2012; Sasarman et al., 2010). SLIRP becomes unstable and is degraded in the absence of LRPPRC and both proteins seem to act

synergistically to promote mRNA stability (Ruzzenente et al., 2012). Mutations in the gene encoding the LRPPRC protein cause the French Canadian type of Leigh syndrome (LSFC), a severe form of infantile neurodegeneration associated with deficient oxidative phosphorylation (Mootha et al., 2003; Mourier et al., 2014; Xu et al., 2004). The pathophysiology of the mitochondrial dysfunction in LSFC patients is not fully understood but likely involves defective regulation of mRNA stability, mRNA maturation and translation.

Mitochondrial tRNA modification

All mammalian mitochondrial tRNAs have rather high A and U content, and some tRNAs such as $tRNA^{S(AGY)}$ have non-canonical secondary structures. Others, such as $tRNA^{D}$ and $tRNA^{F}$, lack the conventional stabilizing intramolecular interactions between the D-T loops. This inherent poor stability makes tRNAs in mammalian mitochondria more sensitive to processing and modification defects than, for example, bacterial tRNAs. There are more than 200 disease-related mutations of tRNA genes in mtDNA and more than half of them can be regarded as definitely pathogenic (Yarham et al., 2010). The majority of these mutations are in positions subcritical to core tRNA function and their pathogenicity is the result of hindrance of the proper recognition by the mitochondrial tRNA-modification machinery.

Modifications of tRNAs can be divided into two subgroups: those that are important for the overall structure and those that are important for decoding (i.e., codon-anticodon recognition). One of the important modifications for the overall structure of the mitochondrial tRNAs, m1A9 or m1G9, is introduced already when the 5´ processing of the tRNA occurs, as discussed above. However, the relative order of the ensuing modifications is currently unknown. To this end, the interdependence of different modifications should be studied using cell lines or mouse models with knockdowns or knockouts of tRNA-modification enzymes. In the following section, we shall focus on modifications that are related to human disease and that are located in the anticodon stem-loop.

In several mitochondrial tRNAs, a wobble-position uridine is modified by a rather large adduct on the C5 atom of the uracil base. The modification, τm^5U (Figure 3), is needed for the correct codon-anticodon pairing for several mitochondrial tRNAs (Kurata et al., 2003). Specifically, the modification has been shown to stabilize U-G wobble pairing, at least when bound to a bacterial ribosome (Kurata et al., 2008). Taurine is the major part of this adduct and is connected to the uracil-base C5 through a methylene moiety (Figure 3). The taurine moiety is dietary (Suzuki et al., 2002), while the carbon source of the methylene moiety is unknown. The modification is unique for animal mitochondria, although there are similar adducts ($cmnm^5$) in bacteria and yeast mitochondria (Figure 3). Whereas the mechanism of taurine modification of animal mitochondria tRNA is unknown, the mechanism of $cmnm^5$ modification (Figure 3) in bacteria and yeast mitochondria is well studied. In the bacterial case, a complex of two proteins (MnmE and MnmG) is sufficient and necessary to use a glycine and tetrahydrofolate derivative to make $cmnm^5$. In yeast, it is clear that the presence of $cmnm^5$ in mitochondrial tRNA requires MSS1 and MTO1 (Umeda et al., 2005), although the biochemical activity of this protein complex remains to be reconstituted in vitro. Homologs to MSS1 and MTO1 are also present in humans (hGTPBP3 and hMTO1), but it is unclear if a suitable tetrahydrofolate derivative is available as a co-substrate in human mitochondria. It is therefore uncertain, how the first step of the modification, which gives rises to the methylene moiety, is carried out. Defects in taurine modification are implicated in mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes syndrome (MELAS) as one causative mutation in $tRNA^{L(UUR)}$ prevents recognition by the tRNA-taurine modification system (Kirino et al., 2005; Yasukawa et al., 2000). Out of the pool of taurine-modified mitochondrial tRNAs, three (E, K and Q) are further modified with a thiolation of the 2-position on the wobble τm^5U to make τm^5s^2U (Figure 3). A similar thiolation but then on a canonical U–base is catalyzed by thiouridylase MnmA in bacteria (Ikeuchi et al., 2006). MTU1 is the mitochondrial homolog of MnmA and knockdown of MTU1 diminished detectable s2U and reduced oxidative phosphorylation (Umeda et al., 2005). Point mutations

in MTU1 have been identified in patients with acute infantile liver failure. The identified point mutations rendered either amino acid changes in MTU1 or exon skipping and samples from the patients showed loss of 2-thiolation in the mitochondrial tRNAs for E, K and Q (Zeharia et al., 2009).

Another enzyme involved in modifications in the anticodon stem-loop is pseudouridine synthase 1 (PUS1), which targets a large range of cellular RNAs, both cytosolic and mitochondrial. Specifically, U27 and U28 in several mitochondrial tRNAs (K, M, L, I and D) are pseudouridylated by PUS1 at either one or both positions. These pseudouridine modifications can potentially stabilize and preorder the anticodon stem loop in a confirmation amenable for the correct function on the ribosome (Agris, 2008). The structural-functional aspects of the active site of pseudouridine synthases are conserved in all classes of this large enzyme family (Ericsson et al., 2004) and characterized by a conserved aspartate residue. Different point mutations that are close in sequence or space to the active site in PUS1 have been found in mitochondrial myopathy lactic acidosis, and sideroblastic anemia (MLASA) patients (Fernandez-Vizarra et al., 2007; Patton et al., 2005).

In the cytosol of eukaryotes, there are two main species of $tRNA^M$ that are dedicated to either translation initiation or elongation. The mitochondrial genome, however, encodes only one species of $tRNA^M$. Intriguingly, this single species of $tRNA^M$ not only decodes AUG but also the canonical isoleucine codon AUA in initiation and elongation. Furthermore, mitochondrial $tRNA^M$ also decodes AUU and AUC but only if they are the initiating codons, otherwise they are decoded as isoleucine as in the canonical code (Fearnley and Walker, 1987). Specifically, the genes of ND1, ND2, ND3 and ND5 in human mitochondria utilize non-AUG start codons (Figure 1). To enable the proper codon-anticodon recognition of these non-canonical methionine codons, a formylation of the wobble C34 has been suggested and has, in the case of AUA, been shown to be needed (Bilbille et al., 2011; Moriya et al., 1994). The source of the formyl group and the responsible enzyme(s) for this critical modification remain unclear.

Aminoacyltransferase activity in mitochondria

Just as in the eukaryotic cytosol and the bacterial cytosol, tRNAs in mitochondria are specifically charged with amino acids by aminoacyl-tRNA synthetases (aaRS). However, a few aspects of this charging process are idiosyncratic to mitochondria. There are 19 aaRSs in mitochondria, all encoded in the nuclear genome and, similarly to the cytosolic case in eukaryotes and bacteria, they can be divided into two classes (Class I and Class II) based on their primary sequences. There is no mitochondrial aaRS for Q. Instead, the aminoacylation of $tRNA^{Q}$ follows an indirect pathway with misaminoacylation with E followed by a transamidation to Q by the heterotrimeric mitochondrial amidotransferase hGatCAB (Nagao et al., 2009).

In general, it is very important that tRNAs are properly recognized by their cognate aaRSs and recurring mischarging of tRNAs severely reduces the overall translational fidelity. The mitochondrial aaRSs are unusual in their relative lack of discriminatory capacity and also, in some cases, disobeying the universal tRNA identity rules (Bonnefond et al., 2005; Giegé et al., 1998). It is likely that this is a consequence of the reduced T- and D-loop sequences of mitochondrial tRNAs. As an example, $tRNA^{Q}$ is easily misacylated by the aaRS for S (Shimada et al., 2001). However, there appears to be a strong discriminatory mechanism in place against wrongly charged tRNAs in the binding step to elongation factor Tu (mtEFTu) (Nagao et al., 2007), although the molecular mechanism for this selection remains elusive. In fact, a multitude of mitochondrial diseases originates from mutations in mitochondrial aaRSs, but defects in aminoacylation can also be caused by mutations in the tRNA substrate such as those observed for $tRNA^{I}$ and $tRNA^{K}$ (Degoul et al., 1998; Sissler et al., 2004).

As discussed above, mitochondria only have a single gene that encodes $tRNA^{M}$ and a nucleus-encoded mitochondrial methionyl-tRNA formyltransferase (MTFMT) formylates a subset of $tRNA^{M}$ for use in translation initiation whereas the non-formylated species is used for translation elongation (Tucker et al., 2011). Recessive mutations inactivating the MTFMT gene cause severe oxidative phosphorylation deficiency and Leigh syndrome in children (Tucker et al., 2011).

Mitoribosome biogenesis

The mitoribosome biogenesis pathway can be divided into several distinct steps: (i) synthesis of 12S and 16S rRNA by transcription of mtDNA; (ii) nuclear gene expression to produce the mitoribosomal proteins of the small (MRPS) and large (MRPL) subunit, including possible posttranslational modifications of some of these proteins; (iii) post-transcriptional modification of the rRNAs by methylation, 2´-O-ribose methylation and pseudouridylation of specific residues; (iv) assembly of 16S rRNA and MRPL proteins to form the large subunit (LSU) of the mitoribosome; (v) assembly of 12S rRNA and MRPS proteins to form the small subunit (SSU) of the mitoribosome; and (vi) assembly of the mature SSU and LSU to functional mitoribosomes. It should be noted that several of these steps may partly coincide as some MRPs likely assemble with the rRNAs already during the transcription process (Bogenhagen et al., 2014).

In nuclear and bacterial ribosome biogenesis, the initial rRNA processing and some modification steps are largely co-transcriptional (Henras et al., 2008; Kaczanowska and Rydén-Aulin, 2007). The rRNA methyltransferases RNMTL1, MRM1, and MRM2 were recently found in speckles that contained newly transcribed mitochondrial RNA (Lee et al., 2013), supporting a co-transcriptional role in rRNA modification. Whether or not these structures are the same as the MRGs containing MRPP1-3 remains to be determined. In contrast, other rRNA modifications likely occur late in the biogenesis process and require the presence of rRNA/MRP assembly intermediates that are specifically recognized by rRNA-modification enzymes (Metodiev et al., 2009; Metodiev et al., 2014).

Translating mitoribosomes in yeast are reported to be tethered to the inner mitochondrial membrane by MRP20, which is a homolog to bacterial L23 and human MRPL23. In yeast, MRP20 is necessary for the formation of a ribosomal sub-complex with other proteins to achieve proper mitoribosome assembly and function (Kaur and Stuart, 2011). This suggests that the LSU of the mitoribosome is assembled on the inner mitochondrial membrane (Figure 4 and 5).

Modifications of 16S rRNA and biogenesis of the LSU

It was observed early that the large subunit of mammalian mitoribosomes is, compared to its bacterial and cytosolic counterparts, quite scarcely modified (Dubin, 1974). Three ribose methylations (human 16S numbering Gm^{1145}; Um^{1369} Gm^{1370}) have been reported from experiments in hamster cells (Baer and Dubin, 1981). In yeast, the enzymes responsible for the corresponding two first methylations in the large mitoribosomal rRNA (Gm^{2270}; Um^{2791}) have been identified as MRM1 (Pet56p) and MRM2, respectively (Sirum-Connolly and Mason, 1993; Pintard et al., 2002). Methylation by MRM1 is crucial for LSU (yeast 21S) stability and the mitochondrial function is compromised without this modification (Sirum-Connolly and Mason, 1993). Likewise, MRM2 in yeast encodes an RNA-modifying enzyme (Um^{2791}) and LSU production is slightly reduced upon MRM2 deletion (Pintard et al., 2002). Furthermore, the human homologs of MRM1 and MRM2 were recently shown to localize to mitochondria (Lee et al., 2013) but whether they carry out their presumed function (i.e., 2´-O-ribose methylation of G1145 and U1369, respectively) has not yet been verified. However, the enzyme responsible for the third observed 2´-O-ribose methylation of 16S rRNA, Gm1370, was recently identified by the Bogenhagen group as RNMTL1, a methyltransferase of the SPOUT class (Lee et al., 2013).

Pseudouridinylation was not observed in the initial studies of the large mitochondrial rRNA in hamster cells (Dubin and Taylor, 1978) although it could later be observed at U1397 in mouse and human large mitochondrial rRNA using more sensitive methods (Ofengand and Bakin, 1997). The enzyme responsible for the corresponding modification in yeast mitochondria (Pus5) has a range of human homologs and it is currently unclear which, if any, of these perform the modification (Ansmant et al., 2000).

The MTERF3 protein is predicted to bind nucleic acids (Spåhr et al., 2010) and studies of *Mterf3* knockout mice have shown that MTERF3 is essential for embryogenesis and that its loss leads to a dramatic upregulation of mitochondrial transcription accompanied by severe respiratory chain deficiency (Park et al., 2007). In a subsequent study, it was found

that knockout and downregulation of dmMTERF3 in fruit flies not only activates mitochondrial transcription, but also leads to impaired assembly of the LSU of the mitoribosome (Wredenberg et al., 2013). Similar to the findings in flies, the biogenesis of the LSU and mitochondrial translation are severely impaired in *Mterf3* knockout mice (Wredenberg et al., 2013). RNA immunoprecipitation experiments have shown that both MTERF3 and dmMTERF3 specifically interacts with 16S rRNA in mice and in flies, respectively (Wredenberg et al., 2013). Whether MTERF3 interacts with a modifying enzyme to promote some specific type of rRNA modification or has another function in the biogenesis of the LSU is currently unknown.

The C7orf30 protein interacts with the LSU of the mitoribosome (Wanschers et al., 2012) and promotes its biogenesis (Rorbach and Minczuk, 2012). The C7orf30 protein has been reported to interact with MRPL14 and downregulation of either of these two proteins leads to the formation of an LSU lacking several MRPLs that cannot be properly assembled into a monosome thereby resulting in severely impaired mitochondrial translation (Fung et al., 2013).

Modifications of 12S rRNA and biogenesis of the SSU

Five modifications have been identified in 13S rRNA of the SSU of the hamster mitoribosome (Baer and Dubin, 1981) and these are likely conserved in all mammals. These modifications were mapped to the mouse mitochondrial ribosome sequences (Van Etten et al., 1980) and included two adenine dimethylations ($m^6{}_2A^{937}$ and $m^6{}_2A^{938}$), one uracil methylation (m^5U^{425}) and two cytosine methylations (m^4C^{840} and m^5C^{841} or m^5C^{842}) (Baer and Dubin, 1981).

The presence of two dimethylated adenines in a stem-loop structure at the 3´-end of the rRNA of the SSU is highly conserved in all domains of life (Shutt and Gray, 2006). In *E. coli,* the lack of the enzyme responsible for this modification, KsgA, leads to resistance to the aminoglycoside kasugamycin and growth retardation (Helser et al., 1971; Poldermans et al., 1980). Mouse knockout studies show that loss of the *Tfb1m* gene leads to embryonic lethality

and complete absence of $m^6{}_2A^{937}$and $m^6{}_2A^{938}$ of the 12S rRNA in mouse mitochondria (Metodiev et al., 2009). Without these modifications the levels of the assembled SSU of the mitoribosome are drastically reduced and mitochondrial translation is severely impaired (Metodiev et al., 2009).

The mitochondrial cytosine methyltransferase NSUN4 belongs to the same family of m5C-methyltransferases as the bacterial rRNA m5C-methyltransferases RsmB, RsmF and YccW (Cámara et al., 2011). In contrast to the bacterial homologs, NSUN4 lacks RNA binding domain extensions and instead it forms a stable complex with MTERF4 that targets the NSUN4/MTERF4 complex to the LSU of the mitoribosome (Cámara et al., 2011; Spåhr et al., 2012; Yakubovskaya et al., 2012). Knockout of either MTERF4 (Cámara et al., 2011) or NSUN4 (Metodiev et al., 2014) is embryonic lethal and appears to affect a late step in ribosomal biogenesis as both the SSU and LSU remain stable but do not interact to form a functional monosome. Recent sequencing of cDNA generated from bisulfite-treated mouse mitochondrial rRNA identified two cytosine methylations (m^4C^{840} and m^5C^{842}) in 12S rRNA (Metodiev et al., 2014) corresponding to the modification sites previously identified in hamster mitochondria (Baer and Dubin, 1981). The m^4C^{840} modification in mice has an E. coli homolog (m^4C^{1402}) that has been proposed to regulate translational precision through functional and conformational control of the P-site (Kimura and Suzuki, 2010). In *Nsun4* knockout mice, the m^5C^{842} modification of 12S rRNA is absent whereas the m^4C^{840} modification is preserved (Metodiev et al., 2014). Remarkably, the knockout of MTERF4 does not affect the m^5C^{842} modification despite the fact that NSUN4 and MTERF4 form a very stable heterodimeric complex (Spåhr et al., 2012; Yakubovskaya et al., 2012). This has led to a model in which a free fraction of NSUN4 is proposed as responsible for the creation of the m^5C^{842} modification of the 12S rRNA, whereas the NSUN4/MTERF4 complex has an essential role in the final assembly step of the monosome by binding the LSU to interact with the SSU (Metodiev et al., 2014). It is possible that the m^5C^{842} modification of the 12S rRNA provides a quality control mark to ensure that only fully mature SSUs can interact with the LSU in the final assembly step of the monosome (Metodiev et al., 2014). It is important to

note that the adenine dimethylations ($m^6{}_2A^{937}$and $m^6{}_2A^{938}$) of 12S rRNA are established independently of NSUN4 (Metodiev et al., 2014), which indicates that they may occur at an earlier stage of the biogenesis of the SSU. Also the protein encoded by C4orf14 has been reported to be necessary for SSU biogenesis, although the molecular mechanisms are not understood (He et al., 2012).

The mammalian mitoribosome

Mitoribosomes were found in mitochondrial isolates as early as the mid-'50s, but due to problems with proper mitochondrial isolation, their more detailed characterization required another ten years of methods' development (O'Brien and Kalf, 1967).

The complete mammalian mitoribosome (55S) is made up of the SSU (28S) and the LSU (39S). The SSU contains the 12S rRNA and more than 29 proteins, 15 of which do not have any obvious bacterial counterparts, whereas the LSU contains the 16S rRNA and 50 proteins, 20 of which have no obvious bacterial counterpart (Sharma et al., 2003). This means that the mammalian mitochondrial ribosome contains a mass ratio of RNA to protein of about 1:2, whereas the mass ratios of bacterial and the eukaryotic cytosolic ribosomes is about 2:1 (Agrawal and Sharma, 2012). The mitoribosome has a proteobacterial origin, and during evolution, certain rRNA segments have been lost whereas the share of protein has increased and, as discussed below, ribosomal proteins have in some regions replaced evolutionarily lost rRNA segments.

About half of the MRPs do not have any bacterial homolog identifiable by sequence alone, although it is probable that structural-functional aspects have been conserved in the absence of sequence homology. Nevertheless, it is clear that there are some unique proteins in the mitoribosome that have been acquired over the evolutionary period as a consequence of rRNA component loss. All mammalian MRPs are nuclear-encoded and most contain a mitochondrial localization sequence on the N-terminus (O'Brien, 2002).

Structure description

The structure of the whole bovine mitoribosome has been determined by the Rajenda Agrawal group using cryo-EM at relatively low (13.5Å) resolution (Sharma et al., 2003) and this was for long the only available structural information on mammalian mitoribosomes. Very recently, however, the Agrawal work was complemented by the 4.9 Å resolution EM-structure of the LSU of the pig mitoribosome by the of Nenad Ban group (Greber et al., 2014) (Figure 4). A pertinent feature of the EM-structures is that the rRNA is almost completely covered with protein. This is in stark contrast to the structures of the bacterial and the eukaryotic cytosolic ribosome, in which the protein is found in only small patches on the rRNA. Another difference from the bacterial and the eukaryotic cytosolic ribosomes is that there is considerable space between the MRPs on the mitochondrial ribosome with several passages available for bulk matrix solvent. This is one explanation for the fact that the sedimentation rate is lower for the mitoribosome (55S) than for *E. coli* (70S), although the mass of the mitochondrial ribosome is considerably larger at 2.7 MDa compared to 2.3 MDa for the *E. coli* ribosome.

The LSU and SSU are connected by 15 inter-subunit bridges, of which only six are conserved to the bacterial homologs, whereas the rest differ in their chemical or spatial arrangement (Sharma et al., 2003; Kaushal et al, 2014). The mitoribosome subunits are mainly joined by protein-protein bridges in contrast to the bacterial counterpart, wherein most interconnection is by RNA-RNA bridging. Furthermore, the mRNAs that enter the SSU do so through an mRNA entrance lined with conserved mitochondrion-specific proteins (Sharma et al., 2003). In fact, in mammals it is unknown how the more-or-less leaderless mRNAs are recognized and transported to this gate. It has been shown that the addition of just a few nucleotides 5´ to the translation start practically abolishes the translation of mitochondrial mRNAs that are normally devoid of a 5´ UTR (Figure 1) (Christian and Spremulli, 2010). This is in stark contrast to the mRNA requirements of both bacterial and eukaryotic cytosolic ribosomes, and it points to fundamental differences in mitochondrial translation initiation in comparison with translation initiation in these systems.

All of the translation products from the mammalian mitoribosome are membrane proteins and hence there are unique opportunities for adaptation and specialization of the mammalian mitoribosome for the task of translating membrane proteins. Indeed, it appears that the mitoribosome is normally bound to the inner membrane, removing the need for cycling between free and membrane-bound states, which occurs with the bacterial and eukaryotic cytosolic ribosomes (Liu and Spremulli, 2000). Furthermore, evidence is accumulating in favor of the claim that the polypeptides are inserted co-translationally into the inner membrane (Gruschke and Ott, 2010).

From the EM-structures of the mitoribosome, it is apparent that on top of the conventional polypeptide exit site (PES), there is another channel (polypeptide accessible site [PAS]) some 25 Å from the PES where the newly formed peptide can also pass out from the ribosome (Greber et al., 2014; Sharma et al., 2003). Indeed, the human Oxa1L, which is important for insertion of proteins into the mitochondrial inner membrane has been reported to interact with the PAS but not with the conventional PES (Haque et al., 2010). It cannot be excluded that whether PAS or PES is used is translation-product dependent.

From the higher resolution structure of the LSU from the Ban group, it was apparent that the ring of proteins MRPL23, MRPL47, MRPL22, MRPL24 and MRPL17 typically found around the PES in homologous ribosomes has an outer layer of proteins in the mammalian mitoribosome (Greber et al., 2014). Several of these outer layer proteins could be modeled into the electron density (Figure 4) and their interactions could be validated through crosslinking and mass spectrometry. Out of these novel outer-layer proteins, perhaps the most interesting is MRPL45, which shows strong homology to the membrane-associated TIM44. Furthermore, MRPL45 is ideally positioned as to act as a membrane anchor of LSU on the inner mitochondrial membrane to position the PES and PAS towards the membrane insertion machinery. In addition, MRPL45 forms a strong novel interaction with MRPL24 thereby replacing the rRNA that has the L24-fixating role in the bacterial ribosome. This is only one example of a situation in which the reductively evolved 16S rRNA has left large voids on the

surface of the LSU and the functionality of the reduced rRNA has been taken up by mitoribosomal proteins.

On the other side of the LSU, at the central proturberance (CP), there are major differences compared to the bacterial ribosome. Although, this was clear already from the lower-resolution structure of the whole mitoribosome by the Agrawal group, the higher resolution in the newer LSU EM-structure permits the modeling of several proteins into the electron density of the CP. Here, MRPL52 takes on a prominent role, as it connects the 39S body with the CP with a 50-residue-long helix (Figure 4). This interaction replaces in part the 5S RNA in bacterial and eukaryotic cytosolic ribosomes. Interestingly, a small piece of RNA is found to bind MRPL18 in a similar fashion as the β-domain of 5S RNA bind L18 in the bacterial and eukaryotic cytosolic homologues (Figure 4). The identity of this piece of RNA is currently unknown. The recent 3.2 Å resolution EM structure of the yeast LSU also shows that the 5S rRNA is absent in the central protuberance of the LSU, but in contrast to the porcine LSU there is no other shorter RNA present at this location (Amunts et al., 2014). Instead, the central protuberance of the yeast LSU consists entirely of mitochondria-specific ribosomal proteins (Amunts et al., 2014).

Mitochondrial translation

Similar to bacterial and cytosolic ribosomes, mitochondrial translation is a multistep process requiring several different factors for initiation, elongation, termination and recycling (Figure 5). The mitochondrial translation process will only be briefly summarized here as a detailed review of this process has recently been published (Christian and Spremulli, 2012). Mitochondria contain two initiation factors (IF), denoted mtIF2 and mtIF3, whereas a homolog of the highly conserved IF1 is lacking. However, there is a 37 amino acid insertion domain in mtIF2 and this insertion was able to functionally replace IF1 in *E. coli* (Gaur et al., 2008). Furthermore, the inserted peptide sequence does bind on the eubacterial ribosome at a site where normally the eubacterial IF1 would bind (Yassin et al., 2011). In the first step, mtIF3 binds the SSU and dissociates the monosome to release the LSU. The SSU mtIF3

complex then interacts with mtIF2, mRNA and tRNA carrying formylated methionine ($tRNA^{fM}$). After the formation of this initiation complex, where $tRNA^{fM}$ is bound to the start codon of the ORF of the mRNA, mtIF2 hydrolyzes its bound GTP to GDP and both initiation factors are released as the LSU binds to start translation (Figure 5).

The elongation phase ensues after the completion of the initiation, when mtEFTu, which forms a ternary complex with GTP and aminoacylated tRNA, enters the A-site of the mitoribosome. Base pairing of the tRNA anticodon with the codon contained in the mRNA leads to conversion of GTP to GDP and release of mtEFTu. The ribosome then catalyzes peptide bond formation, whereby the peptide chain of the tRNA present at the P-site is transferred to the newly entered aminoacylated tRNA of the A-site, thus leading to prolongation of the peptide chain by one amino acid. Next, binding of elongation factor G1 (mtEFG1) catalyzes a translocation step that moves the tRNA with the attached peptide chain from the A- to the P-site simultaneously moving the deacylated tRNA from the P to the E site. The cycle is then repeated as mtEFTu again interacts with the ribosome to bring in the next aminoacylated tRNA.

At the end of the ORF, a stop codon is present and no aminoacylated tRNA can bind to continue peptide synthesis. Instead translation termination factors will interact with the stop codon to release the nascent peptide chain from the ribosome (Chrzanowska Lightowlers et al., 2011). In this context, it should be noted that the genetic code in mammalian mitochondria is somewhat different from the universal code and there are four different stop codons in mammalian mitochondria (UAA, UAG, AGA and AGG) (Anderson, et al., 1981). Remarkably, seven of the nine UAA stop codons are not fully encoded by mtDNA, but, instead, only generated after the addition of one or two A residues during polyadenylation (Anderson, et al., 1981). Mammalian mitochondria contain four translation termination factor homologs called mtRF1, mtRF1a, ICT1 and C12orf65 (Antonicka et al., 2010; Richter et al., 2010; Soleimanpour-Lichaei et al., 2007), but the specific roles of each of these factors is at present not fully understood. Three of the factors mtRF1a, ICT1 and C12orf65 contain a classical GGQ sequence motif in their active sites that catalyzes hydrolysis of the peptidyl-

tRNA bond. It has been reported that mtRF1a binds the nine UAA and the two UAG codons in mitochondrial transcripts to terminate translation (Soleimanpour-Lichaei et al., 2007). The non-standard stop codons AGA and AGG are present in the COI and ND6 transcripts, respectively (Figure 1), and are preceded by U in humans. Translation pausing at these codons followed by a -1 frame shift has been reported to create a UAG stop codon in both of these transcripts to allow mtRF1a to bind (Temperley et al., 2010b). However, recent homology modeling has questioned this concept and instead argues that ICT1 directly binds the AGA and AGG stop codons (Lind et al., 2013). After the completion of translation termination and release of the nascent peptide chain, the ribosome needs to be recycled through the disassembly of the two subunits and release of the mRNA. The ribosomal recycling factors 1 (mtRRF1) and 2 (mtRRF2, also known as elongation factor G2 or mtEFG2) are involved in this GTP-dependent process in mammalian mitochondria (Christian and Spremulli, 2012).

The pleiotropic effects of impaired mitochondrial translation

After the discovery of pathogenic single large deletions of human mtDNA (Holt et al., 1988), it soon became evident that one or more tRNA genes are always deleted (Mita et al., 1990). The affected patients are always heteroplasmic (Mita et al., 1990) and a fraction of >60% mutated mtDNA is needed to impair mitochondrial translation (Hayashi et al., 1991). Children with high levels of deleted mtDNA in multiple tissues typically develop a multisystem disease with symptoms such as sideroblastic anemia, pancreatic insufficiency, diabetes and renal failure, whereas adults often have a more limited tissue distribution of deleted mtDNA and develop a predominantly neuromuscular phenotype with symptoms such as progressive external ophthalmoplegia, myopathy, ataxia and deafness (Larsson and Clayton, 1995). Also heteroplasmic point mutations in tRNA genes are common causes of mitochondrial disease and today we know >200 such mutations (Blakely et al., 2013). Although the pathogenicity of half of these mutations can be questioned, there are still many mutations where overwhelming evidence shows that they cause human disease (Blakely et al.,

2013). The phenotypes caused by different tRNA point mutations vary tremendously and specific point mutations can cause remarkably different phenotypes in affected patients. One example is the m.3243A<G mutation in $tRNA^{L(UUR)}$, which can cause MELAS syndrome (Goto et al., 1990), progressive external ophthalmoplegia (Moraes et al., 1993), and deafness and diabetes (van den Ouweland et al., 1992). Another example is the m.8344A>G mutation in $tRNA^{K}$ (Shoffner et al., 1990), which in some individuals can cause a severe type of mitochondrial neurodegeneration called the myoclonus epilepsy with ragged-red muscle fibers syndrome (MERRF). However, in other individuals of the same pedigree, large lipomas of the neck as the only manifestation of the m.8344A>G mutation (Holme et al., 1993; Larsson et al., 1992). This phenotypic pleiotropism caused by pathogenic point mutations of mtDNA-encoded tRNA genes is largely unexplained and it has been speculated that the tissue distribution of mutant mtDNA and the nuclear genetic background of the mutation carrier are important determinants of the phenotype.

Given the extensive knowledge that has accumulated over the last 20 years about the extreme variability in clinical manifestations caused by mtDNA mutations affecting tRNA genes, it is perhaps not so surprising that also mutations in nuclear genes controlling mitochondrial translation have also been shown to result in a tremendous variability of clinical symptoms. A variety of mainly autosomal recessive mutations that impair mitochondrial translation are known and some examples of mutated genes include those that encode mRNA stability and polyadenylation factors (LRPPRC and mtPAP), tRNA-modification enzymes (PUS1, MTU1 and MTO1), enzymes that aminoacylate tRNAs (DARS2, RARS2, EARS2, MARS2, FARS2, AARS2, YARS2, SARS2, HARS2 and LARS2), mitoribosomal proteins (MRPS16, MRPS22, MRPS28, MRPL3, MRPL12 and MRPL44), translation elongation and termination factors (mtEFTu, mtEFG1, mtEFTs and C12orf65), an enzyme for formylation of methionine (MTFMT) and translational activators of specific transcripts (TACO1) (Boczonadi and Horvath, 2014; Rötig, 2011). The resulting diseases are often lethal with a neonatal or infantile onset, and several recent reviews provide a detailed discussion about the severe clinical manifestations (Boczonadi and Horvath, 2014;

Rötig, 2011). In several instances there is a relatively strong correlation between the mutated gene and the resulting clinical phenotypes, including neuropathological findings, as exemplified mutations in the different genes encoding the enzymes that aminoacylate tRNAs (Rötig, 2011).

So why does impaired mitochondrial translation lead to such a variability of clinical manifestations? In some instances, this is due to poorly understood specific molecular events initiated by the mutated gene. In other cases, genetic modification of the resulting phenotype is likely to be an important determinant. It should be noted that genetic modification of the phenotype is also quite common in other types of genetic disease and even an archetypal inherited disease such as sickle cell anemia, caused by a homozygous mutation that exchanges a single amino acid in hemoglobin, show a substantial variation in the severity of symptoms between different patients (Lettre, 2012). The complexity of the mitochondrial translation machinery means that a mutated, dysfunctional component will have direct and indirect molecular interactions with a large number of other components. All of these interactions can be subject to genetic modifications and can thereby influence the overall impact on mitochondrial protein synthesis. Furthermore, tissue-specific differences in the abundance of different components of the mitochondrial translation machinery and tissue-specific differences in the dependence on oxidative phosphorylation will also have major impact on the overall phenotype.

Concluding remarks

It is becoming increasingly clear that translational regulation is a major determinant of mammalian mtDNA expression, which is essential for oxidative phosphorylation. The complexity of the mitochondrial translation machinery is remarkable, given that the purpose is to synthesize only 13 proteins of the oxidative phosphorylation system. Translation of the mtDNA-encoded mRNAs is dependent on 24 mtDNA-encoded genes (2 rRNA and 22 tRNAs) and at least one hundred nuclear-encoded genes that encode ribosomal proteins, translation factors, tRNA-modifying enzymes, ribosomal biogenesis factors, tRNA-aminoacyl

transferases and other factors. A large number of mainly autosomal recessive human diseases are caused by defective mitochondrial translation and affected patients often have neonatal or infantile onset often with very severe disease phenotypes. Furthermore, mutations of mtDNA often impair mitochondrial translation to cause a mosaic pattern of severe respiratory chain deficiency in disease and ageing. Within the next few years, we can expect a broader and more detailed molecular knowledge of the mitochondrial translation machinery. In this regard, an atomic structure of the mammalian mitoribosome would provide important mechanistic insights. It stands clear that a better understanding of the regulation of mitochondrial translation should enable the development of specific treatments aimed at ameliorating the mitochondrial dysfunction found in mitochondrial disease, age-associated diseases and ageing itself.

Legends to figures

Figure 1

The gene content of mammalian mtDNA and encoded mRNAs

The mammalian mtDNA contains only one longer non-coding region, denoted the displacement (D-) loop region, which contains regulatory sequences needed for mtDNA replication and transcription. The mtDNA encodes 2 rRNAs (red boxes), 22 tRNAs (green boxes) and 11 mRNAs with 13 open reading frames (ORFs, blue boxes). The mRNAs contain no or very short 5´ non-coding sequences (yellow boxes) preceding the first translation initiation codon (AUA, AUU or AUG). Furthermore, most mRNAs lack 3´ non-coding sequences (yellow boxes) between the stop codon (AGA, AGG, UAA and UAG) and the short poly A tail. Two of the mRNAs (ND4/ND4L and ATP6/ATP8) are bicistronic and each contains two partly overlapping ORFs. The structure of the human mRNAs are shown in the right panel.

Figure 2

Posttranscriptional maturation of mtDNA-encoded RNAs

Each strand of mtDNA is transcribed as a long polycistronic transcript that is subsequently processed to release the individual mRNAs, tRNAs and rRNAs. The mitochondrial RNase P consists of three subunits (MRPP1-3) and cleaves the primary transcript at the 5´-end of tRNAs. MRPP1-2 are responsible for an N1-methylation of mitochondrial tRNAs. The tRNAs are released by cleavage at their 3´-ends by RNase Z (ELAC2). Yellow dots indicate RNA modifications.

Figure 3

RNA modifications present in mitochondria

Nucleobases with modifications that are found in mitochondrial RNA and discussed in the text. The base modifications are highlighted with a lighter background. $Cmnm^5U$ is present

only in bacteria and yeast mitochondria but is shown for comparison to the mammalian mitochondrial taurine-based modifications.

Figure 4

Structure of the large subunit of the mammalian mitoribosome

Rendered from the 4.9 Å EM-structure of the large subunit of the porcine mitoribosome (Greber et al., 2014). rRNA is colored in light-beige and ribosomal proteins that are not specifically denoted are colored in green. The RNA found to be bound to MRPL18 in the structure is denoted 'RNA X'.

Figure 5

Biogenesis of the mammalian mitoribosomes and the translation cycle

The biogenesis of the mitoribosome requires that the 12S and 16S rRNAs are modified and assembled together with the ribosomal proteins. The large subunit of the mitoribosome is believed to be anchored to the inner mitochondrial membrane and the translation cycle requires several factors for initiation, elongation and termination. The membrane anchoring of the mitoribosome is believed to facilitate insertion of newly synthesized proteins into the inner mitochondrial membrane. The recycling of the mitoribosome is not shown.

Acknowledgements

Support for this research was provided by the Swedish Research Council 2011-6510 to B.M.H. and by European Research Council (ERC) advanced investigator grant, the Deutsche Forschungsgemeinschaft (SFB829), and a Swedish Research Council 2013-2859 grant to N.G.L.

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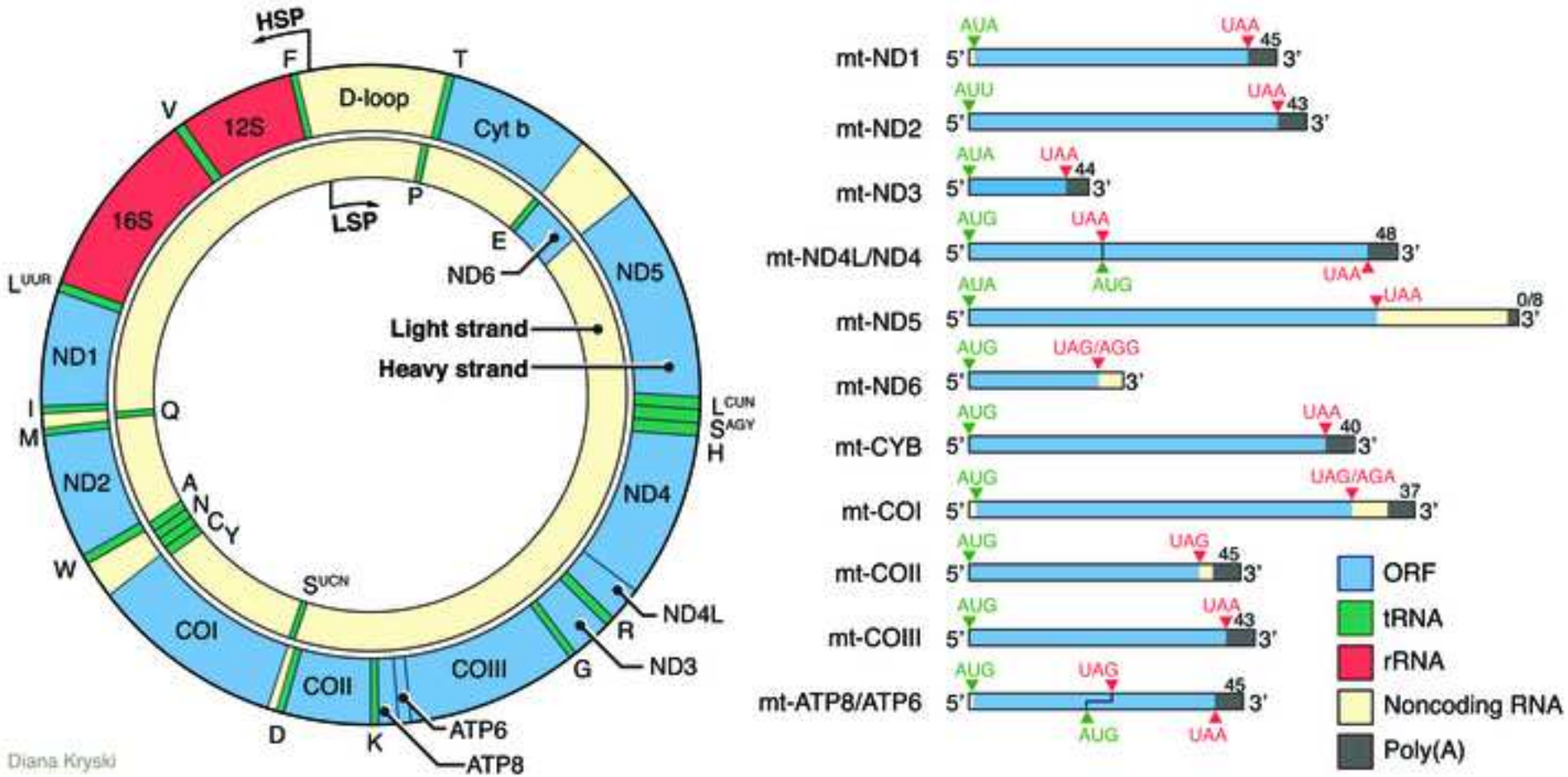

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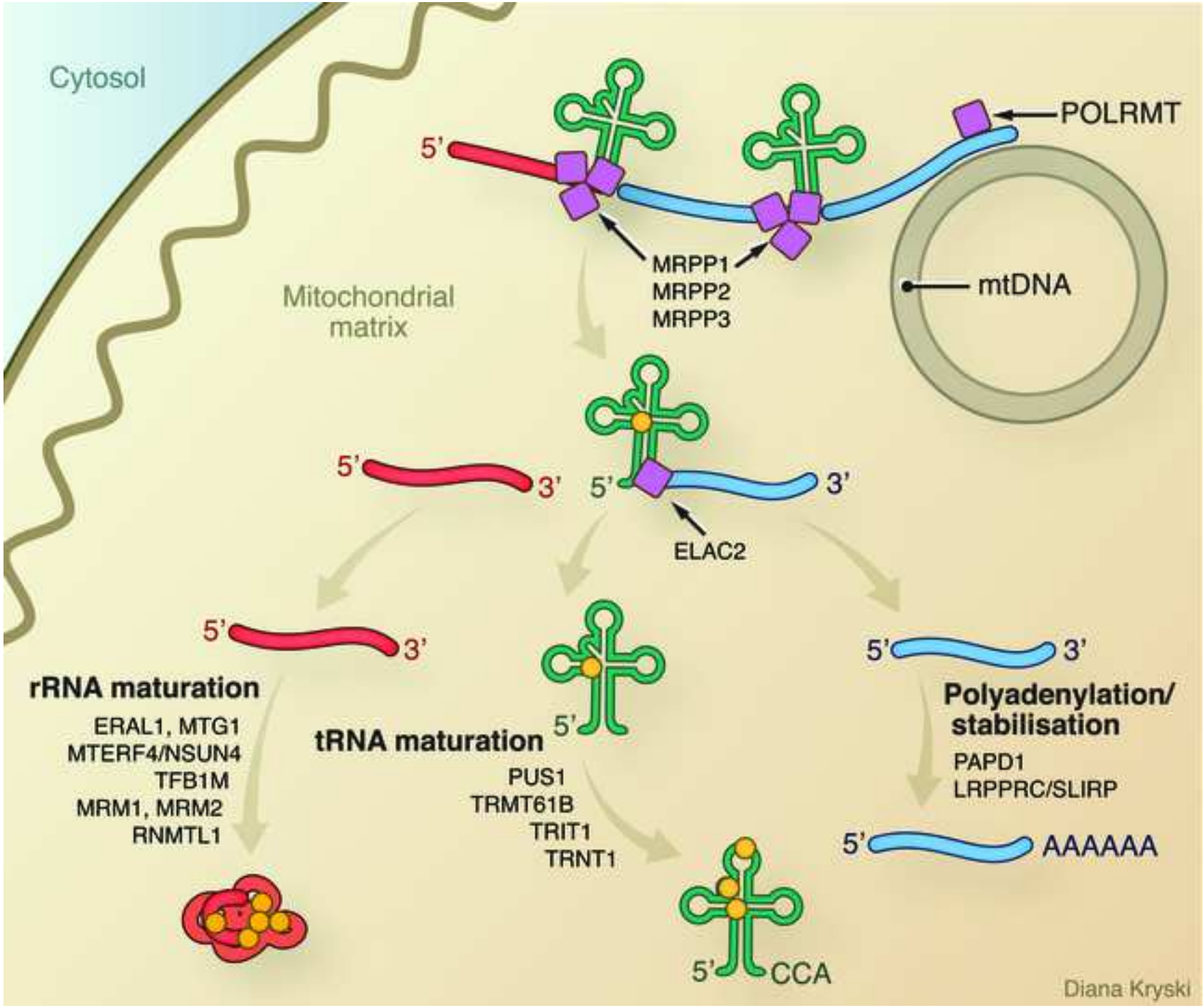

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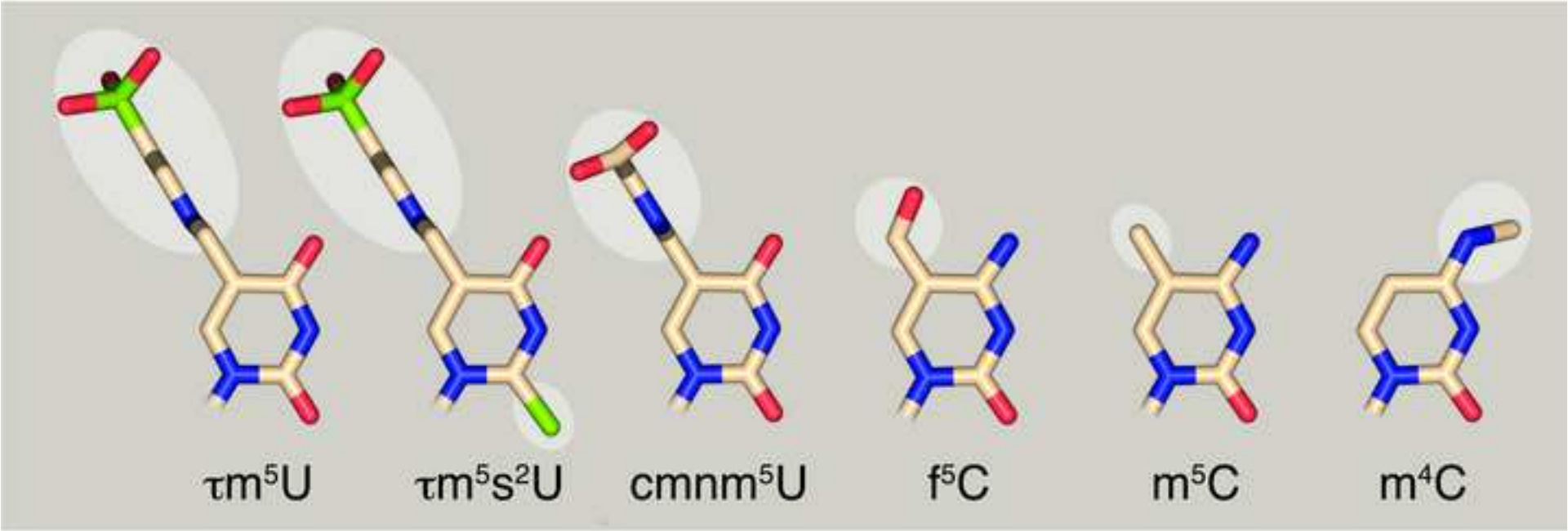

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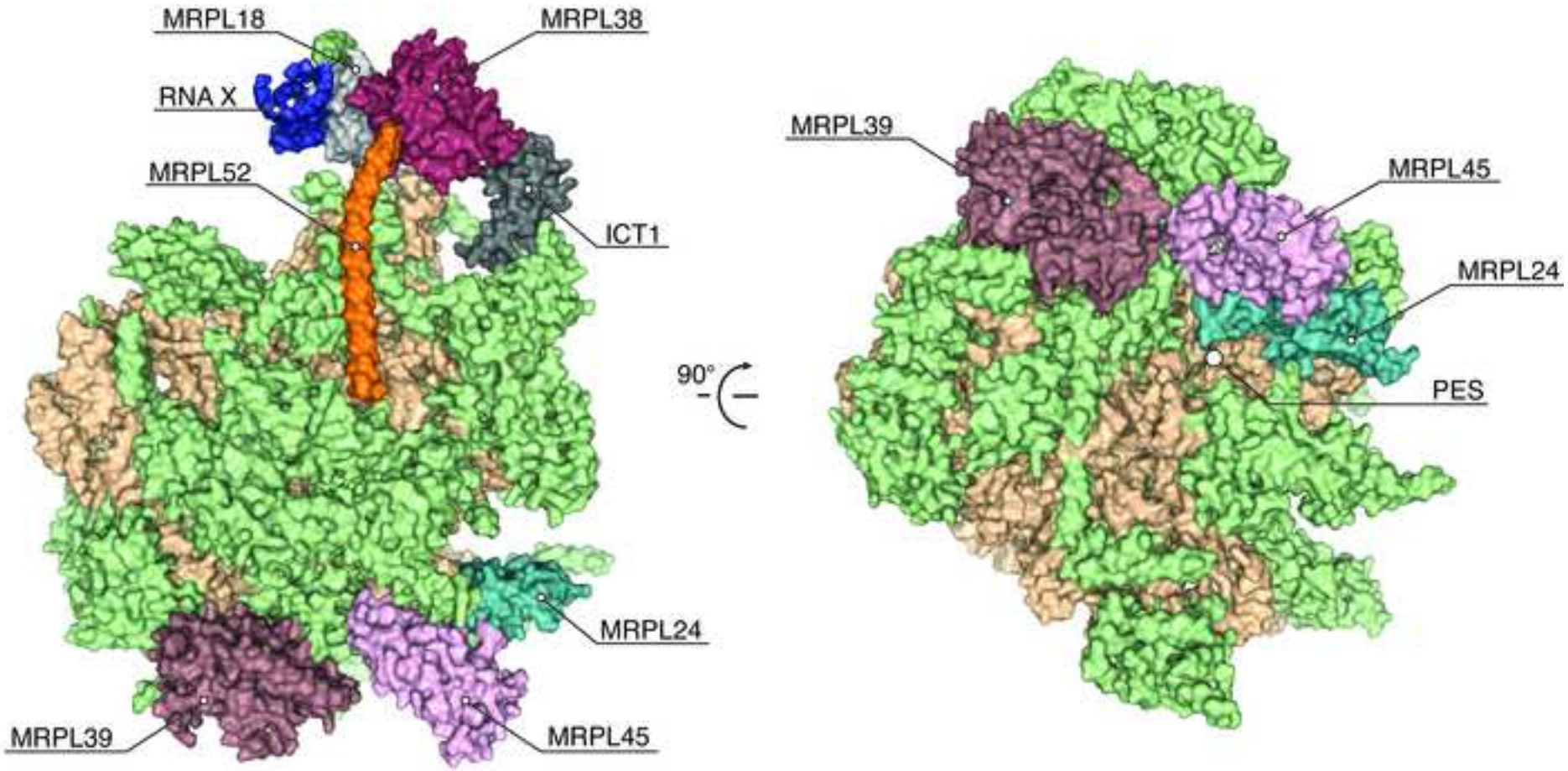

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