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REVIEW

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Mechanisms of mammalian mitochondrial transcription

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Abstract

Numerous age-related human diseases have been associated with deficiencies in cellular energy production. Moreover, genetic alterations resulting in mitochondrial dysfunction are the cause of inheritable disorders commonly known as mitochondrial diseases. Many of these deficiencies have been directly or indirectly linked to deficits in mitochondrial gene expression. Transcription is an essential step in gene expression and elucidating the molecular mechanisms involved in this process is critical for understanding defects in energy production. For the past five decades, substantial efforts have been invested in the field of mitochondrial transcription. These efforts have led to the discovery of the main protein factors responsible for transcription as well as to a basic mechanistic understanding of the transcription process. They have also revealed various mechanisms of transcriptional regulation as well as the links that exist between the transcription process and downstream processes of RNA maturation. Here, we review the knowledge gathered in early mitochondrial transcription studies and focus on recent findings that shape our current understanding of mitochondrial transcription, posttranscriptional processing, as well as transcriptional regulation in mammalian systems.

KEYWORDS

mitochondrial transcription, mitochondrial gene expression, POLRMT, TEFM, MTERF1, TFAM, TFB2M, mitochondrial RNA processing

1 | INTRODUCTION

Mammalian mitochondria perform numerous essential cellular functions including heme and phospholipid biogenesis,[1] the regulation of intracellular calcium signaling, apoptosis,[2] reactive oxygen species production,[3] and most importantly, energy production through oxidative phosphorylation. These processes are directly or indirectly linked to mitochondrial gene expression, the process by which mitochondrial DNA (mtDNA) is transcribed into RNA and subsequently translated into protein. The mammalian mitochondrial double stranded circular genome is ~16.5 Kilobases (Kb), encoding 13 polypeptide genes, 22 tRNAs for translation, and 2 ribosomal RNAs (rRNAs; Figure 1a). The rRNAs are key constituents of the mitochondrial ribosomes, exclusively dedicated to the translation of the 13 mitochondrial gene products.[4,5] Mitochondria need their own transcription and translation machineries because these two processes are mechanistically distinct from nuclear transcription or translation.[6]

This review will focus on mammalian mitochondrial transcription, covering findings spanning the last five decades and emphasizing recent observations not covered in previous reviews. We review the genomic organization of mitochondrial transcription, the current models for the layout of mitochondrial promoters, our molecular understanding of the transcription machinery, and the process of transcription, posttranscriptional processing as well as transcriptional regulation.

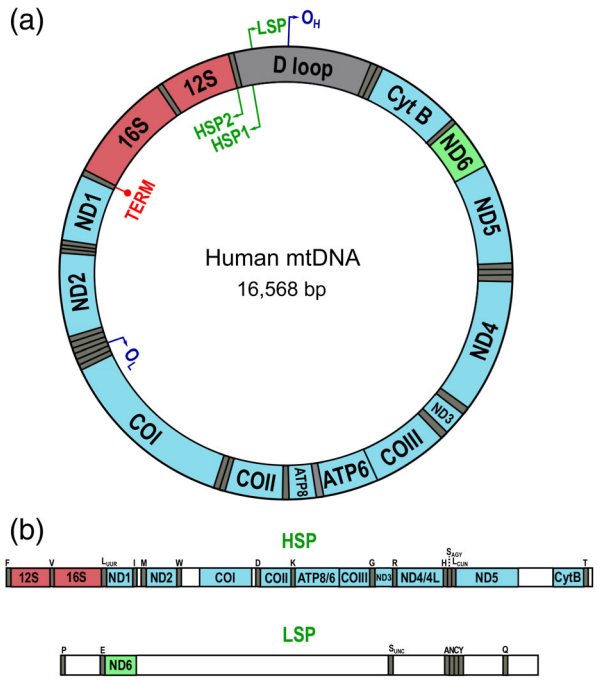

FIGURE 1 Organization of mitochondrial transcription. (a) Scheme of the mitochondrial genome indicating key functional loci as well as the different gene products. rRNAs (red) and most mRNAs (blue) are coded in the H-strand, while a single mRNA (ND6; green) is coded by the L-strand. tRNA genes are show in gray. (b) Scheme of the full-length polycistronic mitochondrial transcripts indicating the gene products encoded by each transcript

2 | MITOCHONDRIAL GENOMIC STRUCTURE: HEAVY VERSUS LIGHT STRANDS

The human mitochondrial genome (mtDNA) is circular and composed of two strands, which were labeled heavy and light strands (H-strand and L-strand, respectively). The H-strand denotes its high content in purine nucleotides compared to the L-strand (owing mostly to a strong imbalance in the guanine/cytosine ratio), making the strands separable by using density centrifugation in alkaline $CsCl_2$ gradients.[7] Early mitochondrial transcription studies using DNA–RNA hybridization experiments showed that most mtRNAs hybridized with one of the DNA strands, suggesting an asymmetry in the utilization of both strands.[8,9] The mtDNA was first sequenced in 1981 by Anderson and colleagues.[4] This made it clear that both strands encode genes that are transcribed, although very few genes are encoded in the L-strand compared to the H-strand. The H-strand encodes 12 of the 13 polypeptides, the 2 ribosomal RNAs (12S and 16S) and 14 tRNAs, while the L-strand encodes one polypeptide, eight tRNAs, and is the source of primer synthesis for mtDNA replication[10] (Figure 1b).

It is important to note that numerous other mammalian genomes have been sequenced, and that their genomic organization appears highly conserved with the exception of minor rearrangements in tRNA genes.[11] However, in vivo and in vitro functional studies have predominantly been carried out using the human or mouse systems. Thus, although the mechanisms of gene expression are expected to be conserved in all mammals, it is not possible to rule out that differences might exist across species.

3 | mtDNA PROMOTERS

Early mitochondrial gene expression studies supported the presence of a single promoter on each strand leading to the transcription of two polycistronic transcripts that are later processed into single RNAs.[12] The two promoters are found in the displacement loop (D-loop) region of mtDNA.[4] Termed heavy strand promoter (HSP) and light strand promoter (LSP), HSP and LSP initiate transcription of H-strand and L-strand, respectively. This model of mitochondrial transcription was supported by the work of Aloni and Attardi, who used pulse labeling as well as RNA–DNA hybridization followed by electron microscopy to show that the mtDNA is symmetrically transcribed from both strands.[13] These early findings were strengthened by the research from three independent groups. These groups showed that the region of mtDNA around the origin of replication contains an initiation site for L-strand transcription and an initiation site for H-strand transcription.[14–16]

Subsequently, Montoya et al. carried out mapping experiments on the human mtDNA to identify the precise sites of transcription initiation on the H-strand and L-strand. They radioactively labeled the 5′ termini of mitochondrial poly(A) containing RNAs using a capping enzyme and mapped those sites by DNA–RNA hybridization. They identified two mapping sites for the capped 5′ ends: one on the H-strand upstream of the tRNA-Phe gene (HSP, now known as HSP1; Figure 1a) and another on the L-strand near the 5′ terminus of the 7S RNA coding sequence (LSP; Fig. 1a). They then confirmed these mapping sites using the 5′ ends of newly synthesized transcripts from in vitro mtDNA transcription experiments.[17] Surprisingly, their mapping experiments identified another initiation site on the H-strand near the 5′ terminus of the 12S rRNA gene (now known as HSP2; Figure 1a). Thus, they proposed a three-promoter model, consisting of LSP on the L-strand and HSP1/HSP2 on the H-strand. The two sites on the H-strand were interpreted to be responsible for two distinct transcription events: HSP1 primary transcripts would originate at position 561, 16 bp upstream of the tRNA-Phe gene[18,19] and terminate at the 3′ end of the 16S rRNA,[19] consisting of the tRNA-Phe, the 12S rRNA, the tRNA-Val, and the 16S rRNA[20]; HSP2 transcripts would originate at position

646, 2 bp upstream of the 12S rRNA gene[19] and proceed to generate near full-length transcripts, although the precise 3′ end of this transcript was not determined.[17,18] This interpretation is however not consistent with later evidence (see Section 7). Research from Clayton's lab also supported the existence of two HSPs.[21,22] However, it is worth noting that the existence of HSP2 has been contested for various reasons. Transcription from HSP2 was demonstrated in cell-based studies and remained primarily unreproducible in vitro using recombinant proteins, raising the question of whether it is a functional promoter in vivo.[23] In vitro HSP2 transcription was later independently achieved by two research groups, and shown to be highly dependent on the reaction conditions: POLRMT and TFB2M were necessary and sufficient to initiate transcription from HSP2 and the presence of TFAM inhibited transcription from this site,[24,25] suggesting that TFAM could participate in a feedback mechanism to regulate transcription from the two strands of mtDNA. However, the experimental conditions used to observe initiation from HSP2 in vitro have been suggested to be conducive to unspecific initiation.[26] Additional support for the existence of HSP2 comes from recent genomic analysis of mitochondrial RNAs.[27] However, the existence and functional significance of HSP2 initiation remain open questions in the field.

4 | TRANSCRIPTION MACHINERY

The mammalian mitochondrial transcription machinery is mechanistically unique. Although it is not possible to rule out the existence of additional yet unidentified factors, the core transcription machinery consists of an RNA polymerase (RNAP; POLRMT), an initiation factor (TFAM), an activator (TFB2M), an elongation factor (TEFM), and a termination factor (MTERF1). All these proteins are highly conserved in mammals (although TFB2M displays a larger degree of divergence), suggesting that they play similar roles across species.

Our understanding of the mechanism of transcription mostly derives from biochemical and structural observations, but the models are also influenced by the phenotypes observed upon genetic manipulation of the relevant genes. Transcription initiation from all three promoters, LSP, HSP1, and HSP2 can be recapitulated in vitro using only two protein components of the transcription machineries: POLRMT and TFB2M.[24,25,28–30] However, substantial evidence suggests that TFAM is a core component of mitochondrial transcription initiation in vivo (see below). Both in vitro and in vivo evidence is consistent with a role for TEFM supporting transcriptional elongation. Finally, strong evidence supports a role for MTERF1 as a transcription termination factor.

5 | TRANSCRIPTION INITIATION

Efficient initiation in vitro depends on the presence of POLRMT and the two initiation factors, TFAM and TFB2M. Our mechanistic understanding of the process has been drastically affected by two crystal structures of the initiation complex on the two mitochondrial promoters (LSP and HSP).[31] We will begin by reviewing the roles and properties of each of the proteins currently known to be involved in the initiation process: POLRMT, TFAM, and TFB2M.

5.1 | POLRMT

Perhaps the most widely studied of the mitochondrial transcription related enzymes, POLRMT is at the heart of the mitochondrial RNA transcription apparatus. The human form of POLRMT was initially discovered by screening the expressed sequence tags database. The protein (1230 residues) was found to be similar to mitochondrial RNAPs from other eukaryotes and to RNAPs from several bacteriophages.[32] The single subunit POLRMT does not transcribe nuclear genes and has an exclusively mitochondrial role.[33] It is solely responsible for transcription of the circular mitochondrial genome. Furthermore, POLRMT is believed to have another essential role, generating replication primers for DNA replication.[34]

The catalytic domain of POLRMT is highly similar to the phage T7 RNAP.[32] Like T7 RNAP, POLRMT has DNA-dependent RNAP activity in vitro on synthetic substrates.[35,36] It is also able to promote RNA synthesis on a premelted substrate.[37] However, unlike T7 RNAP, it is incapable of initiating promoter-dependent in vitro transcription.[35] A crystal structure of POLRMT (residues 105–1230) identified three structural features that explain why it is by itself unable to facilitate promoter-dependent transcription.[38] Although, like T7 RNAP, the catalytic domain of POLRMT adopts a right-hand fold with palm, fingers and thumb subdomains (Figure 2a), it is missing promoter-recognition elements and displays key structural differences in regions that facilitate promoter melting in T7 RNAP.

In addition to the T7 RNAP core, POLRMT contains a flexible N-terminal extension, which shows no homology with T7 RNAP. This N-terminal extension is dispensable for promoter-independent transcription. However, it is essential for transcriptional initiation, likely because it mediates an interaction between POLRMT and the initiation factor TFAM.[40] Although the structure of the entire N-terminal domain has not been solved, the POLRMT crystal structure shows that it contains a PPR domain Figure 2a) that makes some contacts with the DNA backbone in the promoter region. This is the only interaction between POLRMT and the DNA template, thus helping to explain why TFAM is required for POLRMT recruitment.[41]

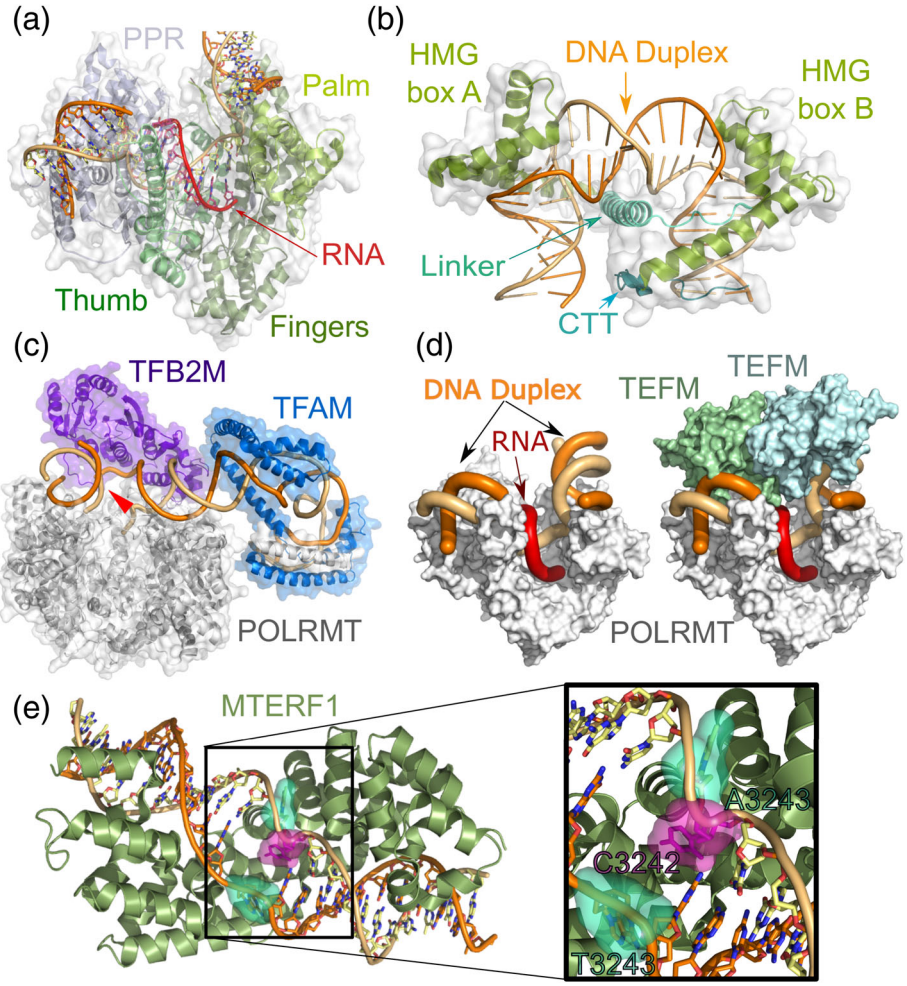

FIGURE 2 Structures of the mitochondrial transcription machinery. (a) POLRMT (PDBID: 3SPA) adopts a canonical right-hand fold with fingers, palm, and thumb subdomains. The POLRMT molecular surface is in transparent gray. The fingers are green, the thumb is dark green, the palm is light green, and the N-terminal and PPR domains are blue. The DNA duplex is show in red-orange. (b) TFAM (PDBID: 3TMM) is composed of two HMG box domains and is able to induce a 180° bend in the DNA. The TFAM molecular surface is transparent, the two HMG boxes (a and b) are shown in green, the linker is light blue and the C-terminal tail (CTT) is blue. The DNA duplex backbone is shown in orange ribbon and the bases are represented in sticks. (c) The transcription initiation complex is formed by TFAM (blue), TFB2M (magenta), and POLRMT (gray). TFAM bends the DNA upstream of the transcription start site (red arrow) and recruits POLRMT. TFB2M binds the nontemplate strand and stabilizes the open promoter complex. (d) A TEFM dimer binds to the elongating POLRMT and acts as a sliding clamp, enhancing its processivity. The structure of an elongation complex (PDBID: 5OLA) is shown without (left) and with TEFM (right), highlighting how the TEFM dimer covers the upstream and downstream DNA duplex (orange) as well as the nascent RNA strand (red). (e) MTERF1 (PDBID: 3MVA) makes extensive contacts with the termination sequence and promotes unwinding of the DNA duplex and base-flipping of three nucleotides, both bases of the A3243 base pair (mutated in MELAS[39]) as well as C3242

Thus, although POLRMT is fully active as an RNAP, initiation activity depends on the presence of two protein cofactors: TFAM and TFB2M. Furthermore, although POLRMT is a processive enzyme, able to synthesize long RNA fragments in vitro, the production of longer RNA transcripts seems to require its association with the elongation factor TEFM[42] (see below).

5.2 | TFAM

TFAM is a multifunctional high mobility group (HMG) box protein that plays essential roles in mitochondria. First discovered in 1985 by separation of fractional pools of protein,[43] TFAM was found to facilitate specific transcription, primarily on the L-strand. The role of TFAM as a critical factor for promoter selection during initiation of mitochondrial transcription and efficient promoter recognition by POLRMT has since been confirmed.[44–47] TFAM activates transcription by specifically binding immediately upstream of transcriptional start sites.[31] TFAM plays a second essential role in mitochondria. Owing to its ability to bind mtDNA with no sequence specificity, it coats the entire genome[48] and facilitates its compaction into nucleoids.[49] It has been proposed that TFAM covers the entire surface of a single copy of the mtDNA to form a nucleoid.[50]

Consistent with its key roles in transcription and mtDNA maintenance, TFAM deletion is embryogenically lethal, accompanied by a reduction in mtRNA levels and a severe respiratory chain deficiency.[51,52] Its expression level is also known to regulate mammalian mtDNA copy number.[52] However, the mechanism by which it modulates mtDNA copy number is unknown: on the one hand transcription is necessary for replication primer formation, but TFAM coating is also thought to protect mtDNA from damage.

Crystal structures of TFAM in complex with different mtDNA substrates show that it is comprised of two HMG boxes and a C-terminal tail.[53,54] TFAM has the remarkable ability to induce mtDNA into forming a U-shaped turn (Figure 2b), where each of the singular HMG boxes introduces a 90° turn. These induced turns of the DNA are crucial for interactions with the remainder of the transcription apparatus (see below). Although it is not clear whether this conformation is also relevant to its DNA coating activity, it is tempting to speculate that it may play a role in organizing and compacting the mitochondrial genome.

5.3 | TFB2M

TFB2M was discovered in 2002 as a factor key to assist POLRMT with the process of initiation of mitochondrial RNA transcription.[35] Interestingly, TFB2M is highly similar to a group of highly conserved rRNA methyltransferases.[55] In fact, TFB2M appears to conserve an independent methyltransferase activity of unclear function,[56] but this activity is not necessary for mitochondrial transcription.[57] TFB2M is required for promoter-dependent POLRMT transcription in vitro. A crystal structure of TFB2M shows that it adopts a methyltransferase fold and is highly similar to the TFB1M mitochondrial rRNA methyltransferase.[41,58]

5.4 | Mechanism of initiation

TFAM appears to be the only protein in the initiation complex that can specifically bind to the promoter[59] and thus TFAM binding has been proposed as the initial step in initiation.[31,60,61] Because TFAM and POLRMT can form a direct interaction, TFAM is then believed to recruit POLRMT to the transcription start site. As TFAM is known to coat the mitochondrial genome and its binding mode appears identical at nonpromoter sites,[62] it is not clear why this interaction is restricted to the mitochondrial promoters. In this respect, although the POLRMT–TFAM interaction is of low affinity, it can take place in the absence of DNA,[60] suggesting that perhaps the initial binding event might involve a preformed TFAM–POLRMT complex. This is consistent with the low affinity of POLRMT–TFAM complexes to DNA observed in vitro[61] and might explain the importance of POLRMT for promoter specificity.[59]

The preinitiation complex formed by TFAM and POLRMT is unable to initiate transcription in the absence of TFB2M. TFB2M is recruited to the preinitiation complex and plays a critical role in facilitating DNA opening, binding the promoter and specifically the nontemplate strand, as well as inducing conformational changes in POLRMT that stabilize the melted promoter complex[31] (Figure 2c). The order of events during initiation cannot be established with certainty, and it is important to note that an alternative model for transcription initiation has suggested that a POLRMT–TFAM preinitiation complex might not exist in vivo and that instead TFAM might recruit a POLRMT–TFB2M complex.[61]

It has been suggested that the mitochondrial promoters are differentially regulated, and this seems to be the case at least in vitro.[63] The structures of the initiation complex on both the HSP and LSP promoters however suggest a similar organization of the initiation complex[31] and thus whether differential regulation takes place in vivo is still an open question.

6 | TRANSCRIPTIONAL ELONGATION

Once transcription initiation takes place and POLRMT switches to elongation mode, TFB2M likely dissociates,[37] and TEFM is believed to associate with POLRMT to drive transcription elongation.[64] Knockdown of TEFM by RNA interference show reduced levels of H- and L-strand promoter-distal mitochondrial transcripts, and is accompanied by respiratory deficiencies in human cells.[64] Knockdown of TEFM in mouse cells confirms that it is essential for transcriptional elongation and generation of full-length transcripts.[65]

TEFM forms a dimer in solution and potently enhances the processivity of POLRMT in vitro by substantially increasing its affinity to DNA.[42] The TEFM crystal structure confirms that it dimerizes and reveals that each monomer contains two tandem helix–hairpin–helix domains that are dispensable to enhance POLRMT processivity as well as an RNase H-like fold that mediates the activities relevant to transcriptional elongation. Moreover, dimerization is essential for its association with POLRMT.[66] A crystal structure of a transcription elongation complex showing TEFM complexed with POLRMT shows that TEFM binds POLRMT near the RNA exit channel, forming a sliding clamp that presumably increases the processivity of POLRMT (Figure 2d).[66] Importantly, binding of TEFM is incompatible with TFB2M binding, and consistently TEFM is unable to associate with POLRMT during initiation.[66] Furthermore, the transition to elongation appears to involve substantial rearrangements in the topology of the DNA upstream of the POLRMT active site.[31]

The increase in processivity resulting from association of TEFM and POLRMT can overcome transcriptional termination occurring at various sites in the mtDNA and facilitate bypass of oxidative lesions.[40] Particularly relevant is termination taking place at the conserved sequence block II region because of the generation of RNA G-quadruplexes,[40,66] which has been linked to the generation of replication primers.[67] This led to the suggestion that TEFM might be key to regulate a switch between mitochondrial transcription and replication, whereby POLRMT elongation in the absence of TEFM would result in the formation of RNA replication primers.[67] However, TEFM knockouts do not display an increase in mtDNA replication.[65] Instead, the knockout results in an increase in premature termination as well as a subsequent decrease in formation of replication primers. Further processing by RNase H of longer, TEFM-mediated transcripts is required for their use as replication primers.[68] Moreover, the loss of TEFM can negatively affect mitochondrial RNA processing.[65] Additional work is required to explain the potential connection between mitochondrial transcription elongation and RNA processing.

7 | TRANSCRIPTION TERMINATION

The first studies of mitochondrial transcription termination were motivated by the early conclusion that the rRNA genes situated in the H-strand proximal region of HSP are transcribed at 15–60 times higher rates than the more distal H-strand protein coding genes.[69] Although the higher steady-state levels of mitochondrial rRNAs are now understood to be a consequence of higher stability rather than transcription rates,[28] subsequent studies of the 3′ ends of the 12S and 16S rRNAs led to the suggestion that their synthesis was mediated by a transcription termination event.[70,71] This led to the discovery of mTERF (now known as MTERF1), a protein factor capable of protecting a 28 base pair segment of mtDNA at the 3′ end of the 16S rRNA and mediate transcription termination[72] (TERM in Figure 1a). Subsequent cloning of this factor[73] led to the realization that it is a member of a family of nucleic-acid binding proteins (MTERF proteins) that play various roles related to gene expression in mitochondria and chloroplasts[74,75] and display extreme flexibility in their ability to accommodate a wide range of interactions with nucleic acids.

MTERF1 has been shown to terminate transcription in vitro with very high efficiency.[76] Although original models of transcription termination by MTERF1 proposed that it is responsible for termination of HSP2-driven transcription to facilitate synthesis of rRNAs,[19] these models are not supported by any in vivo evidence, and are inconsistent with in vitro biochemical studies.[76,77] Later knockdown[78] and knockout[28] experiments strongly support the notion that MTERF1 does not play a role in termination of HSP-driven transcription and is instead responsible for termination of transcription originating at LSP. This is also consistent with structural studies supporting termination polarity. The crystal structure of MTERF1 bound to the DNA termination sequence shows that MTERF1 binds DNA through a unique mechanism that involves a repeated module, the MTERF motif, but also partial unwinding of the DNA double helix, the melting of two base pairs, and eversion of three nucleotides[77] (Figure 2e). Sequence recognition by MTERF1 is based on specific recognition of guanine residues in the target sequence by the MTERF modules, and these interactions are prevented by multiple pathogenic mutations.[79]

MTERF1 appears to play roles beyond LSP termination: it has been shown to mediate more general pausing of RNA transcription by POLRMT[80] as well as pausing of mtDNA replication.[80,81] Finally, although MTERF1 might account for LSP termination, termination of HSP transcription occurs through a mechanism that is still poorly understood, appears to be MTERF1-independent, and might involve additional proteins.[82]

8 | RNA PROCESSING

Mitochondrial primary transcripts must undergo processing for the release of mature and functional mtRNAs. This processing encompasses cleavage, polyadenylation, addition of the cytosine, cytosine, adenine (CCA) tail to tRNAs as well as other base posttranscriptional modifications. It has been proposed that all these processes take place in a compartmentalized area of the mitochondrial matrix known as the mitochondrial RNA granule.[83,84] Ojala et al., first proposed the tRNA punctuation model, whereby cleavage of primary full-length transcripts occurs at junctions between mRNAs or rRNAs and tRNAs.[20,85] This model has been widely accepted and proven to generally agree with deep sequencing analysis.[86] However, deep sequencing also revealed unexpected complexity in RNA processing, including noncanonical processing events[87] as well as multiple types of aberrant processing including polyadenylation of some tRNAs.[86]

Cleavage of polycistronic transcripts is carried out by specific enzymes. Cleavage of tRNAs at their 5′ and 3′ ends is performed by RNase P and RNase Z, respectively, releasing individual or bicistronic RNA species to undergo further maturation (Figure 3). RNase P is composed of three subunits (MRPP1, MRPP2, and MRRP3) and is surprisingly devoid of an RNA component.[88] Cleavage at tRNA 3′ ends is performed by RNase Z (ELAC2).[89,90] RNase Z was shown to preferentially process precursors already processed by RNase P.[89,90] Four genes have been identified that are not flanked on both sides by tRNAs and therefore must undergo noncanonical processing. For instance, the mitochondrial cytochrome c oxidase 1 (COX1) is flanked at its 5′ end by

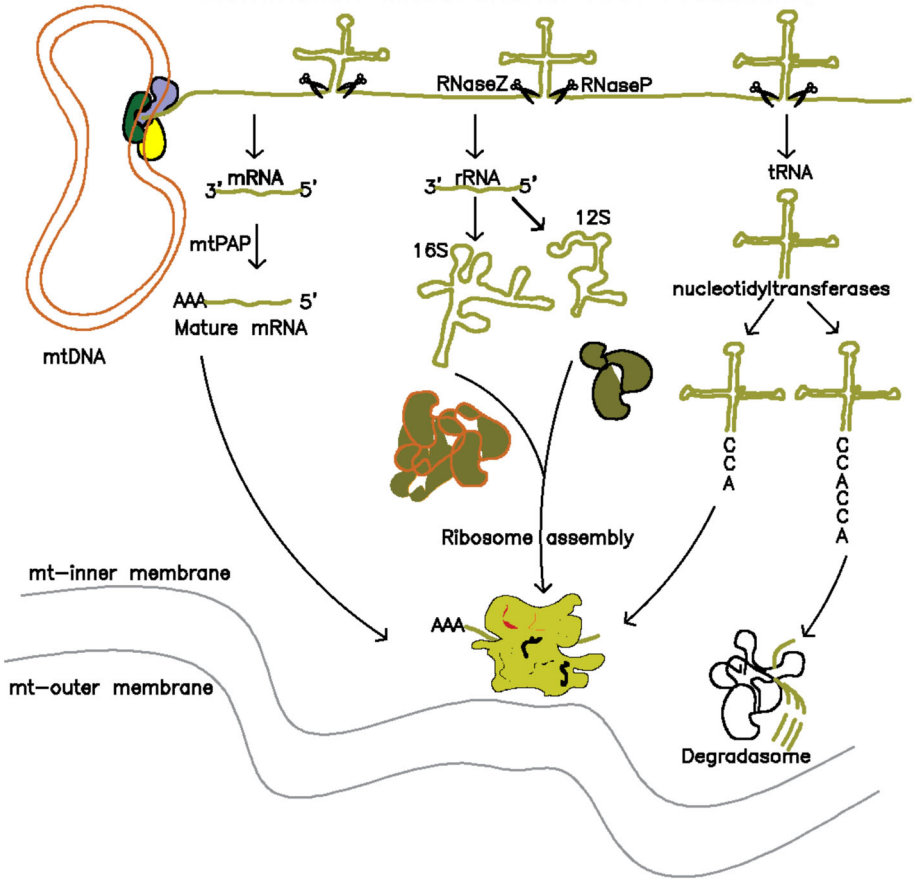

FIGURE 3 Overview of mammalian mitochondrial RNA processing. RNA processing is believed to take place co-transcriptionally. Cleavage by RNases P and Z liberates individual mRNAs, tRNAs and rRNAs. These RNAs then undergo different modifications (see text) resulting in maturation and utilization in downstream processes (i.e., ribosome assembly and translation) or, alternatively, in degradation of unstable RNAs

noncoding RNA (ncRNA) and at its 3′ end by tRNA-Cysteine.[90] The ncRNA upstream of COX1 is thought to adopt a tRNA-like structure providing a basis for processing by RNase P at its 5′ end and processing by RNase Z at its 3′ end.[90,91] Unlike COX1, three other transcripts junctions (ND6-ncRNA, ND5-CYB, and RNA14-COX3) do not depend on RNase P or Z for processing and the specific mechanisms by which they are processed are still not clear. Moreover, not all mitochondrial transcripts are cleaved into individual RNA molecules. Some bicistronic transcripts have been observed including ATP8-ATP6, ATP8/6-COX3, and ND4L-ND4.[90,92,93] Knockdown of any of the previously described mtRNA processing proteins did not affect these junctions.[92]

After cleavage, individual or bicistronic transcripts undergo further modification before becoming mature and functionally active. Similar to nuclear mRNAs, most mtRNAs are polyadenylated at their 3′ ends. The human mitochondrial polyA polymerase is responsible for catalyzing polyadenylation of mtRNA.[94] The mammalian mitochondrial rRNAs contain very short polyA tails, while mRNAs contain ~40–50 adenine bases.[94] The role of mtRNA polyadenylation is controversial. For example, polyadenylation seems to stabilize subunits of complex IV (COX1, COX2, and COX3),[94] destabilize subunits of complex I (ND1 and ND2),[95] but does not affect ND3.[94]

Finally, a common tRNA modification is the addition of a CCA tail at their 3′ termini to facilitate attachment of the amino acid group used for translation. CCA tail addition is catalyzed by enzymes belonging to the nucleotidyltransferase superfamily.[96] Unlike polyadenylation, CCA addition is associated with clear specific functions: in addition to being central to tRNA maturation, a double CCA tail can be added to unstable tRNA species to target them for degradation[97] (Figure 3). In addition to the processes of RNA cleavage, RNA polyadenylation and CCA tail addition, numerous specific and nonspecific posttranscriptional modification must take place during RNA maturation. These are not discussed in this review.

9 | TRANSCRIPTIONAL REGULATION

Mitochondria depend on nuclear-encoded genes for all processes of gene expression. Although there is limited understanding on how mitochondrial transcription is regulated, there is significant evidence from tissue studies that the process is regulated directly and indirectly by nuclear transcription factors.[98] Nuclear transcription factors have been localized in mammalian mitochondria where they either bind

the mtDNA D-loop or mtDNA genes to modulate mtDNA transcription. Thyroid hormone/receptor has been observed in vivo in the mitochondrial matrix[99,100] and shown in vitro to bind to the D-loop and 12S rRNA gene where it regulates mtRNA synthesis.[101,102] Similarly, D-loop binding by cAMP response element binding protein has been observed and thought to promote mtDNA gene expression.[103–106] In vivo, mouse mtDNA D-loop binding was also shown for the signal transducer and activator of Transcription 3,[107] the nuclear DNA chromatin modulator MOF acetyl transferase,[108] and the osteogenic cell line-specific transcription modulator NFATc1 in human mesenchymal stem cells.[109] D-loop binding has additionally been reported for the DNA methyltransferase Dnmt1, believed to play a role in reducing mtDNA transcript levels.[110] Other non-D-loop binding nuclear transcription regulators were reported to influence mitochondrial transcription by directly binding on mitochondrial gene coding sequences, which included the myocyte enhancer factor 2D[111] and the transcription factors c-Jun, JunD, and CEBP.[112]

In addition to regulating transcription by binding directly to the mtDNA, nuclear transcription regulators can modulate expression or activity of the mitochondrial transcriptional machinery. Nuclear respiratory factors, NRF-1 and NRF-2 control expression of TFB2M and POLRMT.[113,114] PGC1 family factors, including PGC1-α, PGC1-β, and PRC in turn regulate the activity of a variety of transcription factors including NRF-1,[115,116] NRF2,[117,118] and Yin Yang 1 (YY1),[119] all believed to influence mitochondrial gene expression. Surprisingly, PGC1-α also forms a complex with TFAM at the mouse mtDNA D-loop to increase mitochondrial gene expression during high energy demands.[120]

Importantly, additional direct mechanisms appear to modulate mitochondrial transcription. The mitochondrial ribosomal protein L12 (MRPL12) seems to be a positive regulator of the mitochondrial transcription process by controlling POLRMT stability.[121] MRPL12 expression correlates with the steady-state levels of mitochondrial transcripts.[122,123] There is growing evidence suggesting that mitochondrial transcription is also regulated in response to changing mitochondrial ATP concentrations: high ATP levels have been shown to inhibit the activity of POLRMT both in isolated mitochondria and in vitro,[124] while intermediate ATP levels activate transcription and very low ATP levels prevent transcription initiation.[125] Finally, mtDNA methylation has been proposed to be associated with mitochondrial transcription regulation.[126–128] Recently, a mechanism has been proposed where mtDNA methylation leads to posttranslational modification of TFAM, resulting in increased nucleoid compaction, limiting mtDNA accessibility to POLRMT and TFB2M.[129] Despite these observations, we do not have a comprehensive picture of the mechanisms that control mitochondrial transcription, and additional research is needed to increase our understanding of mitochondrial transcription regulation.

10 | CONCLUDING REMARKS

Mammalian mitochondrial transcription studies are gaining momentum, especially given the wealth of structural information acquired in recent years. These studies have solidified our mechanistic understanding of the transcription process, although numerous mechanistic questions remain unanswered. Furthermore, recent advances in next-generation sequencing technology have facilitated a new approach to study mitochondrial RNA biology in an unprecedented way. This has already facilitated strong advances in our understanding of posttranscriptional processing, and it is likely that RNA sequencing approaches will continue to provide insight into mitochondria RNA biology. A large number of observations point to numerous mechanisms that regulate mitochondrial transcription. Understanding how this process is regulated is the next frontier in our understanding of the transcription process and will be the key to help us appreciate how alterations in transcription contribute to human disease.

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