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Mitochondrial transcription

How does it end?

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The structure of the mitochondrial transcription termination factor (MTERF1) provides novel insight into the mechanism of binding, recognition of the termination sequence and conformational changes involved in mediating termination. Besides its functional implications, this structure provides a framework to understand the consequences of numerous diseases associated with mitochondrial DNA mutations.

Key words: POLRMT, MTERF, mitochondrial transcription termination, base-flipping, polarity, mitochondria, gene expression

Abbreviations: HSP, heavy strand promoter; LSP, light strand promoter; MTERF, mitochondrial transcription termination factor; TFAM, transcription factor A; TFB2M, transcription factor 2

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Deficiencies in mitochondrial gene expression lead to mitochondrial pathologies and play a key role in aging and the onset of age related diseases.[1] Mitochondrial transcription is central to gene expression and is also intimately associated with replication and mitochondrial DNA maintenance. Recent advances have clarified the mechanisms involved in transcriptional initiation,[2,3] but our understanding of the mechanisms controlling transcriptional regulation is still relatively poor. The recent publication of a 2.2-Angstrom structure of human MTERF1[4] provides insight into the mechanisms of mitochondrial transcription termination and the role of MTERF proteins in transcriptional control in mitochondria. Here we describe the possible implications of this structure on our understanding of transcriptional regulation in mitochondria, discuss how the structural information influences our views on the mechanism of mitochondrial termination in the context of other termination systems and review new data that link defects in MTERF1 function with the pathogenesis of mitochondrial diseases.

Human mitochondria contain their own 16 kb circular double-stranded genome responsible for expression of 13 subunits of the respiratory chain, the 22 tRNAs necessary for their translation and the two rRNAs necessary for assembly of the mitochondrial ribosome.[5] Expression of the mitochondrial genome is essential for mitochondrial function and tightly dependent on nuclear gene expression: most genes involved in mitochondrial gene expression are encoded by the nuclear genome and after translation the protein products are imported into the mitochondria.[6] Mitochondrial gene expression is therefore subject to regulation in the nucleus, but local regulation also takes place in the mitochondria. Transcription is a key step in the process of gene expression and appears to be highly regulated at the local mitochondrial level.[7,8] Mitochondrial transcription generates polycistronic transcripts that encompass a large part of the mitochondrial genome.[7,9] In vitro experiments have demonstrated that mitochondrial transcription originates from two promoters, a heavy strand promoter (HSP) and a light strand promoter (LSP) and involves the RNA polymerase, POLRMT and two transcription factors, TFAM and TFB2M.[2,7,9] POLRMT contains a divergent N-terminal domain of unknown function, but its C-terminal domain, containing the catalytic polymerase domain, is highly similar to the bacteriophage T7 RNA polymerase.[10] Despite this similarity, T7 RNA polymerase is able to recognize the promoter, unwind the DNA and initiate transcription by itself, whereas POLRMT requires TFAM and TFB2M for initiation.[2] The role of these factors is still debated, but TFAM may function in DNA helix unwinding and recruit

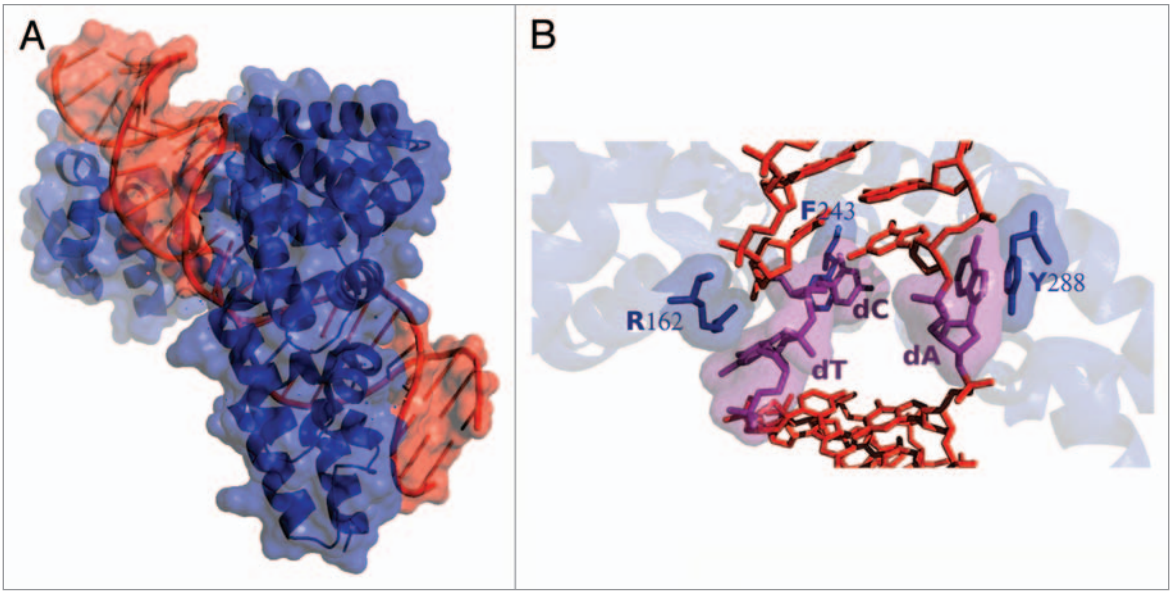

Figure 1. (A) Structure of the mitochondrial transcription termination factor MTERF1 (blue) bound to the leu-tRNA mitochondrial DNA termination sequence (red). (B) Stacking interactions with MTERF1 residues (blue) stabilize three DNA bases (purple) in an extrahelical position. The molecular surface is transparent.

POLRMT and TFB2M to the promoter sequence,[7,9] while TFB2M appears to melt the promoter and stabilize the open promoter complex.[3]

Additional factors have been suggested to provide a supplementary layer of control over transcriptional activity. Recent evidence has highlighted the role of MTERF proteins in the control of mitochondrial gene expression. Three of these proteins (MTERF1-3) have been demonstrated to influence the transcriptional process.[4,11,12] MTERF1 was originally identified in mitochondrial extracts as a factor promoting termination at a specific site in the leucine tRNA gene[13] and was later shown to be sufficient to mediate transcriptional termination in vitro.[14] Because the leucine tRNA is immediately downstream of the mitochondrial rRNA genes, it was suggested that termination by MTERF1 controls the ratio of rRNA to transcript mRNA,[8] although so far no in vivo evidence has supported this role. In addition to the binding site at the leucine tRNA, alternative binding sites in mitochondrial DNA have been reported for MTERF1.[15] One of these sites is adjacent to the HSP, leading to the idea that MTERF1 can couple transcriptional initiation and termination. Consistent with the ability of MTERF1 to bind to the initiation site, it was suggested that MTERF1 could mediate formation of a transcriptional loop between the initiation and termination sites that facilitates rRNA synthesis.[16] It has been proposed that the formation of this loop involves one MTERF1 molecule binding to the HSP and termination sequence of the same DNA molecule and facilitates the rapid recycling of the transcription machinery due to the close proximity of the initiation and termination sites.[16] This model would explain the larger quantity of rRNA transcripts to mRNA transcripts in the mitochondria. However, the existence of alternative MTERF1 binding sites is controversial. Park et al.[11] have not been able to reproduce binding of MTERF1 to the HSP. Thus, more in vivo and in vitro studies are needed to verify if other MTERF1 binding sites are present in the mitochondrial genome.

The structure of MTERF1 reveals that the protein has an all-α-helical structure that binds as a monomer to a 22 bp termination sequence in the mitochondrial leu-tRNA gene.[4] The structure is modular and configured around a motif of two α-helices and a 3_{10} helix repeat (the MTERF repeat), which is similar in structure to other all-α-helical domains such as the ARM and HEAT domains[17] or the RNA binding PUF domain.[18] The different MTERF repeats constitute a helical fold that allows MTERF1 to bind along the major groove of the double-stranded DNA containing the recognition sequence. The extensive interaction surface between protein and DNA indicates that the MTERF1 fold is dedicated to dsDNA binding and the fact that MTERF1 is structurally similar to MTERF3 (rmsd of 2.7 Å for 218 C-α atoms), even though the latter was crystallized in the absence of substrate,[19] strengthens the conclusion that MTERF proteins have evolved to bind nucleic acids. Upon binding its target sequence, MTERF1 alleviates the DNA duplex twist and promotes duplex melting and base-flipping, leading to a novel and unique DNA binding mode (**Fig. 1A**). Although the mechanism by which it promotes base-flipping is not yet clear, MTERF1 stabilizes three nucleotides in an extrahelical conformation via stacking interactions with three protein residues (Phe243, Tyr288 and Arg162; see **Fig. 1B**). These three side chains are essential to maintain the conformation observed in the crystal structure and, while MTERF1 can still bind to the termination sequence in their absence, the affinity for the termination site is significantly reduced. Moreover, the ability of the mutant MTERF1 to promote transcriptional termination in vitro is dramatically decreased. Besides these stacking interactions, the large majority

of contacts established between MTERF1 and DNA involve the phosphate backbone of the recognition sequence, and are therefore largely electrostatic and nonspecific. Sequence specificity appears to be mostly determined by a small number of key interactions between six arginine residues and guanine bases in the termination sequence. This type of major groove interaction is frequently seen in sequence-specific DNA binding proteins and eliminating even a single one of these interactions can drastically affect both DNA binding and transcription termination.[4] Interestingly, this mechanism of sequence recognition implies that while the interaction between MTERF1 and its binding sequence involves contacts with 20 base pairs, only six of 40 bases appear to be actively recognized by the protein. Moreover, since the extensive interactions imply that the whole MTERF1 fold is involved in binding the termination sequence (**Fig. 1A**), it is not immediately apparent how a single MTERF1 molecule could simultaneously bind both the HSP initiation and termination sites in the proposed transcriptional loop model.[16] Nevertheless, it cannot be ruled out that more than one MTERF1 protein or other factors may mediate the interaction of these two sites and facilitate the formation of a DNA loop.

The MTERF1 crystal structure therefore suggests a binding mechanism that involves the establishment of sequence-specific interactions for sequence recognition followed by DNA unwinding. Unwinding would then presumably destabilize the central base pairs of the recognition sequence and facilitate subsequent base flipping in order to stabilize the protein on DNA. Since termination in vitro is dependent on base flipping, the ability of MTERF1 to promote termination appears to be at least partially dependent on the strength of the interaction between MTERF1 and the termination sequence. This would suggest a model of transcription in which MTERF1 simply acts as a "roadblock," interfering with transcriptional elongations, and is consistent with the fact that MTERF1 terminates transcription bidirectionally and can arrest elongation by heterologous polymerases (reviewed in ref. 20 and our own unpublished results). However, at least in vitro, termination displays a clear polarity: MTERF1 is much more efficient when terminating transcription originating from the light strand promoter than the heavy strand promoter.[4,14] This is perhaps a result of most of the observed protein-DNA interactions being established with the light strand[4] (the strand transcribed from the LSP promoter) and the higher affinity of MTERF1 for this strand[21] and perhaps suggests a mechanism in which MTERF1 might transiently bind to single-stranded DNA. Evidence arguing against this model shows that the measured affinity of MTERF1 for single-stranded DNA is extremely low (reviewed in ref. 21 and our own unpublished observations). Nevertheless, except for the asymmetrical distribution of interactions, no obvious structural feature provides an explanation for the polarity of termination.

Some insight into the mechanistic basis of polarity can perhaps be obtained by considering how termination is achieved in other systems. There are striking similarities between MTERF1-dependent termination in mitochondria and replication termination in *E. coli*. In *E. coli,* replication termination involves Tus, a DNA binding protein that is an asymmetric monomer much like MTERF1 and tightly binds DNA through a base-flipping mechanism.[22,23] Moreover, Tus-mediated replication termination is highly polar and occurs efficiently only in one orientation. It has been shown that specific residues on Tus can interact with the helicase DnaB when it approaches in one orientation, blocking the progressing replication fork and terminating replication (**Fig. 2A**).[24] While it is possible that the unique conformation of MTERF1 on DNA might be responsible for the orientation dependence of the termination activity, it is tempting to speculate that perhaps interactions between MTERF1 and mitochondrial POLRMT, analogous to those observed between Tus and DnaB, might determine the polarity of termination events (**Fig. 2B**). Additional experiments will be needed to understand if protein-protein interactions between MTERF1 and POLRMT indeed play a role in termination and whether other proteins can modulate termination polarity in vivo.

The strong polarity observed in termination assays in vitro and the lack of in vivo evidence supporting a role in HSP termination argue that MTERF1-mediated HSP termination might in fact only be a minor event and that the main transcriptional termination event involving MTERF1 is termination of LSP transcription at the leu tRNA site. This hypothesis seems consistent with the fact that no additional genes are encoded by the light strand beyond the leu tRNA. Recent observations in HEK293 cells appear to support this notion, since alteration of the levels of MTERF1 were strongly correlated with an alteration of the ratio of LSP transcripts upstream and downstream of the leu tRNA termination site.[25] It is possible that LSP termination at this site is important to prevent the accumulation of antisense transcripts that might otherwise interfere with the assembly of the rRNAs into ribosomes. This would imply that MTERF1 function is, as originally thought, important for ribosome biogenesis, although by an unexpectedly different mechanism.

The picture of mitochondrial termination is obviously not complete when only considering termination at the leucine tRNA site, as HSP transcription needs to progress past this site to transcribe most genes and is ultimately terminated at the distal site. Termination at the distal site is yet uncharacterized in human mitochondria. Since MTERF1 predominantly binds to the leu-tRNA site, distal termination is not thought to depend on MTERF1, raising the question of how termination is achieved at that site. Interestingly, POLRMT appears to be able to utilize a mechanism similar to bacterial rho-independent termination to generate the RNA primers necessary for mitochondrial replication.[26] In T7 bacteriophages transcription termination occurs as a consequence of the polymerase interacting with spontaneously generated double-stranded RNA hairpins and falling off DNA because of the weak affinity of T7 RNA polymerase for double stranded structures[27] (**Fig. 2C**). In the case of POLRMT, stable G-quadruplex structures can form in the nascent RNA. These structures then mediate transcription termination in a way reminiscent of RNA hairpins in

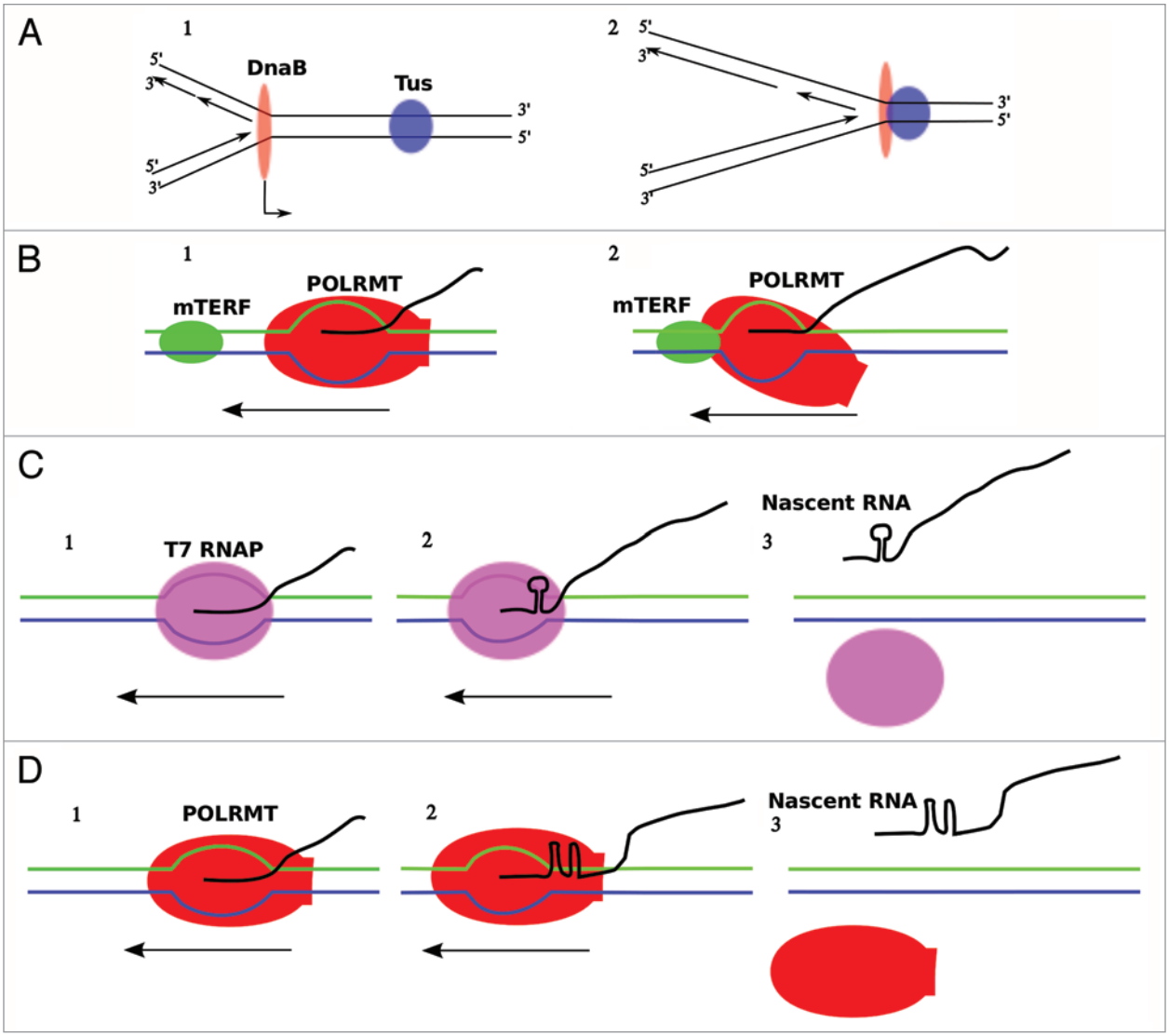

Figure 2. (A) *E. coli* replication is terminated when the helicase DnaB (orange), is blocked by termination factor Tus (blue). (B) MTERF1 (green) may interact with POLRMT (red) and mediate transcription termination. Specific protein-protein interactions might explain termination polarity. (C) A hairpin loop is formed in the nascent RNA (black) terminating transcription in a mechanism similar to rho-independent termination, destabilizing the RNAP—RNA interaction. Large arrows indicate direction of transcription. (D) Mitochondrial RNA polymerase (POLRMT-red) terminates transcription via the formation of a G-quadruplex structure in the nascent RNA (black) in a mechanism similar to the termination process shown in (C).

rho-independent termination (**Fig. 2D**). It is therefore possible that a similar mechanism is responsible for termination of transcription at the HSP distal termination site. It however cannot be excluded that MTERF1 might also play a role in termination at that site. Even though they have not been functionally characterized, alternative MTERF1 binding sites have been described in the proximity of the distal site.[15] Finally, it is possible that proteins different from MTERF1 might contribute to HSP termination.

Many mitochondrial DNA mutations result in altered gene expression and therefore contribute to mitochondrial pathologies. Most of these mutations are though to result in either defects in mitochondrial DNA maintenance or defects in mitochondrial translation. Pathogenic defects in mitochondrial translation are frequently associated with mutations in tRNA genes, which represent a large proportion of mitochondrial DNA mutations. Interestingly, the leucine tRNA (UUR) gene contains the highest number of identified mutations of any mitochondrial tRNA gene. Not surprisingly, several of these mutations occur in the MTERF1 binding sequence. Some of these mutations have been shown in vitro to affect transcription termination. The A3243G mutation, associated with mitochondrial encephalopathy lactic acidosis and stroke (MELAS) is important because of its prevalence and because it has been shown to affect transcription termination in vitro.[28] However, later in vivo studies showed that the A3243G mutation does not affect the balance between mitochondrial HSP transcripts upstream and downstream of the termination sequence and suggested that tRNA defects and not transcriptional alterations are the main cause of the pathogenic alteration.[20] Because the

transcript ratios measured in those studies would only provide a measure of HSP termination, it is possible that defects in LSP termination might exist in A3243G carriers and contribute to pathogenesis. After the MTERF1 structure was solved, revealing the basis for MTERF1 sequence specificity, transcription termination assays performed on seven other mutations within the termination sequence revealed effects on transcriptional termination. Of these, two showed an effect that was significantly stronger than that observed with the A3243G mutation.[4] These mutations include the G3249A mutation that causes a variant of Kearns-Sayre syndrome as well as the G3242A mutation, associated with an uncharacterized mitochondrial disorder.[4] Although tRNA mutations are generally thought to functionally affect the mature tRNA, these in vitro observations raise the possibility that the pathogenic effects of these mutations are related to their effect on MTERF1 binding. Additional experiments will be needed in order to understand how these mutations affect MTERF1 activity and whether these effects indeed contribute to pathogenesis. In considering the effects of these mutations it is important to keep in mind that, in addition to potential effects in transcription, MTERF1 appears to be able to modulate replication and therefore affect mitochondrial DNA maintenance.[15,30]

In summary, recent advances in the field begin to shed light on some of the most uncharacterized aspects of the mitochondrial transcription process. The recent MTERF1 crystal structure provides insight into the mechanism of mitochondrial transcription termination, reveals unexpected structural similarities with other DNA binding proteins, highlights similarities with other models of transcription termination and suggests that some of the tRNA mutations in the leucine tRNA gene might affect transcriptional regulation by MTERF1. Further studies will need to clarify the in vivo role of MTERF1 and other MTERF proteins, the different mechanisms controlling transcriptional elongation in mitochondria and how alterations in mitochondrial transcription contribute to human disease.

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