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Review

MTERF factors: a multifunction protein family

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Abstract

The MTERF family is a large protein family, identified in metazoans and plants, which consists of four subfamilies, MTERF1, 2, 3 and 4. Mitochondrial localisation was predicted for the vast majority of MTERF family members and demonstrated for the characterised MTERF proteins. The main structural feature of MTERF proteins is the presence of a modular architecture, based on repetitions of a 30-residue module, the mTERF motif, containing leucine zipper-like heptads. The MTERF family includes transcription termination factors: human mTERF, sea urchin mtDBP and *Drosophila* DmTTF. In addition to terminating transcription, they are involved in transcription initiation and in the control of mtDNA replication. This multiplicity of functions seems to flank differences in the gene organisation of mitochondrial genomes. MTERF2 and MTERF3 play antithetical roles in controlling mitochondrial transcription: that is, mammalian and *Drosophila* MTERF3 act as negative regulators, whereas mammalian MTERF2 functions as a positive regulator. Both proteins contact mtDNA in the promoter region, perhaps establishing interactions, either mutual or with other factors. Regulation of MTERF gene expression in human and *Drosophila* depends on nuclear transcription factors NRF-2 and DREF, respectively, and proceeds through pathways which appear to discriminate between factors positively or negatively acting in mitochondrial transcription. In this emerging scenario, it appears that MTERF proteins act to coordinate mitochondrial transcription.

Keywords: MTERF protein family; mtDNA replication; mtDNA transcription; transcription termination factor.

Introduction

Mitochondria are cytoplasmic organelles of endosymbiotic origin, which developed from an α-proteobacterium (1). During evolution, most of the bacterial genes moved from the organelle genome to the nuclear genome. In animal cells, mitochondrial DNA (mtDNA) is an example of extreme genetic economy. It consists of a circular molecule of approximately 16 kbp, lacks introns, and contains only one major non-coding sequence, which is the most variable part of the genome in size and sequence, and which includes the regulatory signals for transcription and replication. Animal mtDNA (Figure 1) encodes only 13 subunits of the respiratory complexes and RNAs (two ribosomal RNAs and 22 tRNAs) required for their translation. Therefore, the production and assembly of the respiratory complex subunits require the coordinated expression of the two genomes, which is based on signalling pathways between nucleus and cytoplasm (4, 5).

A considerable amount of knowledge has been produced on the mechanism of mtDNA transcription, particularly in mammals (6). In humans, transcription of the heavy (H) strand requires the HSP promoter, located in the 1000-bp-long D-loop non-coding region (NCR), and proceeds through two partially overlapping units (Figure 2). The ribosomal unit, starting at the initiation site I_{H1} (placed in HSP) and ending at the 3′ end of the 16S rRNA gene, is responsible for the synthesis of the two rRNAs, $tRNA^{Phe}$ and $tRNA^{Val}$. The messenger unit, starting at the initiation site I_{H2}, located two nucleotides upstream of the 5′ end of the 12S rRNA gene, covers almost the entire H-strand and produces a polycistronic molecule, the processing of which originates all the H-strand encoded mRNAs and the remaining 12 tRNAs. I_{H1} is more frequently used than I_{H2}, thus resulting in a higher rate of rRNA transcription (7). Transcription of the light (L) strand starts from the LSP promoter (Figure 2), also located in the D-loop region, 150 bp from HSP, and produces only eight tRNAs, ND6 mRNA and the H-strand replication primer.

Although their gene content remained similar, during evolution mitochondrial genomes of animals from various phyla and orders underwent profound variations in gene organisation, that are likely to be reflected in differences in transcription mechanisms.

In the mitochondrial genome of the sea urchin *Paracentrotus lividus* (Echinoid) (Figure 1), the ribosomal genes are separated by a region of 3.3 kbp containing a cluster of 15 tRNA genes and the genes for ND1 and ND2; the main NCR is very short (132 bp) and is located in the tRNA gene cluster downstream of the 12S rRNA gene (8). In this organism, mitochondrial transcription is presumed to proceed via multiple and partially overlapping transcription units, the initiation sites of which are probably located near six small AT-rich sequences scattered along the genome (see Figure 1) (8, 9).

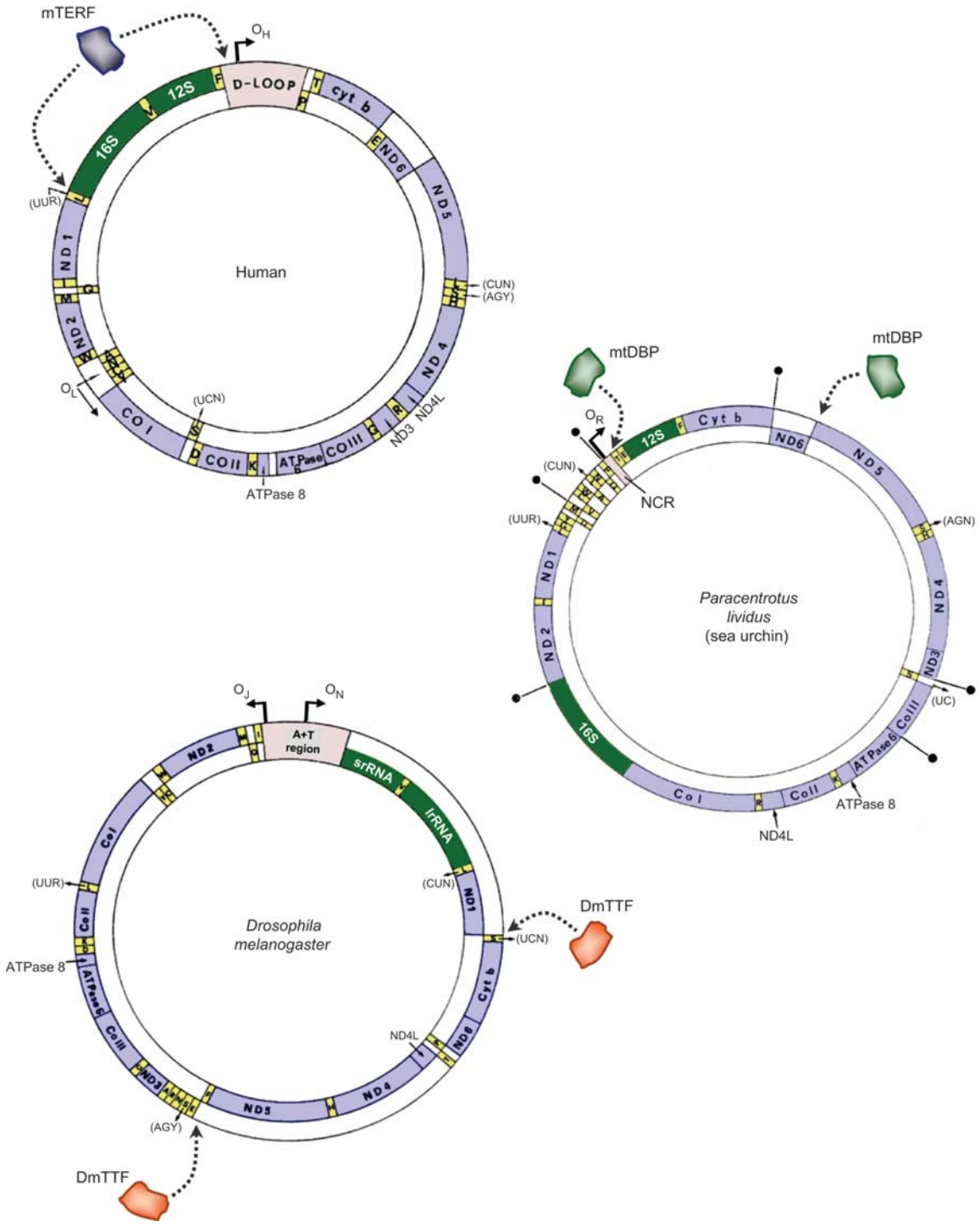

Figure 1 Map of mitochondrial genome in three metazoans, showing the cognate transcription termination factor.
Blue, green and yellow: genes encoding the 13 polypeptides of the respiratory complexes, the two rRNAs and the 22 tRNAs, respectively. Human mtDNA: O_H and O_L mark the replication origin of H- and L-strand, respectively, according to the conventional strand-asymmetric model of mtDNA replication (2). Transcription termination factor mTERF is shown to contact its binding sites. Sea urchin mtDNA: O_R marks the replication origin of the leading strand (3). Black dots: position of the six small AT-rich sequences, perhaps acting as transcription promoters. Position of the two sites contacted by mtDBP is shown. *Drosophila melanogaster* mtDNA: O_J and O_N mark the replication origin of major and minor coding strands, respectively. Transcription termination factor DmTTF is shown to contact its binding sites. The large non-coding AT-rich region (approx. 4.6 kbp) is not to scale.

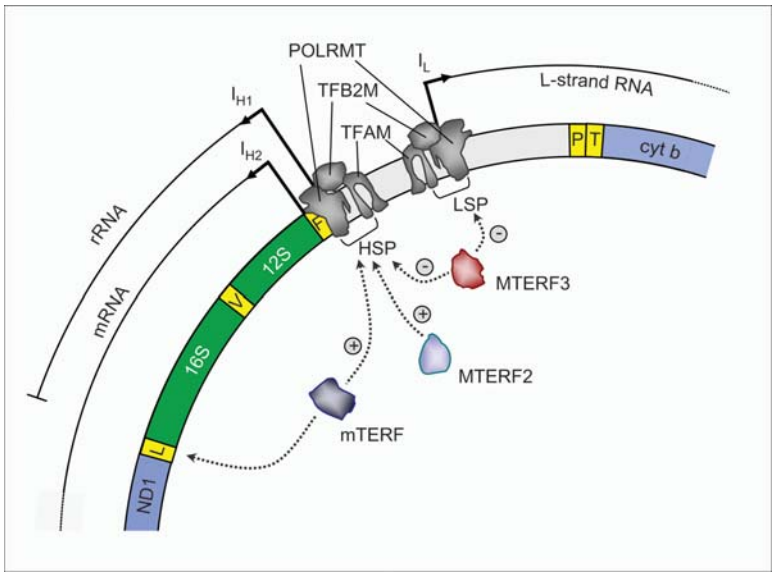

Figure 2 Schematic representation of transcription regulatory regions and factors in human mtDNA. Light grey: D-loop region containing promoters HSP and LSP. Transcription initiation is assisted by factors TFAM and TFB2M. Transcription of rDNA region initiates at I_{H1} and ends at the 3′ end of 16S rRNA gene. Transcription termination is mediated by mTERF which binds inside $tRNA^{Leu(UUR)}$ gene. Simultaneous binding of mTERF to promoter and termination sites causes rDNA looping and allows recycling of POLRMT between both regions. I_{H2} directs transcription of entire H-strand, giving rise to a polycistronic RNA precursor molecule. I_L promotes transcription of L-strand DNA, generating a polycistronic transcript and RNA primers for H-strand replication. Transcription repressor MTERF3 and transcription activator MTERF2 are shown to contact sequences in the promoter region. Symbols ⊕ and ⊖: activation and repression, respectively.

The mtDNA of *Drosophila melanogaster* (Figure 1) is approximately 19.5 kbp long, and the AT-rich main NCR, approximately 4.6 kbp, accounts for the large size of the genome. Unlike human and sea urchin mitochondrial genes, *Drosophila* genes are almost equally distributed between the two strands of mtDNA, forming four clusters located alternatively on the two strands (10). According to early mapping data of mature and precursor mitochondrial RNAs, *Drosophila* mtDNA would be transcribed by multiple transcription units starting at the 5′ ends of the gene blocks and terminating at their 3′ ends (11).

The basal mitochondrial transcription machinery has been well characterised in mammals (2, 12); it consists of a phage-like mitochondrial RNA polymerase (POLRMT) and factors TFAM and TFB2M (Figure 2). TFAM belongs to the family of the high mobility group (HMG) proteins, which are known to bind DNA with low or no sequence specificity. In addition to behaving as an architectural element, the factor specifically binds two mtDNA sequences located upstream of HSP and LSP and stimulates transcription (13, 14). Although TFB2M, a protein homologous to bacterial rRNA methyltransferases, does not show specific DNA binding activity, it is able to activate transcription initiation (15). By combining recombinant POLRMT, TFAM and TFB2M, the human basic mitochondrial transcription system has been reconstituted *in vitro*, which is able to support specific transcription initiation at the H- and L- strand promoters (16). A recent study reported a detailed model for the organisation of the three-component transcription initiation complex, according to which TFB2M contacts promoter DNA near the transcription initiation site and establishes interactions with TFAM, POLRMT and the first nucleotide of the RNA transcript (17).

Although the number of transcription units and the number and position of promoters and termination sites of invertebrate mtDNAs are probably different from those of mammals, mammalian POLRMT, TFAM and TFB2M show evident orthologues in sea urchin and in *Drosophila* (18–20). This suggests that a common transcription initiation core machinery operates in animal mitochondria. However, previous and very recent studies indicate that mitochondrial transcription in animals requires several additional protein factors, such as termination factors and positive and negative modulators. Intriguingly, all these factors show a common evolutionary origin, because they are all members of a large, complex protein family, the MTERF family, whose name derives from human mTERF, the first mitochondrial transcription termination factor to be characterised.

The MTERF protein family in metazoans

The MTERF proteins have been identified in metazoans and plants, but not in fungi. The evolutionary relations of MTERF proteins in metazoans, described by Linder and coworkers (21), indicate the existence in vertebrates of four MTERF paralogue genes, defining four sub-families, named MTERF1, 2, 3 and 4 (Figure 3). Sub-families MTERF1, which includes human mTERF, and MTERF2 are unique to vertebrates; sub-families MTERF3 and MTERF4 also

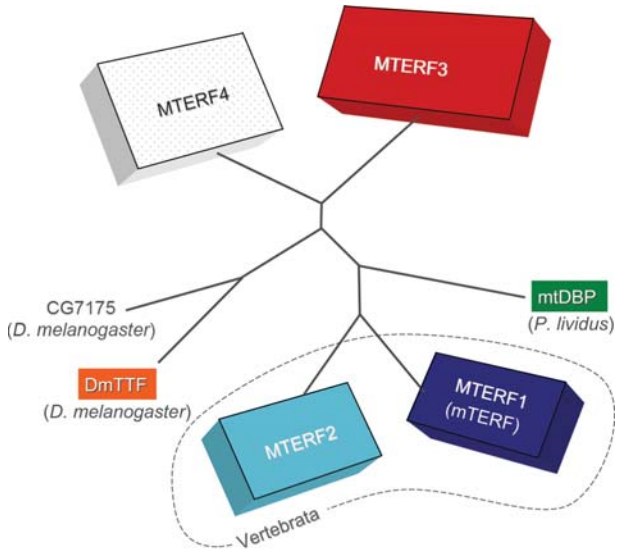

Figure 3 Schematic diagram illustrating the main phylogenetic relations in MTERF protein family.
Boxes represent the four MTERF sub-families. Proteins belonging to MTERF1, which include human transcription termination factor mTERF, and to MTERF2 sub-families are found only in vertebrates; proteins belonging to sub-families MTERF3 and MTERF4, the latter still uncharacterised, are found in all metazoans. Neither *P. lividus* mtDBP nor *D. melanogaster* DmTTF are included in any sub-family; nor is the still uncharacterised *D. melanogaster* protein CG7175.

include members from insects and worms, and therefore probably represent the ancestral MTERF genes in metazoans. Some other MTERF proteins, such as mtDBP and DmTTF, are not included in any of the four defined sub-families (see below) (Figure 3).

Mitochondrial localization was predicted by means of bioinformatics tools for the vast majority of the MTERF family members (21), and experimentally demonstrated for all characterised MTERF proteins.

Many MTERF proteins share a modular architecture characterised by the repetition, in a variable number, of a 30-residue string called the mTERF motif (22, 23). The high conservation of a proline residue at position 8, and of a leucine or another hydrophobic amino acid residue at positions 11, 18, 25 suggests the existence of three leucine zipper (LZ)-like heptads X_3LX_3 inside the mTERF motif.

Mitochondrial transcription termination factors

Human transcription termination factor mTERF

Human mTERF is a 342-residue protein (mature form), first purified and characterised in the late 1980s. It was shown to bind specifically a 28-nt sequence located immediately downstream of the 3′ end of the 16S rRNA gene, within the $tRNA^{Leu(UUR)}$ gene (Figures 1 and 2) and to arrest *in vitro* POLRMT progression (24, 25). According to these properties, mTERF was proposed to control the transcription of the H-strand ribosomal unit through a termination event at the 3′ end of the 16S rRNA gene. More recently, it was demonstrated that recombinant mTERF, bound to its site within the $tRNA^{Leu(UUR)}$, terminates transcription in a bi-directional way, showing even higher efficiency when POLRMT proceeds in the direction of L-strand transcription (26). This mTERF property is consistent with the absence of genes on the L-strand, downstream of the factor binding site.

It was also shown that native mTERF causes not only transcription arrest but also transcription activation at the I_{H1} site, where a novel protein binding site was detected (27) (Figures 1 and 2). One single mTERF molecule was demonstrated to be able to contact the canonical termination site and the I_{H1} site simultaneously, causing looping-out of rDNA. This would give rise to recycling of POLRMT from the termination site to the I_{H1} initiation site, thus accounting for the higher rate of transcription of rDNA compared with mRNA genes and for the higher (40–50-fold) steady-state level of rRNAs with respect to mRNAs (7). This model provides the basic molecular mechanism for regulation of the rRNA/mRNA ratio. The ability to interact with the I_{H1} region is much more evident for the natural purified protein than for the recombinant version, indicating that post-translational modifications or co-purifying binding cofactor(s) might increase the affinity of mTERF for the I_{H1} region.

The termination role of mTERF, although supported by many *in vitro* findings, has never been demonstrated *in vivo*. Alteration of mTERF level in human cultured cells did not produce substantial changes in the level of mitochondrial transcripts but rather affected replication pausing at the canonical binding sequence as well as at new, weaker, binding sites, mainly identified in the D-loop region (28). Therefore, mTERF might play a role in modulating mtDNA replication.

Sea urchin transcription termination factor mtDBP

mtDBP is a 348-residue DNA binding protein (mature form) which binds with high affinity two sites of sea urchin *P. lividus* mtDNA, one in the NCR at the 3′ end of the short D-loop structure and the other at the 3′ ends of the oppositely transcribed ND5 and ND6 genes (Figure 1) (29, 30). The ability of mtDBP to terminate transcription was demonstrated *in vitro* by a minimal reconstituted, promoter-independent transcription system containing recombinant sea urchin proteins mtDBP and POLRMT (18). In this system, transcription terminates in the NCR by means of two alternative modes depending on the direction of transcription. In one case, termination is strictly promoted by mtDBP; in the other, it occurs independently of the bound protein, within its binding site. mtDBP also shows contra-helicase activity which probably serves to regulate replication by controlling D-loop expansion, because, in the region contacted by mtDBP, synthesis of the leading strand stops prematurely to generate the D-loop (31). Transcription through the mtDBP-DNA complex in the opposite direction of leading-strand replication causes mtDBP to be dislodged, so that helicase impairment is abrogated and resumption of DNA replication would occur. mtDBP, with its bi-functional activity, could therefore

be the molecular device that regulates possible interplay between mtDNA transcription and replication in sea urchin mitochondria.

Drosophila transcription termination factor DmTTF

DmTTF is the *Drosophila* homologue of human mTERF and sea urchin mtDBP. The protein specifically binds two short homologous non-coding sequences located at the end of blocks of genes transcribed in the opposite direction, where transcription termination sites were previously predicted (11, 32). One DmTTF binding site is located between the $tRNA^{Glu}$ and $tRNA^{Phe}$ genes and the other between the $tRNA^{Ser(UCN)}$ and ND1 genes (Figure 1).

The role of DmTTF as transcription termination factor has been extensively addressed. *In vitro* analysis has assessed the ability of recombinant DmTTF to arrest the progression of human POLRMT on a chimeric DNA template containing human HSP promoter and the protein binding site (33).

In vivo experiments have evaluated the effect of DmTTF knock-down and over-expression in D.Mel-2 cells. Cells depleted in DmTTF display substantial alteration of the steady-state level of several mitochondrial sense and antisense transcripts (34). In particular, the abundance of transcripts mapping on both strands downstream of DmTTF binding sites is higher than in normal cells, thus indicating that DmTTF depletion relives the block to transcription. This finding strongly confirms that, *in vivo*, the protein bound to both sites arrests the progression of POLRMT moving on both strands. Surprisingly, the level of transcripts mapping between the AT-rich region and the protein binding sites is decreased in DmTTF-depleted cells. An intriguing explanation of this result is that, similarly to mTERF, DmTTF might also function as a transcription activation factor. Hence, its depletion could result in diminished transcription of those genes located immediately downstream of the AT-rich region, where transcription promoters are presumably placed. However, it is not possible to exclude that the observed decrease in transcription might be owing to reduced availability of POLRMT molecules which, in DmTTF-depleted cells, are engaged in aberrant transcription extending beyond the termination sites.

The effect of DmTTF over-expression on mitochondrial transcription was analysed by measuring the content of ND5 and cyt b mRNAs, two transcripts mapping downstream of the protein binding site on either strand (23). As expected, and fitting the role of the protein as transcription terminator, DmTTF over-expression diminished the level of both mRNAs.

Transcription termination factors: structural, functional and evolutionary considerations

The MTERF protein phylogenetic tree (21) clearly shows that sea urchin mtDBP and *Drosophila* DmTTF, although evolutionarily more closely related to MTERF1 and MTERF2 than to MTERF3 and MTERF4 sub-families, do not belong to a definite sub-family (Figure 3). The so-called transcription termination factors mTERF, mtDBP and DmTTF appear less clustered than the members of the MTERF3 and MTERF4 sub-families. Sequence alignment of the three proteins produces a 5% amino acid identity and 28% amino acid similarity (32), both much lower than those of members of the MTERF3 sub-family (see below), thus indicating that the primary structure of transcription termination factors is variable. This is confirmed by the observation that even DmTTF orthologues from other insect species display large amino acid insertions or deletions with respect to the *Drosophila* protein (Roberti, unpublished observations).

Human mTERF, sea urchin mtDBP and *Drosophila* DmTTF display mTERF motifs typical of MTERF family members, including the LZ-like heptads. However, the three proteins contain different numbers of mTERF motifs, and only some of them are located in corresponding positions (23). The presence of LZ heptads inside mTERF factor, together with N- and C-terminal basic domains, was described several years ago by Attardi's group (25). Leucine-zipper motifs were suggested to establish intramolecular interactions aimed at bringing the terminal basic domains into close proximity and to expose them to DNA. Although similar domains and arrangement can be predicted for mtDBP (30), DmTTF, despite the presence of the LZ motifs, lacks evident terminal basic domains (32).

The structural variability of transcription termination factors seems to be related their functional flexibility. Their shared capacity to arrest the progression of POLRMT seems to have evolved into different purposes as their cognate binding sites changed location on the mitochondrial genome. By means of the looping mechanism, mTERF would control transcription of the H-strand ribosomal unit at the level of termination and initiation (27), thus contributing toward determining the higher level of rRNAs with respect to mRNAs. The protein could also be responsible for L-strand transcription termination at the canonical binding site (26). Also in sea urchin and *Drosophila* mitochondria, the level of rRNAs is higher than that of mRNAs; however, unlike human mTERF, the invertebrate proteins mtDBP and DmTTF do not bind at the 3′ end of the ribosomal genes and do not seem to directly regulate the rRNA level which could depend on post-transcriptional events (9, 11, 30, 32). In invertebrates, it is probable that termination factors act at strategic sites mainly in coordinating the passage of the transcription machineries moving on opposite strands. In this way forced transcription pausing, owing to head-on collisions of the machineries, would be avoided and genome integrity safeguarded (35–37). In principle, it cannot be excluded that such a role might also be played by the human factor mTERF.

As indicated by transcript level analysis in knock-down cells, DmTTF could also be implicated in activating transcription initiation (34). This function might require a POLRMT recycling mechanism involving DNA looping generated by the simultaneous interaction of DmTTF, alone or together with other factors, with one canonical binding site and a still unidentified site in the AT-rich region, near the transcription promoters.

The finding that human mTERF and sea urchin mtDBP also play roles in mtDNA replication, by regulating replication pausing in several regions of the genome, and by negatively controlling D-loop expansion, respectively, further substantiates the concept that mitochondrial transcription termination factors are multi-function proteins.

In conclusion, during animal evolution, the precise function, or at least the binding site position, of these transcription/replication termination factors might have been adjusted to match the requirements of transcription and replication mechanisms imposed by changes in the gene organisation of mitochondrial genomes. As the multiple functions of these factors probably also require interactions with other proteins, it cannot be excluded that, in addition to stabilising the tertiary structure of the proteins, the LZ heptads of the mTERF motifs could also serve to interact with auxiliary partners.

In silico analysis reveals that *Caenorabditis elegans* and *Caenorabditis briggsae* possess MTERF3 and MTERF4, but not a putative homologue of the transcription termination factors (21). It could therefore be argued either that worms do not need a transcription termination factor or that the termination function is somehow implemented by another protein. It is intriguing to observe that, in worms, the genes are all located on the same strand of mtDNA, a situation which might imply a simplified transcription mode not requiring a protein regulating the movement of transcription machineries.

Mitochondrial transcription repressors: a conserved function in metazoans?

Mitochondrial transcription seems to require much finer tuning than that exerted by the initiation and termination factors. This became evident after the characterisation of the mammalian MTERF3 factor by Park et al. (38). This protein (approx. 350 residues in the mature form) is located in mitochondria, expressed ubiquitously, and is essential for embryonic development, because homozygous MTERF3 knockout mouse embryos died in mid-gestation. Tissue-specific gene inactivation produced mice with a shortened life-span and also caused (only in the heart) mitochondrial proliferation associated with evident mitochondrial dysfunction, resulting in reduced level and activity of complex I, III and IV. Mitochondrial dysfunction is ascribed to aberrant mtDNA transcription, mainly consisting in increased initiation at both promoters HSP and LSP. It was also shown that MTERF3 interacts with the promoter region of mtDNA (Figure 2). On the basis of these observations, a role as mitochondrial transcriptional repressor was suggested for MTERF3. Because no sequence-specific binding to mtDNA was observed, it was hypothesised that MTERF3 binding to the promoter region is mediated by protein-protein interactions. It is noteworthy that no effect was observed by adding recombinant MTERF3 to the basal *in vitro* transcription system or to mitochondrial extracts immunodepleted of the protein. These findings support the hypothesis that to play its role, MTERF3 requires participation of additional factor(s) which might be contacted through the mTERF motifs. Although the most obvious function of the negative regulator MTERF3 is to adjust mitochondrial DNA expression finely in response to physiological demands, Park et al. did not exclude the possibility that the protein simply serves to avoid collision of transcription complexes.

MTERF3 function has also been characterised in *Drosophila* by analysing the effect of its over-expression in D.Mel-2 cells (23). It was observed that the level of three mitochondrial transcripts, srRNA, COI and ND2, mapping on either strand immediately downstream of the of AT-rich control region, was decreased; a role as negative regulator of mitochondrial transcription was therefore also proposed for the *Drosophila* factor. The observed decrease in *de novo* mitochondrial protein synthesis in MTERF3-depleted cells could be ascribed to a concomitant down-regulation of TFB1M, a factor involved in mitoribosome biogenesis (22, 23, 39).

MTERF3 proteins can be clearly identified in worms, insects, echinoderms and vertebrates, and display a more highly conserved primary structure than that of the transcription termination factors, because multiple alignment of human, sea urchin and *Drosophila* MTERF3 shows 20% amino acid identity and 32% similarity. The conservation of five mTERF motifs in corresponding positions is also evident going from *C. elegans* to humans (22), suggesting that MTERF3 is subjected to stronger functional constraints than the termination factors. The probable conservation of MTERF3 function in all metazoans, in spite of the ample variation in mtDNA gene arrangement, leads to the speculation that such a function cannot be closely linked to a peculiar transcription mode.

Mitochondrial transcription activator: a function restricted to vertebrates?

The scenario of mtDNA transcription has very recently acquired a new player: MTERF2. This protein (also known as mitochondrial transcription termination factor-like or mTERFL) appears to be unique to vertebrates and, in humans, displays 29% amino acid identity and 52% similarity with mTERF. It therefore appears to be a recent gene duplication event in the vertebrate ancestor lineage which originated MTERF1 and MTERF2.

MTERF2 was originally described as a protein the expression of which is inhibited by the addition of serum in serum-starved cultured cells, whereas that of mTERF is induced (40). As reported by Pellegrini et al. (41), the mature form of human MTERF2 (350 residues) localises to the mitochondrial matrix, where it is found in formaldehyde-cross-linked nucleoids. Wenz and et al. have extensively characterised MTERF2 (42). Loss of MTERF2 in homozygote knockout mice produces much less dramatic effects than that of MTERF3, because it does not affect the life-span, and only causes defects in muscle and brain performance, particularly in animals whose OXPHOS system is challenged by a ketogenic diet. In muscle tissue, this malfunctioning is

associated with an evident decline in content and activity of the respiratory complexes. The defect is compensated neither by enhanced mitochondrial proliferation nor by up-regulation of the other MTERF factors and of the proteins of the basal transcription machinery. Concerning the effects on transcription, loss of MTERF2 causes a generalised decrease of transcripts mapping on both strands. Wenz et al. (42) reported that MTERF2 specifically binds mtDNA in the HSP promoter region (Figure 2), a region directly or indirectly also contacted by mTERF and MTERF3. Interactions of MTERF2 with those factors have been shown, and are probably mediated by the LZ-like heptads.

All these findings point to the role of MTERF2 in activating transcription initiation, a function that is antithetical to that of MTERF3 and apparently less essential, because MTERF2 knockout mice survive. The observation that MTERF2 is restricted to vertebrates suggests that its function is related to the peculiar gene arrangement and transcription mode of mtDNA in these organisms. For example, MTERF2 might cooperate with mTERF in generating the loop structure of the ribosomal transcription unit. However, because binding of MTERF2 to the HSP region seems to influence transcription in both directions, an alternative role for MTERF2 could be to avoid interference, owing to steric hindrance, between the transcription initiation machineries bound to the close proximal HSP and LSP promoters. Lastly, it cannot be excluded that MTERF2 operates in modulating mitochondrial transcription, and consequently mitochondrial biogenesis, in response to physiological demands according to the sophisticated pathways which are peculiar to highly evolved vertebrates.

According to the MTERF protein phylogenetic tree, insects provide another example of an MTERF protein unique to one animal group. In insect lineage a gene duplication event occurred which, in *Drosophila*, generated transcription terminator DmTTF and a still uncharacterised factor reported as CG7175 in the FlyBase database and predicted to have a mitochondrial localisation. Therefore, in the course of evolution, insects also seem to have gained a specific protein acting in mitochondrial biogenesis, probably at the level of the transcription process.

Regulation of expression of MTERF proteins

The biogenesis and function of mitochondria require the closely coordinated expression of nuclear and mitochondrial genomes. Therefore, it is very important to clarify the mechanisms that regulate the expression of nuclear genes encoding mitochondrial proteins which are, in turn, involved in the expression of mtDNA.

In mammals many nuclear genes encoding mitochondrial proteins are regulated by various combinations of nuclear transcription factors, such as NRF-1, NRF-2, Sp1, YY1 and ERRα. Moreover, three co-activators of the PGC-1 family (PGC-1α, PGC-1β and PRC) mediate the response of some of the above transcription factors to environmental stimuli (5).

Activation of the expression of transcription initiation factors TFAM and TFB2M by NRF-1 and NRF-2 was already known (43, 44), but only recently have data been produced on the regulation of MTERF proteins by NRF-2.

Figure 4A shows the location of the predicted NRF-1 and NRF-2 binding sites in the promoter of human MTERF proteins. *In vitro* and *in vivo* strategies indicated that NRF-2 positively regulates the expression of mTERF by cooperatively binding two tandemly arranged sites, whereas it has no control over the MTERF3 gene, the promoter of which lacks predicted binding sites (46). *In silico* analysis predicted an NRF-1 site in the MTERF3 promoter, although experimental data tended to exclude *in vivo* binding of the factor (46). Therefore, it appears that MTERF3 is not under NRF-1 control. Two tandemly arranged NRF-2 sites have been predicted in the promoter of MTERF2, suggesting that also the positive modulator of mitochondrial transcription is subjected to NRF-2 control. In addition, NRF-1 and NRF-2 sites are present in the promoter of the still uncharacterised MTERF4 factor.

These findings, together with those regarding TFAM and TFB2M, suggest that control by NRF-2 is a common feature of transcription-activating proteins, and indicates that they are all subjected to regulatory pathways mediated by the PGC-1 family co-activators and triggered by the need for an enhanced mitochondrial biogenesis. Conversely, control of transcription repressor MTERF3 could be subjected to different pathways which probably respond to diverse environmental or metabolic signals.

In *Drosophila*, DREF is a key transcription factor which, in addition to regulating the expression of genes acting in nuclear DNA replication and cell cycle control (47), activates the expression of proteins involved in mtDNA replication and maintenance, such as the mitochondrial single-stranded DNA-binding protein, the accessory subunit of mitochondrial DNA polymerase, and TFAM (19, 48, 49). Figure 4B shows the location of the predicted DRE sites in the promoter of *Drosophila* MTERF proteins. *In vitro* and *in vivo* strategies, recently demonstrated that DREF positively regulates the expression of DmTTF (50). DRE elements can be detected in the promoter of MTERF4 and of the insect-specific protein CG7175, but not in the promoter of MTERF3. Therefore, also in *Drosophila*, the expression of the transcription repressor could be controlled by different pathways than those of the other MTERF proteins, suggesting that similar strategies operate to regulate mitochondrial biogenesis in mammals and insects.

Expert opinion

It has recently become evident that mitochondrial genome expression requires the cooperation of many proteins, among which an important role is played by MTERF family factors. This complex family, comprising transcription termination factors, includes several paralogue members generated by duplication events which, during evolution, allowed mito-

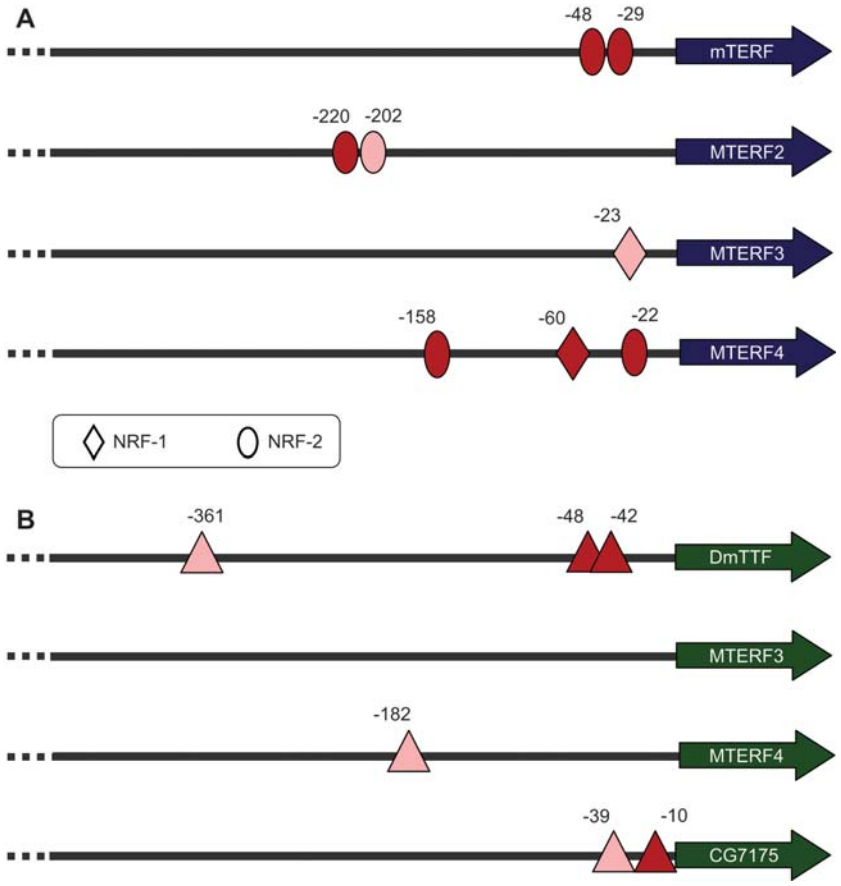

Figure 4 Location of binding sites for transcription regulatory factors predicted by MAPPER tool (45) in the promoter proximal region of genes coding for MTERF family proteins.
Position numbers refer to the transcription start site of each gene, as deduced from mRNA sequence extracted by Ensembl search engine. Dark and light red symbols: high and low scoring sites, respectively. (A) Binding sites for NRF-1 and NRF-2 in human gene promoters. (B) Binding sites for DREF in *Drosophila* gene promoters.

chondria to gain more sophisticated functions regarding transcription, as well as the replication process.

In mammals, the fine tuning of mtDNA transcription seems to be ensured by the concerted action of initiation factors TFAM and TFB2M, termination factor mTERF, repressor MTERF3 and activator MTERF2, all contacting the promoter region, although in some cases not directly. However, information to date is not yet sufficient to unravel the complex network of protein-protein and/or DNA-protein interactions which are established in the promoter region of mammalian mtDNA. For example, it is not clear whether all the involved proteins bind simultaneously to mtDNA, or whether they do so in a mutually exclusive way. Concerning the question of MTERF gene regulation, it appears that, in both mammals and *Drosophila*, transcription activators and repressors follow different regulatory patterns.

Outlook

Research in the field of MTERF proteins is very promising, both in further in-depth characterisation of already studied factors and in determining the role of those still uncharacterised. Therefore, the near future will provide information on the role of MTERF4 in all metazoans and of protein CG7175 in *Drosophila*. The elucidation of the function of still uncharacterised MTERF proteins will contribute further information, allowing us to decipher the molecular mechanisms of mtDNA transcription and replication. In addition, because the involvement of MTERF proteins in mitochondrial-based human pathologies cannot be excluded, a careful search for mutations occurring in the MTERF genes of candidate patients is desirable.

Highlights

- In metazoans, mitochondrial transcription requires a phage-like POLRMT and several accessory factors, many of which belong to the MTERF protein family.
- The MTERF family includes the well-characterized transcription termination factors, proteins which have structural and functional flexibility.

- Human mTERF and sea urchin mtDBP appear to act in mtDNA transcription as well as in replication.
- Transcription termination probably accomplishes various purposes according to the different gene organisation of mitochondrial genomes.
- The MTERF family includes the transcription activator MTERF2, a protein restricted to vertebrates, and the transcription repressor MTERF3, a protein found in all metazoans; both contact mtDNA in the promoter region.
- The complex network of protein-protein interactions (e.g., initiation factors and MTERF family proteins) in the promoter region of human mtDNA must still be clarified.
- The role of MTERF4 and CG7575 in mtDNA transcription and, possibly, replication should also be established.
- Expression of MTERF protein genes seems to be subjected to similar regulatory patterns in human and *Drosophila*.
- Light should be thrown on the regulatory mechanisms underlying MTERF3 expression.

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