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New DNA-Binding Activity of Rat Mitochondrial Transcription Termination Factor (mTERF)

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The molecular mechanisms involved in the regulation of the balance between rRNA and mRNA in mitochondria are poorly understood. The mitochondrial transcription termination factor (mTERF) was highlighted as a potential transcription-control point. In this study, rat mTERF has been expressed *in vitro* and in *Escherichia coli*. The mature protein, in addition to the expected specific DNA–binding capacity for the sequence required for termination, has a new DNA-binding activity, and is able to bind to rat mitochondrial promoter region. This finding suggests communication between transcription initiation and termination regions. However, the results of a competition experiment argue against the formation of a complex between rat mTERF and the termination probe and promoter probe simultaneously, although it remains to be investigated whether another factor(s) might be involved in this interaction. In addition, recombinant human mTERF is also able to bind to human mitochondrial promoter region.

Key words: mitochondrial transcription, mitochondrial transcription termination factor, mtDNA, mTERF, recombinant protein.

Abbreviations: mTERF, mitochondrial transcription termination factor; mtDNA, mitochondrial DNA; RT, rat termination region probe; HLP, promoter region probe; NSP, non-specific probe.

Expression of mammalian mitochondrial DNA (mtDNA) is initiated at three different points located in the D-loop region, one for the L-strand (I_L) and two for the H-strand (I_{H1} and I_{H2}) (*1–3*). According to this dual H-strand transcription model, when transcription initiates at the I_{H1} site, it normally stops at the border of the 16S rRNA gene due to the action of the mitochondrial transcription termination factor (mTERF), and directs the synthesis of the two rRNAs (16S and 12S) and two tRNAs. mTERF is a DNA-binding protein that protects a 28 bp region within the $tRNA^{Leu(UUR)}$ gene at the position immediately adjacent to the region downstream of the 16S rRNA gene (*4*, *5*). The cloning of human mTERF has provided evidence for a novel DNA-binding motif in which three leucine zippers are necessary to bring two widely separated basic domains into contact with the mTERF target DNA sequence (*6*). When the polymerase initiates at the I_{H2} site (about 20 times less active than I_{H1}), it is able to read through the mTERF-dependent termination, transcribing almost the whole mtDNA H-strand [2 tRNAs and mRNAs for 12 polypeptides that are subunits of the oxidative phosphorylation system (OXPHOS)] Therefore, the rates of synthesis of rRNA and mRNA, besides being regulated at the level of transcription initiation, can be explained by transcription termination at the mTERF-binding site (*4*, *7*). Recently, a new model of mTERF regulation by phosphorylation has been proposed (*8*).

In the present work, we report that the mature rat mTERF protein expressed *in vitro* or in *Escherichia coli* exhibits DNA-binding capacity not only for the sequence required for transcription termination, but also for the promoter region. Furthermore, human mTERF also exhibits DNA-binding capacity for the promoter region.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—The plasmid used to produce r-mTERFm, h-mTERFm and h-mtTFAm by *in vitro* translation and the pLEX-His-mTERF vector used to express his-mTERF in bacteria with a 14–amino acid histidine tag (MPRGSHHHHHHGMA) at the N-terminus have been described before (*8*, *9*). These constructs have a deletion of the sequence that encodes the mitochondrial target sequence.

Expression of Recombinant Proteins—In vitro expression: The proteins r-mTERFm, h-mTERFm and h-mtTFAm were expressed in the TNT-coupled transcription-translation system (Promega), which uses a rabbit reticulocyte lysate, following the manufacturer's protocols. When necessary, [^{35}S]methionine (1,000 Ci/mmol, Amersham) was used to label proteins, and the incorporation of the radioactive label and the amount of protein synthesized were calculated.

Bacterial expression: The rat protein his-mTERF was expressed in bacteria using the P_L Expression System (Invitrogen) following the manufacturer's protocols. *Escherichia coli* GI724 (Invitrogen) transformed with

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pLEX-His-mTERF was grown in induction medium [6 g/liter Na_2HPO_4, 3 g/liter KH_2PO_4, 0.5 g/liter NaCl, 1 g/liter NH_4Cl, 0.2% (w/v) amicase, 0.5% (w/v) glucose, 1 mM $MgCl_2$, and 100 μg/ml ampicillin] to an OD_{550} of 0.5 at 30°C. The expression was induced at 37°C for 4 h by the addition of 100 μg/ml tryptophan. Bacteria were harvested and analyzed by 10% (w/v) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie Brilliant Blue staining.

Purification of recombinant rat his-mTERF from E. coli: The purification of rat his-mTERF was carried out as described previously (*10*). Following the induction of expression, bacterial pellets were isolated, dissolved in buffer 1 [6 M guanidinium chloride, 20 mM Tris-HCl (pH 7.9), and 500 mM NaCl] and bound to Ni^{2+}-loaded HiTrap® affinity columns (Pharmacia-Biotech). The columns were washed with buffer 2 [6 M urea, 20 mM Tris-HCl (pH 7.9), 500 mM NaCl, and 20 mM imidazole], and the urea was removed with buffer 3 [20 mM Tris-HCl (pH 7.9), and 150 mM NaCl]. The soluble protein (F4) was eluted from the column with buffer 4 [20 mM Tris-HCl (pH 7.9), 150 mM NaCl, and 50 mM EDTA] and the insoluble aggregates (F5) were recovered with buffer 1 containing 50 mM EDTA. These aggregates were renatured as previously described (*11*). Four volumes of cold acetone (–20°C) were added to the F5 eluate, which was allowed to precipitate for 30 min at –20°C. The sample was then centrifuged for 10 min at 10,000 × *g*, and the acetone supernatant was poured off. The acetone precipitate was allowed to dry for 10 min and then dissolved in 1/100 fold the initial volume of 6 M guanidinium chloride in dilution buffer [50 mM Tris-HCl (pH 7.8), 20% (v/v) glycerol, 0.1 mg/ml BSA, 150 mM NaCl, 1 mM DTT, and 0.1 mM EDTA]. The pellet was dissolved thoroughly and allowed to stand at room temperature for 20 min. The solution was then diluted 50-fold with dilution buffer and permitted to renature for 3 h at room temperature (R). Proteins were analyzed by 10% (w/v) SDS-PAGE with Coomassie Brilliant Blue staining. The protein content was determined by densitometric comparison with marker proteins.

DNA Binding Assay—DNA-binding activity to the rat termination site was determined by electrophoretic mobility-shift assays (EMSA) using the RT probe, which corresponds to the rat mtDNA in the 16S rRNA/Leucyl-tRNA boundary region [L-strand: GG AAG TTA TTA GGG TGG CAG AGC CAA GTA ATT GCG TAA GA-2686, and H-strand: GG TCT TAC GCA ATT ACT TGG CTC TGC CAC CCT AAT AAC TT-2649, using the numbering system of Gadaleta (*12*)]. When necessary, the annealed probe was labeled by filling in the 3′-recessed ends with Klenow enzyme and [α-^{32}P]dCTP. Various amounts of protein were incubated at 25°C for 15 min in 20 μl of reaction mixture containing 10 nM probe, 25 mM Hepes-KOH (pH 7.8), 50 mM KCl, 12.5 mM $MgCl_2$, 0.05% (v/v) Tween-20, 1 mM DTT, 20% (v/v) glycerol, 0.1 μg/μl poly(dI-dC).poly(dI-dC) (added as non specific competitor DNA) and 0.25 μg/μl BSA. After incubation, the reaction mixtures were loaded onto a native 5% (w/v) polyacrylamide gel in Tris-glycine buffer, and electrophoresed at 4°C as described (*13*). In another kind of experiment, the rat mTERF proteins were subjected to electrophoretic mobility shift assay using a rat-HLP probe, which is a PCR product of 116 bp containing the rat mitochondrial promoter region (between 16147–16262). A PCR product of 103 bp between 14521 and 14623 of rat mtDNA was used in some experiments as a non-specific probe (NSP). Finally, the DNA-binding activities of h-mTERFm and h-mtTFAm were studied by EMSA using a human-HLP probe [PCR product of 157 bp containing the human mitochondrial promoter region between 367–523 (*14*)]. In the experiments with h-mtTFAm, the binding mixture did not contain poly(dI-dC).poly(dI-dC). When necessary, the probes were labeled by filling in the 3′-recessed ends with Klenow enzyme and [α-^{32}P]dCTP. Either [^{35}S]methionine-labeled protein and unlabeled probe or unlabeled protein and 3′-^{32}P–labeled probe were used, as specified in the text.

Estimation of the Apparent Equilibrium Dissociation Constant (K_d)—DNA-binding reactions containing known concentrations of labeled probe and recombinant protein were carried out. The bound and free oligonucleotides were fractionated by mobility shift assay, and the amounts were determined by densitometry. The apparent dissociation constant (K_d) corresponding to the dissociation in the equilibrium (DNA + Protein ↔ DNA:Protein) was estimated by the equation: K_d = [DNA] × [Protein]/[DNA:Protein].

RESULTS

Expression of Recombinant Rat mTERF—In order to analyse the functional capacity of rat mTERF, the protein was expressed *in vitro* and in bacteria. The recombinant r-mTERFm was synthesized in a coupled transcription/translation system from a construct having an N-terminal deletion of the fragment that codifies the putative mitochondrial target sequence (Fig. 1A). Furthermore, a full-length rat mTERF for the mature protein was expressed as a histidine amino terminal-tagged fusion protein using the pLEX-His vector in *Escherichia coli* GI724, as described under "EXPERIMENTAL PROCEDURES." When bacteria were grown at 37°C in the presence of tryptophan, the His-tag recombinant protein (his-mTERF) was produced with an apparent molecular mass of ~40 kDa (Fig. 1B, left panel). This recombinant his-mTERF protein was purified by affinity chromatography through Ni^{2+} resin (Fig. 1B, right panel). The soluble fraction (F4) contained the ~40 kDa protein and two extra protein bands of approximately 47 and 30 kDa. The extra protein whith the higher molecular mass might come from the host *E. coli* strain (*15*), while the smaller one could be an N-terminal degradation fragment of his-mTERF that is formed during the purification process (*16*) or some other contaminant from *E. coli*. The insoluble fraction (F5) was rich in his-mTERF protein, with a very low content of extra bands. This fraction was acetone precipitated and renaturated (R) in the presence of BSA (*11*). The soluble fraction comprised about one-fourth and the insoluble fraction about three-fourths of the purified protein.

Binding of Rat mTERF to the Rat Termination Site—The DNA-binding ability of recombinant rat mTERF was tested by mobility shift assay, in which r-mTERFm and his-mTERF were probed with DNA containing the putative rat mTERF binding site (RT probe, see Fig. 2A). The recombinant mature protein, r-mTERFm, was able to

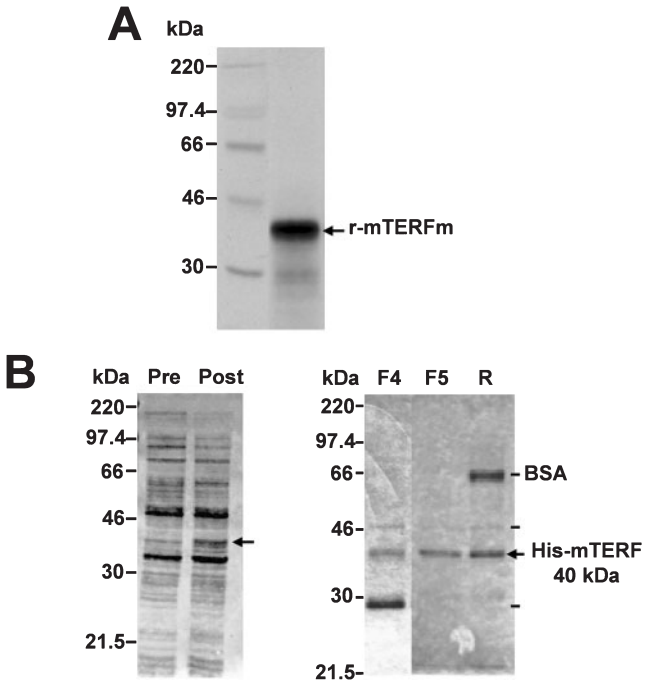

Fig. 1. **Rat mTERF expression.** (A) SDS-PAGE analysis of r-mTERFm synthesized in the presence of rabbit reticulocyte lysate and [^{35}S]methionine. (B) SDS-PAGE analysis of rat his-mTERF expressed in bacteria and purified. Total proteins from Pre- and Post-induction cultures are shown in the left panel. The soluble protein (F4), insoluble aggregates (F5) (both eluted from Ni^{2+} column), and renatured protein (R) are shown in the right panel. The position of molecular mass markers is indicated.

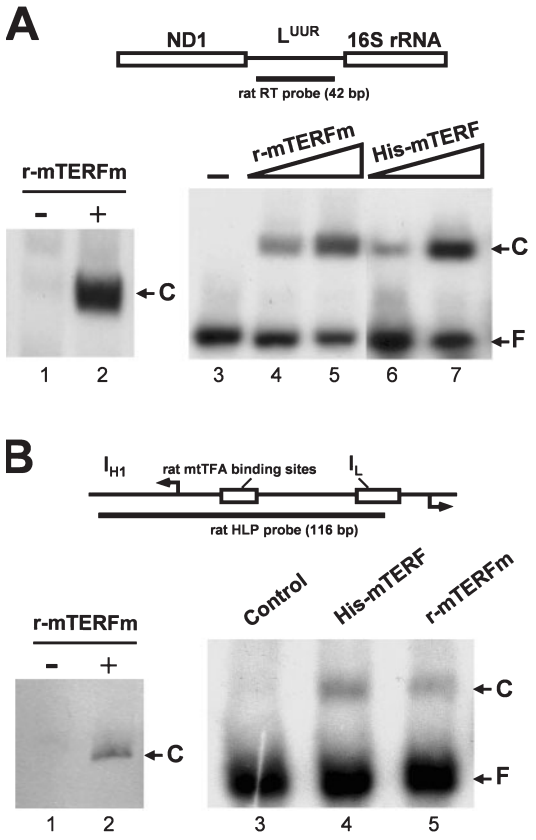

Fig. 2. **DNA-binding activities of recombinant rat mTERF.** (A) DNA-binding to the termination site. Schematic diagram of the rat RT probe used is shown. Mobility shift assay using ^{35}S-labeled r-mTERFm and unlabeled probe (lane 2) or ^{32}P-labeled RT probe and r-mTERFm [lanes 4 (10 nM) and 5 (20 nM)] or rat his-mTERF [lanes 6 (30 nM) and 7 (150 nM)]. (B) DNA-binding to the promoter region. Schematic diagram of the rat-HLP probe used is shown. Mobility shift assay using ^{35}S-labeled r-mTERFm and unlabeled probe (lane 2) or ^{32}P-labeled rat-HLP probe and rat his-mTERF [lane 4 (250 nM)] or r-mTERFm [lane 5 (20 nM)] unlabeled. C, protein-DNA complex; F, free probe.

bind the ^{32}P-labeled RT probe to produce a single retarded band (Fig. 2A). Increasing the amount of protein resulted in an increase in the intensity of the retarded band, without the appearance of any more slowly moving secondary band (lanes 4 and 5). The apparent dissociation constant (K_d) of r-mTERFm for the RT probe was estimated to be ~17 nM. The specificity of the r-mTERFm interaction with RT probe was futher confirmed by using protein labeled with [^{35}S]methionine. As shown in Fig. 2A (lane 2), r-mTERFm produced a single and specific retarded band. Meanwhile, an unbound protein with an isoelectric point (pI) of ~9.48 migrated toward the negative electrode (cathode) and escaped from the gel, because at pH 7.8, the protein has a net positive charge (Fig. 2A, lane 1). Moreover, to analyze the functional capacity of the his-mTERF, the DNA binding activity of the renatured protein (R) was determined. The protein also bound the RT probe to produce a single and specific retarded band (Fig. 2A, lanes 6 and 7) with an apparent K_d of ~210 nM. The estimated values of K_d are in agreement with those previously calculated (*8*).

Binding of Rat mTERF to the Rat Mitochondrial Promoter Region—The dual H-strand transcription model suggests a relationship between transcription initiation and termination events. Therefore, in order to test whether or not the recombinant rat mTERF is able to interact with the transcription initiation region, DNA binding assays were carried out using a 116 bp probe that contains mitochondrial rat promoters (rat-HLP probe, see Fig. 2B). When [^{35}S]methionine-labeled r-mTERFm was used, a single retarded band was produced (Fig. 2B, lane 2). The binding capacity of his-mTERF and r-mTERFm was also demonstrated using the ^{32}P-labeled DNA probe (lanes 4 and 5). These assays, as with the DNA binding assays described above, were carried out in the presence of 100 μg/ml of poly(dI-dC).poly(dI-dC) as nonspecific competitor DNA, which indicated the specificity of the interaction between rat mTERF and the mitochondrial promoter region (see below). The apparent K_d of his-mTERF and r-mTERFm for rat-HLP were estimated to be ~1,300 nM and ~190 nM, respectively.

The occurrence of one binding site upstream and another downstream from the rDNA genes raises the possibility that mTERF might interact with both sequences simultaneously and thus bring the terminator to the vicinity of the promoter by looping out the pre-rRNA coding sequence. To test this model, rat his-mTERF was incubated with the ^{32}P-labeled RT probe in the presence of cold rat-HLP probe (Fig. 3A). The possibility of a double binding capacity of rat mTERF with the termination region and the promoter region at the same

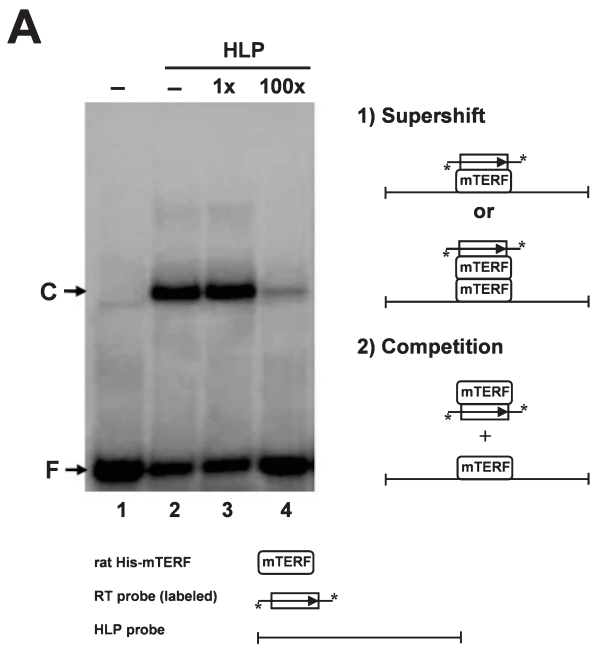

Fig. 3. **Competition assays.** (A). Mobility shift assay using 7.5 nM ^{32}P-labeled RT probe in the absence (lane 1) or presence (lanes 2–4) of 225 nM rat his-mTERF. Increasing amounts of rat-HLP probe were added to the reaction mixture. Schematic illustration of two theoretical possibilities, supershift or competition, are indicated in the right panel. (B) Mobility shift assay using 10 nM ^{32}P-labeled RT probe in the absence (lane 1) or presence (lanes 2–6) of 10 nM r-mTERFm. Increasing amounts of RT (lanes 3 and 4), rat-HLP (lanes 5 and 6) or NSP (lanes 7 and 8) unlabeled probe were added to the reaction mixture. C, protein-DNA complex; F, free probe.

time, whether as a monomer or as a dimer, to form a loop *in vivo*, should produce a supershift in the mobility shift assays. In the other case, it should produce competition between both binding sites (Fig. 3A, right panel). Increasing the amount of rat-HLP probe over a 100-fold molar range produced a decrease in the intensity of the retarded band (competition) and no more slowly moving secondary band was observed, as would be expected in the case of a supershift. The same result was obtained when r-mTERFm was used (data not shown). This result argues against the simultaneous formation of a complex of rat mTERF with the termination probe and promoter probe.

In order to show that rat mTERF binds specifically to the promoter region, a random sequence of rat mtDNA

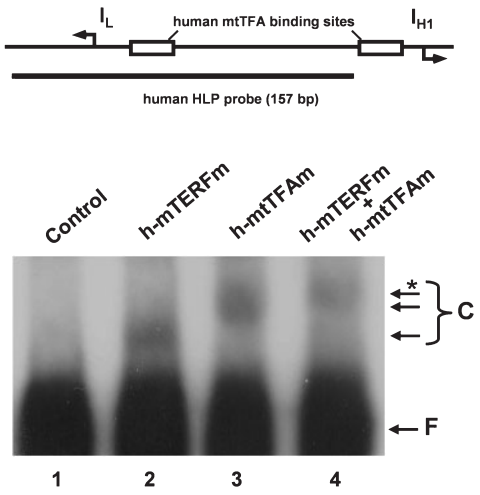

Fig. 4. **DNA-binding activities of recombinant human mTERF and mtTFA.** Schematic diagram of the human-HLP probe used is shown. Mobility shift assay using ^{32}P-labeled human-HLP probe (lane 1) plus h-mTERFm (lane 2) or h-mtTFAm (lane 3) or both proteins (lane 4). C, protein-DNA complex; F, free probe. The asterisk indicates the supershift band.

was used in competition experiments (Fig. 3B). Mobility-shift assays with the ^{32}P-labeled RT probe and r-mTERFm produced a DNA-protein complex (Fig. 3B, lanes 2–8). The protein-DNA complex was unaffected by the addition of a non-specific competitor (NSP probe), that is, a fragment of 103 bp containing part of the cytochrome b gene sequence (Fig. 2B, lanes 7 and 8). However, the displaced band almost disappeared when the binding reaction contained an excess of cold RT probe (Fig. 2B, lanes 3 and 4). Similar results were obtained with the rat-HLP probe (Fig. 2B, lanes 5 and 6), but with less extension, in agreement with the differences in K_d. These data show the failure of rat mTERF to bind other random sequences in the mtDNA, and, therefore, the specificity of the interaction of the protein with the termination (RT probe) and promoter (rat-HLP probe) region.

To investigate whether human mTERF is also able to bind to the human mtDNA promoter region, the protein h-mTERFm was expressed *in vitro*. For this analysis, the human-HLP probe (a region equivalent to rat-HLP) was used (Fig. 4). A mobility-shift experiment in the absence of recombinant proteins did not produce any DNA-protein complex (Fig. 4, lane 1). However, when h-mTERFm was used, a slower migrating band was observed (Fig. 4, lane 2), indicating that human mTERF also binds to the promoter region of human mtDNA. The HLP probes contain an mtTFA-binding site and, as expected, the recombinant h-mtTFAm was able to bind to the human-HLP probe (Fig. 4, lane 3), producing a more retarded band than that produced by h-mTERF, because mtTFA binds to DNA as a tetramer (*17*). Finally, when both recombinant proteins were used simultaneously, a supershift was observed (Fig. 4, lane 4), indicating that the proteins do not compete for the same binding site, and, therefore, both proteins bind to the promoter region at different sites.

DISCUSSION

In the present work, the expression of rat mTERF has provided significant insight into the function of this factor. The recombinant mature protein expressed *in vitro* in a rabbit reticulocyte lysate system (r-mTERFm) or expressed in a bacterial system and purified (rat his-mTERF) has the expected specific DNA-binding capacity for the termination region (Fig. 2A) (*4*, *8*).

The most important result presented in this study is the ability of rat mTERF to bind to the promoter region (Fig. 2B). This interaction is specific since it cannot be prevented by poly(dI-dC)·poly(dI-dC) at very high concentrations, which can disrupt the interaction of nonspecific DNA-binding proteins such as mitochondrial transcription factor A (mtTFA or TFAM) (data not shown). This conclusion is also supported by the failure of rat mTERF to bind other random sequences in the mtDNA (Fig. 3B). Moreover, human mTERF is also able to bind to the human promoter region (Fig. 4). However, the rat mTERF apparent equilibrium dissociation constant (K_d) estimated for binding to the promoter region is between 5- and 10-fold higher than the one for binding to the termination region. Additionally, two binding sites for mtDBP (mitochondrial transcription termination factor from sea urchin *Paracentrotus lividus*) (*18*) and for DmTTF (mitochondrial transcription termination factor from *Drosophila melanogaster*) (*19*) have also been described.

The new DNA-binding activity of mTERF opens the question of whether the protein plays an important role in the formation of an initiation complex as a component of the basal transcription initiation machinery. Nevertheless, this role has been previously ruled out for human mTERF (*9*). Moreover, the human mitochondrial transcription factors B1 (mtTFB1 or TFB1M) (*20*) and B2 (mtTFB2 or TFB2M) (*21*) have been cloned recently, and the basal human mtDNA transcription machinery has been fully reconstituted *in vitro* with mitochondrial RNA polymerase (mtRPOL), mtTFA, mtTFB1 or mtTFB2.

On the other hand, the characterization of novel H-strand coded polyadenylated RNAs, mapping to the D-loop regions of rat, human and mouse mtDNA (*22*), opens the possibility of a transcription termination site in the D-loop. Nevertheless, the fact that the 3′ ends of these poly(A)-RNAs map immediately upstream from the $tRNA^{Phe}$ gene, outside of HLP probes sequence where mTERF is bound, together with the recent identification of the two 45 kDa and 70 kDa proteins involved in this transcription termination (*23*), serve to rule out mTERF as a transcription termination factor in the promoter region.

The regulation of mitochondrial transcription can be operated either at the level of initiation, termination, or both. In particular, the transcription initiation rate at two alternative H-strand sites would determine the synthesis ratio of rRNAs *versus* mRNAs, and the regulation of the balance between rRNA and mRNA seems to play an important role in the control of mitochondrial protein synthesis (*24*). On the basis of *in vivo* data it has been proposed that transcripts from the second initiation site of the H-strand (I_{H2}) are refractive to promote termination by mTERF and transcribe all genes downstream of the termination site (*1*, *2*). In addition, conditions that clearly modify the mitochondria synthesis ratio mRNA/rRNA *in organello* do not affect the binding and occupancy of the rat mTERF target sequence as determined by footprinting analysis (*25*). This finding suggests communication between the transcription initiation and termination regions. From the competition experiment with the termination probe (Fig. 3A) it can be deduced that there is no simultaneous interaction between the promoter and termination regions mediated only by mTERF, but it remains to be investigated whether another factor(s) could be involved in this interaction.

One possible function of the binding of mTERF to the promoter is to establish a defined DNA structure of the H-strand promoter that facilitates binding and interaction of basal transcription factors in order to yield an increase in transcription initiation from I_{H1}. A similar function has been described for the mouse transcription termination factor TTF-I; *i.e.*, the activation of murine rDNA transcription by RNA polymerase I requires the binding of TTF-I to the promoter-proximal terminator, which triggers nucleosome remodeling (*26*).

Although it is clear that mTERF binds to mtDNA in the promoter region, the affinity for this region is 5- 10-fold lower than the affinity for the termination region. This difference in affinities could indicate that the site in the promoter region is not occupied *in vivo*, although it depends of the concentration of mTERF in mitochondria. Furthermore, the affinities could be modified by interaction with other mitochondrial proteins. A critical question raised by this result is whether mTERF has a physiological function in the promoter region. Future work will be aimed at assessing the *in vivo* relevance of this finding, at dissecting the putative role of mTERF in the promoter region, as well as at identifying the new site as accurately as possible by footprinting combined with point mutagenesis to identify both the protected region and the contacts critical for recognition. This will allow a direct comparison between the new and old sites.

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REFERENCES

1. Montoya, J., Christianson, T., Levens, D., Rabinowitz, M., and Attardi, G. (1982) Identification of initiation sites for heavy strand and light strand transcription in human mitochondrial DNA. *Proc. Natl Acad. Sci. USA* **79**, 7195–7199
2. Montoya, J., Gaines, G.L., and Attardi, G. (1983) The pattern of transcription of the human mitochondrial rRNA genes reveals two overlapping transcription units. *Cell* **34**, 151–159
3. Bogenhagen, D.F., Applegate, E.F., and Yoza, B.K. (1984) Identification of a promoter for transcription of the heavy strand of human mtDNA, *in vitro* transcription and deletion mutagenesis. *Cell* **36**, 1105–1113
4. Kruse, B., Narasimhan, N., and Attardi, G. (1989) Termination of transcription in human mitochondria: identification and purification of a DNA binding protein factor that promotes termination. *Cell* **58**, 391–397
5. Daga, A., Micol, V., Aebersold, R., and Attardi, G. (1993) Molecular characterization of the transcription termination factor from human mitochondrial. *J. Biol. Chem.* **268**, 8123–8130

6. Fernandez-Silva, P., Martinez-Azorin, F., Micol, V., and Attardi, G. (1997) The human mitochondrial transcription termination factor (mTERF) is a multizipper protein but binds to DNA as a monomer, with evidence pointing to intramolecular leucine zipper interactions. *EMBO J.* **16**, 1066–1079
7. Christianson, T.W., and Clayton, D.A. (1988) A tridecamer DNA sequence supports human mitochondrial RNA 3′-end formation in vitro. *Mol. Cell. Biol.* **8**, 4502–4509
8. Prieto-Martin, A., Montoya, J., and Martinez-Azorin, F. (2004) Phosphorylation of rat mitochondrial transcription termination factor (mTERF) is required for transcription termination but not for binding to DNA. *Nucleic Acids Res.* **32**, 2059–2068
9. Prieto-Martin, A., Montoya, J., and Martinez-Azorin, F. (2001) A study on the human mitochondrial RNA polymerase activity points to existence of a transcription factor B-like protein. *FEBS Lett.* **5003**, 51–55
10. Holzinger, A., Phillips, K.S., and Weaver, T.E. (1996) Single-step purification/solubilization of recombinant proteins: application to surfactant protein B. *Biotechniques* **20**, 804–808
11. Hager, D.A. and Burgess, R.R. (1980) Elution of proteins from sodium dodecyl sulfate polyacrilamide gels, removal of sodium dodecyl sulfate, and renaturation of enzymatic activity: results with sigma subunit of *Escherichia coli* RNA polymerase, wheat germ topoisomerase and other enzymes. *Anal. Biochem.* **109**, 76–86
12. Gadaleta, G., Pepe, G., DeCandia, G., Quagliariello, C., Sbisa, E., and Saccone, C. (1989) The complete nucleotide sequence of the Rattus norvegicus mitochondrial genome: cryptic signals revealed by comparative analysis between vertebrates. *J. Mol. Evol.* **28**, 497–516
13. Fernandez-Silva, P., Micol, V., and Attardi, G. (1996) Mitochondrial DNA transcription initiation and termination using mitochondrial lysate from cultured human cells. *Methods Enzymol.* **264**, 129–139
14. Anderson, S., Bankier, A.T., Barrel, B.G., de Bruijn, M.H.L., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., *et al.* (1981) Sequence and organization of the human mitochondrial genome. *Nature* **290**, 457–465
15. Hengen, P. (1995) Purification of His-Tag fusion proteins from *Escherichia coli*. *Trends Biochem. Sci.* **20**, 285–286
16. Nam, S.C. and Kang, C. (2001) Expression of cloned cDNA for the human mitochondrial RNA polymerase in *Escherichia coli* and purification. *Protein Expr. Purif.* **21**, 485–491
17. Antoshechkin, I., Bogenhagen, D.F., and Mastrangelo, I.A. (1997) The HMG-box mitochondrial transcription factor xl-mtTFA binds DNA as a tetramer to activate bidirectional transcription. *EMBO J.* **16**, 3198–3206
18. Loguercio Polosa, P., Roberti, M., Musicco, C., Gadaleta, M.N., Quagliariello, E., and Cantatore, P. (1999) Cloning and characterization of mtDBP, a DNA-binding protein which binds two distinct regions of sea urchin mitochondrial DNA. *Nucleic Acids Res.* **27**, 1890–1899
19. Roberti, M., Loguercio, P.L., Bruni, F., Musicco, C., Gadaleta, M.N., and Cantatore, P. (2003) DmTTF, a novel mitochondrial transcription termination factor that recognises two sequences of Drosophila melanogaster mitochondrial DNA. *Nucleic Acids Res.* **31**, 1597–1604
20. McCulloch, V., Seidel-Rogol, B.L., and G.S., S. (2002) A human mitochondrial transcription factor is related to RNA adenine methyltransferases and binds S-adenosylmethionine. *Mol. Cell. Biol.* **22**, 1116–1125
21. Falkenberg, M., Gaspari, M., Rantanen, A., Trifunovic, A., Larsson, N.-G., and Gustafsson, C.M. (2002) Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. *Nat. Genet.* **31**, 289–294
22. Vijayasarathy, C., Zheng, Y.M., Mullick, J., Basu, A., and Avadhani, N.G. (1995) Identification of a stable RNA encoded by the H-strand of the mouse mitochondrial D-loop region and a conserved sequence motif immediately upstream of its polyadenilation site. *Gene Expr.* **4**, 125–141
23. Camasamudram, V., Fang, J.-K., and Avadhani, N.G. (2003) Transcription termination at the mouse mitochondrial H-strand promoter distal site requires an A/T rich sequence motif and sequence specific DNA binding proteins. *Eur. J. Biochem.* **270**, 1128–1140
24. Wiesner, R.J., Aschenbrenner, V., Ruegg, J.C., and Zak, R. (1994) Coordination of nuclear and mitochondrial gene expression during the development of cardiac hypertrophy in rats. *Amer. J. Physiol.* **267**, C229–235
25. Enriquez, J.A., Fernandez-Silva, P., Garrido-Perez, N., Lopez-Perez, M.J., Perez-Martos, A., and Montoya, J. (1999) Direct regulation of mitochondrial RNA synthesis by thyroid hormone. *Mol. Cell. Biol.* **19**, 57–670
26. Längst, G., Blank, T.A., Becker, P.B., and Grummt, I. (1997) RNA polymerase I transcription on nucleosomal templates: TTF-I induces chromatin remodelling and relieves transcriptional repression. *EMBO J.* **16**, 760–768