Human mitochondrial mTERF wraps around DNA through a left-handed superhelical tandem repeat

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The regulation of mitochondrial DNA (mtDNA) processes is slowly being characterized at a structural level. We present here crystal structures of human mitochondrial regulator mTERF, a transcription termination factor also implicated in replication pausing, in complex with double-stranded DNA oligonucleotides containing the rRNALeuUUR gene sequence. mTERF comprises nine left-handed helical tandem repeats that form a left-handed superhelix, the Zurdo domain.

Figure 1 The mTERF Zurdo domain. (a) Repeats I–IX consist of three helices, H1, H2 and H3, shown here for TERF-I. In the mTERF crystal, one protein molecule binds two symmetry-related oligonucleotides, DNA1 (orange) and DNA2 (gray), related by ~36°. N and C, N- and C-terminal ends. (b) Explanation of left- and right-handedness. Left, starting from point 1 and following the numbers, the TERF superhelix curves to the left (bottom). Right, the Armadillo (ARM) repeat shows right-handed tracing. (c) Ball-and-stick representation on TERF-VII of residues mutated and numbered according to the mTERF repeats alignment and logo (see Supplementary Fig. 1).

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also comprising positions 34, 37 and 38 from H2 and 46 and 48 from H3. This hydrophobic core spans the entire protein. In all tandem repeats except the first, logo position 26 (usually glycine), between H1 and H2, shows main chain conformation angles corresponding to a left-handed helix. In addition, the main chain kinks by about 90° at position 44 (usually a proline at the beginning of H3). Glycine and proline tend to disrupt regular secondary-structure elements and here confer the triangular shape of the TERF repeat. Finally, positions 23, 28 and 51 are involved in N- or C-terminal capping of helices H1, H2 and H3, respectively. All these conserved residue define the overall structure of the mTERF repeat (Fig. 1c).

Protein variants mTERF and mTERF ΔN crystallized in complexes with a 15-mer and a 12-mer dsDNA, respectively, which included the sequence of the tRNA\textsubscript{Lys} gene\textsuperscript{13} (Fig. 2). In both crystals, which belong to different space groups, two crystallographically related, identical oligonucleotides (DNA1 and DNA2) are bound by two distinct positively charged regions of a single protein moiety (Fig. 2a and Supplementary Fig. 2). In the full-length complex, repeats I–VI contact one half of DNA1, whereas repeats VIII and IX plus helix C approach the other half of DNA2. DNA1 and DNA2 do not contact each other and are oriented at an angle of 36° (Fig. 1a), in agreement with previous assays that estimated a bending of the termination site of 35° upon protein binding\textsuperscript{14}. The only repeat not engaged in DNA binding, TERF-VII, lacks the polar residue at logo positions 50/51 that is involved in DNA contacts in the other repeats (Supplementary Fig. 1b).

This feature suggests for repeat VII a hinge role between the N- and C-terminal DNA-binding subdomains.

The full-length protein is engaged in 19 nonspecific interactions with both dsDNA backbone phosphates (Supplementary Fig. 1b). Eight of the residues involved further perform C-capping of α-helices at the protein-DNA interface, thus diminishing the repulsion between peptide-bond carbonyls and DNA phosphates. N-terminal repeats I–VI contribute, through residues at logo positions 39, 45/49 and 50/51, to backbone recognition. Two conserved guanines (G12 from strand C and G3 from B) of the binding sequence\textsuperscript{2} are positioned for contacts with Arg169 and Arg202, respectively (Fig. 2b and Supplementary Fig. 2a,b). An adjacent conserved guanine (G4 from strand B) is weakly contacted by Glu165. In the C-terminal subdomain, residues from repeats VIII and IX (logo positions 9 and 13 of H1 and 45 and 51 of H3) contact the phosphate backbone of DNA2 and surround Arg387 from helix C, which interacts through the major groove with three bases thereof (Fig. 2c and Supplementary Fig. 2a,c). Additional contacts contribute to stabilizing the termini of DNA1 and DNA2 within the concave protein surface. Overall, the mTERF residues involved in DNA contacts are conserved throughout the vertebrate MTERF1 subfamily members\textsuperscript{4}, suggesting that similar complexes could be formed by these proteins.
We obtained full-length mTERF in complex with longer dsDNA oligonucleotides (up to 29 base pairs (bp), Fig. 2d), which showed higher stability but did not crystallize. To assess whether a continuous dsDNA bridging DNA1 and DNA2 could be bound by mTERF, we analyzed a complex of the protein with a DNA of 28 bp by small-angle X-ray scattering (SAXS) in solution (Fig. 2d and Supplementary Methods). Based on the crystallographic structure, we constructed 30 models by translating a canonical 28-bp B-DNA in a stepwise base pair manner from DNA1 to DNA2 sites, respecting the crystalline protein–DNA contacts and introducing the required bending. The model that best fit the experimental data showed the concave surface of mTERF wrapping around the major groove of a continuous DNA that protruded on one side of the protein solenoid (Supplementary Fig. 3). The quality of the fit validates the model for the particle in solution. The DNA sequence is coherent with the sequence of DNA1 and suggests that the C-terminal domain slightly rearranges to accommodate the DNA. This intrinsic flexibility of the domain is supported by the consistent, slight variation of the angle between repeats when comparing the mTERF and mTERF ΔN structures, whose pitches differ by 13 Å (Fig. 3a). The N-terminal excision abolishes the interactions with bases of DNA1, which is displaced to form an unbent pseudocontinuous DNA fiber by base stacking with DNA2 (Fig. 3). If C-terminal subdomain repeats VII–IX plus helix C of the two structures are superimposed, the respective DNA2-phosphate positions match perfectly (Fig. 3). However, the mTERF ΔN structure unambiguously shows DNA2 bound in opposite orientations, with Arg387 contacting different bases in the major groove (Fig. 3b and Supplementary Fig. 2d). Furthermore, mTERF ΔN in complex with a 15-bp DNA yielded a structure in space group C2221, where two protein moieties symmetrically contacted a single, two-fold–disordered DNA molecule. Overall, this underpins the nonspecific binding capacity of the mTERF ΔN variant to a DNA major groove.

In summary, nine left-handed helical mTERF repeats of three helices each give rise to a twisted solenoid that binds a continuous bent dsDNA. This suggests a similar binding mode, causing DNA bending, to the mTDNA termination site in vivo. A number of sequence-specific proteins have been shown to cause an alteration in DNA structure upon binding15. The only transcriptional termination factor reported to exert this effect is DNA polymerase I–specific transcription termination factor I, which binds DNA as a monomer and induces a bending of 40° (ref. 16), but no protein–DNA complex structure exists for comparison. The structure of the Zurdo domain is likely relevant to other TERF family members, which notwithstanding must have member-specific features to accomplish their particular functions.

Accession codes. Protein Data Bank: Coordinates and structure factors for mTERF and the mTERF ΔN variant have been deposited under accession codes 3N6S and 3N7Q, respectively.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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AUTHOR CONTRIBUTIONS

M.S. and M.C. provided materials and infrastructure. N.J.-M., H.T.J. and the rest of the authors participated in manuscript writing and discussion. M.S. designed and supervised the project.

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