2014-08-04

TFAM forces mtDNA to make a U-turn

Hällberg, B Martin; Larsson, Nils-Göran

Nat Struct Mol Biol. 2011 Nov 4;18(11):1179-81.
Springer Nature
http://doi.org/10.1038/nsmb.2167
http://hdl.handle.net/10616/42132

TFAM Forces mtDNA to Make a U-turn

B. Martin Hallberg[1] and Nils-Göran Larsson[2]

[1]Department of Cell and Molecular Biology, Karolinska Institutet, Box 285, 171 77 Stockholm, Sweden, [2]Max Planck Institute for Biology of Ageing, Robert-Koch-Str. 21, 50931 Köln, Germany.

Correspondence to:

martin.hallberg@ki.se or larsson@age.mpg.de

The mammalian mitochondrial transcription factor A (TFAM) is encoded in the nucleus and imported into mitochondria, where it functions as an activator of mtDNA transcription and packages mtDNA into DNA-protein aggregates called mitochondrial nucleoids. Two X-ray crystallography studies in this issue reveal that TFAM shapes mtDNA into a sharp U-turn, thereby providing a molecular mechanism for its dual roles in the expression and maintenance of mtDNA.

TFAM was discovered in the mid-1980s as an activity, which enabled *in vitro* reconstitution of transcription initiation from human mtDNA promoters using mitochondrial protein extracts[1]. In conjunction with a partially purified mitochondrial RNA polymerase (POLRMT) fraction, purified TFAM was found to activate *in vitro* transcription from the two major mtDNA promoters: the heavy and light strand promoters (HSP and LSP)[1]. Footprinting and methylation interference experiments revealed that TFAM binds to specific sites upstream of both the HSP and LSP transcription start sites[2]. In addition, subsequent cloning and sequencing of the human *TFAM* gene demonstrated that TFAM belongs to the high mobility group (HMG) box family of proteins[3]. TFAM contains a leader peptide that is cleaved after import to the mitochondrial matrix, producing the mitochondrial isoform; this consists of two high mobility group (HMG) boxes separated by a linker and a charged carboxy-terminal tail[3]. Similar to other HMG box proteins, TFAM is able to bind, wrap, and bend DNA without any obvious sequence specificity[4]. In contrast, the carboxy-terminal tail of TFAM is critical for activation of promoter-specific mtDNA transcription[5]. TFAM's budding yeast homolog (Abf2) consists of two HMG-boxes

separated by a short linker, but it has no charged carboxy-terminal tail. Consequently, Abf2 is dispensable in mitochondrial transcription, but it plays an essential role in yeast mtDNA maintenance[6]. The budding yeast basal mtDNA transcription machinery is comprised of the mitochondrial RNA polymerase (RPO41) and an interacting specificity factor (Mtf1)[7]. Identification of the mammalian homolog to Mtf1, denoted TFB2M, led to the development of a fully recombinant *in vitro* transcription system and demonstrated that the basal machinery required for promoter-specific initiation of mammalian mtDNA transcription consists of only three proteins: POLRMT, TFB2M, and TFAM[8].

Studies of knockout mice clarified that TFAM is essential for mtDNA maintenance[9] and that increased transgenic expression of TFAM results in an increase in the number of mtDNA copies *in vivo*[10]. Confocal microscopy studies have found that TFAM is distributed in a punctuate pattern within the mitochondrial network, colocalizes with mtDNA and is present in every mitochondrial nucleoid[11]. Consistent with these observations, biochemical purification of nucleoids has shown TFAM to be an abundant protein component[12]. The role of TFAM in mtDNA organization is further supported by atomic force microscopy studies that demonstrate that it can compact DNA into nucleoid-like structures[13]. Some data support the idea that the binding of TFAM to mtDNA is cooperative[13], which may imply that high affinity TFAM-binding sites initiate a cascade of TFAM binding on the mtDNA molecule. Current estimates from mammalian cell lines and mouse tissues indicate that TFAM is present at 1 copy per 15–20bp of mtDNA and it is thus likely to fully coat mtDNA[10,14] (Fig. 1). Super-resolution microscopy has revealed that the apparent mean size of nucleoids is ~100 nm in mammals[14]. By combining imaging and

quantitative molecular approaches, it has been demonstrated that most nucleoids in human fibroblasts contain one single copy of mtDNA[14].

This issue presents two structures of TFAM bound to LSP[15,16], both of which unexpectedly reveal that TFAM binding imposes a dramatic U-turn upon mtDNA (Fig. 1, Fig. 2A). TFAM is part of the ubiquitous family of HMG box proteins that are exclusively found in eukaryotes, and previous co-crystal structures of DNA-HMG boxes have displayed a sharply bent DNA with residues from the HMG box intercalating between bases, thereby breaking up the DNA stacking. The HMG box family can display both sequence-specific and non sequence-specific DNA binding. TFAM belongs to the subgroup of tandem HMG box proteins, with two HMG boxes separated by a linker. Interestingly, the N-terminal HMG box of TFAM has the same affinity for the LSP promoter DNA as the full-length protein, indicating that a large part of the affinity is localized within that HMG-box[16]. However, the strong bending of the DNA is dependent on both the linker and the C-terminal HMG box, making the reversal in the DNA's direction dependent on the cooperative binding of both HMG boxes and the intervening linker. Each of the two HMG boxes cause the DNA to bend nearly 90 degrees, and the positively charged residues of the linker helix interact with the minor groove of a nearly straight B-form DNA between the DNA bent by the two HMG boxes. The net result is a total reversal in the direction of the DNA helical axis. Interestingly, fluorescence-resonance transfer (FRET) experiments show that non-specific binding of TFAM also results in dramatic DNA bending[15], a feature of obvious use to the architectural function of TFAM in compacting the mitochondrial nucleoid.

In fact, the manner in which TFAM bends the DNA displays a striking similarity to that of the prokaryotic heat unstable nucleoid (HU) and integration host

factor (IHF) proteins (Fig. 2B). The HU/IHF proteins organize and compact the bacterial nucleoid by introducing U-turns into the DNA, just as TFAM is predicted to do in the mitochondrial nucleoid (Fig. 1, Fig. 2A,B). It currently remains unclear whether TFAM alone is sufficient to compact and organize mtDNA into nucleoids. It is possible that other yet unidentified proteins have specific functions in organizing the TFAM-coated mtDNA into higher order structures important to the nucleoid's final organization.

Moreover, TFAM may play a mechanistic role in mtDNA transcription that is similar to the role of TATA-box binding protein (TBP) in nuclear transcription. The binding of TBP to the TATA-box causes dramatic bending of DNA (Fig. 2C), which results in the recruitment of a number of proteins necessary for transcription initiation at nuclear promoters. Remarkably, TFAM appear to share both the architectural role of the HU/IHF proteins and TBP's role of organizing the transcription initiation complex. In fact, similar to the role of TBP, the specific interaction between TFAM and the mitochondrial promoters, which results in the dramatic bending of mtDNA, is likely of central importance in the activation of mitochondrial transcription, as it results in positioning the carboxy-terminal tail of TFAM in close proximity to the transcription start site occupied by the POLRMT-TFB2M complex[17] (Fig. 1). The carboxy-terminus of TFAM plays a critical role in transcription activation[5] and has been reported to interact with both TFB2M and the structurally similar TFB1M protein[18]. These putative interactions must be evaluated further, particularly as TFB1M likely is not involved in transcription, because TFB1M functions as an rRNA methyltransferase essential to mitochondrial ribosomal biogenesis *in vivo*[19]. What is clear, though, is that TFB2M interacts directly with promoter DNA near

the transcription start site during transcription initiation[17]. The structure of human mitochondrial RNA polymerase (POLRMT) has recently been solved and shows strong similarity to the pol I family of polymerases, in particular to T7 RNA polymerase[20]. The structure is without bound DNA and the domains of POLRMT predicted to be involved in promoter binding and melting of DNA are either disordered or have a different orientation in comparison to equivalent domains in T7 RNA polymerase. Interestingly, the POLRMT structure reveals an N-terminal pentatricopeptide repeat domain (PPR) of novel structure that appears to sequester a loop binding the promoter, which corresponds to the AT-rich recognition loop in T7 RNA polymerase. The sequestering of this AT-rich recognition loop of the PPR-domain of POLRMT may be dependent on the formation of the complete initiation complex with DNA, TFAM, and TFB2M. Alternatively, the sequestering of the AT-rich recognition loop may provide a mechanism whereby other factors bind the PPR domain to regulate mitochondrial transcription. A tentative model of the TFAM-POLRMT complex around the LSP promoter does not exclude that the C-terminal tail of TFAM interacts directly with POLRMT (Fig 2D).

In summary, the novel structures of TFAM offer exciting insights into the roles of this protein in the regulation of transcription and organization of mtDNA. As always in science, an important and novel result, such as the X-ray crystallographic structure of TFAM, opens up a vast array of follow-up questions. A future challenge involves understanding exactly how TFAM stimulates transcription initiation. The recently uncovered structure of POLRMT[20] paves the way to the structure of the complete basal mtDNA transcription machinery bound to LSP, which may answer this question. It is now clear that TFAM organizes mtDNA by introducing repeated

U-turns, thereby leading to DNA compaction. Is the binding of TFAM to mtDNA, in essence, unspecific, or are there preferential binding sites, besides LSP and HSP? If preferential interaction sites exist, what are the rules that govern the positioning of TFAM? Moreover, is the architectural role of TFAM related purely to local compaction, or is TFAM involved in the formation of higher-order structures similar to the chromatin in the nucleus? Is there a remodeling complex that removes TFAM to make mtDNA accessible for replication and repair? These and many more outstanding questions will likely be answered within the next few years.

Legends to figures

Figure 1.

TFAM is a dual function protein needed for the transcription and packaging of mtDNA. Schematic representation of a mammalian cell with the mitochondrial network containing multiple mitochondrial nucleoids (left). TFAM bends mtDNA at the promoter and is necessary for transcription initiation (upper right). Proposed model for TFAM in packaging mtDNA into nucleoids (lower right).

Figure 2. The structure of TFAM and relationship to other proteins involved in DNA compaction and transcription. Cartoon representation of: **A.** TFAM in complex with 28bp LSP DNA[15]. **B.** HU/IHF in complex with non-specific DNA[21]. **C.** Human TBP in complex with the TATA box DNA[22]. **D.** Tentative model of the interaction between TFAM and POLRMT at the LSP. TFAM with N-terminal HMG domain colored in green, linker in blue, C-terminal HMG domain in yellow and C-terminal tail in red. POLRMT with the PPR domain in blue, N-terminal domain in light grey, C-terminal domain dark grey and bound part of an N-terminal extension in pink.

References:

1 Fisher, R. P. & Clayton, D. A. A transcription factor required for promoter recognition by human mitochondrial RNA polymerase. Accurate initiation at the heavy- and light-strand promoters dissected and reconstituted in vitro. *J. Biol. Chem.* **260**, 11330-11338 (1985).

2 Fisher, R. P., Parisi, M. A. & Clayton, D. A. Flexible recognition of rapidly evolving promoter sequences by mitochondrial transcription factor 1. *Genes Dev.* **3**, 2202-2217 (1989).

3 Parisi, M. A. & Clayton, D. A. Similarity of human mitochondrial transcription factor 1 to high mobility group proteins. *Science* **252**, 965-969 (1991).

4 Fisher, R. P., Lisowsky, T., Parisi, M. A. & Clayton, D. A. DNA wrapping and bending by a mitochondrial high mobility group-like transcriptional activator protein. *J. Biol. Chem.* **267**, 3358-3367 (1992).

5 Dairaghi, D. J., Shadel, G. S. & Clayton, D. A. Addition of a 29 residue carboxyl-terminal tail converts a simple HMG box-containing protein into a transcriptional activator. *J. Mol. Biol.* **249**, 11-28 (1995).

6 Zelenaya-Troitskaya, O., Newman, S. M., Okamoto, K., Perlman, P. S. & Butow, R. A. Functions of the high mobility group protein, Abf2p, in mitochondrial DNA segregation, recombination and copy number in Saccharomyces cerevisiae. *Genetics* **148**, 1763-1776 (1998).

7 Matsunaga, M. & Jaehning, J. A. A mutation in the yeast mitochondrial core RNA polymerase, Rpo41, confers defects in both specificity factor interaction and promoter utilization. *J Biol Chem* **279**, 2012-2019 (2004).

8 Falkenberg, M. *et al.* Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. *Nat. Genet.* **31**, 289-294 (2002).

9 Larsson, N. G. *et al.* Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. *Nat. Genet.* **18**, 231-236 (1998).

10 Ekstrand, M. I. *et al.* Mitochondrial transcription factor A regulates mtDNA copy number in mammals. *Hum. Mol. Genet.* **13**, 935-944 (2004).

11 Garrido, N. *et al.* Composition and dynamics of human mitochondrial nucleoids. *Mol Biol Cell* **14**, 1583-1596 (2003).

12 Wang, Y. & Bogenhagen, D. F. Human mitochondrial DNA nucleoids are linked to protein folding machinery and metabolic enzymes at the mitochondrial inner membrane. *J Biol Chem* **281**, 25791-25802 (2006).

13 Kaufman, B. A. *et al.* The mitochondrial transcription factor TFAM coordinates the assembly of multiple DNA molecules into nucleoid-like structures. *Mol Biol Cell* **18**, 3225-3236 (2007).

14 Kukat, C. *et al.* Super-resolution microscopy reveals that mammalian mitochondrial nucleoids have a uniform size and frequently contain a single copy of mtDNA. *Proc Natl Acad Sci U S A* **108**, 13534-13539, (2011).

15 Ngo, H. B., Kaiser, J. T. & Chan, D. C. Tfam, a mitochondrial transcription and packaging factor, imposes a U-turn on mitochondrial DNA. *Nat. Struct. Mol. Biol.* **18**, 1290-1296 (2011).

16 Rubio-Cosials, A. *et al.* Human mitochondrial transcription factor A induces a U-turn structure in the light strand promoter. *Nat. Struct. Mol. Biol.* **18**, 1281-1289 (2011).

17 Sologub, M., Litonin, D., Anikin, M., Mustaev, A. & Temiakov, D. TFB2 is a transient component of the catalytic site of the human mitochondrial RNA polymerase. *Cell* **139**, 934-944 (2009).
18 McCulloch, V. & Shadel, G. S. Human mitochondrial transcription factor B1 interacts with the C-terminal activation region of h-mtTFA and stimulates transcription independently of its RNA methyltransferase activity. *Mol Cell Biol* **23**, 5816-5824 (2003).
19 Metodiev, M. D. *et al.* Methylation of 12S rRNA is necessary for in vivo stability of the small subunit of the mammalian mitochondrial ribosome. *Cell Metab* **9**, 386-397 (2009).
20 Ringel, R. *et al.* Structure of human mitochondrial RNA polymerase. *Nature* (2011).
21 Mouw, K. W. & Rice, P. A. Shaping the Borrelia burgdorferi genome: crystal structure and binding properties of the DNA-bending protein Hbb. *Mol Microbiol* **63**, 1319-1330 (2007).
22 Nikolov, D. B. *et al.* Crystal structure of a human TATA box-binding protein/TATA element complex. *Proc Natl Acad Sci U S A* **93**, 4862-4867 (1996).

Figure 1:

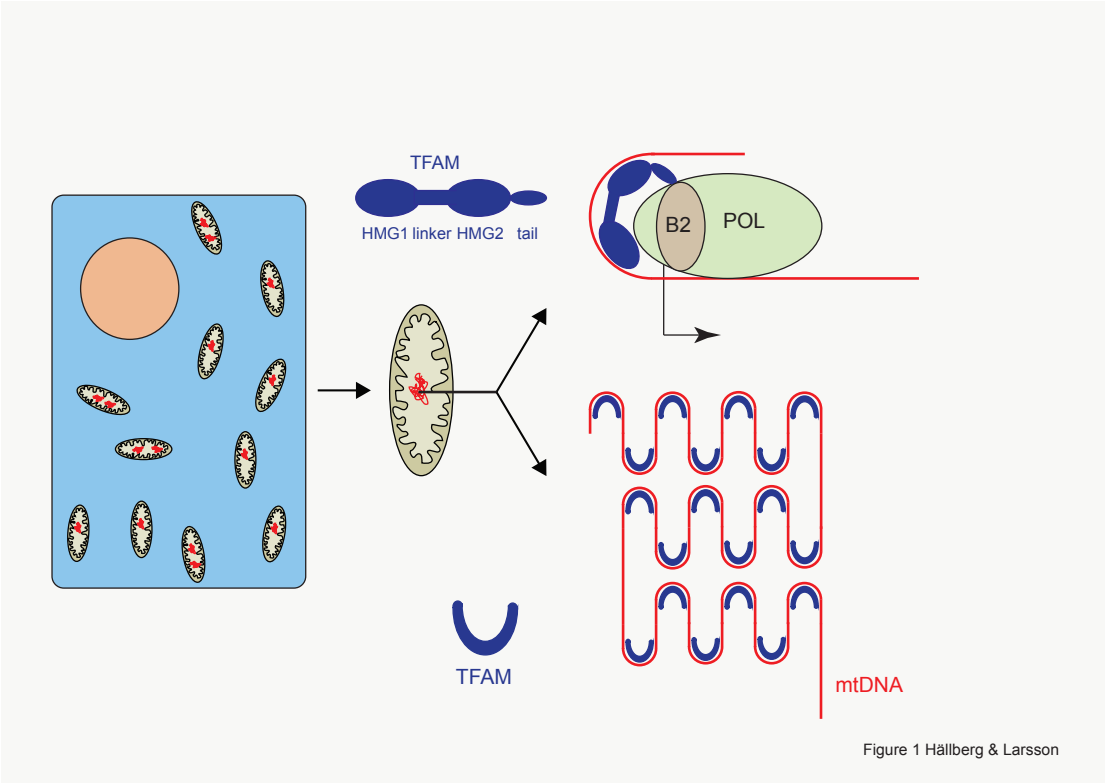

Figure 2: