



**Karolinska  
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**Institutionen för Laboratoriemedicine**

# Linking Transcription to DNA Replication in Mammalian Mitochondria

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## ABSTRACT

Most of the adenosine phosphate (ATP) used to drive cellular processes is produced through oxidative phosphorylation (OXPHOS). At the heart of this process is the electron transport chain, which contains a number of protein complexes located in the inner membrane of mitochondria. In addition to about 90 nuclear encoded gene products, the electron transport chain also contains 13 subunits encoded by mitochondrial DNA (mtDNA). This double-stranded, circular DNA genome is therefore essential for proper OXPHOS function; mutations, deletion, or depletion of mtDNA can cause mitochondrial dysfunction and human disease.

The mtDNA molecule is replicated and transcribed by molecular machineries that are distinct from those present in the nucleus. Many of the components are related to proteins identified in bacteria and bacteriophages, in keeping with the prokaryotic origin of the mitochondrion. The transcription machinery in mammalian mitochondria contains three essential factors: mitochondrial RNA polymerase (POLRMT), mitochondrial transcription factor A (TFAM), and mitochondrial transcription factor B (TFB2M). Recently, the role of TFAM as a core initiation factor has been questioned. In this thesis, we demonstrate that both TFAM and TFB2M are required for mitochondrial transcription. We also demonstrate that TFB1M, a TFB2M paralog, does not function as a bona fide transcription factor. Even if TFAM is an essential factor for transcription initiation *in vitro*, the absolute requirement of TFAM can be circumvented by experimental conditions that stimulate DNA breathing at the promoter. We suggest a possible explanation for this effect, by demonstrating that TFAM can introduce negative supercoils and cause conformational changes in mtDNA that facilitates promoter recognition and transcription initiation by POLRMT and TFB2M.

Our studies also demonstrate that a substantial fraction of the transcription events initiated from the light strand promoter (LSP) are prematurely terminated at a conserved sequence element, CSBII. The 3'-ends of these transcripts correspond to prominent RNA-DNA transition sites mapped *in vivo*, strongly suggesting that they are used to prime mitochondrial DNA synthesis.

The mitochondrial transcription termination factor (MTERF1) was originally identified as a regulator of rRNA transcription. MTERF1 binds to a 28-bp mtDNA sequence downstream of the 16S rRNA gene. MTERF1 supposedly terminates transcription at this location to produce more rRNAs than the mRNAs species encoded by down stream sequences on the same strand. In this thesis, we explore an alternative role for MTERF1 as a regulator of mtDNA replication. We demonstrate that binding of MTERF1 causes a pause in the progression of the mitochondrial replication fork in one direction, but not in the other. The effect is explained by MTERF1's ability to counteract the DNA unwinding activity of the mitochondrial DNA helicase, TWINKLE. Based on our findings, we speculate that MTERF1 can coordinate synthesis of the two mtDNA strands.

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