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# **Structural-functional studies of mitochondrial matrix proteins**

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## **List of scientific papers:**

- I. The amino terminal extension of mammalian mitochondrial RNA polymerase ensures promoter specific transcription initiation.  
Posse V, Hoberg E, Dierckx A, **Shahzad S**, Koolmeister C, Larsson NG, Wilhelmsson LM, Hällberg BM and Gustafsson C;  
Nucleic Acids Research, 2014, 42; 3638-3647.
- II. TEFM is a potent stimulator of mitochondrial transcription elongation *in- vitro*.  
Posse V, **Shahzad S**, Falkenberg M, Hällberg BM and Gustafsson CM.  
Nucleic Acids Research, 2015, 43:2615-24.
- III. Structure and degradation mechanism of the Human Mitochondrial Lon protease  
**Shahzad S**, Hernandez CP, Falkenberg M and Hällberg BM  
*Manuscript*.

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# Structural-functional studies of mitochondrial matrix proteins

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## Abstract

The mitochondrion is the powerhouse of the eukaryotic cell. Most of the energy required to carry out cellular processes is generated inside mitochondria via the process of oxidative phosphorylation. The machinery required for oxidative phosphorylation is encoded by both the nuclear and the mitochondrial genome. The cellular energy production will thus collapse in the absence of mitochondrial gene expression. The mitochondrial RNA polymerase, POLRMT, together with two transcription factors, TFAM and TFB2m, initiate mitochondrial transcription. However, the exact mechanistic details of mitochondrial-transcription initiation are unclear. Furthermore, the transcription by POLRMT is non-processive, and it prematurely terminates after 150 nucleotides in a conserved sequence block region, CSBII. This indicates that accessory factors are required for a complete transcription event.

In Paper I, we investigated the transcription initiation in mitochondria and proposed a model. In addition, we were able to demonstrate that an N-terminal extension (NTE) in POLRMT plays a role in the transition from the initiation to the elongation phase, possibly by undergoing a conformational change. Upon the deletion of NTE, the POLRMT is hyperactive, and together with TFB2m, it can carry out non-specific transcription events. Thus, we conclude that the NTE is necessary for promoter-specific transcription initiation. Once the transcription is initiated, the POLRMT enters the elongation phase.

In Paper II, we were able to characterize the *in-vitro* role of a transcription-elongation factor, TEFM. TEFM increases the processivity of the POLRMT by allowing it to bypass road blocks, such as the CSBII, and DNA lesions, such as apurinic or apyrimidinic sites. We furthermore suggested that TEFM may be involved in the regulation of mitochondrial transcription and replication in mammalian mitochondria.

For mitochondrial homeostasis, the integrity of proteins in the mitochondrial matrix must be maintained, and this is achieved through the mitochondrial protein quality control (PQC) system. Lon is the major mitochondrial matrix protease and is essential for mitochondrial PQC. Lon belongs to the AAA<sup>+</sup>-protease family and is a homo-hexamer that uses energy from ATP hydrolysis to recognize, bind, and translocate its substrate into a proteolytic chamber.

In Paper III, we presented the structure of a full-length human mitochondrial Lon determined by single-particle cryo-EM to a resolution of 3.6 Å. We showed that the human Lon, in its ADP-bound form, has its six protomers arranged in an open helical conformation with an 8 Å translational shift. The highly flexible N-terminal domains of every first and fourth protomer dimerizes, thereby giving rise to a unique arrangement that strengthens the oligomerization. At the same time, the arrangement provides new structural motifs for inter-protomer communication. Based on our analysis, we propose a hand-over-hand model, using three protomers, for substrate translocation by Lon, and our results can be generalized to the broad family of LonA AAA<sup>+</sup> proteases.

**Keywords:** Mitochondria, mtDNA, transcription, protein quality control, Lon, AAA<sup>+</sup>, cryoEM

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