

Molecular insights into mitochondrial transcription and its role in DNA replication

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet, kommer att offentlig försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, fredagen den 13 januari 2017, klockan 09.00

av Viktor Posse

Fakultetsopponent:
Professor Gerhart Wagner
Uppsala universitet, Sverige

Avhandlingen baseras på följande delarbeten:

- I. **Posse V**, Hoberg E, Dierckx A, Shahzad S, Koolmeister C, Larsson NG, Wilhelmsson LM, Hällberg BM and Gustafsson CM. The amino terminal extension of mammalian mitochondrial RNA polymerase ensures promoter specific transcription initiation. *Nucleic Acids Res.* 2014. 42(6): 3638-47
- II. **Posse V** and Gustafsson CM. Human mitochondrial transcription factor B2 is required for promoter melting during initiation of transcription. *Manuscript*
- III. **Posse V**, Shahzad S, Falkenberg M, Hällberg BM and Gustafsson CM. TEFM is a potent stimulator of mitochondrial transcription elongation in vitro. *Nucleic Acids Res.* 2015. 43(5): 2615-24
- IV. **Posse V**, Al-Behadili A, Uhler JP, Falkenberg M and Gustafsson CM. The molecular mechanism of DNA replication initiation in human mitochondria. *Manuscript*

**SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR BIOMEDICIN**



Molecular insights into mitochondrial transcription and its role in DNA replication

Viktor Posse

Avdelningen för medicinsk kemi och cellbiologi, institutionen för biomedicin, Sahlgrenska akademien, Göteborgs universitet, Sverige.

Abstract

The mitochondrion is an organelle of the eukaryotic cell responsible for the production of most of the cellular energy-carrying molecule adenosine triphosphate (ATP), through the process of oxidative phosphorylation. The mitochondrion contains its own genome, a small circular DNA molecule (mtDNA), encoding essential subunits of the oxidative phosphorylation system. Initiation of mitochondrial transcription involves three proteins, the mitochondrial RNA polymerase, POLRMT, and its two transcription factors, TFAM and TFB2M. Even though the process of transcription has been reconstituted *in vitro*, a full molecular understanding is still missing. Initiation of mitochondrial DNA replication is believed to be primed by transcription prematurely terminated at a sequence known as CSBII. The mechanisms of replication initiation have however not been fully defined. In this thesis we have studied transcription and replication of mtDNA.

In the first part of this thesis we demonstrate that the transcription initiation machinery is recruited in discrete steps. Furthermore, we find that a large domain of POLRMT known as the N-terminal extension is dispensable for transcription initiation, and instead functions in suppressing initiation events from non-promoter DNA. Additionally we demonstrate that TFB2M is the last factor that is recruited to the initiation complex and that it induces melting of the mitochondrial promoters.

In this thesis we also demonstrate that POLRMT is a non-processive polymerase that needs the presence of the elongation factor TEFM for processive transcription. TEFM increases the affinity of POLRMT for an elongation-like RNA-DNA template and decreases the probability of premature transcription termination. Our data also suggest that TEFM might be of importance for mitochondrial replication initiation, since it affects termination at CSBII.

In the last part of this thesis we study the RNA-DNA hybrids (R-loops) that can be formed by the CSBII terminated transcript. We characterize these R-loops and demonstrate that they can be processed by RNaseH1 to form replicative primers that can be used by the mitochondrial replication machinery.

Keywords: Mitochondrion, mtDNA, transcription, DNA replication