Molecular insights into mitochondrial transcription and its role in DNA replication

Akademisk avhandling

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SAHLGRENSKA AKADEMIN
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Molecular insights into mitochondrial transcription and its role in DNA replication

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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