Initiation of mammalian mitochondrial DNA replication

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska Akademin vid
Göteborgs universitet kommer att offentligen försvaras i
Hörsal Ivan Östholm, Medicinaregatan 13, Göteborg,
fredagen den 25 april 2014 kl. 9.00

av

Elisabeth Jemt

Fakultetsopponent:
Professor Aleksandra Trifunovic
Institute of Genetics, Cologne University, Cologne, Germany

This thesis is based on the following studies:

I. Mitochondrial RNA polymerase is needed for activation of the origin of light-strand DNA replication.
Fusté JM, Wanrooij S, Jemt E, Granycome CE, Cluett TJ, Shi Y, Atanassova N, Holt IJ, Gustafsson CM, Falkenberg M.
Mol Cell. 2010 Jan 15; 37(1): 67-78

II. The mitochondrial DNA helicase TWINKLE can assemble on a closed circular template and support initiation of DNA synthesis.
Jemt E, Farge G, Bäckström S, Holmlund T, Gustafsson CM, Falkenberg M.
Nucleic Acids Res. 2011 Nov; 39(21): 9238-49.

III. MTERF1 binds mtDNA to prevent transcriptional interference at the light-strand promoter but is dispensable for rRNA gene transcription regulation.
Terzioglu M, Ruzzenente B, Harmel J, Mourier A, Jemt E, López MD, Kukat C, Stewart JB, Wibom R, Meharg C, Habermann B, Falkenberg M, Gustafsson CM, Park CB, Larsson NG.
Cell Metab. 2013 Apr 2; 17(4): 618-26

IV. A conserved sequence element is involved in termination of mitochondrial DNA replication and transcription.
Jemt E, Persson Ö, Mehmedovic M, López M, Shi Y, Freyer C, Samuelsson T, Falkenberg M
Manuscript

Initiation of mammalian mitochondrial DNA replication

Elisabeth Jemt

Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, Göteborg, Sweden

ABSTRACT

Mitochondria produce most of the adenosine triphosphate required in a eukaryotic cell and they contain their own genome. The mitochondrial DNA (mtDNA) is a double stranded circular molecule that codes for proteins required for cellular respiration and RNA molecules involved in translation of these proteins. Replication of the mtDNA is therefore essential for cell viability and the aim of this thesis has been to understand the molecular mechanisms of mtDNA replication.

In general, initiation of DNA replication involves a series of steps including recognition of an origin of replication, loading of replicative helicases, and synthesis of an RNA primer that can be used by DNA polymerases to initiate DNA synthesis. We have studied this process in mammalian mitochondria and demonstrate that the mitochondrial RNA polymerase (POLRMT) synthesizes the RNA primer required for initiation of lagging strand replication at the origin of light strand (O_L). We have reconstituted, and in detail characterized, O_L-dependent initiation of lagging strand replication *in vitro* using purified POLRMT and core factors of the mitochondrial replisome.

We have also addressed how the TWINKLE helicase is loaded during initiation of leading strand replication. TWINKLE is a ring-shaped helicase and must be opened up to accommodate DNA in its central channel. Many helicases require specialized loading factor to assemble onto DNA, but we find that TWINKLE can function without such a factor. In the presence of the other components of the mitochondrial replisome, we show that TWINKLE can assemble on a DNA template resembling the mtDNA *in vivo* and support primer dependent initiation of DNA synthesis.

Most mtDNA replication initiation events are prematurely terminated and do not result in duplication of the entire mtDNA molecule. We address the mechanisms responsible for this termination event and identify a highly conserved sequence with palindromic character located immediately downstream of the premature mtDNA replication termination site. Interestingly, transcription initiated at the heavy strand promoter (HSP) is also terminated at this region, suggesting that the termination sequence functions in a bidirectional manner. Based on the results of *in vitro* biochemistry and cell culture experiments, we propose that a *trans*-acting factor binds to the palindromic sequence and simultaneously directs termination of both mtDNA transcription and replication.

MTERF1 binds specifically to an mtDNA sequence just downstream of the ribosomal RNA transcription unit. The function of MTERF1 has been debated and to elucidate its functional role *in vivo*, we here characterize an Mterf1 knock-out mouse model. We find that MTERF1 is non-essential and that the protein acts to prevent the transcription machinery from interfering with the downstream light strand promoter (LSP), an incidence that may disturb expression of coding genes, but also the formation of primers required for initiation of mtDNA replication.

Keywords: mitochondria, mtDNA, DNA replication

ISBN: 978-91-628-8980 -7

http://hdl.handle.net/2077/34846