Göteborg, 2021

Molecular insights into primer removal during mtDNA replication

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i sal 2119, Hus 2, hälsovetarbacken, Arvid Wallgrens backe, Göteborg

Onsdagen den 24 mars 2021, klockan 13:00

Av **Ali Al-Behadili**

Fakultetsopponent:

Professor Stefan Björklund

Umeå Universitet, Sverige

Avhandlingen baseras på följande delarbeten

I. RNase H1 directs origin-specific initiation of DNA replication in human mitochondria.

Posse, V., **A. Al-Behadili**, J. P. Uhler, A. R. Clausen, A. Reyes, M. Zeviani, M. Falkenberg ,C. M. Gustafsson. *PLOS Genetics*, 2019; 15(1):e1007781.

II. A two-nuclease pathway involving RNase H1 is required for primer removal at human mitochondrial OriL.

Al-Behadili, A., J.P. Uhler, A.-K. Berglund, B. Peter, M. Doimo, A. Reyes, S. Wanrooij, M. Zeviani, M. Falkenberg. *Nucleic acids research*, 2018;46(18):9471-83.

III. *In vitro* characterization of EXOG as a component of a mitochondrial oligonucleotide degradation pathway.

Al-Behadili, A., D. Erdinc, J.P. Uhler, I. Atanassov, T. J. Nicholls, M. Falkenberg. *Manuscript*, 2021.

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR BIOMEDICIN

GÖTEBORGS UNIVERSITET

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ABSTRACT

Mitochondria are vital for cell survival, and the primary producers of ATP, the energy currency used for various metabolic processes. Mitochondria are unique from other cellular compartments because they have their own genomes of circular small double-stranded DNA (mtDNA) of approximately 16.6 kbp in size. The mtDNA is highly compact, containing no introns and little non-coding DNA. MtDNA has two non-coding regions: one large region known as the control region or the non-coding region that contains the promoters for transcription of (LSP and HSP) and the origin of replication of the H strand (OriH), and a smaller region containing the origin of replication for the L-strand (OriL). MtDNA is replicated by a set of replication factors distinct from those needed for DNA replication in the nucleus. A fundamental step in mtDNA replication is the processing of the RNA primers needed for replication initiation.

In this thesis, we could demonstrate that Ribonuclease H1 (RNase H1) is essential for the process of replication initiation at OriH. We could also elucidate the role of RNase H1 during primer removal and ligation at the mitochondrial origin of light-strand DNA synthesis (OriL) and explain the pathogenic consequences of disease-causing mutations in RNase H1.These findings have taken the field of mitochondrial DNA transcription and replication forward and generated knowledge to build further research.

In the last project, we studied EXOG, a mitochondrial exonuclease. We demonstrated that EXOG could interplay with RNase H1 and other mitochondrial nucleases *in vitro* and identified a possible pathway for EXOG to function in.

Keywords: mtDNA, RNA primer, RNase H1

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