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# Pathogenic mechanisms affecting mitochondrial DNA replication and transcription

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer att offentligen försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, torsdag den 25 Maj 2023, klockan 13.00

av **Meenakshi Singh**

Fakultetsopponent:
Professor Sjoerd Wanrooij
Umeå Universitet, Sverige

**Avhandlingen baseras på följande delarbeten**

I. Ribonucleotides embedded in template DNA impair mitochondrial RNA polymerase progression. **Meenakshi Singh**, Viktor Posse, Bradley Peter, Maria Falkenberg and Claes M. Gustafsson. *Nucleic Acids Research*, 2022, Vol. 50, No. 2, 989–999. https://doi.org/10.1093/nar/gkab1251

II. POLRMT mutations impair mitochondrial transcription causing neurological disease. Monika Oláhová, Bradley Peter, Zsolt Szilagyi, Hector Diaz-Maldonado, **Meenakshi Singh**, Ewen W. Sommerville, Emma L. Blakely, Jack J. Collier, Emily Hoberg, Viktor Stránecký, Hana Hartmannová, Anthony J. Bleyer, Kim L. McBride, Sasigarn A. Bowden, Zuzana Korandová, Alena Pecinová, Hans-Hilger Ropers, Kimia Kahrizi, Hossein Najmabadi, Mark A. Tarnopolsky, Lauren I. Brady, K. Nicole Weaver, Carlos E. Prada, Katrin Õunap, Monica H. Wojcik, Sander Pajusalu, Safoora B. Syeda, Lynn Pais, Elicia A. Estrella, Christine C. Bruels, Louis M. Kunkel, Peter B. Kang, Penelope E. Bonnen, Tomáš Mráček, Stanislav Kmoch, Gráinne S. Gorman, Maria Falkenberg, Claes M. Gustafsson and Robert W. Taylor. *Nature Communications*, 2021, Vol. 12, No. 1, 1135. https://doi.org/10.1038/s41467-021-21279-0

III. 5-Fluorouracil and 6-Thioguanine impair mitochondrial DNA replication. **Meenakshi Singh,** Louise Jenninger, Emily Hoberg, Laleh Arabinian, Xuefeng Zhu, Claes M. Gustafsson and Maria Falkenberg. *Manuscript to be submitted.*

**SAHLGRENSKA AKADEMIN**
**INSTITUTIONEN FÖR BIOMEDICIN**

GÖTEBORGS UNIVERSITET

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# Pathogenic mechanisms affecting mitochondrial DNA replication and transcription

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**Abstract**

Mitochondria are cytoplasmic organelles fundamental to life and health. In mitochondria, energy from the food we eat are converted into adenosine triphosphate (ATP), which in turn is used as a source of chemical energy to drive a multitude of cellular reactions. Owing to its endosymbiotic origin, the mitochondrion contains its own genetic material, a circular double stranded DNA molecule (mtDNA) of about 16.6 kb. The mitochondrial genome contains 37 genes, which codes for 13 protein components of the oxidative phosphorylation system, 2 ribosomal RNAs that are required for mitochondrial ribosome biogenesis, and a set of 22 transfer RNAs.

The enzymatic systems needed to replicate and transcribe mtDNA are distinct from those present in the nucleus. The objective of this thesis is to characterize pathogenic mechanisms affecting mtDNA replication and transcription in human cells. In collaboration with clinical colleagues, we report that mutations in the gene coding for mitochondrial RNA polymerase (POLRMT) can cause mitochondrial dysfunction and neurological disease. Using *in vitro* biochemistry and cell biology approaches, we find that the identified mutations cause deleterious effects on mitochondrial transcription, which in turn impair biogenesis of the oxidative phosphorylation system.

Mammalian mitochondria lack systems for ribonucleotide excision repair and mtDNA therefore contains relatively high levels of embedded ribonucleotides, which can be even higher in patients suffering from genetic disorders associated with imbalanced nucleotide pools. We demonstrate that embedded ribonucleotides can cause problems for the transcribing POLRMT, causing premature termination of mitochondrial transcription. We suggest that these effects can contribute to the phenotypes associated with nucleotide pool disorders.

Others have demonstrated that nucleoside analogues used to treat retroviral infections can cause a progressive accumulation of somatic mtDNA mutations. We investigated effects of two nucleoside analogues commonly used to treat childhood cancer, 5-Fluorouracil (5-FU) and 6-Thioguanine (6-TG). Using a reconstituted mtDNA replication system, with highly purified components, we demonstrate that both 5-FU and 6-TG impair the activity of mitochondrial DNA polymerase γ *in vitro*. We also find that these compounds can cause mtDNA replication stalling in cells. Taken together, our data suggest that 5-FU and 6-TG have the potential to cause mutations, but future studies of mtDNA isolated from patients treated with these compounds are required to validate this idea.

**Keywords:** mtDNA, ribonucleotides, nucleoside analogues, POLRMT

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