Göteborg, 2021

Ribonucleotides in DNA

- application in genome-wide DNA polymerase tracking and physiological role in eukaryotes

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,

Fredagen den 11 juni 2021, klockan 13:00

av **Katrin Kreisel**

Fakultetsopponent: **Professor Grant W. Brown**

University of Toronto, Canada

Avhandlingen baseras på följande delarbeten

I. **Kreisel, K**, Engqvist, MKM, Kalm, J, Thompson, LJ, Boström, M, Navarrete, C, McDonald, JP, Larsson, E, Woodgate, R, Clausen, AR. DNA polymerase η contributes to genome-wide lagging strand synthesis. *Nucleic Acids Research*, 2019; 47(5): 2425-2435

II. **Kreisel, K**, Engqvist, MKM, Clausen, AR. Simultaneous mapping and quantitation of ribonucleotides in human mitochondrial DNA. *Journal of Visualized Experiments* 2017; 129: e56551

III. Wanrooij, PH, Tran, P, Thompson, LJ, Carvalho, G, Sharma, S, **Kreisel, K**, Navarrete, C, Feldberg, A, Watt, DL, Nilsson AK, Engqvist, MKM, Clausen, AR, Chabes, A. Elimination of rNMPs from mitochondrial DNA has no effect on its stability. *Proceedings of the National Academy of Sciences of the United States of America* 2020; 117(25): 14306-14313

IV. **Kreisel, K**, Kalm, J, Bandaru, S, Ala, C, Akyürek, L, Clausen, AR. Stably incorporated ribonucleotides in murine tissues: quantitation, base identity and distribution in nuclear and mitochondrial DNA. *(to be submitted)*

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR BIOMEDICIN

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Ribonucleotides in DNA - application in genome-wide DNA polymerase tracking and physiological role in eukaryotes

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Abstract

The genetic code in the eukaryotic cell is stored in DNA, which is more stable than RNA. Replication fidelity and DNA repair mechanisms are in place to preserve the information encoded. Despite DNA polymerases' discrimination against ribonucleotides, they are frequently incorporated into DNA and even in the presence of efficient ribonucleotide removal pathways, ribonucleotides may remain stably incorporated in the DNA. Ribonucleotides can be used as a marker of DNA replication enzymology by using HydEn-seq, a next-generation sequencing technique for the genome-wide mapping of ribonucleotides. I aimed to elucidate the activities of the translesion synthesis DNA polymerase η in yeast. By using a steric gate variant that incorporates more ribonucleotides and by tracking those ribonucleotides, I determined a lagging strand preference dependent on its C-terminus in Paper I. Moreover, I was interested in the physiological role of incorporated ribonucleotides and used an extension of the HydEn-seq method outlined in Paper II, to map and quantitate ribonucleotides simultaneously. By investigating ribonucleotide incorporation into mouse mitochondrial DNA (mtDNA) in Paper III, we found that ribonucleotides are acquired mostly up until adulthood and are not connected to age-related mtDNA instability. To gain a more comprehensive view on incorporated ribonucleotides in the DNA of mammals, I mapped and quantitated incorporated ribonucleotides in nuclear DNA (nDNA) and mtDNA from murine blood, bone marrow, brain, heart, kidney, liver, lung, muscle and spleen in Paper IV. I found tissue-dependent variations in the number and the identity of incorporated ribonucleotides and marked differences between nDNA and mtDNA. The ribonucleotide distribution in both types of DNA was non-random and in nDNA affected by the proximity of genomic features, which in most cases increased the number of embedded ribonucleotides locally.

The thesis extends the knowledge of DNA polymerase η's activity and the physiological role that incorporated ribonucleotides play in DNA. This more detailed characterization of the incorporated ribonucleotides genome-wide is a basic requirement for the understanding of diseases associated with genome instability, such as certain types of cancers or Aicardi-Goutières syndrome.

Keywords: Ribonucleotides, DNA instability, DNA polymerase eta, nuclear DNA, mitochondrial DNA

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