Molecular and structural studies of proteins required for mitochondrial DNA maintenance

Akademisk avhandling

Som för avläggande av medicine doktorexamen vid Sahlgrenska Akademin, Göteborgs Universitet, kommer att offentligen försvaras i hörsal Gösta Sandels, Medicinaregatan 9, Göteborg

Fredagen den 20 Mai 2022, kl 13:00

Av

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Stony Brook University, New York, USA

Avhandlingen baseras på följande arbeten:

I Bradley Peter, Geraldine Farge, **Carlos Pardo-Hernández**, Stefan Tångefjord and Maria Falkenberg. Structural basis for adPEO-causing mutations in the mitochondrial TWINKLE helicase. *Human Molecular Genetics*, 2019, Vol.28, No.7 1090-1099.

II Pedro Silva-Pinheiro†, **Carlos Pardo-Hernández†**, Aurelio Reyes, Lisa Tilokani, Anup Mishra, Raffaele Cerutti, Shuaifeng Li, Dieu-Hien Rozsivalova, Sebastian Valenzuela, Sukru A. Dogan, Bradley Peter, Patricio Fernández-Silva, Aleksandra Trifunovic, Julien Prudent, Michal Minczuk, Laurence Bindoff, Bertil Macao, Massimo Zeviani, Maria Falkenberg† and Carlo Viscomi†. **DNA polymerase gamma mutations that impair holoenzyme stability cause catalytic subunit depletion.** *Nucleic Acids Research*, 2021, Vol.49, No.9 5230-5248.

III Genís Valentín Gesé†, Saba Shahzad†, **Carlos Pardo-Hernández†**, Anna Wramstedt, Maria Falkenberg and B. Martin Hällberg. A dual allosteric pathway drives human mitochondrial Lon. *Manuscript submitted to eLife,* 2022.

IV **Carlos Pardo-Hernández†**, Mariella T. Simon†, Shaya S. Eftekharian, Alexander Stover, Daniel D. Nguyen, Bradley Peter, Wei-Lin Huang, Lauren O'Grady, Inderneel Sahai, Raymond Wang, Susanne M. Rafelski, Claes Gustafsson, Maria Falkenberg† and Jose E. Abdenur†. Novel mutations in the substrate binding domain of the mitochondrial matrix protease LONP1 are a cause of mitochondrial disease. *Manuscript,* 2022.

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ABSTRACT

Mitochondria are membrane-bound organelles that produce the majority of ATP used to drive metabolic processes in eukaryotic cells. A unique feature of mitochondria is the existence of a separate mitochondrial DNA (mtDNA), which codes for thirteen proteins needed for oxidative phosphorylation (OXPHOS). The double-stranded mtDNA is copied by a dedicated enzymatic machinery, which includes mitochondrial DNA polymerase γ (POLγ) and a set of accessory proteins. Mitochondria also harbor transcription and translation machineries, which are distinct from those in the nucleus. Mutations that affect factors required for mtDNA maintenance or expression can cause mitochondrial disease.

In the present thesis, we have characterised a subset of proteins which play an important role in replication and transcription of mtDNA, both from a structural and a functional point of view. We have studied mitochondrial helicase TWINKLE and analysed the molecular consequences of disease-causing mutations in this protein. By means of electron microscopy, we have correlated structural changes caused by these mutations, with functional consequences on the protein activity. Our findings demonstrate that mutations impair TWINKLE's ability to undergo large-scale conformational changes required for proper function. We have also studied one of the most prevalent mitochondrial disease-causing mutations affecting POLγ, A467T. Using a combination of biochemical characterization and in vivo studies using a murine model, we demonstrate that the mutation impairs interactions between the catalytic and accessory subunits of POLγ. As a result, POLγ dissociates and the catalytic subunit is degraded by LONP1, a protein involved in maintaining mitochondrial matrix protein homeostasis. Details of LONP1 function is further characterised in the two other studies. In these works, we use cryo electron microscopy (cryo-EM) to determine the structure of full-length, human mitochondrial LONP1. In combination with a range of biochemical experiments we use this structural information to establish the mechanisms by which LONP1 can degrade mitochondrial proteins. We also analyse the functional consequences of two LONP1 mutations causing human disease. In vitro analysis demonstrated that the mutations impaired LONP1's ability to bind and degrade substrates in an ATP-dependent manner.

In summary, our work has provided new structural and functional information about POLγ, TWINKLE, and LONP1 – three key factors required for proper mitochondrial function. Our work has also characterised disease-causing mutations affecting these proteins and established the molecular consequences of these alterations.

Keywords: Mitochondria, mtDNA, LONP1, POLγ, mtDNA maintenance, mitochondrial disease, TWINKLE

ISBN 978-91-8009-671-3 (PRINT) ISBN 978-91-8009-672-0 (PDF)
http://hdl.handle.net/2077/70924