

# Structural studies of mitochondrial DNA polymerase $\gamma$

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet kommer att offentlig försvaras i hörsal Arvid Carlsson, Medicinargatan 3, Göteborg, onsdagen den 17 december 2025, klockan 9.00

av Sebastian Valenzuela

Fakultetsopponent:

Professor Hauke S. Hillen

University Medical Center Göttingen, Tyskland

## Avhandlingen baseras på följande delarbeten

- I. **Valenzuela S**, Zhu X, Macao B, Stamgren M, *et al.* (additional 24 authors), Giroux S, Gustafsson CM, Falkenberg M. Small molecules restore mutant mitochondrial DNA polymerase activity. *Nature*. 2025;642(8067):501-7.
- II. Corrà S\*, Zuppardo A\*, **Valenzuela S\***, Jenninger L\*, *et al.* (additional 14 authors), Zhu X, Falkenberg M, Viscomi C. Modelling *POLG* mutations in mice unravels a critical role of POL $\gamma$ B in regulating phenotypic severity. *Nature Communications*. 2025;16(1):4782.
- III. **Valenzuela S\***, Hoberg E\*, Sillamaa S, Stamgren M, Pardo-Hernández C, Jenninger L, Macao B, Zhu X, Săcultanu M, Miralles Fusté J, Keating TA, Giroux S, Gustafsson CM, Falkenberg M. Structural basis for allosteric activation of human POL $\gamma$  via polymerase-state stabilization. *Manuscript*.

\*Contributed equally.

# Structural studies of mitochondrial DNA polymerase $\gamma$

Sebastian Valenzuela

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## Abstract

Mitochondria are essential eukaryotic organelles that generate most of the cell's adenosine triphosphate (ATP), the energy currency used to power cellular activities. Because mitochondria are descendants of once free-living bacteria that formed an endosymbiotic relationship with an archaeal host cell, mitochondria contain a small but well-preserved genome. Mitochondrial DNA (mtDNA) encodes 13 proteins that are crucial for ATP production, and proper maintenance of this genome is therefore essential for the cell. mtDNA replication is carried out by DNA polymerase  $\gamma$  (POL $\gamma$ ), a heterotrimeric complex composed of the catalytic subunit POL $\gamma$ A (*POLG*) and the accessory dimer POL $\gamma$ B (*POLG2*). Pathogenic *POLG* variants are among the most common causes of inherited mitochondrial disease, yet the underlying mechanisms remain poorly defined, and no effective therapies exist. This thesis integrates biochemical analysis, cryogenic electron microscopy (cryo-EM), cell assays, and mouse models to expand the mechanistic understanding of POL $\gamma$  function and dysfunction.

In Paper I, we identified small molecules that can restore polymerization activity in mutant POL $\gamma$  complexes, both *in vitro* and in patient-derived fibroblasts. Our findings position these compounds as potential therapeutic candidates for *POLG*-related disease. In Paper II, we generated and characterized mouse models to study common disease-causing *POLG* variants. *In vitro*, mouse Poly displays greater catalytic efficiency than the human enzyme, which results in milder phenotypes in mice. This observation is in part due to a more potent mouse accessory subunit, and our findings establish POL $\gamma$ B as a critical determinant of phenotypic severity in *POLG* mouse models. In Paper III, we combined cryo-EM and biochemical assays to elucidate how the small-molecule modulators identified in Paper I allosterically activate POL $\gamma$  by stabilizing it in the polymerase state.

Collectively, these studies provide important mechanistic insight into POL $\gamma$  function and dysfunction, establish characterized mouse models, and lay the foundation for developing targeted therapies to treat mitochondrial disorders caused by *POLG* mutations.

**Keywords:** POL $\gamma$ , mitochondria, mtDNA, DNA replication

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