

## The Accessory Subunit of Mammalian DNA Polymerase $\gamma$ Is a Functional Homodimer

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**Introduction:** Mitochondrial DNA is replicated and repaired by a nuclear-encoded DNA polymerase, Poly $\gamma$ , distinct from the polymerases that replicate and repair nuclear DNA. Poly $\gamma$  is composed of two subunits, a catalytic subunit of 125-140 kDa and an accessory subunit of 35-51 kDa. The small subunit, Poly $\gamma$ B, has been characterized as a processivity factor for the polymerase [1-4]. Upon interaction with the catalytic subunit, Poly $\gamma$ B increases the affinity of the polymerase for DNA and promotes tighter nucleotide binding, increasing the polymerization rate.

**Methods and Materials:** The crystal structure was solved at a resolution of 1.95 Å using multiple anomalous diffraction (MAD) of a crystal of selenomethionine Poly $\gamma$ B in combination with data of a native crystal to 2.25 Å resolution and non-crystallographic symmetry averaging. Crystals were analyzed at the National Synchrotron Light Source at the Brookhaven National Laboratory, beam lines X26c and X25. Mouse SeMet Poly $\gamma$ B crystals belong to space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> with unit cell dimensions a=96.62 Å, b=133.42 Å, c=135.04 Å and  $\alpha=\beta=\gamma=90^\circ$ . Crystals were measured at three wavelengths close to the Se absorption edge chosen to maximize f' and delta f' of Se. Data collection for the isomorphous native crystals was carried out at a wavelength of 1.1 Å.

Deletions mutants of human Poly $\gamma$ B lacking solvent exposed loops and hairpins were designed based on the structure, expressed and purified. They were characterized by native gel electrophoresis in the presence of different DNA constructs and/or Poly $\gamma$ A and by glycerol gradient sedimentation.

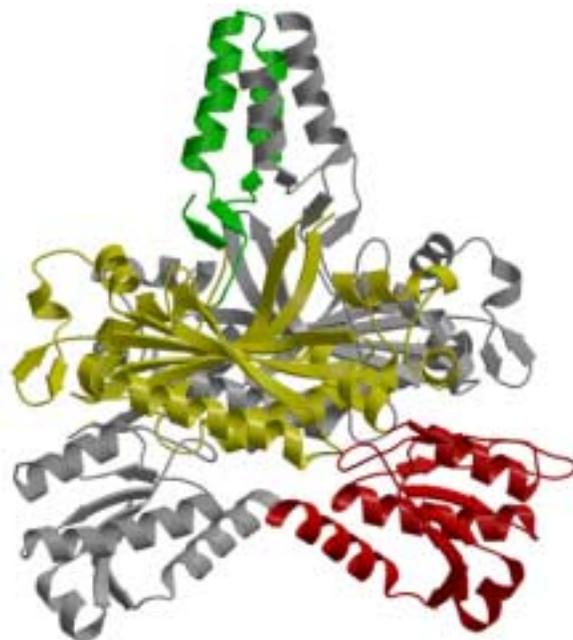
**Results:** The 1.95 Å crystal structure of mouse Poly $\gamma$ B shows high similarity to the glycyl-tRNA synthetase fold. However, residues thought to be required for tRNA synthetase activity are not conserved in Poly $\gamma$ B. Poly $\gamma$ B forms a homodimer stabilized by a unique intermolecular four-helix bundle. A human Poly $\gamma$ B mutant lacking the four-helix bundle failed to dimerize in solution, lost its Poly $\gamma$ A-stimulating activity but retained the ability to bind with Poly $\gamma$ A to a primer-template construct, indicating that the functional holoenzyme contains two molecules of Poly $\gamma$ B. Two mutants lacking surface  $\beta$ -hairpin motifs retained activity as a processivity factor, but lost the ability to bind folded ssDNA.

**Conclusions:** We report the first evidence that the accessory subunit of mitochondrial DNA polymerase, Poly $\gamma$ B, is functional as a homodimer and associates with one copy of the catalytic subunit in a heterotrimeric holoenzyme. The evolutionary relationship of Poly $\gamma$ B to aminoacyl tRNA synthetases is reflected in conservation of nucleic acid binding properties, since surface loops involved in tRNA recognition by aaRS appear to be important for the interaction of Poly $\gamma$ B with folded ssDNA. As a processivity factor, Poly $\gamma$ B exhibits unique properties. It is clearly distinct from the sliding clamps like PCNA and has little structural similarity to thioredoxin. The ability of Poly $\gamma$ B to bind DNA suggests a parallel to the herpes virus DNA pol processivity factor, UL42, although the detailed DNA binding properties and structures differ considerably.

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**Figure 1** Fold and domain structure of Poly $\gamma$ B. Domain 1 (yellow) and domain 3 (red) are similar to the domains of type IIa aminoacyl tRNA synthetases. Domain 2 (green) forms an intermolecular four-helix bundle required for DNA-binding and Poly $\gamma$ A stimulation.