

## Structural determinants of polymerase fidelity and nucleotide discrimination in mammalian DNA pol $\beta$

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### DNA polymerase $\beta$ mutator mutants

Pol  $\beta$  is one of the smallest eukaryotic polymerases. It is a simple, well-characterised system to study the correlation between polymerase structure and fidelity of DNA synthesis. Pol $\beta$  plays a crucial role in base excision repair (BER) as it removes 5'-deoxyribose phosphate and fills in short lesions in DNA (short patch synthesis). Furthermore, pol $\beta$  is implicated in nucleotide excision repair. There is also evidence to suggest a meiotic function for pol $\beta$ .

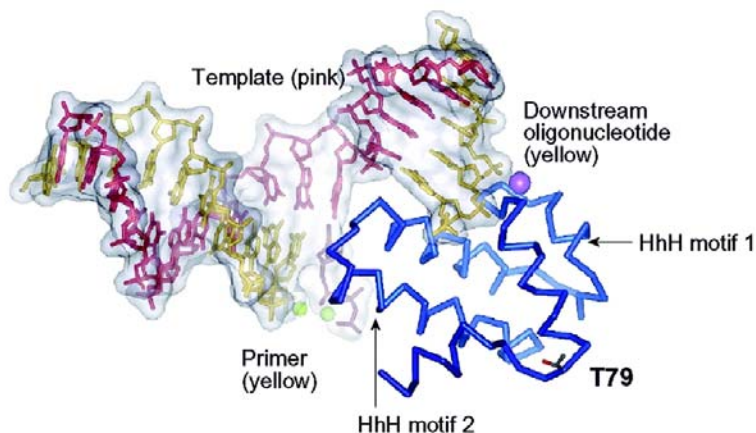
For these studies, pol $\beta$  mutants (for example F272L, M282L, and T79S) are being identified using an *in vivo* genetic screen based on the replacement of the DNA *polA* gene in *E.coli* with the mammalian *pol\beta* gene. Mutator phenotypes were isolated using a Trp<sup>+</sup> reversion assay from a library of random pol $\beta$  mutants. Pol $\beta$  variants that produce Trp<sup>+</sup> revertants in significant numbers and frequencies over wild type pol $\beta$  are isolated, transfected into the *E.coli* SC18-12 strain and characterised biochemically and structurally.

### Polymerase fidelity

Transient-state kinetic methods were used to investigate the mechanism of the intrinsic mutator activity of F272L, M282L and T79S. In the case of F272L, the kinetic data show that the mutant produces error rates at a frequency 10-fold higher than that of wild type both *in vivo* and in *in vitro* assays. The mutant displays an increase in the frequency of frameshift mutations as well as in the rate of misincorporation. A G:A mismatch occurs 4 times more frequently in the F272L mutant than in wild-type Pol $\beta$ .

For M282L, biochemical studies indicate an overall reduction in fidelity (11-fold decrease in dNTP substrate discrimination at the level of ground-state binding and 3-fold increase in nucleotide discrimination during the structural step and/or chemical step). Thermal and chemical equilibrium unfolding studies were used to determine the overall stability of the mutant Pol $\beta$ . The CD spectra indicate that the reduced fidelity appears to be related to a distinct increase in protein stability.

Thr-79 is located in the N-terminal 8kDa domain of pol $\beta$  and has no contact with either the DNA template or the incoming dNTP substrate. The T79S mutant produced 8-fold more multiple mutations in the HSV1-TK assay than wild-type Pol $\beta$ . Surprisingly, T79S is a misincorporation mutator only when using a 3'-recessed dsDNA substrate. In the presence of a single nucleotide-gapped DNA substrate, T79S displays an antimutator phenotype when catalyzing DNA synthesis opposite template C and has similar fidelity as wild type opposite templates A, G, or T. Thr79 forms a hydrogen bonding network within the helix-hairpin-helix motifs of the 8kDa domain that is important for positioning the DNA within the active site.



Interestingly, a change to Ser appears to disrupt this hydrogen bonding network and results in an enzyme that is unable to bend the DNA into the proper geometry for accurate DNA synthesis.

### **Structural studies**

X-ray crystallographic methods are used to investigate the structural basis of the decreased DNA synthesis fidelity in these pol $\beta$  mutator mutants. Residue 272 is located on the DNA binding surface of pol $\beta$  and a mutation at this position is not expected to result in a large conformational change of the entire molecule. The loss of the aromatic ring in the F272L mutant, however, appears to produce a geometrically less constrained dNTP-binding pocket. It also triggers a cascade of side chain movements indicating increased flexibility in the dNTP at the groundstate (prior to the structural or chemical step). This increase appears to be responsible for the decrease in dNTP affinity and fidelity.

In wild type pol $\beta$ , Met-282 is located in the interior of the C-terminal domain and does not directly interact with the substrates. When mutated to Leu, residues 283, 293, and 295 shift to accommodate the reduction in side chain volume. In comparison with the wild type structure, the M282L mutant produces a collapsed, more densely packed hydrophobic core, which in turn results in a polymerase with enhanced protein stability. The increase in protein stability was corroborated by equilibrium unfolding studies in the presence of urea.

### **Publications:**

Maitra, M., Gudzelak, A., Li, S.X., Matsumoto, Y., Eckert, K.A., Jager, J. & Sweasy JB. (2002) Thr79 is a residue that governs the fidelity of DNA pol $\beta$  by helping to position the DNA within the active site. *J Biol Chem.* **277**, 35550-60

Shah, A.M., Conn, D.A., Li, S.X., Capaldi, A., Jaeger, J. & Sweasy, J. (2001) A DNA polymerase beta mutator mutant with reduced nucleotide discrimination and increased protein stability. *Biochemistry* **40**, 11372-81.

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### **Funding:**

We thank the NIH for support