Bulk DNA synthesis during replication occurs in a highly faithful and efficient manner primarily through the combined actions of three DNA polymerases: DNA pols α, δ and ε. Yeast Pols δ and ε and human Pol δ are highly accurate due to having a high intrinsic fidelity combined with an associated proofreading exonuclease activity. Although the N-terminal catalytic portion of polymerase ε is highly conserved throughout all eukaryotes, human Pol ε is relatively poorly understood.

Here we present the expression and purification of recombinant catalytic half of human DNA Pol ε. We determined the fidelity of human Pol ε using the lacZ forward mutation assay, which is able to measure error rates for all twelve possible base-base mismatches, as well as for frameshift mutations, in a large number of sequence contexts. Error rates for base pair substitutions and single-base frameshifts as well as the error spectrum of exonuclease-deficient human Pol ε, which reflect the intrinsic selectivity of the enzyme, shows that the human enzyme is more accurate than yeast enzyme, but has a similar error specificity.

Mutator mutants of DNA polymerases that have unique error signatures while retaining wild type catalytic activity have proven useful for determining the precise roles of those pols in vivo. Toward this goal, we mutated an active site residue of human Pol ε, M630, known to generate a unique error signature in yeast. We show that the M630G derivative of human Pol ε has strongly reduced replication fidelity. Similar to the yeast Pol ε mutant, the human M630G mutant has significantly elevated rates of T to A transversions, while retaining wild type catalytic activity. Importantly, the exonuclease-proficient M630G mutant is still able to correct its own errors, but has a unique error signature. Thus, M630G human Pol ε may be a useful tool in determining the precise in vivo role of Pol ε in DNA replication and in studying how disruption of the normal replication machinery can lead to mutagenesis and human disease including cancer.