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Evidence for Extrinsic Exonucleolytic Proofreading

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Abstract

Exonucleolytic proofreading of DNA synthesis errors is one of the major determinants of genome stability. However, many DNA transactions that contribute to genome stability require synthesis by polymerases that naturally lack intrinsic 3' exonuclease activity and some of which are highly inaccurate. Here we discuss evidence that errors made by these polymerases may be edited by a separate 3' exonuclease, and we consider how such extrinsic proofreading may differ from proofreading by exonucleases that are intrinsic to replicative DNA polymerases.

Keywords

proofreading; DNA replication fidelity; DNA repair; mutagenesis; base substitutions; DNA polymerase; exonuclease

THREE FIDELITY STEPS

Three processes strongly contribute to high DNA replication fidelity: the nucleotide selectivity of DNA polymerases, proofreading of polymerization errors by the 3' exonuclease activities intrinsic to some polymerases, and post-replication DNA mismatch repair. Our understanding of polymerase nucleotide selectivity (see ref. 1 and refs. therein) and DNA mismatch repai2 has improved tremendously over the past decade. At the same time, many DNA polymerases have been discovered that lack intrinsic 3' exonuclease activity, such that they cannot proofread their own mistakes.3 Moreover, several of these enzymes have much lower nucleotide selectivity than do the major replicative DNA polymerases (reviewed in ref. 4). Nonetheless, these naturally exonuclease-deficient polymerases participate in many DNA transactions that ultimately contribute to genome stability (reviewed in refs. 3 and 5). This raises the question of whether errors made by an exonuclease-deficient DNA polymerase can be removed by the exonuclease activity of a separate protein.

PROOFREADING OF ERRORS MADE BY DNA POLYMERASES WITH INTRINSIC 3' EXONUCLEASE ACTIVITY

The bulk of chain elongation during replication is often conducted by DNA polymerases that have an intrinsic 3' exonuclease activity within the same peptide chain as the polymerase.

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Classical examples are bacteriophage T7 and T4 DNA polymerases and eukaryotic DNA polymerases δ and ε (pol δ/ε). When their polymerase active sites are bound to a correctly paired duplex primer-template, efficient incorporation of the next correct dNTP occurs. However occasional misinsertion can create a terminal mismatch, slowing incorporation of the next correct nucleotide and providing an opportunity for the primer terminus to fray. The resulting single stranded DNA then partitions to the exonuclease active site, which is designed to bind single stranded DNA for excision of the misinserted nucleotide. The competition between the polymerase and exonuclease activities determines the efficiency of proofreading (Fig. 1A and see ref. 6). Movement of the nascent strand between the two active sites may occur intra-molecularly (Fig. 1A), i.e., without complete enzyme dissociation from the DNA.7 Proofreading may also occur inter-molecularly, i.e., when misinsertion is followed by polymerase dissociation and the error is excised upon binding of the single-stranded primer terminus directly to the exonuclease active site.6,7 Partitioning between polymerization and excision can be attenuated by amino acid substitutions located between the active sites (e.g., see ref. 8) or located at the exonuclease active site.9 Also, increasing the concentration of the next correct nucleotide to be incorporated will increase the probability of mismatch extension, thereby decreasing proofreading efficiency.10 Proofreading by intrinsic exonucleases is believed to enhance the fidelity of normal replication by between 10- and 1000-fold, depending on the mismatch (see ref. 4 and refs. therein). Intrinsic proofreading is also likely to enhance the fidelity of certain repair reactions. For example, pols δ and ε participate in recombination, in DNA mismatch repair and in gap filling synthesis during base excision repair and nucleotide excision repair.3,5

PROOFREADING OF ERRORS MADE BY AN EXONUCLEASE DEFICIENT BACTERIAL REPLICASE

Some replicases contain the polymerase and proofreading exonuclease active sites in separate proteins. The classic example is *E. coli* DNA polymerase III holoenzyme (reviewed in ref. 11), whose polymerase and 3' exonuclease activities are in the α and ε subunits respectively. Although encoded by distinct genes, these subunits are physically associated in a manner that allows the ε subunit to efficiently proofread misinsertions generated by the exonuclease-deficient α subunit during replication.12 As previously pointed out,13 having proofreading activity on a separate protein may be advantageous under some circumstances. For example, when a bacteria population is in a stressful environment, those bacteria most fit to survive the stress may arise from a subpopulation that has a mutator phenotype (e.g., see refs. 14 and 15, and refs. therein). Theoretically, such a mutator phenotype could result from replication transiently catalyzed without using the proofreading subunit.

EVIDENCE THAT ERRORS MADE BY POL α ARE PROOFREAD BY POL δ

Replication in eukaryotes requires naturally exonuclease-deficient pol α to synthesize 20–30 nucleotides during initiation of each ~250 base Okazaki fragment on the lagging strand, after which a switch to synthesis by pol δ/ε occurs. Given that pol α has a base substitution error rate of ~10^{-4},[16] this could introduce many thousands of mismatches into the human genome during each replication cycle. This led us to test whether the intrinsic exonuclease activity of yeast pol δ and/or pol ε might proofread replication errors made by pol α during replication of the lagging strand in vivo. We found[17] that a weak mutator phenotype of a yeast strain harboring a *pol1-L868M* allele that encodes an error-prone form of pol α[18] was synergistically increased (Fig. 2) upon inactivation of the 3' exonuclease of pol δ (*pol3-5DV* allele). These data and an earlier study showing that the 3' exonuclease activity of calf thymus pol δ can excise mismatches generated by calf thymus pol α during DNA synthesis in vitro,[19] are consistent with a model (Fig. 1B) wherein the 3' exonuclease of pol δ proofreads errors generated by pol α during initiation of Okazaki fragments in vivo. These

data add to accumulating evidence[20] that pol δ participates in lagging strand DNA replication. Moreover, this could be another example wherein errors made by an exonuclease-deficient DNA polymerase are proofread by an exonuclease activity present in a separate protein.

How the competition for termini between the polymerase active site in pol α and the exonuclease active site in pol δ is coordinated during putative extrinsic proofreading is not known. Coordination between the two active sites could differ from that which occurs during intra-molecular partitioning for polymerases with an intrinsic exonuclease activity. It may be more akin to inter-molecular proofreading involving intervening polymerase dissociation (Fig. 1A). Pol δ access to mismatched primer termini generated by pol α could be modulated by accessory proteins (e.g., RPA, RFC, PCNA) that help to coordinate the normal switch between pol α and pol δ (and/or pol ε?) that occurs during synthesis of each Okazaki fragment. Thus, the efficiency of this type of extrinsic proofreading may not be the same as that determined for polymerases with intrinsic proofreading activity. For this reason, other DNA transactions may contribute to the strong synergy between the *pol1-L868M* and *pol3-5DV* mutations observed in vivo. For example, L868M pol α is promiscuous for mismatch extension,[17] such that some of the observed synergy could result from L868M pol α extension of mismatches generated by exonuclease-deficient pol δ during replication. Arguing against this possibility is the fact that mutagenesis in a pol3-5DV strain does not depend on pol ζ, which is renowned for promiscuous mismatch extension.[21,22] Another possibility is suggested by the fact that errors made by L868M pol α are subject to mismatch repair[17,18] and the exonuclease activity of pol δ is implicated in the excision step of mismatch repair.[23] Thus partially defective mismatch repair due to loss of pol δ exonuclease activity could contribute to some of the synergy (Fig. 2) between L868M pol α and 5DV pol δ. The fact that mismatch repair remains active in the *pol1-L868M-pol3-5DV* strain[17,18] argues against this possibility. So too does the fact that no synergy was observed between L868M pol α and exonuclease-deficient pol ε (ref. [17] and Fig. 2), whose exonuclease is also implicated in mismatch repair.[23] This lack of synergy also implies that pol ε is denied access to mismatched termini generated by L868M pol α. It remains to be seen whether pol ε might proofread errors made by pol α and/or another polymerase under other replication conditions where the roles of pol δ and pol ε may differ.[20] For example, pol ε may have a larger role in replication of heterochromatin,[24] or in replication under aberrant circumstances, e.g., when replication is slowed due to DNA damage, due to a defective polymerase[25] or perhaps even due to overproduction of an inaccurate polymerase.[26]

PROOFREADING DURING TRANSLESION DNA SYNTHESIS?

One solution to replication fork stalling at a DNA lesion is translesion synthesis (TLS). TLS is catalyzed by exonuclease-deficient DNA polymerases[22] that have much lower fidelity than pol α.4 A separate exonuclease could possibly catalyze intermolecular proofreading of errors made during lesion bypass at a replication fork (Fig. 1C). Consistent with this hypothesis, pol δ and pol ε can remove errors made by pol ε during bypass of a *cis-syn* TT dimer in vitro.27 This pol η-dependent TLS reaction is known to reduce susceptibility to skin cancer (reviewed in Ref. 22). This is interesting because mice deficient in the exonuclease activity of pol δ have an increased risk of skin cancer.28 Theoretically, intermolecular proofreading during TLS could also operate during DNA repair, e.g., removal of inter-strand crosslinks that involves both pol δ and the exonuclease-deficient TLS enzyme pol ζ.29

Proofreading of errors made during TLS may depend on whether the lesion retains base coding potential. For example, bypass of lesions that retain relatively normal base coding, like the 3' T of a *cis-syn* TT dimmer,[30] may provide the discrimination between “correct”

and “incorrect” insertion needed for proofreading to enhance fidelity. On the other hand, certain lesions perturb coding potential in a manner that precludes the discrimination needed for proofreading. Examples include incorporation of any nucleotide opposite an abasic site, incorporation of dAMP opposite uracil resulting from cytosine deamination in a photodimer and incorporation of dAMP opposite 8-oxo-G, which is perceived by some polymerases as a correct event.[31]

PROOFREADING DURING SINGLE NUCLEOTIDE BASE EXCISION REPAIR?

Base excision repair (BER) protects cells against the lethal and mutagenic effects of DNA damage by correctly replacing damaged bases with undamaged bases. Three exonuclease-deficient DNA polymerases (pol β, pol λ, pol ι) have been implicated in gap-filling synthesis during mammalian single-nucleotide base excision repair (snBER).[32],33 These polymerases all lack intrinsic proofreading activity and are less accurate than the major replicative polymerases. Nonetheless, the number of DNA bases subject to BER due to base modification by normal cellular processes like depurination, deamination, oxidation and alkylation has been estimated to exceed 10,000 per day.34 One possible mechanism to improve the fidelity of snBER is proofreading by a separate exonuclease (Fig. 1D). Several candidate exonucleases could perform this task, such as a TREX exonuclease[35],[36] or the exonuclease activities of pol δ or pol ε, enzymes implicated in long patch BER. An additional candidate is another snBER enzyme, the apurinic endonuclease. Apurinic endonuclease has a 3' exonuclease activity that can preferentially excise terminal mismatches.[37] The 3' exonuclease of the yeast apurinic endonuclease Apn1 has recently been implicated in the repair of 8-oxo-guanine in vivo.[38] Consistent with the possibility of proofreading by apurinic endonuclease during snBER (Fig. 1D) is the observation that the fidelity of a snBER reaction involving pol β and the apurinic endonuclease APE is 3- to 8-fold higher than is the fidelity of gap-filling by pol β alone.[39] If APE proofreading is indeed responsible for this higher fidelity, this would represent proofreading of 67–87% of mismatches. This efficiency is modest compared to the efficiency of editing by DNA polymerases with intrinsic exonucleases, where the contribution of proofreading to fidelity depends on the rate of excision of the misinserted nucleotide relative to the rate of extension of the mismatched primer terminus (Fig. 1A). Proofreading of errors made by exonuclease-deficient polymerases like pol β during single-nucleotide BER could differ in at least two ways. Since only one nucleotide is incorporated, there is no need for further extension, such that proofreading efficiency should not depend on the concentration of the next correct nucleotide. Proofreading independent of dNTP concentration could be advantageous for BER reactions that occur throughout the cell cycle regardless of whether dNTP concentrations are as high as in S phase or as low as in G_1 phase. Also, once the mismatched DNA is generated, the competition for this nicked, mismatched terminus is between the active site in the ligase that seals the nick (and/or the dRP lyase) and the active site APE. Interestingly, these two proteins are separated in the single-nucleotide BER reaction pathway by the polymerase (Fig. 1D). Finally, as mentioned above, proofreading by a separate exonuclease may allow regulation of BER fidelity. For example, while proofreading BER errors could help to suppress mutagenesis resulting from genome wide DNA damage, it may be counterproductive for putative uracil-initiated BER reactions thought to promote somatic hypermutation of immunoglobulin genes.[40] In like manner, it could be counterproductive for repair efficiency and cell survival if an exonuclease digested mismatched primer termini during attempted microhomology mediated end joining of double-strand DNA breaks by exonuclease-deficient pol λ and pol μ.[41]

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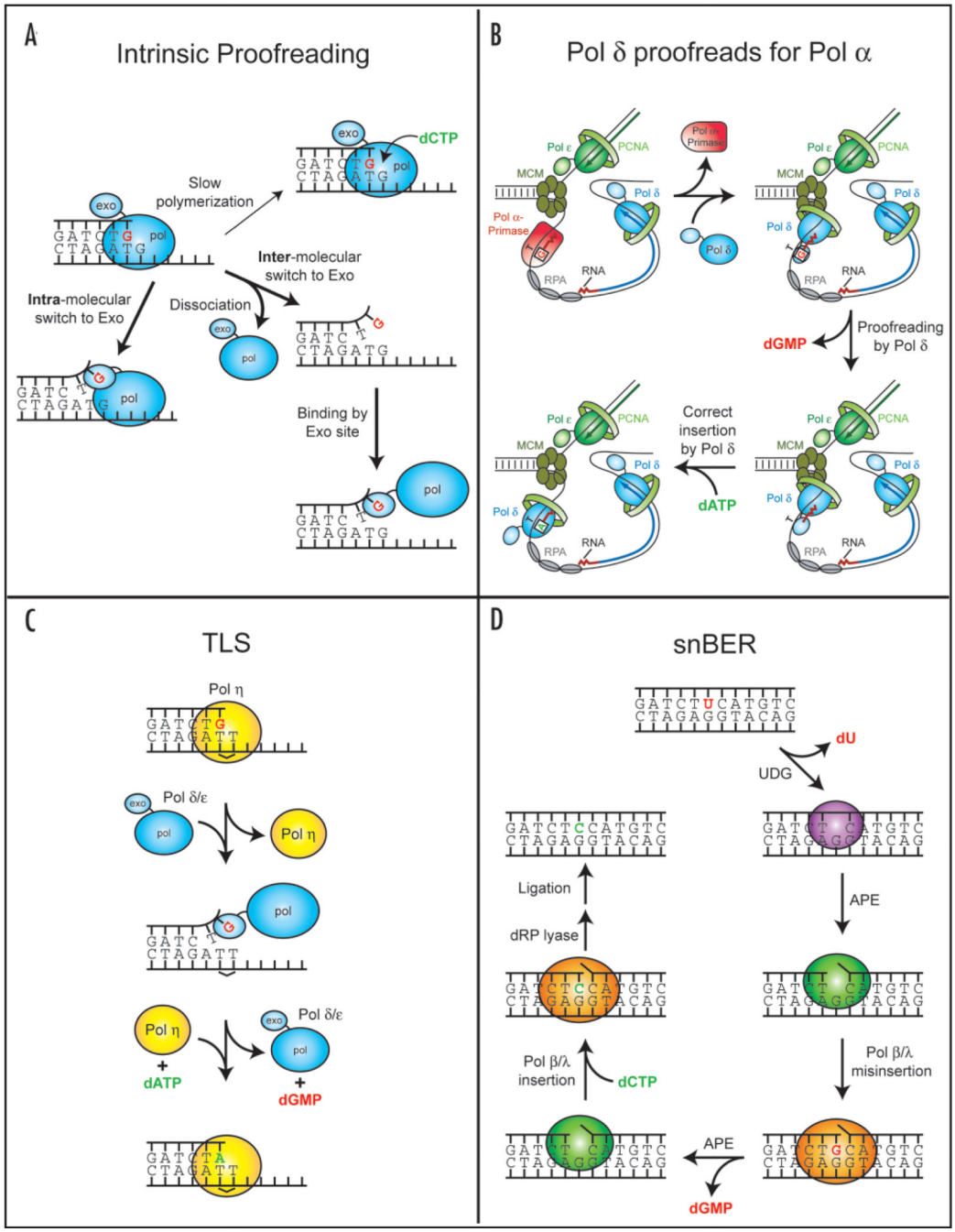

Figure 1.
Models for involvement of proofreading in various DNA transactions. See text for description. The image in Panel B is adapted from (Ref. 20).

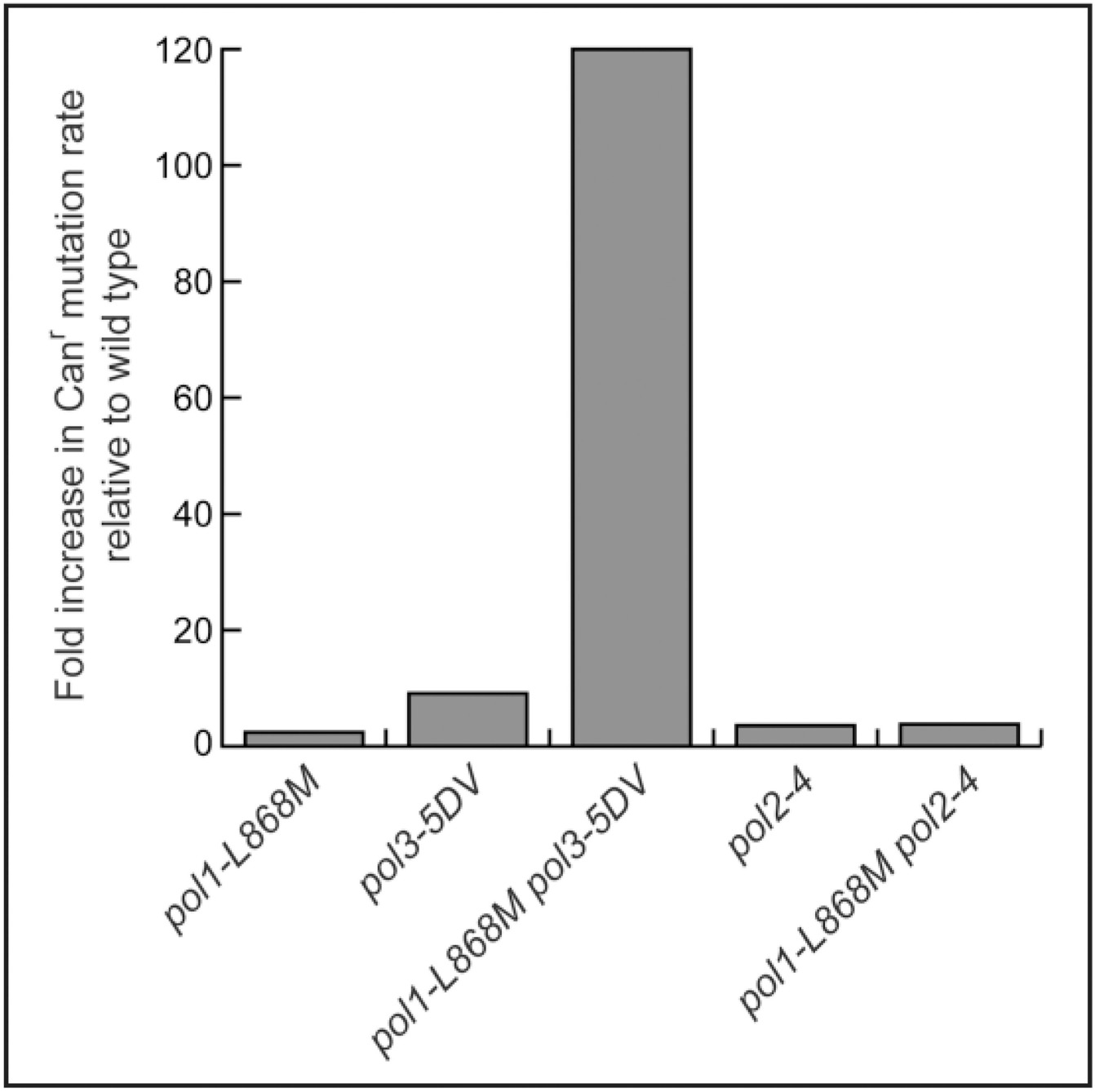

Figure 2.
Mutational synergy between *pol1-L868M* and *pol3-5DV*. The values plotted are taken from Table 3 in reference [17].