DNA polymerase δ proofreads errors made by DNA polymerase ε

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During eukaryotic replication, DNA polymerases ε (Polε) and δ (Polδ) synthesize the leading and lagging strands, respectively. In a long-known contradiction to this model, defects in the fidelity of Polε have a much weaker impact on mutagenesis than analogous Polδ defects. It has been previously proposed that Polδ contributes more to mutation avoidance because it proofreads mismatches created by Polε in addition to its own errors. However, direct evidence for this model was missing. We show that, in yeast, the mutation rate increases synergistically when a Polε nucleotide selectivity defect is combined with a Polδ proofreading defect, demonstrating extrinsic proofreading of Polε errors by Polδ. In contrast, combining Polδ nucleotide selectivity and Polε proofreading defects produces no synergy, indicating that Polε cannot correct errors made by Polδ. We further show that Polδ can remove errors made by exonuclease-deficient Polε in vitro. These findings illustrate the complexity of the one-strand–one-polymerase model where synthesis appears to be largely divided, but Polδ proofreading operates on both strands.

DNA replication | extrinsic proofreading | DNA polymerase δ | DNA polymerase ε

The most widely accepted model of eukaryotic DNA replication proposed in the 1990s suggests that Polα-primase synthesizes short RNA–DNA primers at the origins and at the beginning of the Okazaki fragments, Polε synthesizes the leading strand, and Polδ completes the lagging strand (1). During the three decades that passed since the landmark publication by Morrison et al., numerous reports have contributed evidence for the participation of Polε and Polδ in leading and lagging strand replication, respectively. Genetic studies detected strand-specific increases in mutagenesis in yeast and human cells carrying inaccurate Polε or Polδ variants (2–6). More sensitive assays monitoring ribonucleotide incorporation into DNA by Polε or Polδ variants with relaxed sugar selectivity confirmed ribonucleotide accumulation in the leading strand in Polε mutants and in the lagging strand in Polδ mutants (7, 8). Polδ but not Polε was shown to proofread errors made by Polα (9) and participate in the maturation of Okazaki fragments on the lagging strand (10, 11). At the same time, Polε but not Polδ interacts with the Cdc45-MCM-GINS helicase on the leading strand (12). While the roles of Polδ in the synthesis of the leading strand near replication origins and termination zones have recently been detected (13–16), these stretches of Polδ synthesis appear to account for a relatively minor fraction of the leading strand (~18%, ref. 16). Overall, a bulk of evidence supports the originally proposed division of labor with Polε and Polδ predominantly replicating opposite DNA strands.

In contradiction to this model, Polδ fidelity defects have long been known to have a greater impact on mutagenesis than analogous Polε defects. Both Polε and Polδ contribute to mutation avoidance via their intrinsic nucleotide selectivity conferred by the polymerase domain and the proofreading activity located in a separate exonuclease domain. The exonuclease activity of both polymerases can be abolished by alanine substitutions at the conserved carboxylate residues in the ExoI motif FDIET/C (17, 18). The resulting mutator phenotype of the Polδ-exo$^-$ variant is an order of magnitude stronger than the phenotype of the analogous Polε-exo$^-$ variant (2, 17–25). Furthermore, haploid yeast deficient in Polδ proofreading do not survive when DNA mismatch repair (MMR) is also inactivated with the death attributed to an excessive level of mutagenesis (26). In contrast, yeast lacking both proofreading by Polε and MMR are viable, and while the mutation rate in these strains is high, it does not reach the lethal threshold (19, 21, 22, 25, 27). Similarly, when identical tyrosine to alanine substitutions were made in the conserved region III of the polymerase domains (Polδ-Y708A and Polε-Y831A), the Polδ variant produced a much stronger mutator effect than the analogous Polε variant (28). To explain the controversy between the accepted fork model and the disparity of Polδ and Polε effects on mutagenesis, a hypothesis has been entertained that Polδ proofreads errors made by Polε in addition to its own errors, thus, contributing more significantly to mutation avoidance. This hypothesis, discussed in multiple publications (2, 29–31), stems from the original observation by Morrison and Sugino that the combination of Polδ and Polε proofreading defects results in a synergistic increase in mutation rate (19). The synergy implies that the exonucleases of Polε and Polδ act on the same pool of replication errors and could potentially mean Polε correcting errors made by Polδ, Polδ correcting errors made by Polε, or both polymerases proofreading for each other. In general, the possibility of extrinsic proofreading has been demonstrated in multiple in vivo and in vitro studies. Initial experiments showed that errors made by purified calf thymus Polα could be corrected by the ε subunit of *Escherichia coli* DNA polymerase III or by Polδ (32, 33). Several mammalian autonomous exonucleases have also been shown to

Significance

Polδ and Polε are the two major replicative polymerases in eukaryotes, but their precise roles at the replication fork remain a subject of debate. A bulk of data supports a model where Polε and Polδ synthesize leading and lagging DNA strands, respectively. However, this model has been difficult to reconcile with the fact that mutations in Polδ have much stronger consequences for genome stability than equivalent mutations in Polε. We provide direct evidence for a long-entertained idea that Polδ can proofread errors made by Polε in addition to its own errors, thus, making a more prominent contribution to mutation avoidance. This paper provides an essential advance in the understanding of the mechanism of eukaryotic DNA replication.

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increase the fidelity of Polα in vitro (34–36). Both *E. coli* and eukaryotic replicative polymerases can excise nucleotides incorporated by translesion synthesis polymerases at sites of DNA damage (37, 38). With respect to the extrinsic proofreading capabilities of Polδ and Polε in vivo, several studies have been illuminating. As already mentioned, Polδ but not Polε has been shown to proofread errors made by an error-prone Polα variant in yeast (9). Furthermore, Polδ exonuclease defects are almost completely recessive, indicating that wild-type Polδ can efficiently proofread errors created by Polδ-exo$^-$ (18, 26, 31). On the other hand, the mutant allele encoding Polε-exo$^-$ is semidominant, suggesting that wild-type Polε does not correct errors *in trans* (31, 39). Flood et al. (31) further investigated extrinsic proofreading by Polδ and Polε using transformation of yeast cells with oligonucleotides that, when annealed, create a 3′-terminal mismatch. These experiments showed that Polδ but not Polε can proofread *in trans* and that the exonuclease of Polδ can act on oligonucleotides annealed to both leading and lagging strands. However, it remained unknown whether the exonuclease of Polδ could proofread errors generated by Polε during normal chromosomal replication.

To answer this question, we used yeast strains harboring a nucleotide selectivity defect in one polymerase, Polδ or Polε, and a proofreading defect in the other. We compared mutation rates between the corresponding single and double mutants to determine whether the proofreading activity of one polymerase acts in series or in parallel with the nucleotide selectivity of the other. We also used an in vitro replication system to investigate whether Polδ can excise mismatched primer termini generated by exonuclease-deficient Polε. Our results show that Polδ can correct errors made by Polε, but Polε cannot correct errors made by Polδ. This observation provides direct evidence that the remarkably mild in vivo consequences of severe Polε fidelity defects are explained by the compensatory proofreading by Polδ. These findings support a replication fork model wherein synthesis on leading and lagging strands is primarily accomplished by separate polymerases, but proofreading is more dynamic and can be performed by the exonuclease of Polδ on both strands.

Results

Polδ Proofreads Errors Made by Polε, but Polε Does Not Proofread Errors Made by Polδ In Vivo. The synergistic interaction between the exonucleases of Polε and Polδ has been previously demonstrated using the *pol2-4* and *pol3-01* alleles, which result in the replacement of two catalytic carboxylates in the ExoI motif of the respective polymerase with alanines (FDIET/C → FAIAT/C; ref. 19). The *pol3-01* mutation, however, may have consequences beyond simply destroying the exonuclease of Polδ as its extremely strong mutator phenotype has been reported to be partially dependent on the activation of the S-phase checkpoint (40), and a different allele, *pol3-D520V*, exists that also eliminates the exonuclease activity but is a weaker mutator (10). We started by verifying that the synergy between Polε and Polδ could still be detected when the *pol3-D520V* allele is used instead of *pol3-01* to produce exonuclease-deficient Polδ. While the *pol2-4 pol3-01* double mutant haploids were inviable due to a catastrophically high mutation rate (19), the *pol2-4 pol3-D520V* haploids survived (*SI Appendix*, Fig. S1). The mutation rate in the *pol2-4 pol3-D520V* strains increased synergistically as compared with the single *pol2-4* and *pol3-D520V* mutants (*SI Appendix*, Table S1), consistent with the idea that the exonucleases of Polδ and Polε act on the same pool of replication errors. We next ascertained that this synergistic interaction is not due to the *pol3-D520V* mutation disrupting MMR. If the exonuclease of Polδ is essential for functional MMR, combining *pol3-D520V* with a MMR defect would yield no further increase in mutation rate beyond the effect of *pol3-D520V* alone. On the other hand, if Polδ proofreading and MMR act in series, a synergistic increase in mutation rate would be expected in the double mutants. Haploid yeast deficient in MMR and harboring *pol3-D520V* are not viable (41); therefore, we assessed the epistatic relationship between *pol3-D520V* and MMR deficiency in diploid strains, which can tolerate a higher level of mutagenesis. We used the *MSH6* deletion to inactivate MMR as the Msh6-dependent pathway is primarily responsible for the repair of single-base mismatches (42), which is the predominant type of replication errors generated by exonuclease-deficient Polδ and Polε (43–45). Diploids homozygous for both *pol3-D520V* and *msh6* mutations showed a strong synergistic increase in mutation rate as compared with the single *pol3-D520V* and *msh6* mutants (*SI Appendix*, Table S2). A similar synergistic increase in mutagenesis in *pol3-D520V/pol3-D520V msh6/msh6* diploids was observed by Flood et al. for base substitutions at a single nucleotide position in the *TRP5* gene (31). We recapitulate and expand these earlier findings by using the forward mutagenesis reporter *CAN1* that can detect a variety of base substitutions and indels in many DNA sequence contexts as well as the *his7-2* frameshift reporter that is particularly sensitive to MMR defects. Together, these data demonstrate that *pol3-D520V* does not confer a MMR defect. Thus, the synergy between *pol2-4* and *pol3-D520V* indicates proofreading of the same errors by Polε and Polδ. It also shows that the *pol3-D520V* allele provides an adequate model for the extrinsic proofreading studies described below.

Next, we investigated whether Polδ proofreads errors made by Polε by combining a nucleotide selectivity defect in Polε (*pol2-M644G*) with a proofreading defect in Polδ (*pol3-D520V*). The *pol2-M644G* confers a change in the polymerase domain of Polε, which causes promiscuity during nucleotide incorporation without compromising proofreading (3). The *pol2-M644G* strains, therefore, accumulate a high number of Polε-specific errors. We observed a synergistic increase in mutation rate in the double *pol2-M644G pol3-D520V* mutants (Table 1). This synergy indicates that the nucleotide selectivity of Polε and the proofreading activity of Polδ act consecutively to prevent replication errors and, thus, Polδ proofreads errors made by Polε in vivo. In a reciprocal experiment, we combined a Polδ nucleotide selectivity defect (*pol3-L612M*) with a Polε proofreading defect (*pol2-4*) to determine whether Polε can proofread errors made by Polδ. Similar to *pol2-M644G*, *pol3-L612M* increases the rate of nucleotide misincorporation by Polδ without impacting exonuclease activity (46). In contrast to the *pol2-M644G pol3-D520V* combination, the *pol3-L612M pol2-4* combination resulted in only an additive increase in the mutation rate in the double mutant compared to the single *pol3-L612M* and *pol2-4* mutants (Table 2). The additive interaction indicates that Polδ nucleotide selectivity and Polε exonuclease activity act in parallel nonoverlapping pathways, and, therefore, Polε does not proofread errors made by Polδ.

Polδ Removes Mismatched Primer Termini Created by Polε In Vitro. We next developed an in vitro assay to examine whether Polδ can excise mismatched primer termini generated by exonuclease-deficient Polε. In this assay, purified yeast Polε-exo$^-$ is allowed to synthesize DNA on a 9-kb single-stranded circular template in the presence of accessory proteins proliferating cell nuclear antigen (PCNA), replication factor C (RFC), replication protein A (RPA), and highly imbalanced dNTP concentrations. dCTP and dTTP are provided at their physiological S-phase concentrations (47, 48), dATP is at ∼1/5 of its S-phase concentration, and dGTP is in vast excess (∼150-fold) over its S-phase concentration (Fig. 1*A*). The dNTP imbalance results in a high rate of incorrect nucleotide incorporation, which inhibits synthesis because Polε-exo$^-$ cannot proofread these errors. Although it is capable of extending mismatched primer termini (43), the number of mismatches under these conditions is overwhelming, and the continuous need to extend them delays synthesis to a point where no long products (>2.5 kb) are produced (*SI Appendix*, Fig. S2). In contrast, the dNTP imbalance is not inhibitory to exonuclease-proficient wild-type Polε, indicating that inhibition of Polε-exo$^-$ is due to the lack

Table 1. Synergistic interaction of Polε nucleotide selectivity and Polδ proofreading defects

Genotype	*CAN1* mutation			*his7-2* reversion		
	Mutation rate ($\times10^{-7}$)		Fold increase over wild type	Mutation rate ($\times10^{-8}$)		Fold increase over wild type
POL2 POL3	2.5	(2.1–2.9)	1.0	0.83	(0.70–0.97)	1.0
pol2-M644G POL3	9.7	(8.2–12)	3.9	1.4	(1.0–1.6)	1.7
POL2 pol3-D520V	19	(16–21)	7.6	8.0	(7.0–9.6)	9.6
pol2-M644G pol3-D520V	92	(77–110)	37	13	(11–15)	16

Mutation rates are medians for at least 18 cultures from two to three independently constructed strains of the same genotype. The 95% confidence intervals are shown in parentheses.

of proofreading (*SI Appendix*, Fig. S2). After several minutes of inefficient synthesis attempts by Polε-exo$^-$, Polδ was added to the reactions, and its ability to assist with the removal of mismatched termini and rescue DNA synthesis was measured by the accumulation of long products (Fig. 1*A*). We found that the addition of Polδ rescued synthesis significantly (Fig. 1 *B* and *C*), indicating that Polδ efficiently corrected misinsertions made by Polε-exo$^-$. Importantly, the restoration of synthesis in this system could only be due to Polδ acting on Polε-exo$^-$-generated primer termini as, in our reaction conditions, all originally available primers are extended by Polε-exo$^-$ before Polδ is added (Fig. 1*B* and *SI Appendix*, Fig. S2). The polymerase exchange could conceivably involve physical interaction between Polδ and Polε or simply reflect binding of Polδ to mismatched primer termini vacated by Polε. While our in vitro assay cannot distinguish between these possibilities, it shows that the biochemical properties of Polε are consistent with its ability to yield to Polδ in the context of ongoing DNA synthesis in the presence of accessory replication proteins.

Discussion

The accepted model for eukaryotic DNA replication is not easily reconciled with the stronger mutator effects of Polδ variants in comparison with analogous Polε variants. It has been proposed that Polδ can proofread errors made by Polε in addition to its own errors, which would explain its more prominent contribution to mutation avoidance. Currently available data suggest that, indeed, Polδ but not Polε can readily proofread errors *in trans* (9, 18, 26, 31, 39). However, evidence that Polδ can proofread DNA synthesized by Polε at the replication fork has been lacking. Using inaccurate variants of Polδ and Polε, here we demonstrate that incorrect nucleotides incorporated by Polε are efficiently removed by the exonuclease of Polδ, but Polε cannot remove nucleotides misincorporated by Polδ (Fig. 2). This conclusion is supported by the following observations. i) Mutation rate increases synergistically when the Polε nucleotide selectivity defect is combined with Polδ proofreading defect. ii) Only an additive increase in mutagenesis is observed when the Polδ nucleotide selectivity defect is combined with the Polε proofreading defect. iii) Mismatched primer termini generated by Polε-exo$^-$ can be proofread by Polδ in an in vitro replication system.

Polδ Is a Versatile Extrinsic Proofreading Enzyme. Multiple studies suggested that Polδ is more efficient at extrinsic proofreading than Polε. Polδ can remove mismatches generated by Polα both in vitro and in vivo (9, 33). Since Okazaki fragments are all initiated by exonuclease-deficient Polα, there is a clear need for extrinsic proofreading by the lagging strand polymerase, whereas there is less of a need for Polε to carry this out on the leading strand. Indeed, Polε does not appear to correct errors made by Polα in vivo (9). It is particularly interesting to note the recent evidence that initial leading strand synthesis is performed by Polδ (13–15), which further diminishes the need for extrinsic proofreading of Polα-generated errors by Polε on the leading strand. Additionally, the semidominance of the *pol2-4* mutation and almost complete dominance of *POL3* over the *pol3-01* and *pol3-D520V* mutations demonstrates that only Polδ can remove errors inserted by a different polymerase molecule (18, 26, 31, 39). The removal of 3′-terminal mismatches during oligonucleotide-mediated transformation by Polδ but not Polε (31) also suggests that Polδ is much better suited to extrinsic proofreading than Polε. Finally, this study provides evidence that Polδ proofreads errors made by Polε in vivo, while Polε cannot proofread for Polδ.

Thus, the competition of Polδ and Polε exonucleases for correcting the same pool of replication errors originally demonstrated by Morrison and Sugino in the 1990s (19) is apparently one sided. Perhaps the different properties and regulatory mechanisms of the two polymerases leave them appropriately suited to their own specialized roles. Polε is a component of the replication initiation complex where it associates with origins during the G1/S phase transition (49, 50). Polε remains bound to the moving helicase via the C terminus of its catalytic subunit Pol2 as the N terminus copies the leading strand (3, 12). A flexible region between the two halves of Pol2 allows the polymerase to dissociate from the DNA while remaining bound to the replication machinery (51). This association with the helicase indicates that Polε may not be free to carry out extrinsic proofreading, but the flexibility of the N terminus could allow a different polymerase access to the 3′ end of the leading strand. On the other hand, dissociation and reassociation of Polδ with the primer terminus occurs routinely during lagging strand synthesis, and Polδ is loaded much faster than Polε onto the PCNA-primer-template junction (52). Thus, the high efficiency of Polδ at correcting errors made by Polε may result from a

Table 2. Additive interaction of Polδ nucleotide selectivity and Polε proofreading defects

Genotype	*CAN1* mutation			*his7-2* reversion		
	Mutation rate ($\times10^{-7}$)		Fold increase over wild type	Mutation rate ($\times10^{-8}$)		Fold increase over wild type
POL2 POL3	2.5	(2.1–2.9)	1.0	0.83	(0.70–0.97)	1.0
pol2-4 POL3	7.6	(6.8–8.7)	3.0	6.3	(5.6–6.9)	7.6
POL2 pol3-L612M	11	(9.7–13)	4.4	5.0	(4.1–5.9)	6.0
pol2-4 pol3-L612M	17	(16–18)	6.8	8.9	(7.6–11)	11

Mutation rates are medians for at least 18 cultures from two to three independently constructed strains of the same genotype. The 95% confidence intervals are shown in parentheses.

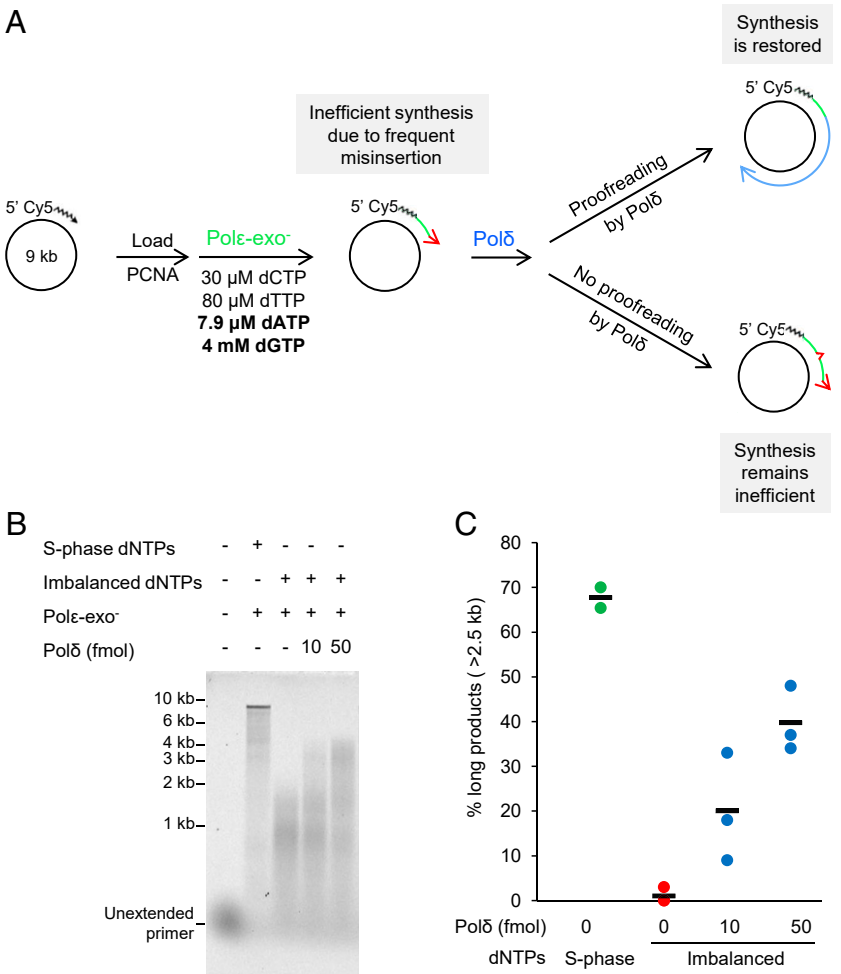

Fig. 1. Errors made by Polε-exo⁻ are removed by Polδ in vitro. (*A*) Schematic of polymerase rescue assay. A Cy5-labeled primer (wavy black line) annealed to single-stranded plasmid template M13/CAN1(1-1560-F) was extended by purified Polε-exo⁻ (green line) in the presence of highly imbalanced dNTPs. dNTP concentrations below or above the normal S-phase concentrations are indicated in bold font. Synthesis is inefficient under these conditions due to frequent nucleotide misincorporation (shown in red). Polδ was then added to the reactions, and its ability to assist Polε-exo⁻ with the removal of misincorporated nucleotides was monitored by the restoration of DNA synthesis (blue line). For experimental details, see the *Materials and Methods* section. (*B*) Analysis of M13/CAN1(1-1560-F) replication products by electrophoresis in a 1% alkaline agarose gel. The primer was elongated by Polε-exo⁻ for 7 min, followed by synthesis with 0, 10, or 50 fmol of Polδ for an additional 3 min. (*C*) Quantification of long products (above 2.5 kb) from *B*.

combination of two factors: the high proclivity of Polε to yield to another polymerase and the greater flexibility and robustness of Polδ when associating with new primer termini.

Genome Stability Requires Redundancy of Replication Fidelity Mechanisms. The overlap in replication and repair mechanisms is essential to prevent lethal and pathogenic mutations and ensure the stability of DNA. For example, several DNA glycosylases function in base excision repair such that, when one is compromised, the others can compensate (53). Multiple translesion synthesis polymerases provide redundant mechanisms of lesion bypass (54, 55). Cancer cells in which one DNA repair pathway has been compromised become resistant to DNA-damaging therapeutic drugs, in part, due to the redundancy that exists to repair the damage and prevent mutations. Targeting a redundant repair pathway in combination with a DNA damaging agent is a promising approach to overcome resistance (56). A recent example is the inclusion of nucleoside analog 5-NIdR, an inhibitor of translesion synthesis with temozolomide in the treatment of homologous-recombination-impaired tumors to promote cancer cell death (57, 58).

The redundancy that serves to protect the genome is also found in the DNA replication process. It is well established that three different mechanisms, nucleotide selectivity, exonucleolytic proofreading, and MMR, act to prevent and correct replication errors. A combination of nucleotide selectivity and proofreading defects in Polδ results in a catastrophically high mutation rate incompatible with life in haploid yeast (59), indicating that proofreading normally compensates for reduced nucleotide selectivity. Haploid yeast deficient in Polδ proofreading require functional MMR for survival (26). Recent work has demonstrated that polymerase fidelity and MMR can compensate for defects in cellular metabolism that lead to dNTP pool imbalances and help maintain a normal low mutation rate despite the abnormal dNTP levels (60, 61). Extrinsic proofreading of Polε errors by Polδ shown here as well as proofreading of Polα errors by Polδ shown previously (9) is yet another mechanism of redundancy to prevent accumulation of DNA replication errors.

The redundancy in replication fidelity mechanisms has implications for human cancer biology. Mutations in the *POLE* gene, which encodes the catalytic subunit of Polε in humans, are found in 5–8% of sporadic colorectal and endometrial cancers and define a unique subset of these cancers with a so-called ultramutated phenotype (62). The *POLE* mutations predominantly affect the exonuclease domain of Polε and cause strong mutator and cancer susceptibility

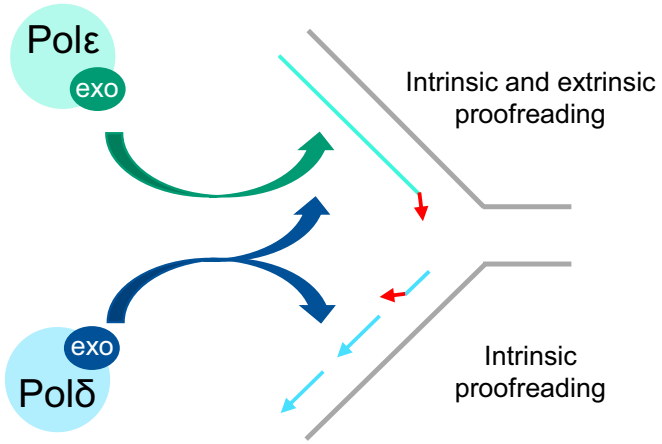

Fig. 2. Interplay of Polε and Polδ proofreading and synthesis activities at the replication fork. Polε replicates the leading strand and proofreads its own errors. Polδ replicates the lagging strand but can remove errors made by Polε in addition to its own errors.

phenotypes in model systems (39, 63, 64). Although MMR defects are also common in colorectal and endometrial tumors, strong *POLE* mutators are never seen in combination with MMR deficiency, suggesting that MMR is critical to keep the mutation rate at a level compatible with cell survival. Curiously, mutations affecting the exonuclease domain of Polδ are seen much less frequently in sporadic tumors. While never explicitly tested, it is possible that these result in much stronger mutator phenotypes that hamper cell proliferation, and *POLE*-mutant cancers survive because extrinsic proofreading by Polδ helps reduce the number of errors to a tolerable level. Studies in mouse models suggested that the relative contributions of Polδ and Polε proofreading activities to replication fidelity and cancer prevention could vary depending on the cell and tissue types as well as the developmental stage. In a MMR-deficient background, both Polδ and Polε proofreading defects are lethal, but embryos lacking Polδ proofreading die earlier than those lacking Polε proofreading (65). In a MMR-proficient background, a Polδ proofreading defect leads to a significantly earlier onset of cancer than the analogous defect in Polε (65–67). These observations are reminiscent of the stronger effects of Polδ mutations in yeast, although dramatic differences in the spectrum of tumors in Polδ vs. Polε mutant mice preclude accurate comparison of cancer susceptibility. A combination of Polδ and Polε proofreading defects, however, greatly accelerates the development of tumors characteristic of Polδ proofreading deficiency (65), consistent with the idea that tumors in Polδ proofreading-deficient mice result, in part, from Polε errors. Curiously, neither the stronger effects of Polδ exonuclease nor synergy between Polδ and Polε was detected when the mutation rate was measured in fibroblast cell lines derived from the mutant embryos (65). These studies illuminate the complexity of the mammalian developmental and tissue biology and highlight the importance of investigating possible cooperation of Polδ and Polε exonucleases in cancer-relevant cells and tissues.

Materials and Methods

Yeast Strains and Plasmids. All *Saccharomyces cerevisiae* strains used in this study are derivatives of E134 (*MAT*α *ade5-1 lys2::InsE$_{A14}$ trp1-289 his7-2 leu2-3,112 ura3-52*) (21, 68) and 1B-D770 (*MAT***a** *ade5-1 lys2-Tn5-13 trp1-289 his7-2 leu2-3,112 ura3-4*) (68). The plasmid used to construct *pol2-M644G* mutants was p173, a *URA3*-based yeast integrative vector containing a BamHI-BspEI C-terminal fragment of *POL2* (69) in which the *pol2-M644G* mutation was created by site-directed mutagenesis (3). It was kindly provided to us by Youri Pavlov (University of Nebraska Medical Center). The *pol2-M644G* mutation was introduced into E134 by transformation with this plasmid linearized with BsrGI, followed by selection for the loss of the plasmid backbone on medium containing 5-fluoroorotic acid (the integration–excision procedure). To construct *pol3-D520V* and *pol3-L612M* mutants, we used p170, a *URA3*-based integrative plasmid containing an EcoRV-HindIII C-terminal fragment of *POL3* (70) in which the *pol3-D520V* and *pol3-L612M* were created by site-directed mutagenesis (10, 71). These p170 derivatives were also provided by Youri Pavlov. The mutations were introduced into 1B-D770 by integration–excision of BseRI-linearized p170 with the D520V mutation and HpaI-linearized p170 with the L612M mutation. The *pol2-4* mutation was introduced into E134 by integration–excision of BamHI-linearized YIpJB1 (17). Single-mutant *pol2* and *pol3* haploids were crossed to make double-heterozygous diploids, which were then sporulated, and tetrads were dissected to obtain double-mutant *pol2 pol3* haploids. The presence of *pol2* and *pol3* mutations was confirmed by Sanger sequencing prior to mutation rate measurements.

The haploid strains TM30 (the same as 1B-D770 but *CAN1::Kl.LEU2*) and TM44 (the same as E134 but *can1*Δ*::loxP*) (47) were used to construct diploid strains homozygous for *pol3-D520V*, *msh6::kanMX* or both mutations as well as the isogenic wild-type diploids. Crosses of TM30 and TM44 derivatives produce diploids with a single copy of *CAN1* linked to a selectable marker, *Kluyveromyces lactis LEU2*. In this system, recessive *can1* mutations can be scored on medium lacking leucine and containing canavanine. The selection for leucine prototrophy discriminates against cells that acquire resistance to canavanine due to a loss of the entire *CAN1::Kl.LEU2* locus by mitotic recombination, and nearly all Leu$^+$ Can^r colonies result from intragenic mutations in *CAN1* (47). To construct the *pol3-D520V/pol3-D520V msh6::kanMX/msh6::kanMX* diploids, we first transformed both TM30 and TM44 with a BseRI-linearized p170 plasmid containing the *pol3-D520V* mutation. In this way, integration of the plasmid into the chromosomal *POL3* locus places the *pol3-D520V* mutation in a truncated, nonexpressed portion of *POL3*. Then, we deleted *MSH6* in these strains by transformation with a PCR-generated DNA fragment containing the *kanMX* cassette flanked by short sequence homology to *MSH6* (72). We crossed derivatives of TM30 and TM44 harboring the deletion of *MSH6* and the integrated nonexpressed *pol3-D520V* mutation to obtain diploids. Finally, we selected for cells that had lost the p170 plasmid from both chromosomes simultaneously on medium containing 5-fluoroorotic acid and used Sanger sequencing to find clones homozygous for the *pol3-D520V* mutation, now present in the full-length expressed alleles. Isogenic single-mutant diploids (*pol3-D520V/pol3-D520V* or *msh6::kanMX/msh6::kanMX*) and wild-type controls were constructed similarly, omitting the *MSH6* disruption step, the p170-*pol3-D520V* transformation step, or both.

Mutation Rate Measurements. The rate of *CAN1* forward mutation and *his7-2* reversion was measured by fluctuation analysis as described previously (73). Briefly, multiple independent cultures of each strain were grown from single colonies in liquid medium overnight. Appropriate dilutions were plated on complete and selective medium, and colonies counted to obtain the mutant frequency (the total number of mutants in the culture divided by the total number of viable cells in the culture). The mutation rate was calculated from the mutant frequency using the Drake equation (74). The mutation rate reported for each strain is the median mutation rate for at least 18 cultures from two or more independently constructed clones of the same genotype. The Wilcoxon–Mann–Whitney test was used to determine whether differences between the mutation rates are statistically significant.

Proteins. Preparations of four-subunit *S. cerevisiae* Polε, Polε-exo$^-$, three-subunit Polδ, PCNA, and RPA used in this work have been described (45, 47, 75). Purified yeast RFC was kindly provided by Peter Burgers (Washington University School of Medicine).

In Vitro Replication Assay. Singly primed circular DNA substrates for in vitro replication assays were prepared by annealing the Cy5-labeled oligonucleotide P50-M13 (Cy5-5′-AAGGAATCTTTGTGAGAAAACTGTGAAAGAGGATGTAACAGGGATGAATG-3′) to the M13/CAN1(1-1560-F) single-stranded DNA (76) by incubating the primer and template at a ratio of 1:1 in the presence of 150 mM NaAc at 92 °C for 2 min and then cooling slowly to room temperature (~2 h). The 10-μL replication reactions contained 40 mM Tris·HCl pH 7.8, 8 mM $MgAc_2$, 125 mM NaAc, 1 mM DTT, 0.2 mg/mL bovine serum albumin, 1 mM ATP, dNTPs at S-phase concentrations (30 μM dCTP, 80 μM dTTP, 38 μM dATP, and 26 μM dGTP) (47, 48) or imbalanced concentrations (30 μM dCTP, 80 μM dTTP, 7.9 μM dATP, and 4 mM dGTP), 2 nM singly primed M13/CAN1(1-1560-F), 790 nM RPA, 2 nM RFC, 21 nM PCNA, 50 nM Polε or Polε-exo$^-$, and, when indicated, 1 or 5 nM wild-type Polδ. RPA was the first protein added to the reaction, followed by a 1-min incubation at 30 °C, then RFC and PCNA were added, followed by another 1-min incubation at

30 °C. Replication was initiated by the addition of Polε. For the extrinsic proofreading assay, replication by Polε-exo$^-$ was allowed to proceed for 7 min after which Polδ was added, and the reaction was incubated for an additional 3 min. Reactions were stopped by the addition of 1 μL of 500 mM ethylenediaminetetraacetic acid (EDTA) and 1 μL of 10% sodium dodecyl sulfate, incubated with 2 μL of 20 mg/mL Proteinase K (ThermoFisher Scientific) at 55 °C for 1 h and purified by phenol/chloroform extraction and ethanol precipitation. DNA pellets were dissolved in 20 μL ddH_2O and mixed with 4 μL of 6× alkaline loading buffer containing 300 mM NaOH, 6 mM EDTA, 18% (wt/vol) Ficoll, 0.15% (wt/vol) bromocresol green, and 0.25% (wt/vol) xylene cyanol. The reaction products were separated in a 1% alkaline agarose gel at 70 V for 20 h in a cold room. Quantification was performed by fluorescence imaging on a Typhoon system (GE Healthcare). Percent synthesis was calculated as a percentage of total pixel intensity of the lane using ImageQuant software (v2003.02).

Data Availability. All data used to reach the conclusions are presented fully within the article and *SI Appendix*.

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1. A. Morrison, H. Araki, A. B. Clark, R. K. Hamatake, A. Sugino, A third essential DNA polymerase in *S. cerevisiae*. *Cell* **62**, 1143–1151 (1990).
2. P. V. Shcherbakova, Y. I. Pavlov, 3′→5′ exonucleases of DNA polymerases ε and δ correct base analog induced DNA replication errors on opposite DNA strands in *Saccharomyces cerevisiae*. *Genetics* **142**, 717–726 (1996).
3. Z. F. Pursell, I. Isoz, E. B. Lundström, E. Johansson, T. A. Kunkel, Yeast DNA polymerase ε participates in leading-strand DNA replication. *Science* **317**, 127–130 (2007).
4. S. A. Nick McElhinny, D. A. Gordenin, C. M. Stith, P. M. Burgers, T. A. Kunkel, Division of labor at the eukaryotic replication fork. *Mol. Cell* **30**, 137–144 (2008).
5. A. A. Larrea *et al*., Genome-wide model for the normal eukaryotic DNA replication fork. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 17674–17679 (2010).
6. E. Shinbrot *et al*., Exonuclease mutations in DNA polymerase epsilon reveal replication strand specific mutation patterns and human origins of replication. *Genome Res.* **24**, 1740–1750 (2014).
7. Y. Daigaku *et al*., A global profile of replicative polymerase usage. *Nat. Struct. Mol. Biol.* **22**, 192–198 (2015).
8. A. R. Clausen *et al*., Tracking replication enzymology in vivo by genome-wide mapping of ribonucleotide incorporation. *Nat. Struct. Mol. Biol.* **22**, 185–191 (2015).
9. Y. I. Pavlov *et al*., Evidence that errors made by DNA polymerase α are corrected by DNA polymerase δ. *Curr. Biol.* **16**, 202–207 (2006).
10. Y. H. Jin *et al*., The 3′→5′ exonuclease of DNA polymerase δ can substitute for the 5′ flap endonuclease Rad27/Fen1 in processing Okazaki fragments and preventing genome instability. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 5122–5127 (2001).
11. P. Garg, C. M. Stith, N. Sabouri, E. Johansson, P. M. Burgers, Idling by DNA polymerase δ maintains a ligatable nick during lagging-strand DNA replication. *Genes Dev.* **18**, 2764–2773 (2004).
12. L. D. Langston *et al*., CMG helicase and DNA polymerase ε form a functional 15-subunit holoenzyme for eukaryotic leading-strand DNA replication. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 15390–15395 (2014).
13. J. T. P. Yeeles, A. Janska, A. Early, J. F. X. Diffley, How the eukaryotic replisome achieves rapid and efficient DNA replication. *Mol. Cell* **65**, 105–116 (2017).
14. V. Aria, J. T. P. Yeeles, Mechanism of bidirectional leading-strand synthesis establishment at eukaryotic DNA replication origins. *Mol. Cell* **73**, 199–211 (2018).
15. M. A. Garbacz *et al*., Evidence that DNA polymerase δ contributes to initiating leading strand DNA replication in *Saccharomyces cerevisiae*. *Nat. Commun.* **9**, 858 (2018).
16. Z. X. Zhou, S. A. Lujan, A. B. Burkholder, M. A. Garbacz, T. A. Kunkel, Roles for DNA polymerase δ in initiating and terminating leading strand DNA replication. *Nat. Commun.* **10**, 3992 (2019).
17. A. Morrison, J. B. Bell, T. A. Kunkel, A. Sugino, Eukaryotic DNA polymerase amino acid sequence required for 3′—5′ exonuclease activity. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9473–9477 (1991).
18. M. Simon, L. Giot, G. Faye, The 3′ to 5′ exonuclease activity located in the DNA polymerase δ subunit of *Saccharomyces cerevisiae* is required for accurate replication. *EMBO J.* **10**, 2165–2170 (1991).
19. A. Morrison, A. Sugino, The 3′→5′ exonucleases of both DNA polymerases δ and ε participate in correcting errors of DNA replication in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **242**, 289–296 (1994).
20. P. V. Shcherbakova, V. N. Noskov, M. R. Pshenichnov, Y. I. Pavlov, Base analog 6-*N*-hydroxylaminopurine mutagenesis in the yeast *Saccharomyces cerevisiae* is controlled by replicative DNA polymerases. *Mutat. Res.* **369**, 33–44 (1996).
21. H. T. Tran, J. D. Keen, M. Kricker, M. A. Resnick, D. A. Gordenin, Hypermutability of homonucleotide runs in mismatch repair and DNA polymerase proofreading yeast mutants. *Mol. Cell. Biol.* **17**, 2859–2865 (1997).
22. H. T. Tran, D. A. Gordenin, M. A. Resnick, The 3′→5′ exonucleases of DNA polymerases δ and ε and the 5′→3′ exonuclease Exo1 have major roles in postreplication mutation avoidance in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **19**, 2000–2007 (1999).
23. T. Ohya *et al*., The DNA polymerase domain of pol(ε) is required for rapid, efficient, and highly accurate chromosomal DNA replication, telomere length maintenance, and normal cell senescence in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **277**, 28099–28108 (2002).
24. Y. I. Pavlov, S. Maki, H. Maki, T. A. Kunkel, Evidence for interplay among yeast replicative DNA polymerases α, δ and ε from studies of exonuclease and polymerase active site mutations. *BMC Biol.* **2**, 11 (2004).
25. M. B. Lee *et al*., Defining the impact of mutation accumulation on replicative lifespan in yeast using cancer-associated mutator phenotypes. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 3062–3071 (2019).
26. A. Morrison, A. L. Johnson, L. H. Johnston, A. Sugino, Pathway correcting DNA replication errors in *Saccharomyces cerevisiae*. *EMBO J.* **12**, 1467–1473 (1993).
27. J. A. St Charles, S. E. Liberti, J. S. Williams, S. A. Lujan, T. A. Kunkel, Quantifying the contributions of base selectivity, proofreading and mismatch repair to nuclear DNA replication in *Saccharomyces cerevisiae*. *DNA Repair (Amst.)* **31**, 41–51 (2015).
28. Y. I. Pavlov, P. V. Shcherbakova, T. A. Kunkel, *In vivo* consequences of putative active site mutations in yeast DNA polymerases α, ε, δ, and ζ. *Genetics* **159**, 47–64 (2001).
29. Y. I. Pavlov, P. V. Shcherbakova, DNA polymerases at the eukaryotic fork-20 years later. *Mutat. Res.* **685**, 45–53 (2010).
30. L. N. Williams, A. J. Herr, B. D. Preston, Emergence of DNA polymerase ε antimutators that escape error-induced extinction in yeast. *Genetics* **193**, 751–770 (2013).
31. C. L. Flood *et al*., Replicative DNA polymerase δ but not ε proofreads errors in *Cis* and in *Trans*. *PLoS Genet.* **11**, e1005049 (2015).
32. F. W. Perrino, L. A. Loeb, Proofreading by the ε subunit of *Escherichia coli* DNA polymerase III increases the fidelity of calf thymus DNA polymerase α. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3085–3088 (1989).
33. F. W. Perrino, L. A. Loeb, Hydrolysis of 3′-terminal mispairs in vitro by the 3′—5′ exonuclease of DNA polymerase δ permits subsequent extension by DNA polymerase α. *Biochemistry* **29**, 5226–5231 (1990).
34. K. R. Brown, K. L. Weatherdon, C. L. Galligan, V. Skalski, A nuclear 3′-5′ exonuclease proofreads for the exonuclease-deficient DNA polymerase α. *DNA Repair (Amst.)* **1**, 795–810 (2002).
35. N. V. Belyakova *et al*., Proof-reading 3′–>5′ exonucleases isolated from rat liver nuclei. *Eur. J. Biochem.* **217**, 493–500 (1993).
36. P. Huang, Excision of mismatched nucleotides from DNA: A potential mechanism for enhancing DNA replication fidelity by the wild-type p53 protein. *Oncogene* **17**, 261–270 (1998).
37. S. D. McCulloch *et al*., Enzymatic switching for efficient and accurate translesion DNA replication. *Nucleic Acids Res.* **32**, 4665–4675 (2004).
38. R. P. Fuchs, S. Fujii, Translesion synthesis in *Escherichia coli*: Lessons from the NarI mutation hot spot. *DNA Repair (Amst.)* **6**, 1032–1041 (2007).
39. D. P. Kane, P. V. Shcherbakova, A common cancer-associated DNA polymerase ε mutation causes an exceptionally strong mutator phenotype, indicating fidelity defects distinct from loss of proofreading. *Cancer Res.* **74**, 1895–1901 (2014).
40. A. Datta *et al*., Checkpoint-dependent activation of mutagenic repair in *Saccharomyces cerevisiae pol3-01* mutants. *Mol. Cell* **6**, 593–603 (2000).
41. Y. H. Jin *et al*., The multiple biological roles of the 3′→5′ exonuclease of *Saccharomyces cerevisiae* DNA polymerase δ require switching between the polymerase and exonuclease domains. *Mol. Cell. Biol.* **25**, 461–471 (2005).
42. G. T. Marsischky, N. Filosi, M. F. Kane, R. Kolodner, Redundancy of *Saccharomyces cerevisiae MSH3* and *MSH6* in *MSH2*-dependent mismatch repair. *Genes Dev.* **10**, 407–420 (1996).
43. P. V. Shcherbakova *et al*., Unique error signature of the four-subunit yeast DNA polymerase ε. *J. Biol. Chem.* **278**, 43770–43780 (2003).
44. J. M. Fortune, C. M. Stith, G. E. Kissling, P. M. Burgers, T. A. Kunkel, RPA and PCNA suppress formation of large deletion errors by yeast DNA polymerase δ. *Nucleic Acids Res.* **34**, 4335–4341 (2006).
45. X. Xing *et al*., A recurrent cancer-associated substitution in DNA polymerase ε produces a hyperactive enzyme. *Nat. Commun.* **10**, 374 (2019).
46. S. A. Nick McElhinny, C. M. Stith, P. M. Burgers, T. A. Kunkel, Inefficient proofreading and biased error rates during inaccurate DNA synthesis by a mutant derivative of *Saccharomyces cerevisiae* DNA polymerase δ. *J. Biol. Chem.* **282**, 2324–2332 (2007).
47. T. M. Mertz, S. Sharma, A. Chabes, P. V. Shcherbakova, Colon cancer-associated mutator DNA polymerase δ variant causes expansion of dNTP pools increasing its own infidelity. *Proc. Natl. Acad. Sci. U.S.A.* **112**, E2467–E2476 (2015).
48. N. Sabouri, J. Viberg, D. K. Goyal, E. Johansson, A. Chabes, Evidence for lesion bypass by yeast replicative DNA polymerases during DNA damage. *Nucleic Acids Res.* **36**, 5660–5667 (2008).
49. S. Muramatsu, K. Hirai, Y. S. Tak, Y. Kamimura, H. Araki, CDK-dependent complex formation between replication proteins Dpb11, Sld2, Pol ε, and GINS in budding yeast. *Genes Dev.* **24**, 602–612 (2010).
50. O. M. Aparicio, D. M. Weinstein, S. P. Bell, Components and dynamics of DNA replication complexes in *S. cerevisiae*: Redistribution of MCM proteins and Cdc45p during S phase. *Cell* **91**, 59–69 (1997).
51. J. Sun, Z. Yuan, R. Georgescu, H. Li, M. O'Donnell, The eukaryotic CMG helicase pumpjack and integration into the replisome. *Nucleus* **7**, 146–154 (2016).
52. O. Chilkova *et al*., The eukaryotic leading and lagging strand DNA polymerases are loaded onto primer-ends via separate mechanisms but have comparable processivity in the presence of PCNA. *Nucleic Acids Res.* **35**, 6588–6597 (2007).

53. S. Boiteux, S. Jinks-Robertson, DNA repair mechanisms and the bypass of DNA damage in *Saccharomyces cerevisiae*. *Genetics* **193**, 1025–1064 (2013).
54. S. Sharma, C. M. Helchowski, C. E. Canman, The roles of DNA polymerase ζ and the Y family DNA polymerases in promoting or preventing genome instability. *Mutat. Res.* **743–744**, 97–110 (2013).
55. J. G. Jansen *et al*., Redundancy of mammalian Y family DNA polymerases in cellular responses to genomic DNA lesions induced by ultraviolet light. *Nucleic Acids Res.* **42**, 11071–11082 (2014).
56. N. Hosoya, K. Miyagawa, Targeting DNA damage response in cancer therapy. *Cancer Sci.* **105**, 370–388 (2014).
57. J. S. Choi, A. Berdis, Combating resistance to DNA damaging agents. *Oncoscience* **5**, 134–136 (2018).
58. J. S. Choi, S. Kim, E. Motea, A. Berdis, Inhibiting translesion DNA synthesis as an approach to combat drug resistance to DNA damaging agents. *Oncotarget* **8**, 40804–40816 (2017).
59. A. J. Herr, S. R. Kennedy, G. M. Knowels, E. M. Schultz, B. D. Preston, DNA replication error-induced extinction of diploid yeast. *Genetics* **196**, 677–691 (2014).
60. C. M. Manhart, E. Alani, DNA replication and mismatch repair safeguard against metabolic imbalances. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 5561–5563 (2017).
61. T. T. Schmidt *et al*., Alterations in cellular metabolism triggered by *URA7* or *GLN3* inactivation cause imbalanced dNTP pools and increased mutagenesis. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E4442–E4451 (2017).
62. S. R. Barbari, P. V. Shcherbakova, Replicative DNA polymerase defects in human cancers: Consequences, mechanisms, and implications for therapy. *DNA Repair (Amst.)* **56**, 16–25 (2017).
63. S. R. Barbari, D. P. Kane, E. A. Moore, P. V. Shcherbakova, Functional analysis of cancer-associated DNA polymerase ε variants in *Saccharomyces cerevisiae*. *G3 (Bethesda)* **8**, 1019–1029 (2018).
64. H. D. Li *et al*., Polymerase-mediated ultramutagenesis in mice produces diverse cancers with high mutational load. *J. Clin. Invest.* **128**, 4179–4191 (2018).
65. T. M. Albertson *et al*., DNA polymerase ε and δ proofreading suppress discrete mutator and cancer phenotypes in mice. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 17101–17104 (2009).
66. R. E. Goldsby *et al*., Defective DNA polymerase-δ proofreading causes cancer susceptibility in mice. *Nat. Med.* **7**, 638–639 (2001).
67. R. E. Goldsby *et al*., High incidence of epithelial cancers in mice deficient for DNA polymerase δ proofreading. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 15560–15565 (2002).
68. P. V. Shcherbakova, T. A. Kunkel, Mutator phenotypes conferred by *MLH1* overexpression and by heterozygosity for *mlh1* mutations. *Mol. Cell. Biol.* **19**, 3177–3183 (1999).
69. J. M. Kirchner, H. Tran, M. A. Resnick, A DNA polymerase ε mutant that specifically causes +1 frameshift mutations within homonucleotide runs in yeast. *Genetics* **155**, 1623–1632 (2000).
70. R. J. Kokoska *et al*., Destabilization of yeast micro- and minisatellite DNA sequences by mutations affecting a nuclease involved in Okazaki fragment processing (*rad27*) and DNA polymerase δ (*pol3-t*). *Mol. Cell. Biol.* **18**, 2779–2788 (1998).
71. L. Li, K. M. Murphy, U. Kanevets, L. J. Reha-Krantz, Sensitivity to phosphonoacetic acid: A new phenotype to probe DNA polymerase δ in *Saccharomyces cerevisiae*. *Genetics* **170**, 569–580 (2005).
72. A. L. Goldstein, J. H. McCusker, Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* **15**, 1541–1553 (1999).
73. M. R. Northam, H. A. Robinson, O. V. Kochenova, P. V. Shcherbakova, Participation of DNA polymerase ζ in replication of undamaged DNA in *Saccharomyces cerevisiae*. *Genetics* **184**, 27–42 (2010).
74. J. W. Drake, A constant rate of spontaneous mutation in DNA-based microbes. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7160–7164 (1991).
75. D. L. Daee, T. M. Mertz, P. V. Shcherbakova, A cancer-associated DNA polymerase δ variant modeled in yeast causes a catastrophic increase in genomic instability. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 157–162 (2010).
76. M. R. Northam *et al*., DNA polymerases ζ and Rev1 mediate error-prone bypass of non-B DNA structures. *Nucleic Acids Res.* **42**, 290–306 (2014).