HHS Public Access

Author manuscript

DNA Repair (Amst). Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

DNA Repair (Amst). 2015 July ; 31: 41–51. doi:10.1016/j.dnarep.2015.04.006.

Quantifying the contributions of base selectivity, proofreading and mismatch repair to nuclear DNA replication in *Saccharomyces cerevisiae*

Jordan A. St Charles, **Sascha E. Liberti**, **Jessica S. Williams**, **Scott A. Lujan**, and **Thomas A. Kunkel***

Laboratory of Molecular Genetics and Laboratory of Structural Biology, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC 27709, United States

Abstract

Mismatches generated during eukaryotic nuclear DNA replication are removed by two evolutionarily conserved error correction mechanisms acting in series, proofreading and mismatch repair (MMR). Defects in both processes are associated with increased susceptibility to cancer. To better understand these processes, we have quantified base selectivity, proofreading and MMR during nuclear DNA replication in *Saccharomyces cerevisiae*. In the absence of proofreading and MMR, the primary leading and lagging strand replicases, polymerase ε and polymerase δ respectively, synthesize DNA *in vivo* with somewhat different error rates and specificity, and with apparent base selectivity that is more than 100 times higher than measured *in vitro*. Moreover, leading and lagging strand replication fidelity rely on a different balance between proofreading and MMR. On average, proofreading contributes more to replication fidelity than does MMR, but their relative contributions vary from nearly all proofreading of some mismatches to mostly MMR of other mismatches. Thus accurate replication of the two DNA strands results from a non-uniform and variable balance between error prevention, proofreading and MMR.

Keywords

Replication fidelity; DNA polymerase; Genome stability; Mutation rate

1. Introduction

In an unperturbed eukaryotic cell cycle, high nuclear DNA replication fidelity is achieved through the sequential operation of three processes: the selectivity of three replicative DNA polymerases (replicases) for inserting correct nucleotides into properly aligned DNA substrates, exonucleolytic proofreading of mismatches made during replication, and DNA mismatch repair (MMR) of rare errors that escape proofreading. In *Escherichia coli*, where DNA polymerase III is the primary replicase for both DNA strands, the relative contribution

*Corresponding author. Tel.: +1 919 541 2644; fax: +1 919 541 7613. kunkel@niehs.nih.gov (T.A. Kunkel).

Conflict of interest

The authors declare that they have no conflict of interest with this work.

of these processes to replication fidelity has been determined by comparing spontaneous mutation rates in a wild type strain to rates in strains deficient in either proofreading by Pol III, in MMR, or in both error correction mechanisms [1]. A similar approach has been applied to replication of the *Saccharomyces cerevisiae* nuclear genome [2,3], but in a less comprehensive manner and at a time when the identities of the major leading and lagging strand replicases were uncertain. Now however, compelling evidence using base substitution patterns (see [4] and references therein) and more recently using strand-specific ribonucleotide incorporation [5–8] as biomarkers of replicase actions indicates that the leading strand is primarily replicated by DNA polymerase ε (Pol ε), the product of the yeast *POL2* gene. Synthesis of the nascent lagging strand involves limited synthesis by Pol α (*POL1*), which is naturally deficient in 3′-exonucleolytic activity and cannot proofread mismatches, which it generates at a rate of about 10^{-4} *in vitro* [9]. Synthesis by Pol α is followed by extensive synthesis by Pol δ (the product of the yeast *POL3* gene). When this knowledge of strand specific replicase activity is combined with use of a mutational reporter gene placed close to a well-studied replication origin, it is now possible to deduce the identity of the mismatches that are being generated, proofread or corrected by MMR during nuclear DNA replication in a yeast cell.

Unlike Pol α, the catalytic subunits of Pol ε [10] and Pol δ [11] have 3′-exonuclease activity for proofreading their own errors. Moreover, there is evidence to suggest that the exonuclease activity of Pol δ, but not that of Pol ε, likely proofreads errors made by Pol α during lagging strand replication [12], and more recent evidence that Pol δ can proofread errors made by Pol ε [13]. Not only is proofreading more complicated in yeast as compared to *E. coli*, but so too is eukaryotic MMR more complicated than in *E. coli*. This is because two complexes of eukaryotic *M*ut*S H*omologs are present for MMR. Single base–base mismatches that can result in base substitutions are primarily repaired by Msh2–Msh6 (MutSα), with Msh2–Msh3 (MutSβ) having but a much smaller role [14,15]. However, MutSα and MutSβ can both participate in repairing insertion-deletion (indel) mutations containing one or two unpaired bases, and MutSβ has primary responsibility for repairing mismatches containing multiple unpaired bases [16].

In this study, we have examined the contribution of all three replication fidelity processes to genome stability in budding yeast. The complexity described above necessitates the use of several yeast strains to quantify the contributions of nucleotide selectivity, proofreading and MMR to leading and lagging strand DNA replication fidelity. Here we use the *URA3* reporter gene that scores all types of substitutions in many different sequence contexts. We compare mutation rates in a wild type strain to rates in strains defective in proofreading by Pol δ (*pol3-5DV*, subsequently referred to as *pol3-exo*$^-$) [17], or defective in proofreading by Pol ε (*pol2-04*, subsequently referred to as *pol2-exo*$^-$) [10], in both cases resulting from mutations in their exonuclease active sites. Rates in MMR-proficient strains are compared to rates in *msh6*Δ strains that cannot repair the vast majority of single base-base mismatches due to inactivation of MutSα. However, in these strains, most indel mismatches are repaired by MutSβ, thus reducing the possibility of error catastrophe due to lethal indels in strains lacking both proofreading and MMR. Here we have determined mutation rates and mutational spectra in all strains, and used these data to calculate rates for each type of base

substitution. Pairwise comparisons of rates among strains then allowed us to estimate the contributions of the three fidelity processes to different single base-base mismatches in different sequence contexts, and to do so for leading and lagging strand replication. The results indicate that on average, proofreading contributes more to replication fidelity than MMR, but the relative importance of proofreading and MMR for correcting replication errors varies widely. In the absence of both MMR and proofreading, Pols δ and ε generate errors *in vivo* at much lower rates than have been measured *in vitro*, suggesting that additional fidelity mechanisms may operate at the replication fork *in vivo*.

2. Methods

2.1. Strain construction

The identities and sources of the strains used in this study are listed in Supplementary Table 1. For this study, we used *pol2-04* (Pol ε) and *pol3-5DV* (Pol δ) mutants that are subsequently referred to as *pol2-exo*$^-$ and *pol3-exo*$^-$, respectively. In the *pol2-04* mutant, alanines were substituted for D290 and E292 in the 3′-exonuclease active site, inactivating 3′-exonuclease activity but leaving polymerase activity similar to that of wild type Pol ε [10]. The homologous allele for Pol δ is *pol3-01* (D321A, E323A [18]), but this allele is lethal in combination with *msh6*Δ [19]. We therefore used the *pol3-5DV* mutant, in which a valine was substituted for D520 in the exonuclease active site to inactivate 3′-exonuclease activity but leave polymerase activity similar to that of wild type Pol δ [20].

2.2. Mutation rate measurements and mutational spectra

Spontaneous mutation rates at the *URA3* locus were measured by fluctuation analysis as described [21,22]. *URA3* mutation spectra were obtained by sequencing the *URA3* gene in collections of independent 5-fluoroorotic acid-resistant (5-FOA^R) colonies. Each 5-FOA^R colony from the double mutant strains was obtained from an independent spore. Genomic DNA was isolated from 5-FOA^R colonies, the *URA3* gene was PCR-amplified and the DNA product was sequenced. Rates for each type of mutation are calculated as the total number of each type of mutation divided by the total number of 5-FOA^R mutants sequenced and then multiplied by the total mutation rate. For individual types of base substitutions, the substitution rate per base pair per generation was calculated by dividing the mutation rate by the number of sites in the *URA3* gene where that event is known to result in 5-FOA^R (Supplementary Fig. 1). The contributions of base selectivity, proofreading and mismatch repair to replication fidelity were calculated as described below.

3. Results and discussion

3.1. Approach

We measured mutation rates in yeast strains that were wild type for proofreading and MMR, deficient in proofreading (*pol2-exo*$^-$ or *pol3-exo*$^-$) only, deficient in MMR of base-base mismatches (*msh6*Δ), or deficient in proofreading and MMR (*pol2-exo*$^-$ *msh6*Δ or *pol3-exo*$^-$ *msh6*Δ). Because the double mutant strains are highly mutable and rapidly accumulates mutations that could modulate mutation rates, we minimized the number of generations used to measure rates by sporulating diploid strains that were heterozygous for proofreading.

Spore viability for the *POL2/pol2-exo⁻msh6*Δ/*MSH6* and *POL3/pol3-exo⁻ msh6*Δ/*MSH6* strains were 94% and 96%, respectively. The *pol2-exo⁻ msh6*Δ mutants have normal colony size and the *pol3-exo⁻ msh6*Δ mutants are smaller than normal (Supplementary Fig. 2). 60–100 independent, double mutant spore colonies were used to measure the rate of resistance to 5-FOA, which primarily scores mutations in the *URA3* gene, including substitutions resulting from all 12 possible single base mismatches at numerous locations in the coding sequence (Supplementary Fig. 1). In the strains used here, *URA3* is located about 2000 base pairs from *ARS306*, an early-firing and efficient replication origin on chromosome 3. The next closest origin (*ARS307*) is about 35,000 base pairs away, such that the replication fork originating at *ARS306* will generate the majority of errors in *URA3*, an interpretation strongly supported by recent ribonucleotide mapping studies [5–8]. In this situation, the coding sequence depicted in Supplementary Fig. 1 is the template for lagging strand synthesis, and the complementary non-coding sequence (not shown) is the template for leading strand replication.

3.2. Mutation rates and specificity

In the wild type strain, the base substitution rate for resistance to 5-FOA is low, and close to the unbiased substitution rate for the whole yeast genome [4]. This fact, and the observation that the vast majority of the 5-FOA-resistant mutants have mutations in *URA3* (Table 1), underscores the utility and reliability of *URA3* as a reporter for genome stability. Compared to the wild type strain, mutation rates in the *pol2-exo⁻*, *pol3-exo⁻* and *msh6*Δ single mutant strains are elevated by 19- to 45-fold (Fig. 1A). These increases are consistent with earlier studies in yeast demonstrating that defects in proofreading or MMR alone elevate mutation rates (see [23,24] and references therein). The mutation rates in the *pol2-exo⁻ msh6*Δ and *pol3-exo⁻ msh6*Δ double mutant strains are much higher than the sum of the single mutant strains (Fig. 1A, right). Again, the results are consistent with earlier studies [18,25] indicating that proofreading and MMR act in series to correct replication errors by Pols δ and ε.

Also, the mutation rate in the *pol3-exo⁻ msh6*Δ strain is higher than that in the *pol2-exo⁻ msh6*Δ strain. Given evidence that the exonuclease activity of Pol δ, but not that of Pol ε, proofreads errors made by Pol α[12], and evidence that Pol δcan also proofread errors made by Pol ε [13], the higher mutation rate in the *pol3-exo⁻ msh6*Δ strain could be due to loss Pol δ proofreading of errors made by any of the three replicases, whereas loss Pol ε may only proofread its own errors. The different mutation rates in the *pol3-exo⁻ msh6*Δ and *pol2-exo⁻ msh6*Δ strains could also be related to differences in activation of the S phase checkpoint in proofreading-deficient strains (see [26] and references therein).

To determine rates for individual substitutions, mutational spectra were obtained by sequencing the *URA3* gene in collections of independent 5-FOA^R colonies. The spectra for wild type *versus msh6*Δ, for *pol2-exo⁻ versus pol2-exo⁻ msh6*Δ, and for *pol3-exo⁻versus pol3-exo⁻ msh6*Δ strains are shown in Figs. 2, 3 and 4, respectively. Table 1 lists the number of occurrences and the rate per generation for each type of substitution, both before and after correcting the rate for the known number of detectable sites in *URA3* (from Supplementary Fig. 1). From these rates, and knowing which strand acts as the template for the majority of

leading or lagging strand replication, we calculated the contributions of base selectivity, proofreading and MMR for total substitutions (Fig. 1B) and for substitutions resulting from formation of each of the 12 possible single base mismatches generated during leading and lagging strand replication (Table 2). The contribution of MMR was calculated by dividing the total base substitution rate in the *exo*$^-$ *msh6*Δ strains by the corresponding base substitution rates in the *exo*$^-$strains. The contribution of proofreading to replication fidelity was determined by dividing mutation rates in the *exo*$^-$ *msh6*Δ strains by the corresponding rates in the *msh6*Δ strain. Apparent base selectivity was calculated as the inverse of the mutation rate for the two *exo*$^-$ *msh6*Δ strains that lack both proofreading and MMR. We also examined the effects of DNA sequence context by calculating rates and fidelity factors for substitutions at specific base pairs in *URA3* (Fig. 5). The latter examples focus on mismatches generated when dTTP, the dNTP present at the highest concentration in yeast [27], is misinserted opposite template G, C or T.

3.3. Contribution of MMR to replication fidelity

Dividing the total base substitution rate in the *pol2-exo*$^-$ *msh6*Δ strain (78×10^{-9}, Table 1) by the corresponding base substitution rate in the *pol2-exo*$^-$ strain (1.2×10^{-9}) estimates the contribution of MMR to the fidelity of replication of the nascent leading strand. This comparison indicates that on average, MMR corrects 64 of every 65 single base-base mismatches generated by Pol ε in the absence of its intrinsic exonuclease activity (Fig. 1B, right-most dark gray bar, and Table 2). The corresponding comparison of the *pol3-exo*$^-$ *msh6*Δ strain to the *pol3-exo*$^-$ strain indicates even greater MMR efficiency for lagging strand errors, where 249 of every 250 mismatches are corrected. These mismatches are generated during lagging strand replication by Pol δ lacking its intrinsic exonuclease activity, as well as by Pol α, which lacks an intrinsic proofreading activity but whose mistakes may sometimes be edited by Pol δ [12]. These results confirm that MMR efficiently corrects replication errors in both nascent strands. They further imply that MMR is somewhat more efficient at correcting lagging strand mismatches as compared to leading strand mismatches. The latter interpretation is similar to that derived from previous studies examining mismatches containing modified bases [28,29], and from studies of mutator replicases rendered inaccurate by replacing an amino acid in the polymerase active site while leaving the proofreading exonuclease active site unperturbed (see [4,21,22,30–32] and references therein). An important difference between the present study and the earlier reports is that here we monitor mismatches involving unmodified bases in cells where a proofreading exonuclease has been inactivated but the polymerase active site and nucleotide selectivity remain wild type. In all these studies, MMR appears to be more efficient at correcting lagging strand errors as compared to leading strand errors, a fact that we previously suggested may reflect a higher density of signals for MMR in the lagging strand, possibly including PCNA [33] and the 5′-ends of Okazaki fragments in the discontinuously replicated lagging strand [32]. The idea that MMR is somewhat more efficient at correcting lagging strand replication errors due to a higher density of 5DNA ends is further supported by studies showing that Exonuclease 1 (Exo1), a 5′-exonuclease, contributes more to MMR of lagging as compared to leading strand [34,35], and that Exo1 makes a larger contribution to MMR of errors made by Pol α as compared to errors made by Pol δ, which are always more distal to 5′-DNA ends of Okazaki fragments [35].

On average, transition mismatches are repaired more efficiently than are transversion mismatches (Table 2). This result is consistent with similar observations on MMR in *E. coli* [1]. Nonetheless, a general conclusion that transition mismatches are repaired more efficiently than transversion mismatches in yeast is unwarranted, because apparent MMR efficiencies vary widely among the 12 mismatches. For example, in the *pol2-exo*$^-$ background, one of the four transversion mismatches (template C-incoming dTTP, 490-fold MMR correction factor, Table 2) is repaired more efficiently than any of the four purine–pyrimidine mismatches that result in transitions. Moreover, this same pyrimidine–pyrimidine mismatch (C-dT, 490-fold) is repaired much more efficiently than another pyrimidine–pyrimidine mismatch (T-dT, 5.8-fold). MMR efficiency also varies in the *pol3-exo*$^-$ background, but less so (range of 16-fold, Table 2). In some cases, the efficiency of repairing a mismatch containing the same two bases varies depending on which base is in the template and which base is the incoming nucleotide. The largest such difference is in the *pol2-exo*$^-$ background, where MMR repairs 489 of 490 template C–dT mismatches but only 21 of 22 T–dC mismatches (Table 2). Similar but smaller MMR biases are also observed in the *pol3-exo*$^-$ background (Table 2). Theoretically, these variations may reflect the binding affinity of yeast MutSα for mismatches of different composition, which vary more than 10-fold [36], or they may reflect MMR events downstream of MutSα binding.

3.4. Contribution of proofreading to replication fidelity

The contribution of proofreading to replication fidelity was determined by dividing mutation rates in the *pol2-exo*$^-$ *msh6*Δ or *pol3-exo*$^-$ *msh6*Δ strain by the corresponding rates in the *msh6*Δ strain. When all substitutions are considered, the average contribution of proofreading to replication fidelity *in vivo* is 160-fold for Pol ε and 1000-fold for Pol δ (Fig. 2, middle, and Table 2). These results establish that, on average, (i) proofreading contributes several-fold more than MMR to both leading and lagging strand replication fidelity *in vivo*, and (ii) proofreading of lagging strand mismatches is more efficient than proofreading of leading strand mismatches (Fig. 1B). Just as for MMR, proofreading efficiency also varies widely (Table 2). In the *pol2-exo*$^-$ background, transversion mismatches are proofread four times more efficiently than are transition mismatches (300-fold *versus* 73-fold), whereas the opposite is true in the *pol3-exo*$^-$ background (250 *versus* 1500). In the *pol2-exo*$^-$ background, proofreading efficiency among the mismatches varies by more than 100-fold, from 910-fold for T–dT to only 7-fold for G–dA in the *pol2-exo*$^-$ strain, and from 5800-fold for T–dG to 28-fold for G–dA. Mismatch symmetry also matters, as exemplified by the 11-fold difference in proofreading efficiency between a C–dT and T–dC mismatch in the *pol3-exo*$^-$ background (250 *versus* 2800).

3.5. Variations in the relative importance of proofreading and MMR

Replication errors in the *URA3* open reading frame are non-uniformly distributed (Figs. 2–4). For example, among 178 independent 5-FOA^R colonies from the *pol2-exo*$^-$ *msh6*Δ strain (Table 1), 43 colonies contained a G to T substitution at base pair 679 (Fig. 3). By comparison, a combined total of only 39 G to T substitutions was observed at all 56 other G-C base pairs in *URA3* where this substitution results in resistance to 5-FOA (Supplementary Fig. 1). These substitutions reflect uncorrected misinsertion of dTTP opposite template C during leading strand replication by Pol ε. The existence of this and other such hotspots

provides the opportunity to quantify the relative contributions of proofreading and MMR to correcting individual mismatches of the same base composition and symmetry. In Fig. 5, average proofreading and MMR correction factors for all substitutions (panels A and B) are compared to average correction factors for two mismatches (panels C and D), and to site-specific examples of these same mismatches (panels E and F). All of these mismatches involve the misinsertion of a dTTP, the dNTP present at the highest concentration in yeast [27], and whose misincorporation is responsible for the greatest proportion of substitutions. In the *pol2-exo*$^-$ strain, misinsertion of dTTP opposite template C at base pair 345 is corrected more efficiently by proofreading than by MMR, whereas the opposite is true for the same mismatch but in a different sequence context at base pair 679 (Fig. 5E). Similarly in the *pol3-exo*$^-$ strain, misinsertion of dTTP opposite template G at base pair 368 is corrected more efficiently by proofreading than by MMR, but the opposite is true for the same error but at base pair 764 (Fig. 5F). These examples clearly illustrate that even when mismatches of the same composition and symmetry are considered, the relative contributions of proofreading and MMR to genome stability strongly depend on the local sequence environment. Possible explanations for these observations come from studies showing that proofreading depends on local base-base stacking interactions and on the concentrations of correct dNTPs to be incorporated after misinsertion (reviewed in [24]). Both parameters affect partitioning between polymerization to embed the mismatch in the DNA duplex and thus protect it from removal, *versus* fraying of the primer terminus to permit movement of the error to the exonuclease active site for removal. Compared to proofreading, these parameters may be less relevant to MMR, whose efficiency may depend more on the affinity of MutSε to bind a mismatch, the flexibility of a mismatched DNA duplex, and/or steps further downstream in the MMR pathway. The observations showing that the relative contributions of proofreading and MMR to replication fidelity vary by mismatch composition, sequence environment, replicase and DNA strand (Table 2, Fig. 5) illustrate the difficulty in predicting (i) the consequences of defects in these two processes on specific genes or other functional non-coding sequences in the genome, and (ii) the relative importance of proofreading *versus* MMR in preventing the mutations that drive tissue-specific tumorigenesis [23,37–42].

3.6. Contribution of base selectivity to replication fidelity

Base selectivity was calculated here as the inverse of the mutation rate for the *pol2-exo*$^-$ *msh6*Δ or *pol3-exo*$^-$ *msh6*Δ strains that lack proofreading and MMR. Note that mismatches in the *pol3-exo*$^-$*msh6*Δ strain can be caused by either Pol δ or Pol α, such that the calculated selectivity values are estimates of “apparent” base selectivity, which could be different *in vivo* (discussed further below). The calculated selectivity values show that on average, Pol ε and Pol δ create only one mismatch that results in a base substitution for every 1.3×10^7 or 2.0×10^6 correct bases incorporated, respectively (Fig. 1B). Thus base selectivity appears to be by far the largest contributor to the fidelity of both leading and lagging strand replication. Interestingly, while Pol ε discriminates about equally well against transition and transversion mismatches (fidelity factors of 2.1×10^7 and 1.4×10^7 correctly replicated bases per mismatch, respectively, Table 2), Pol δ discriminates against transversions (blue events in Fig. 4) better than transitions (red events in Fig. 4) by 17-fold (Table 2, 17×10^6 *versus* 1×10^6). Among different mismatches generated in the *pol2-exo*$^-$ background,

selectivity against A–dG mismatches (≥2.6 × 10^8) is 60-fold higher than against C–dT mismatches (4.3 × 10^6), whereas in the *pol3-exo*$^-$ background, selectivity against G–dG mismatches (9.2 × 10^7) is 140-fold higher than against G–dT mismatches (6.7 × 10^5). These results imply that, during nuclear DNA replication *in vivo*, (i) apparent base selectivity is higher for leading than lagging strand replication, and (ii) the two major nuclear replicases have substantially different misincorporation specificities regarding mismatch composition (Table 2) and sequence context (different hotspots in Figs. 3 and 4).

When the dNTPs responsible for the substitutions in Table 1 are totaled irrespective of the template base, dTTP is misincorporated more often than the other three dNTPs, and during replication of both strands. This fact is interesting because the intracellular concentration of dTTP in yeast is about 2 to 3-fold higher than the concentrations of the other three dNTPs [27]. This suggests that this natural dNTP pool imbalance influences replication error rate and error specificity by Pols ε and α, which has implications in mammalian cells where dNTP pools are also naturally imbalanced [43,44].

The rank order of selectivity against certain mismatches is somewhat unexpected. Previous studies in a variety of systems led to the idea that the most commonly formed base–base mismatch is G–T, whereas pyrimidine–pyrimidine mismatches, especially C–dC, are rarely formed. This generally appears to be the case for lagging strand replication, where selectivity against G–dT and T–dG is at least 10-fold lower than for the other 10 mismatches, and where selectivity against C–C and the other pyrimidine–pyrimidine mismatches is very high (Table 2). However, this does not appear to be the case for leading strand replication, where the lowest selectivity is for the C–dT mismatch, and selectivity against this and two of the other three pyrimidine–pyrimidine mismatches (T–dC and T–dT) is similar to selectivity against G–dT and T–dG mismatches. Differences in the error specificity of Pol ε and Pol δ are still apparent, albeit reduced, even after subtracting the events at the hottest of the substitution hot spots in the spectra (*e.g.*, transversions at base pairs 279, 679 and 686 in Fig. 3). These data suggest that the active sites of the two major nuclear replicases impose different structural, kinetic and/or thermodynamic constraints on formation of mismatches of the same composition.

3.7. *In vivo* influences promoting increased replication fidelity

Proofreading-deficient Pol ε and Pol δ generate only one error for every 12,000,000 or 2,000,000 correct bases replicated *in vivo*, respectively (Fig. 1B). Both values are much higher than the base selectivity of proofreading-deficient yeast Pol ε and Pol δ measured during DNA synthesis *in vitro* (black lines in bars in Fig. 1B), where one error is observed for every 4.200 and 7700 correct bases incorporated, respectively [45,46]. These differences are large, 2900-fold for Pol ε and 280-fold for Pol α. There are several non-exclusive possibilities for these differences. One fact that may be relevant is that the DNA synthesis reaction mixtures *in vitro* contain all four dNTPs at a concentration of 100 μM, whereas dNTP concentrations in yeast are lower and slightly imbalanced [27,47]. Other reaction parameters *in vitro* may be less than optimal for high selectivity, including the pH and magnesium concentration, both of which are known to affect the fidelity of DNA synthesis *in vitro* [48–50]. Replication accessory proteins not yet examined *in vitro* could also

improve fidelity *in vivo*, although studies to date have not revealed large increases in nucleotide selectivity by accessory proteins.

A perhaps more interesting possibility for the higher "apparent" base selectivity *in vivo* is extrinsic proofreading. Biochemical [51] and genetic evidence [2,12] suggest that errors made by naturally exonuclease-deficient Pol α, whose primary role is in lagging strand replication, can be proofread by Pol δ. Thus it is possible that in the absence of only one of the two proofreading activities intrinsic to Pol ε and Pol δ, the exonuclease that remains intact in one replicase can proofread mismatches generated by the other major, but proofreading-deficient replicase [2,12]. Indeed, a recent study [13] provides evidence that Pol δ proofreads errors made by Pol ε. Theoretically, extrinsic proofreading may also be catalyzed by other 3′-exonucleases (e.g., see [52]). To the extent that extrinsic proofreading may occur during replication in yeast, this implies that (i) the actual base selectivity of the replicases *in vivo* could be substantially lower than calculated here, and therefore more in line with the estimates from studies *in vitro*, and (ii) the base selectivity calculated here for lagging strand replication may be a mixture of the base selectivity of Pol α plus Pol ε, because both polymerases contribute to the mature lagging strand (see [21,53], and more recently [5,8]).

Also of interest is genetic evidence suggesting that Pol ε does not efficiently proofread errors made by Pol α [12] or errors made by proofreading-deficient Pol ε [13]. Given the participation of Pol δ in several other DNA transactions in cells, it may be that Pol δ is generally more efficient at extrinsic proofreading than is Pol ε. This could account for the 10-fold greater difference in apparent base selectivity *in vivo versus in vitro* mentioned above (2900-fold for the leading strand, 280-fold for the lagging strand). Possibly relevant here is evidence that Pol ε has a strong interaction with the CMG (Cdc45, Mcm2-7, and GINS) complex, while Pol δ has a very weak interaction with the same complex [54]. In contrast, Pol δ has a strong interaction with PCNA, which stimulates its processivity. It is possible that if mismatches created by Pol ε during leading strand replication cannot be proofread by its intrinsic exonuclease, Pol ε can transiently dissociate from the terminal mismatch to allow Pol δ proofreading, and then Pol ε may re-engage and continue leading strand replication [54,55]. Still other possibilities may lie in the identity of yet-to-be disclosed suppressors of mutability in MMR-deficient *pol2-4* strains, leading to the suggestion that factors in addition to proofreading and MMR influence leading-strand DNA replication fidelity [56].

3.8. Complementarity among the three DNA replication fidelity processes

The observation that the two major nuclear replicases have substantially different base selectivity against different mismatches means that the two downstream error correction processes must meet different challenges in order to achieve high fidelity replication of both DNA strands. That proofreading and MMR have evolved to effectively meet these different challenges is illustrated by the following observations. (i) Average base selectivity is lower during lagging strand replication, but average proofreading and MMR efficiencies are higher, whereas the opposite holds for leading strand replication (Fig. 1B). (ii) On average, transitions are generated at a 20-fold higher rate in the *pol3-exo*$^{-}$ *msh6*Δ strain (1300×10^{-7},

Table 1) as compared to the *pol2-exo*$^-$ *msh6*Δ strain (64×10^{-7}), and this difference is counterbalanced by the fact that proofreading of transition mismatches is 20-fold more efficient on the lagging strand (Table 2, correction factor 1500-fold, as compared to the leading strand, 73-fold). (iii) As compared to the above example, average selectivity against transversion mismatches is similar during leading and lagging strand replication, and so too are proofreading and MMR efficiencies (Table 2). (iv) Selection against a C–dT mismatch is relatively low in the *pol2-exo*$^-$ background, and highly efficient proofreading and MMR counterbalance this low selectivity. (*v*) Conversely, high selectivity against certain mismatches, such as C–dA, A–dC, G–dA, is associated with lesser contributions of proofreading and MMR. These observations, in combination with earlier studies of engineered variants of replicases harboring polymerases active site point mutations (see [4] and references therein), support the idea that the eukaryotic replicases, their attendant 3′-exonucleases, and MMR have coevolved to most efficiently correct replication errors made at the highest rates and in the most risky sequence contexts, in order to accurately replicate both DNA strands of the nuclear genome. This idea, and the fact that the contribution to replication fidelity of base selectivity, proofreading and MMR all strongly depend on mismatch composition and symmetry, the replicase, and the strand and sequence context in which the mismatch resides, likely contribute to defining the composition of nuclear genomes. The balancing act among the three replicases may partly underlie differences in life span and tissue-specific tumorigenesis in mice encoding proofreading deficient alleles of Pols ε and δ [57]. These differences may also be relevant to the nature and number of mutations that are thought to drive tissue-specific tumor development in humans harboring mutations in the exonuclease motifs of Pols ε and δ [23,37–42].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Dmitry Gordenin and members of the Genome Integrity and Structural Biology Laboratory at the NIEHS for helpful discussions on experiments, and Kin Chan and Deepa Singh for valuable comments on the manuscript. This work was supported by the Division of Intramural Research of the National Institutes of Health, National Institute of Environmental Health Sciences, by Projects Z01 ES065070 and Z01 ES065089 to TAK.

References

1. Schaaper RM. Base selection, proofreading, and mismatch repair during DNA replication in *Escherichia coli*. J Biol Chem. 1993; 268:23762–23765. [PubMed: 8226906]
2. Morrison A, Sugino A. The 3′ → 5′ exonucleases of both DNA polymerases delta and epsilon participate in correcting errors of DNA replication in *Saccharomyces cerevisiae*. Mol Gen Genet. 1994; 242:289–296. [PubMed: 8107676]
3. Greene CN, Jinks-Robertson S. Spontaneous frameshift mutations in *Saccharomyces cerevisiae*: accumulation during DNA replication and removal by proofreading and mismatch repair activities. Genetics. 2001; 159:65–75. [PubMed: 11560887]
4. Lujan SA, Clausen AR, Clark AB, MacAlpine HK, MacAlpine DM, Malc EP, Burkholder AB, Fargo DC, Gordenin DA, Kunkel TA. Heterogeneous polymerase fidelity and mismatch repair bias genome variation and composition. Genome Res. 2014; 24:1751–1764. [PubMed: 25217194]
5. Clausen AR, Lujan SA, Burkholder AB, Orebaugh CD, Williams JS, Clausen MF, Malc EP, Mieczkowski PA, Fargo DC, Smith DJ, Kunkel TA. Tracking replication enzymology in vivo by

genome-wide mapping of ribonucleotide incorporation. Nat Struct Mol Biol. 2015; 22:185–191. [PubMed: 25622295]

6. Koh KD, Balachander S, Hesselberth JR, Storici F. Ribose-seq: global mapping of ribonucleotides embedded in genomic DNA. Nat Methods. 2015; 12:251–257. [PubMed: 25622106]
7. Daigaku Y, Keszthelyi A, Muller CA, Miyabe I, Brooks T, Retkute R, Hubank M, Nieduszynski CA, Carr AM. A global profile of replicative polymerase usage. Nat Struct Mol Biol. 2015; 22:192–198. [PubMed: 25664722]
8. Reijns MA, Kemp H, Ding J, de Proce SM, Jackson AP, Taylor MS. Lagging-strand replication shapes the mutational landscape of the genome. Nature. 2015; 518:502–506. [PubMed: 25624100]
9. Kunkel TA, Hamatake RK, Motto-Fox J, Fitzgerald MP, Sugino A. Fidelity of DNA polymerase I and the DNA polymerase I-DNA primase complex from *Saccharomyces cerevisiae*. Mol Cell Biol. 1989; 9:4447–4458. [PubMed: 2555694]
10. Morrison A, Bell JB, Kunkel TA, Sugino A. Eukaryotic DNA polymerase amino acid sequence required for 3′–5′ exonuclease activity. Proc Natl Acad Sci U S A. 1991; 88:9473–9477. [PubMed: 1658784]
11. Simon M, Giot L, Faye G. The 3′ to 5′ exonuclease activity located in the DNA polymerase delta subunit of *Saccharomyces cerevisiae* is required for accurate replication. EMBO J. 1991; 10:2165–2170. [PubMed: 1648480]
12. Pavlov YI, Frahm C, Nick McElhinny SA, Niimi A, Suzuki M, Kunkel TA. Evidence that errors made by DNA polymerase alpha are corrected by DNA polymerase delta. Curr Biol. 2006; 16:202–207. [PubMed: 16431373]
13. Flood CL, Rodriguez GP, Bao G, Shockley AH, Kow YW, Crouse GF. Replicative DNA polymerase delta but not epsilon proofreads errors in cis and in trans. PLoS Genet. 2015; 11:e1005049. [PubMed: 25742645]
14. Glaab WE, Risinger JI, Umar A, Kunkel TA, Barrett JC, Tindall KR. Characterization of distinct human endometrial carcinoma cell lines deficient in mismatch repair that originated from a single tumor. J Biol Chem. 1998; 273:26662–26669. [PubMed: 9756907]
15. Harrington JM, Kolodner RD. *Saccharomyces cerevisiae* Msh2–Msh3 acts in repair of base-base mispairs. Mol Cell Biol. 2007; 27:6546–6554. [PubMed: 17636021]
16. Modrich P. Mechanisms in eukaryotic mismatch repair. J Biol Chem. 2006; 281:30305–30309. [PubMed: 16905530]
17. Jin YH, Garg P, Stith CM, Al-Refai H, Sterling JF, Murray LJ, Kunkel TA, Resnick MA, Burgers PM, Gordenin DA. The multiple biological roles of the 3′ → 5′ exonuclease of *Saccharomyces cerevisiae* DNA polymerase delta require switching between the polymerase and exonuclease domains. Mol Cell Biol. 2005; 25:461–471. [PubMed: 15601866]
18. Morrison A, Johnson AL, Johnston LH, Sugino A. Pathway correcting DNA replication errors in *Saccharomyces cerevisiae*. EMBO J. 1993; 12:1467–1473. [PubMed: 8385605]
19. Herr AJ, Ogawa M, Lawrence NA, Williams LN, Eggington JM, Singh M, Smith RA, Preston BD. Mutator suppression and escape from replication error-induced extinction in yeast. PLoS Genet. 2011; 7:e1002282. [PubMed: 22022273]
20. Jin YH, Obert R, Burgers PM, Kunkel TA, Resnick MA, Gordenin DA. The 3′ → 5′ exonuclease of DNA polymerase delta can substitute for the 5′ flap endonuclease Rad27/Fen1 in processing Okazaki fragments and preventing genome instability. Proc Natl Acad Sci U S A. 2001; 98:5122–5127. [PubMed: 11309502]
21. Nick McElhinny SA, Gordenin DA, Stith CM, Burgers PM, Kunkel TA. Division of labor at the eukaryotic replication fork. Mol Cell. 2008; 30:137–144. [PubMed: 18439893]
22. Pursell ZF, Isoz I, Lundstrom EB, Johansson E, Kunkel TA. Yeast DNA polymerase epsilon participates in leading-strand DNA replication. Science. 2007; 317:127–130. [PubMed: 17615360]
23. Kane DP, Shcherbakova PV. A common cancer-associated DNA polymerase epsilon mutation causes an exceptionally strong mutator phenotype, indicating fidelity defects distinct from loss of proofreading. Cancer Res. 2014; 74:1895–1901. [PubMed: 24525744]
24. Reha-Krantz LJ. DNA polymerase proofreading: multiple roles maintain genome stability. Biochim Biophys Acta. 2010; 1804:1049–1063. [PubMed: 19545649]

25. Tran HT, Gordenin DA, Resnick MA. The 3′ → 5′ exonucleases of DNA polymerases delta and epsilon and the 5′ → 3′ exonuclease Exo1 have major roles in postreplication mutation avoidance in *Saccharomyces cerevisiae*. Mol Cell Biol. 1999; 19:2000–2007. [PubMed: 10022887]
26. Williams LL, Marjavaara L, Knowels GM, Schultz EM, Fox EJ, Chabes A, Herr AJ. dNTP pool levels modulate mutator ohenotypes of error-prone DNA polymerase epsilon variants. Proc Natl Acad Sci U S A. 2015 (in press).
27. Nick McElhinny SA, Watts BE, Kumar D, Watt DL, Lundstrom EB, Burgers PM, Johansson E, Chabes A, Kunkel TA. Abundant ribonucleotide incorporation into DNA by yeast replicative polymerases. Proc Natl Acad Sci U S A. 2010; 107:4949–4954. [PubMed: 20194773]
28. Pavlov YI, Mian IM, Kunkel TA. Evidence for preferential mismatch repair of lagging strand DNA replication errors in yeast. Curr Biol. 2003; 13:744–748. [PubMed: 12725731]
29. Kow YW, Bao G, Reeves JW, Jinks-Robertson S, Crouse GF. Oligonucleotide transformation of yeast reveals mismatch repair complexes to be differentially active on DNA replication strands. Proc Natl Acad Sci U S A. 2007; 104:11352–11357. [PubMed: 17592146]
30. Larrea AA, Lujan SA, Nick McElhinny SA, Mieczkowski PA, Resnick MA, Gordenin DA, Kunkel TA. Genome-wide model for the normal eukaryotic DNA replication fork. Proc Natl Acad Sci U S A. 2010; 107:17674–17679. [PubMed: 20876092]
31. Lujan SA, Williams JS, Pursell ZF, Abdulovic-Cui AA, Clark AB, Nick McElhinny SA, Kunkel TA. Mismatch repair balances leading and lagging strand DNA replication fidelity. PLoS Genet. 2012; 8:e1003016. [PubMed: 23071460]
32. Nick McElhinny SA, Kissling GE, Kunkel TA. Differential correction of lagging-strand replication errors made by DNA polymerases {alpha} and {delta}. Proc Natl Acad Sci U S A. 2010; 107:21070–21075. [PubMed: 21041657]
33. Pavlov YI, Newlon CS, Kunkel TA. Yeast origins establish a strand bias for replicational mutagenesis. Mol Cell. 2002; 10:207–213. [PubMed: 12150920]
34. Hombauer H, Campbell CS, Smith CE, Desai A, Kolodner RD. Visualization of eukaryotic DNA mismatch repair reveals distinct recognition and repair intermediates. Cell. 2011; 147:1040–1053. [PubMed: 22118461]
35. Liberti SE, Larrea AA, Kunkel TA. Exonuclease 1 preferentially repairs mismatches generated by DNA polymerase alpha. DNA Repair (Amst). 2013; 12:92–96. [PubMed: 23245696]
36. Marsischky GT, Kolodner RD. Biochemical characterization of the interaction between the *Saccharomyces cerevisiae* MSH2–MSH6 complex and mispaired bases in DNA. J Biol Chem. 1999; 274:26668–26682. [PubMed: 10480869]
37. Cancer Genome Atlas. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012; 487:330–337. [PubMed: 22810696]
38. Church DN, Briggs SE, Palles C, Domingo E, Kearsey SJ, Grimes JM, Gorman M, Martin L, Howarth KM, Hodgson SV, Kaur K, Taylor J, Tomlinson IP. The NSECG Collaborators. DNA polymerase epsilon and delta exonuclease domain mutations in endometrial cancer. Hum Mol Genet. 2013; 22:2820–2828. [PubMed: 23528559]
39. Palles C, Cazier JB, Howarth KM, Domingo E, Jones AM, Broderick P, Kemp Z, Spain SL, Guarino E, Salguero I, Sherborne A, Chubb D, Carvajal-Carmona LG, Ma Y, Kaur K, Dobbins S, Barclay E, Gorman M, Martin L, Kovac MB, Humphray S, Consortium C, Consortium WGS, Lucassen A, Holmes CC, Bentley D, Donnelly P, Taylor J, Petridis C, Roylance R, Sawyer EJ, Kerr DJ, Clark S, Grimes J, Kearsey SE, Thomas HJ, McVean G, Houlston RS, Tomlinson I. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. Nat Genet. 2013; 45:136–144. [PubMed: 23263490]
40. Shinbrot E, Henninger EE, Weinhold N, Covington KR, Goksenin AY, Schultz N, Chao H, Doddapaneni H, Muzny DM, Gibbs RA, Sander C, Pursell ZF, Wheeler DA. Exonuclease mutations in DNA polymerase epsilon reveal replication strand specific mutation patterns and human origins of replication. Genome Res. 2014; 24:1740–1750. [PubMed: 25228659]
41. Yoshida R, Miyashita K, Inoue M, Shimamoto A, Yan Z, Egashira A, Oki E, Kakeji Y, Oda S, Maehara Y. Concurrent genetic alterations in DNA polymerase proofreading and mismatch repair in human colorectal cancer. Eur J Hum Genet. 2011; 19:320–325. [PubMed: 21157497]

42. Shlien A, Campbell BB, de Borja R, Alexandrov LB, Merico D, Wedge D, Van Loo P, Tarpey PS, Coupland P, Behjati S, Pollett A, Lipman T, Heidari A, Deshmukh S, Avitzur N, Meier B, Gerstung M, Hong Y, Merino DM, Ramakrishna M, Remke M, Arnold R, Panigrahi GB, Thakkar NP, Hodel KP, Henninger EE, Goksenin AY, Bakry D, Charames GS, Druker H, Lerner-Ellis J, Mistry M, Dvir R, Grant R, Elhasid R, Farah R, Taylor GP, Nathan PC, Alexander S, Ben-Shachar S, Ling SC, Gallinger S, Constantini S, Dirks P, Huang A, Scherer SW, Grundy RG, Durno C, Aronson M, Gartner A, Meyn MS, Taylor MD, Pursell ZF, Pearson CE, Malkin D, Futreal PA, Stratton MR, Bouffet E, Hawkins C, Campbell PJ, Tabori U. Combined hereditary and somatic mutations of replication error repair genes result in rapid onset of ultra-hypermutated cancers. Nat Genet. 2015; 47:257–262. [PubMed: 25642631]
43. Kunz BA, Kohalmi SE, Kunkel TA, Mathews CK, McIntosh EM, Reidy JA. International Commission for Protection Against Environmental Mutagens and Carcinogens. Deoxyribonucleoside triphosphate levels: a critical factor in the maintenance of genetic stability. Mutat Res. 1994; 318:1–64. [PubMed: 7519315]
44. Traut TW. Physiological concentrations of purines and pyrimidines. Mol Cell Biochem. 1994; 140:1–22. [PubMed: 7877593]
45. Fortune JM, Pavlov YI, Welch CM, Johansson E, Burgers PM, Kunkel TA. *Saccharomyces cerevisiae* DNA polymerase delta: high fidelity for base substitutions but lower fidelity for single- and multi-base deletions. J Biol Chem. 2005; 280:29980–29987. [PubMed: 15964835]
46. Shcherbakova PV, Pavlov YI, Chilkova O, Rogozin IB, Johansson E, Kunkel TA. Unique error signature of the four-subunit yeast DNA polymerase epsilon. J Biol Chem. 2003; 278:43770–43780. [PubMed: 12882968]
47. Chabes A, Georgieva B, Domkin V, Zhao X, Rothstein R, Thelander L. Survival of DNA damage in yeast directly depends on increased dNTP levels allowed by relaxed feedback inhibition of ribonucleotide reductase. Cell. 2003; 112:391–401. [PubMed: 12581528]
48. Eckert KA, Kunkel TA. High fidelity DNA synthesis by the *Thermus aquaticus* DNA polymerase. Nucleic Acids Res. 1990; 18:3739–3744. [PubMed: 2374708]
49. Eckert KA, Kunkel TA. Fidelity of DNA synthesis catalyzed by human DNA polymerase alpha and HIV-1 reverse transcriptase: effect of reaction pH. Nucleic Acids Res. 1993; 21:5212–5220. [PubMed: 7504813]
50. Eckert KA, Kunkel TA. Effect of reaction pH on the fidelity and processivity of exonuclease-deficient Klenow polymerase. J Biol Chem. 1993; 268:13462–13471. [PubMed: 8390464]
51. Perrino FW, Loeb LA. Hydrolysis of 3′-terminal mispairs in vitro by the 3′–5′ exonuclease of DNA polymerase delta permits subsequent extension by DNA polymerase alpha. Biochemistry. 1990; 29:5226–5231. [PubMed: 2166556]
52. Nick McElhinny SA, Pavlov YI, Kunkel TA. Evidence for extrinsic exonucleolytic proofreading. Cell Cycle. 2006; 5:958–962. [PubMed: 16687920]
53. Niimi A, Limsirichaikul S, Yoshida S, Iwai S, Masutani C, Hanaoka F, Kool ET, Nishiyama Y, Suzuki M. Palm mutants in DNA polymerases alpha and eta alter DNA replication fidelity and translesion activity. Mol Cell Biol. 2004; 24:2734–2746. [PubMed: 15024063]
54. Georgescu RE, Langston L, Yao NY, Yurieva O, Zhang D, Finkelstein J, Agarwal T, O'Donnell ME. Mechanism of asymmetric polymerase assembly at the eukaryotic replication fork. Nat Struct Mol Biol. 2014; 21:664–670. [PubMed: 24997598]
55. Kunkel TA, Burgers PM. Delivering nonidentical twins. Nat Struct Mol Biol. 2014; 21:649–651. [PubMed: 24997601]
56. Williams LN, Herr AJ, Preston BD. Emergence of DNA polymerase epsilon antimutators that escape error-induced extinction in yeast. Genetics. 2013; 193:751–770. [PubMed: 23307893]
57. Albertson TM, Ogawa M, Bugni JM, Hays LE, Chen Y, Wang Y, Treuting PM, Heddle JA, Goldsby RE, Preston BD. DNA polymerase epsilon and delta proofreading suppress discrete mutator and cancer phenotypes in mice. Proc Natl Acad Sci U S A. 2009; 106:17101–17104. [PubMed: 19805137]

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.dnarep.2015.04.006

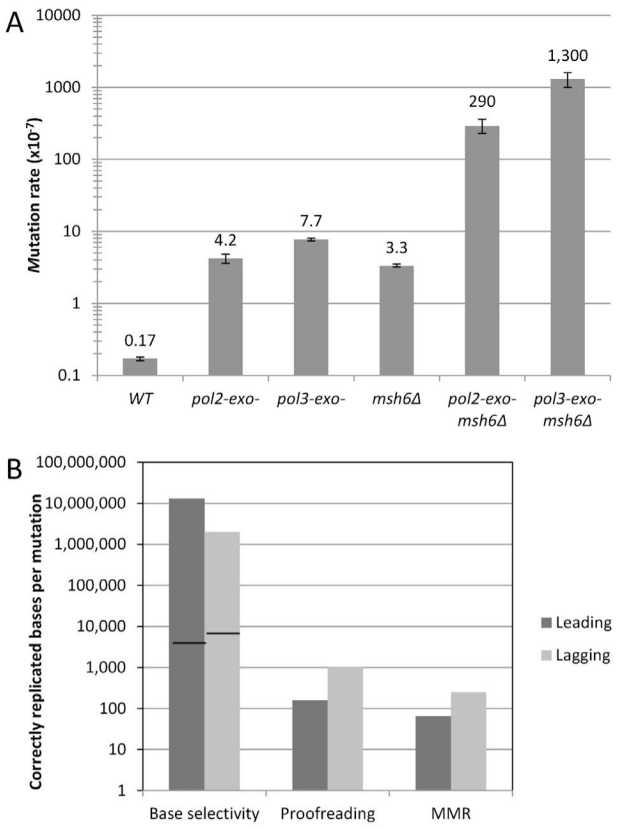

Fig. 1.

Mutation rates and fidelity factors for base selectivity, proofreading, and MMR on both the leading and lagging strands. (A) Mutation rates were measured at the *agp1::URA3-OR1* locus in WT, *pol2-exo*$^-$, *pol3-exo*$^-$, *msh6*Δ, *pol2-exo*$^-$ *msh6*Δ, *and pol3-exo*$^-$ *msh6*Δ strains. Please note that the *y*-axis is on a logarithmic scale. (B) The apparent fidelity factors for base selectivity, proofreading, and MMR are depicted for the leading strand (dark gray) and the lagging strand (light gray). The *y*-axis values are number of correctly replicated or repaired bases per mutation and is on a logarithmic scale. The lines within the bars for base selectivity indicate the base selectivity of Pol ε[46] and Pol δ [45] measured *in vitro*.

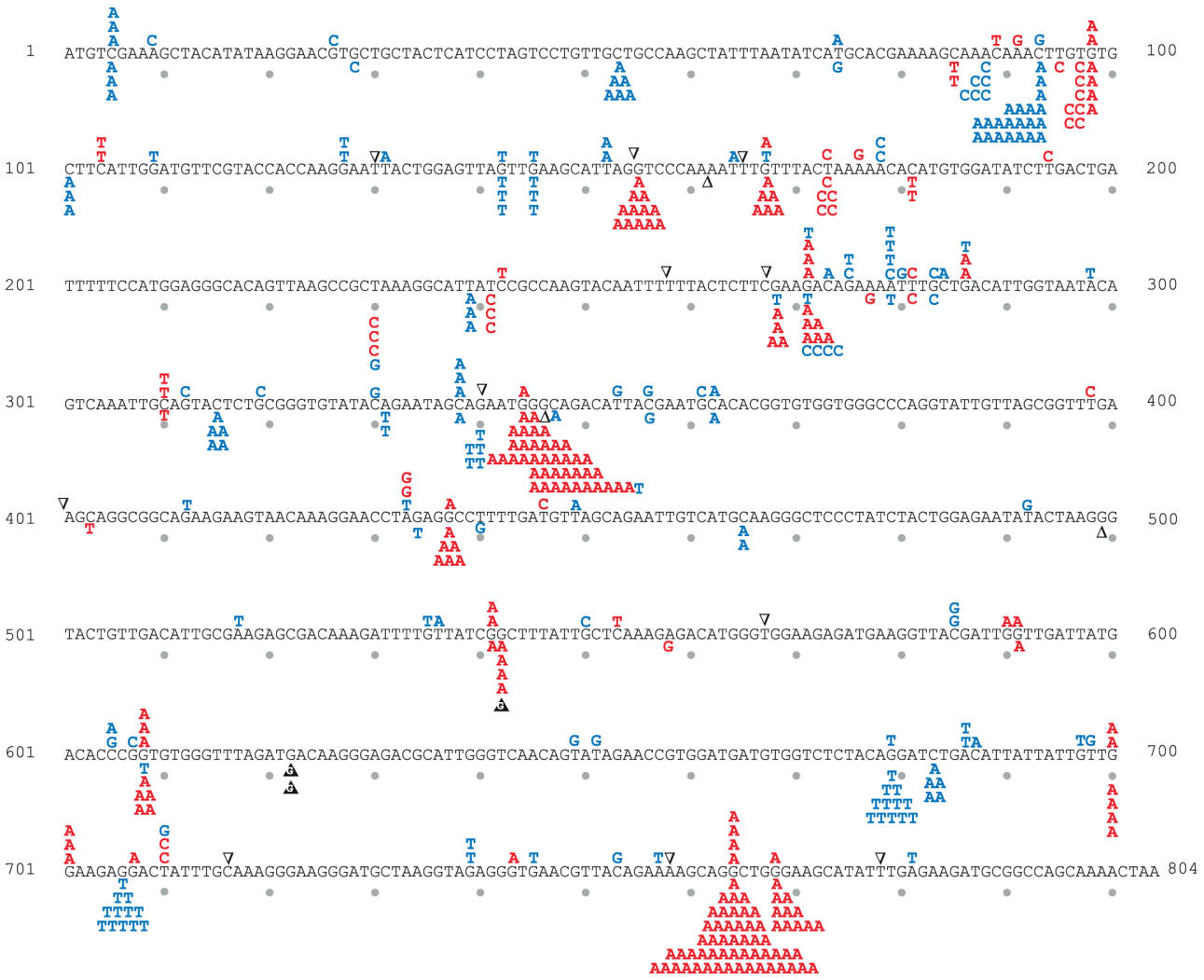

Fig. 2.

URA3 mutation spectra for the wild type and *msh6*Δ strains. Mutations observed in the wild type and *msh6*Δ strains are shown above and below the *URA3* coding sequence, respectively. Red letters are transitions, blue letters are transversions, open black triangles are single base pair deletions and filled triangles with a letter are single base pair insertions.

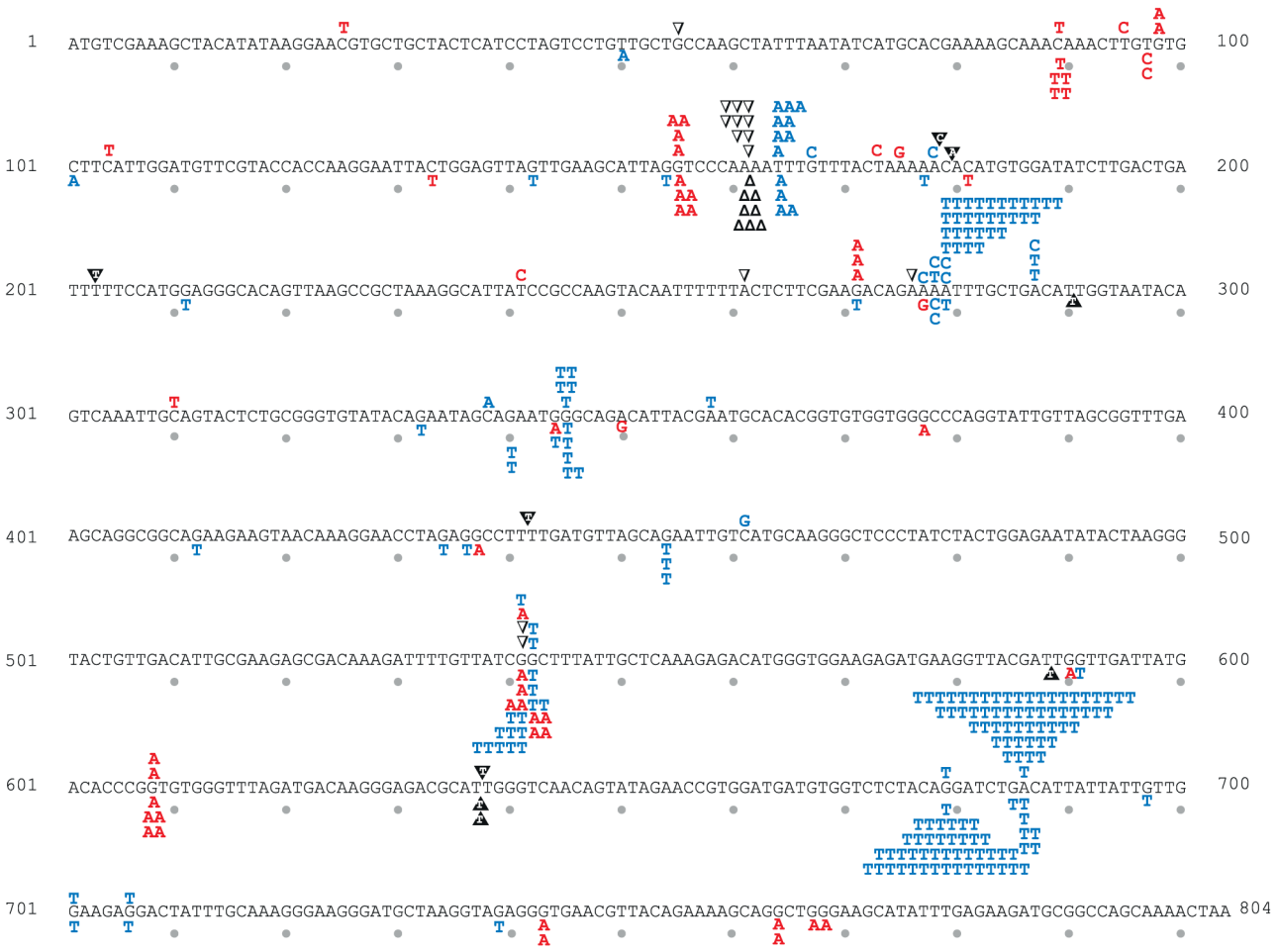

Fig. 3.

URA3 mutation spectra for *pol2-exo*$^{-}$ and *pol2-exo*$^{-}$ *msh6*Δ strains. As in Fig. 2.

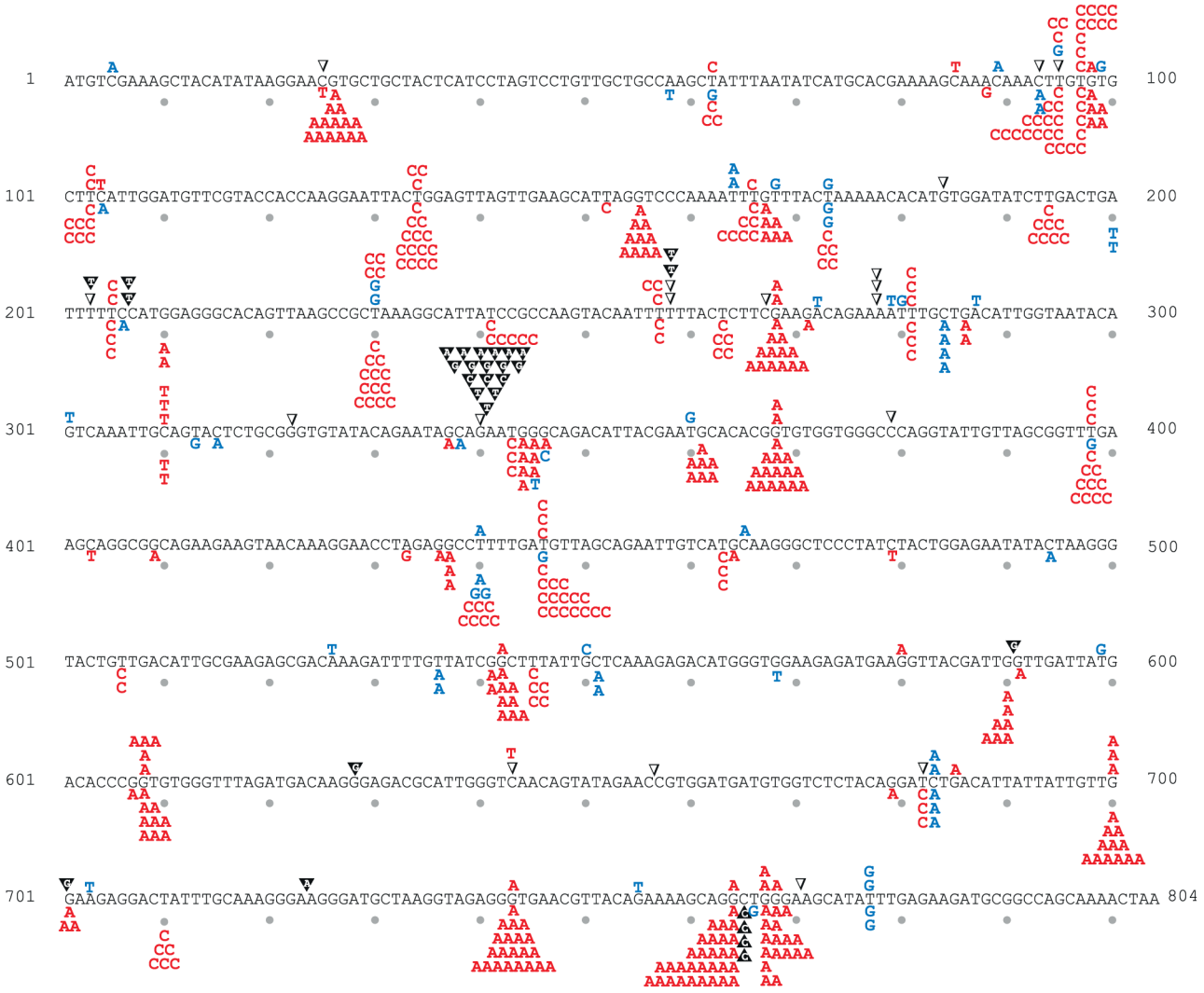

Fig. 4.

URA3 mutation spectra for *pol3-exo*$^{-}$ and *pol3-exo*$^{-}$ *msh6*Δ strains. As in Fig. 2.

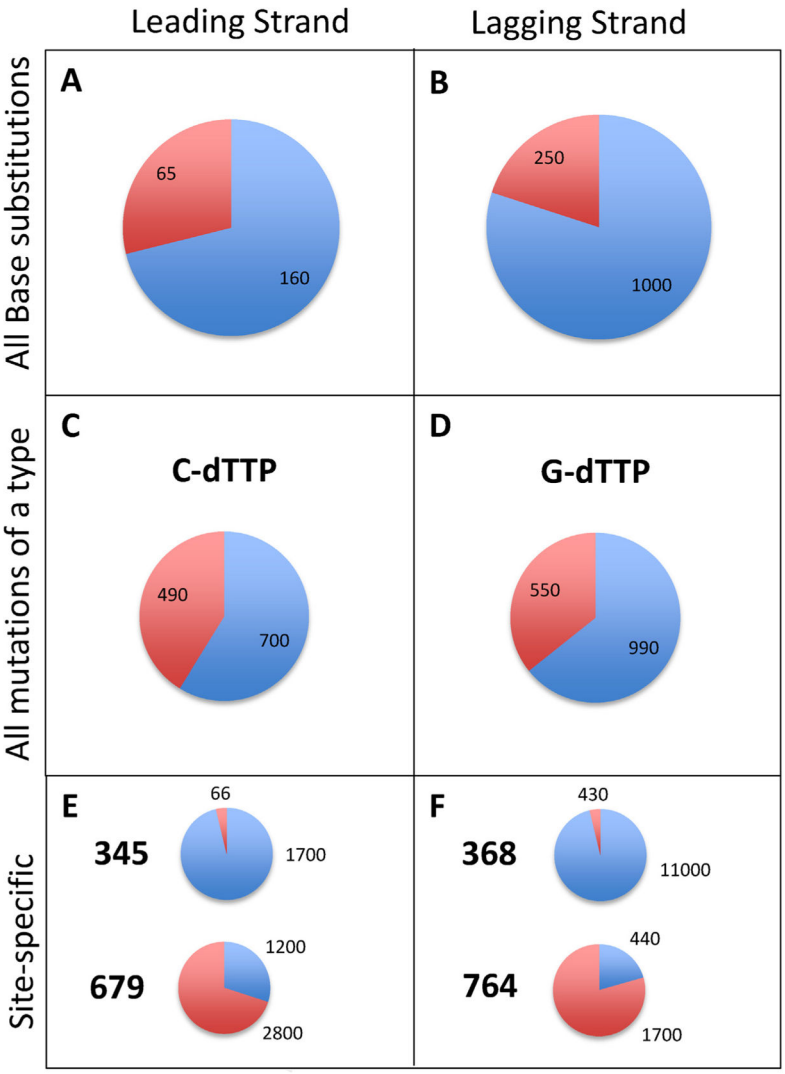

Fig. 5.

The contributions of proofreading and MMR to correcting base-base mismatches. Results on the left are for the *pol2-exo*$^-$ strains, and results on the right are for the *pol3-exo*$^-$ strains. The contributions of proofreading and MMR to correcting: (A) and (B) total base-base mismatches, (C) total C-dTTP mismatches (D) total G-dTTP mismatches, (E) individual C-dTTP mismatches at *URA3* base pairs 345 and 679, and (F) individual G-dTTP mismatches at *URA3* base pairs 368 and 764. The numbers superimposed within or above the pie slices indicate the error correction factors, either for proofreading (in blue) or MMR (in red).

Table 1

Total and specific mutation rates in six yeast strains.

		WT			*msh6Δ*			*pol2-exo⁻*			*pol2-exo⁻ msh6Δ*			*pol3-exo⁻*			*pol3-exo⁻ msh6Δ*		
Mutation rate (×10^{-7})		**0.17**			**3.3**			**4.2**			**290**			**7.7**			**1300**		
95% Confidence intervals		**(0.16–0.18)**			**(3.2–3.5)**			**(3.6–4.8)**			**(230–260)**			**(7.3–8.0)**			**(1000–1600)**		
# 5FOA Resistant clones sequenced		**211**			**691**			**170**			**178**			**123**			**361**		
			Mutation rates (×10^{-9})			**Mutation rates (×10^{-9})**			**Mutation rates (×10^{-9})**			**Mutation rates (×10^{-9})**			**Mutation rates (×10^{-9})**			**Mutation rates (×10^{-9})**	
	Target size[a]	**Total**	**Mutation type[b]**	**Per base[c]**	**Total**	**Mutation type[b]**	**Per base[c]**	**Total**	**Mutation type[b]**	**Per base[c]**	**Total**	**Mutation type[b]**	**Per base[c]**	**Total**	**Mutation type[b]**	**Per base[c]**	**Total**	**Mutation type[b]**	**Per base[c]**
Base substitutions	285	122	9.8	0.034	292	140	0.49	139	340	1.2	137	22000	78	91	570	2	399	140000	500
Transitions	130	47	3.8	0.029	182	87	0.67	20	49	0.38	39	6400	49	64	400	3.1	363	130000	1000
A to G	18	4	0.32	0.018	2	0.96	0.053	1	2.5	0.14	2	330	18	0	***6.3***	***0.35***	2	720	40
T to C	27	7	0.56	0.021	20	9.6	0.35	3	7.4	0.27	2	330	12	37	230	8.6	154	55000	2100
C to T	36	11	0.89	0.025	6	2.9	0.08	4	9.9	0.27	7	1100	32	6	38	1	5	1800	50
G to A	49	25	2	0.041	154	74	1.5	12	30	0.61	28	4600	93	21	130	2.7	202	73000	1500
Transversions	225	75	6	0.027	110	53	0.23	119	290	1.3	98	16000	71	27	170	0.75	36	13000	58
A to T	39	9	0.73	0.019	3	1.4	0.037	91	220	5.8	8	1300	33	5	31	0.8	3	1100	28
T to A	51	7	0.56	0.011	3	1.4	0.028	8	20	0.39	5	810	16	3	19	0.37	3	1100	21
T to G	42	8	0.64	0.015	3	1.4	0.034	0	***2.5***[d]	***0.059***	0	***160***	***3.9***	11	69	1.6	11	4000	94
A to C	25	5	0.4	0.016	6	2.9	0.11	6	15	0.59	2	330	13	0	***6.3***	***0.25***	0	***360***	***14***
C to A	38	11	0.89	0.023	48	23	0.6	1	2.5	0.065	1	160	4.3	5	31	0.82	16	5800	150
G to T	58	19	1.5	0.026	40	19	0.33	11	27	0.47	82	13000	230	2	13	0.22	2	720	12
C to G	20	6	0.48	0.024	1	0.48	0.024	1	2.5	0.12	0	***160***	***8.1***	0	***6.3***	***0.31***	0	***360***	***18***
G to C	33	10	0.81	0.024	6	2.9	0.087	1	2.5	0.075	0	***160***	***4.9***	1	6.3	0.19	1	360	11

[a] Number of base pairs in URA3 where this substitution confers 5-FOA resistance, from Supplementary Fig. 1.

[b] Rates are the total mutation rate times the proportion of the observed mutations of that type (left-most column) among the total 5-FOA^R clones sequenced.

[c] Mutation rate per phenotypically detectable base pair.

[d] Italicized values in bold are “less than or equal to” rates for events not seen, calculated based on the maximum mutation rate had one event been observed.

Table 2

Contributions of selectivity, proofreading and MMR to replication fidelity.

	Leading strand			Lagging strand		
	Base selectivity	**Proofreading**	**MMR**	**Base selectivity**	**Proofreading**	**MMR**
	12000000	160	65	2000000	1000	250
Transitions	21000000	73	130	1000000	1500	330
T-dG	55000000	340	130	490000	5800	240
A-dC	83000000	34	44	25000000	750	>=120
G-dT	32000000	400	120	670000	990	550
C-dA	11000000	62	150	20000000	630	48
Transversions	14000000	300	54	17000000	250	77
T-dT	30000000	910	5.8	47000000	750	58
A-dA	63000000	570	41	36000000	750	35
A-dG	>=260000000	=<110	*a*	>=69000000	=<130	*a*
T-dC	77000000	110	22	11000000	2800	58
G-dA	230000000	7.1	66	81000000	38	58
C-dT	4300000	700	490	6600000	250	180
G-dG	>=120000000	=<340	=<66	92000000	130	58
C-dC	>=200000000	=<57	=<66	>=56000000	=<750	[b]

[a] Too few events detected to quantify a contribution.