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Mol Microbiol. Author manuscript; available in PMC 2010 December 1.

Published in final edited form as:
Mol Microbiol. 2009 December ; 74(5): 1114–1127. doi:10.1111/j.1365-2958.2009.06921.x.

Role of *Escherichia coli* DNA Polymerase I in chromosomal DNA replication fidelity

Karolina Makiela-Dzbenska[1], **Malgorzata Jaszczur**[1], **Magdalena Banach-Orlowska**[1,†], **Piotr Jonczyk**[1], **Roel M. Schaaper**[2], and **Iwona J. Fijalkowska**[1,*]

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, Warsaw, Poland. [2]Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA.

Summary

We have investigated the possible role of *E. coli* DNA polymerase I in chromosomal replication fidelity. This was done by substituting the chromosomal *polA* gene by the *polAexo* variant containing an inactivated 3'→5' exonuclease, which serves as a proofreader for this enzyme's misinsertion errors. Using this strain, activities of Pol I during DNA replication might be detectable as increases in the bacterial mutation rate. Using a series of defined *lacZ* reversion alleles in two orientations on the chromosome as markers for mutagenesis, 1.5- to 4-fold increases in mutant frequencies were observed. In general, these increases were largest for *lac* orientations favoring events during lagging strand DNA replication. Further analysis of these effects in strains affected in other *E. coli* DNA replication functions indicated that this *polAexo* mutator effect is best explained by an effect that is additive compared to other error-producing events at the replication fork. No evidence was found that Pol I participates in the polymerase switching between Pol II, III and IV at the fork. Instead, our data suggest that the additional errors produced by *polAexo* are created during the maturation of Okazaki fragments in the lagging strand.

Introduction

Chromosomal DNA replication is a highly accurate process. The fidelity of this process relies on faithful DNA synthesis by the replicative DNA polymerases. *Escherichia coli* possesses 5 distinct DNA polymerases. Replication of the chromosome is performed primarily by DNA polymerase III holoenzyme (HE), which is an asymmetric, dimeric, 17-subunit complex that is capable of coordinated high-speed and high-fidelity synthesis of leading and lagging strand at the replication fork (McHenry, 1991; Kelman & O'Donnell, 1995; McHenry, 2003; O'Donnell, 2006; Pomerantz and O'Donnell, 2007). The α, ε and θ subunits of HE constitute the polymerase core. The α subunit, responsible for polymerase activity, is encoded by the *dnaE* gene (Gefter *et al*., 1971). The ε subunit, encoded by the *dnaQ* gene (Takano *et al*., 1986), contains the 3' → 5' exonuclease that proofreads mismatches made by α subunit. The small (8 kDa) θ-subunit primarily serves as a stabilizer for ε subunit (Taft-Benz and Schaaper, 2004). Additionally, HE contains a pair of sliding-clamp processivity factors (β_2), one for each core, as well as the seven-subunit DnaX complex ($\tau_2\gamma\delta\delta'\chi\psi$) (O'Donnell *et al*., 2001; McHenry, 2003) responsible for loading and unloading the processivity clamp.

*For correspondence. iwonaf@ibb.waw.pl; Tel. (+48) 22 592 1113; fax (+48) 22 658 4636.
†Present address: International Institute of Molecular and Cell Biology, Warsaw, Poland

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While the role of Pol III in chromosomal replication is clearly established, the precise role(s) of the additional DNA polymerases are still being investigated, including a possible role in chromosomal replication and its fidelity. DNA polymerase II (Pol II) encoded by the *polB* (*dinA*) gene belongs to the B-family of polymerases. Like eukaryotic replicative B family polymerases, Pol II is a high fidelity enzyme possessing 3' → 5' exonuclease activity (Cai *et al*., 1995). The constitutive intracellular concentration of Pol II (30–50 molecules/cell) is comparable to the estimated concentration of Pol III HE (30 molecules/cell) (McHenry and Kornberg, 1977; Qiu and Goodman, 1997). However, upon induction of the SOS response, Pol II levels can be further increased sevenfold (Qiu *et al*., 1997; Bonner *et al*., 1988, 1990). Our group (Banach-Orlowska *et al*., 2005) and others (Bonner *et al*., 1988; Escarceller *et al*., 1994; Foster *et al*., 1995; Rangarajan *et al*., 1997, 1999; Foster, 2000; Napolitano *et al*., 2000) have previously demonstrated that Pol II can perform certain functions at the replication fork. One such proposed function is to substitute for Pol III when HE has difficulties extending a mismatched primer terminus (Banach-Orlowska *et al*., 2005), thus serving as a proofreader for Pol III, as also suggested by Curti *et al*. (2008).

Two other polymerases, Pol IV and Pol V, are members of the Y family of DNA polymerases; they lack a proofreading activity and are generally considered low-fidelity DNA polymerases (Goodman, 2002). Both are expressed at elevated levels during SOS induction. Pol V (*umuDC* gene product) (Reuven *et al*., 1999; Tang *et al*., 1999) is the major polymerase involved in damage-induced mutagenesis. It performs translesion synthesis, creating mutations targeted at DNA damage sites, but also produces untargeted mutations at non-damaged DNA sites (Ichikawa-Ryo and Kondo, 1975; Fijalkowska *et al*., 1997). Importantly, Pol V is not significantly expressed in non-induced cells.

Pol IV is encoded by the *dinB* gene (Wagner *et al*., 1999). The basal level of Pol IV in normal, uninduced cells is relatively high (250 molecules per cell) (Kim *et al*., 2001). Pol IV can also participate in translesion synthesis (TLS), alone or in combination with Pol V, depending upon the nature of the lesion and its local sequence context (Napolitano *et al*., 2000; Wagner *et al*., 2002). However, despite its relative high basal level, Pol IV does not contribute significantly to the chromosomal mutation rate in growing cells (Kuban *et al*., 2004; Wolff *et al*., 2004).

DNA Polymerase I, encoded by the *polA* gene, the first DNA polymerase discovered, is the most abundant polymerase in *E. coli* (approximately 400 molecules per cell) (Kornberg and Baker, 1992). The Pol I polypeptide has two functional domains: a large domain (Klenow fragment) that contains the 5'→3' polymerase and 3'→5' proofreading exonuclease, and a small fragment that contains a 5'→3' exonuclease activity (Joyce and Grindley, 1984). The latter activity enables Pol I to remove RNA primers that were used to initiate the downstream Okazaki fragment (Okazaki *et al*., 1971). Other well-known functions of Pol I are to participate in DNA repair and recombination. During DNA repair, Pol I fills in DNA gaps that result from the removal of DNA lesions (for a review, see Friedberg *et al*., 1995, 2006). Δ*polA* strains are inviable when grown in rich medium, but their viability can be restored by providing the 5'→3' exonuclease function *in trans* (Joyce and Grindley, 1984).

The mechanisms regulating the involvement of multiple DNA polymerases in DNA replication are a subject of active interest (Shcherbakova and Fijalkowska, 2006; Fuchs and Fujii, 2007; Jarosz *et al*. 2007; Lehmann *et al*., 2007; Curti *et al*., 2008). Evidence has accumulated indicating that DNA polymerases can collaborate and substitute for each other not only during translesion synthesis, but also during ongoing DNA synthesis. For example, Pol II was proposed to be able to act as proofreader for HE-produced misinsertion errors (Banach-Orlowska *et al*., 2005). These experiments made use of *polBex1* mutants defective in the Pol II 3' proofreading activity, which were demonstrated to display a modest mutator

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effect. This mutator effect increased synergistically when combined with certain *dnaE* or *dnaQ* mutator alleles (Banach-Orlowska *et al*., 2005), suggesting that the level of Pol III-made errors is a determining factor for the mutagenic action of the proofreading-deficient Pol II. It was postulated that Pol II occasionally has access to mismatched 3' termini created by Pol III; these mismatches will normally be removed by Pol II by means of its proofreading activity but will be extended into replication errors by the proofreading-deficient version of the enzyme. The same model readily allows for the second postulated role of Pol II, namely protection of mismatched 3' termini against the mutagenic action of Pol IV. The latter (proofreading-deficient) polymerase is capable of producing mutations as evidenced by the mutator activity observed when the enzyme is overproduced (Kim *et al*., 1997; Wagner and Nohmi, 2000; Kuban *et al*., 2005). Likewise, this Pol IV mutator phenotype is proposed to result from the extension of mismatched primer termini created by Pol III HE (Kuban *et al*., 2005).

The data discussed above suggest that there is competition between Pol II, Pol III, and Pol IV [and in SOS-induced cells, also Pol V (Fijalkowska *et al*., 1989)] for a terminal mismatch that may become accessible to other polymerases upon dissociation of Pol III (Mo and Schaaper, 1996; Pham *et al*. 1998, 1999). In the present study, we have addressed the possible role of Pol I in this competition and, hence, in determining the chromosomal error rate. As Pol I is an abundant polymerase, contains a 3' proofreading activity and, like other polymerases, is capable of interacting with the β processivity clamp (Bonner *et al*., 1992; López de Saro and O'Donnell, 2001; Pham *et al*., 2001; Wagner *et al*., 2001; López de Saro *et al*., 2003; Sutton and Duzen, 2006), a role for this enzyme may be envisaged. To investigate this issue, we created a proofreading-deficient variant of Pol I (*polAexo* mutant) (Bebenek *et al*., 1990) and investigated whether this deficiency leads to increased mutant frequencies in a variety of genetic backgrounds. Based on the results we conclude that Pol I does affect the chromosomal mutation rate. However, this effect is not achieved by directly competing with Pol II, Pol III or Pol IV. Instead, we postulate that the role of Pol I at the replication fork is primarily the faithful filling of Okazaki fragment gaps associated with removal of RNA primers in the lagging strand.

Results

Experimental system for studying the fidelity role of Pol I

To study the possible role of Pol I in replication fidelity, we constructed strains in which the chromosomal *polA* gene was replaced by an error-prone form, *polA-D424A* (*polAexo*) (see Experimental Procedures for the construction). In this mutant, residue Asp424 in the Exo I motif of the 3' exonuclease domain is replaced by Ala. Biochemical experiments on this mutant polymerase have shown the enzyme to be specifically defective in the 3'→5' exonucleolytic proofreading activity, while retaining full DNA synthesis activity (Bebenek *et al*., 1990). *In vivo*, this substitution is expected to increase the error rate of Pol I during DNA synthesis in the cell. The underlying assumption for our experiments was that in a background of high-fidelity DNA synthesis even a small amount of error-prone DNA synthesis by the exonuclease-defective variant might be detectable as an increase in the bacterial mutation frequency.

In order to measure mutagenesis, we used a previously described *lacZ* reversion system employing pairs of strains in which a *lacZ* mutational target resides on the chromosome in the two possible orientations relative to the direction of replication (Fijalkowska *et al*., 1998). This approach permits analysis of the results in terms of potentially differential effects during leading and lagging strand replication, as discussed (Fijalkowska *et al*., 1998; Maliszewska *et al*., 2000; Gawel *et al*., 2002a b; Banach-Orlowska *et al*., 2005; Kuban *et al*., 2006; Gawel *et al*., 2008). In many of these experiments, the strains used are also mismatch-

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repair deficient (*mutL*) to more directly measure the effects on uncorrected replication errors.

In Table 1 we present the results of several experiments aimed at showing the effect of the *polAex*o allele on mutant frequencies for a set of four *lac* alleles, as well as on the frequency of rifampicin or nalidixic-acid resistant mutants. The *lac* alleles used permit the specific scoring of G·C→A·T, G·C→T·A, A·T→T·A, or A·T→G·C base substitutions, respectively (Cupples and Miller, 1989). For each allele, the mutant frequency was measured for the two chromosomal orientations (R and L) of the *lac* target (Fijalkowska *et al*., 1998). As before (Fijalkowska *et al*., 1998), significant differences are observed in the mutant frequency between the two orientations, particularly in the mismatch-repair-defective background (Table 1B), which have been interpreted to reflect the differential fidelity of leading and lagging strand replication (Fijalkowska *et al*., 1998). To illustrate one example, for the *lac* G·C→A·T allele, in section B, there is a 3-fold difference between the two orientations: 150 $\times 10^{-8}$ (L-orientation) versus 43×10^{-8} (R-orientation) (Table 1B). As G·C→A·T mutations are assumed to be mediated primarily by G·T mispairings (rather than C·A), the location of the template G is in the leading strand for the L-orientation and in the lagging strand for the R-orientation (Fijalkowska *et al*., 1998), thus leading to the conclusion that for this *lac* allele lagging-strand replication is several fold more accurate than leading-strand replication. Using similar reasoning for the three other *lac* alleles, the primary strand whose fidelity is measured for each *lac* orientation can be deduced (see Fijalkowska *et al*., 1998 and the Legend to Table 1 for more details). Based on these data, reproduced in multiple studies from our laboratory, we have suggested that on the *E. coli* chromosome, lagging strand replication is more accurate. Thus with regard to the present issue of the role of Pol I, the use of this *lac* system provides additionally an opportunity to investigate any strand-specific effects of Pol I.

Expected signature(s) for Pol I-mediated errors

Previous studies have investigated the *in vitro* fidelity of the D424A exonuclease-deficient form of Pol I Klenow fragment, assaying overall error rates or the rates of misinsertion and mismatch extension in various sequence contexts (Bebenek *et al*., 1990; Joyce *et al*., 1992). In general, the observations conform to the pattern observed with many other DNA polymerases (Fijalkowska *et al*., 1998). Among transition mismatches, T·G and G·T are more frequent than the complementary A·C and C·A at both the misinsertion and extension step. For transversion mismatches the data are more varied, but generally adhere to the observations for other DNA polymerases, but with one exception. It was found that Pol I generates A·A mismatches at relatively high frequency, similar to or higher than the reciprocal T·T frequency, which has been assumed to be preferred by Pol III (Fijalkowska *et al*., 1998). Thus, for the *lacZ* allele that reverts through A·T→T·A transversion, any synthesis by Pol I (D424A) might lead to an apparent strandedness that is the opposite of what is routinely observed in our assay (see below).

polAexo mutator effects

The experiments of Table 1 in both mismatch-repair proficient (A) and mismatch-repair-deficient (*mutL*) strains (B) show that the presence of the *polAexo* allele indeed increases several of the mutant frequencies, although by modest extents. In the *mutL*$^+$ background, the *polAexo* allele increases the mutant frequency for the G·C→T·A allele 4.1-fold specifically for the R(lagging) orientation. For the A·T→T·A allele the frequency was enhanced 1.6- to 2.7-fold for both orientations. We also measured the frequency of mutants resistant to the antibiotics rifampicin and nalidixic acid. The Rif^R frequency was increased 1.5-fold, while no effect was detected for the Nal^R mutants. In the *mutL* background (B) increases were observed of similar nature and extent. For the *lac* G·C→T·A allele the increase is again

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specific for the R(lagging) orientation, while for the *lac* A·T→T·A allele both orientations are affected. In this case we also observe a small, but statistically significant increase for the A·T→G·C transition for the L(lagging) orientation. Interestingly, no increase could be detected for the Rif^R (or Nal^R) mutations (Table 1B). Table 1 contains representative experiments, but the modest but significant effects of *polAexo* were observed in many repeated experiments.

polAexo effects in the *dnaE915* antimutator background

In previous studies investigating the mutator effects of an exonuclease-deficient Pol II allele (*polBex1*), increased effects of the Pol II proofreading defect were noted when the experiment was performed in strains containing the *dnaE915* allele (Rangarajan *et al*., 1997; Banach-Orlowska *et al*., 2005). This allele represents an antimutator variant of DNA Polymerase III (Fijalkowska *et al*., 1993), although its precise mechanism is not yet established. It was argued that the reduction in background mutation frequency in the antimutator strain made it easier to observe the mutator effect resulting from the Pol II proofreading defect (Rangarajan *et al*., 1997; Banach-Orlowska *et al*., 2005). Likewise, the mutator effect of the *polAexo* variant might be more clearly noted in the *dnaE915* background, and we have pursued this possibility.

The results of these experiments (Table 1C) confirm the previous observations (Banach-Orlowska *et al*., 2005) that *dnaE915* significantly lowers the mutant frequency for each of the *lac* alleles, particularly for the two transitions (G·C→A·T and A·T→G·C). Further, the antimutator effect is clearly dissimilar for the two strands, the strongest reductions occurring for the presumed leading strand (Banach-Orlowska *et al*., 2005). Although not the direct subject of the present study, it is likely that this strand asymmetry of the *dnaE915* antimutator will be helpful in better understanding the differential fidelity mechanisms for leading and lagging strand replication in *E. coli*. The results show that, indeed, in the *mutL dnaE915* background several of the mutator effects of *polAexo* are increased (Table 1C). For example, a 3.8-fold increase in the frequency of G·C→A·T transitions is observed for the R(lagging) orientation (Table 1C) (compared to 1.6-fold in *mutL*) (Table 1B). For the G·C→T·A transversion, a 2.6-increase for the R(lagging) orientation of the G·C→T·A transversion is observed. For the *lac* AT→TA allele both *lac* orientations are enhanced (3.4- and 32-fold), while the two orientations of the A·T→G·C allele are enhanced by 2.6- and 1.5-fold for L (lagging) and R (leading), respectively.

The combined results for both *mutL* and *mutL dnaE915* indicate not only a significant mutator effect of the *polAexo* allele, but also that the effects are unequal for the two DNA strands. Overall, the effect seems to be largely specific for the lagging strand. The one exception may be the case of the A·T→T·A transversions, where both orientations are enhanced and, in fact, the L(leading) orientation is most strongly enhanced. However, this assignment of "leading" is based on assumed T·T mispairing being responsible for the majority of A·T→T·A transversions. As indicated above, Pol I has been shown to have a strong tendency to produce A·A mispairings (Joyce *et al*., 1992). Hence, the strong enhancement of mutations in the L orientation by *polAexo* is most likely fully consistent with a preferred action of Pol I in the lagging strand.

Comparison of *polAexo* with *polBex*

The experiments of Table 1 also include for comparison the results with the *polBex* mutant and the *polAexo polBex* double mutant. The mutator effects of *polAexo* and *polBex* are generally comparable in magnitude, although some differences are seen. In the *mutL dnaE915* background, most of the effects of *polBex* are slightly larger, and include a clear (4-fold) effect on the frequency of Rif^r mutations. *PolBex* effects are also observed for both

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orientations of the *lac* alleles, consistent with our proposal that Pol II can operate in both strands (Banach-Orlowska *et al*., 2005).

An interesting picture emerges when looking at the results for the *polAexo polBex* double mutator strains (Table 1, last column). It appears that the two mutator effects operate independently and that, quantitatively, the joint mutator effect might be most easily described by simple additivity of the two separate mutant frequencies. This suggests that the pathways by which Pol I and Pol II contribute to replication fidelity are separate and do not involve a common intermediate and, hence, reflect a lack of direct competition between the two polymerases. The next series of experiments was conducted to more precisely delineate the possible role of Pol I.

Pol I does not process mismatches created by Pol III HE

One model that we have advanced for the involvement of accessory polymerases in the DNA replication process, especially its fidelity, is a switching of polymerases when Pol III HE makes a misinsertion error (Fijalkowska *et al*., 1987; Maliszewska-Tkaczyk *et al*., 2000; Banach-Orlowska *et al*., 2005; Kuban *et al*., 2005; Gawel *et al*., 2008). As mismatches are generally difficult to extend, a temporary stalling may occur which may provide an opportunity for additional polymerases to gain access to the DNA growing point. This interference may be mutagenic if access is gained by an exonuclease deficient enzyme or antimutagenic if access is gained by a proofreading-proficient enzyme. This mechanism has been used to explain the mutator effect of Pol IV overexpression, as well as the back-up proofreading role that Pol II may play for Pol III (Banach-Orlowska *et al*., 2005; Kuban *et al*., 2005; Gawel *et al*., 2008). Experiments in support of this mechanism have generally employed the use of additional deficiencies in Pol III (DnaE) and its accessory subunits, such as the Pol III proofreading subunit (DnaQ), the Pol III τ subunit (DnaX), and the Pol III β subunit (DnaN). In the present study, we conducted similar experiments with the *polAexo* allele.

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In Table 2, we analyze the effect of *polAexo* in a series of *dnaQ, dnaE, dnaN, and dnaX* mutants. Some of these were investigated in the *mutL* background, while others, due to strain construction constraints, where examined in the *mutL*$^{+}$ background. The studies were also conducted specifically with the *lac* G·C→T·A allele in the lagging orientation, as this configuration generally displays one of the stronger effects for the *polAexo* allele. Two *dnaQ* mutator mutants were used (*dnaQ928* and *dnaQ49*), which carry a defect in the Pol III epsilon subunit (Takano *et al*., 1986;Taft-Benz and Schaaper, 1998). Two *dnaE* alleles were used (*dnaE486 and dnaE511*), carrying defects in the Pol III α subunit resulting in a mutator phenotype (Vandewiele *et al*., 2002). The *dnaX36* mutant carries a defect in the Pol III τ subunit that leads to a very specific mutator effect (enhancement of transversions and −1 frameshift mutations) (Pham *et al*., 2006;Gawel *et al*., 2008). Finally, the *dnaN159* allele represents a defect in the β subunit of HE, which also specifies a mutator phenotype that has been ascribed to an altered polymerase usage (Sutton, 2004;Sutton and Duzen, 2006). In parallel, we compared the results of the *polAexo* mutation with those of the *polBex1* mutation. The general logic of these experiments is that if Pol I were to extend mismatches created by the mutator HEs, a synergistic (ie, more than additive or even multiplicative) effect of the *dna* and *polAex* mutator effects is predicted. On the other hand, if the *dna* and *polAex* alleles were to act entirely separate, then the combined mutator effect would likely be simply the sum of the individual effects.

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Table 2 shows that the interaction of *polAexo* with each of these replication/fidelity defects is very slight or even absent; all *polAex*-mediated enhancements of the *dna* mutator effects are less than 2-fold. Instead, it appears that simple additivity of the various mutator effects is the most straightforward interpretation. For example, for the *mutL dnaN159* strain, the

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enhancement of the mutant frequency by the *polAexo* allele is 4.2×10^{-8} (9.8×10^{-8} *minus* 5.6×10^{-8}), which corresponds reasonably to the *polAex* effect in the *mutL dnaN*$^{+}$ strain (4.6×10^{-8} *minus* $1.4 \times 10^{-8} = 3.2 \times 10^{-8}$). Overall, these data suggest that role of Pol I is not related to any of the error-producing pathways studied here. This finding contrasts to very substantial interactions displayed by the *polBex1* allele seen in the same experiment (see also Banach-Orlowska *et al*., 2005). We conclude that the mode of mutation production by *polAexo*, in contrast of that of *polBex*, is unlikely related to the extension by Pol I of Pol III-mediated misinsertion errors.

Competition between accessory polymerases

In Table 3 we present results of one additional experiment in which we analyzed the effects of the *polAexo* deficiency in strains lacking Pol II, Pol IV, or both. It was argued that if Pol I were to compete with either polymerase, then the effect of *polAexo* might be amplified in such deficient backgrounds. The results clearly indicate that the *polAexo* effect is not changed in either the Δ*polB*, Δ*dinB*, or Δ*polB* Δ*dinB* backgrounds (Table 3). In the *mutL* background, the deletion of Pol II, Pol IV, or both, does not significantly affect the mutant frequency for the tested G·C→T·A allele, as observed before (Kuban *et al*., 2006; unpublished data), and the mutator effect of the *polAexo* allele remains unaltered (2.7- to 3.5-fold effect, Table 3A). An additional set of measurements was performed in the *dnaE486* background, which was previously found to be suitable for demonstrating increased participation of Pol II and Pol IV (Banach-Orlowska *et al*., 2005;Kuban *et al*., 2005;Curti *et al*., 2008). This experiment, performed in the *mutL*$^{+}$ background, clearly demonstrates that even under conditions where Pol II and Pol IV actively contribute to determining the mutation rate, the effect of *polAexo* still remains unchanged (0.8-to 1.6-fold effect, Table 3B).

Lack of interaction of *polAexo* with Nucleotide Excision Repair (NER)

Pol I is the primary polymerase to fill in the gaps during NER, and the possibility that the *polAexo* mutator effect results from the error-prone filling of those gaps should be considered. In addition, Hasegawa *et al*. (2008) recently proposed an active role of NER in the production of spontaneous mutations, based on their observation that *uvrA* and *uvrB* deficiencies led to a *reduction* in spontaneous mutations. It was proposed that the NER system frequently engages in unnecessary "repair" of undamaged DNA and that the gapfilling synthesis by Pol I is responsible for a large fraction of spontaneous mutations (Hasegawa *et al*., 2008). To test this hypothesis in our present system we investigated the *polAexo* effect in strains deficient in NER. In Table 4 we present results for the *uvrA* and *uvrC* strains, along with the single mutant control. The experiments were conducted in both mismatch-repair proficient (Table 4A) and deficient backgrounds (Table 4B). Our data clearly indicate that the NER deficiency does not affect the *polAexo* mutator effect. In the *uvr* backgrounds, the mutant frequencies for the G·C→A·T and G·C→T·A *lac* alleles are essentially unchanged, and, most importantly, the magnitude of the *polAexo* mutator effect is unaltered and remains confined to the lagging strand orientation. The NER-deficiency did also not significantly change the *polAexo* mutator effect for the *lac* A·T→T·A transversion and did not affect the level of the *lac* A·T→G·C transitions (data not shown). Likewise, no altered *polAexo* effects were noted in the Δ*uvrB* background (not shown). Therefore, we conclude that the additional mutations observed in the *polAexo* strains are not a result of errors made by Pol I in excision repair tracts.

Discussion

Two major roles for DNA polymerase I have been established previously: (i) the maturation of Okazaki fragments by removal of the RNA primers in the lagging strand and filling the

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resulting gaps, and (ii) filling excision repair tracts during excision repair (NER and BER). In addition to those roles, another possible function of Pol I that we investigated in this work is its active role in polymerase switching at the replication point, analogously to the proposed roles of Pol II and Pol IV. In particular, a correction of Pol III-mediated misinsertion errors by proofreading-proficient polymerases such as Pol I (and Pol II) might constitute a chromosomal fidelity mechanism. Our observations of a mutator effect associated with the exonuclease-deficient form of Pol I indicate that Pol I plays a fidelity role under normal wild-type conditions. This fidelity role appears to be modest, up to about 4-fold, depending on the base-pair substitution investigated. With regard to the precise mechanism underlying this fidelity function, three findings are most relevant: (i) preferential mutator effects for lagging strand mutational events, (ii) lack of synergism between the *polAexo* mutator effect and other tested polymerase or replication defects, and (iii) apparent lack of competition of Pol I with the accessory polymerases Pol II and Pol IV. Our overall conclusion based on the current data and analysis following below is that this fidelity role is likely limited to the error-free processing Okazaki fragments and associated gap-filling, and does not relate to any direct polymerase exchanges at the replication fork nor involves excessive NER activity.

The lagging strand preference of the *polAexo* effect is obviously consistent with the established role of Pol I in processing the Okazaki fragments. Assuming an average Okazaki fragment length of 1000 nucleotides and the length of RNA primers of 10–20 nt, Pol I is likely responsible for 1% to 2% of all synthesis in the lagging strand. While a relatively small fraction, this mode of synthesis, if significantly less accurate than that of the Pol III HE, may still contribute measurably to the overall error rate. For example, if the exonuclease-deficient Pol I would be 50-fold less accurate than the proofreading-proficient Pol III HE, a not unreasonable assumption, then a 2% contribution may produce a doubling of the chromosomal error rate.

We have previously presented evidence suggesting that other DNA polymerase defects or disturbances can lead to mutator effects specific for the lagging strand, such as the overproduction of Pol V (Maliszewska-Tkaczyk *et al*., 2000) or Pol IV (Kuban *et al*., 2005; Gawel *et al*., 2008) or from the proofreading-deficient form of Pol II (Banach-Orlowska *et al*., 2005; Gawel *et al*., 2008). These results were interpreted to indicate preferential (although not exclusive, such as in the case of Pol II) access of these polymerases to the lagging strand. In all cases, the model considered that the action of these polymerases occurred on terminal mismatches created by misinsertions made by the replicative Pol III HE. These mismatches might be particularly accessible to alternative polymerases in the lagging strand due to the increased dissociative character of HE in this strand. The present study was undertaken to probe whether Pol I might also be involved in this type of terminal error processing. The results do not support this idea: the observed lack of synergism of the *polAexo* mutator with other replication-associated mutator effects as well as the lack of competition of Pol I with Pol II and/or Pol IV, suggest clearly that the action of Pol I resides in a distinctly different pathway from that envisioned for Pol II and Pol IV. While the latter three enzymes may compete with Pol III for mispairs at the replication point, this is not the case for Pol I. Instead, the operation of Pol I in remedying Okazaki fragment gaps provides a pathway that is separate from the polymerase switching at the replication point and readily accounts for the nonsynergistic (i.e., additive) effects of the *polAexo* mutator, as well as, of course, its preference for the lagging strand.

Our results with the NER-deficient *uvr* strains do not support a significant contribution to spontaneous mutation by Pol I-mediated gap filling during NER, a pathway recently proposed (Hasegawa *et al*., 2008). Notably, no significant differences were observed in the mutant frequencies (for either pol^{+} or *polAexo* strains) when comparing NER^{+} and NER^{-}

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strains. Secondly, it is unlikely that the action of Pol I during NER-mediated gap filling will lead to a strand bias favoring the lagging strand. Finally, most of our experiments were performed in the mismatch-repair-defective background, where uncorrected replication errors are expected to largely outnumber mutations from other sources. Obviously, errors made by Pol I during Okazaki fragment filling (in contrast to errors made during NER away from the replication point), are expected to be subject to correction by MMR, as is observed (see Table 1–Table 4).

To possibly address the discrepancy with the Hasegawa *et al*. (2008) study, it is worth pointing out that, in addition to any strain background effects, there are at least three significant differences between the two studies. Hasegawa *et al*. (2008) analyzed rifampicin-resistant mutants, occurring at a large number of sites in the *rpoB* gene. Secondly, they used exclusively mismatch-repair-proficient strains. Thirdly, the role of Pol I was assayed by expressing the *polAexo* gene from a multicopy plasmid, likely leading to significant overexpression. Each of these factors may affect the outcome and interpretation, and further studies will be needed to clarify these issues. Nevertheless, our present analysis of the strand-specific mutability of a series of *lac* alleles in the mismatch-repair defective background provides solid support for our proposed role of Pol I at the replication fork.

In Fig. 1 we present a scheme incorporating the current results on Pol I as well as previous data on the participation and contribution of other DNA polymerases to *E. coli* chromosomal replication fidelity (Kuban *et al*., 2004;Banach-Orlowska *et al*., 2005;Kuban *et al*., 2005). While Pol III, as the major replicative enzyme, provides for high overall fidelity, occasional participation by Pol II can lead to a further reduction in error rate by functioning as a back-up proofreader in cases where HE has difficulty negotiating a terminal mismatch (Banach-Orlowska *et al*., 2005;Gawel *et al*., 2008). Under those conditions, Pol II also acts to prevent access by Pol IV, whose action would lead to an increase in the chromosomal error rate. The precise mechanism underlying the hand-off to Pol II is not yet clear but may involve the action of the Pol III τ subunit (Pham *et al*., 2006;Gawel *et al*., 2008). The apparent exclusion from this process of Pol I, present in abundant quantities in the cell, as indicated here also suggests that this hand-off is not likely a simple mass-action driven process. When overproduced, Pol IV and Pol V can also gain access to the fork, leading to increased error rates. Their contributions seems largely confined to the lagging strand, and one may presume that there is a fundamental difference in the ways by which accessory polymerases obtain access to the two strands. Finally, Pol I is limited to the filling of Okazaki fragments generated during lagging strand DNA synthesis, a role for which it is well adapted due to its associated 5'→3' exonuclease. Its 3'→5' (proofreading) exonuclease ensures that this gapfilling occurs with sufficient accuracy so as not to produce an increase in the overall replication error rate.

Experimental procedures

Media

Solid and liquid media were as described (Fijalkowska and Schaaper, 1995). Minimal plates were supplemented with 0.4% glucose or 0.4% lactose as a carbon source and 50 $\mu g\ ml^{-1}$ amino acids, as required. Antibiotics were added as follows: tetracycline, 12.5 $\mu g\ ml^{-1}$; chloramphenicol, 24 $\mu g\ ml^{-1}$; spectinomycin, 20 $\mu g\ ml^{-1}$; kanamycin 50 $\mu g\ ml^{-1}$; ampicillin, 25 $\mu g\ ml^{-1}$; rifampicin, 100 $\mu g\ ml^{-1}$; nalidixic acid, 30 $\mu g\ ml^{-1}$.

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Construction of plasmids

Generally, plasmids were constructed according to the standard protocols as described by Sambrook *et al*. (2001). Propagation of plasmids was performed in *E. coli* DH5α. PCR products were confirmed by DNA sequencing.

Cloning of the *polA* gene

Genomic DNA from strain MC4100 was used as a template to clone the *polA* gene by PCR amplification (using *Pfu* Turbo polymerase) with primers: UP-polA: 5'-CGCTTAAAGCTTTTGTCATTGATGTAG-3' (*Hind*III site underlined) and LOW-polA: 5'-GCATAGGGAATTCTAATAGCCATCAC-3' (*EcoR*I site underlined). The 4.1-kb *Hind*III/*EcoR*I fragment was then inserted into the chloramphenicol-resistance low-copy plasmid pHSG576 (Takeshita *et al*. 1987) resulting in plasmid ppolA (~7.7 kb). Correctness of the construct was confirmed by sequencing with several primers homologous to the sequence of *polA* gene.

Construction of the *polAD424A* (*polAexo*) allele

To create plasmid ppolAex containing the exonuclease-deficient D424A mutation in *polA* (positions 1270–1272), PCR amplification was performed using plasmid ppolA as a template and as primers D424A-F, 5'-CAAAACCTGAAATACGCGCGCGGTATTCTGGCGAACTACGGC-3', and D424A-R, 5'-GCCGTAGTTCGCCAGAATACCGCGCGCGTATTTCAGGTTTTG-3'. The PCR reaction was performed using the Stratagene Site-Directed Mutagenesis Kit and protocols provided by the manufacturer. Replacement of the GAT codon by GCG at *polA* positions 1270–1272 leads to substitution of Asp424 by Ala (D424A). Among transformants, the presence of the D424A mutation was verified by sequencing the *polA* region. The D424A alteration also leads to the loss of a *Pvu*I restriction site.

Gene replacement cassette for substitution of chromosomal *polA*+ by *polAexo*

The cassette used for replacing the chromosomal *polA* gene by the *polAexo* variant contained the *cat* gene from transposon Tn*9* inserted into the intergenic region between the *polA* gene and the adjacent *spf* gene. The cassette was assembled in three sections, in linear order: (i) a KpnI-EcoRI fragment containing the *polA*+ or *polA*(*exo*) gene, (ii) an EcoRI-Hind III fragment gene containing the *cat* gene from transposon Tn*9*, and (iii) a HindIII-BamHI fragment containing part of the *spf* gene, which is located downstream of *polA* on the chromosome. The *polA* alleles were amplified by PCR from plasmid ppolA or ppolAex, respectively, using as primers: UP-polA, 5'-GGTACCCTCTCATACCAGCTGGCGACG-3' (KpnI_site underlined) and LOW-polA, 5'-GCATAGGGAATTCTAATAGCCATCAC-3' (EcoRI_site underlined). The resulting KpnI-EcoRI PCR products (~2.1 kb) were cloned into pBluescript II SK (+) (Stratagene) using the KpnI and EcoRI sites, yielding plasmids pSKpolA and pSKpolAD424A, respectively. The *cat* gene of transposon *Tn*9 was PCR amplified using plasmid pHSG576 (Takeshita *et al*. 1987) as a template and as primer pair: up_Tn9 5'-CTAGAATTCGCGCCGAATAAATACCTGTGACG-3' (*EcoR*I site underlined) and lw_Tn9 5'-GCACTTTTTGTAATTTTTTTCAGTTGTTGCATAGGAAGCTTAACTGGCCTCAGGCAT TTGAG-3' (HindIII site underlined) resulting in 1097-bp PCR product. Primer lw_Tn9 is partially homologous to the *spf* gene (double underline). The third fragment was prepared from chromosomal DNA of strain MC4100 by amplification of the *spf* gene downstream of *polA* using as primers: up_spf (homologous to lw_Tn9) 5'-GTTAAGCTTCCTATGCAACAACTGAAAAAAAATTACAAAAAGTGC-3' (HindIII site underlined) and lw_spf 5'-GCAGGATCCTTTCTTGCCTTAATGCTTGTGCC-3' (BamHI site underlined), yielding a 311-bp PCR product. The 1097-bp and 311-bp PCR fragments

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were then joined by an amplification reaction using primers up_Tn9 and lw_spf described above, yielding a 1408-bp Tn*9*-*spf* fragment. This fragment was ligated into EcoRI/BamHI digested plasmids pSKpolA and pSKpolAD424A, yielding plasmids pSKpolAint and pSKpolAD424Aint, carrying the complete gene replacement cassettes. KpnI/BamHI restriction of pSKpolAint and pSKpolAD244Aint yielded the linear cassettes used for replacement (see below).

Gene replacement

To replace the chromosomal *polA*$^+$ gene with the *cat*-containing *polA*$^+$ or *polAexo* cassette described above, we used a modification of the method of Datsenko and Wanner (2000). Strain BW25141 carrying plasmid pKD46 (providing the λ-Red recombination proteins) was transformed with the linear KpnI-BamHI DNA fragments described above. Upon transformation of the fragment into BW25141 (pKD46), homologous recombination between the *polA* and *spf* regions of chromosome and the fragment led to integration of the *cat*-carrying cassette into the chromosome, permitting selection of the desired recombinants by chloramphenicol resistance. P1 transduction using P1*virA* was then used to transfer the new alleles into strain KA796 (Schaaper *et al*., 1985). The resulting strains were proven to carry the *polA*$^+$ or *polAexo* allele by sequencing of the *polA* gene. Replacement of the chromosomal *polA* region by the Tn*9cat*-containing cassette was confirmed by two PCR reactions. The presence of the D424A mutation was confirmed by single-colony PCR using as primers 5'-CGGACTGGATACGCTGTATGC-3' (forward) and 5'-CTTCCAGTACCTCTTCCGACG-3' (reverse), yielding a ~1.2 kb product. As the D424A alteration leads to the loss of a PvuI restriction site, the PCR product was digested with PvuI. Only in case of the wild-type *polA*$^+$ allele the digestion results in 646-bp and 574-bp products. The second colony PCR was performed using one primer internal to the Tn*9cat* sequence (5'-AACGTGGCCAATATGGAC-3' forward) and a second primer complementary to a sequence in the adjacent *yihA* gene (5'-ACACGCTGACTAACCAGA-3' reverse) (gene order *polA-cat-spf-yihA*). A ~1.2-kb PCR product was obtained only if the integration cassette was introduced into the expected chromosomal position of the *polA* locus.

Expression analysis

To check whether expression of the *polA* gene from the *cat*-containing chromosomal construct was altered in any way from the normal chromosomal configuration, we performed real-time Quantitative PCR analysis of *polA* mRNA. These experiments are detailed in the Supporting Information. The results (Fig. S1) indicate that expression of the *polA* gene is unaffected by the presence of the nearby *cat* gene. Expression of the *polAexo* gene was slightly reduced to 76 % of that of the *polA*$^+$ gene, possibly within the margin of error.

Strain constructions

For the purpose of mutagenesis studies, the new KA796-derived strains containing the *polA*$^+$ or *polAexo (polAD424A)* alleles (see above) were used as donors in P1*virA*-mediated transductions into a set of *lacZ* tester strains. These strains, described before (Fijalkowska *et al*., 1998), contain defined *lacZ* alleles in the two possible orientations on the chromosome. With the one exception indicated below, they are derivatives of MC4100 [Δ(*argF-lac*)*169*] containing the *lac* operon derived from strains CC102, CC104, CC105, and CC106 (Cupples and Miller, 1989) inserted into the phage λ attachment site in two orientations (Fijalkowska *et al*., 1998). The four *lacZ* missense alleles allow scoring of mutagenesis *via* reversion to *lac*$^+$ by a specific base substitution mutation: G·C→A·T, G·C→T·A, A·T→T·A, or A·T→G·C, respectively (Cupples and Miller, 1989).

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The *mutL*::Tn*5* or *mutL*::Tn*10* markers, specifying a deficiency in mismatch repair, were introduced by transduction from strains NR9559 (Fijalkowska and Schaaper, 1995) or NR9161 (Schaaper *et al*., 1989) using selection for kanamycin or tetracycline resistance, respectively. The *polB1* deletion allele [Δ(*araD-polB*)::Ω] was transferred by transduction from strain SH2101 (Escarceller *et al*., 1994) using spectinomycin resistance as a selective marker. The *polBex1* derivatives were created as described (Banach-Orlowska *et al*., 2005). The *dinB*::*kan* marker was introduced by transduction from YG7207 (Kim *et al*., 1997).

The *dnaQ49* and *dnaQ928* alleles, specifying Pol III proofreading defects, were introduced by transduction from strain NR9695 (Schaaper and Cornacchio, 1992) or NR11641 (Taft-Benz and Schaaper, 1998), respectively, using selection for tetracycline resistance and scoring for mutator phenotype (~ 40% linkage of *dnaQ* with *zae-502*::Tn*10*).

The *dnaN159* (TS) allele, specifying a defect in the HE β-clamp, was transferred from strain HC194, obtained from the Coli Genetic Stock Center (Yale University). First, the *dnaN* allele was linked in HC194 with transposon *zid-501*::Tn*10* (Singer *et al*. 1989), yielding strain NR11824 (*dnaN159*, *zid-501*::Tn*10*). NR11824 was then used as donor for subsequent transfers of *dnaN159* (selection for tetracycline resistance and scoring for impaired growth at 42°C).

The *dnaE486* (TS) and *dnaE511* (TS) alleles were transferred from strains CS115 and AR2 (Vandewiele *et al*., 2002), respectively, kindly provided by Dr. R. Woodgate (NICHHD, NIH). Selection was for tetracycline resistance conferred by transposon *zae-502*::Tn*10*, followed by screening for impaired growth at 42°C (~ 40% linkage).

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The *dnaE915* (antimutator) allele was transferred from strain NR9905, in which *dnaE915* is flanked by two nearby markers, *zae-502*::Tn*10* and *zae*::Tn*10d-Cam* (Fijalkowska *et al*. 1993). An intermediate strain containing *dnaE915* linked only to *zae-502*::Tn*10* was constructed, which was then used to transfer *dnaE915* into the desired *lacZ* strains carrying *polA*$^{+}$ or *polAexo*, using tetracycline resistance. In all cases, the presence of *dnaE915* allele was confirmed by sequencing.

The Δ*uvrA*::mini-Tn*10kan*, Δ*uvrB*::mini-Tn*10kan* and Δ*uvrC*::mini-Tn*10kan* mutations were transferred from strains JD25374, JD26189, and JD26681, respectively, obtained from the National Institute of Genetics, Japan (http://www.shigen.nig.ac.jp/ecoli/strain/top/top.jsp). Selection was for kanamycin resistance, followed by screening for UV sensitivity.

Experiments with the *dnaX36* allele were performed in a slightly different strain background. Strain NR11927 (*ara*, *thi*, Δ*prolac*, *mutL*::Tn*5*, *dnaX36*), identical to the previously described strain NR11928 (Pham *et al*., 2006), was transduced to carry the *lac* operon of strain CC104 in two orientations in the λ *att* site (Fijalkowska *et al*., 1998) using ampicillin resistance selection, followed by introduction of the *polAexo* allele.

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Mutant frequency measurements

Mutant frequencies were determined by starting, for each strain, 10–20 cultures (2 ml of LB) from single colonies and growing them to saturation at 37°C (30°C or 34°C, where indicated) with agitation. Colonies were taken from two or three independent *lac* integrants for each *lac* orientation and usually from several independent transductants (e.g., *polA*$^{+}$, *polAexo*, etc.) for each integrant. As a *polA*$^{+}$ control for all *polAexo* experiments, the analogously constructed *polA*$^{+}$ strains were used that also carried the *cat* cassette (see construction, above). For *lac* reversions assays appropriate dilutions were plated on minimal glucose plates to determine the total cell count and on minimal lactose plates to determine

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the number of *lac*$^{+}$ mutants. For the forward mutagenesis assays toward rifampicin or nalidixic acid resistance, appropriate dilutions were plated on LB plates to determine the total cell count and on LB plates containing rifampicin or nalidixic acid to determine the number of antibiotic-resistant mutants. To calculate mutant frequencies, the number of mutants per plate was divided by the total number of cells. The non-parametric Mann-Whitney criterion (Sokal and Rohlf, 1981) was applied to the mutant frequency distributions for the purpose of comparing any given set of two strains using Statistica 5.5 (StatSoft) analysis software.

Acknowledgments

We thank Drs. Aneta Kaniak, Adrianna Skoneczna and Jaroslaw Ciesla for support with the PCR experiments, and Drs. Thomas Kunkel and Mark Itsko of the NIEHS for their careful reading of the manuscript for this paper. This work was supported by grant 2 PO4A 061 30 (to K.M., M.J., I.J.F. and P.J.) from the Polish Ministry of Science and Higher Education, and by funds allocated to project number Z01 ES065086 of the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

References

Banach-Orlowska M, Fijalkowska IJ, Schaaper RM, Jonczyk P. DNA polymerase II as a fidelity factor in chromosomal DNA synthesis in *Escherichia coli*. Mol Microbiol. 2005; 58:61–70. [PubMed: 16164549]

Bebenek K, Joyce CM, Fitzgerald MP, Kunkel TA. The fidelity of DNA synthesis catalyzed by derivatives of *Escherichia coli* DNA polymerase I. J Biol Chem. 1990; 265:13878–13887. [PubMed: 2199444]

Bonner CA, Randall SK, Rayssiguier C, Radman M, Eritja R, Kaplan BE, et al. Purification and characterization of an inducible *Escherichia coli* DNA polymerase capable of insertion and bypass at abasic lesions in DNA. J Biol Chem. 1988; 263:18946–18952. [PubMed: 3058691]

Bonner CA, Hays S, McEntee K, Goodman MF. DNA polymerase II is encoded by the DNA damage-inducible *dinA* gene of *Escherichia coli*. Proc Natl Acad Sci USA. 1990; 87:7663–7667. [PubMed: 2217198]

Bonner CA, Stukenberg PT, Rajagopalan M, Eritja R, O'Donnell M, McEntee K, et al. Processive DNA synthesis by DNA polymerase II mediated by DNA polymerase III accessory proteins. J Biol Chem. 1992; 267:11431–11438. [PubMed: 1534562]

Cai H, Yu H, McEntee K, Kunkel TA, Goodman MF. Purification and properties of wild-type and exonuclease-deficient DNA polymerase II from *Escherichia coli*. J Biol Chem. 1995; 270:15327–15335. [PubMed: 7797520]

Cupples CG, Miller JH. A set of *lacZ* mutations in *Escherichia coli* that allow rapid detection of each of the six base substitutions. Proc Natl Acad Sci USA. 1989; 86:5345–5349. [PubMed: 2501784]

Curti E, McDonald JP, Mead S, Woodgate R. DNA polymerase switching: effects on spontaneous mutagenesis in *Escherichia coli*. Mol Microbiol. 2008; 71:315–331. [PubMed: 19019142]

Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci USA. 2000; 97:6640–6645. [PubMed: 10829079]

Escarceller M, Hicks J, Gudmundsson G, Trump G, Touati D, Lovett S. Involvement of *Escherichia coli* DNA polymerase II in response to oxidative damage and adaptive mutation. J Bacteriol. 1994; 176:6221–6228. [PubMed: 7928992]

Fijalkowska IJ, Dunn RL, Schaaper RM. Mutants of *Escherichia coli* with increased fidelity of DNA replication. Genetics. 1993; 134:1023–1030. [PubMed: 8375645]

Fijalkowska IJ, Schaaper RM. Effects of *Escherichia coli* DNA antimutator alleles in a proofreading-deficient *mutD5* strain. J Bacteriol. 1995; 177:5979–5986. [PubMed: 7592352]

Fijalkowska IJ, Dunn RL, Schaaper RM. Genetic requirements and mutational specificity of the *Escherichia coli* SOS mutator activity. J Bacteriol. 1997; 179:7435–7445. [PubMed: 9393709]

Fijalkowska IJ, Jonczyk P, Maliszewska-Tkaczyk M, Bialoskorska M, Schaaper RM. Unequal fidelity of leading and lagging strand DNA replication on the *Escherichia coli* chromosome. Proc Natl Acad Sci USA. 1998; 95:10020–10025. [PubMed: 9707593]

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Friedberg, EC.; Walker, GC.; Siede, W. DNA Repair and Mutagenesis. Washington, DC: ASM Press; 1995.

Friedberg, EC.; Walker, GC.; Siede, W.; Wood, R.; Schultz, RA.; Ellenberger, T. DNA Repair and Mutagenesis. 2nd edn.. Washington, DC: ASM Press; 2006.

Foster PL. Adaptive mutation in *Escherichia coli*. Cold Spring Harbor Symp. Quant Biol. 2000; 65:21–29. [PubMed: 12760017]

Foster PL, Gudmundsson G, Trimarchi JM, Cai H, Goodman MF. Proofreading-defective DNA polymerase II increases adaptive mutation in *Escherichia coli*. Proc Natl Acad Sci USA. 1995; 92:7951–7955. [PubMed: 7644519]

Fuchs RP, Fujii S. Translesion synthesis in *Escherichia coli*: lessons from the NarI mutation hot spot. DNA Repair. 2007; 6:1032–1041. [PubMed: 17403618]

Gawel D, Jonczyk P, Bialoskorska M, Schaaper RM, Fijalkowska IJ. Asymmetry of frameshift mutagenesis during leading and lagging-strand replication in *Escherichia coli*. Mutat Res. 2002a; 501:129–136. [PubMed: 11934444]

Gawel D, Maliszewska-Tkaczyk M, Jonczyk P, Schaaper RM, Fijalkowska IJ. Lack of strand bias in UV-induced mutagenesis in *Escherichia coli*. J Bacteriol. 2002b; 184:4449–4454. [PubMed: 12142415]

Gawel D, Pham PT, Fijalkowska IJ, Jonczyk P, Schaaper RM. Role of accessory DNA polymerases in DNA replication in *Escherichia coli*: analysis of the *dnaX36* mutator mutant. J Bacteriol. 2008; 190:1730–1742. [PubMed: 18156258]

Gefter ML, Hirota Y, Kornberg T, Wechsler JA, Barnoux C. Analysis of DNA polymerases II and III in mutants of *Escherichia coli* thermosensitive for DNA synthesis. Proc Natl Acad Sci USA. 1971; 68:3150–3153. [PubMed: 4943556]

Goodman MF. Error-prone repair DNA polymerases in prokaryotes and eukaryotes. Annu Rev Biochem. 2002; 70:17–50. [PubMed: 12045089]

Hasegawa K, Yoshiyama K, Maki H. Spontaneous mutagenesis associated with nucleotide excision repair in *Escherichia coli*. Genes Cells. 2008; 13:459–469. [PubMed: 18429818]

Ichikawa-Ryo H, Kondo S. Indirect mutagenesis in phage L by ultraviolet pre-irradiation of host bacteria. J Mol Biol. 1975; 97:77–92. [PubMed: 1100853]

Jarosz DF, Beuning PJ, Cohen SE, Walker GC. Y-family DNA polymerases in *Escherichia coli*. Trends Microbiol. 2007; 15:70–77. [PubMed: 17207624]

Joyce CM, Grindley ND. Method for determining whether a gene of *Escherichia coli* is essential - application to the *polA* gene. J Bacteriol. 1984; 158:636–643. [PubMed: 6233260]

Joyce CM, Sun XC, Grindley ND. Reactions at the polymerase active site that contribute to the fidelity of *Escherichia coli* DNA polymerase I (Klenow fragment). J Biol Chem. 1992; 267:24485–24500. [PubMed: 1447195]

Kelman Z, O'Donnell M. DNA polymerase III holoenzyme: structure and function of a chromosomal replicating machine. Annu Rev Biochem. 1995; 64:171–200. [PubMed: 7574479]

Kim SR, Maenhaut-Michel G, Yamada M, Yamamoto Y, Matsui K, Sofuni T. Multiple pathways for SOS-induced mutagenesis in *Escherichia coli*: an overexpression of *dinB/dinP* results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA. Proc Natl Acad Sci USA. 1997; 94:13792–13797. [PubMed: 9391106]

Kim SR, Matsui K, Yamada P, Gruz P, Nohmi T. Roles of chromosomal and episomal *dinB* genes encoding Pol IV in targeted and untargeted mutagenesis in *Escherichia coli*. Mol Genet Genom. 2001; 266:207–215.

Kornberg, A.; Baker, TA. DNA Replication. 2nd edn.. New York: Freeman; 1992.

Kuban W, Jonczyk P, Gawel D, Malanowska K, Schaaper RM, Fijalkowska IJ. Role of *Escherichia coli* DNA polymerase IV in *in vivo* replication fidelity. J Bacteriol. 2004; 186:4802–4807. [PubMed: 15231812]

Kuban W, Banach-Orlowska M, Bialoskorska M, Lipowska A, Schaaper RM, Jonczyk P, Fijalkowska IJ. Mutator phenotype resulting from DNA polymerase IV overproduction in *Escherichia coli*: preferential mutagenesis on the lagging strand. J Bacteriol. 2005; 187:6862–6866. [PubMed: 16166552]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Kuban W, Banach-Orlowska M, Schaaper RM, Jonczyk P, Fijalkowska IJ. Role of DNA polymerase IV in *Escherichia coli* SOS mutator activity. J Bacteriol. 2006; 188:7977–7980. [PubMed: 16980447]

Lehmann AR, Niimi A, Ogi T, Brown S, Sabbioneda S, Wing JF, et al. Translesion synthesis: Y-family polymerases and the polymerase switch. DNA Repair. 2007; 6:891–899. [PubMed: 17363342]

López de Saro FJ, O'Donnell M. Interaction of ®-sliding clamp with MutS, ligase and DNA polymerase I. Proc Natl Acad Sci USA. 2001; 98:8376–8380. [PubMed: 11459978]

López de Saro FJ, Georgescu RE, Goodman MF, O'Donnell M. Competitive processivity-clamp usage by DNA polymerases during DNA replication and repair. EMBO J. 2003; 22:6408–6418. [PubMed: 14633999]

Maliszewska-Tkaczyk M, Jonczyk P, Bialoskorska M, Schaaper RM, Fijalkowska IJ. SOS mutator activity: unequal mutagenesis on leading and lagging strands. Proc Natl Acad Sci USA. 2000; 97:12678–12683. [PubMed: 11050167]

McHenry CS. DNA polymerase III holoenzyme. Components, structure, and mechanism of a true replicative complex. J Biol Chem. 1991; 266:19127–19130. [PubMed: 1918028]

McHenry CS, Kornberg A. DNA polymerase III holoenzyme of *Escherichia coli*. Purification and resolution into subunits. J Biol Chem. 1997; 252:6478–6484. [PubMed: 330531]

McHenry CS. Chromosomal replicases as asymmetric dimers: studies of subunit arrangement and functional consequences. Mol Microbiol. 2003; 49:1157–1165. [PubMed: 12940977]

Mo JY, Schaaper RM. Fidelity and error specificity of the alpha catalytic subunit of *Escherichia coli* DNA polymerase III. J Biol Chem. 1996; 271:18947–18953. [PubMed: 8702558]

Napolitano R, Janel-Bintz R, Wagner J, Fuchs RP. All three SOS-inducible DNA polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis. EMBO J. 2000; 19:6259–6265. [PubMed: 11080171]

O'Donnell M, Jeruzalemi D, Kuriyan J. Clamp loader structure predicts the architecture of DNA polymerase III holoenzyme and RFC. Curr Biol. 2001; 11:R935–R946. [PubMed: 11719243]

O'Donnell M. Replisome architecture and dynamics in *Escherichia coli*. J Biol Chem. 2006; 281:10653–10656. [PubMed: 16421093]

Okazaki R, Arisawa M, Sugino A. Slow joining of newly replicated DNA chains in DNA polymerase I-deficient *Escherichia coli* mutants. Proc Natl Acad Sci USA. 1971; 68:2954–2957. [PubMed: 4943548]

Pham PT, Olson MW, McHenry CS, Schaaper RM. The base substitution and frameshift fidelity of *Escherichia coli* DNA polymerase III holoenzyme *in vitro*. J Biol Chem. 1998; 273:23575–23584. [PubMed: 9722597]

Pham PT, Olson MW, McHenry CS, Schaaper RM. Mismatch extension by *Escherichia coli* DNA polymerase III holoenzyme. J Biol Chem. 1999; 274:3705–3710. [PubMed: 9920922]

Pham P, Bertram JG, O'Donnell M, Woodgate R, Goodman MF. A model for SOS-lesion targeted mutations in *E. coli*. Nature. 2001; 409:366–370. [PubMed: 11201748]

Pomerantz RT, O'Donnell M. Replisome mechanics: insights into a twin DNA polymerase machine. Trends Microbiol. 2007; 15:156–164. [PubMed: 17350265]

Pham PT, Zhao W, Schaaper RM. Mutator mutants of *Escherichia coli* carrying a defect in the DNA polymerase III τ subunit. Mol Microbiol. 2006; 59:1149–1161. [PubMed: 16430690]

Qiu Z, Goodman MF. The *Escherichia coli polB* locus is identical to *dinA*, the structural gene for DNA polymerase II. Characterization of Pol II purified from a *polB* mutant. J Biol Chem. 1997; 272:8611–8617. [PubMed: 9079692]

Rangarajan S, Gudmundsson G, Qiu Z, Foster PL, Goodman MF. *Escherichia coli* DNA polymerase II catalyzes chromosomal and episomal DNA synthesis *in vivo*. Proc Natl Acad Sci USA. 1997; 94:946–951. [PubMed: 9023362]

Rangarajan S, Woodgate R, Goodman MF. A phenotype for enigmatic DNA polymerase II: a pivotal role for pol II in replication restart in UV-irradiated *Escherichia coli*. Proc Natl Acad Sci USA. 1999; 96:9224–9229. [PubMed: 10430924]

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Reuven NB, Arad G, Maor-Shoshani A, Livneh Z. The mutagenesis protein UmuC is a DNA polymerase activated by UmuD', RecA, and SSB and is specialized for translesion replication. J Biol Chem. 1999; 274:31763–31766. [PubMed: 10542196]

Sambrook, J.; Russell, DW. Molecular Cloning. A laboratory manual. Third edition. Irwin, N.; Janssen, KA., editors. Vol. Vol.1. Cold Spring Harbor Laboratory Press; 2001.

Schaaper RM, Danforth BN, Glickman BW. Rapid repeated cloning of mutant *lac* repressor genes. Gene. 1985; 39:181–189. [PubMed: 4092929]

Schaaper RM, Cornacchio R. An *Escherichia coli dnaE* mutation with suppressor activity toward mutator *mutD5*. J Bacteriol. 1992; 174:1074–1082.

Schaaper RM, Bond BI, Fowler RG. A·T→C·G transversions and their prevention by the *Escherichia coli mutT* and *mutHLS* pathways. Mol Gen Genet. 1989; 219:256–262. [PubMed: 2693944]

Shcherbakova PV, Fijalkowska IJ. Translesion synthesis DNA polymerases and control of genome stability. Front Biosci. 2006; 11:2496–2517. [PubMed: 16720328]

Singer M, Baker TA, Schnitzler G, Deischel SM, Goel M, Dove W, et al. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol Rev. 1989; 53:1–24. [PubMed: 2540407]

Sokal, RR.; Rohlf, FJ. Biometry. 2nd edn.. San Francisco, CA: Freeman; 1981. p. 429-445.

Sutton MD. The *Escherichia coli dnaN159* mutant displays altered DNA polymerase usage and chronic SOS induction. J Bacteriol. 2004; 186:6738–6748. [PubMed: 15466025]

Sutton MD, Duzen JM. Specific amino acid residues in the beta sliding clamp establish a DNA polymerase usage hierarchy in *Escherichia coli*. DNA Repair. 2006; 5:312–323. [PubMed: 16338175]

Taft-Benz SA, Schaaper RM. Mutational analysis of the 3'→5' proofreading exonuclease of *Escherichia coli* DNA polymerase III. Nucleic Acids Res. 1998; 26:4005–4011. [PubMed: 9705512]

Taft-Benz SA, Schaaper RM. The theta subunit of *Escherichia coli* DNA polymerase III: a role in stabilizing the epsilon proofreading subunit. J Bacteriol. 2004; 186:2774–2780. [PubMed: 15090519]

Takano K, Nakabeppu Y, Maki H, Horiuchi T, Sekiguchi M. Structure and function of *dnaQ* and *mutD* mutators of *Escherichia coli*. Mol Gen Genet. 1986; 205:9–13. [PubMed: 3540531]

Takeshita S, Sato M, Toba M, Masahashi W, Hashimoto-Gotoh T. High-copy-number and low-copy-number plasmid vectors for *lacZ* alpha-complementation and chloramphenicol- or kanamycin-resistance selection. Gene. 1987; 61:63–74. [PubMed: 3327753]

Tang M, Shen X, Frank EG, O'Donnell M, Woodgate R, Goodman MF. UmuD'(2)C is an error-prone DNA polymerase, *Escherichia coli* pol V. Proc Natl Acad Sci USA. 1999; 96:8919–8924. [PubMed: 10430871]

Wagner J, Fujii S, Gruz P, Nohmi T, Fuchs RP. The β clamp targets DNA polymerase IV to DNA and strongly increases its processivity. EMBO Rep. 2001; 1:484–488. [PubMed: 11263491]

Wagner J, Etienne H, Janel-Bintz R, Fuchs RP. Genetics of mutagenesis in *E. coli*: various combinations of translesion polymerases (Pol II, IV and V) deal with lesion/sequence context diversity. DNA Repair. 2002; 1:159–167. [PubMed: 12509262]

Wagner J, Gruz P, Kim SR, Yamada M, Matsui K, Fuchs RP, Nohmi T. The *dinB* gene encodes a novel *E. coli* DNA polymerase, DNA pol IV, involved in mutagenesis. Mol. Cell. 1999; 4:281–286. [PubMed: 10488344]

Wagner J, Nohmi T. *Escherichia coli* DNA Polymerase IV mutator activity: genetic requirements and mutational specificity. J Bacteriol. 2000; 182:4587–4595. [PubMed: 10913093]

Wolff E, Kim M, Hu K, Yang H, Miller JH. Polymerases leave fingerprints: analysis of the mutational spectrum in *Escherichia coli rpoB* to assess the role of polymerase IV in spontaneous mutation. J Bacteriol. 2004; 186:2900–2905. [PubMed: 15090533]

Vandewiele D, Fernandez de Henestrosa AR, Timms AR, Bridges BA, Woodgate R. Sequence analysis and phenotypes of five temperature sensitive mutator alleles of *dnaE*, encoding modified α-catalytic subunits of *Escherichia coli* DNA polymerase III holoenzyme. Mutat Res. 2002; 499:85–95. [PubMed: 11804607]

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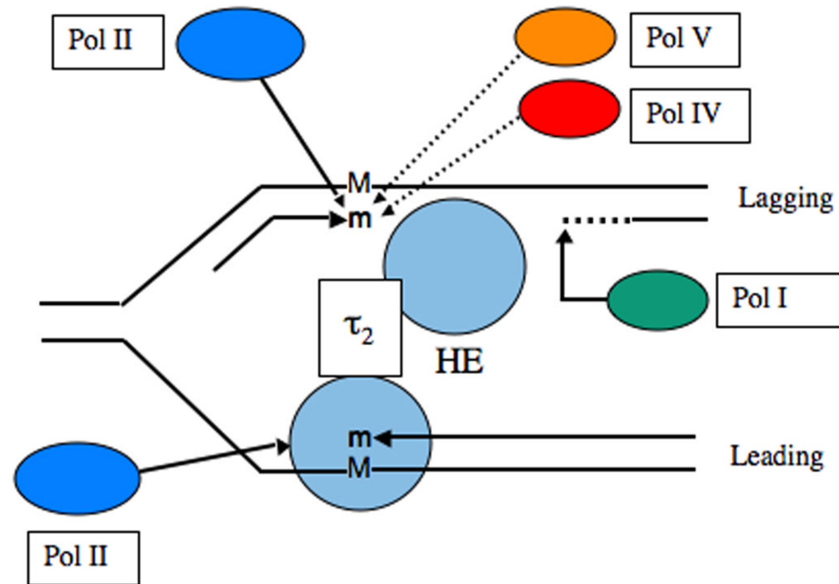

Fig. 1.
Participation of DNA polymerases at replication fork in *E. coli*. Shown is a scheme delineating events that might occur upon Pol III HE creating a 3' terminal mismatch (M·m) by misinsertion in lagging or leading strand. The HE is depicted in a simplified form as two Pol III cores connected by the dimeric τ subunit. The most likely outcome of a misinsertion is removal of the misinserted nucleotide by the Pol III proofreading activity. However, occasionally proofreading might not occur, and the resulting stalling of HE may provide an opportunity for other polymerases to participate. Here, we have shown a dissociation of the Pol III core from the mismatch in the lagging strand, which may occur in a manner similar to the dissociation normally occurring at the end of an Okazaki fragment. This provides an opportunity for Pol II to act as a back-up proofreader (Banach-Orlowska *et al*., 2005) or for Pol IV or Pol V to extend the mismatch (mutator activity). Polymerase exchange is also possible in the leading strand, but possibly through a different mechanism that strongly favors action by Pol II. The function of Pol I appears limited to fixing the Okazaki fragment gap, generally in an error-free way. See Text for more details.

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Table 1

Mutator effects of *polAexo* and *polBex* alleles in various strain backgrounds.[a]

lac allele[b]	*lac* orientation (strand)[c]	*lac* → *lac*$^+$ (per 10^8)			
		Pol$^+$	*polAexo*	*polBex*	*polAexo polBex*
A. *mutL*$^+$					
G·C → A·T	R(lagging)	2.5 ±0.7	2.3 ±0.9 [0.9]	ND	ND
	L(leading)	2.1 ±0.8	1.8 ±0.8 [0.8]	ND	ND
G·C → T·A	R(lagging)	0.8 ±0.7	3.3 ±1.0 [**4.1**]	ND	ND
	L(leading)	1.8 ±1.0	1.8 ±0.9 [1.0]	ND	ND
A·T → T·A	R(lagging)	0.9 ±0.5	1.5 ±0.5 [**1.6**]	ND	ND
	L(leading)	0.6 ±0.5	1.6 ±0.5 [**2.7**]	ND	ND
A·T → G·C	L(lagging)	0.1 ±0.1	0.1 ±0.1 [1.0]	ND	ND
	R(leading)	0.1 ±0.2	0.1 ±0.1 [1.0]	ND	ND
Rif^R		1.2 ±0.9	1.8 ±1.3 [**1.5**]	ND	ND
Nal^R		0.2 ±0.2	0.2 ±0.3 [1.0]	ND	ND
B. *mutL*					
G·C→A·T	R(lagging)	43 ±11	69 ±19 [1.6]	50 ±24 [1.2]	79 ±26 [1.8]
	L(leading)	150 ±35	167 ±26 [1.1]	200 ±41 [1.3]	163 ±38 [1.1]
G·C→T·A	R(lagging)	1.9 ±0.8	3.9 ±1.6 [**2.1**]	4.5 ±3.2 [**2.4**]	6.1 ±1.6 [3.2]
	L(leading)	3.8 ±0.7	2.6 ±0.8 [0.7]	3.6 ±1.4 [0.9]	3.3 ±1.6 [0.9]
A·T→T·A	R(lagging)	1.0 ±0.7	1.7 ±1.0 [**1.7**]	1.6 ±0.9 [**1.6**]	ND
	L(leading)	3.0 ±1.3	5.5 ±4.0 [**1.8**]	2.4 ±1.2 [0.8]	ND
A·T→G·C	L(lagging)	17 ±3	23 ±5 [**1.4**]	26 ±12 [**1.6**]	ND
	R(leading)	48 ±10	48 ±9 [1.0]	36 ±22 [0.8]	ND
Rif^R/10^8		277 ±115	244 ±166 [0.9]	311 ±170 [1.1]	261 ±74 [0.9]
C. *mutL dnaE915*					
G·C→A·T	R(lagging)	9.0 ±3.6	34 ±31 [**3.8**]	63 ±32 [**6.4**]	86 ±26 [8.7]
	L(leading)	30 ±4	29 ±3.0 [1.0]	67 ±13 [**2.2**]	51 ±12 [1.7]
G·C→T·A	R(lagging)	1.6 ±0.6	4.1 ±0.6 [**2.6**]	6.0 ±2.0 [**3.8**]	8.8 ±2.8 [5.5]

lac allele[b]	*lac* orientation (strand)[c]	*lac* → *lac*+ (per 10^8)			
		***Pol*+**	***polAexo***	***polBex***	***polAexo polBex***
	L(leading)	0.7 ±0.3	0.7 ±0.3 [1.0]	2.5 ±0.9 [**3.6**]	2.9 ±0.8 [4.1]
A·T→T·A	R(lagging)	0.5 ±0.6	1.7 ±3.4 [**3.4**]	3.8 ±1.9 [**7.6**]	ND
	L(leading)	0.3 ±0.4	9.5 ±4.4 [**32**]	2.2 ±0.7 [**7.3**]	ND
A·T→G·C	L(lagging)	7.0 ±1.1	18 ±2.4 [**2.6**]	35 ±7 [**5.0**]	ND
	R(leading)	5.5 ±2.0	8.2 ±3.1 [1.5]	12 ±7 [**2.2**]	ND
$Rif^R/10^8$		62 ±29	87 ±35 [1.4]	250 ±190 [**4.0**]	290 ±130 [4.7]

[a] The three strain backgrounds are: (A) mismatch-repair proficient (*mutL*+), (B) mismatch-repair deficient (*mutL*::Tn*5*), and (C) mismatch-repair deficient (*mutL*::Tn*5*) plus *dnaE915* (antimutator allele, see text). Mutant frequencies are based on the median value for 10–20 independent cultures (see Experimental procedures). In [brackets] the *polAexo* or *polBex* mutator effect defined as the increase in mutant frequency relative the *pol*+ strain.

Bold numbers indicate statistically significant values ($P < 0.05$, see Experimental procedures).

ND – not determined.

[b] *lac* indicates the *lac* allele which reversion is being tested (see text).

[c] The L and R orientations of the *lac* operon are as defined in Fijalkowska *et al*. (1998). The DNA strand in parentheses denotes the strand in which the presumed dominant mispair occurs for each of the four base substitutions (G·T for G·C→A·T, C·T for G·C→T·A, T·T for A·T→T·A, and T·G for A·T→G·C) (template base indicated first). See text and Fijalkowska *et al*. (1998) for a discussion.

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Table 2

Effect of *polAexo* and *polBex* on *lac* G·C→T·A mutant frequency in strains with impaired Pol III HE.[a]

Strain	***lac*** **→** ***lac***$^{+}$ **(per** 10^{8}**)**		
	polA^{+}	***polAexo***	***polBex***
mutL^{+}	0.4 ± 0.2	1.3 ± 0.3	1.6 ± 1.0
mutL^{+} *dnaQ928*	9.2 ± 2.2	11.6 ± 1.4	16 ± 7.0
mutL^{+} *dnaE486*	2.8 ± 1.4	4.0 ± 1.6	251 ± 23
mutL^{+} *dnaE511*	2.0 ± 1.4	3.9 ± 1.8	163 ± 35
mutL	1.4 ± 0.7	4.6 ± 2.3	8.4 ± 11
mutL dnaQ49	44 ± 23	42 ± 12	120 ± 11
mutL dnaN159	5.6 ± 1.7	9.8 ± 3.2	34 ± 7.0
mutL dnaX36	56 ± 12	81 ± 16	ND

[a]The experiments were performed with the *lac* G·C→T·A allele in the R(lagging) orientation. Mismatch repair deficient strains were *mutL*::Tn*5*. Mutant frequencies were determined as described in Experimental Procedures. Each entry is based on the median value for 10–20 independent cultures grown at 30°C (*dnaQ49*, *dnaN159*), 34°C (*dnaQ928*, *dnaE486*, *dnaE511*), or 37°C *(dnaX36)*.

ND – not determined.

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Table 3

Competition between Pol I, Pol II and Pol IV DNA polymerases.[a]

Strain	*lac* orientation (strand)	*lac* G·C→T·A (per 10^8)		*polAexo* effect
		polA+	*polAexo*	
A. *mutL strains*				
dnaE+	R(lagging)	3.3 ± 0.9	9.1 ± 2.8	**2.7**
dnaE+ Δ*dinB*	R(lagging)	4.0 ± 1.2	11.5 ± 1.3	**2.9**
dnaE+ Δ*polB*	R(lagging)	3.0 ± 1.2	10.5 ± 2.7	**3.5**
dnaE+ Δ*dinB* Δ*polB*	R(lagging)	4.6 ± 1.8	12.4 ± 2.7	**2.7**
B. *mutL*+ strains				
dnaE486	R(lagging)	4.2 ± 1.7	5.9 ± 2.2	1.4
dnaE486 Δ*dinB*	R(lagging)	2.7 ± 1.3	3.1 ± 1.4	1.1
dnaE486 Δ*polB*	R(lagging)	8.4 ± 2.5	6.3 ± 3.9	0.8
dnaE486 Δ*dinB* Δ*polB*	R(lagging)	0.8 ± 0.6	1.3 ± 0.7	**1.6**

[a]The experiments were performed with the *lac* G·C→T·A allele in the R(lagging) orientation. The *mutL* strains were *mutL*::Tn*10*. Mutant frequencies were determined as described in Experimental procedures. Each entry is based on the median value for 10 independent cultures grown at 37°C (*dnaE*+) or 34°C (*dnaE486*). No statistically significant effects are observed for the Δ*polB*, Δ*dinB*, or Δ*polB* Δ*dinB* strains in the *polA*+ *dnaE*+ series (both *mutL*+ and *mutL*) ($P > 0.05$). The corresponding differences in the *polA*+ *dnaE486* series are significant ($P < 0.05$).

Statistically significant *polAexo* mutator effects are indicated in **bold**.

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Table 4

Mutator effects of proofreading-deficient *polAexo* mutant in NER-deficient background.[a]

		lac **orientation (strand)**	***lac*** **→ *lac*⁺ (per 10^8)**		***polAexo* effect**
			***polA*⁺**	***polAexo***	
A. *mutL*⁺ strains					
uvr⁺	G·C→A·T	R(lagging)	0.6 ± 0.3	0.5 ± 0.3	0.8
		L(leading)	0.8 ± 0.4	0.5 ± 0.2	
	G·C→T·A	R(lagging)	1.0 ± 0.7	2.4 ± 1.0	**2.4**
		L(leading)	1.0 ± 0.4	0.9 ± 0.4	
uvrA	G·C→A·T	R(lagging)	1.4 ± 0.6	1.1 ± 0.9	0.8
		L(leading)	1.1 ± 0.4	1.3 ± 0.4	
	G·C→T·A	R(lagging)	1.3 ± 0.6	3.3 ± 0.9	**2.5**
		L(leading)	1.4 ± 0.5	1.45 ± 0.6	
uvrC	G·C→A·T	R(lagging)	1.2 ± 0.5	1.2 ± 0.6	1.0
		L(leading)	1.0 ± 0.4	1.0 ± 0.5	
	G·C→T·A	R(lagging)	0.85 ± 0.6	2.2 ± 0.5	**2.6**
		L(leading)	0.86 ± 0.4	1.7 ± 1.4	
B. *mutL* strains					
uvr⁺	G·C→A·T	R(lagging)	33 ±15	39 ± 20	1.2
		L(leading)	139 ± 28	153 ± 13	
	G·C→T·A	R(lagging)	1.4 ± 0.5	4.1 ± 1.5	**2.9**
		L(leading)	4.1 ± 0.9	2.8 ± 1.2	
uvrA	G·C→A·T	R(lagging)	20 ± 7	26 ± 5	1.3
		L(leading)	129 ± 18	149 ± 16	
	G·C→T·A	R(lagging)	2.1 ± 0.8	4.4 ± 1.2	**2.1**
		L(leading)	3.2 ± 1.6	3.9 ± 1.7	
uvrC	G·C→A·T	R(lagging)	21 ± 6	37 ± 6	**1.8**
		L(leading)	126 ± 22	124 ± 27	
	G·C→T·A	R(lagging)	2.1 ± 0.6	5.8 ± 2.8	**2.7**
		L(leading)	3.6 ± 0.9	3.6 ± 2.0	

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[a]Mutant frequencies were determined as described in Experimental Procedures. Each entry is based on the median value for 10 independent cultures grown at 37°C. The mismatch-repair-deficient strains were *mutL*::Tn*10*. The *polAexo* effect was calculated dividing the frequency of lac^+ mutants in the *polAexo* and $polA^+$ strains. All *polAexo* mutator effects shown in bold were statistically significant ($P < 0.05$, see Experimental procedures).