Published in final edited form as:
Mutat Res. 2011 July 1; 712(1-2): 28–32.

Proofreading deficiency of Pol I increases the levels of spontaneous *rpoB* mutations in *E. coli*

K. Makiela-Dzbenska[a], P. Jonczyk[a], R. M. Schaaper[b], I. J. Fijalkowska[a,*]

[a]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106 Warsaw, Poland

[b]National Institute of Environmental Health Sciences, PO Box 12233, Research Triangle Park, North Carolina 27709, USA

Corresponding author. Tel. +48 22 592 1113; fax: +48 22 658 4636. E-mail address: iwonaf@ibb.waw.pl (Iwona J. Fijalkowska)

ABSTRACT

The fidelity role of DNA polymerase I in chromosomal DNA replication in *E. coli* was investigated using the *rpoB* forward target. These experiments indicated that in a strain carrying a proofreading-exonuclease-defective form of Pol I (*polAexo* mutant) the frequency of *rpoB* mutations increased by about 2-fold, consistent with a model that the fidelity of DNA polymerase I is important in controlling the overall fidelity of chromosomal DNA replication. DNA sequencing of *rpoB* mutants revealed that the Pol I exonuclease deficiency lead to an increase in a variety of base-substitution mutations. A *polAexo* mutator effect was also observed in strains defective in DNA mismatch repair and carrying the *dnaE915* antimutator allele. Overall, the data are consistent with a proposed role of Pol I in the faithful completion of Okazaki fragment gaps at the replication fork.

Keywords

DNA replication fidelity; DNA polymerase; Mutagenesis; Proofreading; Okazaki fragment

1. Introduction

Faithful replication of chromosomal DNA is fundamentally important for all organisms. For that reason it is important to investigate in detail the mechanisms that are responsible for the high fidelity of DNA replication that is generally observed. One level of accuracy is achieved at the replicating DNA polymerase through its insertion fidelity and associated proofreading activity. Following DNA synthesis, DNA mismatch repair systems provide further fidelity improvement by removing polymerase errors that escaped the proofreading step. An additional feature that may contribute to overall replication fidelity is the participation of accessory DNA polymerases, of which many have been shown to exist. In certain cases this contribution may lead to improved

fidelity, when the accessory DNA polymerase is accurate (e.g., proofreading-proficient polymerases), while in other cases this may lead to lower fidelity when the polymerase is error-prone. Examples of both effects have been demonstrated [1-7].

The *E. coli* model system has been one particularly useful system to investigate the role of such accessory DNA polymerases using a variety of genetic approaches [1-3, 6-9]. *E. coli* contains 5 different DNA polymerases of which DNA polymerase III is the main replicative polymerase responsible for chromosomal duplication (DNA polymerase III holoenzyme, HE). DNA Polymerase II can play an important fidelity role by acting as a back-up proofreader for errors created by Pol III [1, 7, 10]. In contrast, Pol IV and Pol V, when present in amplified amounts, can reduce replication fidelity. In each of these cases, it has been proposed that these DNA accessory polymerases can compete with Pol III HE for the growing point. This may occur particularly when progress of the replication fork is temporarily stalled by a Pol III-generated error (terminal mismatch) [1-3, 7, 10]. The precise mechanism of polymerase switching at the replication point is an active area of research. One interesting feature uncovered from these studies is that the contribution of the accessory DNA polymerase appears to be generally largest for the lagging strand [1, 2, 7, 10, 11].

One recent study from our laboratory [12] focused on the role of DNA polymerase I, encoded by the *polA* gene, which was the first polymerase discovered in *E. coli* [13]. This polymerase has two associated exonucleases: a 5' $\rightarrow$ 3' exonuclease and a 3' $\rightarrow$ 5' exonuclease, with the latter fullfilling a proofreading function. Pol I has been assigned two major functions: in gap filling during DNA excision repair processes and in the maturation of Okazaki fragments through removal of RNA primers and the filling of the resulting lagging-strand gaps. Despite these established functions, a role for Pol I in the fidelity of chromosomal DNA replication is still

to be determined. As Pol I is the most abundant polymerase in *E. coli* (approximately 400 molecules per cell) [14], a contribution to the production or removal of replication errors must be considered. Several studies have reported on the role of Pol I in spontaneous mutagenesis [15-19]. As a rule, these experiments have investigated the mutagenic consequences (a mutator effect) of the lack of Pol I. While the details of how such mutator effects occur in the absence of Pol I are not clear, the experiments clearly indicate that the presence of Pol I is important for maintaining lower overall mutation rates. Our recent approach to better identify the precise role of Pol I employed the use of a strain containing a proofreading-deficient (but polymerase-proficient) form of Pol I (*polAexo* mutant). The advantage of using this strain is that it allows one to potentially detect even small amounts of DNA synthesis by Pol I through an increase in the bacterial mutation rate, as the proofreading defect would convert any normally error-free synthesis by Pol I into an error-prone contribution.

A previous study with the *polAexo* strain using a *lacZ* reversion system to monitor mutagenesis indicated that this strain has a mutator phenotype and that, therefore, Pol I does indeed have a fidelity role [12]. This fidelity role is generally modest, about 2- to 4-fold, depending on the type of base substitution monitored. The effects of *polAexo* allele appeared preferential for the lagging strand and did not appear to involve competition with any of the other DNA polymerases. On this basis, we concluded that Pol I does not participate in any polymerase switching at the replication fork. Instead, the fidelity role of Pol I is likely achieved by error-free DNA synthesis during completion of Okazaki fragments in the lagging strand [12].

As our previous work analyzed *polAexo* effects by monitoring a set of base-pair substitutions at a single site in the *lacZ* gene, we have extended our investigation by analyzing forward mutagenesis to rifampicin resistance. Such Rif^r mutants occur at a large number of sites in the *rpoB* gene encoding the β subunit of RNA polymerase. This system was previously shown

to be a useful tool for the analysis of the specificity of mutagenesis and underlying mechanisms [8, 15, 20].

2. Materials And Methods

2.1. E. coli strains and media.

To investigate the contribution of polymerase I DNA to fidelity we used a strain carrying a chromosomal mutation in the 3'→5' exonuclease domain of Pol I DNA. This mutant, *polAexo*, carrying the D424A mutation that inactivates the ability of Pol I to proofread, was constructed as described in [12]. A DNA mismatch repair deficiency in these strains was created by P1 transduction using the *mutL*::Tn*5* marker [21] using selection for kanamycin. The *dnaE915* allele [22] was introduced by P1 transduction using linkage with the *zae-502*::Tn*10* transposon (~ 60% linkage with *dnaE*) and confirmed by sequencing of a ~900 bp product obtained from PCR performed with set of primers: up 5'-GGTATCGCAGATCATCACCT-3' and down 5'-GTTGCTTAGCCATCTCTTCC-3'. All of the *E. coli* K-12 strains used in this study were derivatives of MC4100, grown on LB agar plates supplemented with appropriate antibiotics (as described in [12]).

2.2. Mutant frequency determination.

Each strain was diluted from a frozen stock culture and plated on the appropriate antibiotic-containing LB agar plates. Plates were incubated at 37°C overnight. Single colonies were picked and used to inoculate independent 2 ml LB cultures. Cultures were grown with agitation for 24 h at 37°C. Appropriate dilutions were spread on LB plates to determine the total cell count and on LB agar plate containing 100 μg/ml rifampicin or 30 μg/ml nalidixic acid to determine the number of antibiotic-resistant mutants. For determining the frequency of nalidixic

acid-resistant mutants in the wild-type (*mutL*$^+$) background, cultures were first concentrated 10-fold by centrifugation before plating on the selective plates. Mutant frequencies were calculated by dividing the number of mutants per plate by the total number of cells. For mutant frequency determinations, 20-30 cultures were started for each strain from single colonies and for 24 hr at 37°C with agitation. Each experiment was repeated 4-5 times (2 times for the Nal^r experiments). The non-parametric Mann-Whitney criterion [23] 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.

For spectral analysis of *rpoB* (Rif^r) mutants, several hundreds of independent LB cultures were grown in parallel for each strain, and 0.1 ml was plated on an LB Rif plate. One Rif^r colony was picked randomly from each plate to ensure independence of the mutants. To avoid possible bias based on colony size, a protocol was developed in which the colony closest to a predetermined spot was chosen from each plate. In this manner, about 200 independent Rif^r colonies were obtained for each of the *polA*$^+$ and *polAexo* derivatives of the *mutL*$^+$ strain; ~360 colonies for each strain in the *mutL* background; and 180 colonies for the *mutL dnaE915* strains. Each mutant was restreaked on another LB Rif plate. The *polAexo* mutator effect (fold increase) for each base substitution (e.g. A·T→G·C) was calculated dividing the frequency of Rif^r mutants in *polAexo* over *polA*$^+$.

2.3. DNA sequencing.

The protocol used to identify *rpoB* mutations in the various strain backgrounds is described below. Two clusters of the *rpoB* gene were considered (as in [20]). Colony PCR was performed in a 96-well microtiter plate on individual colonies. A 953-bp region of the *rpoB* gene (Cluster I) was amplified using the PCR primers rpoB1frw (5'-GAA TGT CAA ATC CGT GGC GT-3') and rpoB1rev (5'-CCA ACC GCA GAC AAG TCA TA-3') and a 885-bp region of the

rpoB gene (Cluster 2) was amplified using the PCR primers rpoB2frw (5'-CGT CGT ATC CGT TCC GTT GG-3') and rpoB2rev (5'-TTC ACC CGG ATA CAT CTC GTC-3'). Amplification was performed by denaturation at for 5 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 1.5 min at 72°C, followed by a 7 min final extension step at 72°C. Primers SEK1rpoB (5'-GAA GGC ACC GTA AAA GAC AT-3') and SEK2rpoB (5'-CGT GTA GAG CGT GCG GTG AAA-3') were used to determine the nucleotide sequence of the target region of *rpoB* in the two PCR amplicons. Nucleotide sequences were aligned and analyzed using the BioEdit Sequence Alignment Editor (free on-line program).

3. Results

3.1 Mutator effect of the polAexo allele in the mismatch-repair proficient background.

The construction of a strain of *E. coli* containing a chromosomal replacement of the wild-type *polA*$^{+}$ gene by the proofreading-deficient *polAexo* variant (carrying the D424A mutation) has been described [12]. Here, this strain was used to investigate the frequency and nature of rifampicin-resistant mutants in comparison to the wild-type strain. The data in Table 1 showed that the Pol I proofreading deficiency cause an about 2-fold increase in the level of spontaneous Rif^{r} mutations (experiment 1). A 9-fold increase was observed when scoring for nalidixic acid-resistant mutations. These experiments were performed multiple times and gave consistent results. These results support our previous suggestion that Pol I performs a role in controlling the level of DNA replication errors. This role likely involves faithful synthesis by this polymerase, whose contribution is revealed here by the conversion of the enzyme into its inaccurate, proofreading-defective form. Below, we describe the precise nature of the *polAexo*-induced *rpoB* mutations.

3.2. Spectra of rpoB mutations in wild-type and proofreading-defective Pol I strains in mismatch-repair proficient background.

Sequencing of the *rpoB* gene for a large number of independent Rif^r mutants yielded 181 and 207 independent *rpoB* mutations for the *polA*$^+$ and *polAexo* strains, respectively. This initial set was done in the mismatch-repair-proficient background. The spectrum of mutations for the *polA*$^+$ strain resembles previous spectra from wild-type controls [20]. It revealed mutations distributed along the whole *rpoB* gene (Table 2), consisting of both transitions (107/181 or 59%) and transversions (74/181 or 40%) with several clear hot spots. Major hot spots for transitions are A·T→G·C at position 1547, accounting for more than 8% of all mutations, and G·C→A·T at positions 1576 (13%), 1592 (~12%), and 1691 (7%). Two transversion hotspots were seen, both A·T→T·A, at positions 1538 (> 12% of all mutations) and 1577 (7%).

The presence of the *polAexo* allele resulted in increases in virtually all classes of base substitutions, although certain individual sites show significantly larger effects. Overall, the *polAexo* mutator increased transitions 1.8-fold and transversions 1.9-fold. The strongest effects were observed for the A·T→G·C transitions at positions 1534 and 1538 (~5-fold and 6.5-fold increase, respectively) and for the G·C→A·T transition at 1546 (3-fold increase). As a result of these increases, the A·T→G·C transition at 1534 represents ~ 9% (18/207) of the total number of mutations in the *polAexo* strain but only 3% (6/181) in the *polA*$^+$ strain. Likewise, the A·T→G·C transition at 1538 represents 2% of all mutations in the *polAexo* strain, compared to 0.5% in the *polA*$^+$ strain. For the transversions, a greater than 3-fold mutator effect of the *polAexo* allele is seen for G·C→T·A at positions 436,1535 and 1537, and greater than 2-fold for A·T→T·A at position 1547, 1598, and 1714.

3.3. Mutational effects of the polAexo allele in the mismatch-repair defective mutL background.

In Table 1, we also describe the results of experiments performed in the mismatch-repair defective *mutL* background (experiment 2). In general, this background is useful for detection of uncorrected DNA replication errors [1, 10, 24, 25]. In this series of experiments we also included the *dnaE915* antimutator allele [21, 22], which is useful for specifically lowering the contribution of errors made by DNA polymerase III holoenzyme [1, 8], thereby facilitating detection of errors contributed by other DNA polymerases, such as Pol I. Lack of DNA mismatch repair strongly increased the frequency of observed Rif^r (165-fold) and Nal^r mutants (2800-fold) (Table 1, experiment 2). Interestingly, the mutator effect of *polAexo* was not readily detectable in the *mutL* background, but a significant effect could be observed for the Rif^r mutants in the *mutL dnaE915* background (1.5-fold). While this fold increase is modest, it was consistent over multiple (n = 5) experiments ($p<0.05$). The likely explanation for our ability to see this *polAexo* effect in the *dnaE915* background is the significant reduction of Rif^r mutations (~4-fold) by the *dnaE* antimutator allele.

DNA sequencing of the Rif^r mutants for these strains yielded several noteworthy results (Table 3). The spectrum of *rpoB* mutations in the *mutL* strain differs significantly from that of the $mutL^+$ strain, being dominated by A·T→G·C and G·C→A·T transitions (over 90% of all mutations) as observed in other studies [20], and is consistent with transition errors being the predominant source of uncorrected replication errors by Pol III HE [25, 26]. Base-pair substitutions were located at 16 sites with seven prominent hot spots (defined as sites where 20 or more mutations occurred). As expected from the overall frequency data (Table 1), the spectrum

of mutations in the *mutL polAexo* strain is nearly identical to that of the *mutL polA*$^+$ strain (Table 3).

Significant changes in the spectra were observed in the *dnaE915* antimutator background (Table 3). Most obviously, the ratio of A·T→G·C versus G·C→A·T transitions is altered, 212:127 in *dnaE*$^+$ versus 76:97 in *dnaE915*. Thus, the antimutator effect is significantly larger for the A·T→G·C transitions than for the reciprocal G·C→A·T transitions. Furthermore, in this background, we are able to observe spectral differences between *polA*$^+$ and *polAexo* strains (Table 3). Notably, the *polAexo* effect appears specific for the A·T→G·C transitions, which are reduced 1.8-fold, whereas the frequency of the G·C→A·T remains unchanged.

4. Discussion

The results from this work confirm and expand our previous conclusions regarding the role of DNA Polymerase I in chromosomal replication fidelity, which were based on a limited set of *lacZ* reversion assays [12]. Here our analysis applies to a large number of sites in the chromosomal *rpoB* target. The data in the *rpoB* target show that substitution of wild-type Pol I by its exonuclease-deficient form increases the mutation rate significantly. This indicates that Pol I performs DNA synthesis in *E. coli* that is normally error free, but becomes error prone when its exonuclease proofreading activity is inactive. It was argued previously that this DNA synthesis by Pol I occurs mainly at the replication fork, although not at the actual growing point but instead during gap-filling DNA synthesis as part of the Okazaki fragment maturation.

The types of base substitution errors produced in the *polAexo* strain are various, with both types of transitions as well as the transversions being enhanced. Nevertheless, some specificity

emerges. The increase in transitions (overall 1.8-fold) was slightly larger for the A·T→G·C (2.8-fold) than for the G·C→A·T (1.4-fold). The increase for the transversions was ~1.9-fold.

The preferred analysis for the role of accessory DNA polymerases in DNA replication is in mismatch-repair defective strains. This type of analysis was indeed pursued for the case of the *lacZ* reversion alleles [12]. It was shown that Pol I while conducting DNA synthesis at the replication fork did not compete with other DNA polymerases; instead, the effects appeared merely additive. In the present case, no *polAexo* effect could be detected in the mismatch-repair defective *mutL* background. The simplest explanation for this phenomenon is that in the *rpoB* target, due to the very strong increase of Rif^r mutants in the mismatch-repair defective background (> 160-fold) due to Pol III HE-mediated errors, the contribution of the Pol I-mediated errors cannot be readily detected. Nevertheless, when the number of Pol III HE-mediated errors was reduced using the *dnaE915* antimutator allele, the *polAexo* effect could again be detected.

One alternative analysis of the role of Pol I in spontaneous mutagenesis was put forward by Hasegawa *et al*. [27]. These authors proposed that a significant fraction of spontaneous mutations arise from repair synthesis (on undamaged DNA) by Pol I in gratuitous *uvrABCD*-dependent Nucleotide Excision repair (NER). This result arose from the observation that *uvrAB*-defective strains showed reduced frequencies of Rif^r (*rpoB*) mutations. Addition of a plasmid expressing the exonuclease-deficient Pol I DNA polymerase further increased this *uvrAB*-dependent mutator effect. However, our previous study did not reveal any *uvrABC*-dependent mutator effect using *lac* mutations [12], suggesting that at least in our strains no such mechanism operated. Furthermore, the spectrum of spontaneous *rpoB* mutants in the Hasegawa *et al*. study [27] differed significant from other published spontaneous *rpoB* spectra [15, 20] as well as from

our present spectrum. Specifically, the *rpoB* mutations disappearing from the spectrum in the *uvr* mutants appeared to constitute a subgroup of mutations that is not present in other spontaneous *rpoB* spectra. Therefore, the significance of the *uvrAB*-dependent antimutator effect for these mutations remains to be determined. One aspect that is shared by our current model and that of Hasegawa *et al*. [27] is that the contribution of Pol I to errors is assumed to be additive with regard to those made by Pol III (and possibly other polymerases) at the growing point. Nevertheless, one important distinction between the two types of additivity is the susceptibility to DNA mismatch repair. In the case of excision repair gaps, which occur most often in fully methylated DNA, no effect of mismatch repair is to be expected. In contrast, a significant effect of MMR is to be expected in the case of Pol I filling Okazaki fragments gaps. The data in Table 1 indicate that the contribution of PolAexo-mediated errors is 1.5×10^{-8} (3.4×10^{-8} *minus* 1.9×10^{-8}) in the *mutL*$^{+}$ strain, whereas this is 40×10^{-8} (121×10^{-8} *minus* 81×10^{-8}) in the *mutL dnaE915* strain. While the exact value of these contributions may be quite uncertain, there is no question that, quantitatively, the *polAexo* mutator effect is significantly larger in the mismatch-repair-defective (*dnaE915*) background. Hence, these mutations are subject to correction by mismatch repair, and this is consistent with our model of Pol I performing the bulk of its DNA synthesis at the replication fork.

In conclusion, the present data provide strong support for the proposed role of DNA Polymerase I in maintaining a high level of chromosomal DNA replication fidelity, most likely fulfilled by the faithful processing of Okazaki fragments.

Acknowledgements

The authors thank Drs. S. Lujan and J. Stone of the NIEHS for their critical reading of the manuscript and helpful comments. This research was supported grant N301 030034 from the

Polish Ministry of Science and Higher Education (to I. J. F. and K.M.) and by project no. Z01 ES065086 of the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

References

[1] M. Banach-Orlowska, I.J. Fijalkowska, R.M. Schaaper, P. Jonczyk, DNA polymerase II as a fidelity factor in chromosomal DNA synthesis in *Escherichia coli*, Mol Microbiol, 58 (2005) 61-70.

[2] W. Kuban, M. Banach-Orlowska, M. Bialoskorska, A. Lipowska, R.M. Schaaper, P. Jonczyk, I.J. Fijalkowska, Mutator phenotype resulting from DNA polymerase IV overproduction in *Escherichia coli*: preferential mutagenesis on the lagging strand, J Bacteriol, 187 (2005) 6862-6866.

[3] W. Kuban, P. Jonczyk, D. Gawel, K. Malanowska, R.M. Schaaper, I.J. Fijalkowska, Role of *Escherichia coli* DNA polymerase IV in *in vivo* replication fidelity, J Bacteriol, 186 (2004) 4802-4807.

[4] S.D. McCulloch, T.A. Kunkel, The fidelity of DNA synthesis by eukaryotic replicative and translesion synthesis polymerases, Cell Res, 18 (2008) 148-161.

[5] Y.I. Pavlov, P.V. Shcherbakova, DNA polymerases at the eukaryotic fork-20 years later, Mutat Res, 685 45-53.

[6] D. Gawel, M.D. Hamilton, R.M. Schaaper, A novel mutator of *Escherichia coli* carrying a defect in the *dgt* gene, encoding a dGTP triphosphohydrolase, J Bacteriol, 190 (2008) 6931-6939.

[7] D. Gawel, P. Jonczyk, I.J. Fijalkowska, R.M. Schaaper, The *dnaX36* mutator of *Escherichia coli*: effects of the DNA Polymerase III holoenzyme τ subunit on chromosomal DNA replication fidelity, J Bacteriol. (In press)

[8] S. Rangarajan, G. Gudmundsson, Z. Qiu, P.L. Foster, M.F. Goodman, *Escherichia coli* DNA polymerase II catalyzes chromosomal and episomal DNA synthesis *in vivo*, Proc Natl Acad Sci U S A, 94 (1997) 946-951.

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

[10] D. Gawel, P.T. Pham, I.J. Fijalkowska, P. Jonczyk, R.M. Schaaper, Role of accessory DNA polymerases in DNA replication in *Escherichia coli*: analysis of the *dnaX36* mutator mutant, J Bacteriol, 190 (2008) 1730-1742.

[11] M. Maliszewska-Tkaczyk, P. Jonczyk, M. Bialoskorska, R.M. Schaaper, I.J. Fijalkowska, SOS mutator activity: unequal mutagenesis on leading and lagging strands, Proc Natl Acad Sci U S A, 97 (2000) 12678-12683.

[12] K. Makiela-Dzbenska, M. Jaszczur, M. Banach-Orlowska, P. Jonczyk, R.M. Schaaper, I.J. Fijalkowska, Role of *Escherichia coli* DNA polymerase I in chromosomal DNA replication fidelity, Mol Microbiol, 74 (2009) 1114-1127.

[13] I.R. Lehman, M.J. Bessman, E.S. Simms, A. Kornberg, Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from *Escherichia coli*, J Biol Chem, 233 (1958) 163-170.

[14] A. Kornberg, T.A. Baker, DNA Replication, 2 ed., Freeman, New York, 1992.

[15] E. Curti, J.P. McDonald, S. Mead, R. Woodgate, DNA polymerase switching: effects on spontaneous mutagenesis in *Escherichia coli*, Mol Microbiol, 71 (2009) 315-331.

[16] D.F. Fix, P.A. Burns, B.W. Glickman, DNA sequence analysis of spontaneous mutation in a *polA1* strain of *Escherichia coli* indicates sequence-specific effects, Mol Gen Genet, 207 (1987) 267-272.

[17] M. Imai, Y. Tago, M. Ihara, M. Kawata, K. Yamamoto, Role of the 5' --> 3' exonuclease and Klenow fragment of *Escherichia coli* DNA polymerase I in base mismatch repair, Mol Genet Genomics, 278 (2007) 211-220.

[18] Y. Tago, M. Imai, M. Ihara, H. Atofuji, Y. Nagata, K. Yamamoto, *Escherichia coli* mutator (Delta)polA is defective in base mismatch correction: the nature of *in vivo* DNA replication errors, J Mol Biol, 351 (2005) 299-308.

[19] K.K. Vaccaro, E.C. Siegel, The frameshift mutability of *polA1* and *recA1* derivatives of mutator strains of *Escherichia coli*, Mutat Res, 42 (1977) 443-446.

[20] L. Garibyan, T. Huang, M. Kim, E. Wolff, A. Nguyen, T. Nguyen, A. Diep, K. Hu, A. Iverson, H. Yang, J.H. Miller, Use of the *rpoB* gene to determine the specificity of base substitution mutations on the *Escherichia coli* chromosome, DNA Repair (Amst), 2 (2003) 593-608.

[21] I.J. Fijalkowska, R.M. Schaaper, Effects of *Escherichia coli dnaE* antimutator alleles in a proofreading-deficient *mutD5* strain, J Bacteriol, 177 (1995) 5979-5986.

[22] I.J. Fijalkowska, R.L. Dunn, R.M. Schaaper, Mutants of *Escherichia coli* with increased fidelity of DNA replication, Genetics, 134 (1993) 1023-1030.

[23] R.R. Sokal, F.J. Rohlf, Biometry, 2 ed., Freeman, San Francisco, 1981.

[24] I.J. Fijalkowska, P. Jonczyk, M.M. Tkaczyk, M. Bialoskorska, R.M. Schaaper, Unequal fidelity of leading strand and lagging strand DNA replication on the *Escherichia coli* chromosome, Proc Natl Acad Sci U S A, 95 (1998) 10020-10025.

[25] P.T. Pham, W. Zhao, R.M. Schaaper, Mutator mutants of *Escherichia coli* carrying a defect in the DNA polymerase III τ subunit, Mol Microbiol, 59 (2006) 1149-1161.

[26] R.M. Schaaper, Base selection, proofreading, and mismatch repair during DNA replication in *Escherichia coli*, J Biol Chem, 268 (1993) 23762-23765.

[27] K. Hasegawa, K. Yoshiyama, H. Maki, Spontaneous mutagenesis associated with nucleotide excision repair in *Escherichia coli*, Genes Cells, 13 (2008) 459-469.

Table 1

Mutant frequencies (mutants per 10^8 cells) for resistance to rifampicin (Rif^r) and nalidixic-acid (Nal^r) for wild-type (*polA*$^+$) and *polAexo* strains in various backgrounds

Experiment	Strain	Rif^r	Nal^r
1	*mutL*$^+$ *polA*$^+$	1.9	0.03
	mutL$^+$ *polAexo*	3.4 **(1.8 x)**	0.27 (**9.0 x**)
2	*mutL polA*$^+$	311	83
	mutL polAexo	321 (1.0 x)	85 (1 x)
	mutL dnaE915 polA$^+$	81	ND
	mutL dnaE915 polAexo	121 (**1.5 x**)	ND

Values within parentheses indicate the increase compared to the corresponding *polA*$^+$ strain (mutator effect). Frequencies are the averages for 20-30 independent cultures (see Section 2). Bold numbers indicate statistically significant differences ($P < 0.05$, see Section 2). ND = not done.

Table 2

Number (#) and mutant frequencies (MF) of sequenced transitions and transversions in the *rpoB* gene in mismatch-repair-proficient *polA*$^+$ (wild-type) and *polAexo* strains

Class of mutation	*rpoB* position	wild-type		*polAexo*	
		#	MF	#	MF
Base substitutions		181	(1.86)	207	(3.4)
Transitions		107	(1.09)	120	(1.97)
A·T→G·C		29	(0.298)	50	(0.821)
	1532	3	(0.030)	4	(0.065)
	1534	6	(0.061)	18	(0.295)
	1538	1	(0.010)	4	(0.065)
	1547	15	(0.154)	21	(0.344)
	1598	4	(0.041)	3	(0.049)
G·C→A·T		78	(0.801)	70	(1.149)
	1535	8	(0.082)	0	(0.000)
	1546	8	(0.082)	16	(0.262)
	1565	1	(0.010)	0	(0.000)
	1576	24	(0.246)	21	(0.345)
	1586	3	(0.030)	0	(0.000)
	1592	21	(0.216)	21	(0.345)
	1691	13	(0.133)	12	(0.197)
Transversions		74	(0.760)	87	(1.428)
G·C→T·A		18	(0.185)	23	(0.377)
	436	2	(0.020)	4	(0.065)
	1535	0	(0.000)	2	(0.033)
	1537	1	(0.010)	2	(0.033)
	1546	6	(0.061)	4	(0.065)
	1576	7	(0.072)	9	(0.148)
	1592	2	(0.020)	2	(0.033)
A·T→T·A		42	(0.431)	52	(0.854)
	1538	22	(0.226)	26	(0.427)
	1547	2	(0.020)	3	(0.049)
	1577	13	(0.133)	13	(0.213)
	1598	0	(0.000)	2	(0.033)
	1714	5	(0.051)	8	(0.131)
A·T→C·G		9	(0.092)	7	(0.115)
	1538	3	(0.030)	2	(0.033)
	1714	6	(0.061)	2	(0.033)
	1715	0	(0.000)	3	(0.049)
G·C→C·G		5	(0.051)	5	(0.082)
	1576	5	(0.051)	5	(0.082)

Values within parentheses indicate the frequencies (per 10^8 cells) of individual or classes of mutations. These frequencies were calculated by multiplying their number relative to the total number of sequenced mutations by the overall mutant frequencies of Table 1. The fold increase (mutator effect) represents the ratio of mutant frequencies in *polAexo* over *polA*$^+$.

Table 3

Number (#) and frequency (MF per 10^8 cells) of sequenced base-pair substitutions in the *rpoB* gene for *polA*$^+$ and *polAexo* strains in the mismatch-repair-deficient *mutL* background[a]

Class of Mutation	*rpoB* position	*mutL* #	*mutL* (MF)	*mutL polAexo* #	*mutL polAexo* (MF)	*mutL dnaE915* #	*mutL dnaE915* (MF)	*mutL dnaE915polAexo* #	*mutL dnaE915polAexo* (MF)
Base substitutions		359	(311)	360	(321)	179	(81)	175	(121)
Transitions		339	(293)	353	(315)	173	(78)	165	(114)
A·T→G·C		212	(184)	224	(200)	76	(34.4)	92	(63.6)
	1532	27	(23.4)	27	(24.1)	8	(3.62)	6	(4.14)
	1534	30	(26.0)	32	(28.5)	32	(14.5)	49	(33.9)
	1538	37	(32.1)	37	(33.0)	9	(4.07)	11	(7.6)
	1547	118	(102)	128	(114)	26	(11.8)	24	(16.6)
	1552	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.69)
	1598	0	(0.0)	0	(0.0)	1	(0.45)	1	(0.69)
G·C→A·T		127	(110)	129	(115)	97	(43.9)	73	(50.5)
	1535	1	(0.86)	1	(0.89)	0	(0.0)	1	(0.69)
	1546	55	(47.6)	59	(52.6)	75	(33.9)	46	(31.8)
	1576	25	(21.7)	32	(28.5)	4	(1.81)	4	(2.76)
	1586	8	(6.93)	8	(7.13)	10	(4.52)	16	(11.1)
	1592	11	(9.53)	7	(6.24)	3	(1.35)	3	(2.07)
	1691	27	(23.4)	22	(19.6)	5	(2.26)	3	(2.07)
Transversions		20	(17.32)	7	(6.24)	6	(2.71)	10	(6.91)
G·C→T·A		3	(2.59)	2	(1.78)	4	(1.81)	8	(5.53)
	436	1	(0.86)	0	(0.0)	0	(0.0)	0	(0.0)
	1546	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.69)
	1576	2	(1.73)	1	(0.89)	3	(1.35)	3	(2.07)
	1592	0	(0.0)	1	(0.89)	1	(0.45)	4	(2.76)
A·T→T·A		16	(13.9)	5	(4.45)	2	(0.90)	2	(1.38)
	1538	1	(0.86)	0	(0.0)	0	(0.0)	1	(0.69)
	1577	1	(0.86)	1	(0.89)	0	(0.0)	0	(0.0)
	1598	3	(2.59)	0	(0.0)	0	(0.0)	0	(0.0)
	1714	11	(9.53)	4	(3.56)	2	(0.90)	1	(0.69)

A·T→C·G		1	(0.86)	0		0		0	
	1715	1	(0.86)	0	(0.0)	0	(0.0)	0	(0.0)

[a] Values within parentheses indicate the frequencies (per 10^8 cells) of individual or classes of mutations (see legend to Table 2).