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# Mutator Phenotype Resulting from DNA Polymerase IV Overproduction in *Escherichia coli*: Preferential Mutagenesis on the Lagging Strand

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**We investigated the mutator effect resulting from overproduction of *Escherichia coli* DNA polymerase IV. Using *lac* mutational targets in the two possible orientations on the chromosome, we observed preferential mutagenesis during lagging strand synthesis. The mutator activity likely results from extension of mismatches produced by polymerase III holoenzyme.**

Errors of DNA replication constitute one major potential source of spontaneous mutations (4). DNA replication is a generally accurate process, the final fidelity being often described as the product of three serial steps: base selection, exonucleolytic proofreading, and postreplicative mismatch repair (26), leading to an average mutation rate in the range of $10^{-10}$ mutations per base pair replicated. Duplication of the *Escherichia coli* chromosome is performed by polymerase (Pol) III holoenzyme (HE). HE is a dimeric, 17-subunit complex capable of high-speed, high-fidelity DNA synthesis, copying, in a coordinated fashion, the leading and lagging strand of the replication fork (for a review, see reference 18).

In addition to Pol III, *E. coli* contains four additional DNA polymerases, whose cellular roles are a subject of ongoing studies, including their possible role in overall replication fidelity and mutation production. Of particular interest is DNA Pol IV (for a review, see reference 10). Pol IV, the product of the *dinB* gene (35), is considered a low-fidelity enzyme, due in part to the lack of a 3′ (proofreading) exonuclease. Pol IV is induced as part of the SOS response (12). However, its basal level in normal cells is fairly high, as much as 250 molecules per cell (14), compared to an estimated 30 molecules of HE (19), which raises questions about its role under normal conditions. Pol IV has been shown to participate in mutagenesis in resting cells (adaptive mutagenesis) (7, 20, 33). However, in growing cells the loss of Pol IV does not significantly affect the level of chromosomal spontaneous mutations (15, 21, 38), indicating that Pol IV has limited access to the replication growing point under these active growth conditions. In contrast, *lacZ* reversion on an F′ episome was significantly reduced in growing cells lacking Pol IV (15). Importantly, Pol IV overproduction from a multicopy *dinB* plasmid was shown to result in a generalized mutator phenotype (13, 36). These combined results suggest that Pol IV has, at least in principle, access to the replication fork and may contribute to mutation.

The precise mechanisms by which accessory polymerases, such as Pol IV, may obtain access to the replication point are of interest. One important question is whether any involvement of accessory polymerases is equal in both DNA strands or whether it occurs with a certain preference in either the leading or lagging strand. Our laboratory has previously developed a system that allows comparison of the fidelity of replication in the two DNA strands on the *E. coli* chromosome (5). The results with this system have indicated that the fidelity of replication is unequal between the two strands (5, 8, 9, 17). Specifically, for base substitution mutations, lagging-strand replication was deduced to be more accurate than leading-strand replication (5). In the present study, we investigated whether the mutator activity resulting from overproduction of Pol IV is also biased toward one of the strands.

**Differential mutator effects of Pol IV in the two replicating strands.** To assay the Pol IV mutator activity (13, 36), we constructed two plasmids containing the *dinB* gene encoding Pol IV. pMO4, a medium-copy-number plasmid carrying *dinB* and its cognate promoter, carries the SacI-EcoRI DNA fragment of *dinB* plasmid pYG768 (kindly provided by T. Nohmi) in the SacI/EcoRI site of the chloramphenicol resistance plasmid pHSG398 (31), a derivative of plasmid pBR322. pLO1, a low-copy *dinB* plasmid carries the 1.56-kb SacI-EcoRI DNA fragment from pYG768 in the SacI/EcoRI site of pSC101-derived plasmid pWSK129 (37) conferring kanamycin resistance.

Our assay for assaying mutational strand biases is based on comparison of the mutability of a *lacZ* gene in pairs of strains containing the *lac* operon in the two possible orientations relative to the direction of replication (5). In this comparison, any particular *lacZ* sequence will be replicated by the leading-strand machinery in one orientation and by the lagging-strand machinery in the other. Therefore, any differential mutability of the target gene between the strains within the pair is most directly interpreted as reflecting a difference in fidelity of lead-

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TABLE 1. Lac reversion in strains carrying *dinB* plasmids pMO4 or pLO1: *dinB* mutator activity and effect of *lac* orientation[a]

| Expt and *lac* allele | *lac* orientation | Mismatch and strand | Plasmid | $lac^+$ mutants/plate | Titer ($10^8$) | $lac^+$ mutants/$10^8$ | Mutator effect[b] |
|---|---|---|---|---|---|---|---|
| Expt A: with medium-copy-no. plasmid pMO4 | | | | | | | |
| G · C→A · T | R | G · T, lagging | Vector | 94 | 51 | 18 | |
| | | | pMO4 | 130 | 12 | 110 | 6.1 |
| | L | G · T, leading | Vector | 485 | 51 | 95 | |
| | | | pMO4 | 270 | 12 | 225 | 2.4 |
| G · C→T · A | R | C · T, lagging | Vector | 3 | 43 | 0.69 | |
| | | | pMO4 | 9 | 11 | 8.2 | 12 |
| | L | C · T, leading | Vector | 5 | 34 | 1.5 | |
| | | | pMO4 | 2 | 13 | 1.5 | 1.0 (NS) |
| A · T→T · A | R | T · T, lagging | Vector | 5 | 59 | 0.85 | |
| | | | pMO4 | 9 | 13 | 6.9 | 8.1 |
| | L | T · T, leading | Vector | 6 | 51 | 1.2 | |
| | | | pMO4 | 1 | 12 | 0.83 | 0.7 (NS) |
| A · T→G · C | R | T · G, leading | Vector | 130 | 67 | 19 | |
| | | | pMO4 | 130 | 20 | 65 | 3.4 |
| | L | T · G, lagging | Vector | 46 | 58 | 7.9 | |
| | | | pMO4 | 155 | 20 | 78 | 9.9 |
| Expt B: with low-copy-no. plasmid pLO1 | | | | | | | |
| G · C→A · T | R | G · T, lagging | Vector | 110 | 64 | 17 | |
| | | | pLO1 | 310 | 60 | 52 | 3.1 |
| | L | G · T, leading | Vector | 230 | 63 | 36 | |
| | | | pLO1 | 390 | 61 | 64 | 1.7 |
| G · C→T · A | R | C · T, lagging | Vector | 5 | 58 | 0.86 | |
| | | | pLO1 | 18 | 46 | 3.9 | 4.5 |
| | L | C · T, leading | Vector | 6 | 60 | 1.0 | |
| | | | pLO1 | 6 | 51 | 1.2 | 1.2 (NS) |
| A · T→T · A | R | T · T, lagging | Vector | 6 | 63 | 0.95 | |
| | | | pLO1 | 16 | 61 | 2.6 | 2.7 |
| | L | T · T, leading | Vector | 11 | 61 | 1.8 | |
| | | | pLO1 | 19 | 56 | 3.4 | 1.9 |

[a] Strains are *mutL* derivatives of MC4100 (Δ*lac*) containing defined *lac* operons (3) in the phage lambda attachment site (5). The strains indicated in group A, carrying medium-copy *dinB* plasmid pMO4 or vector pHSG398 (31), are *mutL*::Tn*5* (6). Those in group B, carrying low-copy *dinB* plasmid pLO1 or vector pWSK129 (37), are *mutL*::Tn*10* (27). Each entry is based on the medium value for 15 independent cultures. The number of mutants per plate corresponds to 0.1 ml of undiluted culture plated on the minimal-lactose plates.

[b] The indicated mutator effects are statistically significant ($P < 0.02$) based on the nonparametric Mann-Whitney criterion (28) applied to the mutant frequency distribution of individual cultures, except where indicated as not significant (NS).

ing- and lagging-strand synthesis (5). The two orientations of the *lac* operon in each pair have been designated, arbitrarily, R (right) or L (left) (5). For the current experiments we used a total of four strain pairs each containing a different *lacZ* allele, allowing investigation of four defined base-pair substitutions: two transitions (G · C→A · T and A · T→G · C) and two transversions (G · C→T · A and A · T→T · A) (3, 5). The strains used, unless otherwise indicated, were mismatch-repair deficient (*mutL*) in order to prevent interference by the mismatch-repair system, thus allowing a more direct view of DNA replication errors.

The data in Table 1 show that, as observed previously (5, 15, 17), there are significantly different mutant frequencies between the two *lac* orientations in the control strains (vector only). For example, for the G · C→A · T allele, the difference between L and R oriented *lac* genes was >5-fold ($95 \times 10^{-8}$ versus $18 \times 10^{-8}$, Table 1, expt A). Smaller, but significant differences are observed for the G · C→T · A and A · T→G · C alleles (2.1- and 2.5-fold, respectively), a finding consistent with numerous previous measurements on these alleles (5, 15, 17). The table also indicates the strand in which the favored mispair is located for either R or L orientation. For each of the four *lac* base substitutions, the lowest mutant frequency is seen when this mispair is present in the lagging strand.

The presence of the pMO4, the medium-copy $dinB^+$ plasmid, increased the reversion frequency of all four *lacZ* alleles (Table 1, expt A), thus reproducing the Pol IV mutator activity first noted by Kim et al. (13). Interestingly, calculation of the mutator effect for the two *lac* orientations shows that the Pol IV mutator effect is unequal for the two orientations (Table 1,

final column). The effect is largest (6- to 12-fold) for the R orientation of the G · C→A · T, G · C→T · A and A · T→T · A allele and for the L orientation of the A · T→G · C allele compared to only 1.0- to 3.4-fold increases for the opposite orientations. In each case, the larger Pol IV effect occurs for the orientation that was lowest in the control strain. Thus, the Pol IV mutator effect is largest for the lagging strand.

Cells possessing plasmid pMO4 had reduced cell counts (by ~3-fold) in the overnight cultures (stationary phase), a finding indicative of some deleterious effects of Pol IV overproduction (Table 1, expt A). In contrast, overexpression from low-copy plasmid pLO1 did not produce any negative effects on the cell titers (Table 1, expt B). As expected, the mutator effects with this plasmid were less than with pMO4. Nevertheless, pLO1 significantly increased the frequencies of G · C→A · T, G · C→T · A, and A · T→T · A revertants (Table 1, expt B). As before, the effects are strand specific, with the largest effect seen for the lagging strand.

**Pol IV overproduction in strains containing *dnaQ* or *dnaE* mutator alleles.** The mutator effect due to Pol IV overexpression can be interpreted most directly in one of two ways. In one interpretation, the increased number of mutations results from errors made by Pol IV itself. Since Pol IV is error prone (32, 35), any synthesis by this enzyme may be expected to yield additional mutations. However, a large amount of DNA synthesis by Pol IV would be required to account for the observed mutant frequency increase. For example, assuming that Pol IV is 100-fold less accurate than Pol III, a 10-fold mutator effect would require 10% of the chromosome to be replicated by Pol IV. This seems not very likely. In the second model, the Pol IV mutator activity results from error-prone extension of mismatches made by Pol III HE. This model is attractive because terminal mismatches present a likely occasion where polymerase exchange might take place. Pol III HE, like other polymerases, may have difficulty continuing synthesis from a mismatched 3′ terminus and may dissociate from (or is competed off) the primer-template. Pol IV could then continue synthesis from the mismatch, fixing the potential mutation. To test this second hypothesis, we investigated the Pol IV mutator effect in certain *dnaQ* or *dnaE* mutator strains. An increase in error rate in these mutators, due to either defective proofreading (*dnaQ*) or defective polymerization (*dnaE*), will increase the frequency of terminal mispairs available for Pol IV to act on, leading to a proportional increase in mutant frequencies. Conversely, if the Pol IV effect were to be independent of Pol III-mediated errors, a simple additive effect is predicted. We selected the *lac* G · C→T · A transversion allele to test this hypothesis, because it proved particularly responsive to the *dinB* effect (Table 1). These experiments were conducted in the *mutL*$^+$ background.

The *dnaQ928* mutator carries a G17S amino acid substitution in the Pol III ε proofreading subunit, resulting in catalytic deficiency and mutator activity (30). In combination with plasmid pLO1, the *dnaQ928* mutator effect was significantly increased: 3.2-fold for the R-oriented construct and 1.7-fold for the L-oriented construct. These effects, while moderate, are clearly greater than additive, i.e., the combined mutator effects are greater than expected from simple addition of the pLO1 and *dnaQ928* mutant frequencies.

Similar effects were observed when pLO1 was combined with the *dnaE486* and *dnaE511* alleles (Table 2). The *dnaE* alleles carry a single amino acid substitution (S885P and T260I for *dnaE486* and *dnaE511*, respectively) in the Pol III α subunit (34). Both exhibit a temperature-sensitive growth phenotype, as well as a spontaneous mutator activity. The precise molecular basis of their mutator phenotype is unknown but may involve increased error production or decreased ability to withstand competition with error-prone polymerases (34). The experiments were performed at 34°C at which no significant temperature sensitivity problems were observed, although their mutability increased significantly between 30 and 34°C (data not shown). Relative to the wild-type control, *dnaE486* and *dnaE511* increased spontaneous mutagenesis 40- and 12-fold, respectively, in the R orientation or by 9- and 6-fold, respectively, in the L orientation (Table 2). The presence of pLO1 further increased the mutant frequencies significantly: 3.5- and 18-fold for *dnaE486* and *dnaE511* in the R orientation and 1.9- and 7.9-fold in the L orientation. These effects are clearly more than additive compared to the single pLO1 and *dnaE* mutant frequencies, and in some cases they are nearly multiplicative. The results with *dnaQ928* and the two *dnaE* alleles clearly supports the hypothesis that Pol IV enhances the production of mutations through extension from Pol III produced errors.

The results of Table 2 also show that loss of Pol IV (Δ*dinB*::*kan* strains) significantly reduces the mutator activity of the *dnaE* mutators. This indicates that in these strains the basal level of Pol IV is a significant contributor to the mutator effect. Roughly 50% of the mutations in these strains may be considered to result from Pol IV action. This contrasts with the situation in wild-type strains, where essentially no effect of the

TABLE 2. Effect of Pol IV overproduction on *lac* G · C→T · A mutant frequencies in R or L *lac* orientations in *dnaQ* or *dnaE* mutator strains[a]

| Genotype | Plasmid | Lac$^+$/10$^{8b}$ | |
|---|---|---|---|
| | | R | L |
| Wild type | Vector | 0.2 | 0.3 |
| Wild type | pLO1 | 1.5 (7.5) | 0.8 (2.7) |
| *dinB*::*kan* | —[c] | 0.15 | 0.3 |
| *dnaQ928* | Vector | 5.0 | 20 |
| *dnaQ928* | pLO1 | 16 (3.2) | 34 (1.7) |
| *dnaQ928 dinB*::*kan* | — | 3.5 | 22 |
| *dnaE486* | Vector | 7.9 | 2.8 |
| *dnaE486* | pLO1 | 28 (3.5) | 5.4 (1.9) |
| *dnaE486 dinB*::*kan* | — | 2.8 | 1.7 |
| *dnaE511* | Vector | 2.3 | 1.9 |
| *dnaE511* | pLO1 | 42 (18) | 15 (7.9) |
| *dnaE511 dinB*::*kan* | — | 1.1 | 1.0 |

[a] The strains are as described in Table 1, except that they are *mutL*$^+$. The Δ*dinB*::*kan* allele was transduced from YG7207 (13), the *dnaQ928* allele was transduced from NR11641 (30), and the *dnaE486* and *dnaE511* alleles were transduced from strains CS115 and AR2, respectively (34). pLO1 is the low-copy-number *dinB*$^+$ plasmid described in Table 1 and in the text. The vector was pWSK129 (37).

[b] Each mutant frequency entry is based on the median value for 15 independent cultures grown at 34°C. Shown in parentheses is the fold mutator effect of pLO1 (*dinB*$^+$).

[c] —, no plasmid.

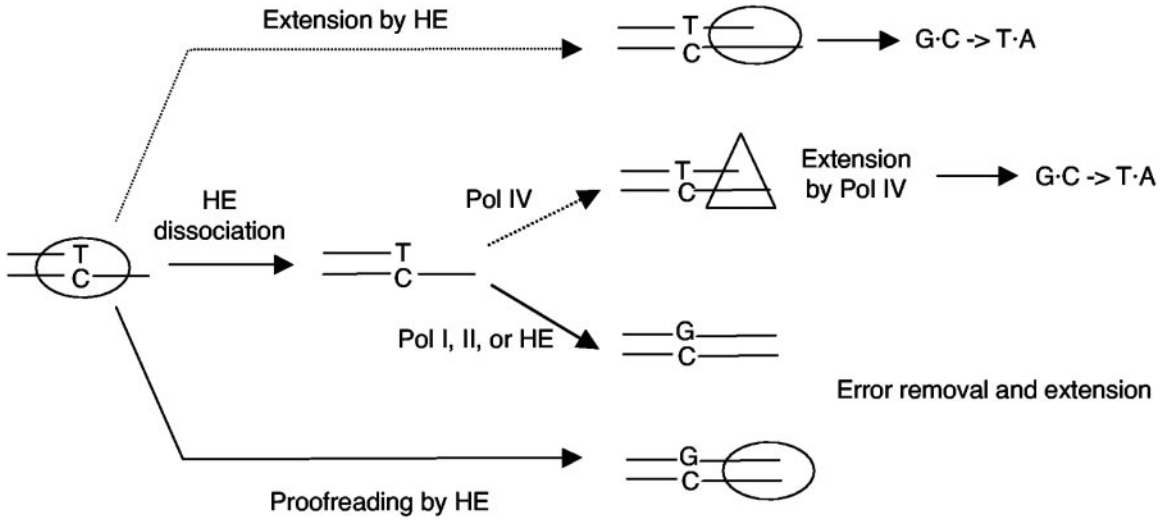


FIG. 1. Diagram showing the possible fates of a terminal mismatch created by Pol III HE. The mismatch can be extended by HE to yield a mutation (G · C→T · A in this case) (top) or removed by HE via proofreading (bottom). However, as a third pathway, HE may dissociate (middle), setting up a competition between the various polymerases, including HE itself. Extension by Pol IV is likely to lead to a mutation; extension by Pol I, II, or HE is likely to lead to removal of the mismatch. The model as presented here does not address whether HE dissociation is a passive process or a coordinated one. Stippled lines indicate the less-likely pathways under normal conditions. However, in the presence of excess Pol IV, this pathway can become a major contributor to mutagenesis.

Pol IV basal levels can be detected (Table 2), as also demonstrated previously (15, 21, 38).

**Access of Pol IV to the replication fork.** Our findings indicate that the Pol IV mutator activity acts unequally on the two strands and enhances preferentially mutations originating in the lagging strand. Implicit in the latter point is that the assigned mispairs through which the observed *lac* mutations occur do not change (for example, C · T instead of G · A in the case of the G · C→T · A transversions) when normal and Pol IV-overproducing conditions are compared. Since Pol III is proposed to generate the responsible mispairs in either case, this assumption is likely valid. The preferential effect of Pol IV in the lagging strand is analogous to that observed for overexpression of DNA polymerase V in a *recA730* strain, where lagging-strand mutagenesis is preferentially enhanced in a similar fashion (17). Presumably, lagging-strand replication creates a greater opportunity for Pol IV and Pol V to participate. This increased access is likely related to the greater probability of polymerase dissociation in this strand at the sites of Pol III-generated terminal mispairs. Nevertheless, while lagging-strand mutagenesis is clearly most strongly enhanced (from 6- to 11.7-fold, depending on the *lacZ* allele; Table 1), the effect of Pol IV is not exclusive to the lagging strand. We also observe an up to 3-fold increase for the opposite orientation, presumably reflecting the effect of Pol IV in the leading strand (Table 1).

In vitro data on numerous DNA polymerases, including Pol III (22, 24, 25, 32), have shown that extension from terminal mismatches is generally a difficult feat, presumably due to the aberrant geometry of the primer terminus, which does not fit well in the polymerase active site (for a review, see reference 16). The temporary stalling that ensues provides increased opportunity for competing processes, such as proofreading (error removal) or polymerase dissociation (11). It is an open question whether any such dissociation, if occurring, is simply a passive or a controlled event. Regardless of this question, our data indicate that there is likely to be competition between the various polymerases for the terminal mismatch. We have diagrammed this competition in Fig. 1. Upon HE dissociation, the primer terminus may be subsequently bound by a proofreading-capable enzyme, such as Pol I, Pol II, or Pol III itself, leading to excision of the mismatch. However, if Pol IV obtains access to the mismatch, it is likely to be fixed as a potential mutation. Increasing the relative amount of Pol IV will increase the chances of the error-prone fixing of the mismatch proportionately, as seen in the present study.

In the case of the proofreading-impaired *dnaQ928* mutant (Table 2), the proofreading defect will simply increase the number of terminal mismatches available for entering the dissociation pathway, where they will become a substrate for the competing enzymes, including Pol IV. The biochemical basis for the mutator effect of the two *dnaE* temperature-sensitive mutants, *dnaE486* and *dnaE511*, has not been fully established (34). They may suffer from increased frequency of misincorporation and/or increased dissociation at terminal mismatches. In either case, there will be increased numbers of mismatches available as substrate for Pol IV. Consistent with this, the mutator phenotypes of *E. coli mutD5* and *dnaE1336* were shown to be partially *dinB* dependent (23, 29). In further support of the model of Fig. 1, we have recently shown that also DNA Pol II is able to participate in chromosomal replication (1). In contrast to Pol IV, Pol II plays an antimutagenic role by editing Pol III-mediated mispairs. It also competes effectively with Pol IV, limiting access of the latter to the replication point (1).

In summary, our data indicate that *E. coli* Pol IV is able to access the replication fork when Pol III HE has difficulty processing the terminal mismatches that it has created. Although not detectable in our study, this "rescue" activity of Pol IV can be readily extended to the general case of rescuing a stalled

HE in the absence of any mismatches. As discussed (2, 10), Pol IV may be well suited for this kind of activity based on its affinity for aberrant or nonstandard primer termini.

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