JOURNAL OF BACTERIOLOGY, Mar. 2008, p. 1730–1742 Vol. 190, No. 5
0021-9193/08/$08.00+0 doi:10.1128/JB.01463-07

Role of Accessory DNA Polymerases in DNA Replication in *Escherichia coli*: Analysis of the *dnaX36* Mutator Mutant[▽]

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Received 10 September 2007/Accepted 13 December 2007

The *dnaX36*(TS) mutant of *Escherichia coli* confers a distinct mutator phenotype characterized by enhancement of transversion base substitutions and certain (−1) frameshift mutations. Here, we have further investigated the possible mechanism(s) underlying this mutator effect, focusing in particular on the role of the various *E. coli* DNA polymerases. The *dnaX* gene encodes the τ subunit of DNA polymerase III (Pol III) holoenzyme, the enzyme responsible for replication of the bacterial chromosome. The *dnaX36* defect resides in the C-terminal domain V of τ, essential for interaction of τ with the α (polymerase) subunit, suggesting that the mutator phenotype is caused by an impaired or altered α-τ interaction. We previously proposed that the mutator activity results from aberrant processing of terminal mismatches created by Pol III insertion errors. The present results, including lack of interaction of *dnaX*36 with *mutM*, *mutY*, and *recA* defects, support our assumption that *dnaX36*-mediated mutations originate as errors of replication rather than DNA damage-related events. Second, an important role is described for DNA Pol II and Pol IV in preventing and producing, respectively, the mutations. In the system used, a high fraction of the mutations is dependent on the action of Pol IV in a (*dinB*) gene dosage-dependent manner. However, an even larger but opposing role is deduced for Pol II, revealing Pol II to be a major editor of Pol III mediated replication errors. Overall, the results provide insight into the interplay of the various DNA polymerases, and of τ subunit, in securing a high fidelity of replication.

The mechanisms by which cells produce mutations, or try to avoid making them, are of significant research interest. Mutations may occur from replication errors, as DNA replication proceeds with high but not infinite accuracy. While the fidelity of individual DNA polymerases, including their base insertion fidelity and proofreading ability, has been investigated in detail (for reviews, see references 43 and 44), recent emphasis has shifted to the fidelity of the chromosomal replisomes, the multisubunit complexes that perform the simultaneous replication of leading and lagging strands. Specific issues of interest are the contribution of the various replisomal subunits, the mechanisms underlying the differential fidelity of leading and lagging strand replication, and the involvement of the additional DNA polymerases that have been discovered in recent years.

In the model system *Escherichia coli*, chromosomal replication is performed by the 17-subunit protein complex DNA polymerase III (Pol III) holoenzyme (HE) (49, 50, 56). HE is organized into several functional modules: two Pol III core units (one for each strand), two β-clamp processivity factors, and the DnaX complex. Each Pol III core is made up of three subunits (α, ε, and θ), in which α is the DNA polymerase, ε is the proofreading subunit (3′→5′ exonuclease), and θ is a stabilizing factor for the ε subunit (37, 82). Each β-clamp is a dimer of identical subunits (β_2) in the shape of a ring, tethering the core units to the DNA through an interaction with the α subunit. This interaction with the β-clamp is responsible for high processivity of DNA synthesis, which is particularly important for synthesis of the leading strand (38). The seven-subunit DnaX-complex ($\tau_2\gamma\delta\delta'\chi\psi$) contains an ATP-powered clamp-loader activity ($\gamma\delta\delta'\chi\psi$) responsible for assembly of the β clamps onto DNA, which needs to be done repeatedly on the lagging strand. The τ_2 component of the DnaX complex serves essentially as an organizing center of HE. Each τ subunit interacts with the α subunit of a Pol III core, coupling the leading- and lagging-strand polymerases (22, 34, 49, 51, 56, 77) and also binds the $\gamma\delta\delta'\chi\psi$ clamploading complex (49, 56, 57). In addition to being the structural scaffold of HE, τ also plays important regulatory roles. For example, it interacts with the DnaB helicase, regulating the speed of the replication fork (10, 21), and triggers the dissociation of the lagging-strand polymerase at the end of Okazaki fragments through its interaction with the α subunit (45, 46, 51). Recently, an HE containing three τ subunits (instead of two τ's and one γ) and three Pol III core molecules has also been described and may be a biologically relevant species (50).

In the present study, we were particularly interested in the role of τ subunit in the control of chromosomal replication fidelity. The τ protein is the 71.1-kDa (643 amino acids) full-length product of the *dnaX* gene. The gene also produces the 47.5-kDa γ subunit, which results from an early termination in the *dnaX* reading frame through a programmed −1 ribosomal frameshift. As a result, γ is identical to the first 430 residues of τ (3, 16, 85). The τ subunit contains five distinct domains, I through V, of which domains I to III correspond to γ, whereas domains IV and V are unique to τ (21–23). Domain IV medi-

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▽ Published ahead of print on 21 December 2007.

ates the interaction of τ with the DnaB helicase (21), while domain V is responsible for the α-τ interaction. Since τ is present as a dimer (or trimer), the α-τ interaction effectively couples two (or three) Pol III core assemblies within the HE, thus facilitating joint synthesis of leading and lagging strands. The α-τ interaction is also responsible for the proper cycling of Pol III on the lagging strand (22, 45, 46, 50).

Previously, our laboratory has investigated the properties of certain *E. coli dnaX* mutants (61). These studies revealed that the temperature-sensitive allele *dnaX36*, encoding a mutant τ subunit containing a Glu-to-Lys change at residue 601 in domain V (4), displayed elevated mutation rates (61). This mutator phenotype suggested that one additional function of τ is to promote the fidelity of the chromosomal replication complex. Interestingly, the mutator effect is characterized by an unusual specificity: transversion base substitutions and (−1) frameshifts in non-run sequences are specifically enhanced. This observation led us to propose a model in which the τ subunit is important for the proper response of HE after certain misincorporation events by α subunit (61). In this model, certain transversion mismatches provide temporary stalling points for HE, which require the action of τ subunit to be resolved in an error-free manner. In the absence of proper α-τ interaction, these mismatches are processed in an error-prone manner, either as transversion base substitutions (by direct extension) or as (−1) frameshifts by misalignment-extension in sequence contexts where the misinserted base is complementary to the next template base. The latter type of misalignment-extension has been observed for many polymerases and represents a preferred way of HE under these circumstances (61).

In the present study, we have further investigated the role of the τ subunit as a fidelity factor. Our focus was on the source of the replication errors occurring in a *dnaX36* strain and on the role of the additional *E. coli* DNA polymerases (Pol I, II, IV, and V), which have been increasingly considered to be potentially important players in various aspects of replication and mutagenesis (1, 17, 20, 28, 35, 41, 50).

MATERIALS AND METHODS

Media. Solid and liquid media have been described previously (14). Minimal medium (MM) was supplemented with 0.4% glucose or lactose as a carbon source, 5 μg of thiamine/ml and, where appropriate, 50 μg of tryptophan/ml. Solid medium contained 1.5% agar (Difco). The XPG plates used for papillation assays are minimal medium plates containing glucose (0.2%), phenyl-β-D-galactopyranoside (P-Gal) (0.5 mg/ml), and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; 40 μg/ml) (14). Where required, antibiotics were added at the following final concentrations: chloramphenicol, 20 μg/ml; kanamycin, 25 μg/ml; rifampin, 100 μg/ml; tetracycline, 12.5 μg/ml; spectinomycin, 50 μg/ml; and streptomycin, 50 μg/ml.

Strain constructions. The strains used in the present study are listed in Table 1. All strains used for the mutagenesis experiments are derivatives of KA796 (*ara thi* Δ*prolac*) (71). Derivative strains were constructed by P1 transduction using P1*virA* and/or by F′ crosses introducing F′*prolac* from strains CC102, CC104, CC105, and CC106 (9). NR13138 is a *trpE9777* derivative of KA796 containing the *trpE9777* marker (75) derived from NR3951 (72). The *dnaX36* allele was introduced either from strain NR12159 based on the close linkage (90%) with *zba*-2321::mini-Tn*10cam* (61), selecting for chloramphenicol resistance, or from strain NR11914 using linkage with $purE^+$ (5%) when transduced into a *purE*::Tn*10* recipient as described previously (61). The presence of *dnaX36* was confirmed by testing for sensitivity to temperatures above 43°C on LB plates lacking NaCl (26). The *mutL*::Tn*10* and *mutL*::Tn*5* alleles were transferred from strains NR9163 and NR9559, respectively (14, 69), selecting for tetracycline or kanamycin resistance. The *recA56* allele was transferred from strain UTH2 (89) based on linkage with nearby *srl*::Tn*10*, selecting for tetracycline resistance, followed by testing for UV sensitivity. The Δ*polB1* allele [Δ(*araD-polB*)::Ω] was transferred by transduction from strain SH2101 (11) using spectinomycin resistance as a selective marker, followed by checking for acquired streptomycin resistance. Strains carrying the *polBex1* allele were constructed as described previously (1) by first converting the recipient strain to *leu*::Tn*10*, followed by conversion to leu^+ *polBex1* using as a donor strain HC101 (*polBex1* leu^+) (18). The presence of the *polBex1* allele was confirmed by backcrossing into a *dnaX36 mutL* strain containing the F′*prolac* from strain CC104 and scoring for hyperpapillation on X-Gal plates. The *dnaX36 polBex1* combination leads to a very strong mutator phenotype (see Results), which is readily visualized on these plates. The *polA1* allele was transferred by transduction from strain MFH210 (25) using linkage with transposon *zig-261*::Tn*10*. The Δ*umuDC*::*cat* allele was transferred from strain RW82 (91), selecting for chloramphenicol resistance. The Δ(*dinB-yafN*)::*kan* allele was transferred from strain YG7207 (35) using selection for kanamycin resistance. The presence of the deletion was confirmed by PCR. The *dnaQ49* and *dnaQ930* alleles were transferred from strains NR9695 (70) and NR9986 (81), respectively, using linkage with transposon insertion *zae-502*::Tn*10*, selecting for tetracycline resistance and checking for mutator phenotype or temperature sensitivity. The sources of the *dnaE915*, *dnaE925*, and *dnaE941* antimutator alleles were NR10173, NR11258, and NR10775, respectively (14, 15, 67), using linkage with *zae-502*::Tn*10* or *zae*::Tn*10d-cam* or both (12). The *mutY*::Tn*10kan* and *mutM*::mini-*tet* alleles were transferred from strains TT101 (54) and PR68 (63), respectively.

Mutant frequency measurements. For each strain a total of 12 to 18 independent LB cultures (2 ml each) were initiated from single colonies (one colony per tube). The colonies were taken from three to nine independent isolates for each strain. Cultures were grown to saturation at 37°C (or 30°C where indicated) on a rotator wheel. The total cell count was determined by plating 0.1 ml of a 10^{-6} dilution on MM-glucose plates (supplemented with tryptophan when required). The number of *lac* revertants was determined by plating 0.1 or 0.05 ml of undiluted cultures on MM-lactose plates. The number of *trpE9777* revertants was determined by plating 0.1 ml of a 10-fold dilution on MM-glucose plates. The number of rifampin-resistant colonies was determined by plating 0.1 ml of 10-fold-diluted cultures on LB plates containing rifampin (100 μg/ml). Plates were incubated for 20 h (LB) or 40 h (MM) at either 37 or 30°C. To calculate mutant frequencies, the number of mutants per plate was divided by the number of total cells. Occasional jackpot cultures were removed from the analysis. Average frequencies with standard errors (SE) were determined by using the statistical software program Prism (GraphPad).

RESULTS

To further our understanding of the *dnaX36* mutator and the mechanism(s) by which the τ subunit may promote fidelity, we studied the interaction of *dnaX36* with a series of DNA repair or replication defects. We evaluated the effects on the *dnaX36*-promoted mutagenesis by using four different mutational assays: reversion of two defined *lac* alleles (9), reversion of the *trpE9777* allele, and acquisition of rifampin resistance (Rif^r). The two *lac* alleles permit specific measurement of a G · C→T · A or A · T→T · A transversion using the F′*prolac* originally present in strains CC104 or CC105 (9). The *trpE9777* allele permits measurement of (−1) frameshift errors by loss of an A · T base pair from a run of six A · T pairs (75). Rifampin resistance measures a variety of base substitutions in the *rpoB* gene (26). Previous studies showed the two indicated *lac* transversion alleles and the *trpE* frameshift allele to be particularly sensitive to the *dnaX36* mutator effect (61). All of the strains used were mismatch repair deficient (*mutL*) to facilitate the analysis of mutagenesis in terms of uncorrected replication errors.

Interaction of *dnaX36* with the *mutM* and *mutY* pathways. We first sought to corroborate our contention that the *dnaX36* mutator effect is related to increased production of replication errors rather than any increased DNA damage processing.

The *mutM* and *mutY* genes play an important role in protecting the cell against the mutagenic effects of 8-oxoguanine

TABLE 1. *E. coli* strains used in this study

Strain	Relevant genotype[a]	Source or reference
Strains used for construction		
CC102	F′CC102	9
CC104	F′CC104	9
CC105	F′CC105	9
CC106	F′CC106	9
HC101	*polBex1*	18
KA796	*ara thi* Δ(*prolac*)	71
MFH210	*polA1 zig-261*::Tn*10*	25
NR9163	*mutL218*::Tn*10*	69
NR9559	*mutL211*::Tn*5*	14
NR9695	*dnaQ49 zae-502*::Tn*10*	70
NR10173	*dnaE915 zae*::Tn*10d-cam*	14
NR10775	*dnaE941 zae*::Tn*10d-cam zae-502*::Tn*10*	67
NR11258	*dnaE925 zae*::Tn*10d-cam*	14
NR11916	*dnaX36 purE*::Tn*10*	61
NR12159	*dnaX36 zba-2321*::mini-Tn*10cam*	61
NR13138	*ara*, *thi*, Δ(*prolac*), *trpE9777*	This study
PR68	*mutY68*::Tn*10kan*	63
RW82	Δ(*umuDC*)*595*::*cat*	91
SH2101	Δ*polB1* [Δ(*araD-polB*)::Ω]	11
TT101	*mutM103*::*mini-tet*	54
UTH2	*recA56 srl-360*::Tn*10*	89
YG7207	Δ(*dinB-yafN*)::*kan*	35
Strains used for mutagenesis[b]		
NR10452	*dnaE915*, F′CC102	This study
NR10454	*dnaE915*, F′CC104	This study
NR10455	*dnaE915*, F′CC105	This study
NR10456	*dnaE915*, F′CC106	This study
NR11102	F′CC102	This study
NR11104	F′CC104	This study
NR11105	F′CC105	This study
NR11106	F′CC106	This study
NR11172	*dnaE941*, F′CC102	This study
NR11174	*dnaE941*, F′CC104	This study
NR11175	*dnaE941*, F′CC105	This study
NR11176	*dnaE941*, F′CC106	This study
NR11939	F′CC104	This study
NR11940	F′CC105	This study
NR11951	*dnaX36*, F′CC104	This study
NR11952	*dnaX36*, F′CC105	This study
NR12193	*dnaQ49*, F′CC104	This study
NR12194	*dnaQ49*, F′CC105	This study
NR12197	*dnaX36 dnaQ49*, F′CC104	This study
NR12198	*dnaX36 dnaQ49*, F′CC105	This study
NR12526	*dnaX36 dnaE925*, F′CC104	This study
NR12527	*dnaX36 dnaE941*, F′CC104	This study
NR12528	*dnaX36 dnaE915*, F′CC104	This study
NR12529	*dnaX36 dnaE925*, F′CC105	This study
NR12530	*dnaX36 dnaE941*, F′CC105	This study
NR12531	*dnaX36 dnaE915*, F′CC105	This study
NR12532	*mutY*, F′CC104	This study
NR12533	*mutM*, F′CC104	This study
NR12534	*dnaX36 mutY*, F′CC104	This study
NR12535	*dnaX36 mutM*, F′CC104	This study
NR12548	*recA56*, F′CC104	This study
NR12549	*recA56*, F′CC105	This study
NR12550	*dnaX36 recA56*, F′CC104	This study
NR12551	*dnaX36 recA56*, F′CC105	This study
NR13153	*trpE9777*, F′CC104	This study
NR13155	*trpE9777*, F′CC104/*dinB*	This study
NR13157	*trpE9777 dinB*, F′CC104	This study
NR13159	*trpE9777 dinB*, F′CC104/*dinB*	This study
NR13256	*trpE9777 dnaX36*, F′CC104	This study
NR13258	*trpE9777 dnaX36*, F′CC104/*dinB*	This study
NR13272	*trpE9777 dnaX36 dinB*, F′CC104	This study
NR13274	*trpE9777 dnaX36 dinB*, F′CC104/*dinB*	This study
NR13276	*trpE9777* Δ*umuDC*, F′CC104	This study
NR13278	*trpE9777 dnaX36* Δ*umuDC*, F′CC104/*dinB*	This study
NR13292	*trpE9777 dnaX36* Δ*umuDC dinB*, F′CC104	This study
NR13296	*trpE9777 dnaX36* Δ*umuDC dinB*, F′CC104/*dinB*	This study
NR13312	*trpE9777* Δ*umuDC*, F′CC104	This study
NR13314	*trpE9777* Δ*umuDC*, F′CC104/*dinB*	This study
NR13316	*trpE9777* Δ*umuDC dinB*, F′CC104	This study
NR13318	*trpE9777* Δ*umuDC dinB*, F′CC104/*dinB*	This study
NR16108	*trpE9777 dnaX36 polBex1*, F′CC104	This study
NR16116	*trpE9777 dnaX36 polBex1 dinB*, F′CC104/*dinB*	This study
NR16159	*trpE9777 dnaX36* Δ*polB*, F′CC104	This study
NR16163	*trpE9777 dnaX36* Δ*polB dinB*, F′CC104/*dinB*	This study
NR16169	*trpE9777 dnaX36 polBex1*, F′CC105	This study
NR16173	*trpE9777 dnaX36 polBex1 dinB*, F′CC105/*dinB*	This study
NR16176	*trpE9777 dnaX36* Δ*polB*, F′CC105	This study
NR16183	*trpE9777 dnaX36* Δ*polB dinB*, F′CC105/*dinB*	This study
NR16226	*trpE9777*, F′CC105	This study
NR16228	*trpE9777 dinB*, F′CC105/*dinB*	This study
NR16246	*trpE9777 dnaX36*, F′CC105	This study
NR16248	*trpE9777 dnaX36 dinB*, F′CC105/*dinB*	This study
NR16878	*trpE9777 polBex1*, F′CC104	This study
NR16879	*trpE9777 polBex1*, F′CC105	This study
NR16889	*trpE9777* Δ*polB*, F′CC104	This study
NR16890	*trpE9777* Δ*polB*, F′CC105	This study
NR17223	*trpE9777 polBex1 dinB*, F′CC104/*dinB*	This study
NR17224	*trpE9777 polBex1 dinB*, F′CC105/*dinB*	This study
NR17225	*trpE9777* Δ*polB dinB*, F′CC104/*dinB*	This study
NR17226	*trpE9777* Δ*polB dinB*, F′CC105/*dinB*	This study
NR17502	*dnaE925*, F′CC102	This study
NR17504	*dnaE925*, F′CC104	This study
NR17505	*dnaE925*, F′CC105	This study
NR17506	*dnaE925*, F′CC106	This study
NR17507	*dnaQ930*, F′CC104	This study
NR17508	*dnaX36 dnaQ930*, F′CC104	This study
NR17509	*dnaQ930*, F′CC105	This study
NR17510	*dnaX36 dnaQ930*, F′CC105	This study

[a] F′CC102, F′CC104, F′CC105, and F′CC106 refer to the F′*prolac* originally present in strains CC102, CC104, CC105, and CC106, which permit measurement of *lac* G · C→A · T, G · C→T · A, A · T→T · A, and A · T→G · C base substitution mutations, respectively (9). The designations F′CC104/*dinB* and F′CC105/*dinB* indicate deletion of the *dinB* gene on the F′ episome. See Materials and Methods for details on the various constructions.

[b] All of these strains are also *ara thi mutL* Δ*prolac*.

(8-oxodG). This base analog is mutagenic due to its ambivalent base-pairing properties and capable of pairing with both C and A during DNA synthesis (74). MutM is a DNA glycosylase capable of removing 8-oxoG from 8-oxodG · dC pairs, whereas MutY is a glycosylase that removes A from 8-oxodG · dA pairs. Lack of either MutM or MutY leads to increased G · C→T · A mutations (53). Since G · C→T · A are among the most frequent mutations induced in a *dnaX36* strain (61),

TABLE 2. Mutability of *dnaX36* in combination with *mutM*, *mutY*, and *recA* deficiencies[a]

Expt	Genotype	No. of revertants/10^8 cells	
		lac G · C→T · A[b]	*lac* A · T→T · A[c]
1	*dnaX*$^+$	1.4	ND
	dnaX36	100	ND
	mutM	5.0	ND
	dnaX36 mutM	90	ND
	mutY	18	ND
	dnaX36 mutY	210	ND
2	*dnaX*$^+$	1.9	0.5
	dnaX36	130	7.6
	recA56	0.8	0.1
	dnaX36 recA56	54	4.0

[a] All strains are also mismatch repair deficient (*mutL*). Revertant frequencies (*lac*→*lac*$^+$) were determined as described in Materials and Methods. ND, not done.

[b] *lac* G · C→T · A reversions were determined by using the strains NR11939 (*dnaX*$^+$), NR11951 (*dnaX36*), NR12533 (*mutM*), NR12535 (*dnaX36 mutM*), NR12532 (*mutY*), NR12534 (*dnaX36 mutY*), NR12548 (*recA56*), and NR12550 (*dnaX36 recA56*).

[c] *lac* A · T→T · A reversions were determined by using the strains NR11940 (*dnaX*$^+$), NR11952 (*dnaX36*), NR12549 (*recA56*), and NR12551 (*dnaX36 recA56*).

we investigated a possible interaction between the *dnaX36* and the *mutM*/*mutY* pathways. The results in Table 2 (experiment 1) show that the single *dnaX*, *mutM*, and *mutY* defects produce significant mutator effects (74-, 3.6-, and 13-fold for the *lac* G · C→T · A allele). However, the double *dnaX36 mutM* or *dnaX36 mutY* strains did not show any major changes (≤2-fold) in the frequency of G · C→T · A transversions compared to the single *dnaX36* strain. Thus, it is unlikely that the strong increase in G · C→T · A transversions in the *dnaX36* strain is related to the presence of 8-oxodG in the DNA.

The *dnaX36* mutator effect is independent of RecA functions. RecA is an essential factor in mediating both homologous recombination and the inducible SOS response. Both aspects of RecA function are involved in modes of mutagenesis, such as adaptive mutagenesis, which requires the RecA recombination function (17), and SOS mutagenesis, which requires both RecA itself and the RecA-regulated error-prone DNA polymerase V (*umuDC* gene product) (80, 84). The results in Table 2 (experiment 2) indicate that the *dnaX36* mutator effect is not obviated by the *recA56*-null mutation. Although the absolute frequencies in the *recA56* strain are reduced by a few fold, a similar reduction is observed for the *dnaX*$^+$ control, leaving the *dnaX36* mutator effect at essentially the same level (60- to 70-fold for G · C→T · A and 15-to 40-fold for A · T→T · A). The reduction in mutant frequencies in *recA56* cells may relate to copy number issues of the F′*prolac* episome, but we have not investigated this further.

The (negative) results with the *recA56* and *mutM* and *mutY* strains support the proposed idea that the *dnaX36* mutator effect results from the processing of normal DNA replication errors rather than the processing of any damaged DNA bases (61). This conclusion is also consistent with the efficient correction of *dnaX36*-generated mutations by the *mutHLS* mismatch repair system, as reported previously (61). We further investigated the generation of these replication errors with regard to the role of each of the *E. coli* DNA polymerases (see below).

Role of Pol I. To investigate the role of DNA Pol I in the *dnaX36* mutator effect, we used the *polA1* allele, a defective allele that lacks polymerase activity but still retains the 3′→5′ exonuclease activity (32, 39). Δ*polA* alleles have been reported but are generally inviable (32), indicating the important role of Pol I in the cell, likely related to its role in removing the RNA primers that initiate Okazaki fragments. Our experiments revealed that it was not possible to create a *dnaX36 polA1* double mutant, even at low temperatures (down to room temperature). This inviability is reminiscent of the observed synthetic lethality of *polA1* with a number of other repair/replication defects, such as *recA*, *recBC*, *uvrB*, and others (39). We conclude that *dnaX36* strains have an increased requirement for DNA polymerase I, which is consistent with some level of DNA replication defect in the *dnaX36*(TS) strain (26). On the other hand, a *dnaX36 polA1* double mutant could be readily obtained by transduction if the recipient cell contained a plasmid carrying the gene for rat Pol β (79). This result (not shown) is consistent with *dnaX36* strains requiring additional DNA polymerizing capacity, possibly because of stalled replication forks and/or an increased number of DNA gaps.

Role of Pol IV and Pol V. *E. coli* Pol IV and Pol V (the products of the *dinB* and *umuDC* genes, respectively) are two accessory polymerases characterized by the lack of 3′ exonuclease (proofreading) activity, and they are generally considered error-prone (83, 86, 87). Both polymerases are expressed in an inducible manner as part of the SOS response. The two polymerases differ in their basal expression levels. Pol IV, when expressed from a single chromosomal gene copy, is present at a level of about 250 copies (35), and this is further increased ~7-fold by SOS induction (36). In contrast, Pol V is considered to be largely absent from normal cells but is strongly induced by the SOS response (92). In view of the error-prone character of these polymerases, we investigated their role in the *dnaX36* mutator effect. Considering the lack of effect of the *recA* deficiency on the *dnaX36* mutator effect (see above), any effect of these polymerases would have to be mediated by their basal levels.

We measured the frequency of *lac* G · C→T · A transversions and of (−1) frameshifts at the *trpE9777* locus. In the strains used, the *lac* gene is located on the F′*prolac* episome (9). Since the *pro-lac* region on the F′ episome also includes the *dinB* gene, the strains used have both chromosomal and episomal copies of *dinB*. We therefore conducted our experiments with a series of four strains, containing (i) the full set of *dinB* copies (chromosomal and episomal), (ii) only the episomal copy, (iii) only the chromosomal copy, or (iv) neither copy. The results of the mutagenesis experiments are presented in Fig. 1. It can be seen that in the *dnaX36* background both *lac* and *trp* reversion strongly depend on *dinB* (i.e., Pol IV activity). Notably, comparing the fully *dinB*-deficient strain to the fully proficient one, ~75% of the *lac* G · C→T · A mutations and 90% of *trpE* frameshift mutations are *dinB* dependent. Loss of *dinB* from the episome causes a stronger effect than loss from the chromosome. This likely reflects the copy number of F′, which is generally greater than one. Using quantitative PCR measurements, we estimated the copy number to be ca. two to three in these strains (data not shown), which is

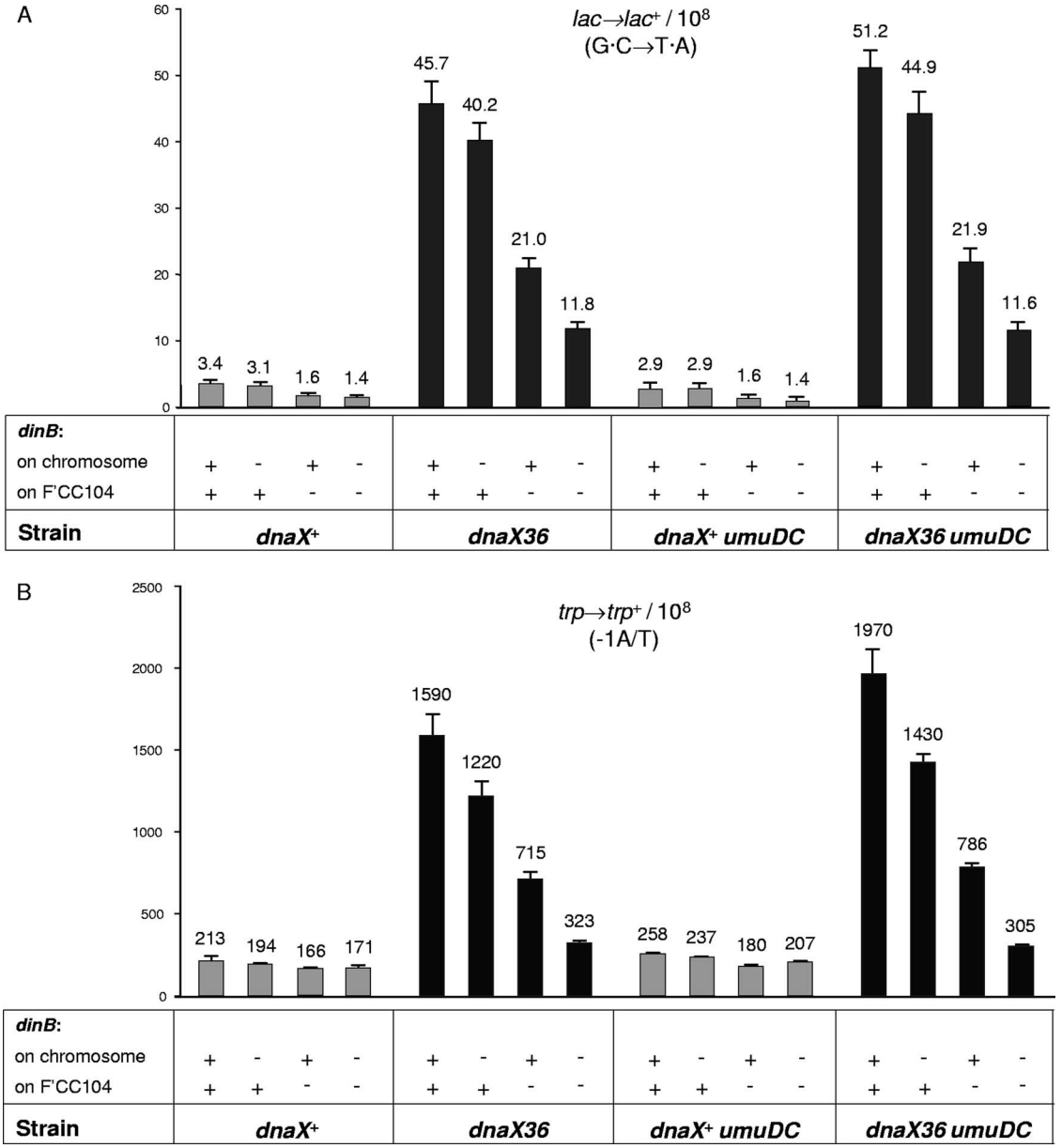

FIG. 1. Effect of Pol IV (*dinB*) and Pol V (*umuDC*) on the *dnaX36* mutator effect. All strains used were mismatch repair deficient (*mutL*). Mutant frequencies were determined as described in the Materials and Methods. (A) Mutant frequencies for *lac* G · C→T · A transversions; (B) mutant frequencies for *trpE9777* reversion (−1 frameshift). The figure shows the results of one representative experiment. These experiments were performed multiple times, which yielded similar results. The *dnaX*$^+$ strains used were NR13153, NR13157, NR13155, and NR13159. The *dnaX36* strains were NR13256, NR13272, NR13258, and NR13274. The *dnaX*$^+$ Δ*umuDC* strains were NR13312, NR13316, NR13314, and NR13318. The *dnaX36* Δ*umuDC* strains were NR13276, NR13292, NR13278, and NR13296. The status of the *dinB* gene, chromosomal and/or episomal, in these strains is indicated in the boxes below the graph.

consistent with reports by others (17, 19, 36). Nevertheless, even in the absence of Pol IV the *dnaX36* mutator effect for the *lac* allele is still significant (eightfold), indicating that there are both Pol IV-dependent and Pol IV-independent components to the *dnaX36* mutator effect.

Figure 1 also includes the results with the Pol V-deficient strains (*umuDC*). It is clear that the *dnaX36* mutator effect is fully *umuDC*-independent regardless of the *dinB* configuration, which is consistent with the results obtained with the *recA56* strain.

Role of Pol II. DNA Pol II is a proofreading-proficient polymerase encoded by the *polB* gene. Like Pol IV, Pol II is an SOS-inducible enzyme with a relatively high basal level (30 to 50 copies per cell), which is increased ~7-fold upon SOS induction (5, 8, 29, 62). Genetic studies have shown that Pol II may be involved in a variety of cellular activities, such as repair of DNA damaged by UV irradiation (48) or oxidation (11), repair of interstrand cross-links (2), lesion bypass (20), replication restart after UV irradiation of *E. coli* (65), adaptive mutagenesis (17, 18), and long-term survival (93). In vitro

studies have shown that Pol II interacts with Pol III accessory proteins (β-clamp and clamp-loading complex) to become competent to synthesize DNA with high fidelity and processivity (6, 27). Most recently, Pol II was shown to be capable of playing a backup role as a proofreader for Pol III-produced misinsertion errors and to protect mismatched 3′ termini against the action of Pol IV (1).

To investigate the possible role of Pol II in the *dnaX36* mutator effect, we used two alleles of *polB*: Δ*polB* and *polBex1* (64). The Δ*polB* is a simple deletion, whereas *polBex1* encodes an error-prone Pol II defective in exonucleolytic proofreading but proficient in DNA synthesis activity (18). This allele is useful because the lack of exonuclease may allow detection of limited amounts of Pol II participation in DNA replication through a mutator effect (1). We also made *polB* strains that are additionally defective in Pol IV (*dinB*). Mutagenesis was tested by *lac* reversion (G · C→T · A and A · T→T · A), *trpE9777* reversion, and forward Rif^r mutagenesis. The results are shown in the panels of Fig. 2. For the *dnaX*$^+$ strains, the Δ*polB* allele did not significantly affect any of the mutant frequencies, a finding consistent with previous findings on Δ*polB* strains (1, 64). In contrast, the *polBex1* strains showed increased *lac* mutagenesis, 12-fold for G · C→T · A and 8-fold for A · T→T · A, although little effect was seen for the *trpE* or Rif^r markers. The mutator effect of *polBex1* for the *lac* alleles parallels that seen by Banach-Orlowska et al. (1), although in that study the *lac* alleles were located on the chromosome instead of the F′ episome. These results are indicative of a role of Pol II in both chromosomal and episomal DNA synthesis.

In the *dnaX36* strains, we observed the expected mutator phenotype for all three tested markers: it was strongest for the *lac* markers (8- and 11-fold) and the *trpE* marker (12-fold) but only moderate (2-fold) for the Rif^r marker (see also reference 61). Interestingly, in the *dnaX36* background, sizable effects were observed for both Δ*polB* and *polBex*. The Δ*polB* mutation increased the G · C→T · A and A · T→T · A *lac* mutant frequency by 5- and 9-fold, respectively. Even stronger effects were found for the *polBex1* allele. The G · C→T · A and A · T→T · A *lac* frequencies were enhanced 16- and 22-fold, respectively, while the frequency of Rif^r mutations was increased 5-fold. These results are indicative of a significantly expanded role of Pol II in DNA synthesis in the *dnaX36* mutant.

When considering the role of Pol IV in these strains, the results clearly reveal that the enhanced mutator effect of the *dnaX36* Δ*polB* strain is almost entirely dependent on Pol IV (*dinB*). Thus, one function of Pol II under these conditions is to prevent access of Pol IV. In contrast, only a small antimutator effect (≤2-fold) was observed in *dnaX36 polBex1* strains upon loss of Pol IV (*dinB*). This is further evidence that the access of Pol IV is limited by the presence of Pol II.

Role of Pol III. Our present results reveal significant polymerase trafficking in *dnaX*$^+$ and, especially, *dnaX36* strains. It was therefore of interest to investigate also the role of Pol III in the *dnaX36* mutator effect. We first examined the role of the Pol III proofreading activity, encoded by the *dnaQ* gene (Pol III ε subunit). We combined d*naX36* with the proofreading-impaired *dnaQ49* or *dnaQ930* allele, which both provide a strong mutator effect in *dnaX*$^+$ strains. The *dnaQ49* (V96G) mutator activity is temperature dependent and results, at least in part, from defective binding of ε subunit to the polymerase (31, 73, 82). The *dnaQ930* (H98Y) allele is a stable allele, and its defect is presumed largely catalytic in nature (81). The *dnaX36 dnaQ49* double mutant proved to be more temperature sensitive than the single *dnaQ49* mutant, and the experiments were therefore performed at 30°C, at which temperature growth was essentially normal. The results in Table 3 (experiments 1 and 2) show that *dnaQ49*, *dnaQ930*, and *dnaX36* individually all display strong mutator phenotypes for the two *lac* reversions. However, in the double *dnaX36 dnaQ49* and *dnaX36 dnaQ930* mutants the mutant frequency is either not substantially altered from that of the single mutants or, at best, consistent with simple additivity of the single mutator effects. Thus, diminishment of the Pol III proofreading does not appear to influence the *dnaX36* mutator effect.

For the Pol III α subunit, we were unable to obtain combinations of *dnaX36* with several *dnaE*(Ts) mutator alleles due to poor viability. However, we were able to obtain combinations of *dnaX36* with three *dnaE* antimutator alleles: *dnaE915*, *dnaE925*, and *dnaE941*. These *dnaE* alleles are characterized by their improved fidelity of chromosomal and episomal DNA replication in mismatch repair-deficient and -proficient cells (12, 14, 66–68). It was proposed that their increase in replication accuracy does not result from improved insertion fidelity of the Pol III α subunit but, instead, from improved processing of the insertion errors once made, either by increased proofreading or by increased dissociation of the polymerase from the terminal mismatch (14, 15). In Table 3, experiments 3 and 4, we reproduced the antimutator effects as observed in the *dnaX*$^+$ background. For this case, we also show the effects for the two *lac* transitions (G · C→A · T and A · T→G · C), since the antimutator effects are typically largest for transitions (at least in mismatch repair-defective strains) (14, 66). Interestingly, the *dnaE* alleles do not produce antimutator effects in the *dnaX36* background. In fact, in several instances a mutator effect is observed, for example, four- to eightfold for *dnaE915* or two- to threefold for the *dnaE941* allele.

These unusual interactions of *dnaX36* with the Pol III proofreading deficiencies (*dnaQ*) and the *dnaE* antimutator alleles should be taken into account when addressing the mechanisms responsible for the *dnaX36* mutator effect.

DISCUSSION

***dnaX36* defect.** In the present study we have further investigated the *dnaX36* mutator. The precise defect in the *dnaX36* mutant is not known but likely involves one or more aspects of the τ-α subunit interaction within HE (61). The *dnaX36* mutation is located in DnaX domain V responsible for interaction with the α subunit (22), and biochemical measurements of the τ-α protein-protein interaction have shown significant impairment in this interaction for several of the domain V mutants (61; C. McHenry, unpublished data). Nevertheless, the in vivo replication defect associated with *dnaX36* is likely to be modest. The mutant grows normally at 37°C, and temperature sensitivity is only apparent at temperatures above 43°C on salt-free media (26). Thus, while the α-τ interaction may be altered or weakened under normal conditions (37°), it is unlikely that it is abolished altogether, and we presume that increased α-τ dissociations happen only occasionally.

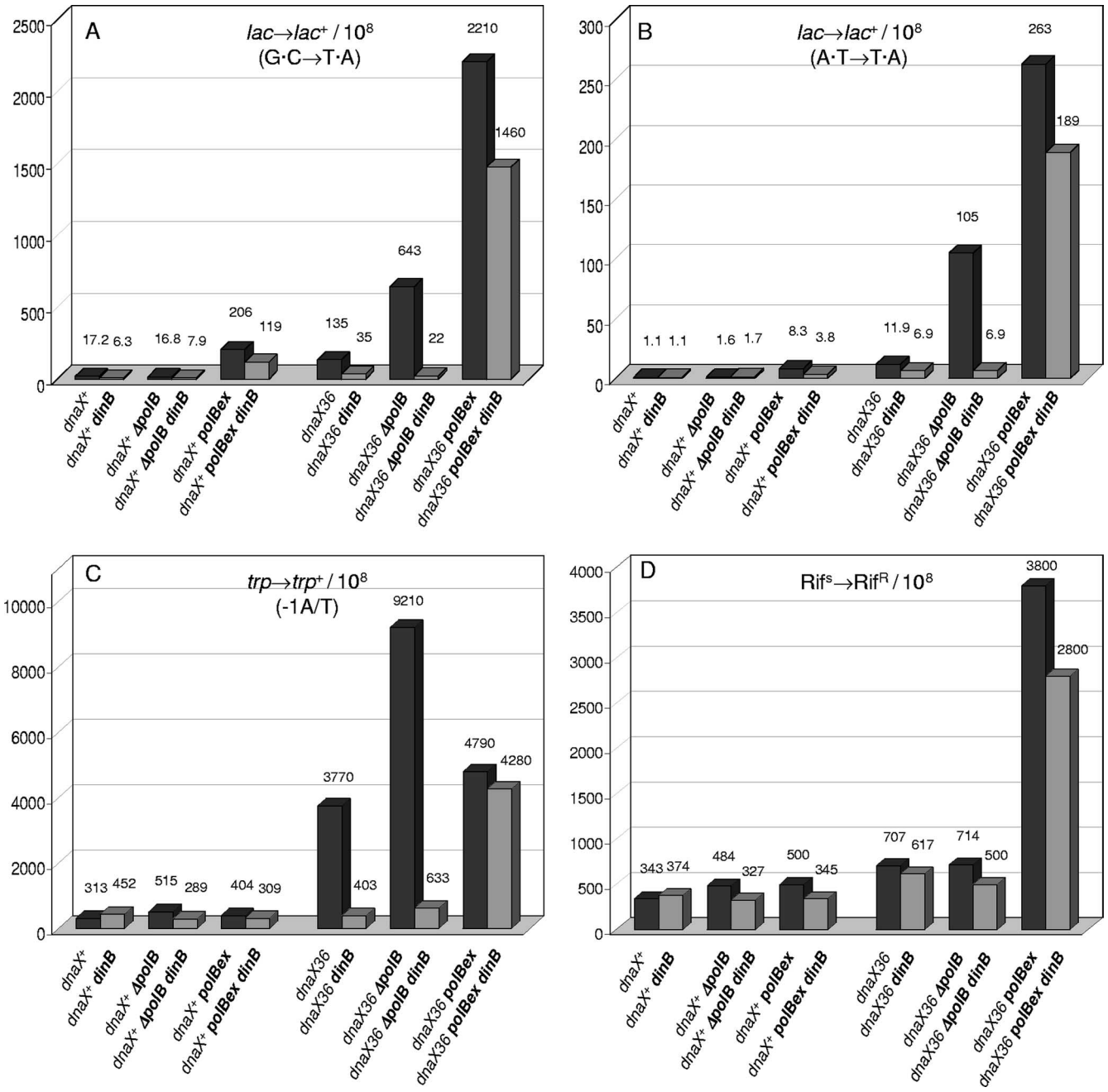

FIG. 2. Effect of Pol II (Δ*polB* and *polBex1*) and Pol IV (*dinB*) on the *dnaX36* mutator activity. The *dinB* strains lack both chromosomal and episomal gene copies. All strains are also mismatch repair deficient (*mutL*). Mutant frequencies were determined as described in Materials and Methods. In this series of experiments, the background mutant frequency for the *lac* G · C→T · A was higher than in previous experiments (Table 2 and Fig. 1). The present experiments were performed at a later time and under slightly different conditions. Nevertheless, within this series of experiments the results were highly consistent and reproducible over several repeats. (A) Mutant frequencies for *lac* G · C→T · A transversions. The strains used were: NR13153 (*dnaX*$^+$), NR13159 (*dnaX*$^+$ *dinB*), NR16889 (*dnaX*$^+$ Δ*polB*), NR17225 (*dnaX*$^+$ Δ*polB dinB*), NR16878 (*dnaX*$^+$ *polBex*), NR17223 (*dnaX*$^+$ *polBex dinB*), NR13256 (*dnaX36*); NR13274 (*dnaX36*, *dinB*); NR16159 (*dnaX36*, Δ*polB*); NR16163 (*dnaX36* Δ*polB dinB*), NR16108 (*dnaX36 polBex*), and NR16116 (*dnaX36 polBex dinB*). The frequencies ± the SE values were 17.2 ± 1.3, 6.3 ± 0.8, 16.8 ± 1.9, 7.9 ± 0.5, 206 ± 13, 119 ± 8, 135 ± 10, 35 ± 5, 643 ± 86, 22 ± 2, 2,210 ± 239, and 1,460 ± 168, respectively. (B) Mutant frequencies for *lac* A · T→T · A transversions. The strains used were NR16226 (*dnaX*$^+$), NR16228 (*dnaX*$^+$ *dinB*), NR16890 (*dnaX*$^+$ Δ*polB*), NR17226 (*dnaX*$^+$ Δ*polB dinB*), NR16879 (*dnaX*$^+$ *polBex*), NR17224 (*dnaX*$^+$ *polBex dinB*), NR16246 (*dnaX36*), NR16248 (*dnaX36 dinB*), NR16176 (*dnaX36* Δ*polB*), NR16183 (*dnaX36* Δ*polB dinB*), NR16169 (*dnaX36 polBex*), and NR16173 (*dnaX36 polBex*, *dinB*). The mutant frequencies ± the SE were 1.1 ± 0.1, 1.1 ± 0.2, 1.6 ± 0.2, 1.7 ± 0.2, 8.3 ± 0.8, 3.8 ± 0.7, 11.9 ± 1.4, 6.9 ± 1.1, 105 ± 14, 6.9 ± 1.2, 263 ± 27, and 189 ± 20, respectively. (C) Mutant frequencies for *trpE9777* reversion. Strains used were as described in panel A. The frequencies ± the SE were 313 ± 19, 452 ± 57, 515 ± 28, 289 ± 24, 404 ± 37, 309 ± 19, 3,770 ± 350, 403 ± 49, 9,210 ± 2,268, 633 ± 98, 4,790 ± 487, and 4,280 ± 332, respectively. (D) Mutant frequencies for Rif^r. The strains used were as described in panel A. The frequencies ± the SE were 343 ± 35, 374 ± 13, 484 ± 24, 327 ± 19, 500 ± 63, 345 ± 14, 707 ± 48, 617 ± 44, 714 ± 92, 500 ± 71, 3,800 ± 550, and 2,800 ± 218, respectively.

TABLE 3. Mutability of *dnaX36* in certain *dnaQ* and *dnaE* backgrounds[a]

Expt[b]	Genotype	No. of revertants/10^8 cells ± SE			
		lac G · C→A · T	*lac* G · C→T · A	*lac* A · T→T · A	*lac* A · T→G · C
1	*dnaX*$^+$	ND	2.5 ± 0.2	0.4 ± 0.1	ND
	dnaX36	ND	130 ± 7	7.3 ± 2	ND
	dnaQ49	ND	125 ± 59	200 ± 30	ND
	dnaX36 dnaQ49	ND	380 ± 39	130 ± 42	ND
2	*dnaX*$^+$	ND	15 ± 1	0.6 ± 0.1	ND
	dnaX36	ND	174 ± 4	17 ± 1	ND
	dnaQ930	ND	185 ± 41	49 ± 7	ND
	dnaX36 dna930	ND	156 ± 19	37 ± 3	ND
3	*dnaX*$^+$	71 ± 4	9.5 ± 0.7	1.0 ± 0.1	24 ± 3
	dnaE915	21 ± 1	6.1 ± 0.3	0.5 ± 0.1	9.8 ± 1.0
	dnaE925	7.2 ± 0.4	5.3 ± 0.4	0.2 ± 0.1	2.0 ± 0.2
	dnaE941	6.2 ± 0.4	3.3 ± 0.2	0.1 ± 0.1	1.8 ± 0.3
4	*dnaX36*	ND	104 ± 9	5.9 ± 0.5	ND
	dnaX36 dnaE915	ND	470 ± 21	47 ± 3	ND
	dnaX36 dnaE925	ND	108 ± 13	10 ± 1	ND
	dnaX36 dnaE941	ND	203 ± 8	20 ± 1	ND

[a] All strains are also mismatch repair deficient (*mutL*). Mutant frequencies were determined as described in Materials and Methods. Experiments 1 and 2 were performed at 30°C. Experiments 2 and 3 were performed at a later time and under slightly different conditions from experiments 1 and 4 and generally display higher frequencies (especially for the *lac* G · C→T · A allele). Nevertheless, within each series of experiments the results were highly consistent. See also the legend to Fig. 2. ND, not done.

[b] Experiment 1 was performed using the strains NR11939 (*dnaX*$^+$), NR11951 (*dnaX36*), NR12193 (*dnaQ49*), and NR12197 (*dnaX36 dnaQ49*), all carrying F′CC104 (G · C→T · A), and with the strains NR11940 (*dnaX*$^+$), NR11952 (*dnaX36*), NR12194 (*dnaQ49*), and NR12198 (*dnaX36 dnaQ49*), all carrying F′CC105 (A · T→T · A). Experiment 2 was performed using the *dnaX*$^+$ and *dnaX36* strains of experiment 1, along with NR17507 (*dnaQ930*) and NR17508 (*dnaX36 dnaQ930*) carrying F′CC104 (G · C→T · A) and with NR17509 (*dnaQ930*) and NR17510 (*dnaX36 dnaQ930*) carrying F′CC105 (A · T→T · A). Experiment 3 was performed using the strains NR11102 (*dnaX*$^+$), NR10452 (*dnaE915*), NR17502 (*dnaE925*), and NR11172 (*dnaE941*), all carrying F′CC102 (G · C→A · T); the strains NR11104 (*dnaX*$^+$), NR10454 (*dnaE915*), NR17504 (*dnaE925*), and NR11174 (*dnaE941*), all carrying F′CC104 (G · C→T · A); the strains NR11105 (*dnaX*$^+$), NR10455 (*dnaE915*), NR17505 (*dnaE925*), and NR11175 (*dnaE941*), all carrying F′CC105 (A · T→T · A); and the strains NR11106 (*dnaX*$^+$), NR10456 (*dnaE915*), NR17506 (*dnaE925*), and NR11176 (*dnaE941*), all carrying F′CC106 (A · T→G · C). Experiment 4 was performed using the strains NR11939 (*dnaX36*), NR12528 (*dnaX36 dnaE915*), NR12526 (*dnaX36 dnaE925*), and NR12527 (*dnaX36 dnaE941*), all carrying F′CC104 (G · C→T · A) and the strains NR11940 (*dnaX36*), NR12531 (*dnaX36 dnaE915*), NR12529 (*dnaX36 dnaE925*), and NR12530 (*dnaX36 dnaE941*), all carrying F′CC105 (A · T→T · A).

Our experiments have demonstrated a dependence of *dnaX36* on the DNA synthesis function of Pol I. Since Pol I generally performs gap-filling reactions, this may indicate an increased number of such gaps in *dnaX36* mutants, presumably produced by premature dissociations of Pol III from the primer terminus. On the other hand, we found that *dnaX36* is fully compatible with a *recA* deficiency. Since a *recA* defect is synthetically lethal with several other DNA processing defects, such as *dam*, *dut*, *rdgB*, and *polA1* (39, 40), this suggests that the number of DNA interruptions in *dnaX36* is likely limited.

We present below arguments that proper α-τ interaction is particularly important when Pol III is temporarily stalled. Stalling may occur when the polymerase encounters certain obstacles such as DNA lesions, secondary structures, or DNA mismatches. For mutagenesis, the behavior of Pol III at mismatches is likely most relevant. On the other hand, events initiated at terminal mismatches are likely only a subfraction of all events for which the τ-α interaction affects DNA replication. Thus, the fact that the *dnaX36-polA1* combination was not viable should not be construed to indicate that Pol I is involved in the mutagenesis process, although this cannot be excluded. Broadly, the mechanisms responsible for the *dnaX36* mutator effect will be considered within two possible models, which may not be mutually exclusive. In the first, HE may be structurally destabilized in the *dnaX36* mutant and dissociate more frequently from the primer terminus due to the impaired α-τ interaction, providing an opportunity for other DNA polymerases to contribute to the overall error rate. In the second model, the *dnaX* mutator effect results more specifically from loss of a "mismatch management" function that we have previously proposed for τ subunit (61).

***dnaX36* mutator and role of accessory DNA polymerases.** An important current finding is the significant role of the accessory polymerases Pol IV and Pol II in the *dnaX36* mutant, either by promoting errors (Pol IV) or by preventing them (Pol II). The intrinsic error-prone potential of Pol IV is well known (35, 41, 83, 88). However, access of Pol IV to the growing point must normally be limited, because the presence or absence of Pol IV does not measurably affect the mutation rate when *dinB* is present as a single chromosomal copy (42, 90). On the other hand, a mutator effect of Pol IV was previously noted for the *lacZ* G · C→T · A allele when *dinB* is present both on F′*prolac* and the chromosome (about three gene copies total) (42). This effect, noted in the form of an ~2.5-fold reduction in mutant frequency in the fully deficient *dinB* strain, is reproduced in the present study (Fig. 1). However, the effect is specific to the *lac* G · C→T · A transversion, and no such effect is observed for the episomal *lac* A · T→T · A transversion or the chromosomal *trpE9777* or *rpoB* (Rif^r) targets. In the *dnaX36* strain, the effect of Pol IV is significantly increased ~4-fold for the G · C→T · A marker) (Fig. 1A and 2A) and is now also observed for the A · T→T · A marker (2-fold) (Fig. 2B), as well as for the chromosomal *trpE* marker (9-fold) (Fig. 1B). Thus, the normally limited access of Pol IV is increased in the *dnaX36* strain.

Likewise, increased effects of Pol II are observed in the *dnaX36* background, a finding indicative of a larger role of this polymerase as well. For example, although deleting *polB* has no effect on the frequency of any of the four tested markers in the *dnaX*$^+$ background, substantial increases in mutant frequencies are observed due to the *polB* deletion in the *dnaX36* background: 4-, 2-, and 9-fold for *lac* G · C→T · A, *lac* A · T →T · A, and *trpE9777*, respectively (Fig. 2). Likewise, large effects were observed for the *polBex1* allele in the *dnaX36* background: 16-, 22-, and 5-fold for *lac* G · C→T · A, *lac* A · T→T · A, and *rpoB* (Rif^r), respectively (Fig. 2). Thus, in addition to playing a demonstrable role in maintaining the fidelity of replication in *dnaX*$^+$ strains (Fig. 2) (1), Pol II plays an increased role in replication fidelity in *dnaX36* strains. Part of this role is to prevent access of Pol IV, as can be readily deduced from the *dnaX36* Δ*polB* strain, whose enhanced mutator phenotype relative to the *dnaX36* strain is entirely dependent on the presence Pol IV, as well as from the *dnaX36 polBex1* strain, whose enhanced mutator phenotype is essentially *dinB* independent (Fig. 2), both results indicating that Pol

IV competes poorly with Pol II. Furthermore, the very strong *dnaX36 polBex1* mutator effect (with or without *dinB*) indicates that the role of Pol II extends beyond simply excluding Pol IV and that Pol II plays an important role in maintaining fidelity in *dnaX36* strains.

Increased or altered DNA polymerase usage in HE has also been reported for certain other *E. coli* strains bearing mutations in Pol III subunits. Increased usage of Pol IV was reported for the *dnaE1336* mutator mutant (76). Likewise, increased usage of Pol IV, as well as an important mutation prevention role for Pol II, were observed for the *dnaE486* and *dnaE511* mutator mutants (1, 41). The *dnaN159* mutant, carrying a defective β-clamp was also reported to display altered polymerase usage (78). The *dnaN159* mutant, like the *dnaX36* mutant, is also dependent on the Pol I polymerase activity for viability (78).

Initial error production by accessory polymerases? Of critical importance regarding the role of Pol III, and of other polymerases, in the *dnaX36* mutator effect is the origin of the errors that lead to the observed mutations. One simple possibility to account for the role of the accessory polymerases would be that during replication Pol III becomes occasionally disengaged from the replication point, and synthesis is then continued by accessory polymerases. This may lead to increased error production when synthesis is performed by an error-prone enzyme such as Pol IV or the exonuclease-deficient form of Pol II. Within such a model, the *dnaX36* mutant simply suffers from increased Pol III disengagement and, hence, increased error production. This mode of mutagenesis is certainly consistent with the decrease in mutagenesis in *dnaX36 dinB* strains and the increase in mutagenesis in *dnaX36 polBex1* strains. It is also consistent with the lack of effects of the *dnaQ* alleles on *dnaX36* since, within this model, the intrinsic Pol III error rate would be irrelevant. Nevertheless, even in the absence of Pol IV (*dnaX36 dinB* strains) there is still a significant residual *dnaX36* mutator effect, at least for the *lac* G · C→T · A mutations (~8-fold, Fig. 1A). A strong *dnaX36* mutator effect is also observed in an alternative experimental system where the *lac* operon is located chromosomally (13) and *dinB* is present in only one copy; under these conditions the *dnaX36* mutator effect is not *dinB* dependent (results to be published elsewhere). Since Pol V is likewise not required (Table 2 and Fig. 1), ongoing DNA synthesis by error-prone polymerases is not an obligatory feature of the *dnaX36* mutator effect. A second concern with this kind of model is that an accounting for observed mutator effects through DNA synthesis by error-prone polymerases requires extensive amounts of synthesis by such enzymes that may not seem realistic (1, 13, 42, 47). For example, assuming that the exonuclease-deficient form of Pol II is roughly 100-fold less accurate than Pol III HE (7), the 100-fold or more mutator effect of the *dnaX36 PolBex1* strains for the *lac* alleles (Fig. 2A,B) would require Pol II to synthesize essentially the entire chromosome. Lower but still significant numbers would apply to error-prone synthesis by Pol IV, which is essentially a low-processivity enzyme.

Initial error production by Pol III. In contrast to the random Pol III dissociations considered above, we consider terminal mispairs created by Pol III misinsertion errors as the more relevant events for the role of τ subunit and the accessory polymerases (1, 13, 42, 47). Terminal mispairs are known impediments for ongoing synthesis (33, 52, 58) and constitute potential stalling points. At such sites, polymerases face a choice among several competing paths, including direct extension from the mismatched primer, transfer of the mismatch to the exonuclease site, or dissociation from the mismatch (with concomitant opportunity for access by other polymerases). The proportioning among these pathways is a major determinant for the ultimate error rate, and τ, as the central, coordinating subunit of HE, is likely a determinant of this proportioning.

In Fig. 3, we diagram several of the possible polymerase pathways at a terminal mismatch created by Pol III. If HE were to continue synthesis from this mismatch a mutation would result (at least in the absence of mismatch repair, as is the case in our study) (line 2). Of course, the by far more likely outcome is removal of the mismatch by the HE proofreading activity (top line). As a third option (line 3), the polymerase may dissociate from the mismatch. This dissociation, permitting access of other DNA polymerases to the growing point, is likely to be rare in *dnaX*$^+$ strains but may occur with increased probability in a *dnaX36* mutant. Binding of the abandoned mismatch by either Pol II or Pol III (or HE) would lead to removal of the mismatch by their exonuclease activities, but binding and extension by Pol IV would be mutagenic. This scenario of enhanced dissociation at terminal mismatches due to increased instability of HE in the *dnaX36* mutant may provide one possible explanation for its mutator effect, while also accounting for the significant role of Pol IV. An alternative model is described below.

A mismatch-management function for τ? One problem with the straightforward dissociation model described above is that all *dnaX36*-induced mutations necessarily result from the action of Pol IV. In the present system this may be the case for the *trpE9777* frameshift mutations but not for the *lac* G · C→T · A transversions for which a significant *dinB*-independent component is noted (Fig. 1 and 2). For example, in the completely *dinB*-deficient lines, the *dnaX36* mutator effect is still 8-fold (11.8 versus 1.4) compared to the 13-fold effect (45.7 versus 3.4) in the fully *dinB*-proficient stain (Fig. 1A). Thus, both Pol IV-dependent and -independent components exist.

The alternative analysis follows from a previously described model in which τ was proposed to function as a sensor for HE molecules stalled "nonproductively" at certain terminal mismatches, and in which the observed mutator effect of *dnaX36* and related domain V *dnaX* mutants resulted from the lack of this τ sensor function (61). The τ sensor function may be considered analogous to the described function of τ in the lagging strand, where τ is capable of sensing that Pol III has reached the end of an Okazaki fragment (45, 46, 51), which triggers the release of Pol III from the primer terminus, enabling HE to resume synthesis at the next Okazaki fragment. Indirect evidence for stalling of HE at terminal mismatches has been obtained from in vitro fidelity studies on HE (59, 60). The stalled HE complexes were termed "nonproductive" since neither extension nor proofreading of the mismatch appeared to take place for long periods of time (59, 60). It was proposed that the resolution of such stalled complexes, if occurring in vivo, would require the sensing action of τ (61). Resolution might occur in the simplest way by enabling the conformational switch to the Pol III exonuclease mode. Alternatively, resolu-

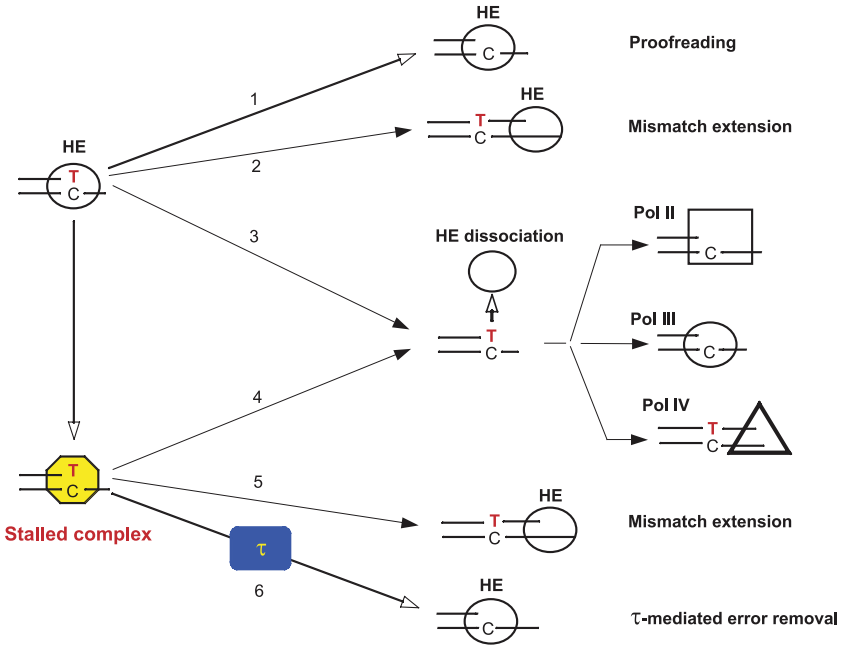

FIG. 3. Diagram depicting the various ways by which a Pol III misinsertion error may be processed in an error-free or error-prone manner. The scheme includes the competition between polymerases that may ensue upon dissociation of a Pol III HE from the mismatch (line 3 or 4), as well as the proposed τ-dependent mode of error removal (61) (line 6). Note that Pol III, while initially unable to proofread the error in the stalled state, may do so effectively upon rebinding, since the latter may occur preferentially using the exonuclease active site (30, 59, 60).

tion could involve dissociation of the nonproductive Pol III from the mismatch in favor of another proofreading polymerase, such as Pol II. Rebinding of the mismatch by Pol III itself would also lead to removal of the mismatch, since initial binding to a primer terminus favors the exonuclease site (30, 60). Evidence has been presented for a possible tool-belt organization of Pol III HE, in which a secondary site on the β-clamp accommodates an additional polymerase capable of switching places with Pol III (28). Furthermore, the recent discovery that HE might contain three τ subunits and three Pol III core modules (50) would allow for a convenient shuttling of the mismatch to the additional Pol III unit, followed by proofreading. It seems reasonable to assume that these various polymerase switches would be facilitated by the action of τ.

The various options emanating from a stalled Pol III complex are outlined in the bottom section of Fig. 3. Line 6 represents the normal τ-mediated mode of error removal as described above. However, in the *dnaX36* mutant, due to impairment of the τ-α interaction, this resolution option may not be operative. One consequence might then be that Pol III is ultimately forced to extend the mismatch, fixing the mutation (line 5). This mode of mismatch extension provides a sufficient explanation for the *dinB*-independent component of the *dnaX36* mutator effect observed in the present study (Fig. 2 and 3). Alternatively, Pol III may ultimately abandon the mismatch even without any assistance of τ (line 4). This (possibly less controlled) dissociation may provide an avenue for Pol IV to increase the error rate, when present in sufficiently large amounts, or for Pol II to remove the mismatch, which appears to be the predominant event.

This model for the *dnaX36* mutator effect is also supported by its unusual specificity, namely, the specific enhancement of transversions and −1 frameshifts at "non-run" sequences (61). Since transversion mismatches (purine · purine or pyrimidine · pyrimidine) are structurally the most distorting and the most difficult to extend (33, 52), these mismatches are the ones most likely to lead to a stalled HE complex. Hence, forced extension of these mismatches yields transversion base substitutions or, in a permissive sequence context, −1 frameshifts by realignment of the mismatched primer base on the next (correct) template base. The latter option, when permitted by the sequence context, is strongly favored by HE over direct extension (55, 59–61).

A hierarchy of polymerases. One significant observation from the present experiments is the exceptionally large role that Pol II appears to play in the maintenance of fidelity in the *dnaX36* mutant. Pol II competes effectively, of course, against Pol IV, but the overall fraction of errors removed by Pol II is particularly notable. For example, for the *lac* G · C→T · A reporter, the *polBex1* allele increases the mutant frequency of the *dnaX36* strain by 16-fold (*dinB*$^+$) and 42-fold (*dinB*) (Fig. 2A), which, within the context of the model of Fig. 3, may be interpreted to mean that in the *polB*$^+$ background 93 to 98% of available Pol III errors will be corrected by Pol II. Similar numbers apply to the *lac* A · T→T · A allele (Fig. 2B) and the Rif^r mutants (Fig. 2D). One (partial) exception is the case of the *trpE9777* frameshift mutations, which are affected significantly (10-fold) by the *polBex* allele only in the *dinB* background. It is possible that the slipped primer-template intermediate(s) that underlie the frameshift mutations are particularly amenable for processing by Pol IV. It should also be noted that in most cases the effect of a *polB* deletion is not as pronounced as that of *polBex1* (Fig. 2A, B, and D). In the Δ*polB* background *dnaX36*-mediated mutagenesis is enhanced

(in a Pol IV-dependent manner), but this increase is not as large as in the *polBex1* background. One might conclude that, in the absence of Pol II, Pol III (or HE itself) is also capable of competing effectively with Pol IV. Thus, a hierarchy of DNA polymerases in addressing abandoned terminal mispairs may be Pol II > Pol III > Pol IV.

Role of proofreading. Of interest regarding the *dnaX36* mutator effect is the apparently lack of a role of the Pol III proofreading. Within the context of a simple dissociation mechanism (Fig. 3, line 3) one might argue that lack of Pol III proofreading would increase the number of mismatches available for dissociation but would not fundamentally alter the chance of Pol IV to access the mismatch once dissociation occurred; therefore, the lack of proofreading would not alter the magnitude of the mutator effect (fold increase). As our experiments with the *dnaQ* mutations indicate, this is not what is observed; instead, the *dnaX* mutator effect is either no longer apparent or at best additive with the *dnaQ* mutator effect (Table 3). Thus, this result might be used to argue against the simple dissociation model. Alternatively, one may argue that in proofreading-impaired backgrounds the dissociation-mediated mutator pathway (Pol IV effect) can become obscured. More precisely, in a limit case of "no proofreading" the flow of mutations through the direct extension pathway (line 2) may be high relative to that through the dissociation pathway (line 3), whereas the latter is ultimately limited by the ability of Pol IV to compete with Pol II and Pol III. An answer to this question would require detailed insight into the precise rate constants of each of the reactions in Fig. 3.

The proofreading observations are more simply explained based on the stalled complex model (lines 4 to 6). The stalled complex is characterized a priori as a nonextending and nonproofreading entity (61). Thus, the flow of mutations through pathways 4 and 5 might be largely independent of the Pol III proofreading. Second, at any time only a fraction of terminal mismatches is expected to result in a stalled complex. Therefore, any proofreading occurring in lines 4 to 6 would run in parallel to the conventional main proofreading pathway (line 1). Therefore, simple additivity of effects may sometimes be expected (Table 3). Finally, in the case of the *dnaE* "antimutators" (Table 3), it is likely that some of these *dnaE* alleles, in addition to increasing proofreading through the conventional pathway (an antimutator effect), are also, due to their catalytic impairment, more likely to end up in a stalled state. They may thus be expected to enhance in some cases the *dnaX36* mutator effect.

ACKNOWLEDGMENTS

We thank Stephanie Nick McElhinny and Jeffrey Stumpf of the National Institute of Environmental Health Sciences for their careful reading of the manuscript.

This study was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences, and by grant 2 PO4A 061 30 (to I.J.F. and P.J.) from the Polish Ministry of Science and Higher Education.

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