JOURNAL OF BACTERIOLOGY, July 2003, p. 3972–3977 Vol. 185, No. 13
0021-9193/03/$08.00+0 DOI: 10.1128/JB.185.13.3972–3977.2003

The *dinB* Operon and Spontaneous Mutation in *Escherichia coli*

Gregory J. McKenzie,[1]† Daniel B. Magner,[2] Peter L. Lee,[1]‡ and Susan M. Rosenberg[1,2,3,4]*

Department of Molecular and Human Genetics,[1] Interdepartmental Graduate Program in Cell and Molecular Biology,[2] and Departments of Biochemistry and Molecular Biology[3] and Molecular Virology and Microbiology,[4] Baylor College of Medicine, Houston, Texas 77030-3411

Received 30 September 2002/Accepted 9 April 2003

Apparently conflicting data regarding the role of SOS-inducible, error-prone DNA polymerase IV (DinB) in spontaneous mutation are resolved by the finding that mutation is reduced by a polar allele with which *dinB* and neighboring *yafN* are deleted but not by two nonpolar *dinB* alleles. We demonstrate the existence of a *dinB* operon that contains four genes, *dinB-yafN-yafO-yafP*. The results imply a role for *yafN*, *yafO*, and/or *yafP* in spontaneous mutation.

Understanding of mutation mechanisms has been altered dramatically by the discovery of a superfamily of error-prone DNA polymerases called the polymerase Y (pol Y) superfamily (23), which is evolutionarily conserved throughout all modern domains of life (reviewed in references 9, 21 and 27). Many of these polymerases can insert bases across from DNA lesions, but this translesion synthesis activity is correlated with diminished fidelity on undamaged DNA templates. *Escherichia coli* contains two DNA polymerases from this family, DNA pol IV and pol V, both of which are upregulated during the SOS DNA damage response. DNA pol IV has a propensity for creating −1 frameshifts at mononucleotide repeats and also G-to-T transversions (12, 28–30). Recently, we found that DNA pol IV (encoded by the *dinB* gene) is required for "stationary-phase" or "adaptive" mutation in a *lac* frameshift reversion assay (19). Stationary-phase mutation in the Lac system occurs in slowly growing or nongrowing Lac$^-$ cells during starvation on lactose medium, and the mutation mechanism differs from spontaneous mutation during exponential growth in its requirements for different proteins (those required for double-strand break repair) and in producing different kinds of mutations, i.e., mostly −1 frameshifts (reviewed in references 8 and 24). We found that DinB (pol IV) is not required for spontaneous reversion of the same *lac* allele during exponential growth (growth-dependent mutation) (19). We also detected no effect of a *dinB* loss-of-function mutation in several other growth-dependent mutation assays (19). This suggests that pol IV does not contribute significantly to spontaneous mutation in rapidly growing cells. However, these observations appear to conflict with the results of others in which a pol IV-deficient mutant displayed a two- to threefold decrease in growth-dependent reversion of the same *lac* allele that we studied (26). In this study, we examined two possible reasons for this apparent discrepancy and found evidence that the source of the differences in results is the different *dinB* alleles used in the two studies. We report that *dinB* is part of a multigene operon and present evidence that one or more of the genes downstream of *dinB* may affect growth-dependent mutation.

dinB is the first of four open reading frames (ORFs) transcribed in the same orientation (see Fig. 1) (1). The functions of the downstream genes, *yafN*, *yafO*, and *yafP*, are unknown. Transcription of all four ORFs is induced by DNA-damaging agents as part of a LexA-regulated SOS response (5). Although *dinB* has a LexA-repressed promoter (12), the last three genes have no obvious promoters (5), suggesting that all four may be transcribed as part of an operon from the *dinB* promoter. There are two major differences between the reported experiments testing the role of DinB (pol IV) in growth-dependent mutation. First, we used a nonpolar *dinB* substitution mutation that affects the polymerase active site (2, 17, 19, 29, 32), whereas the apparently conflicting results were obtained with a *dinB* deletion-insertion allele in which part of *yafN*, as well as *dinB*, is deleted (12, 26). In addition to truncating *yafN*, the deletion-insertion could also have polar effects on downstream genes *yafO* and *yafP*. Second, we reduced contamination of the growth-dependent Lac$^+$ mutant colony counts (mutants formed prior to plating on lactose medium) with stationary-phase mutant colonies (formed continuously after exposure to lactose medium) by using an internally controlled method to determine the time to count colonies (earlier than when stationary-phase mutant colonies are prevalent), as described in detail previously (11). In the other study, mutant colonies were scored at an arbitrary, and later, 43-h time point (26), which could allow inclusion of Lac$^+$ stationary-phase mutant colonies. Because the latter form *dinB*$^+$ dependently (19), this could cause an appearance of *dinB*-dependent, growth-dependent mutation when, in reality, only the stationary-phase mutants contributing to the counts were *dinB* dependent. We hypothesized (19) that the apparently conflicting results arose from allele differences, specifically, from effects of the downstream genes on growth-dependent mutation in the case of the *dinB* deletion-insertion allele, or from the difference in the time of scoring of Lac$^+$ mutant colonies for determination of the mutation rates.

We tested these hypotheses by determining growth-dependent rates of mutation to Lac$^+$ in parallel in strains carrying either the nonpolar *dinB10* allele we used previously or the

* Corresponding author. Mailing address: Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Rm. S809A, Mail Stop BCM225, Houston, TX 77030-3411. Phone: (713) 798-6924. Fax: (713) 798-8967. E-mail: smr@bcm.tmc.edu.

† Present address: Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

‡ Present address: Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77005.

TABLE 1. *E. coli* K-12 strains used in this study

Strain(s)	Description	Reference or source
FC29	Δ(*lac-proAB*)$_{XIII}$ *thi ara*[F′ Δ(*lacI-lacZ*)]	3
FC36	Δ(*lac-proAB*)$_{XIII}$ *thi ara* Rif^r	3
FC40	Δ(*lac-proAB*)$_{XIII}$ *thi ara* Rif^r[F′ *proAB*$^+$ *lacI33*Ω*lacZ*]	3
	AB1157 Δ(*dinB-yafN*)::*kan*	12; via F. Taddei
MG1655	Wild type	1
P90C	Δ(*lac-proAB*)$_{XIII}$	4
SMR3804, SMR3805, SMR3807, SMR3855–SMR3859, SMR3861, SMR3863	10 independent Lac$^+$ revertants of FC40; *dinB*$^+$	20[a]
SMR4511	MG1655 Δ*lacX74 proAB-81*::Tn*10*	This work[b]
SMR4562	Genotype same as that of FC40, independent construction	18
SMR5816	P90C[F′ *proAB-81*::Tn*10 lacI33*Ω*lacZ*]	This work[c]
SMR5830	SMR4562 *dinB10*[F′ *dinB10 proAB*$^+$ *lacI33*Ω*lacZ*][d]	19
SMR5865–SMR5874	10 independent Lac$^+$ revertants of SMR5830	19[a]
SMR5889	SMR4562 Δ*dinB51*[F′ Δ*dinB51 proAB*$^+$ *lacI33*Ω*lacZ*][d]	This work
SMR6111	SMR4562 Δ(*dinB-yafN*)::*kan*[F′ Δ(*dinB-yafN*)::*kan proAB*$^+$ *lacI33*Ω*lacZ*][d]	This work
SMR6356–SMR6365	10 independent Lac$^+$ revertants of SMR6111	This work[a]
SMR7481–SMR7490	10 independent Lac$^+$ revertants of SMR5889	This work[a]
SMR7491	SMR4562 Δ(*yafN-yafP*)*602*[F′ Δ(*yafN-yafP*)*602 proAB*$^+$ *lacI33*Ω*lacZ*][d]	This work
SMR7492–SMR7501	10 independent Lac$^+$ revertants of SMR7491	This work[b]

[a] Spontaneous Lac$^+$ revertants were isolated, one each from 10 independent cultures.
[b] Construction made by P1 transductions with the *proAB-81*::Tn*10* and Δ*lacX74* alleles derived from strains NK5525 (kindly provided by N. Kleckner via the *E. coli* Genetic Stock Center, Yale University) and CH1504 (16), respectively.
[c] Construction made by P1 transduction of *proAB-81*::Tn*10* derived from strain NK5525 (kindly provided by N. Kleckner) into the F′ derived from FC40 and mating into P90C.
[d] The *dinB* and *yafN-yafP* genes are present on both the chromosome and F′, and so homozygous mutant strains were constructed.

Δ(*dinB-yafN*)::*kan* allele (referred to in reference 12 as Δ*dinB*::*kan*) used by others (26) (Table 1). The strains in which the Lac$^+$ mutation rates were assayed carry two copies of the *dinB* and *yaf* genes: one in the chromosome and one in the F′ plasmid carrying the *lac* frameshift allele (19). To move Δ(*dinB-yafN*)::*kan* into both sites, PCR products of the Δ(*dinB-yafN*)::*kan* allele were made with primers BP89 5′-GCATTTCTCAA ACCCTGAAATC-3′ and BP90 5′-AATACCCGCATCCTTA TTCCTT-3′ on template strain AB1157 Δ(*dinB-yafN*)::*kan* and were moved via homologous linear replacement (6) into SMR5833, an FC40 derivative carrying recombination-promoting plasmid pKD46 (6). Isolates were screened for positions of insert on the F′ or chromosome as previously (19). One isolate carrying Δ(*dinB-yafN*)::*kan* on the chromosome was used as a donor for P1 transduction into FC36 to create SMR6095 [genotype FC36 Δ(*dinB-yafN*)::*kan*]. Another isolate, carrying Δ(*dinB-yafN*)::*kan* on the F′ plasmid, was mated into SMR4511 to create SMR6094 {genotype MG1655 *proAB-81*::Tn*10* Δ*lacX74* [F′ Δ(*dinB-yafN*)::*kan proAB*$^+$ *lacI33*Ω*lacZ*]}. These resulting strains were mated to produce homozygous Δ(*dinB-yafN*)::*kan* strain SMR6111 (Table 1). The presence of Δ(*dinB-yafN*)::*kan* on both chromosome and F′ was verified by PCR and mating as described previously (19).

The mutation rates were determined by using fluctuation tests as described previously (19), and mutation rates were calculated by using the Lac$^+$ mutant counts taken at two times: First, in the controlled-time method (e.g., see references 11 and 19), known numbers of cells of control strains isogenic to each strain tested [*dinB*$^+$, *dinB10*, or Δ(*dinB-yafN*)::*kan*, etc.], but carrying a Lac$^+$ reversion mutation, are plated on lactose selective medium in parallel with the fluctuation tests. Colony counts in the fluctuation tests are taken continuously, and the counts corresponding to the time at which 50% of the control colonies have arisen are used for calculation of mutation rates. This method serves the dual functions of reducing contamination with stationary-phase Lac$^+$ mutant colonies (which arise during selection on lactose plates) and also avoiding artificially low observations of mutant colonies in slowly growing strains, which take longer to form colonies under the selective conditions (on lactose plate medium) (11). Second, in the fixed-time method (26), colony counts are taken at 43 h.

For the controlled-time method, 40-culture fluctuation tests were performed with 5-ml cultures that were grown to saturation in M9 medium with 0.1% glycerol, washed three times in M9 wash, and concentrated 10-fold. Between 300 and 350 μl of the cell suspensions was plated with about 2×10^9 FC29 scavenger cells (Table 1), which consume any contaminating nonlactose carbon sources (3), on solid M9 medium with 0.1% lactose, and Lac$^+$ mutant colonies were scored while scoring Lac$^+$ speed-of-growth controls. Mutation rates were calculated by the method of the median (14) using colony counts from the time at which 50% of the Lac$^+$ control colonies were visible and then multiplying by 2 to obtain the numbers of colonies at the time at which 100% of the Lac$^+$ control colonies were visible. The median number of colonies observed ranged between 1 and 15.5 (with a mode of 2) among all of the experiments.

Using the controlled-time method, we found (Table 2 and previously [19]) that the Lac$^+$ mutation rates for the *dinB*$^+$ and *dinB10* strains are indistinguishable from each other at the time when 50% of the control Lac$^+$ colonies have become visible (as shown by overlapping standard errors and failure to reject the null hypothesis of equal rates at $P = 0.97$ by single-factor analysis of variance [ANOVA] and the Tukey test [31]). However, the mutation rate of the Δ(*dinB-yafN*)::*kan* strain is two- to threefold lower than that of the *dinB*$^+$ control (a statistically significant difference at $P = 0.025$ by single-factor ANOVA and the Tukey test). Thus, it appears that the difference between the two sets of previous results (19, 26) can be explained by the different *dinB* alleles used. The Δ(*dinB-*

TABLE 2. Lac$^+$ mutation rate determinations from controlled-time colony counts

dinB allele[a] and expt	Mutation rate (no. of mutations/cell/generation)	Mean mutation rate (±SEM) (no. of mutations/cell/generation)	*P* value for difference from *dinB*$^+$[b]
dinB$^+$			
1	11×10^{-10}	$9.4 (\pm 1) \times 10^{-10}$	
2	8.2×10^{-10}		
3	12×10^{-10}		
4	8.9×10^{-10}		
5	16×10^{-10}		
6	11×10^{-10}		
7	12×10^{-10}		
8	5.8×10^{-10}		
9	5.7×10^{-10}		
10	5.2×10^{-10}		
11	7.6×10^{-10}		
dinB10			
1	6.6×10^{-10}	$8.2 (\pm 1) \times 10^{-10}$	0.97
2	7.9×10^{-10}		
3	10×10^{-10}		
Δ(*dinB-yafN*)::*kan*			
1	3.5×10^{-10}	$5.6 (\pm 0.3) \times 10^{-10}$	0.025
2	5.8×10^{-10}		
3	5.8×10^{-10}		
4	6.8×10^{-10}		
5	6.6×10^{-10}		
6	6.0×10^{-10}		
7	5.5×10^{-10}		
8	4.3×10^{-10}		
9	4.6×10^{-10}		
10	5.4×10^{-10}		
11	7.2×10^{-10}		
Δ*dinB51*			
4	9.0×10^{-10}	$10 (\pm 1) \times 10^{-10}$	0.98
5	8.4×10^{-10}		
6	12×10^{-10}		
7	12×10^{-10}		
Δ(*yafN-yafP*)*602*			
4	6.2×10^{-10}	$8.7 (\pm 1) \times 10^{-10}$	0.96
5	6.8×10^{-10}		
6	3.4×10^{-10}		
7	17×10^{-10}		
8	11×10^{-10}		
9	5.6×10^{-10}		
10	9.3×10^{-10}		
11	10×10^{-10}		

[a] The strains assayed were SMR4562, SMR5830, SMR6111, SMR5889, and SMR7491, and the Lac$^+$ timing control strains for them, plated in parallel, were SMR3804, SMR3805, SMR3807, SMR3855 to SMR3859, SMR3861, SMR3863, SMR5865 to SMR5874, SMR6356 to SMR6365, SMR7481 to SMR7490, and SMR7492 to SMR7501 (Table 1).

[b] *P* values were calculated by using single-factor ANOVA and the Tukey test (31) with SigmaStat 2.03 for Windows by SPSS, Inc., with the mutation rates at three significant figures.

yafN)::*kan* allele decreases the level of growth-dependent mutation significantly.

With the fixed-time colony counts, in which the additional Lac$^+$ mutants that appear by 43 h (including stationary-phase mutants) were included in the mutation rate calculations, the calculated mutation rates were slightly higher (Table 3), as expected. Again, we saw that the rates for the *dinB*$^+$ and Δ(*dinB-yafN*)::*kan* strains were different, but with marginal significance (at only $P > 0.1$ by single-factor ANOVA and the Tukey test), and that *dinB10* was not significantly different from *din*$^+$ ($P > 0.5$). Because the Δ(*dinB-yafN*)::*kan* allele (but not the nonpolar *dinB10* allele) decreased mutation significantly even when adaptive mutants were more stringently eliminated from the colony counts (Table 2), we conclude that genuine growth-dependent mutation was decreased by Δ(*dinB-yafN*)::*kan* but not by *dinB10*.

There are at least two possible reasons for the different phenotypes of the different *dinB* alleles in growth-dependent Lac$^+$ mutation. First, the pol IV protein may play some structural role that is not abolished by the *dinB10* substitution mutation, and thus, the null deletion mutant may have a stronger phenotype. That is, *dinB10* may be a partial-function allele. Alternatively, loss of function of one or more of the *yaf* genes may be responsible for the decrease in mutation in Δ(*dinB-yafN*)::*kan* cells. To address the possibility that *dinB10* is a partial-function allele, we constructed a nonpolar *dinB* null deletion allele, an in-frame deletion of *dinB* that does not affect the *yafN-yafP* genes, Δ*dinB51* (Table 1). First, an in-frame *dinB* deletion-*kan* insertion, Δ*dinB50*::*kan*, was made by PCR using primers dinBwL 5′-GAAATCACTGTATACTTTACCAGTGTTGAGAGGTGAGCAATGCGTAAAATCATTCCGGGGATCCGTCGACC-3′ and dinBwR 5′-TAATAATGCACACCAGAATATACATAATAGTATACATCATAATCCCAGCACTGTAGGCTGGAGCTGCTTC-3′ on template plasmid pKD13 (6) (underlined bases are complementary to the plasmid) and moved via homologous linear replacement (6) into SMR5833 (described above). Isolates were screened for insert positions (19) on the F′ plasmid (SMR5876) or on the chromosome (SMR5875). SMR5878 (genotype FC36 Δ*dinB50*::*kan*) was made by transduction of FC36 with SMR5875 as the donor. Next, the *kan* cassette was removed from SMR5878 as described in reference 6 to create SMR5883 (genotype FC36 Δ*dinB51*). In this allele, codons 5 to 347 of the 351-codon *dinB* gene are replaced with *FRT* (the Flp recombinase

TABLE 3. Lac$^+$ mutation rate determinations from fixed-time (43-h) colony counts

dinB allele[a] and expt	No. of cultures	Mutation rate (no. of mutations/cell/generation)[b]	Mean mutation rate (±SEM) (no. of mutations/cell/generation)
dinB$^+$			
1	40	3.9×10^{-9}	$4.6 (\pm 1) \times 10^{-9}$
2	30	3.3×10^{-9}	
3	40	6.7×10^{-9}	
dinB10			
1	40	1.6×10^{-9}	$2.4 (\pm 0.9) \times 10^{-9}$
2	28	1.3×10^{-9}	
3	40	4.2×10^{-9}	
Δ(*dinB-yafN*)::*kan*			
1	40	1.2×10^{-9}	$1.8 (\pm 0.7) \times 10^{-9}$
2	40	1.1×10^{-9}	
3	40	3.1×10^{-9}	

[a] The strains assayed were SMR4562, SMR5830, and SMR6111 (Table 1).

[b] Determinations of mutation rates were performed by fluctuation tests as described in the text for Table 2, except that the colony counts from which mutation rates were calculated were all taken at a fixed time of 43 h of incubation after plating. Mutation rates were calculated by the method of the median (14).

target site). SMR5876, carrying Δ*dinB50*::*kan* on the F′ plasmid, was mated into SMR4511 to create SMR5877 (genotype MG1655 *proAB-81*::Tn*10* Δ*lacX74*[F′ Δ*dinB50*::*kan proAB*$^+$ *lacI33*Ω*lacZ*]), from which the *kan* cassette was removed to create SMR5882 (genotype MG1655 *proAB-81*::Tn*10* Δ*lacX74* [F′ Δ*dinB51 proAB*$^+$ *lacI33*Ω*lacZ*]). SMR5882 and SMR5883 were mated to produce homozygous Δ*dinB51* mutant strain SMR5889 (Table 1). The presence of Δ*dinB51* on both the chromosome and the F′ plasmid was verified as described previously (19).

With the nonpolar deletion Δ*dinB51*, we found that growth-dependent Lac$^+$ reversion was no different in this strain than in the *dinB*$^+$ strain (Table 2, $P = 0.98$) and so conclude that no (nonredundant) function of the DinB protein is needed for growth-dependent mutation. This supports the alternative possibility that one or more of the *yafN*, *yafO*, or *yafP* gene products contribute to growth-dependent mutation and that the depression of mutation in cells carrying the Δ(*dinB-yafN*)::*kan* deletion-insertion allele results either from the loss of *yafN* by deletion or from polar effects on *yafO* and/or *yafP*. We tested this hypothesis further by determining whether the *yaf* genes are cotranscribed with *dinB*.

To determine whether *dinB-yafN-yafO-yafP* is a transcriptional unit, we used reverse transcriptase PCR (RT-PCR) to detect transcripts. Because all four ORFs are SOS inducible (5), we induced expression of these genes by treating log-phase cultures of *E. coli* MG1655 with 20 μg of bleomycin per ml to induce the SOS response (13). Treatment for 20 min at 37°C resulted in a 30-fold decrease in viability. We isolated RNA from the induced cultures with the Qiagen RNeasy kit and used RT-PCR (25) to detect transcripts containing *dinB*, *yafN*, *yafO*, and *yafP*. Each result was obtained from three independent RNA preparations, and representative data are shown in Fig. 1. For each set of primers (A to E), amplification of chromosomal DNA showed the expected-size product (lane 1), amplification of reverse-transcribed RNA demonstrated the presence of the transcript (lane 2), and a control amplification of RNA without RT showed that the product in lane 2 was not from contaminating DNA (lane 3). Correct identities of the PCR products were verified by restriction mapping (data not shown). Primers within each downstream ORF, *yafN*, *yafO*, or *yafP*, yielded a product with a 5′ primer within the *dinB* gene (Fig. 1), indicating that a single transcript includes the entire *dinB*, *yafN*, and *yafO* genes and at least part of the *yafP* gene (Fig. 1, product E). We conclude that these genes constitute an operon. We were able to amplify a product with primers in *dinB* and the beginning of the *yafP* ORF, but we were unable to amplify with a primer to the 3′ end of *yafP*. However, as shown in lanes D of Fig. 1, we were able to detect a transcript including the 5′ end of *yafO* and the 3′ end of *yafP*, indicating that there is no significant transcription terminator downstream of the *yafP* 5′ end, which we detected in transcripts containing *dinB*. This, coupled with the lack of potential promoter sequences in the *yafN*-to-*yafP* region, implies that *yafP* is also part of the *dinB-yafN-yafO* operon. The results support the possibility that the Δ(*dinB-yafN*)::*kan* allele may confer loss of function not only of the deleted *dinB* and *yafN* genes but also of *yafO* and *yafP* via polar effects.

A *yaf* gene(s) may be required for growth-dependent Lac$^+$ reversion either on its own or only when *dinB* is also absent, as

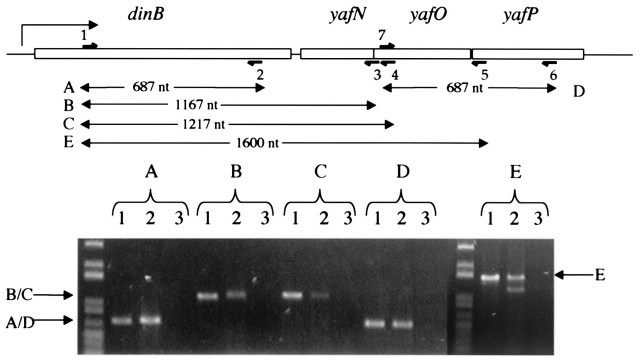

FIG. 1. Messages on the same transcript. RT-PCR was performed on RNA extracted from SOS-induced *E. coli* strain MG1655 (Table 1) with primers (half arrows) complementary to the locations diagrammed at the top. The reactions were run on a 0.75% agarose gel in 1× TAE buffer (25) and stained with ethidium bromide, and a representative photograph is shown. In each set of reactions (A to E) for a given primer pair shown above (A to E), lane 1 shows a PCR performed on genomic DNA to give a size standard. Lane 2 contains the products of an RT-PCR performed on extracted RNA. Lane 3 contains products of a PCR performed on the extracted RNA leaving out the RT to ensure that the products seen are due to amplification of RNA, not contaminating DNA. Primers are complementary to sequences in the locations indicated. Primer 1 is BP381 (5′-ACGCCTA CAAAGAAGCCTCA-3′). Primer 2 is BP382 (5′-GATCAGCTTTAT TCAGCAGC-3′). Primer 3 is BP90 (5′-AATACCCGCATCCTTATT CCTT-3′). Primer 4 is BP375 (5′-ACGCATCAAGTTCCTCTGCT-3′). Primer 5 is BP 377 (5′-TCGCCAGGCTGATAGTTTCT-3′). Primer 6 is BP380 (5′-TTATGCTTGCGTCCACCGTA-3′). Primer 7 is BP378 (5′-AGCAGAGGAACTTGATGCGT-3′). nt, nucleotides.

is the case with the Δ(*dinB-yafN*)::*kan* allele. We constructed strains with only *yafN-yafP* deleted (which are *dinB*$^+$) to test these possibilities. PCR products that create the allele Δ(*yafN-yafP*)*601*::*kan* were made with primers yafNwL 5′-TGTATA TTCTGGTGTGCATTATTATGAGGGTATCACTGTATG CATCGAATTATTCCGGGGATCCGTCGACC-3′ and yafPwR 5′-ATACCAGGCGGGCGTTATTTTCATTGCAA GCTGGATTTAATGTTGCGGTTTTGTAGGCTGGAGCT GCTTC-3′ to amplify plasmid pKD13 (6) and moved via homologous linear replacement (6) into SMR5832 [genotype FC36(pKD46)] and SMR6233 [genotype MG1655(pKD46)] to create SMR6352 [genotype FC36 Δ(*yafN-yafP*)*601*::*kan*] and SMR6353 [genotype MG1655 Δ(*yafN-yafP*)*601*::*kan*], respectively. The *kan* gene was removed from SMR6352 (6) to create SMR7479 [genotype FC36 Δ(*yafN-yafP*)*602*]. This deletion replaces codon 5 of the *yafN* gene through codon 146 of the 150-codon *yafP* gene with a single *FRT* site at the deletion junction. Δ(*yafN-yafP*)*601*::*kan* was transduced into SMR5816 from SMR6353, creating SMR7477 {genotype P90C[F′ Δ(*yafN-yafP*)*601*::*kan proAB*$^+$ *lacI33*Ω*lacZ*]}. The *kan* gene was removed (6), creating SMR7478 {genotype P90C [F′ Δ(*yafN-yafP*)*602 proAB*$^+$ *lacI33*Ω*lacZ*]}. SMR7478 and SMR7479 were mated to produce homozygous Δ(*yafN-yafP*) *602* strain SMR7491 (Table 1).

In the strain with *yafN-yafP* deleted, mutation rates were too variable for us to distinguish whether or not mutation was decreased. In eight mutation rate determinations (Table 2), the strain carrying Δ(*yafN-yafP*)*602* displayed variable mutation rates that are neither significantly different from those of

the isogenic *din*$^+$ *yaf*$^+$ strain ($P = 0.96$) nor significantly different from those of the Δ(*dinB-yafN*)::*kan* isogenic strain ($P = 0.18$). Thus, it remains possible either that a *yaf* gene(s) affects mutation on its own or that a *yaf* gene(s) affects mutation only when *dinB* is also deleted, as is the case for the Δ(*dinB-yafN*)::*kan* strain (Table 2). This possibility, i.e., redundant roles of *dinB* and a *yaf* gene(s) in growth-dependent mutation, would contrast with the nonredundant role of *dinB* in adaptive Lac$^+$ reversion (19).

In summary, we have shown that a polar *dinB* allele causes decreased spontaneous mutation whereas a nonpolar substitution and a nonpolar deletion allele of *dinB* do not and that *dinB* is part of an operon of four genes, *dinB-yafN-yafO-yafP*. All of these genes were shown previously to be LexA controlled and SOS inducible (5). It is likely that the Δ(*dinB-yafN*)::*kan* allele disrupts expression of all four genes, and thus, phenotypes ascribed to *dinB* that were obtained with this allele may result partly or wholly from loss of *yafN*, *yafO*, and/or *yafP* function. This could be important regarding phenotypes previously ascribed to *dinB* reported for the Δ(*dinB-yafN*)::*kan* allele. These include phenotypes in spontaneous mutation (26), translesion synthesis (15, 22), and adaptive mutation (7), although the last has also been demonstrated with the nonpolar *dinB10* allele and so its attribution to *dinB* is not in doubt (19). Here we have shown that one such phenotype, a reported effect of *dinB* on growth-dependent mutation in a Lac reversion assay (26), is likely to result from loss of function of one or more of the *yaf* genes.

What are the functions of the other genes in the *dinB* operon? *yafO* and *yafP* have no informative homologs; however, YafN is highly homologous to the protein type (rather than the RNA type) of antitoxins of bacterial toxin-antitoxin systems (10). These toxin-antitoxin systems increase the stability of plasmids within populations by selectively killing cells that lose the plasmid. The role of chromosomal toxin-antitoxin systems is not clear. In these systems, the antitoxin binds the toxin, which would otherwise cause cell death, and inhibits its function (10). The genes encoding toxin-antitoxin pairs are often found in proximity to each other, but neither YafO nor YafP has homology to a known toxin. Thus, YafN may be an SOS-inducible antitoxin that regulates the function of an as yet unidentified toxin. If found, a phenotype of *yafN* in mutation may result from lack of antitoxin and therefore killing of rare SOS-induced cells that would otherwise experience mutation. Finally, the results suggest that the *yafN*, *yafO*, and/or *yafP* gene products contribute to spontaneous mutation in growing cells. The SOS-regulated nature of their expression makes the mutation phenotype intriguing.

The first two authors contributed equally to this work.

We thank Charles Hill and the *E. coli* Genetic Stock Center for bacterial strains and P. J. Hastings, Megan Hersh, Mary-Jane Lombardo, Joseph Petrosino, Bernard Strauss, and Roger Woodgate for helpful comments on the manuscript.

This work was supported by Department of Defense Breast Cancer Research Program Predoctoral Fellowship DAMD17-99-1-9072 (G.J.M.), an American Society for Microbiology Undergraduate Summer Research Fellowship (P.L.L.), and National Institutes of Health grants T32-GM08231 (D.B.M.) and R01-GM53158.

REFERENCES

1. **Blattner, F. R., G. Plunkett III, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glassner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao.** 1997. The complete genome sequence of *Escherichia coli* K-12. Science **277:**1453–1474.
2. **Boudsocq, F., H. Ling, W. Yang, and R. Woodgate.** 2002. Structure-based interpretation of missense mutations in Y-family DNA polymerases and their implications for polymerase function and lesion bypass. DNA Repair **1:**343–358.
3. **Cairns, J., and P. L. Foster.** 1991. Adaptive reversion of a frameshift mutation in *Escherichia coli*. Genetics **128:**695–701.
4. **Coulondre, C., and J. H. Miller.** 1977. Genetic studies of the *lac* repressor. IV. Mutagenic specificity in the *lacI* gene of *Escherichia coli*. J. Mol. Biol. **117:**577–606.
5. **Courcelle, J., A. Khodursky, B. Peter, P. O. Brown, and P. C. Hanawalt.** 2001. Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. Genetics **158:**41–64.
6. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step gene inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
7. **Foster, P. L.** 2000. Adaptive mutation in *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **65:**21–29.
8. **Foster, P. L.** 1999. Mechanisms of stationary phase mutation: a decade of adaptive mutation. Annu. Rev. Genet. **33:**57–88.
9. **Friedberg, E. C., R. Wagner, and M. Radman.** 2002. Specialized DNA polymerases, cellular survival, and the genesis of mutations. Science **296:**1627–1630.
10. **Grønlund, H., and K. Gerdes.** 1999. Toxin-antitoxin systems homologous with *relBE* of *Escherichia coli* plasmid P307 are ubiquitous in prokaryotes. J. Mol. Biol. **285:**1401–1415.
11. **Harris, R. S., G. Feng, K. J. Ross, R. Sidhu, C. Thulin, S. Longerich, S. K. Szigety, P. J. Hastings, M. E. Winkler, and S. M. Rosenberg.** 1999. Mismatch repair is diminished during stationary-phase mutation. Mutat. Res. **437:**51–60.
12. **Kim, S.-R., G. Maenhaut-Michel, M. Yamada, Y. Yamamoto, K. Matsui, T. Sofuni, T. Nohmi, and H. Ohmori.** 1997. Multiple pathways for SOS-induced mutagenesis in *Escherichia coli*: an SOS gene product (DinB/P) enhances frameshift mutations in the absence of any exogenous agents that damage DNA. Proc. Natl. Acad. Sci. USA **94:**13792–13797.
13. **Köppen, A., S. Krobitsch, B. Thoms, and W. Wackernagel.** 1995. Interaction with recombination hotspot χ converts the RecBCD enzyme of *Escherichia coli* into a χ-independent recombinase by inactivation of the RecD subunit. Proc. Natl. Acad. Sci. USA **92:**6249–6253.
14. **Lea, D. E., and C. A. Coulson.** 1949. The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:**264–285.
15. **Lenne-Samuel, N., R. Janel-Bintz, A. Kolbanovskiy, N. E. Geacintov, and R. P. P. Fuchs.** 2000. The processing of a benzo(a)pyrene adduct into a frameshift or a base substitution mutation requires a different set of genes in *Escherichia coli*. Mol. Microbiol. **38:**299–307.
16. **Lin, R. J., M. Capage, and C. W. Hill.** 1984. A repetitive DNA sequence, *rhs*, responsible for duplications within the *Escherichia coli* K-12 chromosome. J. Mol. Biol. **177:**1–18.
17. **Ling, H., F. Boudsocq, R. Woodgate, and W. Yang.** 2001. Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication. Cell **107:**91–102.
18. **McKenzie, G. J., R. S. Harris, P. L. Lee, and S. M. Rosenberg.** 2000. The SOS response regulates adaptive mutation. Proc. Natl. Acad. Sci. USA **97:**6646–6651.
19. **McKenzie, G. J., P. L. Lee, M.-J. Lombardo, P. J. Hastings, and S. M. Rosenberg.** 2001. SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. Mol. Cell **7:**571–579. (Erratum, **7:**1119.)
20. **McKenzie, G. J., M.-J. Lombardo, and S. M. Rosenberg.** 1998. Recombination-dependent mutation in *Escherichia coli* occurs in stationary phase. Genetics **149:**1163–1165.
21. **McKenzie, G. J., and S. M. Rosenberg.** 2001. Adaptive mutations, mutator DNA polymerases and genetic change strategies of pathogens. Curr. Opin. Microbiol. **4:**586–594.
22. **Napolitano, R., R. Janel-Bintz, J. Wagner, and R. P. P. Fuchs.** 2000. All three SOS-inducible DNA polymerases (pol II, pol IV, and pol V) are involved in induced mutagenesis. EMBO J. **19:**6259–6265.
23. **Ohmori, H., E. C. Friedberg, R. P. P. Fuchs, M. F. Goodman, F. Hanaoka, D. Hinkle, T. A. Kunkel, C. W. Lawrence, Z. Livneh, T. Nohmi, L. Prakash, S. Prakash, T. Todo, G. C. Walker, Z. Wang, and R. Woodgate.** 2001. The Y-family of DNA polymerases. Mol. Cell **8:**7–8.
24. **Rosenberg, S. M.** 2001. Evolving responsively: adaptive mutation. Nature Rev. Genet. **2:**504–515.
25. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

26. **Strauss, B. S., R. Roberts, L. Francis, and P. Pouryazdanparast.** 2000. Role of the *dinB* gene product in spontaneous mutation in *Escherichia coli* with an impaired replicative polymerase. J. Bacteriol. **182:**6742–6750.
27. **Sutton, M. D., and G. C. Walker.** 2001. Managing DNA polymerases: coordinating DNA replication, DNA repair, and DNA recombination. Proc. Natl. Acad. Sci. USA **98:**8342–8349.
28. **Tang, M., P. Pham, X. Shen, J. S. Taylor, M. O'Donnell, R. Woodgate, and M. F. Goodman.** 2000. Roles of *E. coli* DNA polymerases IV and V in lesion-targeted and untargeted SOS mutagenesis. Nature **404:**1014–1018.
29. **Wagner, J., P. Grúz, S. R. Kim, M. Yamada, K. Matsui, R. P. P. Fuchs, and T. Nohmi.** 1999. The *dinB* gene encodes a novel *E. coli* DNA polymerase, DNA pol IV, involved in mutagenesis. Mol. Cell **4:**281–286.
30. **Wagner, J., and T. Nohmi.** 2000. *Escherichia coli* DNA polymerase IV mutator activity: genetic requirements and mutational specificity. J. Bacteriol. **182:**4587–4595.
31. **Zar, J. H.** 1984. Biostatistical analysis, 2nd ed. Prentice Hall, Englewood Cliffs, N.J.
32. **Zhou, B. L., J. D. Pata, and T. A. Steitz.** 2001. Crystal structure of a DinB lesion bypass DNA polymerase catalytic fragment reveals a classic polymerase catalytic domain. Mol. Cell **8:**427–437.