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Genetics

Mutants in the Exo I motif of *Escherichia coli dnaQ*: Defective proofreading and inviability due to error catastrophe

(fidelity of DNA replication/*dnaQ* and *dnaE* genes/*mutD5* mutator/*dnaE* antimutators/saturation of mismatch repair)

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ABSTRACT **The *Escherichia coli dnaQ* gene encodes the proofreading 3′ exonuclease (ε subunit) of DNA polymerase III holoenzyme and is a critical determinant of chromosomal replication fidelity. We constructed by site-specific mutagenesis a mutant, *dnaQ926*, by changing two conserved amino acid residues (Asp-12→Ala and Glu-14→Ala) in the Exo I motif, which, by analogy to other proofreading exonucleases, is essential for the catalytic activity. When residing on a plasmid, *dnaQ926* confers a strong, dominant mutator phenotype, suggesting that the protein, although deficient in exonuclease activity, still binds to the polymerase subunit (α subunit or *dnaE* gene product). When *dnaQ926* was transferred to the chromosome, replacing the wild-type gene, the cells became inviable. However, viable *dnaQ926* strains could be obtained if they contained one of the *dnaE* alleles previously characterized in our laboratory as antimutator alleles or if it carried a multicopy plasmid containing the *E. coli mutL*$^+$ gene. These results suggest that loss of proofreading exonuclease activity in *dnaQ926* is lethal due to excessive error rates (error catastrophe). Error catastrophe results from both the loss of proofreading and the subsequent saturation of DNA mismatch repair. The probability of lethality by excessive mutation is supported by calculations estimating the number of inactivating mutations in essential genes per chromosome replication.**

In most organisms, the fidelity of DNA replication is determined by three main processes: base selection (or insertion fidelity) by the DNA polymerase, editing by the associated 3′ exonuclease activity (proofreading), and postreplicative DNA mismatch repair (1). In the bacterium *Escherichia coli*, base selection is performed by the *dnaE* gene product, the α (i.e., polymerase) subunit of DNA polymerase III holoenzyme, which replicates the chromosome. Proofreading is performed by the *dnaQ* gene product, the ε subunit of the holoenzyme complex. The α and ε subunits associate tightly to form, together with the θ subunit, the pol III core (2). DNA mismatch repair is performed by the *mutH*, *mutL*, and *mutS* gene products in concert with other proteins (3). The fidelity afforded by the sequential action of the three systems approaches the mutation rate in *E. coli*, $\approx 10^{-10}$ per base pair per round of replication (4, 5).

The current study is directed at the precise role of the *dnaQ*-encoded 3′ exonuclease function, including its role as a proofreader. Several types of *dnaQ* mutants have been reported. A *dnaQ* deletion mutant was found to have extremely poor viability unless also containing a stabilizing mutation (*spq*) in the *dnaE* gene (6, 7). The poor viability is assumed to result from reduced DNA replication capacity due to the instability of the DNA replication complex lacking an important subunit (7). This result points to the dual role of the ε subunit: its exonuclease function and its presumed structural role within the replication complex based on its tight binding to both the α and θ subunits (2). An understanding of the specific role of the (proofreading) exonuclease may thus require a mutant that is devoid of exonuclease activity but otherwise normal in its protein–protein interactions. One possible candidate is the *mutD5* mutant (8, 9), which carries a mutation in *dnaQ* (10, 11). *mutD5* is an exceptionally strong mutator, mutation rates being enhanced 10^4- to 10^5-fold in rich medium (8, 9, 12). The high mutability results not only from the proofreading defect (13) but also from the associated impairment of DNA mismatch repair due to saturation of its capacity (12, 14–16). *mutD5* is also dominant in a genetic complementation assay (9, 17, 18), suggesting that it still interacts with the polymerase subunit. On the other hand, *mutD5* is a missense mutation and, while its exonuclease is strongly impaired (19), it may contain some small residual exonuclease activity (ref. 19; P. Pham and R.M.S., unpublished data) that could have important biological consequences.

Amino acid sequence analyses of a large number of DNA polymerase-associated exonucleases has revealed three well-conserved protein regions, termed Exo I, Exo II, and Exo III, which are probably essential for catalytic activity (for review, see ref. 20). The Exo I region contains two highly conserved Asp and Glu residues, which may function in coordination of an essential metal ion (21, 22). Substitution of these two residues in the *E. coli* pol I Klenow fragment yielded an enzyme devoid (<0.01% remaining) of exonuclease activity, while retaining its overall structure (21).

In this study, we demonstrate that *mutD5* contains a Thr → Ile mutation in the Exo I motif, adjacent to the conserved Glu. This location in the Exo I motif is consistent with the strongly impaired exonuclease activity and high mutator activity (19). At the same time, this location is also consistent with the existence of some residual proofreading activity, as complete inactivation may require elimination of the invariant Asp and Glu residues. In this work, we have altered by site-specific mutagenesis the two invariant Glu and Asp residues and have investigated the mutational consequences. Our data show that a strain containing the resulting *dnaQ926* as the sole copy of the gene is inviable. However, viability is restored by introducing one of the *dnaE* antimutator alleles previously isolated in our laboratory (23–25) or by a multicopy plasmid containing the *E. coli mutL*$^+$ gene. These data are consistent with the idea that a *dnaQ926* strain is inviable due to its unacceptably high mutation rate.

MATERIALS AND METHODS

Strains and Media. NR10325 is KA796 (26) but containing F′CC105. F′CC105 is the F′*pro*$^+$*lacIZ* from strain CC105 (27),

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which we have found useful for assessing mutator strength via papillation (28). From NR10325 we created the following *dnaE* antimutator strains by cotransduction of *dnaE* with transposon *zae*::Tn*10d*-Cam (23, 24): NR10975 (*dnaE*$^+$), NR10985 (*dnaE911*), NR10995 (*dnaE915*), and NR11240 (*dnaE925*). The *dnaE* alleles have been described (23, 25). NR11253 [*ara*, *thi*, *Δprolac*, *metB*, *argE*(am), *rif*, *nalA*, *supE*, *recA56*, *srl*::Tn*10*, *dnaE925*, *zae*::Tn*10d*-Cam, F′CC105] was constructed from XA102 (29). Gene replacement (see below) yielded NR11257 (NR11240, but *mutD5*) and NR11269 (NR11240, but *dnaQ926*). From these, we created NR11363 (*mutD5*, *zae-502*::Tn*10*) and NR11364 (*dnaQ926*, *zae-502*::Tn*10*), by introduction of transposon *zae-502*::Tn*10* from JW353 (23). pMQ350 is a pBR322-derived plasmid carrying the *E. coli mutL*$^+$ gene (T. Loh and M. G. Marinus, personal communication). Solid and liquid media (both LB and minimal) were as described (23). XPG plates, used for papillation studies (25), were minimal-medium plates containing glucose (0.2%), phenyl-β-D-galactopyranoside (0.5 mg/ml), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (40 μg/ml) (28).

Site-Specific Mutagenesis. Plasmid pMM5 (5.9 kb) (18) is pBR322 containing a 1.6-kb chromosomal *Eco*RI fragment carrying the *aspV*, *dnaQ*, and *rnhA* genes, in this order (30). pIF20 (7.2 kb) was derived from pMM5 by inserting the Pharmacia Kan Genblock (1.3-kb *Hin*cII fragment) into the *aspV Hin*cII site. The 1.4-kb *dnaQ*-containing *Xma* I/*Eco*47III fragment (sites in Kan and *rnhA*, respectively) from pIF20 containing *dnaQ* was inserted into the *Hin*cII site of M13mp18. Site-specific mutagenesis on *dnaQ* was performed in this vector using the uracil technique described by Kunkel *et al.* (31). To obtain *dnaQ926*, we introduced 2 base-pair changes in *dnaQ* (A→C at position 971 and A→C at position 977) (numbering according to ref. 32) changing Asp-12 and Glu-14 both to Ala. In parallel, we also recreated *mutD5* (C→T at position 968, Thr-16→Ile). The changes were confirmed by sequencing the entire gene. The mutated gene was removed from the phage in two different ways. The 1.4-kb *Xma* I/*Sph* I fragment containing *dnaQ* was used to replace the 2.4-kb *Xma* I/*Sph* I fragment of pMM5, creating plasmids pIF2013 (*dnaQ926*) and pIF2031 (*mutD5*). Alternatively, the 1.0-kb *Pst* I fragment containing *dnaQ* was removed and inserted in the *Pst* I site of pBluescript II SK(−) to yield pIF44 (*dnaQ926*) or pIF45 (*mutD5*). From there the *dnaQ* gene was transferred as a 1.0-kb *Spe* I/*Apa* I fragment into the corresponding sites of plasmid pBIP3 (33) to yield pIF444 (*dnaQ926*) or pIF445 (*mutD5*). All manipulations were done in strain NR11240 (*dnaE925*, *recA56*) and in minimal medium, whenever possible, to minimize the deleterious effects of high mutation rates.

Gene Replacement. Gene replacement was via the method of Slater and Maurer (33). Strain NR11253 containing pIF444 or pIF445 was infected with phage f1R189 and the lysate used to infect strain NR11240. The resulting kanamycin-resistant colonies were tested on XPG plates to identify diploid *dnaQ*$^+$/*dnaQ*$^-$ products (kanamycin-resistant mutators). Diploids were then plated on LB-sucrose to investigate their ability to segregate *dnaQ926* or *mutD5* haploids (kanamycin-sensitive mutators). Most produced only *dnaQ*$^+$ haploids, but one isolate was found for each allele that segregated kanamycin-sensitive mutators. The haploid nature of these segregants was confirmed by PCR amplifying the *dnaQ* gene from single colonies (34) and sequencing the relevant *dnaQ* region. The parental diploids provided a double-banding pattern representative of both *dnaQ*$^+$ and *mutD5* (*dnaQ926*), while the presumed haploids revealed only the expected single (mutant) band. The correct nature of the newly obtained strains was then confirmed by cloning the *dnaQ* gene from NR11269 (*dnaQ926*) and NR11363 (*mutD5*) on a 1.6-kb chromosomal *Eco*RI fragment into pBluescript KS(+) and sequencing the gene in its entirety.

Mutation Frequency Measurements. Mutant frequencies were determined as described (23, 25). Papillation tests on XPG plates were done as described (25) by visually determining the average number of papillae (Lac$^+$ minicolonies that grow out from the surface of a colony). The *lacZ* allele on F′CC105 reverts exclusively by A·T → T·A transversion and its reversion is greatly stimulated in proofreading-deficient strains (25).

RESULTS

DNA Sequencing of *mutD5*. To better interpret the proofreading defect of *mutD5*, we sequenced the *dnaQ* gene from *mutD5* strain NR9455 (35). We found a single base change, C → T at position 967, changing Thr-15 to Ile. This mutation is located in the region of *dnaQ* identified as the Exo I motif (Fig. 1). The mutation does not correspond to that reported by Takano *et al.* (11), who observed two different amino acid substitutions elsewhere in the gene. However, our observation is consistent with the mutation found by E. C. Cox (personal communication), in whose laboratory *mutD5* originated (8). The Exo I motif is thought to represent part of the catalytic domain of the exonuclease (21, 22). The location of the *mutD5* mutation in the catalytic domain is consistent with its dominant mutator phenotype: deficient in proofreading but competent in binding to α subunit, the determinants of the α/ε interaction presumably residing elsewhere in the protein (11). We also noted that the mutation does not involve the invariant residues Asp-12 or Glu-14. Changes at these residues are likely necessary to create a completely deficient phenotype. We decided to create a mutant altered at these residues by site-specific mutagenesis, changing both Asp-12 and Glu-14 to alanine, as was done for the corresponding sites in *E. coli* Klenow fragment (21) and in other enzymes, leading in those cases to essentially completely proofreading-deficient polymerases (reviewed in ref. 20).

***dnaQ926* Is a Strong Dominant Mutator.** Site-specific mutagenesis of the *dnaQ* gene, yielded the pBR322-based plasmid pIF2013, which carries the *dnaQ926* allele containing the desired changes. We also created pIF2031 containing the *mutD5* mutation. When transformed into strain DH1, these plasmids conferred a strong (1000- to 10,000-fold) mutator phenotype in trans (Table 1). *dnaQ926* confers a stronger mutator phenotype than *mutD5*, consistent with the possibility that *mutD5* indeed retains some residual proofreading. The plasmids, especially pIF2013 (*dnaQ926*), also adversely affected the health of the strain, producing a lower final cell density and, in the case of pIF2013, also mainly small, heter-

Human Polε	244-	V	L	A	F	**D**	I	**E**	T	T	K -253
Human Polδ	312-	V	L	S	F	**D**	I	**E**	C	A	G -321
S. cerevisiae Polε	286-	V	M	A	F	**D**	I	**E**	T	T	K -295
S. cerevisiae Polδ	317-	I	M	S	F	**D**	I	**E**	C	A	G -326
S. pombe Polε	265-	V	M	A	F	**D**	I	**E**	T	T	K -274
Herpes simplex	364-	L	M	C	F	**D**	I	**E**	C	K	A -373
T4	108-	V	A	N	C	**D**	I	**E**	V	T	G -117
Φ29	8-	M	Y	S	C	**D**	F	**E**	T	T	T - 17
E. coli Pol I	351-	V	F	A	F	**D**	T	**E**	T	D	S -360
E. coli Pol II	152-	W	V	S	I	**D**	I	**E**	T	T	R -161
E. coli Pol III	8-	Q	I	V	L	**D**	T	**E**	T	T	G - 17
mutD5	8-	Q	I	V	L	**D**	T	**E**	**I**	T	G - 17
dnaQ926	8-	Q	I	V	L	**A**	T	**A**	T	T	G - 17

FIG. 1. The Exo I motif of the 3′ → 5′ exonuclease domain of DNA polymerases. The top 11 entries were taken from the alignments of refs. 20 and 36. *E. coli* Pol III refers to the ε subunit. *mutD5* and *dnaQ926* are the observed or created amino acid substitutions in ε (see text). Ciphers indicate the sequence number of the first and last presented amino acid.

Table 1. Effect of *dnaQ* alleles in trans on cell titer and mutagenesis

Plasmid	Viable cells $\times 10^{-6}$	Rif^r per plate	Rif^r per 10^6 cells
None	120	3	0.03
pBR322	110	4	0.04
pIF2013 (*dnaQ926*)	2	690	347
pIF2031 (*mutD5*)	35	2000	57

The experiment was performed in strain DH1 growing in minimal medium at 30°C. Rif^r denotes mutants resistant to rifampicin. Numbers are averages for three independent transformants.

ogeneous colonies (results not shown). Thus, loss of exonuclease appears to be deleterious, as previously suggested (35). In subsequent experiments (not shown), we noted that mutagenesis by the two plasmids was significantly reduced (and viability significantly improved), if the experiment was performed in a *dnaE925* strain. *dnaE925* was isolated previously as a suppressor of the high mutability of *mutD5* and was shown to be an antimutator in proofreading-proficient strains as well (25). *dnaE925* is probably similar to the series of *dnaE* antimutator alleles isolated as suppressors of the high mutability of *mutL* strains and which have been postulated to replicate their DNA with increased fidelity (23).

A Chromosomal *dnaQ926* Mutant Is Inviable in the *dnaE*$^+$ Background. Replacement of the chromosomal *dnaQ*$^+$ gene with *dnaQ926* was attempted using the method of Slater and Maurer (33). Our initial efforts were unsuccessful. We hypothesized that this could be due to the proofreading defect or the very high mutation rates of this construct. In view of the beneficial effects of the *dnaE925* allele described above, we repeated all steps in the presence of *dnaE925*. In this background, we were successful in obtaining *dnaQ*$^+$/*dnaQ926* and *dnaQ*$^+$/*mutD5* diploids and, from these, *dnaQ926* and *mutD5* haploids. Both the diploids and the haploids proved strong mutators, with *dnaQ926* in most cases decidedly stronger than *mutD5* (Table 2). Viability in the *dnaE925* background was good.

A series of P1 transductions was then performed to test the properties of *dnaQ926* isolates outside the *dnaE925* background. Transposon *zae-502*::Tn*10* conferring tetracycline resistance was inserted into the chromosome between *dnaE925* and *dnaQ926* (or *mutD5*). In transductional crosses, this tetracycline resistance is 30–50% linked to either gene (23, 24). Using these new strains as a donor, tetracycline resistance was transferred to either a wild-type strain or to one of three *dnaE* antimutator strains (*dnaE911*, *dnaE915*, or *dnaE925*) (23, 25). The products were analyzed for mutator phenotype on XPG plates, on which the extent of mutator phenotype can be readily discerned based on the number of papillae (28). Four types of tetracycline-resistant products are expected, each at roughly equal frequency (≈25%) (*dnaQ*$^+$ *dnaE*$^+$, *dnaQ*$^-$ *dnaE*$^+$, *dnaQ*$^+$ *dnaE*$^-$, and *dnaQ*$^-$ *dnaE*$^-$). Starting from *mutD5*, each of these products was readily obtained at the expected frequencies. However, in the case of *dnaQ926*, no *dnaE*$^+$ *dnaQ926* product could be detected among many hundreds of transductants tested, although the *dnaQ926* combinations with *dnaE911*, *dnaE915*, and *dnaE925* were readily obtained. We concluded that the *dnaQ926 dnaE*$^+$ product is inviable. More specifically, the exonuclease deficiency afforded by *dnaQ926* is lethal in our *E. coli* strains unless the cell also contains a suppressor mutation in the *dnaE* gene. Table 3 lists the mutabilities of the various *mutD5* and *dnaQ926* isolates.

Table 2. Mutagenesis in diploid and haploid derivatives of *mutD5* and *dnaQ926*

Type	Genotype		Mutants per 10^6 cells	
	dnaQ	*dnaE*	Rif^r	Nal^r
Diploid	*mutD5*, *dnaQ*$^+$	*dnaE925*	1.4	0.004
Diploid	*dnaQ926*, *dnaQ*$^+$	*dnaE925*	2.4	0.25
Haploid	*mutD5*	*dnaE925*	1.7	0.12
Haploid	*dnaQ926*	*dnaE925*	67	14

All strains are derivatives of NR11240 (*dnaE925*). Frequencies are based on a total of six independent cultures grown in LB at 37°C. For comparison, the mutant frequencies in a wild-type strain are 0.03 and 0.002 per 10^6 for Rif^r and Nal^r, respectively. Rif^r and Nal^r, resistance to rifampicin and nalidixic acid, respectively.

The Cause of Inviability of *dnaQ926*. Based on the present results, the simplest explanation for the inviability of *dnaQ926* is that it is such a strong mutator as to be incompatible with sustained life. In this model, the role of the *dnaE* antimutators is simply to reduce the overall mutability to an acceptable level. However, some other possibilities need also be considered. Inviability could be due to an absolute requirement of the cell for at least a low level exonuclease activity (possibly independent of its role in fidelity). The *dnaQ926* ε subunit, based on its intimate interaction with the polymerase subunit, could also adversely affect DNA polymerization activity, effectively "poisoning" the polymerization capacity. In these alternative scenarios, the function of the *dnaE* alleles (in addition to their antimutator activity) would be to render the DNA polymerase independent of the exonuclease subunit. The experiments described below argue against such possibilities and argue in favor of the excessive error hypothesis.

The strong mutator phenotype of *mutD5* results from both its proofreading deficiency and the accompanying impairment of DNA mismatch repair by saturation (12, 14–16). Evidence for mismatch-repair saturation includes the observation that *mutD5* strains display strongly reduced mutation rates when transformed by a multicopy plasmid containing the *S. typhimurium mutL* gene (16). Presumably, the MutL gene product is most readily depleted under conditions of high levels of replication errors. The possibility of reducing the mutation rate of proofreading-defective strains by enhancing DNA mismatch repair provides a direct way to probe the role of excessive error rates in the lethality of *dnaQ926*. We asked whether *dnaQ926* might be viable in a *dnaE*$^+$ background in the presence of a MutL-overproducing plasmid. This indeed proved the case, except that the *E. coli mutL* gene was required rather than the *Salmonella* gene. Fig. 2 is a photograph of the products of a transduction of *dnaQ926* into a *dnaE*$^+$ strain containing plasmid pMQ350 (*E. coli mutL*$^+$) after transfer to an XPG plate (where the strength of the mutator phenotype can be visually determined). Two types of mutators appear, the *dnaQ926 dnaE925* combination obtained before and the exceptionally strong mutator *dnaQ926 dnaE*$^+$. Table 4 provides

Table 3. Effects of *dnaE* antimutators on *mutD5* and *dnaQ926* strains

Genotype		Mutants per 10^6 cells		
dnaQ	*dnaE*	Rif^r	Nal^r	Str^r
mutD5	+	200	54	3.5
mutD5	*dnaE911*	6.8	1	0.4
mutD5	*dnaE915*	8.7	0.6	0.2
mutD5	*dnaE925*	1.2	0.03	0.009
dnaQ926	+		Inviable	
dnaQ926	*dnaE911*	210	66	38
dnaQ926	*dnaE915*	160	24	33
dnaQ926	*dnaE925*	110	20	30

Strains are NR10325 containing the indicated *dnaQ* and *dnaE* alleles and are products of the transductions described in the text. All frequencies are based on 12 independent cultures growing at 37°C. Experiment shown was performed in LB medium, but similar results were obtained in minimal medium. Rif^r, Nal^r, and Str^r denote resistance to rifampicin, nalidixic acid, and streptomycin, respectively.

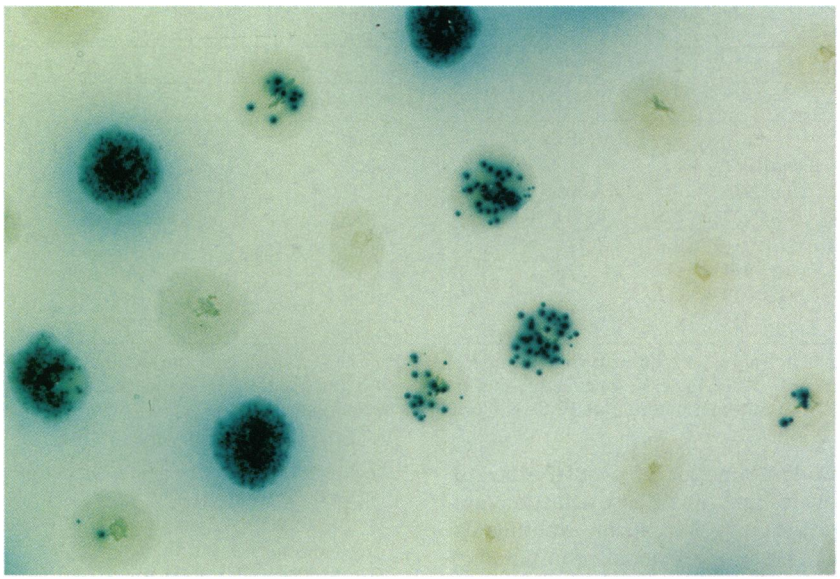

FIG. 2. *dnaQ926* is viable in a pMQ350 (*mutL*$^+$) background. Colonies are the product of a P1 transduction using a *dnaE925*, *zae-502*::Tn*10*, *dnaQ926* strain as a donor, and a *dnaE*$^+$, F′CC105 strain carrying plasmid pMQ350 (*mutL*$^+$) as a recipient. Selection was for tetracycline resistance, followed by transfer of random colonies to an XPG plate. Colonies without papillae are *dnaQ*$^+$, colonies with ≈25 papillae are *dnaE925 dnaQ926*, whereas colonies with very high numbers of papillae are *dnaE*$^+$ *dnaQ926*.

a comparison of the effects of the *E. coli* and *Salmonella mutL* genes, whereas Table 5 shows the relative effects of the three antimutator alleles in the pMQ350 background. Rescue of *dnaQ926* by pMQ350 did not occur in a *mutH*-defective strain (data not shown), suggesting that rescue proceeds through restoration of mismatch repair rather than some nonspecific action of the *mutL* gene product and also eliminating the possibility that lethality results from attempted but uncompleted DNA mismatch repair.

DISCUSSION

The data of this paper show that a defect in the *dnaQ* gene of *E. coli* that impairs and possibly completely eliminates the 3′ exonucleolytic proofreading of the replicating complex is lethal in the *E. coli* strains tested here. Lethality does not result from a requirement of the proofreading capacity *per se* but from a case of error catastrophe. Loss of proofreading causes increased levels of replication errors, which in turn leads to impairment or collapse of the mismatch-repair system, presumably by saturation (12, 16). The combined effects yield mutation rates that are high enough to be incompatible with life.

Table 4. Mutability of *mutD5* and *dnaQ926* strains carrying a plasmid expressing the *mutL* gene from *S. typhimurium* or *E. coli*

Genotype	Mutants per 10^6 cells		Colony size
	Rif^r	Nal^r	
mutD5	100	12	+
mutD5 (pGW1842)	42	2.5	++
mutD5 (pMQ350)	14	<0.01	+++
dnaQ926		Not viable	
dnaQ926 (pGW1842)		Not viable	
dnaQ926 (pMQ350)	77	63	+

Strains are derivatives of NR10325 (with or without indicated plasmid) into which *mutD5* and *dnaQ926* had been transduced from NR11363 and NR11364, respectively. pGW1842 carries the *S. typhimurium mutL* gene (37). pMQ350 carries the *E. coli mutL* gene (T. Loh and M. G. Marinus, personal communication). This experiment is based on 10 cultures grown in minimal medium at 37°C. Increasing number of + signs corresponds to increased colony size, with +++ indicating normal, wild-type size. Rif^r and Nal^r, resistance to rifampicin and nalidixic acid, respectively.

The data further suggest that error processing in *E. coli* may differ from that in eukaryotes in important aspects. In the yeast *S. cerevisiae*, inactivation of the 3′ exonuclease of polymerase δ leads to normally viable cells displaying a moderate mutator effect (38). However, lethality is observed in a double mutant containing both the proofreading defect and a deletion of the PMS1 mismatch-repair gene (38). Thus, significantly greater redundancy may exist in this organism, either at the level of DNA polymerase proofreading, several polymerases being capable of participating in proofreading (36), or at the level of the mismatch-repair proteins, of which numerous homologs are being identified (39).

The maximum level of mutations that can be sustained by any haploid organism is probably close to one mutation in an essential gene per genome per round of replication. It has been argued that this is nearly the case for certain RNA viruses (40): attempts to increase the level of mutations in these organisms rapidly lead to inactivation. We can use our current data to estimate the number of lethal mutations per cell division in *mutD5* and *dnaQ926* strains, using as an example the Rif^r mutant frequency (Table 6). The calculations suggest the occurrence of roughly 9 and 47 uncorrected errors per chromosome per cell division in *mutD5* and *dnaQ926*, respectively, which may respond to about 0.3 mutation per division in an essential gene in *mutD5* and 1.3 per division in *dnaQ926*. While only rough estimates, these calculated levels are consistent with the impaired growth of *mutD5* and the inviability of *dnaQ926*. That *mutD5* cells are close to, but still some distance removed from, lethality is evidenced by the viability of *mutD5 mutL* double mutants (5, 25). These have an ≈3-fold higher mutability than *mutD5* (presumably representing the remaining contribution of mismatch repair in *mutD5*) and display further reduced viability compared to *mutD5* (5, 25).

We have previously used *mutD5* strains to estimate from *in vivo* mutation spectra the relative contributions of base selection and proofreading to *in vivo* DNA replication fidelity (5). On the assumption that *mutD5* was completely deficient in proofreading, we derived an estimate of 3×10^{-6} for insertion fidelity and a 60-fold contribution for proofreading. As indi-

Table 5. Mutability of *dnaQ926* and *mutD5* in different *dnaE* antimutator backgrounds in the presence of plasmid pMQ350 (*mutL*$^+$)

Genotype		Rif^r per 10^6 cells	Lac$^+$/plate		Titer $\times 10^{-7}$, cells per ml
dnaQ	*dnaE*		A·T → T·A	A·T → G·C	
mutD5	+	9.7	4000	79	37
mutD5	*dnaE911*	1.1	154	31	49
mutD5	*dnaE915*	0.8	92	42	38
mutD5	*dnaE925*	0.4	36	3	46
dnaQ926	+	71	8000	320	14
dnaQ926	*dnaE911*	12	1220	61	31
dnaQ926	*dnaE915*	5.7	1080	18	35
dnaQ926	*dnaE925*	2.3	260	13	42

Strains used are as in Table 3, except carrying plasmid pMQ350. Lac reversion is of the *lac* alleles from F′CC105 (A·T → T·A) and F′CC106 (A·T → G·C) (27) as an example of a transversion and a transition, respectively. This experiment is based on 10 cultures grown in minimal medium at 37°C. Rif^r, rifampicin resistance.

cated in that study, a residual amount of proofreading in *mutD5* would lead to an overestimation of base selection and an underestimation of proofreading. The current experiments clearly indicate that *mutD5* possesses residual proofreading, which could contribute as much as a 5-fold increase in fidelity. Thus, base selection fidelity by pol III holoenzyme is more likely to be 1–2 $\times 10^{-5}$ and proofreading could contribute as much as 300-fold.

That mutation accumulation may play an important role in disease has been proposed repeatedly but has been difficult to address experimentally (42–45). For example, a role for mutation accumulation has been suggested with regard to the mechanisms of aging (42–44)—where the term error-catastrophe was first used (42)—and in cancer (45). The bacterial system that we have studied provides a simple model to investigate more quantitatively the correlation between high mutation rates and their deleterious effects.

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Table 6. Estimated lethal mutations per cell division in *mutD5* and *dnaQ926* strains

Event	*mutD5*	*dnaQ926*
Mutant frequency (Rif^r)*	165×10^{-6}	825×10^{-6}
Mutation rate (per cell division)†	18×10^{-6}	100×10^{-6}
Error rate/bp (target ≈10 bp)‡	1.8×10^{-6}	10×10^{-6}
Errors/genome (4.7×10^6 bp)	9	47
Mutations/genome (1/7 detectable)§	1.3	7
Mutations in essential genes (20%)	0.3	1.3

*The Rif^r mutant frequency for the *mutD5* strain is the median of 10 published values (refs. 5, 12, 16, 25, and 35, and this paper). For *dnaQ926* the value cannot be measured directly, but we conservatively estimate it to be ≈5-fold higher than for *mutD5* (from Tables 2 and 4).

†Mutation rate calculated according to Drake (4) using population size $n = 5 \times 10^8$ and 1×10^8 for the two strains.

‡Approximate number of transition base-pair sites in the *rpoB* gene that can yield resistance to rifampicin is 10 (41).

§Estimate based on data from the *lacI* gene (ref. 5, unpublished data). The *lacI*^{d} target contains ≈180 nucleotides at which 137 mutagenic substitutions have been observed. The detectability of a base-substitution error (the predominant error type under these conditions) is therefore $137/(3 \times 180) = 0.26$. The *lacI*^{d} region is ≈1.8-fold more sensitive to substitution mutagenesis than the *lacI* gene in its entirety [roughly one-third of *lacI* mutants reside in one-sixth of the gene (5)]. Thus, $0.26/1.8 = 0.14$ of substitution errors over the entire gene (1080 nucleotides) are expected to generate a deficient phenotype.

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