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Interactions between epsilon, the proofreading subunit of DNA polymerase III, and proteins involved in the SOS response of *Escherichia coli*

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Abstract

Summary—Epsilon, a fidelity subunit of *Escherichia coli* DNA Polymerase III, is encoded by *dnaQ*⁺. *dnaQ49* is a recessive allele that confers temperature-sensitive and salt-suppressible phenotypes for both replication fidelity and viability. SOS mutagenesis in *E. coli* is regulated by LexA and requires activated RecA (RecA*) and the products of the *umuDC* operon, *dnaQ49* strains with various *recA*, *lexA* and *umuDC* alleles were constructed to determine if activities induced as part of the SOS response influence epsilon activity. We found: (1) both UmuDC and RecA* independently enhance the *dnaQ49* mutator phenotype, and (2) expression of RecA* activity in the absence of UmuDC function increases the temperature sensitivity for viability of *dnaQ49*. These results support the hypothesis that RecA and one or both of the UmuDC proteins interact with the replication complex during SOS mutagenesis.

Keywords

Mutagenesis; Replication; *dnaQ*; *recA*; *umuDC*

Introduction

The RecA protein of *Escherichia coli* plays at least three roles in mutagenesis. When activated to its proteolytic state (RecA*) genetically or by DNA damage, it enhances the self-cleavage of the LexA protein, the common repressor of the genes of the *recA*-*lexA* regulon (Little 1984). The induction of these genes, which include the mutagenesis promoting *umuDC* operon as well as *recA* and *lexA*, constitutes the SOS response (reviewed in Walker 1984). RecA* also facilitates the cleavage of UmuD (Burckhardt et al. 1988; Nohmi et al. 1988; Shinagawa et al. 1988), which activates UmuD for its function in mutagenesis (Nohmi et al. 1988). Since a cleavage-independent *umuD* mutant does not restore mutability to a *recA* defective mutant, RecA appears to have at least one more role in mutagenesis (Nohmi et al. 1988).

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It has recently been proposed that the UmuDC proteins increase the stability of the replication complex during translesion DNA synthesis by acting as processivity factors [Battista JF, Nohmi T, Donnelly CE, Walker GC (1988) Role of UmuD and UmuC in UV and chemical mutagenesis. In: Friedberg E, Hanawalt P (eds) Mechanism and consequences of DNA damage processing. Liss, New York, in press; Hevroni D, Livneh Z (1988) Bypass and termination at apurinic sites during replication of single-stranded DNA in vitro: a model for apurinic site mutagenesis. Proc Natl Acad Sci USA 85:5046-5050]

SOS mutagenesis is hypothesized to be mediated by an error-prone polymerase (Radman 1975). That RecA may interact with and modify the replication complex is suggested by the following evidence: (1) *in vivo* RecA is required after DNA damage for the recovery of DNA synthesis (Khidhir et al. 1985; Witkin et al. 1987) and the initiation and maintenance of stable DNA replication (Witkin and Kogoma 1984), and (2) *in vitro* purified RecA protein reduces the fidelity of DNA synthesis by DNA polymerase III (Pol III) holoenzyme, apparently by inhibiting the 3' → 5' exonuclease activity of the epsilon subunit (Fersht and Knill-Jones 1983; Lu et al. 1986). One or both of the gene products of *umuDC* may also interact with the replication complex since: (1) overproduction of UmuDC inhibits DNA synthesis (Marsh and Walker 1985), (2) UmuDC suppresses the inability of a mutant RecA, RecA718, to promote the recovery of DNA synthesis after DNA damage (Witkin et al. 1987), and (3) removal of UV induced blocking lesions by delayed photoreversal allows recovery of mutations in *umuDC*⁻ bacteria, suggesting that UmuDC is involved in the continuation of DNA synthesis from bases mis-inserted opposite DNA lesions (Bridges and Woodgate 1985). Thus, the several direct roles of RecA may be to “target” the lesion, inhibit the proofreading function of Pol III, and interact with and modify the UmuDC proteins which then aid in or promote lesion bypass (Lu et al. 1986; Bridges and Woodgate 1985; Ennis et al. 1985). However, given the complexity of the system many other models can be generated that fit the experimental data.

The *dnaQ* gene encodes epsilon, a fidelity subunit of Pol III (Scheuermann et al. 1983). *dnaQ49* is a recessive allele of *dnaQ*⁺ that confers a temperature-inducible and salt-suppressible mutator phenotype; at higher temperatures in salt-free media, *dnaQ49* strains are non-viable due to inhibition of DNA synthesis (Horiuchi et al. 1978). Piechocki et al. (1986) reported that *dnaQ49*-dependent base substitution mutations were dominated by transversions, the same class of mutation produced when the SOS response is induced in the absence of DNA damage (Miller and Low 1984). They further found that the *dnaQ49* mutator phenotype was independent of *recA* and *umuDC*, but reduced by a *lexA* (*Ind*⁻) (noninducible) allele. They hypothesized that Pol III complexed with the *dnaQ49* gene product is functionally the error-prone SOS polymerase, not requiring the participation of RecA or UmuDC, but requiring some other gene repressed by LexA, to express mutations.

Because the phenotypes conferred by *dnaQ49* are conditional, we reasoned that they could be modulated by other proteins that may normally interact with epsilon. We used the classical method of fluctuation tests (Luria and Delbruck 1943) to reexamine interactions between SOS genes and the *dnaQ49* mutator phenotype. Our results confirmed those of Piechocki et al. (1986) that the mutation rates of *dnaQ49* strains are influenced by the *lexA* genotype. However, we found that: (1) the effect of LexA was largely if not entirely attributable to its regulation of the *umuDC* and *recA* genes, (2) UmuDC⁺ and RecA* independently enhanced the *dnaQ49* mutator phenotype, and (3) the temperature sensitivity for viability of *dnaQ49* was exacerbated by RecA* activity in the absence of UmuDC. These results support the hypothesis that RecA* and one or both of the *umuDC* gene products interact with the replication complex and inhibit proofreading. Furthermore, UmuDC may function to stabilize the replication complex during mutagenic translesion DNA synthesis.

Materials and methods

Bacterial strains and plasmids

All strains used in this study are derivatives of DM1187 [=AB1157 (Bachmann 1987) but *argE*⁺ *recA441* *sulA11* *lexA51* (formerly *tif-1* *sfiA11* *spr-51*) (Mount 1977)]. The various alleles used and their origins are given in Table 1. Strains were constructed by P1_{vir} transduction (Miller 1972). The presence of the desired allele was confirmed by sensitivity or resistance to UV light for *recA* and *lexA*, non-mutability by methyl methanesulfonate for

umuC⁻, or high spontaneous mutation frequency for *dnaQ49* and *mutL*. All strains are *Srl*⁺; *lexA*⁺ and *lexA3* strains are *mal*::Tn5 or *mal*⁺; *dnaQ49* strains are *zae*::Tn10; and *umuC36* strains are *zcf-286*::Tn5.

Media

LB media was as given in Miller (1972). Salt-free LB was as LB but without NaCl. Drugs were added in the following concentrations: tetracycline, 20 mg/l; kanamycin, 45 mg/l; streptomycin, 100 mg/l; rifampicin (rif), 100 mg/l.

Fluctuation tests

Strains were grown overnight in LB broth plus appropriate drugs at 32° C. Cultures were then plated on LB-rif to test for rifampicin resistance (rif^r) and diluted 10⁻⁵ or 10⁻⁶, depending on viability, into salt-free LB broth to give an initial inoculum of 10⁴–10³ cells per ml. Aliquots were then grown at 32° C and 37° C. When saturated, the cultures were plated on LB-rif and titered on LB plates incubated at 32° C. Experiments in which there was a greater than 0.01 probability that the original inoculum contained a rif^r cell were discarded. Mutation rates are based on 9–29 cultures and were calculated by the methods given in Lea and Coulson (1949).

Results

Mutation rates of *dnaQ*⁺ and *dnaQ49* bacteria with various *recA* and *lexA* alleles

As detailed in Table 1, the strains used in these experiments have the following phenotypes: (1) deficient (*recA430*), DNA-damage inducible (*recA*⁺), temperature inducible (*recA441*), and constitutive (*recA730*) for RecA* activity, (2) repressed (*lexA3*), RecA*-inducible (*lexA*⁺), and constitutive (*lexA51*) for expression of the SOS response, and (3) wild type (*dnaQ*⁺) and temperature sensitive (*dnaQ49*) for epsilon activity. The spontaneous mutation rates to rif^r of these strains are given in Fig. 1, which is organized so that the *recA* alleles from left to right have increasing RecA* activity and for each *recA* allele SOS derepression increases from left to right.

Figure 1 a shows the mutation rates of *dnaQ*⁺ strains at 37° C in salt-free LB media. Derepression of the SOS regulon (*lexA51* vs *lexA3*) increased mutation rates of *recA430* and *recA*⁺ strains 3-fold but of strains expressing RecA* (*recA441* and *recA730*) 50- to 85-fold. Since even in the *lexA51* background the mutation rate conferred by *recA730* was still 20-fold greater than that of *recA*⁺, the mutator phenotype of this *recA* allele was largely due to amplified levels of RecA* per se, not to increased expression of other SOS genes due to the cleavage of LexA promoted by RecA*.

Figure 1 b shows the mutation rates of *dnaQ49* derivatives of the same strains at 32° C and 37° C in salt-free LB medium. Although the *dnaQ49* defect increased mutation rates one to three orders of magnitude, derepression of the SOS regulon (*lexA51* vs *lexA3*) further enhanced mutation rates two- to threefold. In addition, RecA* activity (*recA730* and *recA441* at 37° C) resulted in a nearly twofold further increase in mutation rates.

The mutator phenotypes of the SOS genes and *dnaQ49* are more than additive

The absolute magnitudes of the differences in mutation rates conferred by the various *lexA* and *recA* alleles were at least tenfold greater in the *dnaQ49* background than in the *dnaQ*⁺ background. Thus the mutator phenotypes of *dnaQ49* and of the *recA/lexA* alleles were clearly more than additive, suggesting that the proteins induced as part of the SOS response were influencing epsilon activity. However, an alternative explanation is that the loss of proofreading in *dnaQ49* strains could reveal errors induced by SOS expression that are

normally corrected in *dnaQ*⁺ strains. For example, if the mutation rate of the *recA730 lexA51 dnaQ*⁺ strain (Fig. 1 a) reflected only 7% of the potential mutations due to RecA* whereas 93% were normally corrected by proofreading, loss of epsilon activity alone could account for the difference in mutation rates at 37° C between *recA*⁺ *lexA51 dnaQ49* and *recA730 lexA51 dnaQ49* (Fig. 1 b).

If this second hypothesis is correct, the 20-fold difference in mutation rates between *recA730 lexA51 dnaQ*⁺ and *recA*⁺ *lexA51 dnaQ*⁺ (Fig. 1 a) reflects RecA*-induced errors that have escaped both proofreading and a second error correction pathway, mismatch repair (reviewed in Radman and Wagner 1986). Thus if undetected errors were present at tenfold higher levels than observed in *recA730 dnaQ*⁺ cells, these errors should also increase the mutation rate of a derivative defective in mismatch repair (*mutL*⁻). However, as shown in Table 2, the mutation rate of the *recA730 lexA51 mutL::Tn10* strain was only slightly higher than its *recA*⁺ counterpart, a difference consistent with an additive effect of *mutL*⁻ and *recA730*. Although other models are possible (see Discussion), the simplest interpretation of these results is that the differences among the *recA/lexA* derivatives of *dnaQ49* were not due to failure to correct underlying errors, but due to the influence of the various *recA* and *lexA* alleles on the *dnaQ49* phenotype itself.

The separate effects of UmuDC, RecA* and SOS derepression on the *dnaQ49 mutator* phenotype

In Fig. 2 the mutation rates at 32° C and 37° C of all the *dnaQ49* strains are compared to *umuC122::Tn5* derivatives of the *recA*⁺ *dnaQ49* and *recA730 dnaQ49* strains. Because *umuDC*⁺ is essential to the mutagenic process but not to the expression of the SOS response (Kato and Shinoura 1977), *umuC*⁻ strains allow the separate effects of SOS induction, RecA* activity and UmuDC activity to be evaluated. These effects were clearest at 32° C.

As shown in Fig. 2a, in *recA*⁺ strains the *umuC122::Tn5* defect reduced mutation rates 40%–70%, i.e. about half of the mutations due to *dnaQ49* were also dependent on *umuC*⁺. Full induction of the *umuDC*⁺ operon in the *lexA51* strain resulted in a further twofold increase in mutation rate.

RecA* activity enhanced the *dnaQ49* mutator phenotype independently of *umuC* or *lexA* (Fig. 2a). The mutation rate of the *recA730 umuC122::Tn5 lexA3* strain was twofold greater than its *recA*⁺ counterpart; with derepression, this difference increased to threefold. The effect of *umuDC*⁺ appeared to be additive to that of RecA* since when SOS was fully induced (*lexA51*), the magnitude of the difference in mutation rates between *umuC122::Tn5* and *umuC*⁺ derivatives was almost exactly the same in *recA730* as in *recA*⁺ strains. The independence of RecA* and UmuDC⁺ activities in the *dnaQ49* background contrasted with their mutator phenotype in *dnaQ*⁺ strains, which required both the *recA730* and *umuDC*⁺ genotypes (data not shown).

There appeared to be little effect of *lexA* alleles independent of the regulation of *recA* and *umuDC* by LexA. Only the small (1.4-fold) difference in mutation rates between *lexA3* and *lexA51* derivatives of *recA*⁺ *umuC122::Tn5 dnaQ49* could be attributed to the effect of SOS induction per se (Fig. 2 a). The constitutive RecA* activity of *recA730* should result in LexA⁺ cleavage; in confirmation, there was little difference in overall mutation rates between *recA730 lexA*⁺ and *recA730 lexA51*.

The mutation rates of the *recA430* and *recA441* strains confirmed the results obtained with *recA*⁺ and *recA730* (Fig. 2a). At 32° C the RecA441 enzyme is not induced for RecA* activity (Mount 1977), and thus at that temperature *recA441* and *recA*⁺ strains were essentially the same. However, some RecA* activity appeared to contribute to the mutation

rates of both *recA*⁺ and *recA441* strains since the mutation rates of the RecA*-deficient *recA430* strains were 50% lower in *lexA3* and *lexA*⁺ backgrounds. This difference was entirely overcome by derepression of SOS, suggesting either: (1) a constitutive level of LexA cleavage in *recA*⁺ and *recA441* strains was allowing some expression of the *umuDC* operon, or (2) amplified RecA430 was equivalent to RecA⁺, a result consistent with the residual level of RecA* activity retained by RecA430 (Devoret et al. 1983).

As shown in Fig. 2b, most of the differences among the strains observed at 32° C were also evident at 37° C. Since the mutation rates were higher the effects of the SOS genes at 37° C were relatively smaller, although of equal or greater absolute magnitude to their effects at 32° C. There was again little impact of SOS derepression independent of *umuDC*⁺ or RecA*. Although data from the *umuC*⁻ derivatives of *recA730* were not obtainable (see below), it appeared that the magnitude of the increase in mutation rates due to *umuDC*⁺ was equivalent at 37° C and 32° C, but the magnitude of the increase due to RecA* was relatively greater at the higher temperature. The RecA* activity of RecA441 was clearly activated at 37° C since mutation rates in this background were similar to those of *recA730* strains.

recA730* and *umuC*⁻ increase the temperature sensitivity for viability of *dnaQ49

None of the *recA730 umuC122::Tn5 dnaQ49* strains were viable at 37° C in salt-free LB. Survival was somewhat greater on solid media but, as shown in Table 3, the plating efficiency of the *recA730 umuC122::Tn5 lexA51 dnaQ49* strain at 37° C was still three orders of magnitude less than its isogenic controls. This temperature sensitivity was not due to overexpression of the *umuC::Tn5* fusion since *umuC36* strains were likewise non-viable (data not shown).

Discussion

The results presented here demonstrate that both UmuDC⁺ and RecA* independently increase the spontaneous mutation rates of strains with a defective epsilon, DnaQ49. In addition, the temperature sensitivity for viability conferred by *dnaQ49* is increased by expression of RecA* activity in the absence of UmuDC⁺. *dnaQ49* is recessive to *dnaQ*⁺ for both replication fidelity and viability (Maruyama et al. 1983). Both phenotypes of *dnaQ49* are due to a mis-sense mutation that can suppress a dominant mutator allele of *dnaQ*, *mutD51*, in *cis* but not in *trans* (Takano et al. 1986). These observations and the fact that DNA polymerization by the alpha subunit of Pol III in vitro is intrinsically temperature sensitive (Maki and Kornberg 1985) have led Takano et al. (1986) to propose that DnaQ49 is defective in its ability to bind to the alpha subunit of Pol III. The rationale of the experiments presented here was that if SOS-induced proteins interact with Pol III, then they may exacerbate the conditional phenotypes of the mutant DnaQ49. The results obtained support this model.

Comparing *recA*⁺ *umuC122::Tn5 lexA3 dnaQ49* and *recA730 umuC*⁺ *lexA51 dnaQ49* strains at 32° C (Fig. 2a), the combined effects of SOS derepression and RecA* and UmuDC⁺ activity resulted in a 7-fold increase in mutation rate (equal to a 24-fold increase in average mutation frequency). This 7-fold increase was composed of a small effect of SOS derepression per se (1.4 ×) and about equal effects of UmuDC⁺ (3 ×) and RecA* (3 ×). At 37° C (Fig. 2b) the relative increase in mutation rates between the strains was smaller (3-fold) and was almost entirely due to UmuDC⁺ (1.4 ×) and RecA* (1.6 ×).

Our results and conclusions differ in a number of respects from those of a previous study by Piechocki et al. (1986). (1) They found a tenfold lower mutation frequency in *lexA (Ind^r) dnaQ49* vs *lexA*⁺ *dnaQ49* strains whereas we found in *recA*⁺ or *recA430* backgrounds little

difference between *lexA* (*Ind*⁻) *dnaQ49* and *lexA*⁺ *dnaQ49* but equivalently higher mutation rates in *lexA* (*Def*) *dnaQ49* strains. (2) They reported (in a note added in proof) no effect of *umuC*⁻ on the *dnaQ49* mutator phenotype whereas we found that full induction of *umuDC*⁺ increased the mutation rates of *dnaQ49* strains threefold. (3) They found that *recA* (*Def*) alleles did not affect the *dnaQ49* mutator phenotype, which is consistent with our finding that there was little difference between *recA*⁺ *dnaQ49* and *recA430 dnaQ49* strains. However, we further demonstrated that fully-induced RecA* activity increased the mutation rates of *dnaQ49* strains threefold.

In summary, Piechocki et al. (1986) concluded that the *dnaQ49* mutator phenotype was not influenced by RecA or UmuDC but was enhanced by a unknown LexA-repressed gene. In contrast, we conclude that both UmuDC⁺ and RecA* enhance the *dnaQ49* mutator phenotype, that the effects of *lexA* alleles are almost entirely attributable to regulation of *recA* and *umuDC*, and find little evidence for a third gene. While further experimentation is necessary to fully resolve these discrepancies, some may be due to different genetic background or to the large variations in mutation frequencies that obtain at high mutation rates. However, one difference in method - our use of salt-free LB media - is not significant as we confirmed our results in normal LB (data not shown).

Although the obvious interpretation of our results is that RecA* and UmuDC influence epsilon activity, there are alternative explanations. One possibility, that loss of epsilon activity is revealing the underlying errors induced via the SOS pathway, is rendered less likely by the failure of a defect in mismatch repair to likewise reveal these errors (Table 2). This experiment is not definitive since the errors induced by SOS expression may be poorly repaired by mismatch repair but efficiently repaired by proofreading (e. g. see Jones et al. 1987). However, also arguing against this interpretation is the fact that RecA*-dependent mutations in *dnaQ*⁺ strains are also *umuDC*⁺-dependent (Ciesla 1982; Blanco et al. 1982; our observations), whereas RecA* and UmuDC act independently to enhance the mutation rates of *dnaQ49* strains (Fig. 2). Thus the mutant epsilon appears to be revealing separate activities of RecA* and UmuDC that are not evident in *dnaQ*⁺ cells.

While we are attributing the effects of *recA* and *umuC* alleles on the *dnaQ49* phenotypes to RecA* and UmuDC, it is possible that other activities are responsible. For example, the impact of *umuC*⁻ (Fig. 2, Table 3) could be due to loss of UmuC function alone instead of loss of cooperative UmuDC activity. Since RecA* facilitates the cleavage of UmuD (Burckhardt et al. 1988; Nohmi et al. 1988; Shinagawa et al. 1988), the effects of RecA* activity could actually be due to the cleavage product of UmuD. However, UmuD and UmuC probably function together since the phenotypes of *umuD*⁻ and *umuC*⁻ are equivalent (Kato and Shinoura 1977; Elledge and Walker 1983 b) and *umuD*⁺ and *umuC*⁺ fail to complement mutations in their evolutionarily diverged homologues, *mucA* and *mucB* respectively (Perry et al. 1985). Nonetheless, other models incorporating more complex interactions can be devised.

With the above caveats, the simplest interpretation of our results is that RecA* and UmuDC directly interact with epsilon or interact with other subunits of the replication complex and thereby affect epsilon activity. The finding that RecA protein can inhibit the 3' → 5' exonuclease activity of epsilon in vitro (Fersht and Knill-Jones 1983; Lu et al. 1986) strongly suggests that a direct interaction between RecA* and epsilon occurs in vivo. We further theorize that UmuDC may interact with alpha, the product of *dnaE*. Our finding that mutations in *umuC* increase the temperature sensitivity of *dnaQ49* when RecA* is active is reminiscent of a similar increase in temperature sensitivity seen in *dnaE486 dnaQ49* strains (Horiuchi et al. 1981). In addition, suppressors of lethal *dnaQ* null mutants have been found in *dnaE*, i.e. mutations in alpha can relieve its requirement for epsilon (Maurer et al. 1988).

Thus an attractive model is that UmuDC may interact with and stabilize alpha when epsilon activity is inhibited.

It was originally proposed by Villani et al. (1978) that exonucleolytic proofreading is inhibited during SOS mutagenesis, thereby allowing replication to continue past nucleotides misincorporated opposite DNA lesions. Bridges and Woodgate (1985) subsequently proposed that misincorporation and lesion bypass are separate processes since only the latter required UmuDC. *mutD5*, a dominant mutator allele of *dnaQ*, had no effect on UV mutagenesis, leading Woodgate et al. (1987) to conclude that, as predicted, proofreading by epsilon is not active during SOS mutagenesis. However, the *mutD5* defect did not restore UV mutability to *umuC*⁻ bacteria, suggesting that inhibition of proofreading is not sufficient to allow replication past DNA lesions (Woodgate et al. 1987). The dominance of the *mutD5* allele implies that, although defective in proofreading, the MutD5 protein binds alpha at least as tightly as wild-type epsilon (Takano et al. 1986). Thus if SOS mutagenesis involves a modification of the interaction between epsilon and alpha, *mutD5* and *dnaQ*⁺ bacteria may not differ in that interaction. Similar experiments with the *dnaQ49* allele may resolve this issue.

In summary, the results reported here suggest that UmuDC and RecA* interact with the replication complex and inhibit epsilon activity. Thus our data support the hypothesis that SOS mutagenesis involves inhibition of proofreading and a resulting decrease in the replication fidelity of Pol III. However, since most SOS-dependent mutations appear to be targeted to the sites of DNA lesions (Foster et al. 1982; Miller 1982), inhibition of proofreading normally must be transient. The results presented here further suggest that UmuDC may function to stabilize the replication complex during mutagenic translesion DNA synthesis.

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Fig 1 a, b.

Mutation rates of the strains in salt-free LB media: **a** *dnaQ*⁺; **b** *dnaQ*⁴⁹. The *recA* alleles from *left to right* have increasing RecA* activity. For each *recA* allele the bars from *left to right* are the mutation rates of *lexA*₃ [= *lexA* (*Ind*)], *lexA*⁺, and *lexA*₅₁ [= *lexA* (*Def*)] derivatives, Thus within each *recA* background SOS derepression increases from *left to right*

Fig. 2a, b.

The mutation rates of *dnaQ49* strains compared to *umuC*::Tn5 derivatives in salt-free LB media: **a** at 32° C; **b** at 37° C. The *recA* alleles from *left* to *right* have increasing RecA* activity. For each *recA* allele the *bars* from *left* to *right* are the mutation rates of *lexA3* [=lexA(Ind⁻)], *lexA*⁺, and *lexA51* [lexA (Def)] derivatives

Table 1

The alleles used in this study

Allele	Alternative name	Phenotype	Source	Reference
<i>recA</i> alleles				
<i>recA430</i>	<i>lexB30</i>	Deficient in RecA*	GW2010, G. Walker, USA	Elledge and Walker (1983a); Roberts and Roberts (1981)
<i>recA</i> ⁺	–	Wild type	AB1157, G. Walker, USA	Bachmann (1987)
<i>recA441</i>	<i>tif-1</i>	Thermally inducible RecA*	DM1187, E. Eisenstadt, USA	Mount (1977); Castellazzi et al. (1972); Roberts et al. (1978)
<i>recA730</i>	–	Constitutive RecA*	RH4655, G. Maenhaut-Michel, Belgium	Witkin et al. (1982)
<i>lexA</i> alleles				
<i>lexA3</i>	<i>lexA (Ind⁻)</i>	Cleavage resistant repressor	RB800, R. Brent, USA	Mount et al. (1972); Little et al. (1980); R. Brent (personal communication)
<i>lexA</i> ⁺	–	Wild type	AB1157, G. Walker, USA	Bachmann (1987)
<i>lexA51</i>	<i>lexA (Def), spr51</i>	Defective repressor	DM1187, E. Eisenstadt, USA	Mount (1977); Castellazzi et al. (1972); Roberts et al. (1978)
<i>umuC</i> alleles				
<i>umuC122::Tn5</i>	–	Nonmutable	GW2100, G. Walker, USA	Elledge and Walker (1983b)
<i>umuC36</i>	–	Nonmutable	EE134, E. Eisenstadt, USA	Kato and Shinoura (1977); G. Walker (personal communication)
Other alleles				
<i>dnaQ49</i>	–	Thermally inducible mutator, temperature sensitive viability	KH1366, B. Bachmann, USA	Horiuchi et al. (1978; 1981)
<i>mutL::Tn10</i>	–	Mismatch repair defective	ES1484, E. Eisenstadt, USA	Radman and Wagner (1986); E. Siegel (personal communication)

Table 2

Comparison of the mutation rates conferred by *dnaQ49* and *mutL::Tn10* in *recA*⁺ and *recA730* strains

Strain	Rif ^r /generation per 10 ⁸ cells ^a			
	32° C	<i>n</i>	37° C	<i>n</i>
<i>dnaQ</i> ⁺ <i>mut</i> ⁺				
<i>recA</i> ⁺ <i>lexA51</i>	0.56	10	0.65	10
<i>recA730</i> <i>lexA51</i>	10.6	12	11.1	12
<i>dnaQ49</i> <i>mut</i> ⁺				
<i>recA</i> ⁺ <i>lexA51</i>	90	28	266	22
<i>recA730</i> <i>lexA51</i>	134	29	417	22
<i>dnaQ</i> ⁺ <i>mutL::Tn10</i>				
<i>recA</i> ⁺ <i>lexA51</i>	115	10	110	9
<i>recA730</i> <i>lexA51</i>	127	10	135	9

n, number of determinations

^a Cultures were grown in salt-free LB at the designated temperatures

Table 3

The plating efficiencies of *umuC⁻ lexA51 dnaQ49* strains at 32° C and 37° C on salt-free LB media

Strain	Viable cells per ml $\times 10^{-8}$		
	LB	Salt-Free LB	
	32° C	32° C	37° C
<i>umuC⁺ lexA51 dnaQ49</i>			
<i>recA⁺</i>	9.9	6.6	6.9
<i>recA730</i>	13.2	9.5	11.2
<i>umuC122::Tn5 lexA51 dnaQ49</i>			
<i>recA⁺</i>	9.3	5.9	8.5
<i>recA730</i>	6.9	2.5	0.0085