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# Efficient translesion replication in the absence of *Escherichia coli* Umu proteins and 3′–5′ exonuclease proofreading function

**(translesion replication/UV mutagenesis/*umuDC*/SOS response/UVM)**

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**ABSTRACT** **Translesion replication (TR) past a cyclobutane pyrimidine dimer in *Escherichia coli* normally requires the UmuD′$_2$C complex, RecA protein, and DNA polymerase III holoenzyme (pol III). However, we find that efficient TR can occur in the absence of the Umu proteins if the 3′–5′ exonuclease proofreading activity of the pol III ε-subunit also is disabled. TR was measured in isogenic *uvrA6* Δ*umuDC* strains carrying the dominant negative *dnaQ* allele, *mutD5*, or Δ*dnaQ spq-2* mutations by transfecting them with single-stranded M13-based vectors containing a specifically located *cis-syn* T–T dimer. As expected, little TR was observed in the Δ*umuDC dnaQ*$^+$ strain. Surprisingly, 26% TR occurred in UV-irradiated Δ*umuDC mutD5* cells, one-half the frequency found in a *uvrA6 umuDC*$^+$*mutD5* strain. *lexA3* (Ind$^-$) derivatives of the strains showed that this TR was contingent on two inducible functions, one LexA-dependent, responsible for ≈70% of the TR, and another LexA-independent, responsible for the remaining ≈30%. Curiously, the Δ*umuDC* Δ*dnaQ spq-2* strain exhibited only the LexA-independent level of TR. The cause of this result appears to be the *spq-2* allele, a *dnaE* mutation required for viability in Δ*dnaQ* strains, since introduction of *spq-2* into the Δ*umuDC mutD5* strain also reduces the frequency of TR to the LexA-independent level. The molecular mechanism responsible for the LexA-independent TR is unknown but may be related to the UVM phenomenon [Palejwala, V. A., Wang, G. E., Murphy, H. S. & Humayun, M. Z. (1995) *J. Bacteriol.* 177, 6041–6048]. LexA-dependent TR does not result from the induction of pol II, since TR in the Δ*umuDC mutD5* strain is unchanged by introduction of a Δ*polB* mutation.**

Almost all evidence indicates that translesion replication (TR) past a *cis-syn* cyclobutane pyrimidine dimer, or comparable replication-inhibiting DNA lesion, in *Escherichia coli* is entirely dependent on the presence of the UmuD′$_2$C protein complex (reviewed in refs. 1 and 2). Numerous observations support this view, but transfection of a Δ*umuDC* strain with a single-stranded vector carrying a uniquely located T–T dimer provides a particularly clear illustration of this dependency; <0.5% TR occurs in these cells whether the LexA regulon is induced or not (3). Two counter examples have been recorded, however. First, near normal frequencies of temperature sensitive (ts) phage mutations were found in photoreactivated Δ*recA* and *umuC122*::Tn*5* mutants infected with single-stranded S13 virus that had been heavily UV-irradiated (4). In the absence of sequence information for the ts mutations, it is not known whether they possess the characteristics of UV-induced events. Second, ≈25% of the wild-type yield of UV-induced *lacI* mutations was observed in a *umuC122*::Tn*5* mutant (5). The induced spectrum was closely similar to that in the wild-type and unlike the spontaneous spectrum, and the mutations occurred principally at bipyrimidine sites. In view of the disparity between these and other data showing the complete dependence of TR on the Umu proteins, it is likely that these unusual results derive from some unknown factor specific to the particular experimental procedures used.

Why efficient TR should be dependent on the UmuD′$_2$C complex is not known, and indeed the molecular mechanisms underlying this process in general, as well as the functions of many of the proteins involved, are still poorly understood (reviewed in refs. 1 and 2). TR is thought to be carried out usually by DNA polymerase III (pol III) holoenzyme, together with RecA protein and the UmuD′$_2$C complex (6, 7). Experiments with ts mutants in the *dnaE* gene, which encodes the α-subunit of pol III indicate that this enzyme is responsible for most TR (8–13), but there is also evidence to suggest that pol II also may be used sometimes (14, 15). The 10 individual subunits that constitute the pol III holoenzyme, some of which can also associate with pol II (16), are organized into four subassemblies; a τ dimer, which links two core polymerases; the γ clamp-loading complex, composed of the γ-, δ-, δ′, χ-, and Ψ-subunits; the β-dimer subunit constituting the sliding clamp; and the core polymerase composed of the α-catalytic subunit, the ε-subunit encoding the 3′–5′ exonuclease-proofreading activity, and the θ-subunit of unknown function (see refs. 17 and 18 for recent reviews). There is currently no evidence to directly implicate the γ-complex itself in TR, but the β-subunit appears to influence it; overproduction of β reduces UV-induced mutagenesis, a condition that can be alleviated by excess UmuD′C-like proteins (19).

Greatest attention has been given to understanding the function in TR of the three polypeptides of the pol III core. The importance of the catalytic function of the α-subunit is obvious, but whether the stringent selection of correct nucleotides imposed by α is maintained during TR is not known. Of the remaining two subunits of the core, the θ polypeptide does not appear to play any significant part in TR because deletion of *holE*, which encodes θ, has no apparent effect on replication or Weigle reactivation of φX174 phage (20). With respect to the ε-subunit, a variety of evidence suggests that its proofreading activity is suppressed during TR, though how this is achieved and why it is necessary is less clear. Similarly, it is not known whether the ε-subunit possesses other functions im-

Abbreviations: TR, translesion replication; ts, temperature sensitive; pol I, pol II, and pol III, DNA polymerase I, II, and III holoenzyme; AAF, *N*-2-acetylaminofluorene; SOS, derepression of DNA-damage inducible genes.

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portant for TR. An early model (21) suggested that proofreading might be responsible for the inhibition of chain elongation caused by mutagenic lesions and that successful TR might therefore require suppression of proofreading during the bypass step. According to this model, insertion of any nucleotide opposite the damage site triggers the proofreading function because the lesion is incapable of base pairing, thus leading to a futile cycle of nucleotide addition and removal, which could prevent chain elongation. In keeping with this suggestion, increased turnover of dNTPs appears to occur during replication *in vitro* on UV-irradiated templates by purified *E. coli* DNA polymerase I, which also possesses a proofreading function (21). Similar observations also have been made with pol III (22). Furthermore, there is evidence indicating that the RecA/UmuD′C protein complex may interact with ε (23–25) and that the 3′–5′ exonuclease proofreading function of polymerase III is in fact suppressed or inactive during TR (13, 26, 27). However, it is not clear whether suppression of proofreading is needed because correct base pairs cannot be formed. Both thymines in a T–T cyclobutane dimer can hydrogen-bond with adenine (28, 29), for example, and replication past this lesion *in vivo* is >93% accurate (30, 31), even though a dimer can almost completely block replication in the absence of SOS induction, that is, derepression of DNA-damage inducible genes. Overproduction of the ε-subunit has been shown to inhibit UV-induced mutagenesis (32, 33), but such an effect has been subsequently attributed to the DNA-binding properties of ε, rather than its proofreading function (34).

Perhaps the most decisive evidence for the absence of a role for ε in UV-induced TR was reported by Slater and Maurer (27). They found that replication of UV-irradiated φX174 phage DNA was very similar in *dnaQ*$^+$ and Δ*dnaQ* strains of *Salmonella typhimurium*. Their data also indicated that deletion of *dnaQ* did not alleviate the requirement for Umu-like proteins in TR, implying that inhibition of chain elongation may not be exclusively caused by proofreading. It is not certain, however, that the properties of the Δ*dnaQ* strain used in these studies are entirely dependent on loss of the 3′–5′ exonuclease activity because the ε-subunit appears also to possess an important structural role within the core enzyme (35), in addition to the proofreading function. Moreover, Δ*dnaQ* strains also necessarily contain *spq-2*, a *dnaE* mutation (V832G) that is required for viability in these cells (20, 36), and it is not yet known whether the *spq-2* mutation itself influences TR.

One way of studying the individual roles of these proteins in TR is to uncover circumstances in which otherwise essential proteins are not required for this process. During the course of experiments designed to reinvestigate the *in vivo* role of the ε-subunit in TR, we discovered that efficient TR past a T–T *cis-syn* cyclobutane dimer could in fact occur in the absence of UmuDC, or other homologous proteins, if the proofreading activity of the ε-subunit also was absent. A related observation has been made recently in similar studies using a single-stranded vector containing a specifically located *N*-2-acetylaminofluorene (AAF) adduct (37).

## MATERIALS AND METHODS

**Bacterial Strains and Plasmids.** The *E. coli* K-12 strains used in the TR experiments (Table 1) were all derivatives of the *recA*$^+$ *lexA*$^+$ *umuDC*$^+$ *uvrA6* strain, TK603 (38) and, except where noted, all are Δ*umuDC*. The *dnaQ*$^+$ strain RW82 (39) carries the Δ*umuDC595*::*cat* allele, whereas the remaining strains are all derivatives of EC8 (40), a strain that is isogenic to RW82 except that it carries the Δ*umuDC596*::*ermGT* allele in place of the Δ*umuDC595*::*cat* allele. All strains were constructed by generalized P1 transduction with P1*vir* (41). DV02 was constructed by first transducing the *dnaQ*$^+$/*dnaQ903*::*tet* pBIP3 locus (20) from RM4321 into EC8 and selecting for tetracycline and kanamycin resistant colonies. A transductant of this kind was then itself transduced with P1 grown on NR11628 (*spq-2 zae*::Tn10*d*-Cam). The *dnaQ*$^+$/*dnaQ903*::*tet* pBIP3 locus in several Tet$^R$/Kan$^R$/Cam$^R$ colonies was resolved by sucrose selection (42) and by subsequently screening for Tet$^R$ and Kan$^S$ colonies. One colony that had the desired antibiotic resistances, uniform colony morphology (provided by *spq-2*), as well as an elevated spontaneous mutator rate (as a result of the *dnaQ903*::*tet* allele) was chosen for further study.

DV03 was constructed by transducing the *spq-2 zae*::Tn10*d*-Cam locus from NR11628 into EC8 and selecting for chloramphenicol resistance. The presence of the *spq-2* allele (DnaEV832G) (20, 43) was identified by colony hybridization (42) with two probes, one corresponding to the wild-type *dnaE* sequence (5′-GCGAAATCGTGTATGGTATT-3′) and the other corresponding to the *spq-2* sequence (20) (5′-GCGAAATCGGGTATGGTATT-3′). One colony that tested positive with the *spq-2* probe and negative with the *dnaE*$^+$ probe was chosen for further analysis.

In an attempt to analyze the effects of a structurally intact DNA polymerase core, but one that lacked proofreading functions, we considered three potential missense mutant alleles, *dnaQ49* (V96G) (45), *mutD5* (T15I) (46), and *dnaQ926* (D12A and E14A) (46). The DnaQ49 protein is, however, recessive to *dnaQ*$^+$ (45) and may, therefore, exhibit similar

Table 1. Strains used in this work

| Strains | Relevant genotype | Source or reference |
|---|---|---|
| RM4231 | *dnaQ*$^+$/*dnaQ903*::*tet* heteropolid with integrated pBIP3 vector | R. Maurer |
| NR11628 | *spq-2 zae*::Tn10*d*-Cam | R. Schaaper |
| NR9458 | *mutD5 zaf*-13::Tn*10* | R. Schaaper |
| SMH10 | *uvr*A6 | 30 |
| TK603 | *uvrA6* | 38 |
| RW82 | As TK603, but Δ*umuDC595*::*cat* | 39 |
| EC8 | As TK603, but Δ*umuDC*596::*ermGT* | 40 |
| RW532 | As EC8, but *lexA3* (Ind$^-$) | R. Woodgate |
| DV02 | As EC8, but *dnaQ*903::*tet spq*-2 *zae*::Tn10*d*-Cam | This paper |
| DV03 | As EC8, but *spq*-2 *zae*::Tn10*d*-Cam | This paper |
| DV05 | As EC8, but *mutD5 zaf*-13::Tn*10* | This paper |
| DV06 | As EC8, but *dnaQ*903::*tet spq*-2 *zae*::Tn10*d*-Cam *lexA3* (Ind$^-$) | This paper |
| DV08 | As EC8, but Δ*polB*::Ω*Spc* | R. Woodgate |
| DV09 | As EC8, but *mutD5 zaf*-13::Tn*10 spq*-2 | This paper |
| DV10 | As EC8, but *mutD5 zaf*-13::Tn*10* Δ*polB*::ΩSpc | This paper |
| DV12 | As TK603, but *mutD5 zaf*-13::Tn*10* | This paper |
| DV13 | As EC8, but *mutD5 zaf*-13::Tn*10 lexA3* (Ind$^-$) | This paper |

characteristics as the Δ*dnaQ903*::*tet* mutation. *dnaQ926* appears to be completely defective for proofreading functions and is also dominant-negative suggesting that it interacts efficiently with α. Unfortunately, the mutation rate in strains carrying *dnaQ926* allele is so high that it leads to "error catastrophe" and the *dnaQ926* mutation is only viable in combination with antimutator alleles of *dnaE* (46). We therefore chose to analyze the effects of *mutD5*. The MutD5 protein is also a dominant-negative protein that exhibits a greatly reduced exonuclease activity (47). Furthermore, the spontaneous mutation rate is elevated $10^3$- to $10^4$-fold so that it saturates mismatch repair (48). The fact that *mutD5* cells are viable without an extragenic suppressor suggests, however, that the MutD5 protein retains residual proofreading activity but only enough to avoid "error-catastrophe" (46). DV05 was therefore constructed by transducing the *mutD5 zaf-13*::Tn*10* locus from NR9458 into EC8 by selecting for tetracycline resistance and screening for an increased spontaneous mutation rate when grown in rich medium (48, 49). Strain DV06 was constructed in a similar manner to DV02, except that the parental strain was RW532 (a *lexA3* ($Ind^-$) derivative of EC8). The F′IQ plasmid from DH5F′IQ (Life Technologies) was then transferred to RW82, DV02, DV03, DV05, and DV06 by conjugation (41). Several isolates were picked and screened for their ability to support M13 growth. On occasions, and perhaps as a consequence of the high mutator activity, some of the *dnaQ*$^-$ cells failed to support phage growth. To avoid this problem, subsequent strains were made by first mating the F′ into the recipient followed by P1 transduction of the *mutD5* allele. By using such an approach, DV09 was constructed by transducing the *mutD5 zaf13*::Tn*10* locus from NR9458 into DV03F′IQ selecting for tetracycline resistance and an increase in spontaneous mutator activity. Interestingly, the spontaneous mutation rate of DV09 was 2- to 3-fold lower than the isogenic strain DV05, suggesting that *spq-2* encodes an antimutator allele of *dnaE*. DV10 was constructed by similarly moving the *mutD5 zaf13*::Tn*10* locus into DV08F′IQ (DV08 being a Δ*polB*::Ω*spc* derivative of EC8), DV12 by transducing into TK603F′IQ and finally DV13 by transducing into RW532 F′IQ. To minimize the potential of accumulating additional mutations, the *dnaQ*$^-$ strains were maintained as frozen stocks and were used directly as the inoculum for the cultures used in TR experiments. Periodically, we used separate strain isolates of a particular genotype, but on all occasions, the TR results from these independent isolates were similar.

**Single Lesion Vectors.** Single-stranded vectors carrying a unique T–T *cis-syn* cyclobutane dimer were constructed as described (50). In brief, M13 mp7L2 hybrid phage single-stranded DNA was linearized with *Eco*RI by restriction within a small duplex region and then recircularized by annealing with a 51-mer scaffold oligonucleotide, which leaves an 11 nucleotide gap between the vector ends. An 11-mer containing a T–T dimer at a unique site or, for the controls, a dimer-free 11-mer, was then efficiently ligated into this gap. The dimer-containing 11-mer was prepared as described (50), and its purity was ≈ 99.5%. After ligation, the scaffold was removed by heat denaturation in the presence of a 10-fold molar excess of anti 51-mer, with sequence complementary to the scaffold. Five hundred microliters of competent cells of each strain, induced for SOS functions by irradiation with 4 J/m$^2$ or left uninduced, were transfected with 5 ng of dimer-containing construct or an equal amount of control vector and the resulting plaques counted. Because it was highly transformable, only 1.5 ng of DNA was used with strain DV02, and conversely 7.5 ng of DNA was used with the poorly transforming DV03 strain. The frequency of translesion synthesis was estimated by normalizing the number of plaques from lesion-containing construct to that of the control. Hybridization and sequence analysis was used to examine replication events at the T–T dimer target site and to detect a low frequency of plaques resulting from transfection with vectors lacking the 11-mer sequence or from other events not involving translesion synthesis (50). TR frequencies were calculated only from plaque counts resulting from true bypass events.

## RESULTS

**Experimental Approach.** TR was measured by transfecting cells from a set of isogenic Δ*umuDC uvrA6* strains that carried various *dnaQ*, *dnaE*, or *lexA* alleles with a single-stranded vector carrying a specifically located T–T *cis-syn* cyclobutane dimer. Compared with the use of UV-irradiated plasmids or viruses, this tool has the advantage of measuring bypass frequency and other mutagenic parameters individually and for a specified lesion. Its chief advantage, however, is the ability of this method to provide reliable data when the spontaneous mutation frequency is very high, and the sensitivity with which it measures the mutagenic parameters, the result of using a population of vector molecules, which uniformly carry the dimer.

**Two Inducible Functions Permit Efficient TR when Umu Proteins and Proofreading Activity Are both Absent.** During the course of experiments designed to investigate the role of the ε-subunit in TR, we observed the occurrence of 25.5% TR past a T–T dimer in a UV-irradiated *mutD5* strain in which the *umuDC* operon had been deleted and which contained no other homologous Umu-like proteins (Table 2, line 4). This finding was highly surprising because a previous study had shown that a single *cis-syn* cyclobutane dimer was an efficient block to replication in Δ*umuDC* cells (3), and a similar result was observed in the present series of experiments; only 2.4% bypass was observed in UV-irradiated cells of a Δ*umuDC dnaQ*$^+$ strain and 1.0% in unirradiated cells (Table 2, line 3). The efficiency is also surprising; 25.5% TR is one-half that observed in an isogenic *mutD5* strain with a wild-type *umuDC* operon (51.9%, Table 2, line 2) and fully equal to that seen in a *uvrA6* but otherwise wild-type strain (Table 2, line 1).

Table 2. Frequency of replication past a T–T cis-syn cyclobutane dimer in UV-irradiated and unirradiated cells

| Strain | Relevant genotype | No. expts | % Translesion replication | |
|---|---|---|---|---|
| | | | −UV | +UV |
| SMH10 | *umuDC*$^+$ *dnaQ*$^+$ | >20 | <0.5 | 15–25 |
| DV12 | *umuDC*$^+$ *mutD5* | 4 | 5.5 ± 0.6 | 51.9 ± 3.8 |
| RW82 | Δ*umuDC dnaQ*$^+$ | 15 | 1.0 ± 0.1 | 2.4 ± 0.2 |
| DV05 | Δ*umuDC mutD5* | 7 | 17.7 ± 3.4 | 25.5 ± 2.8 |
| DV13 | Δ*umuDC mutD5 lexA3* | 5 | 4.6 ± 0.3 | 10.9 ± 0.6 |
| DV02 | Δ*umuDC* Δ*dnaQ spq-2* | 7 | 3.7 ± 1.5 | 12.9 ± 1.5 |
| DV06 | Δ*umuDC* Δ*dnaQ spq-2 lexA3* | 5 | 2.8 ± 0.6 | 9.4 ± 1.5 |
| DV09 | Δ*umuDC mutD5 spq-2* | 4 | 5.5 ± 0.9 | 16.5 ± 2.5 |
| DV10 | Δ*umuDC mutD5* Δ*polB* | 5 | 14.6 ± 2.6 | 34.2 ± 5.4 |

Most of the TR seen in UV-irradiated Δ*umuDC mutD5* cells was made possible by the combined contributions of two inducible functions, one LexA-dependent and the other LexA-independent. The presence of a LexA-dependent component was shown by introducing a *lexA3* (Ind$^-$) allele, which prevents induction of the LexA regulon, into the Δ*umuDC mutD5* strain. TR drops from 25.5% to 10.9% in UV-irradiated Δ*umuDC mutD5 lexA3* (Ind$^-$) cells, and in unirradiated cells the frequency is only 4.6% (Table 2, line 5). After subtracting the noninduced level of TR (4.6%) from the LexA-independent response, these data suggest that 14.6% TR (70% of the total) of the inducible phenomenon is LexA-dependent and 6.3% (30% of the total) is LexA-independent (LexA-independent response: 10.9 − 4.6 = 6.3; LexA-dependent response: 25.5 − 4.6 − 6.3 = 14.6; 6.3/6.3 + 14.6 = 30%, 14.6/6.3 + 14.6 = 70%). The low amount of TR in the unirradiated Δ*umuDC mutD5 lexA3* (Ind$^-$) strain explains the otherwise puzzling observation of 17.7% TR in unirradiated Δ*umuDC mutD5 lexA*$^+$ cells (Table 2, line 4), which at first sight seems to contradict the conclusion that efficient TR mostly depends on inducible functions. The finding that, in the absence of UV irradiation to the cells, the *lexA3* (Ind$^-$) mutation reduces TR in Δ*umuDC mutD5* cells to approximately one-quarter of the *lexA*$^+$ frequency suggests that the LexA regulon is at least partially induced in *mutD5*. In support of this conclusion, preliminary evidence from Western analysis of extracts from unirradiated Δ*umuDC mutD5 lexA*$^+$ cells harboring a low copy-number plasmid encoding UmuD shows they express appreciable levels of UmuD protein, in contrast to extracts from similar Δ*umuDC dnaQ*$^+$ or Δ*umuDC mutD5 lexA3* (Ind$^-$) cells, in which no UmuD protein can be detected (data not shown). In light of these observations, we refer to the *lexA3*-dependent bypass as inducible, even though ≈60% of the bypass can occur in unirradiated cells, that is, in the absence of an exogenously applied inducing treatment.

**The *spq-2* Mutation Is Phenotypically Similar to the *lexA3* (Ind$^-$) Allele.** In an attempt to discover whether the structural function of the ε-subunit within pol III core also had an effect on TR, we performed similar experiments with a Δ*umuDC* Δ*dnaQ* strain. This strain also necessarily contains the *spq-2* allele, a *dnaE* V832G mutation that is required for viability in these poorly growing cells (18, 34). Only approximately one-half as much TR was seen in the UV-irradiated Δ*umuDC* Δ*dnaQ spq-2* strain, compared with its *mutD5* counterpart (12.9% vs. 25.5%, Table 2, lines 6 and 4), and moreover, as shown by results from a Δ*umuDC* Δ*dnaQ spq-2 lexA3* (Ind$^-$) derivative, the inducible response was for the most part LexA-independent (Table 2, line 6). These observations suggest that the *spq-2* mutation may be phenotypically similar to the *lexA3* (Ind$^-$) allele in these *dnaQ*$^-$ backgrounds. We therefore examined the effect of the *spq-2* mutation on the efficiency of TR in the Δ*umuDC mutD5* strain (Table 2, line 8). The frequency of TR in this strain was substantially decreased by the *spq-2* mutation, both in the UV-irradiated cells and, significantly, in the unirradiated cells (16.5% vs. 25.5%, 5.5% vs. 17.7%, respectively). Comparison between lines 5 and 8 of Table 2 suggest that *spq-2* may be somewhat less effective in reducing TR than the *lexA3* (Ind$^-$) mutation (16.5% vs. 10.9% TR, 5.5% vs. 4.6% TR, in UV-irradiated and unirradiated cells, respectively), and the same may be indicated by comparisons between lines 6 and 7, where addition of the *lexA3* (Ind$^-$) mutation to the Δ*umuDC* Δ*dnaQ spq-2* strain marginally decreases TR, although the statistical uncertainties associated with each of these values prevent a firm conclusion. Similarly, a comparison between lines 6 and 8 might possibly indicate that TR by the catalytic subunit alone is less efficient than TR by a pol III core containing the proofreading defective, but structurally functional, *mutD5* protein, though the effect is at best small and again the data are currently inadequate to substantiate this point. Very little TR is seen in a Δ*umuDC dnaQ*$^+$ *spq-2* strain (data not shown), and this strain is essentially phenotypically identical to RW82. Plasmid-encoded levels of UmuD protein are lower in extracts from Δ*umuDC* Δ*dnaQ spq-2*, as compared with Δ*umuDC* Δ*dnaQ*, cells indicating that the *spq-2* mutation partially suppresses the induction of the LexA regulon (data not shown), though it may additionally have a direct effect on bypass efficiency itself.

## DISCUSSION

Many studies attest to the essential role played by the UmuD′$_2$C complex in replication past mutagenic lesions that can potentially inhibit or delay DNA chain elongation (see refs. 1, 2, 6, and 7). Moreover, of particular significance for the work reported here, it has been well established that very little TR occurs past a T–T *cis-syn* cyclobutane dimer located in a single-stranded vector unless the LexA regulon is induced, to promote formation of the UmuD′$_2$C complex (30, 31). Furthermore, no TR occurs even after LexA regulon is induced if the *umuDC* operon has been deleted (3). We now find, however, that efficient TR past a T–T dimer can in fact take place in a UV-irradiated Δ*umuDC* strain if the 3′–5′ exonuclease proofreading function of the pol III ε-subunit, encoded by *dnaQ*, also is disabled by the *dnaQ* mutation, *mutD5*. The amount of TR observed (26%) is one-half of that seen in an isogenic *mutD5* strain, which carries a wild-type copy of the *umuDC* operon on its chromosome (52%), and is fully equal to the amount commonly seen in a *uvrA6* but otherwise wild-type strain (30, 31).

Because the *lexA3* (Ind$^-$) mutation abolishes only part of the TR, dimer bypass in the Δ*umuDC mutD5* strain appears to be contingent on two inducible functions—one LexA-dependent and responsible for 70% of the TR and the other LexA-independent, which is responsible for the remaining 30%. Exposure of *E. coli* to DNA damaging agents results in the induction of at least 40–50 proteins, approximately 27 of which are transcriptionally regulated by the LexA repressor protein. The remaining proteins are inducible but not directly regulated by LexA (1, 51). Which of these confers a capability for TR on the Δ*umuDC mutD5* strain is not yet known, although experiments to identify them are currently in progress. Whatever their identity, the inducible factors presumably provide a substitute function that directly or indirectly compensates for one (or more) of the missing functions of the UmuD′$_2$C complex. The UmuD′$_2$C complex probably performs multiple functions, such as transient suppression of proofreading efficiency, facilitation of nucleotide insertion, and promotion of DNA chain elongation (1, 2, 6, 7). Partial relief from the requirement for the UmuD′$_2$C complex in TR appears, however, to be conditional on the absence of 3′–5′ exonucleolytic proofreading, so the inducible factor presumably supplies a function other than suppression of proofreading, or one that can operate in the absence of this activity. As a LexA-regulated protein that does not use the ε-subunit, DNA polymerase II is an obvious candidate for the LexA-dependent factor. This enzyme does not carry out the observed TR, however, because the frequency of TR is not decreased when *polB*, which encodes pol II, is deleted in the Δ*umuDC mutD5* strain (Table 2, line 9).

The identity of the LexA-independent inducible factor is also unknown at present but may possibly be related to the UVM phenomenon discovered and investigated by Humayun and coworkers (52, 53). The UVM phenomenon, which occurs in the absence of UmuDC and RecA proteins, was named for the *UV M*odulation of the mutation spectrum induced by an ε cytosine lesion, but, as the authors note, UV irradiation of the cells also enhances TR past this lesion. The molecular mechanism responsible for UVM is still unknown, but recent evidence suggests that the UVM phenotype is constitutive in *mutA* or *mutC* mutant strains (53), which are spontaneous

mutators (54). The mutator phenotype is caused by a particular mutant glycine tRNA, in which the anticodon sequence 3′-CUG replaces the normal 3′-CCG in the *glyV* or *glyW* gene, respectively (55), a change that can lead to the mistranslation of aspartate codons as glycine. Slupska *et al.* (55) suggest that the mutator phenotype results from low level mistranslation of aspartate residues in the ε-subunit of pol III, giving a small fraction (perhaps 1–2%) of cells in which proofreading is transiently deficient. Murphy and Humayun (53) point out that this explanation is unlikely to be correct, however; assuming that the mutator condition is itself responsible for UVM, the fraction of the cell population expressing this phenotype must be much larger than the fraction in which a transient low level of mistranslation of *dnaQ* occurs. They therefore suggest that the mistranslation step occurs upstream of proofreading, perhaps leading to the production of a proofreading suppressor. It would be interesting to determine whether this postulated factor is the product of the *npf* gene, which may also suppress proofreading (37). At the present, exploration of the UVM phenomenon has been concerned more with effects on mutation spectrum, and a mechanism that might enhance TR remains to be developed. Whatever the outcome, however, discovery of the molecular identity of the two inducible functions is likely to provide useful insights into the enzymatic roles played by the UmuD′$_2$C complex and ε-subunit in TR.

Although we do not yet know the identity of the molecular mechanisms underlying the TR that occurs in a Δ*umuDC mutD5* strain, it nevertheless appears likely that both the LexA-independent and -dependent events are nonslippage processes and therefore different from the slippage mechanism responsible for most of the bypass of an AAF-guanine adduct within a GGCG$^{AAF}$CC *Nar*I or GGG$^{AAF}$ sequence, which are also enhanced in the absence of proofreading (37). The T–T dimer was located within a 5′-GCAAGTTGGAG-3′sequence, which intrinsically is unlikely to favor slippage. More particularly, however, slippage in this sequence might be expected to produce either T → G or T → A mutations, but neither was found (Table 3). Cyclobutane dimers are relatively rigid structures capable of correct base pairing, so they probably inhibit formation of a slipped mutagenic intermediate and misalignment. As with TR past this lesion in the presence of Umu proteins and proofreading (30, 31), by far the most common event is the correct insertion of A–A opposite the T–T dimer. When mutation events occur, they are almost exclusively 3′-T → C substitutions, a result that is different from those observed in wild-type strains and whose implications will be discussed in a subsequent publication. Bypass of an AAF adduct within a single-stranded plasmid by a slippage mechanism is strongly enhanced in *mutD5* strains, but bypass of this adduct by a nonslipped mechanism, a process more akin to TR past a dimer, is not increased (37). More particularly, unlike the present results, very little TR occurs in a Δ*umuDC* strain whether *mutD5* or *dnaQ*$^+$ (37), suggesting that the phenomenon we observe is likely to be lesion-specific.

As a result of comparing TR in Δ*umuDC* Δ*dnaQ spq-2* and Δ*umuDC mutD5* strains, we made the surprising observation that the *spq-2* mutation, a V832G allele of *dnaE* that is required for viability in Δ*dnaQ* strains (18, 34), has a phenotype similar to that of *lexA3* (Ind$^-$), which encodes a noncleavable repressor. This similarity may be merely coincidental and occur because an α-catalytic subunit containing the V832G substitution is only approximately one-half as efficient at performing TR as wild-type α. However, the *spq-2* allele closely mimics the *lexA3* (Ind$^-$) mutation in disproportionately diminishing TR in unirradiated *mutD5* cells (Table 2, lines 4 and 8) in which the LexA regulon appears to be partially induced. The original Δ*dnaQ* strain in which the *spq-2* mutation was isolated also may have exhibited SOS induction (36). More particularly, preliminary evidence indicates that the *spq-2* mutation does, in fact, partially reduce levels of the LexA-regulated protein, UmuD, in Δ*umuDC mutD5* cells carrying a plasmid expressing this protein. One possible explanation for these findings is that both *dnaQ* mutant alleles result in a pol III enzyme that tries unsuccessfully to elongate from purine–purine mispairs rather than to edit them (13). Such a reaction is kinetically unfavorable and results in polymerase pausing, which itself is likely to induce the SOS response (56). During the course of this work, we discovered that the *dnaE* allele *spq-2* is an antimutator in a *mutD5* background. If *spq-2* is phenotypically similar to other isolated antimutator alleles, its phenotype most probably derives from more frequent dissociation from mispaired termini rather than continued efforts to extend them (57), perhaps resulting in less polymerase pausing and therefore less SOS induction. Whatever the means by which the *spq-2* allele decreases TR, this effect appears to be restricted to strains lacking UmuDC or Umu-like proteins, because there is no evidence for an *spq-2* mediated decrease in TR or for reduced SOS-induction in *spq-2* mutants that express either the Umu or Muc proteins (data not shown); but again, these findings are commensurate with the proposed action of *dnaE* antimutator alleles and of the Umu proteins (13).

Table 3. Sequence at T–T dimer target site in replicated vectors

| | UV | Sequence at T–T dimer site | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | J/m$^2$ | T–T | T–A | T–C | T–G | ΔT | Other | Total |
| All Δ*umuDC* strains | 0 | 1,119 | 0 | 38 | 0 | 2 | 0 | 1,159 |
| | 4 | 1,785 | 0 | 53 | 0 | 4 | 1 | 1,843 |

At first sight, our observation of efficient TR in Δ*umuDC mutD5* cells might seem to question the need for Umu or Umu-like proteins in bacteria. A variety of evidence (11, 24, 25) indicates that proofreading is in any case at least partially suppressed during TR, apparently fulfilling the precondition necessary for TR to occur in the absence of the UmuD′$_2$C complex. However, even higher TR efficiencies are found in cells capable of producing UmuD′$_2$C, and in the presence of plasmid- encoded MucA′$_2$B, TR efficiencies past a T–T dimer approach 100% (data not shown). At the same time, other lesions may present a greater block to TR than the T–T dimer, and successful bypass may therefore depend to a greater extent on the presence of these protein complexes. Further, because proofreading appears to be at least partly suppressed during TR, it might seem inconsistent that replication of the dimer-containing vector does not occur in uninduced wild-type cells, which contain virtually no UmuD′$_2$C complex. Almost no TR takes place in such cells, however, presumably because initially the cells are phenotypically *dnaQ*$^+$ and suppression of proofreading occurs only after the RecA/UmuD′$_2$C proteins interact with ε and other pol III subunits.

Finally, our observation that the requirement for UmuD′$_2$C is partly relieved by inactivation of proofreading appears to conflict with Slater and Maurer's evidence (27) indicating that this is not the case. However, Slater and Maurer did not investigate the conditions under which we see efficient TR. They investigated the effect of the absence of MucA′B proteins on Weigle reactivation only in a Δ*dnaQ spq-2* strain, and only in the absence of UV-irradiation to the cells, conditions under which we also see almost no TR.

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