Inactivation of DNA proofreading obviates the need for SOS induction in frameshift mutagenesis

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ABSTRACT Translesion synthesis at replication-blocking lesions requires the induction of proteins that are controlled by the SOS system in Escherichia coli. Of the proteins identified so far, UmuD•, UmuC, and RecA• were shown to facilitate replication across UV-light-induced lesions, yielding both error-free and mutagenic translesion-synthesis products. Similar to UV lesions, N-2-acetylaminofluorene (AAF), a chemical carcinogen that forms covalent adducts at the C8 position of guanine residues, is a strong replication-blocking lesion. Frameshift mutations are induced efficiently by AAF adducts when located within short repetitive sequences (25, 42–45). Induction of frameshift mutations involves the formation and subsequent elongation of a slipped mutagenic intermediate (SMI; refs. 43 and 46–48). On the other hand, TLS at these lesions can also occur without slippage in an error-free manner (4, 28). Recent experiments have suggested that DNA-proofreading activity encoded by the dnaQ gene is inoperative during SOS-induced UV mutagenesis (34). The SOS response is not turned on constitutively in dnaQ strains, as neither TLS nor UV mutagenesis is induced in proofreading-deficient strains (34, 35). In addition, overexpression of the dnaQ gene product was found to inhibit both spontaneous and induced SOS mutator activities (36, 37). Indirect evidence has suggested that RecA• (the active RecA filament), UmuC, and UmuD•, the mutagenic form of UmuD, may facilitate some of the steps required for efficient TLS (38, 39). On the other hand, it was suggested that RecA• and UmuD• somehow interact with the e-subunit (40). The cold-sensitivity phenotype in strains overexpressing UmuD• was also taken as evidence for the interaction of these proteins with the replication machinery (41).

We have used plasmid constructions with single dG-AAF, a model lesion that severely hinders DNA replication, to study translesion synthesis and induced mutagenesis (4, 42–44). dG-AAF adducts efficiently induce frameshift mutations when located within short repetitive sequences (25, 42–45). Induction of frameshift mutations involves the formation and subsequent elongation of a slipped mutagenic intermediate (SMI; refs. 43 and 46–48). On the other hand, TLS at these lesions can also occur without slippage in an error-free manner (4, 28). Recently, we have shown that although elongation from the nonslipped intermediate depends on functional umuDC• gene products, elongation from the slipped intermediate is ~25-fold increase in the proportion of TLS events and to a concomitant proportional increase in the induced mutation frequency (4). These observations unambiguously prove that SOS mutagenesis results from an increase in the use of the TLS pathway at the expense of the error-free DA pathways.

Most models for SOS mutagenesis postulate the involvement of a modified version of DNA polymerase III holoenzyme (9–16). Of the SOS-regulated genes, three of them, recA, umuD, and umuC, were found to be essential for UV-induced mutagenesis (17–24). An additional SOS-controlled function, designated Npf for NarI processing factor, was shown to stimulate strongly AAF-induced frameshift mutagenesis independently of recA, umuD, and umuC (25–28).

Both in vivo and in vitro data suggest that incorporation of a nucleotide opposite a lesion is not rate-limiting, whereas elongation from the lesion terminus is (29–32). We can assume that two modifications of the DNA polymerase that seem to facilitate the efficiency of TLS are (i) proofreading inactivation to prevent polymerase idling at the lesion site and (ii) improvement of the ability of the polymerase to elongate from a lesion terminus (29–31, 33). In vivo experiments have suggested that DNA-proofreading activity encoded by the dnaQ gene is inoperative during SOS-induced UV mutagenesis (34). The SOS response is not turned on constitutively in dnaQ strains, as neither TLS nor UV mutagenesis is induced in proofreading-deficient strains (34, 35). In addition, overexpression of the dnaQ gene product was found to inhibit both spontaneous and induced SOS mutator activities (36, 37). Indirect evidence has suggested that RecA• (the active RecA filament), UmuC, and UmuD•, the mutagenic form of UmuD, may facilitate some of the steps required for efficient TLS (38, 39). On the other hand, it was suggested that RecA• and UmuD• somehow interact with the e-subunit (40). The cold-sensitivity phenotype in strains overexpressing UmuD• was also taken as evidence for the interaction of these proteins with the replication machinery (41).

Many mutagens react with DNA by forming covalent adducts that strongly interfere with DNA replication and transcription if left unrepaired. Replication of damaged DNA molecules is achieved via two major strategies: (i) translesion synthesis (TLS), a process during which the replication machinery reads through the lesion with an associated risk of fixing a mutation, and (ii) damage avoidance (DA), a general strategy that takes advantage of the information contained in the complementary strand and thereby avoids the mutagenic risk associated with the TLS pathway. Two models of DA have been proposed (1–3), (i) recombinational repair and (ii) polymerase strand switching.

Recently, we have developed an experimental approach to measure the relative proportion at which TLS and DA pathways are used in Escherichia coli (4) and yeast (5). Our assay involves double-stranded DNA constructions with a genetic strand marker located across from a single N-2-acetylaminofluorene deoxyguanosine (dG-AAF) adduct. In E. coli, in the absence of SOS induction, only ~0.5% of the transformed colonies resulted from a TLS event, in good agreement with the fact that dG-AAF adducts are severe replication blocks in vitro (6–8). (SOS is a cellular response to replication-hindering damage in DNA.) The induction of the SOS response led to a...
Materials and Methods

Materials. Oligonucleotides with single AAF adducts located within sequence context 3G or NarI were purified and characterized as described (Fig. 1; refs. 42 and 43). The oligonucleotides were phosphorylated with T4 polynucleotide kinase and [γ-32P]ATP (150 Ci/mmol; 1 Ci = 37 GBq). The kinased oligonucleotides were repurified with PAGE (20%).

Construction of Double-Stranded and Single-Stranded Plasmids Containing Single Adducts. Double- and single-stranded plasmids containing single AAF adducts were constructed as described (42-44) by using the “gapped-duplex” approach followed by the ligation of a partially complementary oligonucleotide within the gap (Fig. 1). The dG-AAF adduct is located within two distinct sequence contexts, the 3G and NarI. For each context, a pair of parental plasmids is used to build the gapped duplex: for sequence 3G, pUC-Helper and pUC-(3G+3) and for the NarI sequence, pCU-Help and pCU-(NarI+3) (4, 44). The formation of the gapped duplex, ligation of the modified oligonucleotide, and purification of the covalently closed circular plasmids have been described in detail (44). For single-stranded plasmids, the same protocol is implemented except that the parental plasmid that yields the nonadducted strand is propagated in a bacterial strain that allows the incorporation of uracil residues (49, 50). Single-stranded plasmids are derived from these double-stranded constructs by in vitro enzymatic digestion of the strand containing uracil (44). Consequently, the adducted strand is the same in double- and single-stranded plasmids.

Strains. The bacterial strains used in the present work are derivatives of strain JM103: F“ trdD36 lacIq Δ(lacZΔM15 proA”B“/endA1 supE sbcBC thi-1 rpsL Δ(pro-lac). dnaQ alleles were introduced into JM103 or its ΔumuDC derivative by P1 transduction by using the following markers: dnaQ49 zae-502::Tn10 from strain EC2414, mutD5 zaf-13::Tn10 from strain NR9458, and spd26, zaf-13::Tn10 from strain NR11557 (51, 52). The presence of the mutant dnaQ alleles was checked by its mutator phenotype by using the rifampicin-resistance (rif”) assay. All JM103 derivatives are maintained on minimal medium plates and grown in Luria–Bertani medium at 37°C for the TLS experiments unless specified otherwise.

AAF-Induced Mutation Assays. AAF-modified plasmids are introduced into various strains by using electroporation (Bio-Rad Gene Pulser) for double-stranded constructs or the CaCl2 transformation procedure for single-stranded constructs (44). The single AAF adduct (+) is located within sequence 3G (-GGG“-) and NarI (-GGG“CC-) in the beginning of lacZ gene of pUC-derived plasmids. The 3G and NarI constructions have a LacZ+ phenotype that can be reverted to LacZ- by -1 and +2 frameshift-event, respectively (43, 53). Wild-type and mutant transformants are scored on indicator plates containing ampicillin, 5-bromo-4-chloro-3-indolyl β-d-galactoside, and isopropyl β-d-thiogalactoside as white and blue colonies, respectively. For all strains, the SOS response was induced by UV irradiation at 50 J/m2 as described (4). All experiments were performed two or three times. For each data point, between 3,000 and 30,000 colonies were scored. When nonadducted DNA is introduced into proofreading-deficient strains, the spontaneous frequency of blue colonies (i.e., frameshift mutants) is in the range of 2.5 × 10-4.

Colony Hybridization Strand Segregation Analysis Assay. We used 20-mer oligonucleotide probes complementary to each of the two strands in the vicinity of the heteroduplex region to monitor the segregation of the two strands as described (4). Several hundred individual transformants were analyzed for each situation.

Results and Discussion

Purpose and Strategy of the Work. Over the years, we have developed single-stranded and double-stranded DNA plasmids containing single dG-AAF adducts (43, 44, 54). These DNA probes are derived from plasmids containing the ColE1 origin of replication. Single-stranded DNA probes are an unambiguous tool for studying TLS, and double-stranded vectors allow the study of additional tolerance pathways defined as DA mechanisms. These latter mechanisms take advantage of the information contained in the complementary strand and allow the replication of damaged double-stranded DNA molecules without the need for the polymerase to read through the lesion (4).

Similar to many lesions such as UV-induced pyrimidine dimers, AAF adducts severely hinder DNA replication. In non-SOS-induced E. coli cells, TLS at a single dG-AAF adduct, expressed as the efficiency of transformation of a single-stranded vector carrying a single AAF adduct normalized to the corresponding nondamaged vector, ranged between 2% and 4% (44). The poor “survival” of such a singly damaged molecule is increased ~10-fold upon induction of the SOS response (44). Similar data were reported for UV-induced pyrimidine dimers (55, 56). These observations reflect the capacity of SOS factors to enhance the ability of the replication machinery to carry out efficient TLS. For AAF adducts, TLS efficiencies were measured within two different sequence contexts: the NarI (-GGG“CC-) and the 3G (-GGG“-) sequences. Within both sequence contexts, an AAF adduct (G*) located at the 3’ end of the short repeat can promote frameshift mutagenesis efficiently, because of the possibility of forming and extending from an SMI (43, 47, 48). The SMI structure was found to be strongly stabilized by the presence of the AAF adduct (46). The SMI contains a loop formed by one (sequence 3G) and two (sequence NarI) bases yielding ~1 and ~2 frameshift mutations, respectively (Fig. 2). Therefore, the plasmid can be replicated by means of two different TLS pathways (44): (i) elongation from the nonslipped intermediate (nonslipped TLS) yielding the wild-type sequence and (ii) elongation from the slipped intermediate (slipped TLS; Fig. 2). Although both nonslipped and slipped TLS pathways are SOS inducible, their SOS requirements are different. Nonslipped

Fig. 1. Sequence for pUC-3G (A) and pCU-Nar (B) constructions showing the sequence of the 3G and NarI oligonucleotides (gray box), the position of the AAF adduct (+), and the sequence heterology located across from the adduct.
TLS requires functional *umuDC*+ gene products, whereas slipped TLS is *umuDC*-independent but requires another, as yet uncharacterized, SOS function (44).

We decided to investigate the role of DNA proofreading in these two TLS pathways. DNA proofreading is mediated by the *dnaQ* gene product, the *ε*-subunit (57, 58). Protein *ε* possesses a 3′ → 5′ exonuclease activity that is stimulated further by its association with the *dnaE* gene product, the *α*-subunit (59). Together with the *holE* gene product (the *θ*-subunit), the three proteins *εθα* form the so-called DNA polymerase III (PolIII) core assembly. *mutD5* and *dnaQ49*, two major *dnaQ* mutant alleles, have been described (60, 61). Allele *dnaQ49* contains a point mutation in the conserved ExoII domain (Val-96 Gly) and was found to be recessive (62). Another well-characterized allele of *dnaQ*, *mutD5*, is a point mutant in the conserved ExoI domain (Thr-15 Ile; ref. 63) and exhibits a dominant phenotype (62). *MutD5* protein is believed to form a stable PolIII core assembly, whereas the *DnaQ49* protein is believed to destabilize the PolIII core assembly (62). Depending on the conditions of growth (i.e., temperature and rich or minimal medium), both *dnaQ49* and *mutD5* alleles confer a robust spontaneous mutator phenotype (≈10⁻¹⁻⁻ to 10⁻²-fold) to their hosts (60, 61). In addition to *dnaQ49* and *mutD5*, we have used *spd26*, a partial revertant of *mutD5* (52).

Analysis of TLS in DNA-Proofreading-Deficient Strain *dnaQ49* with Single-Stranded DNA Vectors. The efficiency of TLS in a given bacterial cell is defined as the transformation efficiency of the single-adducted vector relative to that of the corresponding unmodified vector. The vectors have been engineered to allow direct determination of the induced frameshift mutation frequencies by using the lacZ*′*-α-complementation assay. Vectors pUC-3G and pCU-Nar carry a lacZ*- allele that reverts to LacZ*⁺* by −1 and −2 frameshift mutations, respectively (43, 53). By using indicator plates containing 5-bromo-4-chloro-3-indolyl β-D-galactoside and isopropyl β-D-thiogalactoside, we calculate the efficiency of nonslipped and slipped TLS (Table 1) from the number of white and blue colonies, respectively. The survival (i.e., the sum of nonslipped and slipped TLS) and the −1 or −2 error rate per TLS event (slipped TLS/total TLS) are also indicated in Table 1.

Nonslipped and slipped TLS efficiencies were measured in strains JM103 (dnaQ49) and JM103 (Δ*umuDC*, dnaQ49) and compared with the values obtained in the corresponding *dnaQ*⁺ strains (28).

Slipped TLS pathway. Strikingly, under SOS− conditions, increased levels of slipped TLS are found in strain *dnaQ49* as compared with the wild-type strain (≈70- and ≈10-fold increase in the *NarI* and 3G sequences, respectively). Compares TLS via nonslipped and slipped elongation are expressed in percentages as the number of white colonies formed per nanogram of undamaged control DNA and represents the survival of the damaged molecule with respect to an undamaged control plasmid. −1 or −2 error rate per TLS event is calculated as the ratio of slipped/total TLS for sequences 3G or *NarI*, respectively.

Table 1. TLS in single-stranded DNA in wild-type and *dnaQ49* strains

<table>
<thead>
<tr>
<th>Measurement</th>
<th><em>NarI</em> (-GGCG*CC-)</th>
<th><em>NarI</em> (-GGG*⁺⁺⁺⁺)</th>
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<tr>
<td></td>
<td><em>dnaQ49</em> Wild type</td>
<td><em>dnaQ49</em> Wild type</td>
</tr>
<tr>
<td>TLS via nonslipped</td>
<td>3.5</td>
<td>3.1</td>
</tr>
<tr>
<td>elongation, %</td>
<td>12</td>
<td>16.7</td>
</tr>
<tr>
<td>TLS via slipped</td>
<td>68</td>
<td>1.03</td>
</tr>
<tr>
<td>elongation, %</td>
<td>75</td>
<td>34.5</td>
</tr>
<tr>
<td>Total TLS, %</td>
<td>71.5</td>
<td>4.1</td>
</tr>
<tr>
<td>Error rate/TLS, %</td>
<td>87.8</td>
<td>51.2</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>67</td>
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</tbody>
</table>

*TLS* via nonslipped and slipped elongation are expressed in percentages as the number of white and blue colonies formed per nanogram of DNA, respectively, divided by the number of white colonies per nanogram of undamaged control DNA. The average value of two to four independent experiments is given. The relative error rate is within 20%. Data in the wild-type strain JM103 are from a previous work (28). Total TLS is the sum of nonslipped and slipped elongations and represents the survival of the damaged molecule with respect to an undamaged control plasmid. −1 or −2 error rate per TLS event is calculated as the ratio of slipped/total TLS for sequences 3G or *NarI*, respectively.
rably increased levels of slipped TLS are found in the wild-type strain upon UV irradiation. Therefore, the SOS function that is required in a wild-type strain for the induction of the slipped TLS pathway can be obviated by DNA-proofreading inactivation. In fact, in the NarI context, slipped TLS is more efficient in strain dnaQ49 under SOS− conditions than in the SOS− induced wild-type strain (68% compared with 34.5%). In contrast, within sequence context 3G, SOS induction in the wild-type strain provides a higher level of slipped TLS than dnaQ49 mutation in the absence of SOS induction (3.1% compared with 1.1%). In both sequence contexts, similar data were obtained in the ΔumuDC, dnaQ49 background (data not shown). It should also be stressed that the slippage reaction seems to be intrinsically much easier within the NarI sequence context than within the 3G sequence context. Indeed, the values of slipped TLS are severalfold higher in the NarI sequence than in the 3G sequence context (see Conclusion).

Nonslipped TLS pathway. Similar efficiencies of nonslipped TLS are found in dnaQ49 and wild-type strains within both sequence contexts under both SOS− and SOS+ conditions (Table 1). Moreover, in the ΔumuDC, dnaQ49 background, even upon SOS induction, nonslipped TLS remains low (~2%) in both sequence contexts (data not shown). Consequently, as found in a wild-type strain (28), nonslipped TLS is also UmuDC+ dependent in the dnaQ49 background.

Survival and error rate per TLS event. In terms of total TLS (i.e., the sum of nonslipped and slipped TLS), it is striking to note that by solely inactivating proofreading, the toxicity of the single AAF adduct within the NarI sequence context is almost abolished; survival is ~70% under SOS− conditions). In fact, under these conditions, virtually all TLS is performed via the slippage reaction, yielding a ~2 error rate per TLS event of ~90%. In the 3G sequence context, as the slippage reaction is much more difficult, the increase in survival is not detectable in the proofreading-deficient strain. However, in terms of ~1 error rate per TLS event, the effect of the dnaQ49 mutation is quite dramatic, yielding an increase in the mutation frequency from 4% in the wild-type strain to 46% in the dnaQ49 background.

Analysis of TLS in DNA-Proofreading-Deficient Strain dnaQ49 with Double-Stranded DNA Vectors. When double-stranded vectors are transformed into bacteria, only a relatively small fraction of the transformants actually result from a TLS event, because most of the transformed colonies result only from the replication of the nondamaged strand (DA event mechanism; ref. 4). The introduction of a small heterology in the double-stranded constructions at the adduct site (Fig. 1) allows us to measure the level of TLS with strand-specific oligonucleotide probes (strand segregation analysis assay; ref. 4). With this assay, it is possible to measure the extent of nonslipped TLS. Slipped TLS can easily be quantified phenotypically as blue colonies (see Material and Methods). It should be stressed that the presence of the AAF adduct in one strand and the small loop in the other strand makes this compound less resistant to both nucleotide excision and mismatch repair (4). Thus, it is possible to conduct these measurements in strains proficient in excision repair and mismatch repair.

Slipped TLS pathway. Under SOS− conditions, slipped TLS is ~10-fold higher in both sequence contexts as compared with the wild-type strain (ref. 4; T. Broschard and R.P.P.F., unpublished results). On the other hand, induction of the SOS response in a wild-type background increases slipped TLS ~4- to 5-fold in the NarI sequence context and ~40-fold in the 3G sequence context. Therefore, proofreading inactivation (dnaQ49, SOS− conditions) yields levels of TLS that are in the same range as those obtained in an SOS-induced wild-type strain; in fact, they are ~2-fold higher and ~3-fold lower in the NarI and 3G sequence contexts, respectively. Therefore, in agreement with the conclusion reached by using single-strand constructions, the effect of SOS induction is obliterated largely by proofreading inactivation.

Nonslipped TLS pathway. Nonslipped TLS is increased 3- to 4-fold in the dnaQ49 strain as compared with the wild-type strain within both sequence contexts. When the SOS response is induced, nonslipped TLS is only slightly increased in the NarI sequence context, whereas it is strongly stimulated in the 3G sequence context (see Conclusion).

Comparative Effect of Different dnaQ Alleles. Overall, the data obtained with single-stranded and double-stranded probes are in good agreement. Indeed, both sets of data show that proofreading inactivation strongly increases slipped TLS in the absence of SOS induction (~10-fold). Under SOS− conditions, a small increase of nonslipped TLS is observed with the double-stranded probes.

As double-stranded probes are easier to construct and more stable, we have used them instead of the single-stranded probes for the comparison of different dnaQ alleles. For these experiments, we used the double-stranded NarI construct in light of its high responsiveness in the dnaQ49 strain (i.e., >20% of mutants even in double-stranded DNA; Table 2). The key question was to check whether the strong effect seen in the dnaQ49 background is related to PolIII core destabilization or to a proofreading-defective PolIII core assembly. Consequently, we conducted a series of experiments in mutD5 strains.

In nonirradiated cells, levels of slipped TLS (20–27%) similar to those obtained with allele dnaQ49 were obtained with allele mutD5 (Table 3). These levels are ~10-fold higher than the levels achieved in the SOS− wild-type strain. The spontaneous mutator phenotype of allele mutD5 depends on the growth conditions, being 10- to 100-fold higher in complete medium (Luria–Bertani) than in minimal medium (60). However, as far as slipped TLS is concerned, no difference between complete- and minimal-medium growth conditions were found (Table 3). We also performed these experiments in a partial revertant of mutD5, designated spd26 (suppressor of mutD). This allele contains mutation Thr-15 → Val instead of the Thr-15 → Ile mutation in mutD5. The spd26 mutator activity is ~10-fold lower than that of mutD5 in minimal medium (52). Allele spd26 sustains levels of slipped TLS as high as allele mutD5. Finally, we introduced allele mutD5 in the non-SOS inducible lexA(Ind)- derivative of strain JM103 to investigate

<table>
<thead>
<tr>
<th>Measurement</th>
<th>dnaQ49</th>
<th>Wild type</th>
<th>dnaQ49</th>
<th>Wild type</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLS via nonslipped elongation, % SOS−</td>
<td>2.3</td>
<td>0.95</td>
<td>0.37</td>
<td>0.15</td>
</tr>
<tr>
<td>TLS via slipped elongation, % SOS−</td>
<td>23.1</td>
<td>0.57</td>
<td>2.6</td>
<td>0.05</td>
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<tr>
<td>Total TLS, % SOS−</td>
<td>37.3</td>
<td>3.2</td>
<td>9.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Error rate/TLS, % SOS−</td>
<td>25.4</td>
<td>1.5</td>
<td>3.2</td>
<td>0.42</td>
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<tr>
<td>Error rate/TLS, % SOS+</td>
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<td>1.7</td>
<td>12</td>
<td>12.4</td>
</tr>
<tr>
<td>Error rate/TLS, % SOS−</td>
<td>91</td>
<td>38</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Error rate/TLS, % SOS+</td>
<td>92</td>
<td>19</td>
<td>78</td>
<td>17</td>
</tr>
</tbody>
</table>

TLS via nonslipped elongation is determined with the strand segregation analysis assay as described in Materials and Methods. TLS via slipped elongation is measured directly from the percentage of colonies that are blue on 5-bromo-4-chloro-3-indolyl β-D-galactoside indicator plates reflecting ~1 and ~2 slippage events for the 3G and NarI sequence contexts (T. Broschard and R.P.P.F., unpublished work). Total TLS is the sum of nonslipped and slipped elongations. ~1 or ~2 error rate per TLS event is calculated as the ratio of slipped/total TLS for sequences 3G or NarI, respectively.

whether proofreading inactivation was directly responsible for the high levels of slipped TLS or whether it might be caused by an indirect effect of mutD5 on the SOS response. Similarly high levels of slipped TLS were achieved in this strain, suggesting that the effect seen in the mutD5 strain is related directly to proofreading inactivation and not mediated by an indirect effect of mutD5 on the SOS response (Table 3).

CONCLUSION

The process of TLS is strongly stimulated by the induction of the SOS response; the actors thus far identified are RecA* and the (UmuD')2, UmuC complex (17–24). On the other hand, an additional factor, Npf, under the control of the SOS response was shown to stimulate strongly frameshift mutagenesis independently of the recA and umuDC gene products (25–28).

The study of TLS at AAF lesions within sequence contexts that are hot spots for induced frameshift mutagenesis led us to identify two pathways, the nonslipped and slipped TLS pathways (28). TLS can be described in terms of the different replication intermediates that are involved. The replication intermediate in which the last nucleotide of the primer is located opposite the lesion in the template will be referred to as a “lesion terminus” (LT). All replication intermediates preceding or succeeding this step will be referred to as prelesion termini (pre-LT) and postlesion termini (post-LT), respectively. Given these definitions, TLS can be viewed as a succession of at least two reactions (pre-LT → LT and LT → post-LT). The progression from one step to the next normally requires a DNA-synthesis step.

Irrespective of the nucleotide that is inserted across from a bulky lesion, the 3′ end of the primer in the LT will be distorted. Consequently, the elongation kinetics will be impaired. During TLS past a single dG-AAF residue, a cytosine residue is incorporated across the AAF adduct (8). In the slipped TLS pathway, the LT isomerizes into a misaligned intermediate, and the newly incorporated cytosine residue is incorporated across the AAF adduct (8). In the study of TLS at AAF lesions within sequence contexts that are hot spots for induced frameshift mutagenesis we see a small but distinct increase in nonslipped TLS past a thymine base (cis-cyclobutane dimer is also formed in the SMI). As the slipped TLS pathway is the LT from which the SMI can be formed. As the slipped TLS pathway remains dependent on an SOS function (i.e., the putative npf function), we reasoned that proofreading avoidance may be essential to ensure that the terminal nucleotide of this intermediate is not degraded by the 3′ → 5′ exonuclease, thus allowing its conversion into the SMI. We tested this hypothesis in proofreading-deficient strains and indeed found that the slipped TLS pathway becomes SOS-independent. The most straightforward interpretation of the data is that the “SOS effect” and the “dnaQ effect” have a common mechanistic basis and that the role of Npf may be to inhibit or exclude the DNA-proofreading pathway during TLS. However, we cannot rule out that the similarity of these two phenotypes is only coincidental.

It is tempting to suggest that proofreading inhibition is important not only for frameshift mutagenesis but for all TLS reactions. In fact, Npf may be effective at the different steps of the TLS reaction to “protect” the primer terminus from exonucleolytic degradation. Indeed, with double-stranded DNA, we see a small but distinct increase in nonslipped TLS in proofreading-deficient strains. Evidence that replication past a thymine/thymine cis-cyclobutane dimer is also stimulated in proofreading-deficient strains has been obtained (C. W. Lawrence and R. Woodgate, personal communication). There is no biochemical evidence yet for proofreading inhibition during the SOS response. In contrast, dnaQ expression was found to be induced slightly during the SOS response (64, 65). Our observation that different levels of proofreading deficiencies (i.e., different dnaQ alleles under different physiological conditions) yield similar levels of slipped TLS is intriguing (Table 3) and may imply that only partial proofreading inhibition is required.

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