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Cellular strategies for accommodating replication-hindering adducts in DNA: Control by the SOS response in *Escherichia coli*

(*N*-2-acetylaminofluorene and aminofluorene adducts/trans-lesion synthesis/damage avoidance/frameshift hot spots/UmuDC)

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ABSTRACT **The replication of double-stranded plasmids containing a single adduct was analyzed *in vivo* by means of a sequence heterology that marks the two DNA strands. The single adduct was located within the sequence heterology, making it possible to distinguish trans-lesion synthesis (TLS) events from damage avoidance events in which replication did not proceed through the lesion. When the SOS system of the host bacteria is not induced, the C8-guanine adduct formed by the carcinogen *N*-2-acetylaminofluorene (AAF) yields less than 1% of TLS events, showing that replication does not readily proceed through the lesion. In contrast, the deacetylated adduct *N*-(deoxyguanosin-8-yl)-2-aminofluorene yields ≈70% of TLS events under both SOS-induced and uninduced conditions. These results for TLS *in vivo* are in good agreement with the observation that AAF blocks DNA replication *in vitro*, whereas aminofluorene does so only weakly. Induction of the SOS response causes an increase in TLS events through the AAF adduct (≈13%). The increase in TLS is accompanied by a proportional increase in the frequency of AAF-induced frameshift mutations. However, the polymerase frameshift error rate per TLS event was essentially constant throughout the SOS response. In an SOS-induced Δ*umuD/C* strain, both TLS events and mutagenesis are totally abolished even though there is no decrease in plasmid survival. Error-free replication evidently proceeds efficiently by means of the damage avoidance pathway. We conclude that SOS mutagenesis results from increased TLS rather than from an increased frameshift error rate of the polymerase.**

Most mutagens and carcinogens react with the bases of DNA by forming covalent adducts that interfere with DNA metabolism if left unrepaired. Repair pathways remove these lesions: excision repair removes damaged bases or nucleotides prior to replication and post-replication repair fills in gaps formed in newly synthesized DNA strands when damaged DNA is replicated. These two major repair pathways are believed to be essentially error-free and to contribute about equally to cell survival. The effect of DNA adducts on replication has been analyzed in *in vitro* assays using damaged single-stranded DNA templates and purified DNA polymerases (1–6). Studies of templates containing a single adduct have shown that some adducts are absolute blocks for *in vitro* DNA synthesis. For example, there is no *in vitro* trans-lesion synthesis (TLS) by most DNA polymerases through *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dGuo-AAF) (1), an adduct formed at the C8 position of guanine by the rodent hepatocarcinogen *N*-2-acetylaminofluorene (AAF) (7). However, *in vivo* double-stranded plasmids containing AAF adducts can replicate in excision-repair deficient hosts in an error-free or error-prone (mutagenic) manner (8–11). These observations confirm the fact that there are efficient mechanisms that rescue a blocked replication fork *in vivo* (12). Two such pathways are TLS involving a modified replisome and damage avoidance (DA) mechanisms that use the information of the complementary strand to rescue the blocked replication fork. In TLS, the SOS-controlled *umuD/C* gene products and the activated form of RecA interact with the blocked DNA polymerase III holoenzyme to achieve synthesis through the modified base (13–15). SOS-mediated TLS increases the mutation frequency (16, 17). On the other hand, DA mechanisms are believed to be error free. A well-known DA model is recombinational repair in which a gap in the newly synthesized strand opposite the damage is patched by recombination using the complementary parental strand from the replicated sister chromatid (12, 18).

TLS and DA mechanisms are poorly understood at the molecular level, and their relative contribution to the survival of damaged DNA is not known. In principle, SOS-mediated increases in mutation can be achieved either by increasing the proportion of TLS relative to DA or by increasing the frequency of errors per TLS event. In line with the latter hypothesis, it is generally believed that UmuD'C proteins and RecA associate with DNA polymerase III to form a "mutasome" that will trigger TLS (19–21). In this paper, we investigate the proportion of TLS and DA events and the modulation of the TLS/DA ratio during the induction of the SOS response. We also relate the replication of damaged plasmids to the blocking capacity of the adduct by comparing plasmids that contain either the replication-blocking adduct dGuo-AAF or its deacetylated form *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dGuo-AF), which only weakly interferes with replication (1, 6, 22).

MATERIALS AND METHODS

Construction of Heteroduplexes Containing Single Adducts. The strategy used to construct heteroduplexes containing a single adduct is based on the formation of a gapped duplex followed by the ligation of a partially complementary oligonucleotide within the gap (Fig. 1*A*). Two parental plasmids, pUC-Helper and pUC-(3G+3), are linearized with restriction enzymes *Hinc*II and *Sca*I, respectively (11). Plasmid pUC-(3G+3) is identical to plasmid pUC-Helper except for an oligonucleotide insert in the *Hinc*II site as shown in Fig. 1*A*. The linear forms of the two parental plasmids are mixed in equal quantities, heat denatured, and incubated at 55°C in buffer: 10^{-2} M Tris·HCl, 150 mM NaCl, 10^{-3} M EDTA (pH 7) to allow for the formation of gapped-duplex structures. The crude gapped-duplex mixture is incubated at 16°C with a 2-fold molar excess of a ^{32}P-labeled 15-mer oligonucleotide that is complementary to the gap except for a 4-nucleotide bulge that

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Abbreviations: AAF, *N*-2-acetylaminofluorene; dGuo-AAF, *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene; dGuo-AF, *N*-(deoxyguanosin-8-yl)-2-aminofluorene; TLS, trans-lesion synthesis; DA, damage avoidance; SSA, strand segregation analysis.

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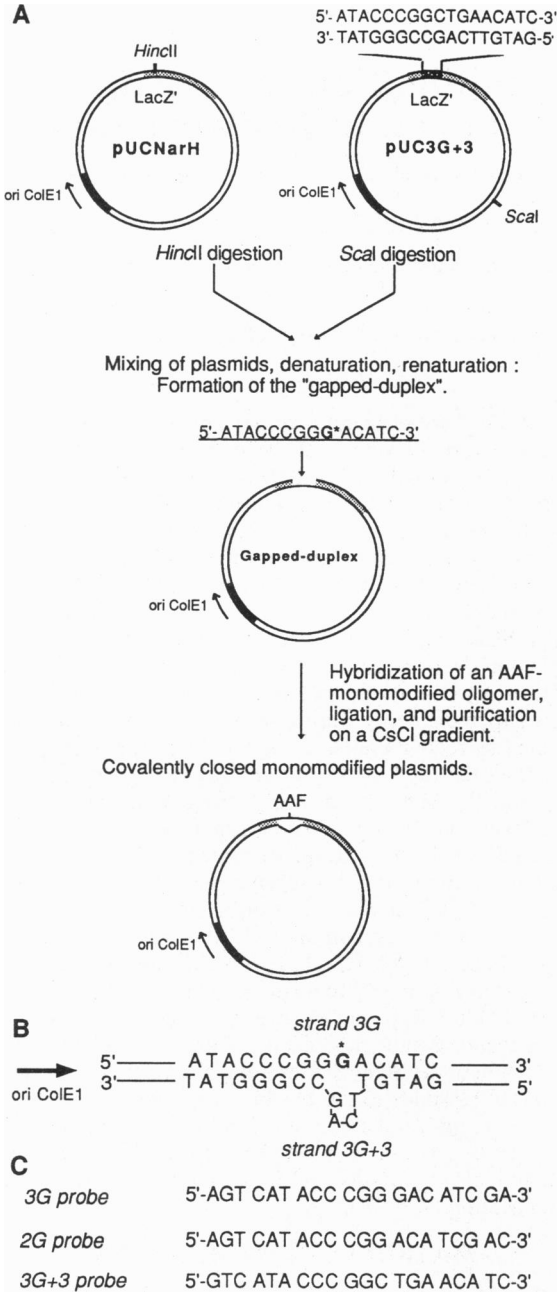

FIG. 1. (*A*) Construction of the gapped-duplex. (*B*) Construction of the heteroduplex showing the adduct site, bulge structure, and direction of the origin of replication. The adducted guanine is in boldface type and noted with an asterisk. As drawn, the AAF adduct is in the template for lagging strand synthesis. (*C*) Sequences of the probes: 3G probes the wild-type strand in which the adduct is initially located; 2G probes the sequence arising from AAF-induced −1 frameshift mutagenesis; 3G+3 probes the nonadducted strand.

forms in the middle (Fig. 1*B*) in the presence of ATP (1 mM) and T4 DNA ligase (New England Biolabs) for 1 min. The covalently closed circles are purified by equilibrium centrifugation on CsCl gradients. The cccDNA was quantified knowing the specific activity of the ^{32}P-labeled oligonucleotide. The oligonucleotide is either nonmodified or contains a single −AAF or −AF adduct on the C8 position of the guanine residue at the 3′ end of a run of three Gs (Fig. 1*B*). The oligonucleotide containing a single AAF adduct was prepared, purified, and characterized as described (10). The oligonucleotide containing a single AF adduct was obtained from the AAF-containing oligonucleotide by a deacetylation reaction as described (6, 23).

Bacterial Strains, Transformation, SOS Induction, and Mutation Assay. The bacterial strains used in the present work are derivatives of strain JM103. Mutant alleles were introduced by P1 transduction using the following markers: *uvrA6*, *lamB* (kan^R), *mutS*, *srl* (tn10), and Δ*umuDC* (Cm^R). The presence of the transduced allele was checked by its corresponding phenotype: UV sensitivity for *uvrA6* increased spontaneous rif^R mutagenesis for *mutS* and induced mutagenesis for the Δ*umuDC* allele. When indicated, the bacterial SOS response was induced by UV irradiation of an exponentially growing bacterial suspension in $MgSO_4$ (10 mM) at the doses indicated. After irradiation, the bacteria were incubated in Luria–Bertani (LB) medium at 37°C for 30 min before transformation to allow for expression of the SOS functions. Transformation with plasmids was achieved by electroporation (Bio-Rad gene pulser). Bacteria were plated on LB agar plates containing ampicillin (100 μg/ml) with or without indicator [5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal)/isopropyl β-D-thiogalactopyranoside (IPTG)] to detect the Lac phenotype. The transformation efficiencies determined as the number of transformants per microgram of DNA have varied by less than 10% in three independent determinations per experimental point. Frameshift mutation (−1) frequencies were calculated as the ratio of blue colonies to the total number of colonies on X-Gal/IPTG indicator plates.

Colony Hybridization Protocol. Oligonucleotide probes (20-mers) were used in a colony hybridization assay to follow the segregation of the two strands of the heteroduplex. Colonies resulting from transformation with the heteroduplex constructions were recovered from plates containing ampicillin, but not X-Gal/IPTG indicator, grown overnight in 200 μl of LB+ ampicillin in 96-well microtitration plates, and transferred onto nylon membranes using a comb that can simultaneously transfer 48 cultures. This protocol produced membranes with highly reproducible colony sizes. The colonies were grown onto the membranes by incubation on top of LB+ ampicillin plates overnight. The oligonucleotide hybridization protocol was performed as described by the manufacturer of the membranes (DuPont), using all three probes, 3G, 3G+3, or 2G, at 58°C (Fig. 1*C*). Filters were analyzed using a Molecular Dynamics PhosphorImager.

RESULTS AND DISCUSSION

The DNA adducts (dGuo-AAF and dGuo-AF) that are used as models in this study are formed when AAF reacts covalently with the C8 position of guanine (24). These adducts have been studied extensively in terms of the structural deformation that they induce in double-stranded DNA (insertion–denaturation model for AAF adducts and outside-binding model for AF adducts) (25–28) and their effect on DNA metabolism. AAF adducts are strong frameshift mutagens *in vivo,* inducing both −1 and −2 frameshift mutations in hotspot sequences (8, 9), whereas AF adducts induce primarily base-pair substitutions, especially G → T transversions (29). AAF adducts strongly block DNA synthesis by several purified DNA polymerases *in vitro*, whereas the same polymerases are able to synthesize through the AF adduct after a brief pause (1, 6, 22). In view of these properties, the C8 AAF adduct can be considered as a paradigm for replication-blocking lesions. Pyrimidine dimers and some other bulky chemical adducts (e.g., polycyclic aromatic hydrocarbons) similarly block replication. On the other hand, AF adducts, although "bulky," interfere only slightly with DNA synthesis *in vitro*.

Strand Segregation Analysis (SSA). Under normal conditions, replication of double-stranded DNA yields two daughter molecules that contain the genetic information derived from each strand. If the parent molecule is a homoduplex, the daughter molecules are identical. If the parent DNA molecule is a heteroduplex, however, two different daughter molecules, each containing the genetic information of one strand, will be

produced. To study the effect of a single adduct on DNA replication, we constructed heteroduplex plasmid molecules containing an insertion of three bases in one strand, thus providing a strand marker. This marker is located across from the adduct site (Fig. 1*B*). Strand specific oligonucleotidic probes (probe 3G and 3G+3, Fig. 1*C*) were used in a colony-hybridization assay to analyze the fate of the two strands during replication *in vivo* (SSA).

The SSA approach was validated using a control construction with no adduct. The adduct-free heteroduplex was introduced into bacteria by electroporation. A mismatch-repair-defective (*mutS*) strain was used to prevent correction of the mismatch (see Table 2). As expected, more than 80% of the colonies responded positively with both probes, indicating that they contained plasmid progeny originating from both strands. About 10% of the colonies contained only strand 3G or 3G+3, most likely reflecting residual mismatch correction (30). Residual mismatch repair does not affect the analysis of adduct-containing constructions because the AAF adduct totally prevents mismatch repair correction even in a *mut*$^+$ strain (data not shown).

A Strand Marker Across from the Adduct Does Not Interfere with Mutagenesis. A single AAF adduct was located within a run of three guanine residues, a previously discovered mutation hotspot in which AAF induces −1 frameshift mutations by a slippage mechanism (10, 31). The run of Gs is in the *lacZ* gene in a +1 reading frame and therefore allows the detection of −1 frameshift mutations as blue colonies on indicator plates (see *Material and Methods*). Mutations are induced most efficiently when the adduct is bound to the guanine at the 3′ end of the run (5′-GGG^{AAF}-3′), suggesting a two-step model that involves the correct insertion of a cytosine across from the adducted G, followed by a misalignment step in which the newly inserted C pairs with the nonadducted G immediately 5′ to the adduct site (10, 31). Elongation from this "slipped mutagenic intermediate" leads to a −1 deletion (see Fig. 4). The mutagenic intermediate is in equilibrium with the nonslipped replication intermediate and is strongly stabilized by the presence of an AAF adduct relative to the nonadducted intermediate (32, 33). This pathway of frameshift mutagenesis has the same genetic requirements as base-substitution mutagenesis induced by UV light: UmuD′, UmuC, and RecA* (34).

The introduction of a strand marker across from the adduct site allows us to study the replication pattern of a damaged DNA molecule *in vivo*. In evaluating the validity of this approach we confirmed that the introduction of the strand marker does not interfere with the process of mutation fixation. The mutation frequency was determined by dividing the number of blue colonies by the total number of colonies (see *Material and Methods*). Table 1 shows that the strand marker has no effect on the mutation frequencies. This result is not unexpected if, as generally accepted, both strands within the replication fork are single stranded. This result reinforces the model in which −1 frameshift mutagenesis occurs by slippage during TLS of the adduct-containing strand.

A Single AAF Adduct Introduces a Strong Bias in the Replication Pattern, Whereas a Single AF Adduct Has Little Effect. Constructions containing either a single dGuo-AAF adduct or its deacetylated derivative dGuo-AF were introduced into strain JM103, *uvrA, mutS* by electroporation and analyzed as described above. The AF adduct had little effect on the replication pattern under both SOS$^-$ and SOS$^+$ conditions, in that ≈70% of the colonies responded positively to both probes (Table 2 and Fig. 2). These "mixed" colonies contain the information of both strands and provide evidence that replication has proceeded through the adduct (i.e., TLS). In contrast, a single dGuo-AAF residue introduces a strong bias in the replication pattern, in that fewer than 1% of the colonies responded to both probes under SOS$^-$ conditions. The striking difference between the AF and AAF adducts with respect to the frequency of TLS events reflects the differential capacity of these two adducts to block the replication fork. Indeed, it was shown *in vitro* that AAF adducts represent absolute blocks for DNA synthesis, whereas AF adducts only induce a delay in the replication kinetics (1, 6, 22). In the case of the AAF adduct, most colonies (98%) responded positively only with probe 3G+3, indicating that they contain only the local information of the strand lacking the adduct. This result suggests that these colonies had undergone a DA mechanism rather than TLS. Several DA mechanisms have been postulated, including post-replication repair and polymerase template switching (18, 35) (see *Discussion*).

Table 1. Frequencies of −1 frameshift mutations induced in constructions with and without a strand marker

	Without strand marker	With strand marker
SOS$^-$	3.7×10^{-4} (52/139,000)	3.2×10^{-4} (20/63,000)
SOS$^+$	182×10^{-4} (310/17,000)	169×10^{-4} (144/8,500)

The −1 frameshift mutation frequencies obtained in strain JM103, *uvrA*, *mutS* with the sequence −GGG^{AAF}-located in the template for lagging strand replication. Numbers in parentheses are actual numbers of mutants over wild-type colonies scored.

Analysis of the Mutant Colonies. We have previously shown that AAF adducts are strong frameshift mutagens within runs of Gs in a forward mutation assay, whereas AF adducts are relatively poor frameshifters (9, 29). In the present work, the −AF adduct induced −1 frameshift mutations at a frequency about 10-fold higher than the background mutation frequency and more than 100-fold lower than the AAF-induced mutation frequency (data not shown). Individual mutant colonies induced by the AAF adduct, detected by their blue coloration on indicator plates, were analyzed by hybridization using probe 3G+3, 3G, and the mutant-specific probe 2G (Fig. 1*C*). Among 87 mutant colonies that were picked randomly, 86 responded positively with probes 3G+3 and 2G, suggesting that frameshift mutations indeed occur during TLS. This result reinforces the proposal that frameshift mutations occur by primer–template misalignment when replication proceeds across the AAF adduct (10). Most colonies (75 of 87) did not hybridize effectively with probe 3G, indicating that most mutants are fixed during the first round of replication and that the damaged strand is then lost. The 12 colonies that responded positively with probe 3G in addition to probes 2G and 3G+3 represent cases in which the damaged strand survived for more than one cycle of replication before mutagenesis occurred.

SOS Mutagenesis Results from Increased TLS Rather than from a DNA Polymerase with Increased Frameshift Error Rate. A dose-dependent increase in the proportion of mixed colonies was found when the cells were irradiated with UV light to induce the SOS functions prior to transformation (Table 2). At an optimal UV dose for the induction of mutations (i.e., 4.5 J/m^2), the proportion of mixed colonies reached 12–13% (Fig. 2). However, most colonies (≈87%) still contained only the information of the nondamaged strand (probe 3G+3) (Table 2), indicating that replication did not occur through the damage. At an intermediate UV exposure (≈3 J/m^2), an intermediate proportion of TLS events were observed (≈4%). The relative proportion of TLS increases with the induction of the SOS response, and there is a concomitant increase in the frequency of −1 frameshift mutations (Table 2).

Assuming that most mutations are fixed during the first replication cycle, the frequency of frameshift errors per TLS event can be estimated by dividing the observed mutant frequency by the relative proportion of TLS events (Table 2). The frequency of frameshift errors per TLS event appears to be almost constant and equal to ≈0.15 ± 0.03 (Table 2 and Fig. 3) under all conditions of SOS induction tested. Thus, regardless of the SOS status, elongation from the slipped primer–template during TLS represents about 15% of the events

Table 2. Analysis of strand segregation of unmodified DNA and constructions containing a single dGuo-AF or dGuo-AAF adduct

Construction/UV irradiation (J/m²)	No adduct/0	dGuo-AF/0	dGuo-AF/4.5	dGuo-AAF/0	dGuo-AAF/3	dGuo-AAF/4.5
TLS (mixed colonies: 3G and 3G+3)	81% (231/287)	73.4% (263/358)	68.3% (245/359)	0.42% (3/712)	3.8% (13/346)	12.4% (44/356)
DA (pure colonies: 3G+3 only)	9.7% (28/287)	19.3% (69/358)	25.6% (92/359)	99.6% (709/712)	96.2% (333/346)	87.6% (312/356)
Pure colonies (3G only)	9.7% (28/287)	7.3% (26/358)	6.1% (22/359)	<0.14% (0/712)	<0.3% (0/346)	<0.3% (0/356)
Observed mutation frequency	ND	ND	ND	4.6×10^{-4} (229/490,000)	68×10^{-4} (92/13,536)	213×10^{-4} (85/3,994)
Frameshift error rate per TLS event	ND	ND	ND	0.11	0.18	0.17

The constructions containing no adduct, a single dGuo-AF, or a single dGuo-AAF adduct were introduced by electroporation into strain JM103, *uvrA*, *mutS* with or without prior induction of the SOS functions by UV irradiation at the dose indicated. A colony responding positively with probes 3G and 3G+3 is a "mixed" colony indicating a TLS event. A colony responding only to probe 3G+3 contains only the information from the nonadducted strand and represents a DA event. The number of colonies responding positively with a given probe or with both probes is divided by the total number of colonies that were analyzed (numbers in parentheses). The observed −1 frameshift mutation frequency is measured as the number of blue colonies divided by the total number of colonies (see *Material and Methods*). The frameshift error rate per TLS event is the observed mutation frequency divided by the proportion of TLS events. The induction of −1 frameshifts by −AF adducts is very weak and has not been determined (ND).

(error-prone TLS leading to −1 frameshift mutation), whereas about 85% of the elongation proceeds from the nonslipped primer–template (error-free TLS) (Fig. 4).

The SOS response appears to modulate the relative proportion of TLS versus DA events without affecting the frequency of frameshift errors per TLS event. The strong induction of −1 frameshift mutations during the SOS response (≈45-fold difference between SOS⁻ and full SOS induction) is largely accounted for by the increase in the relative proportion of TLS (30-fold), and not by an increase in the frequency of frameshift errors per TLS event. Although the hybridization signal is generally uniform among colonies with probe 3G+3, it is of variable intensity with probe 3G (Fig. 2), raising the possibility that uncoupling of the replication of the two strands occurs during TLS with a delay in the replication across the adduct, thus leading to an underrepresentation of the progeny of the damaged strand in the colony. Evidence for uncoupling of the replication of strands in the presence of adducts has been presented (36).

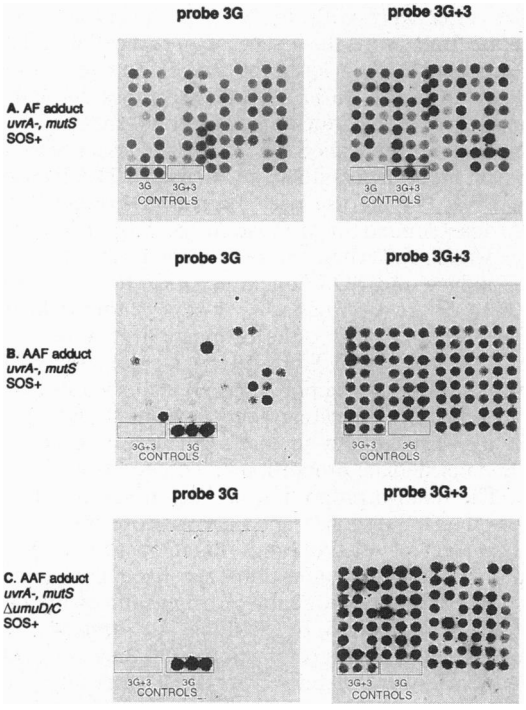

FIG. 2. Colony hybridization with probes 3G and 3G+3: each filter contains three control colonies containing a pure plasmid responding to probes 3G and 3G+3, respectively.

TLS Events and Mutagenesis Are Totally Abolished in a *ΔumuD/C* Strain. The *umuDC* operon is controlled by the SOS response, and its gene products encode proteins involved in mutagenesis (37, 38). We have previously shown that −1 slippage mutagenesis induced by AAF adducts within runs of guanines proceeds by a mutation pathway that exhibits the same genetic requirements as UV-induced base-substitution mutagenesis. Indeed, both the *umuDC* gene products and activated RecA protein (RecA*) are necessary for induction (34). To investigate the role of the umuDC functions in the replication of damaged DNA, we introduced our constructions into a *ΔumuDC* strain. Strand-segregation analysis shows that the *umuDC* gene products are necessary for the SOS⁻dependent increase in TLS. Deleting the *umuDC* operon suppressed both the UV-irradiation-dependent induction of −1 frameshift mutations (Table 3) and the TLS pathway (Table 3 and Fig. 2). It appears that the UmuDC proteins increase slippage-mediated frameshift mutagenesis by increasing the frequency at which the replication machinery copies the damaged strand, and not by increasing the frequency of frameshift errors per TLS event. Under SOS⁻ conditions, most replication events result from DA mechanisms that provide the most efficient way to avoid mutagenesis. The molecular mechanisms by which the information of the nondamaged strand is used remains to be established. SOS functions strongly increase (≈30-fold) TLS events, thus generating a comparable increase in mutation frequency.

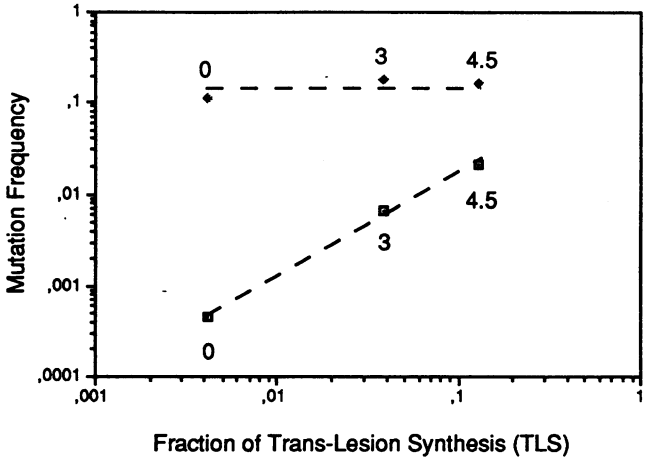

FIG. 3. Observed mutation frequency and frameshift error rate per TLS. The frameshift error rate of the polymerase per TLS was calculated as discussed in the *Results*. The numbers on the points indicate the UV exposure of the cells before electroporation (J/m²).

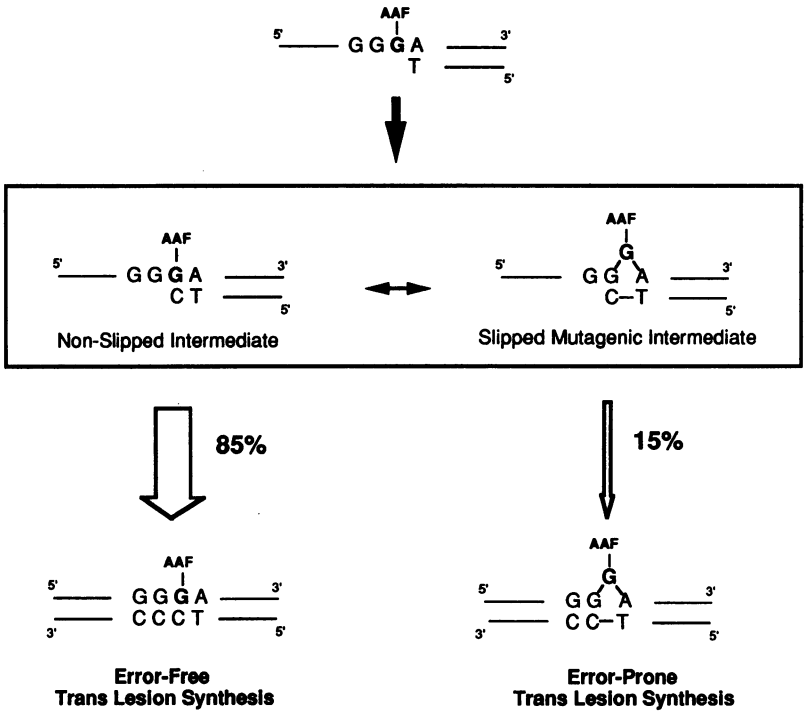

FIG. 4. Mechanism of −1 frameshift mutagenesis. The first step in TLS is the incorporation of a C opposite the adduct. This replication intermediate can either be elongated as such (error-free TLS) or can undergo a slippage event during which the C residue forms a base pair with the next G in the run. Elongation from this slipped mutagenic intermediate gives rise to −1 frameshift mutations (10).

A Single AAF Adduct in a Double-Stranded Plasmid Is Nontoxic. We have compared the transformation efficiency with damaged versus nondamaged plasmids (see *Materials and Methods*). Within an accuracy of ±10%, no toxicity related to the presence of a single AAF adduct was detected in either a umu+ or Δ*umuDC* strain with or without SOS induction. This finding is in full agreement with previous data showing that double-stranded plasmids randomly damaged with AAF adducts or UV lesions are not rescued by SOS induction (39). Double-stranded plasmids thus differ from single-stranded vectors that exhibit increased survival upon SOS induction (40, 41). In addition, a double-stranded plasmid containing as many as 10 AAF adducts in 1 strand but none in the other strand survives about equally well as a nondamaged molecule (36). These results indicate that SOS induction does not increase the survival of damaged double-stranded DNA molecules and that their replication proceeds efficiently provided that one strand remains undamaged.

CONCLUSION

We have investigated the replication in *Escherichia coli* of double-stranded DNA molecules containing a single AAF or AF adduct within a frameshift mutation hotspot. Strand segregation analysis allowed us to determine the proportion of transformed colonies that resulted from replication through the adduct (TLS). Under non-SOS-induced conditions, less than 1% of the colonies with plasmids containing a single AAF adduct were found to result from TLS, whereas 70% had undergone TLS in the case of a single AF adduct. These observations are in agreement with the previously observed capacity of AAF adducts, but not AF adducts, to block DNA synthesis with various DNA polymerases *in vitro*. The induction of the SOS response led to an increase in the proportion of TLS events in AAF-containing plasmid that is proportional to the induced −1 mutation frequency. Therefore, the frameshift error rate of the polymerase per TLS event was estimated to be constant (≈0.15 ± 0.03) throughout the SOS response. Conversely, 85% of TLS events appears to be error free. The high proportion of error-free TLS can be accounted for by the fact that a guanine carrying a C8 adduct still retains its coding properties (22).

Mechanisms of Mutagenesis During TLS. In base-substitution mutagenesis, UmuD′ (the RecA* processed form of UmuD), UmuC, and RecA are believed to interact with polymerase III holoenzyme-producing mutations when elongation proceeds from a mis-inserted nucleotide at the lesion site (21). It has been suggested that the filament of RecA that is formed on the single-stranded DNA region located downstream from the lesion helps UmuD′ and UmuC to be correctly positioned at the lesion site to interact with the DNA polymerase (42). Delayed photoreactivation experiments with UV lesions have suggested that the *umuDC* gene products are not required for the mis-insertion step but are necessary for the elongation step (43–45).

In contrast to base substitution mutagenesis, mis-insertion is not the key step in frameshift mutagenesis at runs, as C is correctly inserted across from the modified guanine. Rather,

Table 3. Analysis of strand segregation using constructions with a single dGuo-AAF adduct in a wild-type or Δ*umuDC* strain of *Escherichia coli*

	SOS⁻ (0 J/m²) *umuD/C*⁺	SOS⁻ (0 J/m²) Δ*umuD/C*	SOS⁺ (4.5 J/m²) *umuD/C*⁺	SOS⁺ (4.5 J/m²) Δ*umuD/C*
TLS (mixed colonies: 3G and 3G+3)	0.42% (3/712)	<0.3% (0/360)	12.4% (44/356)	0.17% (1/585)
DA (pure colonies: 3G+3 only)	99.6% (709/712)	100% (360/360)	87.6% (312/356)	99.8% (584/585)
Pure colonies (3G only)	<0.14% (0/712)	<0.3% (0/360)	<0.3% (0/356)	<0.2% (0/585)
Observed mutation frequency	4.6×10^{-4} (229/490,000)	12×10^{-4} (61/49,600)	213×10^{-4} (85/3,994)	12×10^{-4} (11/8,802)

The constructions containing a single dGuo-AAF adduct were introduced by electroporation into strains JM103, *uvrA*, *mutS* or JM103, *uvrA*, *mutS*, Δ*umuDC* with or without prior induction of the SOS functions by UV irradiation at the dose indicated. The rest of the legend is as in Table 2. The efficiency of the induction of SOS functions in the Δ*umuDC* strain was tested by checking the induction of −2 frameshift mutations within the *NarI* site, a mutation pathway known to be SOS-dependent but *umuDC* independent (9).

the critical event is template–primer misalignment followed by elongation from the slipped mutagenic intermediate (Fig. 4). TLS and mutagenesis are both eliminated in a *ΔumuDC* strain suggesting that the major role of UmuD'C proteins during the SOS response is to allow replication to proceed through the lesion. The frameshift error rate per TLS event appears to be constant. Therefore, the UmuD'C proteins favor, to the same extent, polymerase elongation from the slipped primer template (leading to a −1 frameshift) and from the nonslipped intermediate. The UmuD'C-mediated increase in TLS may be achieved by preventing the polymerase from dissociating at the site of the lesion. Indeed, the possibility that UmuD'C forms an alternative sliding clamp that can to replace the β clamp on damaged DNA has been suggested on the basis of the limited sequence similarities between UmuD and gp45 of bacteriophage T4 and between UmuC and gp44/62 of bacteriophage T4 (46). The gp45 protein is the functional homolog of the β clamp, whereas gp44/62 appears to form a functional analog of the clamp loader complex (γ complex) (47, 48). Thus, while the key steps differ between frameshift- and base-pair substitution mutagenesis, the role of UmuD'C appears to be similar: favoring elongation that fixes the mutations rather than direct involvement in the initiation of mutagenesis.

Tolerance of Damage During Replication of DNA *in Vivo*. Studies of replication in UV-irradiated cells have shown that DNA is initially synthesized in short stretches approximately equal in size to the average spacing between pyrimidine dimers (18, 49). Upon further incubation, the newly replicated DNA reaches lengths that are similar to the replication products in undamaged cells. Models that have been proposed to account for this post-replication repair phenomenon involve either a recombinational mechanism or a polymerase template switch model (18, 35). Both models entail a detour mechanism that uses the nondamaged complementary strand to achieve DNA synthesis past a blocking lesion. In the SSA described in this paper, these events appear as colonies that contain only the information of the nondamaged strand in the vicinity of the adduct site. We have referred to these events as DA events and they represent the majority of colonies resulting from transformation with a plasmid carrying the AAF adduct. Because the strand marker is located across from the adduct site, it is not possible from our data to distinguish events in which the complementary strand is used only locally (e.g., recombinational repair or polymerase strand switching) from those in which the damaged strand is lost entirely. Plasmids with multiple strand markers are now being constructed to address these questions.

The evolutionary origin of SOS mutagenesis is usually rationalized on the basis of either advantageous mutagenesis (50, 51) or increased cellular resistance to DNA damage (20, 52–54). Our results show that during SOS induction the replication strategy of damaged DNA is gradually shifted from a major error-free DA pathway to a minor error-prone TLS pathway with no apparent increase in survival. Thus, our data would appear to favor the advantageous mutagenesis hypothesis. We would argue, however, that discrimination between these alternatives is not straightforward, and our data can be equally compatible with the hypothesis of increased resistance to DNA damage. Resistance may only be achieved, however, under conditions of stress imposed by high densities of DNA damage, when adducts are closely opposed (54).

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