Antagonism of Ultraviolet-Light Mutagenesis by the Methyl-Directed Mismatch-Repair System of *Escherichia coli*

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ABSTRACT

Previous studies have demonstrated that the *Escherichia coli* MutHLS mismatch-repair system can process UV-irradiated DNA *in vivo* and that the human MSH2·MSH6 mismatch-repair protein binds more strongly *in vitro* to photoproduct/base mismatches than to "matched" photoproducts in DNA. We tested the hypothesis that mismatch repair directed against incorrect bases opposite photoproducts might reduce UV mutagenesis, using two alleles at *E. coli lacZ* codon 461, which revert, respectively, via C**C**C → C**T**C and CT**T** → CT**C** transitions. F′ *lacZ* targets were mated from *mut*$^+$ donors into *mutH*, *mutL*, or *mutS* recipients, once cells were at substantial densities, to minimize spontaneous mutation prior to irradiation. In *umu*$^+$ *mut*$^+$ recipients, a range of UV fluences induced *lac*$^+$ revertant frequencies of 4–25 × 10^{-8}; these frequencies were consistently 2-fold higher in *mutH*, *mutL*, or *mutS* recipients. Since this effect on mutation frequency was unaltered by an Mfd$^-$ defect, it appears not to involve transcription-coupled excision repair. In *mut*$^+$ *umuC122*::Tn*5* bacteria, UV mutagenesis (at 60 J/m^2) was very low, but *mutH* or *mutL* or *mutS* mutations increased reversion of both *lacZ* alleles roughly 25-fold, to 5–10 × 10^{-8}. Thus, at UV doses too low to induce SOS functions, such as $Umu_2'D$, most incorrect bases opposite occasional photoproducts may be removed by mismatch repair, whereas in heavily irradiated (SOS-induced) cells, mismatch repair may only correct some photoproduct/base mismatches, so UV mutagenesis remains substantial.

IN most prokaryotes, and in all eukaryotes examined, highly conserved protein systems that recognize DNA mismatches and certain DNA lesions play critical roles in maintenance of genetic stability. These long-patch mismatch-repair systems decrease DNA replication error rates 100- to 1000-fold, by recognizing and correcting base/base and (insertion/deletion)-loopout mismatches that escape proofreading by DNA polymerase (Kornberg and Baker 1992). Genetic analyses, and subsequent comprehensive biochemical characterization *in vitro* (Lahue *et al.* 1989), have elucidated the mismatch-repair pathway in *Escherichia coli*. Homodimers of *E. coli* MutS protein bind preferentially to mismatches; then, MutS and MutL homodimers activate MutH protein to specifically nick unmethylated DNA strands at the nearest adenine-hemimethylated d(GATC) sites, during the interval before adenines in newly replicated d(GATC) sequences are methylated. The activation/nicking process most likely involves a protein/DNA translocation and search process driven by ATP hydrolysis (Allen *et al.* 1997). Thus, MutH directs incision and subsequent excision to the nascent DNA strand specifically, so replication errors are corrected rather than fixed. In a reconstituted system (Lahue *et al.* 1989), excision requires UvrD protein (DNA helicase II) and either 3′-5′ or 5′-3′ single-stranded DNA (ssDNA) endonuclease, depending on the relative orientations of the mismatch and the nicked d(GATC) site (Cooper *et al.* 1993). The replicative polymerase (*E. coli* DNA polymerase III) fills the excision gap. Recombination involving partially diverged DNA sequences is antagonized by mismatch-repair systems, which presumably recognize mismatches in heteroduplex joints (Feinstein and Low 1986; Rayssiguier *et al.* 1989; Petit *et al.* 1991; Worth *et al.* 1994). Accumulating evidence now suggests processing by mismatch-repair systems of DNA molecules containing various DNA lesions. These include ultraviolet-light (UV) photoproducts (Feng *et al.* 1991; Feng and Hays 1995; Mu *et al.* 1997; Wang *et al.* 1999), O^6meG residues (Kat *et al.* 1993; Duckett *et al.* 1996), cisplatin G-G intrastrand cross-links (Duckett *et al.* 1996), adriamycin (Drummond *et al.* 1996) and acetyl-aminofluorene and aminofluorene (AAF/AF) adducts (Li *et al.* 1996), and S^6-methylthioguanine/base mismatches (Swann *et al.* 1996). MutS and MutL activities or their homologs are required for efficient transcription-coupled nucleotide excision repair of cyclobutane pyrimidine dimers (CPDs) in *E. coli* (Mellon and Champe 1995) and human cells (Mellon *et al.* 1996).

The fate of nonreplicating UV-irradiated phage λ chromosomes in *E. coli* deficient in nucleotide excision repair (Uvr$^-$) has provided direct *in vivo* evidence for processing of photoproduct-containing DNA by mismatch-repair proteins. Elevation of homologous recom-

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bination, from nearly undetectable frequencies to as much as 10%, and physical breakdown that resulted in duplex DNA breaks and ssDNA gaps and in loss of biological activity required MutS, MutL, and MutH functions and adenine-undermethylated d(GATC) sites (Feng *et al.* 1991). Further work (Feng and Hays 1995) strongly implicated helicase and ssDNA exonuclease activities involved in mismatch repair (Cooper *et al.* 1993). Studies of photoproduct binding by hMutSα, the human homolog of MutS, have demonstrated specific binding to a variety of mismatched CPDs and [6-4] photoproducts, but not to matched photoproducts (Mu *et al.* 1997; Wang *et al.* 1999).

Mismatch repair, targeted to mismatched photoproducts generated by translesion synthesis during the interval that d(GATC) sites on nascent strands remained unmethylated, would excise incorrect bases rather than the photoproducts. For such a process to antagonize mutagenesis efficiently, the subsequent ssDNA-gap-filling DNA synthesis would have to insert the correct base opposite the template photoproduct that originally provoked the repair process. Correct insertion seems the usual result of synthesis past CPDs in phage ssDNA in *E. coli* (Banerjee *et al.* 1988; Jiang and Taylor 1993) and in human nuclear extracts replicating UV-irradiated SV40-origin plasmids (Carty *et al.* 1993; Thomas and Kunkel 1993). Alternatively, stalled mismatch-repair resynthesis tracts could be extended by recombinational template-switching or gap-filling mechanisms. Finally, failed mismatch-repair resynthesis could lead to DNA degradation, the net result being elimination of mutant chromosomes. In any event, a prediction of these mutagenesis-antagonism models is that UV mutagenesis should be enhanced in *E. coli mutS*, *mutL*, and *mutH* mutants, beyond increases in spontaneous mutation.

To test this prediction we analyzed UV-induced reversion of two *E. coli lacZ* codon-461 alleles, constructed by Cupples and Miller (1989) to revert to *lac*$^+$ only by C**C**C → C**T**C or CT**T** → CT**C** transitions. The targets are thus 3′ pyrimidines in potential photoproduct sites. To minimize culture-to-culture fluctuations in spontaneous mutant frequencies, we mated the F′ *lacZ* targets into *mut*$^-$ cells just before irradiation, when cell densities were already substantial. We find a consistent twofold increase in UV mutagenesis in mismatch-repair-deficient (*umu*$^+$) bacteria and also hitherto unsuspected substantial UmuC-independent UV mutagenesis, which is readily detectable only in *mut*$^-$ cells.

MATERIALS AND METHODS

Bacterial strains and plasmids: *E. coli* K-12 strains employed in the mutation studies are described in Table 1. Transduction with P1 phage employed standard techniques (Miller 1972). Transposon-insertion alleles encoding *cat*, Tn*5*, and Tn*10* were selected on Luria broth (LB) plates containing, respectively, chloramphenicol (35 μg/ml), kanamycin (50 μg/ml), or tetracycline (15 μg/ml). MutS$^-$, MutH$^-$, and MutL$^-$ phenotypes were identified by increased spontaneous resistance to rifampicin (100 μg/ml) in LB plates, and the UmuC$^-$ phenotype was recognized by increased UV sensitivity. Plasmid pMQ315 contains the *mutS*$^+$-encoding *Bgl*II restriction fragment of *E. coli* DNA, inserted into the *Bam*HI site of plasmid pBR322 (Wu and Marinus 1994).

Media and antibiotics: M9 minimal media contained 6 g/liter K_2HPO_4 plus 3 g/liter KH_2PO_4 (pH 7.0), 1 g/liter (19 mm) NH_4Cl, 0.1 mm $CaCl_2$, 1 mm $MgSO_4$, and 0.001% thiamine, plus glucose (0.2%), glycerol (0.2%), or lactose (0.2%). LB plates have been described (Miller 1972). Plates were solidified with 1.5% agar (glucose plates; Difco, Detroit) or 1.5% noble agar (lactose plates; United States Biochemical, Swampscott, MA).

Growth, mating, and irradiation of bacteria: For mutagenesis experiments, bacteria were streaked from frozen-glycerol cultures onto LB plates, and single colonies were used, after no more than 36 hr of growth, to inoculate glycerol-minimal-medium cultures, typically 30 ml, containing 60 μg/ml proline. (Inoculation with older colonies resulted in significantly higher final levels of spontaneous *lac*$^+$ revertants in cultures of *mut*$^-$ strains.) After overnight growth at 37°, cultures of various F$^-$ (*mut*$^+$ or *mut*$^-$) *phr::cat* Δ(*lac-pro*) recipients and *mut*$^+$ F′ (*pro*$^+$ *lacI*$^-$ *lacZ*$^-$) donors were both diluted 1.5-fold with fresh medium, or grown without dilution, to ~3.5×10^8 cells/ml, mixed together, and incubated at 37° with very gentle swirling. (All cultures of F$^-$ recipients contained 60 μg/ml proline.) After a 2-hr mating period, mixtures were washed with glycerol-minimal medium containing chloramphenicol, but no proline, resuspended to 1×10^8 cells/ml in the same medium, and grown for 6–8 hr, to select for *phr::cat* F′ (*pro*$^+$ *lacI*$^-$ *lacZ*$^-$) transconjugants. At the end of selective growth, transconjugants typically represented 80% of the total colony-forming units in the mating mixture, as determined by selective *vs.* nonselective plating.

For UV irradiation, mixtures were diluted with glycerol-minimal medium plus chloramphenicol to yield 2.5×10^8 transconjugants/ml, and 10-ml aliquots were added to uncovered 10-cm plastic petri dishes and irradiated at 2 W/m^2, using 254-nm lamps attenuated by window screen. Unirradiated aliquots were used as controls. Lamp fluences were checked using a Spectronics DRC-100X meter and/or an International Light IL1700 radiometer. To determine surviving fractions, irradiated and unirradiated control cells were spread immediately on glucose-minimal plates containing chloramphenicol, and colonies were scored after 48 hr incubation. Under these conditions, survival frequencies of (unmated) *mutS-*, *mut*$^+$-, and *mutS*$^+$-overproducing (*mutS*$^{+++}$) bacteria (strains LH2519, LH3179, and LH2536, respectively) were very similar to one another: ~25, 17, 10, 5, and 1.3%, at 20, 30, 45, 60, and 90 J/m^2, respectively.

Analysis of mutation in UV-irradiated bacteria: For initial screening of all six *lacZ461* alleles for effects of mismatch repair on UV mutagenesis, *mut*$^+$ and *mutS201*::Tn*5* F′ (*lacZ*) strains were grown as described above without mating and were irradiated and analyzed. In all other experiments, irradiated and unirradiated transconjugant cells were diluted with equal volumes of glycerol-minimal medium containing chloramphenicol and were incubated at 37° with shaking. Cultures were incubated in minimal medium rather than broth to reduce carryover of trace nutrients onto the selective plates, and glycerol was used rather than glucose to ensure maximal expression of the *lacZ* gene. Cells were harvested by centrifugation after 8–10 hr (logarithmic growth was fully restored by 4 hr), resuspended in 0.2 vol of M9 minimal salts, diluted appropriately, and spread on glucose-minimal/chloramphenicol plates to score total (transconjugant) bacteria, or cells were spread directly on scavenged lactose-minimal/chloramphenicol plates to score revertants and were incubated for 48 hr at 37°. [Plates were scavenged 1 day before initiation of

TABLE 1

Bacterial strains

Genotypes of F⁻Δ(*lac-pro*)$_{XIII}$ strains[a]			Genotypes of F′(*lacZ pro*⁺) strains (donors)			
Strain	*mutHLS*[b](*mfd*)	*umuC*[b]	Strain	*mutHLS*[b]	*umuC*[b]	F′*lacZ*[d]
LH2519	*mutS97*	*umu*⁺	LH3138	*mut*⁺	*umu*⁺	*lacZ461-2*
LH2520	*mutH97*	*umu*⁺	LH3140	*mut*⁺	*umu*⁺	*lacZ461-6*
LH2534	*mutS97*	*umuC122*	LH3141	*mutS201*	*umu*⁺	*lacZ461-2*
LH2535	*mutH97*	*umuC122*	LH3143	*mutS201*	*umu*⁺	*lacZ461-6*
LH2536	*mutS*⁺⁺⁺[c]	*umu*⁺	LH3148	*mut*⁺	*umu*⁺	*lacZ461-1*
LH2548	*mut*⁺*(mfd98)*	*umu*⁺	LH3149	*mutS201*	*umu*⁺	*lacZ461-1*
LH2549	*mutS97(mfd98)*	*umu*⁺	LH3150	*mut*⁺	*umu*⁺	*lacZ461-3*
LH3179	*mut*⁺	*umu*⁺	LH3151	*mutS201*	*umu*⁺	*lacZ461-3*
LH3183	*mutL96*	*umu*⁺	LH3152	*mut*⁺	*umu*⁺	*lacZ461-4*
LH3266	*mut*⁺	*umuC122*	LH3153	*mutS201*	*umu*⁺	*lacZ461-4*
LH3269	*mutL96*	*umuC122*	LH3154	*mut*⁺	*umu*⁺	*lacZ461-5*
			LH3155	*mutS201*	*umu*⁺	*lacZ461-5*
			LH3290	*mutL96*	*umuC122*	*lacZ461-6*
			LH3303	*mut*⁺	*umuC122*	*lacZ461-2*
			LH3305	*mut*⁺	*umuC122*	*lacZ461-6*

[a] All derivatives of strain P90C [= *ara* Δ (*lac-proB*)$_{XIII}$ *thi*] (Cupples and Miller 1989), bearing *phr::cat*, were produced by P1 transduction from strain SY1 (Yajima *et al.* 1995).

[b] Alleles (except *mutS*⁺⁺⁺) were introduced by P1 transduction from the following strains: *mfd98*::Tn*5*, previously undesignated allele from strain UNCNOMFD (Selby and Sancar 1995); *mutH97*::Tn*10*, previously undesignated allele and strain from J. Miller; *mutL96*::Tn*10*, previously undesignated allele from strain WF2875 (Feng *et al.* 1991) via strain WF2979A (this lab); *mutS97*::Tn*10*, previously undesignated allele and strain from J. Miller; *mutS201*::Tn*5*, from strain WF2858 (Feng *et al.* 1991) via strain 2943 (this lab); *umuC122*::Tn*5* (Elledge and Walker 1983) from strain DE1845 (from R. Woodgate).

[c] Transformant with plasmid pMQ315 (ampicillin selection).

[d] *lacZ461-1*, *lacZ461-2*, *lacZ461-3*, *lacZ461-4*, *lacZ461-5*, and *lacZ461-6* here designate the respective F′*lacZ* alleles in strains CC101, CC102, CC103, CC104, CC105, and CC106 (Cupples and Miller 1989).

experiments by spreading 5×10^7 bacteria of strain LH3302 (*phr::cat* transductant of Δ*lac* strain FC755) and incubating overnight at 37°. This prevents further mutagenesis on the plates (see results).] Throughout the text, "UV-induced revertant frequencies" implies that *lac*⁺ revertant frequencies were determined for parallel unirradiated cultures and were subtracted from apparent frequencies for UV-irradiated cells. Since unirradiated cultures grew (increased in turbidity) about four times as well as irradiated cultures during the 8-hr postirradiation period, this subtraction may overcorrect slightly for background spontaneous mutant frequencies in the irradiated cells. Revertant frequencies determined after as much as 24 hr of postirradiation growth in liquid medium were the same as 8-hr frequencies.

RESULTS

Design and evaluation of experimental approaches: Our aims were (i) to ensure that most UV mutagenesis (here reversion of *lacZ*⁻ alleles to *lac*⁺) took place during a defined period of postirradiation growth in liquid culture, rather than on selective plates, and (ii) to minimize fluctuations in the background spontaneous mutant frequencies in cultures of mismatch-repair-defective (*mut*⁻) bacteria.

To determine the time required for recovery from UV irradiation and concomitant mutagenesis, we incubated irradiated cells in glycerol-minimal medium and measured total cell masses (turbidity units; Figure 1) and numbers of viable bacteria (data not shown). After a 4-hr recovery period these both began to increase exponentially. However, frequencies of *lac*⁺ revertants after

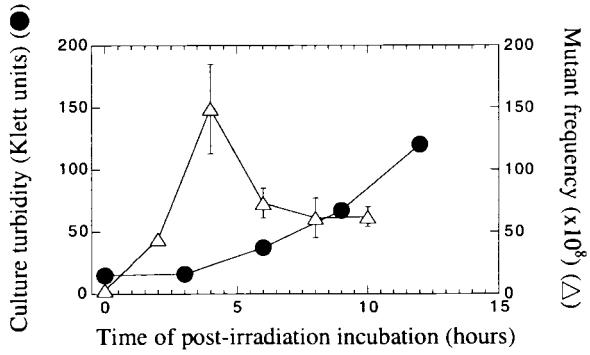

Figure 1.—Time course of UV mutagenesis and recovery from irradiation. Cultures of *phr::cat* F′ (*lacZ461-6*) bacteria (strain LH3140) were grown and irradiated to 60 J/m² in glycerol-minimal medium, diluted to a turbidity of 15 Klett units (~1×10^8 cfu/ml, for unirradiated bacteria), and shaken at 37°. Data correspond to means of turbidity measurements (●) for two independent experiments. Measurements of frequencies of UV-induced *lac*⁺ revertants (△) were essentially as described in materials and methods; data correspond to means for four independent experiments, with standard deviations indicated.

recovery could not be determined unequivocally by spreading on lactose-minimal plates, even when cells were washed several times: the number of visible Lac$^+$ colonies increased every day for at least 5 days, and their number was not proportional to the number of cells spread. These problems were most severe for *mut*$^-$ bacteria. We suspected that trace carbon sources in the lactose-minimal plates were supporting limited slow growth by *lacZ*$^-$ bacteria, the final numbers of cells being more dependent on the amounts of trace nutrients than on the initial numbers of cells, as observed previously (Zieg and Kushner 1977). We also suspected that *lac*$^+$ revertants continued to arise during this slow growth. To eliminate trace nutrients in the plates, we used purified ("noble") agar, high-purity (glucose-free) lactose and scavenged the plates by spreading with 5×10^7 Δ*lac* bacteria and incubating overnight, just before UV mutagenesis experiments. Under these conditions, the number of Lac$^+$ colonies arising was directly proportional to the number of cells spread, and did not change during 2–7 days of incubation on plates.

The low frequency of *lac*$^+$ revertants among cells that were spread immediately on selective plates (Figure 1) indicates that the technique mostly scores mutants that arise during postirradiation incubation in liquid culture. The maximum in revertant frequency after ~4 hr suggests that mutation fixation is nearly complete by then, but that chromosome replication and/or segregation of *lac*$^+$ and *lacZ*$^-$ alleles into daughter cells has not occurred yet. The subsequent 50% decrease in apparent revertant frequency indicates that few additional mutations arise during subsequent rounds of replication. Segregation of parental and daughter strands and cells after mutation of DNA strands would thus double the number of survivors without increasing the number of revertants, as suggested previously (Bridges and Munson 1968). After 6 hr of growth in culture, numbers of both *lac*$^+$ and total cells increase exponentially in parallel, providing a stable mutant frequency (Figure 1 and data not shown).

To screen for effects of mismatch-repair deficiency on UV mutagenesis via different transition and transversion pathways, we employed bacteria encoding on respective F′ episomes the six *lacZ* codon-461 alleles constructed by Cupples and Miller (1989); the designations *lacZ461-1*, *lacZ461-2* . . . *lacZ461-6* used here correspond to their strains CC101, CC102 . . . CC106. Each different allele reverts to a (GAG) codon for glutamate, the only amino acid compatible with β-galactosidase activity (Cupples and Miller 1988), by a different single base substitution. Table 2 shows *lac*$^+$ revertant frequencies (corrected for spontaneous frequencies in the same cultures) induced by a UV dose of 45 J/m^2, in *mut*$^+$ and *mutS201* bacteria bearing each of the six F′(*lacZ*) episomes. For the two *lacZ* alleles where target bases are not in potential dipyrimidine-photoproduct sites, the revertant frequencies ($\times 10^8$) in *mut*$^+$ and *mutS201* derivatives were 48 *vs.* 56 for *lacZ461-4*, and 14 *vs.* 16 for *lacZ461-5* (sense-strand G**C**G → G**A**G and G**T**G → G**A**G transversions, respectively; Table 2, lines 7–10). Thus, neither of these (presumably untargeted) transversions showed a mismatch-repair effect. The two other transversion alleles showed low UV-induced reversion frequencies, although target bases were in potential photoproduct sites, and low mismatch-repair effects: frequencies ($\times 10^8$) in *mut*$^+$ and *mutS201* derivatives were 2.8 *vs.* 4.5 for *lacZ461-1* and 1.6 *vs.* 2.0 for *lacZ461-3* (sense-strand T **T**AG → T **G**AG and T **C**AG → T **G**AG reversions, respectively; Table 2, lines 1 and 2, 5 and 6). However, where the mutation targets were 3′ bases in dipyrimidines, reverting via transitions, revertant frequencies ($\times 10^8$) were high and were consistently twofold higher in *mutS201* derivatives: 73 *vs.* 131 for *lacZ461-2* and 71 *vs.* 159 for *lacZ461-6* (template strand C**C**C → C**T**C and CT**T** → CT**C** reversions, respectively; Table 2, lines 3 and 4, 11 and 12). The *lacZ461-2* and *lacZ461-6* alleles were employed in all subsequent experiments.

Revertant frequencies among unirradiated *mutS* F′ (*lacZ461-2*) and *mutS* F′ (*lacZ461-6*) bacteria fluctuated considerably from culture to culture, perhaps reflecting spontaneous reversion relatively early in colony isolation or culture growth (before irradiation). When the

TABLE 2

UV-induced reversion of *lacZ461* alleles

Relevant genotype		Strain	UV-induced[a] mutant frequency ($\times 10^8$)
lacZ	*mut*		
lacZ461-1	*mut*$^+$	LH3148	2.8 ± 0.6
lacZ461-1	*mutS201*	LH3149	4.5 ± 0.7
lacZ461-2	*mut*$^+$	LH3138	73 ± 10
lacZ461-2	*mutS201*	LH3141	131 ± 16
lacZ461-3	*mut*$^+$	LH3150	1.6 ± 0.3
lacZ461-3	*mutS201*	LH3151	2.0 ± 0.0
lacZ461-4	*mut*$^+$	LH3152	48 ± 5
lacZ461-4	*mutS201*	LH3153	56 ± 6
lacZ461-5	*mut*$^+$	LH3154	14 ± 2
lacZ461-5	*mutS201*	LH3155	16 ± 4
lacZ461-6	*mut*$^+$	LH3140	71 ± 5
lacZ461-6	*mutS201*	LH3143	159 ± 28

[a] Growth of cultures of indicated Δ(*lac-pro*) F′(*lacZ*) bacteria from single colonies, UV irradiation to 45 J/m^2 and subsequent growth for 10 hr in liquid medium (without mating), and measurement of *lac*$^+$ revertant frequencies were as described in materials and methods. For each pair of *lacZ* strains, three cultures each of *mut*$^+$ and *mutS201* strains were grown and analyzed in parallel. There were two or three independent multiculture experiments for each strain. UV-induced *lac*$^+$ revertant frequencies in every culture of an indicated strain, after subtraction of frequencies in respective unirradiated aliquots, were averaged together, since culture-to-culture and experiment-to-experiment variations were of similar magnitudes. Data correspond to means (± standard errors). Mean revertant frequencies ($\times 10^8$) in unirradiated cultures (top to bottom, respectively) were 0.053, 0.054, 0.043, 23, 0.024, 0.020, 0.050, 0.72, 0.46, 0.028, 0.080, 0.032, and 17.

spreads in the respective sets of revertant frequencies observed for a particular strain were measured by calculating the standard deviations from the mean, large culture-to-culture fluctuations in spontaneous mutation frequencies for *mut*$^-$ strains were apparent. Thus, in the experiments shown in Table 2, the standard deviations of the sets of spontaneous-reversion frequencies for *mutS201* strains bearing F′(*lacZ461-1*) . . . F′(*lacZ461-6*) episomes were, respectively, 94, 110, 36, 63, 89, and 88% of the corresponding means. In other experiments, with *umuC122 mutL96* F′(*lacZ461-6*) bacteria (strain LH3290), the set of spontaneous revertant frequencies ($\times 10^8$) combined from several experiments (total n = 16), showed a mean of 50 and a standard deviation of 88, 176% of the mean. In subsequent experiments, therefore, we propagated parallel cultures of F$^-$ *mutS* bacteria or other recipients of interest, and *mut*$^+$ *umuC122* (F′ *lacZ*$^-$) donors, and mated F$^+$ to F$^-$ cultures for 2 hr. Selection for 6–8 hr yielded ~80% *pro*$^+$ chloramphenicol-resistant transconjugants. These were irradiated, grown out, and plated, with continued selection, which increased the transconjugant fraction to 90–95%. When *umu*$^+$ *mut*$^-$ recipients were mated with F′(*lacZ461-6*) donors (see Figures 2 and 3), the set of all spontaneous revertant frequencies ($\times 10^8$) of transconjugant cultures (n = 26 for all cultures of all experiments with *mutS*, *mutL*, and *mutH* recipients) showed a mean of 3.3 and standard deviation 1.0, only 30% of the mean. For F′(*lacZ461-2*) donors, the set of all spontaneous revertant frequencies ($\times 10^8$) for all *umu*$^+$ *mut*$^-$ transconjugant cultures (n = 7) showed mean 3.9 and standard deviation 1.0, only 25% of the mean. In experiments with *umuC122 mut*$^-$ recipients (see Figure 4), the set of all transconjugant spontaneous revertant frequencies ($\times 10^8$) for *lacZ461-6* donors (n = 17) showed mean 2.9, standard deviation 1.5 (52%), and for *lacZ461-2* donors (n = 17), mean 2.7, standard deviation 1.0 (37%).

UV mutagenesis in *umu*$^+$ *mut*$^-$ bacteria: We used the mating technique to compare reversion of *lacZ461-6* mutations in *mutS vs. mut*$^+$ transconjugants at three UV fluences (Figure 2). Revertant frequencies increased with fluence in both strains, but averaged 2.6-fold higher in *mutS97* than in *mut*$^+$ bacteria, in good agreement with the *mutS*/*mut*$^+$ ratio of 2.2 obtained in experiments with single cultures of F′ (*lacZ*) bacteria (no mating; see above). In *mut*$^+$ bacteria harboring a *mutS*$^+$-encoding plasmid, there was a small trend to even lower revertant frequencies.

Figure 3 shows the effects of mismatch-repair deficiencies on *lac461-2* and *lacZ461-6* reversions at a single UV fluence of 30 J/m^2. Revertant frequencies for *mutH*, *mutL*, and *mutS* bacteria were again consistently twice as high as for *mut*$^+$. Both the MutS$^-$ and MutL$^-$ effects might reflect reductions in transcription-coupled nucleotide excision repair (Mellon and Champe 1995), since the *lacZ* gene should be derepressed in these *lacI*-defective bacteria growing in glycerol. However, in strains defective in Mfd activity, which normally couples transcription blockage to template-strand-specific excision repair (Selby *et al.* 1991; Selby and Sancar 1993), a *mutS* mutation again increased *lacZ461-6* T → C reversion, to 2.2 times the *mut*$^+$ value (Figure 4, triangles). The Mfd$^-$ phenotype was confirmed by lower survival at 30 J/m^2 (5% *vs.* 18% for Mfd$^+$) and by transduction of the *mfd*::Tn*5* marker into strain WU3610 and verification of loss of the mutation-frequency-decline phenotype (Witkin 1966).

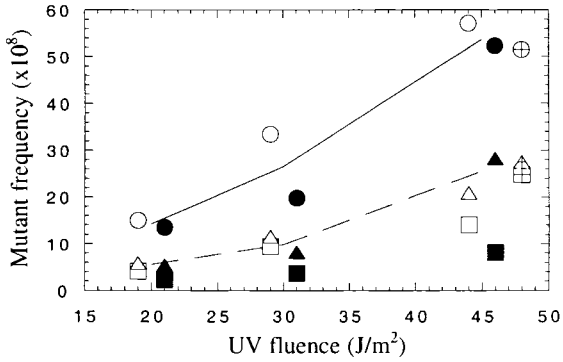

Figure 2.—Dependence of mutant frequency in *umu*$^+$ bacteria on UV dose. Growth and mating of *umu*$^+$ *phr*::*cat* Δ(*lac-pro*) recipients with F′ (*lacZ461-6 pro*$^+$) donors (strain LH3305), selection for transconjugants, UV irradiation to indicated fluences, further growth and plating, and analysis of UV-induced *lac*$^+$ revertant frequencies were as described in materials and methods. For each UV fluence, each open, filled, or crossed symbol corresponds to an independent experiment employing typically three parallel cultures of a particular strain: (open, solid, and crossed circles) *mutS97*, strain LH2519; (open, solid, and crossed squares) *mut*$^+$, strain LH3279; (open, solid, and crossed triangles) *mutS*$^{+++}$, strain LH23305. (For crossed and solid symbols at 45 J/m^2 there were only one or two independent cultures.) Values represent means for the multiple independent cultures for each strain. Some symbols at each UV fluence are off-set for clarity. Solid lines are drawn through means of values corresponding to circles, and dashed lines are drawn through means of triangle values. To estimate standard errors of the mean (SEMs), the set of all values for a particular strain and UV dose, for all cultures and experiments, were combined and analyzed, since experiment-to-experiment variations were not consistently markedly different from culture-to-culture variations. Revertant frequency means (± SEM) ($\times 10^8$) for *mutS97*, *mut*$^+$, and *mutS*$^{+++}$ bacteria, respectively, were as follows: 20 J/m^2, 14.4 (± 0.9), 5.7 (± 0.2), 3.4 (± 0.6); 30 J/m^2, 28.0 (± 3.4), 10.2 (± 0.9), (7.1 ± 1.6); 45 J/m^2, 55.0 (± 1.0), 24.4 (± 4.2), 20.5 (± 2.7). For the set of all data for unirradiated *mutS97* bacteria (n = 14), mean (SEM) was 3.9 (± 0.3) $\times 10^{-8}$ (frequencies for unirradiated *mut*$^+$ and *mutS*$^{+++}$ bacteria were negligible).

UV mutagenesis in UmuC$^-$ Mut$^-$ bacteria: The defining phenotype of *umuC* mutations is a drastic decrease in UV mutagenesis (Kato and Shinoura 1977; Steinborn 1978). Figure 4 shows that UV-induced reversions of *lacZ461-2* and *lacZ461-6* alleles in *umuC*::Tn*5 mut*$^+$ bacteria were almost undetectable (Figure 4), but that inacti-

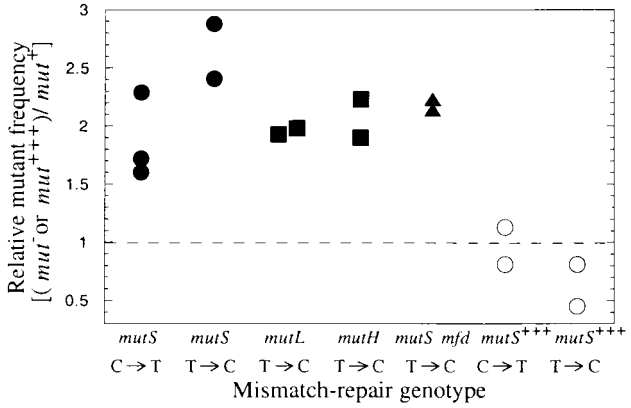

Figure 3.—UV-induced mutation in *umu*$^+$ bacteria. Indicated *umu*$^+$ *phr::cat* Δ(*lac-pro*) recipients and *mut*$^+$ donors of F′ (*lacZ-pro*$^+$) episomes were grown and mated, and transconjugants were selected, irradiated to 60 J/m^2, grown 8 hr, and scored for frequencies of UV-induced *lac*$^+$ revertants, as described in materials and methods. For each indicated genotype, each individual symbol refers to an independent experiment, in which mutant frequencies for two to three parallel cultures of *mut*$^+$ recipients (strain LH3179) were, respectively, compared to frequencies for two to three parallel cultures of one particular mutant recipient: *mutS* (●), strain LH2519; *mutL* (■), LH3183; *mutH* (■), LH2520; *mutS*$^{+++}$ (○), LH2536. [*mut*$^+$ *mfd* recipients (strain LH2548) were compared to *mutS mfd* (▲) recipients (strain LH2549).] For each independent experiment (independent symbol), relative mutant frequency equals mean of mutant frequencies for the *mut*$^-$ (or *mutS*$^{+++}$) cultures divided by the mean for the *mut*$^+$ cultures. Expected reversion pathways for the two F′ (*lacZ*$^-$ *pro*$^+$) donors employed are indicated: C → T, *lacZ461-2* (strain LH3303); T → C, *lacZ461-6* (strain LH3305). For the experiments employing *mut*$^-$ *vs. mut*$^+$ bacteria (solid symbols), mean (± SEM) UV-induced revertant frequencies (× 10^8), of transconjugants for sets of pooled data from all cultures in all repetitions of each particular experiment, were as follows for *mut*$^+$ and *mut*$^-$ strains, respectively: *mutS* C → T, 24.3 (± 1.3) and 13.4 (± 0.3); *mutS* T → C, 28.0 (± 3.4) and 10.2 (± 0.9); *mutL* T → C, 24.8 (± 1.3) and 12.0 (± 0.3); *mutH* T → C, 23.9 (± 1.4) and 11.6 (± 1.0); *mutS mfd* T → C, 30.4 (± 5.3) and 19.2 (± 1.4). Corresponding frequencies (± SEM) (× 10^8) for pooled data for unirradiated *mut*$^-$ bacteria were 3.3 (± 0.4), 4.7 (± 0.3), 3.4 (± 0.4), 4.1 (± 0.4), and 4.4 (± 0.4). Corresponding mean revertant frequencies (± SEM) × 10^8 for *mutS*$^{+++}$ C → T and *mutS*$^{+++}$ T → C were 12.6 ± 1.3 and 7.1 ± 1.6.

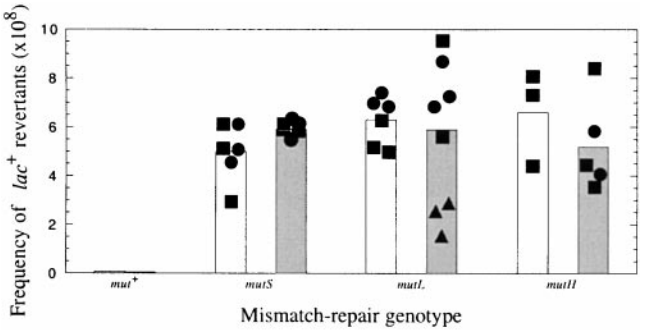

Figure 4.—UV-induced mutation in *umuC122* bacteria. Indicated *umuC122 phr::cat* Δ(*lac-pro*) recipients and *mut*$^+$ donors of F′ (*lacZ461-2 pro*$^+$) [strain LH3303, open bars (C → T reversion)] or F′ (*lacZ461-6 pro*$^+$) [strain LH3305, shaded bars (T → C reversion)] were grown and mated, and transconjugants were selected, irradiated to 60 J/m^2, grown 8 hr, and scored for frequencies of *lac*$^+$ revertants, as described in materials and methods. Individual symbols correspond to individual cultures, and different symbol shapes (■, ●, ▲), respectively, correspond to different independent experiments each employing two or three cultures of the particular indicated strains in parallel. Recipient genotypes indicated: *mut*$^+$, strain LH3265; *mutS*, LH2534; *mutL*, LH3269; *mutH*, LH2535. For the experiments employing *mut*$^-$ bacteria (bars 3–8), respective mean (± SEM) UV-induced and spontaneous transconjugant revertant frequencies (× 10^8) were as follows: *mutS* C → T, 5.0 (± 0.5) and 2.6 (± 0.1); *mutS* T → C, 5.9 (± 0.1) and 2.8 (± 0.6); *mutL* C → T, 5.0 (± 0.4) and 2.7 (± 0.4); *mutL* T → C, 5.6 (± 1.0) and 3.3 (± 0.5); *mutH* C → T, 5.8 (± 1.3) and 1.7 (± 0.5); *mutH* T → C, 5.9 (± 0.8) and 2.9 (± 0.8).

vation of mismatch repair unmasked substantial UmuC-independent UV mutagenesis: UV-induced frequencies of both T → C and C → T reversion induced by 30 J/m^2 were roughly 5 × 10^{-8}, in *mutS*, *mutL*, and *mutH* bacteria. These experiments were repeated, using 100 plates each to analyze revertant frequencies among irradiated *umuC122 mut*$^+$ recipients (strain LH3266) containing F′ *lacZ461-2* or F′ *lacZ461-6* episomes. On the basis of 135 and 121 total Lac$^+$ colonies, respectively, revertant frequencies were 4.0 × 10^{-9} and 2.7 × 10^{-9}; the respective revertant frequencies for *umuC122 mutS*::Tn*10* recipients (strain LH2534) containing the same episomes were 10.7 × 10^{-8} and 7 × 10^{-8}, a 25-fold increase in each case. In earlier experiments not using the mating technique, *mutL umuC122* (F′ *lacZ461-6*) bacteria showed wide fluctuations in spontaneous reversion frequency (see above). Here we analyzed UV mutagenesis only for 7 (of 17) cultures that showed spontaneous revertant frequencies of <10 × 10^{-8} before irradiation; the mean (standard deviation) revertant frequency was 6.7 (± 2.2) × 10^{-8}. UV irradiation of each of these cultures to 60 J/m^2 increased revertant frequencies; the mean was 12.1 (± 6) × 10^{-8}. The apparent UV-specific component, 5.4 × 10^{-8}, was thus in good agreement with values obtained using the mating technique (see above). Revertant frequencies for *mut*$^+$ *umuC122* (F′ *lacZ461-6*) bacteria (no mating) were again <5 × 10^{-9}.

DISCUSSION

The experiments described here support a hypothesis that mismatch-repair systems antagonize UV mutagenesis by excising incorrect bases inserted in nascent strands opposite photoproducts during the course of DNA replication, and replacing them, some or most of the time, with the correct bases. This hypothesis was motivated by observations that human MSH2 · MSH6 protein bound specifically to DNA containing a mismatched cyclobutane pyrimidine dimer (CPD, Py<>Py), *e.g.*, T<>T/AG, or a mismatched pyrimidine-(6-4′)-pyrimidinone photo-

product ([6-4] photoproduct, Py[6-4]Py), *e.g.*, T[6-4]T/AG, but not to DNA containing T<>T/AA or T[6-4]T/AA pairs (Mu *et al.* 1997; Wang *et al.* 1999). Other explanations for these results, especially for the twofold difference between *mut*$^+$ and *mut*$^-$ bacteria seen in *umu*$^+$ strains, might be more efficient excision repair in *mut*$^+$ bacteria, or faster replication (relative to repair) in *mut*$^-$ cells. DNA replication might in principle be faster if not delayed by binding and/or processing of mismatched bases or photoproducts by mismatch-repair proteins, but we are not aware of any reports of faster replication in *mut*$^-$ bacteria. We think it is unlikely that the antagonism of UV mutagenesis by mismatch repair described here simply reflects removal, by MutSL-assisted transcription-coupled excision repair, of premutagenic photoproducts at the C**C**C and T**T** targets in the *lacZ461-2* and *lacZ461-6* template strands, because we observed MutH-dependent and Mfd-independent antagonism of mutagenesis, in contradistinction to the requirements for transcription-coupled repair. Furthermore, the previously reported MutHLS-dependent recombination of repressor-blocked UV-irradiated phage DNA occurred in excision-repair-deficient (Uvr$^-$) bacteria (Feng *et al.* 1991). Clearly, it would be of interest to analyze mismatch-repair effects on UV mutagenesis in Uvr$^-$ bacteria. UV mutagenesis might occur in Uvr$^+$ bacteria during replication, or by insertion of an incorrect nucleotide opposite a nearby second UV photoproduct during DNA synthesis to fill a gap created by excision repair of a first photoproduct in the opposite strand. In the latter case, mismatch repair might prevent fixation of mutations during subsequent excision repair of the second photoproduct. In any event, the constant ratio between UV-induced revertant frequencies for *mut*$^-$ and *mut*$^+$ bacteria seen in Figure 2 as the UV dose increases seems most consistent with a constant efficiency of mismatch repair.

Mismatch repair provoked by photoproduct/base mismatches, as by base/base mismatches, would be expected only in the vicinity of replication forks, where unmethylated d(GATC) sites were still present (Lahue *et al.* 1989). In *un*replicated DNA, therefore, Py<>U/PuG mismatches resulting from the relatively rapid deamination of cytosines in CPDs (Peng and Shaw 1996) would not be processed, whereas Py<>U/PuG mismatches arising in newly replicated DNA might be "corrected" to Py<>U/PuA, actually fixing C → T transitions. Also, if progress of replication forks past template Py<>C photoproducts depended on deamination to Py<>U, so as to facilitate insertion of an adenine nucleotide (Tessman *et al.* 1992), the resulting Py<>U/PuA moiety would not be recognized by MutS (Wang *et al.* 1999). Thus, if deamination of cytosines in CPDs played a major role in UV mutagenesis in the experiments described here, mismatch repair might antagonize UV-induced T → C transitions more efficiently than C → T. However, the apparent mutation-removal efficiencies were approximately the same for UV-induced mutation of the two alleles tested, roughly 50% in *umu*$^+$ and 96% in *umuC122* bacteria. Therefore, most C → T reversions as measured here in *mut*$^-$ *lacZ461-2* bacteria would appear not to have arisen by cytosine-deamination mechanisms, but rather by insertion during DNA replication of adenines opposite photoproducts containing cytosines [perhaps their imino tautomers (Person *et al.* 1974; Jiang and Taylor 1993)]. (Although [6-4] photoproducts may be responsible for some or all of the MutHLS-sensitive UV mutagenesis described here, cytosine deamination is not enhanced in these photoproducts.)

In comparisons among different studies of UV mutagenesis, the specific circumstances under which UV-induced mutations arise and are fixed may be important. In our experiments irradiated cells were diluted and shaken in liquid glycerol-minimal medium, under conditions such that parallel unirradiated cultures grew exponentially. Irradiated cultures resumed exponential growth after ~4 hr, at which time fixation of mutations appeared complete (Figure 1). In the scavenged lactose-minimal plates trace carbon sources appeared not to be available to the *lacZ*$^-$ bacteria for even limited growth and further mutagenesis, since very few Lac$^+$ colonies arose when bacteria were plated immediately after irradiation. Most mutations appeared to arise 2–4 hr after irradiation, although the cultures did not show appreciable growth until 6 hr (Figure 1). Other workers (Crowley and Hanawalt 1998) have shown that for excision-repair-proficient bacteria growing in glucose-minimal medium, removal from the genome overall of CPDs induced by 40 J/m^2 of 254-nm irradiation is essentially complete within 60 min; repair of [6-4] photoproducts is complete in 20 min. Thus, factors other than photoproduct removal would appear to be rate limiting here for resumption of growth and colony-forming proficiency.

Mismatch repair might reduce UV mutagenesis by removing incorrect bases inserted opposite photoproducts during translesion synthesis and/or correcting mismatches from "untargeted" SOS mutagenesis (Kunz and Glickman 1984; Christensen *et al.* 1985; Miller 1985; Caillet-Fauquet and Maenhaut-Michel 1988). Several considerations argue against a hypothesis that most of the UV mutagenesis seen here in *mut*$^-$ *umuC122* bacteria is untargeted.

1. Caillet-Fauquet and Maenhaut-Michel (1988) reported no significant difference between the frequencies of spontaneous reversion to rifampicin resistance of *umuC122 mutS* bacteria that were *recA*$^+$ or those that carried the *recA730* mutation, known to cause an SOS-like activation of RecA protein that increases (untargeted) spontaneous mutation ("SOS mutagenesis"), whereas a *recA270* mutation in *umu*$^+$ *mutS/L* backgrounds increased the mutation fre-

quency 2.5-fold. This is consistent with other reports that SOS mutagenesis requires UmuC function.

2. Fijalkowska *et al.* (1997) observed that a *recA730* mutation increased transversion frequencies (reversions of *lacZ461-4* and *lacZ461-5* mutations) much more than transition frequencies (*lacZ461-2* and *lacZ461-6* reversions), respectively, 2–6-fold *vs.* 20–70-fold, in both *mut*$^+$ and *mutS* bacteria (both *umuC*$^+$). There is no evidence to suggest that this preference might be reversed in *umuC122* bacteria.
3. Previous studies suggest that untargeted mutations amount to only 3–10% of all UV-induced mutations, including those at both putative photoproduct and nonphotoproduct sites (Kunz and Glickman 1984; Christensen *et al.* 1985; Miller 1985).

Thus, although we cannot rule out some contribution of untargeted events to the greatly increased UV-induced transition frequencies seen in *mut*$^-$ derivatives of *umuC122* bacteria, it seems likely that much of this reflects photoproduct-targeted events. In *umu*$^+$ bacteria, the greater-than-first-power dependence of mutant frequency on dose seen in both *mut*$^+$ and *mutS* derivatives (Figure 2) argues in favor of targeted mutagenesis in both strains, since untargeted mutation would be expected to level off at higher doses, once SOS induction was maximal.

Some previous descriptions of UmuC-independent UV mutagenesis in mismatch-repair-proficient bacteria represent special cases not applicable here. Fuchs and co-workers (Napolitano *et al.* 1997) have described SOS-dependent, but UmuC-independent, frameshift mutagenesis at certain hotspots, targeted by 2-acetylaminofluorene adducts, that involves slip mispairing at mononucleotide or dinucleotide repeats at these sequences. The UVM pathway, studied extensively by Humayun (1998), specifically causes mutations by inserting thymine nucleotides opposite etheno-cytosine residues. Although inducible by other kinds of DNA damage, it is independent of RecA and UmuD$_2$′C.

Lawrence and co-workers (Christensen *et al.* 1988) observed substantial UmuC-independent UV mutagenesis when they spread cultures of *mut*$^+$ *uvrA6 umuC122* bacteria, after post-UV-irradiation growth, on nonselective plates, and identified *lacI*$^-$ mutants among other survivors by colony color. However, when they spread the same cultures on scavenged selective (phenyl-galactosidase) plates, similar to the procedure used here, very few colonies (*lacI*$^-$ mutants) were seen. On nonselective plates, mismatch-repair proteins might have declined during colony growth, making cells (*umuC122*) Mut$^-$ phenocopies, as has been reported for stationary-phase (Feng *et al.* 1996) or carbon-starved (Harris *et al.* 1997) bacteria. [P. L. Foster has argued against a hypothesis of low mismatch repair under starvation conditions, however (Foster 1999).] Alternatively, some of these irradiated *uvrA umuC122* bacteria might have become Mut$^-$ phenocopies eventually during incubation on the nonselective plates, due to saturation of mismatch repair (Schaaper and Radman 1989; Cupples *et al.* 1990) by uracil/base mismatches arising from deamination of unrepaired CPD cytosines (Wang *et al.* 1999).

Another source of UmuC-independent UV mutagenesis might be the product of the *dinB* (*dinP*) gene (Ohmori *et al.* 1995), which maps close to *lacZ* and would thus be present in the F′ episomes used here. Although the DinB protein is a UmuC homolog, it is not known whether it interacts with UmuD protein, which should be present at some level in *umuC122* bacteria. It would be of interest to analyze UV mutagenesis in Δ*umuCD mutS* bacteria.

Why is antagonism of UV mutagenesis apparently more complete in *umuC122* than in *umu*$^+$ bacteria? One possibility is that the high numbers of misinsertions in *umu*$^+$ bacteria (corresponding to revertant frequencies of $\sim 50 \times 10^{-8}$ in *mut*$^-$ *umu*$^+$ cells irradiated to 45 J/m^2) saturate the mismatch-repair system, as observed previously in other contexts (Schaaper and Radman 1989; Cupples *et al.* 1990). By this hypothesis, the revertant frequencies in *umuC122 mut*$^-$ cells at 60 J/m^2 ($\sim 5 \times 10^{-8}$) would correspond to misinsertion levels below the saturation threshold. However, the apparent constant efficiency of antagonism of UV mutagenesis by mismatch repair in *umu*$^+$ bacteria, as fluences increase from 20 to 45 J/m^2 (Figure 2), argues against this. Since MutS protein shows lower affinity for photoproduct/base mismatches than for corresponding base/base mismatches (Wang *et al.* 1999; H. Wang and J. B. Hays, unpublished data), high concentrations of MutS might be needed for efficient antagonism of UV mutagenesis. However, a multicopy plasmid encoding the *mutS*$^+$ gene had little effect on mutant frequencies (Figures 2 and 3); simultaneous overproduction of MutS, MutL, and MutH activities has not been tested. A second explanation would be that one class of photoproducts, perhaps CPDs, is responsible for UV mutagenesis in *umuC*$^-$ bacteria, whereas both CPDs and [6-4] photoproducts might target mutations in *umu*$^+$ bacteria. Only mutations targeted by one class might be subject to mismatch repair. However, both T-T CPDs and T-T [6-4] photoproducts mismatched opposite A-G are recognized by MutS or its human homolog hMutSα, and neither photoproduct opposite A-A is recognized (H. Wang and J. B. Hays, unpublished data; Wang *et al.* 1999); *i.e.*, there is no evidence for MutS/hMutSα preference for one mismatched photoproduct over the other. A third explanation might be that in UV-irradiated *umu*$^+$ bacteria, DNA resynthesis associated with mismatch repair triggered by photoproducts might itself result in new misinsertions, opposite photoproducts or at nonphotoproduct sites, by DNA polymerase III (Lahue *et al.* 1989) associated with UmuD′$_2$C and RecA protein.

In bacteria subjected to UV fluences insufficient to induce the SOS response, occasional photoproduct/

base mismatches might arise from DNA replication past UV photoproducts, perhaps facilitated by low-level UmuC-independent constitutive activities that allowed error-prone translesion synthesis. If so, these mismatches would appear to be almost all corrected by the MutHLS system. In heavily DNA-damaged SOS-induced bacteria, however, where a burst of mutagenesis to increase genetic variability has been hypothesized to promote species survival (Echols 1982), inefficient correction of photoproduct/base mismatches, because of error-prone mismatch-repair resynthesis or for other reasons, might be of less concern. One testable prediction of this model is that UV mutagenesis in *mut*⁻ strains independent of UmuC function should not require other SOS functions. Another prediction, that MutS-like proteins specifically bind to mismatched but not to matched photoproducts, has been confirmed for both human hMutSα protein (Mu *et al.* 1997; Wang *et al.* 1999) and *E. coli* MutS protein (H. Wang and J. B. Hays, unpublished observations).

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