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Mutagenesis, by Methylating and Ethylating Agents, in *mutH*, *mutL*, *mutS*, and *uvrD* Mutants of *Salmonella typhimurium* LT2

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Salmonella typhimurium LT2 *mutH*, *mutL*, *mutS*, and *uvrD* mutants were especially sensitive to mutagenesis by both the *recA*$^+$-dependent mutagen methyl methane sulfonate and the *recA*$^+$-independent mutagen ethyl methane sulfonate, but not to mutagenesis by agents such as 4-nitroquinoline-1-oxide and UV irradiation. Similarly, these mutator strains were very sensitive to mutagenesis by the methylating agents *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and *N*-methyl-*N*-nitrosourea. The increased susceptibility to mutagenesis by small alkylating agents due to *mutH*, *mutL*, *mutS*, and *uvrD* mutations was not accompanied by an increased sensitivity to killing by these agents. Various models are discussed in an effort to explain why strains thought to be deficient in methyl-instructed mismatch repair are sensitive to mutagenesis by methylating and ethylating agents.

Several genetic phenomena, including high negative interference (44) and gene conversion of λ heteroduplexes (24, 41, 45), have suggested the existence of a process which converts mismatched base pairs to normal base pairs (26, 38). For such a system to effectively repair mismatches arising as replication errors, it must have some way of recognizing the parental strand so that the correct base pair can be restored. The results of transfection experiments with heteroduplex λ DNAs that differ in the degree of methylation and carry different genetic markers (28) have led to the suggestion that the presence of N^6-methyladenine in the parental strand allows it to be discriminated from the newly synthesized (unmethylated) daughter strand. Such methylation occurs at GATC sequences in both *Escherichia coli* and *Salmonella typhimurium* (7, 10, 17): in *E. coli*, the product of the *dam* gene has been identified as being responsible for this methylation (19). Recently, it has been suggested that methyl-instructed mismatch repair is carried out by the products of the *E. coli mutH*, *mutL*, *mutS*, and *uvrD* genes (6, 8, 9, 24, 27–29).

We have recently isolated and characterized *mutH*, *mutL*, *mutS*, and *uvrD* mutants of *S. typhimurium* that were generated by the insertion of transposable elements Tn*5* and Tn*10* (34). In the course of this work we observed that these spontaneous mutator strains were very sensitive to mutagenesis by both methylating and ethylating agents. In this paper we describe experiments designed to investigate further why mutants thought to be defective in methyl-instructed mismatch repair are also particularly sensitive to mutagenesis by such alkylating agents.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains listed in Table 1 are derivatives of *S. typhimurium* LT2. Mutator strains containing pKM101 were constructed by the method of Mortelmans and Stocker (22), using TA2000 as the donor.

Media and chemicals. Luria broth and M9 minimal glucose medium have been described previously (21). Top agar contained 0.6% agar and 0.5% NaCl. Methyl methane sulfonate (MMS), diethyl sulfate, and 4-nitroquinoline-1-oxide (NQO) were purchased from Aldrich Chemical Co., Milwaukee, Wis. Ethyl methane sulfonate (EMS) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) were obtained from Sigma Chemical Co., St. Louis, Mo. *N*-methyl-*N*-nitrosourea (MNU) was a gift from John Essigman. Dimethyl sulfoxide was obtained from Mallinckrodt, Inc., St. Louis, Mo.

Chemical mutagenesis. Dose-response curves for chemical mutagens were obtained by using a previously described procedure (42). Fresh solutions of MMS, EMS, and diethyl sulfate in dimethyl sulfoxide were prepared immediately before use. NQO and MNU solutions were made in dimethyl sulfoxide and stored as frozen portions at −20°C. MNNG was dissolved in 10 mM sodium acetate (pH 5.0) and stored as frozen portions at −70°C. Frozen solutions were thawed only once and used immediately.

MNNG-induced mutants per survivor were measured by using the following protocols. Cells were grown to a density of 2×10^8 to 4×10^8 cells per ml in

TABLE 1. Bacterial strains[a]

Strain	Genotype	Reference
GW45	*hisG46*	34
TA92	*hisG46* (pKM101)	34
GW1702	*hisG46 mutS121*::Tn*10*	34
GW1714	*hisG46 mutL111*::Tn*10*	34
GW1808	*hisG46 uvrD421*::Tn*5*	34
GW1809	*hisG46 mutB131*::Tn*5*	34
GW1709	*hisG46 srl-211*::Tn*5 recA1 mutS121*::Tn*10*	34
GW1721	*hisG46 srl-211*::Tn*5 recA1 mutL111*::Tn*10*	34
GW1825	*hisG46 srl-202*::Tn*10 recA1 uvrD421*::Tn*5*	34
GW1826	*hisG46 srl-202*::Tn*10 recA1 mutB131*::Tn*5*	34
GW1827	*hisG46 srl-202*::Tn*10 recA1 mutH101*::Tn*5*	34
GW1764	*hisG46 srl-211*::Tn*5 recA1*	34
GW1813	*hisG46 mutS121*::Tn*10 uvrD421*::Tn*5*	34
GW1814	*hisG46 mutL111*::Tn*10 uvrD421*::Tn*5*	34
GW1819	*hisG46 mutS121*::Tn*10 mutH101*::Tn*5*	34
GW1820	*hisG46 mutL111*::Tn*10 mutH101*::Tn*5*	34
TA2000	*purF145* (pKM101)	20

[a] The nomenclature used was taken from Sanderson and Hartman (30), Bachmann and Low (4), and Campbell et al. (5).

minimal glucose medium, pelleted, and resuspended in fresh minimal glucose medium to give a density of 1×10^9 to 2×10^9 cells per ml. Then 1-ml cultures were incubated at 37°C for 5 min to reinitiate growth, and varying amounts of mutagen were added. The cells were then incubated for 10 min at 37°C. Chemically induced revertants were measured by plating 0.1 ml of a 10^{-1} dilution of the cells in 2.5 ml of top agar containing 50 μM histidine. Cell survival was assessed by plating 0.1 ml of a 10^{-6} dilution of the cells under the same conditions used to measure mutagenesis. Slightly concentrated cultures were used in this experiment so that the mutagen could be diluted away before the bacteria were plated. Otherwise, the results would have been complicated by a substantial number of revertants arising from chemical mutagenesis occurring on the plates.

MMS, EMS, and MNNG survival. Cells were grown in minimal glucose medium to a density of 2×10^8 to 4×10^8 cells per ml. To 1 ml of cells 10 μl of MMS, 50 μl of EMS, or 50 μg of MNNG was added (MNNG was added as a 1-mg/ml solution in 10 mM sodium acetate, pH 5.0). Cells were then incubated at 37°C with shaking, and samples of cells were diluted and plated at different times on minimal glucose medium plates supplemented with 50 μg of histidine per ml. Cell survival in treated cultures was compared with cell survival in untreated control cultures.

RESULTS

Mutagenesis by MMS, EMS, and NQO. In addition to their increased spontaneous mutation rates, *mutH*, *mutL*, *mutS*, and *uvrD* strains of *S. typhimurium* also exhibited increased sensitivities to the mutagenic effects of MMS and EMS (Fig. 1); the levels of MMS and EMS used were sublethal for all of the strains. This is not a general property of all *S. typhimurium* mutator strains since a strain carrying a *mutB* mutation (34) had the same sensitivity to MMS and EMS mutagenesis as its *mut*$^+$ parent (data not shown). Moreover, this increased sensitivity of the *mutH*, *mutL*, *mutS*, and *uvrD* strains to chemical mutagenesis is confined to certain classes of mutagens. For example, Fig. 1 indicates that these strains were not more susceptible to mutagenesis by NQO (data shown for only *mutH*).

Although the effects of *mutH*, *mutL*, *mutS*, and *uvrD* mutations on MMS and EMS mutagenesis were qualitatively similar, there were reproducible, quantitative differences among strains carrying these mutations. Strains carrying *mutL* or *mutS* were the most susceptible to mutagenesis, the *uvrD* strain was the least susceptible, and the *mutH* strain was moderately sensitive (Fig. 1).

Figure 1 also shows the dose-response curves for a *mut*$^+$ strain containing plasmid pKM101 (23). The presence of pKM101 in a cell is similar to a mutation in *mutH*, *mutL*, *mutS*, or *uvrD* in that pKM101 enhances some types of chemical mutagenesis (20, 42). However, Fig. 1 clearly shows that the presence of pKM101 altered the cellular responses to a different set of chemical mutagens than deficiencies in *mutH*, *mutL*, *mutS*, and *uvrD* did. pKM101 did not affect EMS mutagenesis but dramatically increased NQO mutagenesis, whereas a mutation in *mutH*, *mutL*, *mutS*, or *uvrD* increased EMS mutagenesis without affecting NQO mutagenesis. Nevertheless, the mutagenic effect of certain agents, such as MMS, can be increased either by pKM101 or by mutations in *mutH*, *mutL*, *mutS*, or *uvrD*.

Mutagenesis by other methylating and ethylating agents. One useful strategy for obtaining clues to the mechanism by which the mutator mutations alter the sensitivity of cells to chemical mutagenesis has been to look at the effects of the mutations on a variety of chemical mutagens. Diethyl sulfate, a weak ethylating agent, was a more potent mutagen for cells carrying mutations in *mutH*, *mutL*, *mutS*, and *uvrD* than for wild-type cells or for cells carrying the *mutB* mutation (data not shown). This result resembles the results discussed above for the related compound, EMS.

Two powerful methylating agents, MNNG

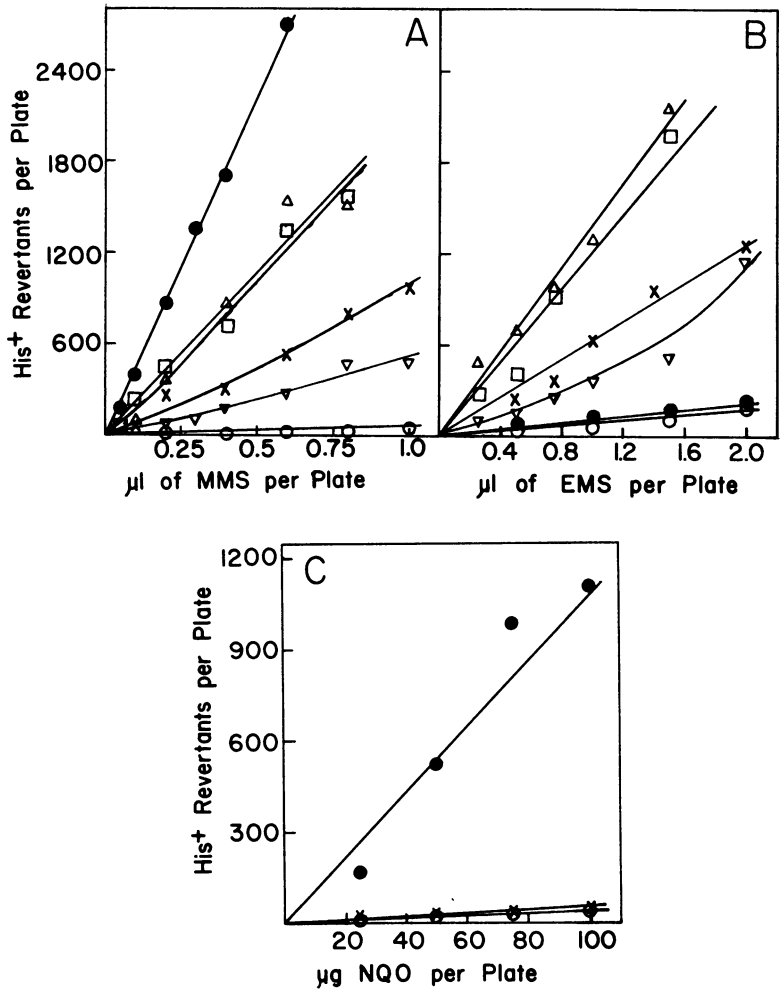

FIG. 1. MMS-, EMS-, and NQO-induced mutagenesis in *S. typhimurium* strains containing insertions in the *mutH*, *mutL*, *mutS*, and *uvrD* genes. Cells were plated onto M9 minimal medium containing limiting histidine and varying amounts of MMS, EMS, or NQO. The spontaneous His$^+$ revertants were substracted for all strains. (A) MMS dose-response curves. (B) EMS dose-response curves. (C) NQO dose-response curves. Symbols: ○, GW45 (*mut*$^+$); ×, GW1810 (*mutH101*); △, GW1714 (*mutL111*); □, GW1702 (*mutS121*); ▽, GW1808 (*uvrD421*); ●, TA92 (*mut*$^+$, pKM101).

and MNU, were also more mutagenic for cells with a deficiency in *mutH*, *mutL*, *mutS*, or *uvrD* than for wild-type cells. However, the shapes of the dose-response curves for MNNG and MNU with wild-type strains (Fig. 2) are different from the shapes of the curves for MMS and EMS (Fig. 1). The MMS and EMS dose-response curves for the wild-type strain and for all of the mutator strains were essentially linear; the increased mutabilities of mutator strains with these chemicals were evident simply as increases in the slopes of the lines.

In contrast, the dose-response curves for the wild-type strain with MNNG and MNU were not linear. With both of these chemicals, very few mutants were induced at low doses, and there was a very steep increase in the accumulation of mutants at higher doses. (There was no significant killing caused by any of the doses used in the experiment shown in Fig. 2.) The non-linearity of the MNNG dose-response curve was observed previously during the development of the Ames *Salmonella* test system (20). Unlike the wild-type strain, *mutH*, *mutL*, *mutS*, and *uvrD* strains yielded large numbers of mutants even at low MNNG or MNU doses, and their dose-response curves were approximately linear. These results raised the possibility that the wild-type strain contains a saturable, accurate repair system which prevents mutagenesis

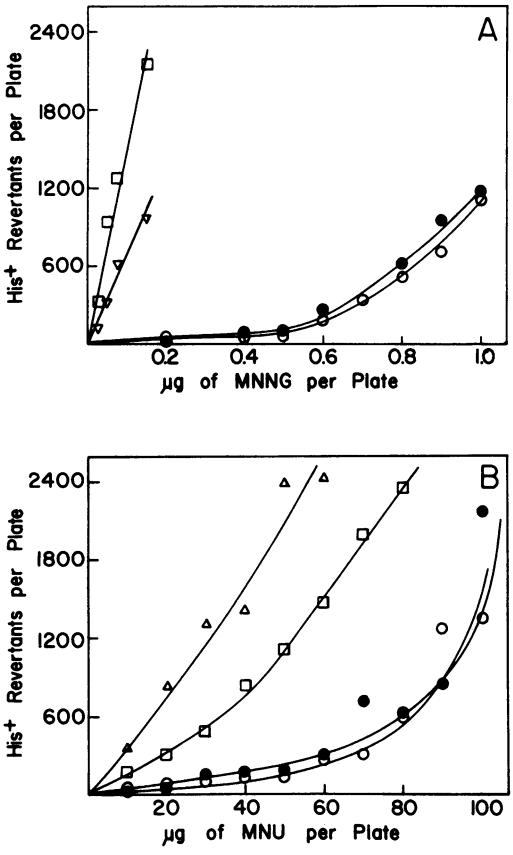

FIG. 2. MNNG- and MNU-induced mutagenesis in *S. typhimurium* strains containing insertions in the *mutH*, *mutL*, *mutS*, and *uvrD* genes. Cells were plated onto M9 minimal medium containing limiting histidine and varying amounts of MNNG or MNU. (A) MNNG dose-response curves. (B) MNU dose-response curves. Symbols: ○, GW45 (*mut*$^+$); ▽, GW1808 (*uvrD421*); △, GW1714 (*mutL111*); □, GW1702 (*mutS121*); ●, TA92 (*mut*$^+$, pKM101).

by MNNG and MNU and that the *mutH*, *mutL*, *mutS*, and *uvrD* strains might be deficient in this repair system.

The plate mutagenesis protocol used to obtain the data in Fig. 1 and 2 was that used widely for the Ames *Salmonella* test (3). Unfortunately, detailed interpretations of the shapes of such dose-response curves are complicated by uncertainties in a number of parameters, including the diffusion time of the mutagen in the agar and the growth rate of the bacteria on the plate. Thus, the susceptibility of cells to MNNG mutagenesis was also examined by using a protocol in which cells in liquid culture were exposed to increasing concentrations of the mutagen for a short period of time, followed by measurement of the frequency of chemically induced mutants per survivor. The shapes of the dose-response curves obtained by this procedure (Fig. 3) showed an interesting difference from the shapes of the dose-response curves in Fig. 2; the curve for the *mutS* strain was not linear but was biphasic, as was the curve for the *mut*$^+$ parental strain. However, the *mutS* strain was clearly more susceptible to MNNG mutagenesis at low doses (0.5 to 1.0 μg/ml) than the parental strain, as suggested by the results in Fig. 2.

Sensitivity of mutator strains to killing by alkylating agents. The increased susceptibility of the mutator strains to mutagenesis by methylating and ethylating agents suggested that the mutations in these strains might alter the processing of premutagenic lesions introduced by these chemicals. One set of genes known to affect the processing of certain premutagenic lesions consists of *uvrA*, *uvrB*, and *uvrC*. In *E. coli*, these genes code for a complex endonuclease (33) which initiates the process of excision repair of pyrimidine dimers and other bulky adducts. *E. coli* or *S. typhimurium* cells carrying *uvr* mutations exhibit increased sensitivities to both the killing and the mutagenesis caused by a variety of DNA-damaging agents (2, 16). This implies that the *uvr*$^+$-dependent excision repair system normally processes not only premutagenic lesions but also lethal lesions. (With some DNA-damaging agents the same lesions may be both lethal and premutagenic.) In contrast to *uvr* mutations, the *mutH*, *mutL*, and *mutS* mutations increased the susceptibility of cells to mutagenesis by MMS, EMS, and MNNG, but did not increase the sensitivity of the cells to

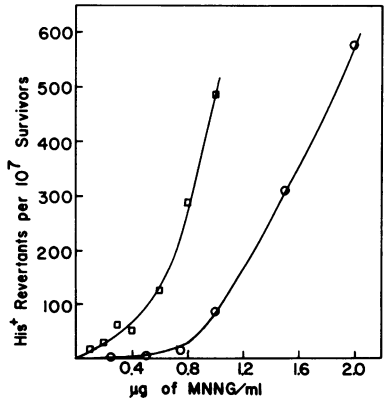

FIG. 3. MNNG-induced mutagenesis in liquid cultures. Exponentially growing cells in liquid medium were treated for 10 min with increasing concentrations of MNNG, and different cell dilutions were plated onto M9 minimal medium containing limiting histidine to measure both mutagenesis and survival. Data are plotted as the number of MNNG-induced revertants per 10^7 survivors versus the dose of MNNG. Spontaneous His$^+$ revertants were subtracted from each data point. Symbols: ○, GW45 (*mut*$^+$); □, GW1702 (*mutS121*).

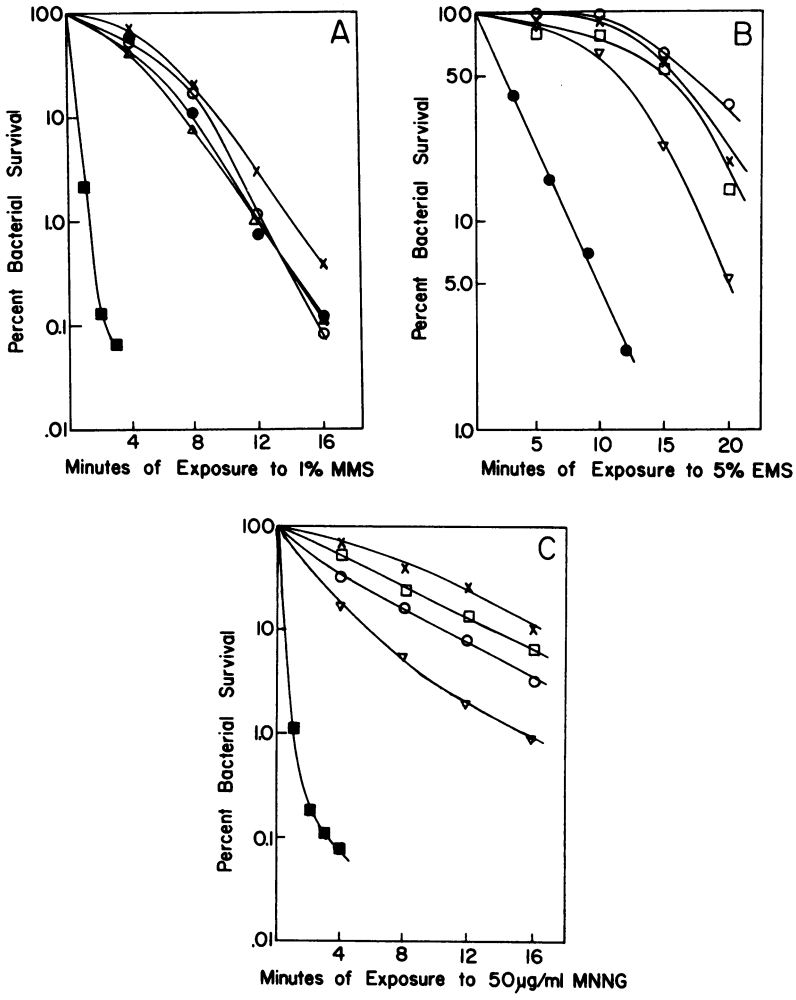

FIG. 4. Sensitivity of *S. typhimurium* mutator strains to killing by small alkylating agents. Exponentially growing cells were treated with a single dose of MMS, EMS, or MNNG in liquid culture. At different times, cells were diluted and plated onto M9 minimal medium to measure survival. (A) MMS killing curves. Symbols: ○, GW45 (*mut*$^+$); ×, GW1810 (*mutH101*); △, GW1714 (*mutL111*); ●, GW1809 (*mutB131*); ■, GW1764 (*recA1*). (B) EMS killing curves. Symbols: ○, GW45 (*mut*$^+$); ×, GW1810 (*mutH101*); □, GW1702 (*mutS121*); ▽, GW1808 (*uvrD421*); ●, GW1764 (*recA1*). (C) MNNG killing curves. Symbols: ○, GW45 (*mut*$^+$); ×, GW1810 (*mutH101*); □, GW1702 (*mutS121*); ▽, GW1808 (*uvrD421*); ■, GW1764 (*recA1*).

killing by these same agents (Fig. 4). In fact, the *mutH* strain reproducibly appeared to be more resistant to killing by MMS and MNNG than the wild-type strain. These results suggest that whatever the mode of action of the *mutH*, *mutL*, and *mutS* gene products, they do not play a significant role in the repair of lethal lesions introduced by MMS, EMS, and MNNG. Similar results have been reported recently for the analogous *E. coli* mutants with respect to MNNG killing (12).

Unlike the other mutators, the *uvrD* strain was somewhat more sensitive to killing by these chemicals than the wild-type strain, but not nearly as sensitive as a *recA* strain (data shown for EMS and MNNG). This is not surprising since the mutation in the *uvrD* gene also confers increased sensitivity to killing by other DNA-damaging agents (34), and it has been postulated that the *E. coli uvrD* gene product functions in another repair pathway(s) independent of *mutH*, *mutL*, and *mutS* (35, 37). The killing curves for the *mutB* strain are identical to those of the parental *mut*$^+$ strain.

Chemical mutagenesis in *mut recA* and *uvrD recA* strains. In both *E. coli* and *S. typhimurium*, the mutagenicity of many chemicals is either totally or partially dependent on a *recA*$^+$ geno-

TABLE 2. MMS mutagenesis in *mut recA* strains

Strain	Relevant genotype	No. of His^+ revertants per plate with no MMS	0.2 μl of MMS		0.4 μl of MMS	
			No. of His^+ revertants per plate[a]	Plating efficiency (%)[b]	No. of His^+ revertants per plate[a]	Plating efficiency (%)[b]
GW1764	*mut*$^+$ *recA1*	14	5	86	4	30
GW1827	*mutH101 recA1*	165	72	64	43	31
GW1721	*mutL111 recA1*	354	127	70	96	47
GW1709	*mutS121 recA1*	249	121	92	165	42
GW1825	*uvrD421 recA1*	295	142	42	133	22
GW1826	*mutB131 recA1*	158	56	100	37	31

[a] Spontaneous His^+ revertants were not subtracted.

[b] Plating efficiency was determined by plating 0.1 ml of a 10^{-6} dilution of an overnight culture under the same conditions used to detect His^+ revertants.

type (20, 46). Thus, a *recA* mutation was introduced into each of the mutator strains, and dose-response curves for several mutagens were obtained. Table 2 shows the number of His^+ revertants per plate over a range of MMS doses for the *recA* derivatives of all of the mutator strains. Plating efficiencies are also included in Table 2 since the *recA* mutation confers sensitivity to MMS killing, as shown in Fig. 4A. For the *mut*$^+$ *recA*$^-$ strain there was a decrease in the number of His^+ revertants per plate as the MMS dose was increased. This result is consistent with the observation of other workers that MMS is a *recA*$^+$-dependent mutagen (20, 40, 42). (The observed decrease in His^+ revertants at higher MMS doses was probably related to the reduction in plating efficiency.) The *recA* derivatives of all of the mutator strains also exhibited a total loss of MMS mutability despite the unusually high sensitivity to MMS mutagenesis of the parental *mutH*, *mutL*, *mutS*, and *uvrD* strains. Thus, the susceptibility to chemical mutagenesis conferred by these mutators is *recA*$^+$ dependent in the case of MMS.

EMS differs from MMS in that EMS is a *recA*$^+$-independent mutagen. As shown in Fig. 5A for the *mut*$^+$ strain, the amount of mutagenesis caused by EMS was virtually unchanged by the introduction of a *recA* mutation. Similarly, the *recA* derivatives of the *mutH*, *mutL*, and *mutS* strains had approximately the same susceptibility to EMS mutagenesis as the parental mutator strains (data shown only for *mutS*). Interestingly, *uvrD* cells became even more sensitive to EMS mutagenesis upon the introduction of a *recA* mutation. Thus, in the case of the *recA*$^+$-independent mutagen EMS, the increased susceptibility to mutagenesis observed

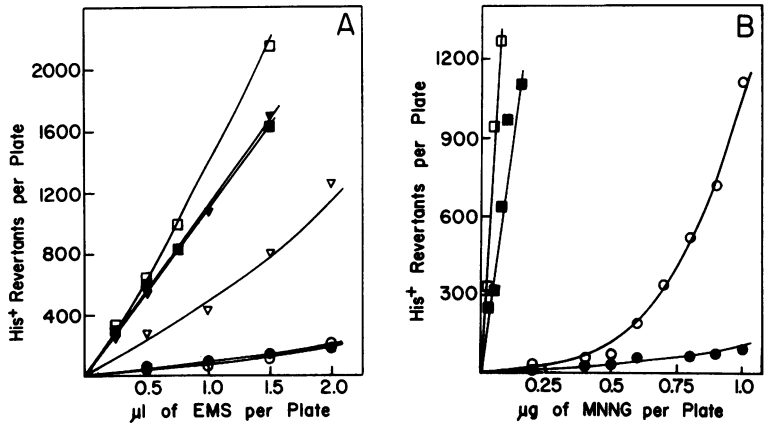

FIG. 5. EMS- and MNNG-induced mutagenesis in *recA*$^-$ derivatives of *S. typhimurium* mutator strains. Cells were plated onto M9 minimal medium containing limiting histidine and varying amounts of EMS or MNNG. (A) EMS dose-response curves. (B) MNNG dose-response curves. Symbols: ○, GW45 (*mut*$^+$); ●, GW1764 (*recA1*); □, GW1702 (*mutS121*); ■, GW1709 (*mutS121 recA1*); ▽, GW1808 (*uvrD421*); ▼, GW1825 (*uvrD421 recA1*).

in *mutH*, *mutL*, *mutS*, and *uvrD* strains did not require a functional *recA* gene product.

As shown in Fig. 5B, MNNG represents yet another class of chemical mutagens with respect to $recA^+$ dependence. The mut^+ $recA^-$ strain was almost as mutable as the mut^+ $recA^+$ parental strain at low MNNG doses, but it lacked the increased sensitivity to MNNG mutagenesis at higher doses (more than 0.5 μg of MNNG per plate). Therefore, MNNG is a partially $recA^+$-dependent mutagen in *S. typhimurium*, which is similar to the observations reported previously in *E. coli* (11). In *mutH*, *mutL*, *mutS*, and *uvrD* strains, the introduction of a *recA* mutation caused a relatively small decrease in susceptibility to MNNG mutagenesis (data shown only for *mutS*).

Analogous results (data not shown) for other chemicals, such as diethyl sulfate and MNU, lend support to the generalization suggested by the experiments described above that the increased sensitivity to chemical mutagenesis caused by deficiencies in *mutH*, *mutL*, *mutS*, and *uvrD* is largely $recA^+$ dependent for mutagens which are $recA^+$ dependent in the mut^+ parental strain and $recA^+$ independent for mutagens which are $recA^+$ independent in the mut^+ genetic background. The difference in $recA^+$ dependence between MMS and EMS might be explained either by differences in the patterns of alkylation by the two compounds (e.g., the amount of N3-alkyladenine versus the amount of O^6-alkylguanine) or by differences in the way in which cells process a particular methyl adduct(s) and its corresponding ethyl adduct(s). However, in the case of two methylating agents, such as MMS and MNNG, a difference in $recA^+$ dependence strongly implies that MNNG and MMS produce different types of premutagenic lesions. As a result, the observation that a mutation in *mutH*, *mutL*, *mutS*, or *uvrD* caused increases in $recA^+$-dependent MMS mutagenesis and in a $recA^+$-independent component of MNNG mutagenesis suggests that these mutations affect the processing of more than one class of premutagenic lesions.

Interaction of *S. typhimurium* mutator strains with pKM101. It has been hypothesized that mutations caused by $recA^+$-dependent mutagens, such as MMS, result from the processing of premutagenic lesions by the cellular error-prone repair system (46). Since the increase in MMS mutagenesis observed in *mutH*, *mutL*, *mutS*, or *uvrD* strains was also $recA^+$ dependent, we wanted to explore further the relationship between error-prone repair and the loss of *mutH*, *mutL*, *mutS*, or *uvrD* function. Unfortunately, an analog of the *E. coli umuC* gene, which probably codes for a protein directly involved in error-prone repair (15), has not been identified yet in *S. typhimurium*, and so we were not able to determine directly whether the increased MMS mutagenesis observed in mutator strains is also $umuC^+$ dependent. However, plasmid pKM101, which appears to increase the capacity of cells to carry out error-prone repair by coding for an analog of *umuC* (43; K. L. Perry, S. J. Elledge, and G. C. Walker, unpublished data), provided an alternative means of studying the relationship between error-prone repair and *S. typhimurium* mutator strains. As shown in Fig. 1A and C, pKM101 greatly enhanced the mutagenicity of MMS, as well as the mutagenicities of other $recA^+$-dependent mutagens.

Figure 6A shows the MMS dose-response curves for the pKM101-containing derivatives of the *mutS*, *mutH*, and mut^+ strains. In general, a mutator strain containing pKM101 exhibited a sensitivity to MMS mutagenesis that was intermediate compared with the sensitivities of the mutator strain without pKM101 and the mut^+ strain containing pKM101. Thus, it appears that the mutators decreased the effect of pKM101 on MMS mutagenesis. This result makes it unlikely that the process by which mutations in *mutH*, *mutL*, *mutS*, and *uvrD* increase MMS mutagenesis is independent of the process (presumably error-prone repair) by which pKM101 increases MMS mutagenesis, since such a model would predict additive effects on MMS mutagenesis; rather, it suggests that the two processes compete for the same lesion or share a common intermediate or both.

EMS and MNNG dose-response curves were also obtained for the pKM101 derivatives of the mutator strains. Since pKM101 has relatively little effect on EMS and MNNG mutagenesis in mut^+ cells, we anticipated that the *mut* (pKM101) strains would behave like the parental *mut* strains. Figures 6B and C show that this prediction was basically true. The *mut* (pKM101) strains appeared to be slightly less sensitive to EMS and MNNG mutagenesis than the *mut* strains, but they more closely resembled the *mut* strains than the mut^+ (pKM101) strain.

Chemical mutagenesis in double mutator strains. Previous studies of spontaneous mutagenesis in *E. coli* and *S. typhimurium* cells carrying mutations in at least two different mutator genes were interpreted as being consistent with a model in which the products of the *mutH*, *mutL*, *mutS*, and *uvrD* genes participate in a common pathway to maintain genetic fidelity (8, 34, 36). Since mutations in the *mutH*, *mutL*, *mutS*, and *uvrD* genes of *S. typhimurium* have qualitatively similar effects on chemical mutagenesis, it is possible that the products of these genes also exert their effects on chemical mutagenesis via a common pathway. To investigate

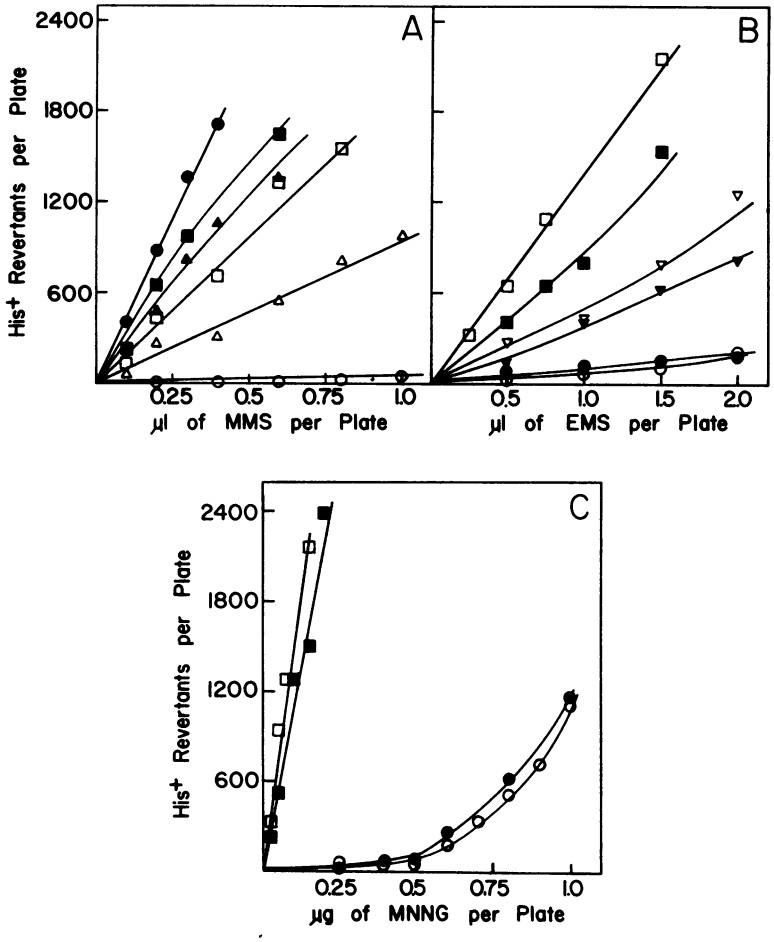

FIG. 6. MMS-, EMS-, and MNNG-induced mutagenesis in *S. typhimurium* mutator strains containing pKM101. Cells were plated onto M9 minimal medium containing limiting histidine and varying amounts of MMS, EMS, or MNNG. (A) MMS dose-response curves. (B) EMS dose-response curves. (C) MNNG dose-response curves. Symbols: ○, GW45 (*mut*$^+$); ●, TA92 (*mut*$^+$, pKM101); △, GW1810 (*mutH101*); ▲, GW1810(pKM101); □, GW1702 (*mutS121*); ■, GW1702(pKM101); ▽, GW1808 (*uvrD421*); ▼, GW1808 (pKM101).

this possibility, we examined the susceptibility of cells containing mutations in two different mutator genes to chemical mutagenesis by a variety of mutagens. If two gene products were involved in two totally unrelated processes, then the effects caused by the deficiency in either gene alone would appear to be additive in the double mutant. Table 3 shows the number of chemically induced mutants caused by single doses of MMS, EMS, and MNNG to the parental mutator strains, as well as to certain double mutants.

The data in Table 3 are not entirely consistent with the simple model that the gene products of *mutH*, *mutL*, *mutS*, and *uvrD* act in a common pathway. For instance, the effects of mutations in *mutH* and *mutS* on MMS, EMS, and MNNG mutagenesis were approximately additive in the *mutH mutS* double mutant, a result supporting the opposite conclusion (that the functions of the *mutS* and *mutH* gene products are independent). However, none of the other double mutants showed such additive effects for EMS, MNNG, or MMS mutagenesis. There were even examples in which a double mutant was less susceptible to chemical mutagenesis than one of the parental mutator strains (e.g., the *mutL uvrD* strain was less sensitive to MMS and MNNG mutagenesis than the *mutL* strain). Thus, the bulk of the evidence supports a model in which the products of the *mutH*, *mutL*, *mutS*, and *uvrD* genes act to prevent certain forms of chemical mutagenesis through participation in a common (or at least related) process(es).

TABLE 3. Comparison of chemical mutagenesis in strains containing an insertion in a single mutator gene with chemical mutagenesis in strains containing two insertions in two different mutator genes

Strain	Relevant genotype	No. of chemically induced His^+ revertants per plate with:[a]		
		0.4 μl of MMS	2.0 μl of EMS	75 ng of MNNG
GW45	*mut*$^+$	14	119	9
GW1810	*mutH101*	418	476	636
GW1714	*mutL111*	1,057	898	1,548
GW1702	*mutS121*	852	791	1,143
GW1808	*uvrD421*	267	419	499
GW1820	*mutH101 mutL111*	695	998	1,693
GW1819	*mutH101 mutS121*	1,162	1,356	2,172
GW1814	*uvrD421 mutL111*	823	1,104	1,110
GW1813	*uvrD421 mutS121*	589	996	1,385

[a] Results were obtained from dose-response curves after subtracting spontaneous revertants.

DISCUSSION

Models to explain the effects of *mutH*, *mutL*, *mutS*, and *uvrD* mutations on chemical mutagenesis by methylating and ethylating agents. The experiments described above represent the beginning of a systematic attempt to explore the roles of the *mutH*, *mutL*, *mutS*, and *uvrD* gene products in reducing the mutagenic effects of exposure to methylating and ethylating agents. The results presented here are somewhat limited in that they are based on the reversion of a single well-characterized missense mutation, *hisG46* (34). However, our results have led to the formulation of several models with testable predictions. Among the many models which could explain the role(s) of the *mutH*, *mutL*, *mutS*, and *uvrD* genes in the avoidance of alkylation mutagenesis, we discuss below only three general classes of models in detail since they do not require postulating an entirely new set of functions for the *mutH*, *mutL*, *mutS*, and *uvrD* gene products in addition to those needed for methylation-instructed mismatch repair. Also implicit in each model is the assumption that all four genes exert their effects on chemical mutagenesis through a common repair pathway, an assumption which is the simplest interpretation of the data presented here and previously (34). The distinctive features of the models are that in model I an alkylated base opposite a normal base is recognized and removed, in model II a normal base opposite a lesion is recognized and removed, and in model III mismatched normal bases that are generated as a consequence of mutagen treatment are recognized and processed.

Model I. In this model, the products of the *mutH*, *mutL*, *mutS*, and *uvrD* genes are viewed as participating in a repair process which recognizes and then removes an alkylated base, such as O^6-methylguanine (O^6MeG). The functions required to carry out such a process could be similar to those required for methyl-instructed mismatch repair. The hypermutability of *mutH*, *mutL*, *mutS*, and *uvrD* mutants with small alkylating agents would then simply be due to an inability to remove efficiently damaged nucleotides in much the same way that *uvrA*, *uvrB*, and *uvrC* mutants are hypermutable with UV and NQO because of deficiencies in excision repair.

Despite its simplicity, the numerous constraints which must be placed on model I in order to reconcile it with the observed experimental results make it seem somewhat unlikely. (i) The lesions repaired would all have to be premutagenic but not lethal since *mutH*, *mutL*, *mutS*, and *uvrD* mutations do not cause an increase in sensitivity to killing by alkylating agents. In this sense such a repair system would differ from the *uvrA*$^+$/*B*$^+$/*C*$^+$-dependent excision repair system. (ii) More than one class of lesion would have to be removed in order to account for the increased sensitivity of *mutH*, *mutL*, *mutS*, and *uvrD* mutants to both MNNG and MMS. (iii) Presumably such a repair system would have to act before DNA replication in order to prevent mutagenesis, whereas methyl-instructed mismatch repair presumably occurs shortly after replication. (iv) The model does not account for the fact that *dam* mutants of *E. coli* exhibit an increased sensitivity to EMS mutagenesis similar to that observed for the *S. typhimurium* mutator strains (9). This property of a *dam* mutant suggests, but does not prove, that strand discrimination is an essential element of a process that acts to prevent EMS mutagenesis.

Model II. This model assumes that at least one component of alkylation mutagenesis results from the misincorporation of a normal nucleotide opposite a premutagenic lesion (targeted mutagenesis) and that the *mutH*, *mutL*, *mutS*, and *uvrD* gene products participate in the removal of the incorrect base or nucleotide, followed by insertion of the correct one. Differences between *recA*$^+$-dependent mutagens and *recA*$^+$-independent mutagens could be rationalized by postulating that *recA*$^+$-dependent mutagens require an error-prone repair activity in order to insert an incorrect nucleotide opposite a lesion (46). This model is also attractive in that repair would occur on the daughter strand after replication, as it does according to the methyl-instructed mismatch repair model.

In the case of MNNG mutagenesis, if one assumes that O^6MeG is the major premutagenic lesion responsible for *recA*$^+$-independent mutagenesis (32), then to be effective, the

system would have to discriminate between O^6MeG · thymine and O^6MeG · cytosine. Both in vivo and in vitro data suggest that procaryotic DNA polymerases have a low capacity for distinguishing between these two base pairs (1, 18). A recent report by Karren and Marinus (14) suggests that mismatch correction can operate at both O^6MeG · thymine and O^6MeG · cytosine base pairs and that this does not lead to a reduced induction of mutation by MNNG. In addition, it would be difficult for this model to account for the effects observed with *recA*$^+$-dependent mutagens if mutagenesis by such agents involved the participation of noncoding lesions (31, 46).

Model III. This model suggests that the gene products of *mutH*, *mutL*, *mutS*, and *uvrD* act to remove mismatched base pairs that have arisen as a consequence of DNA damage without necessarily acting on incorrect nucleotides that are directly opposite a lesion. Model III provides a way of explaining the effects of the *mutH*, *mutL*, *mutS*, and *uvrD* gene products solely in terms of the methylation-instructed mismatch repair model. We consider below the largely *recA*$^+$-independent mutagen MNNG separately from the *recA*$^+$-dependent mutagen MMS.

(i) MNNG mutagenesis. The role of mismatch repair in correcting mismatches arising as a consequence of O^6MeG lesions can be explained by the two-step repair mechanism diagrammed in Fig. 7. We have assumed that *S. typhimurium* contains an O^6MeG-methyl transferase similar to that isolated from *E. coli* (13, 25) but that this protein is produced constitutively at some moderate level in *S. typhimurium* rather than being inducible, as it is in *E. coli*. This assumption is consistent with the nonlinear accumulation of O^6MeG residues in *S. typhimurium* LT2 after MNNG treatment (J. Guttenplan, personal communication), the nature of the mutagenic response of *S. typhimurium* to MNNG (Fig. 2 and 3), and the absence of an inducible adaptive response to MNNG mutagenesis in *S. typhimurium* (P. Schendel, personal communication).

At an appropriate ratio of methyl transferase activity to replication fork movement, some fraction of O^6MeG lesions would be replicated over before they were demethylated, leading to the possible incorporation of a thymine instead of a cytosine. However, although the lesions would still be within the region where GATC sequences are hemimethylated, the O^6MeG could be demethylated to produce a guanine · thymine base pair (Fig. 7). Methyl-instructed mismatch repair could then utilize its ability to discriminate between strands in order to repair the mismatch accurately; a mutation preventing mismatch repair would then make the cells more susceptible to mutagenesis by MNNG. The differences in methyl transferase levels and regulation between *S. typhimurium*

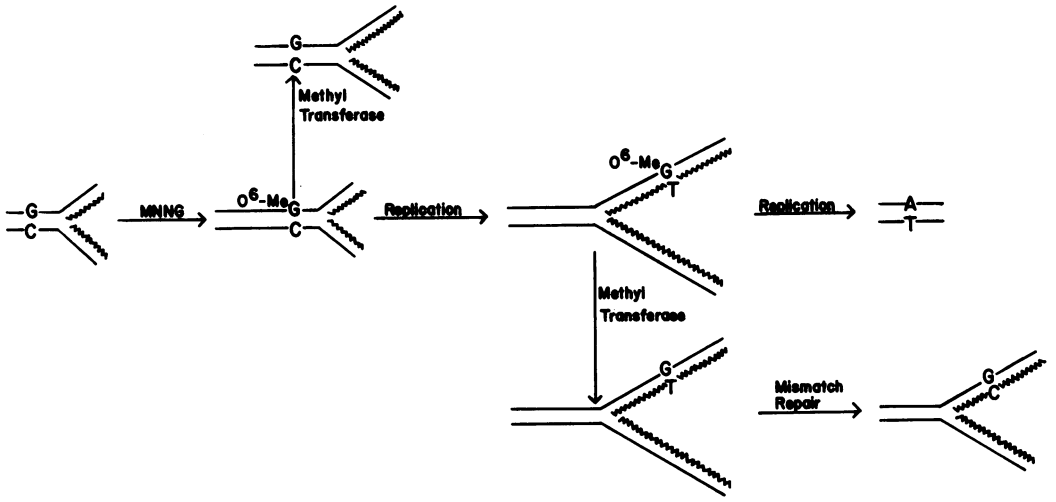

FIG. 7. Model to explain how mutations in *mutH*, *mutL*, *mutS*, and *uvrD* could influence the amount of mutagenesis caused by exposure to MNNG. If methyl transferase levels were very high (e.g., adapted *E. coli*), most O^6MeG lesions would be removed before replication; mismatch repair would not influence mutagenesis. If methyl transferase levels were very low (e.g., unadapted *E. coli*), replication over O^6MeG lesions would result in the misincorporation of thymine; mismatch repair is ineffective in processing these lesions (14). If methyl transferase levels were intermediate, then replication over O^6MeG residues to create O^6MeG · thymine base pairs would occur, but some of these would be demethylated to create guanine · thymine base pairs that are substrates for mismatch repair; in this case loss of mismatch repair capability would result in increased MNNG mutagenesis.

and *E. coli* could account for the fact that *mutH*, *mutL*, *mutS*, and *uvrD* mutations have large effects on MNNG mutagenesis in *S. typhimurium*, whereas these mutations have essentially no effect on MNNG mutagenesis in *E. coli* (38; K. L. Perry and G. C. Walker, unpublished data).

(ii) MMS mutagenesis. Model III can account for the effects of *mutH*, *mutL*, *mutS*, and *uvrD* mutations on *recA*$^+$-dependent mutagenesis by MMS if it is assumed that at least one component of error-prone repair generates mismatched base pairs near the replication fork that can be corrected by methyl-instructed mismatch repair. The incorporation of an incorrect base opposite a normal base would represent "untargeted" mutagenesis in the sense that the incorrect base is not incorporated directly opposite a lesion even though it could conceivably be in the immediate vicinity of a lesion and could have arisen as a consequence of that particular lesion. A number of observations, such as the increased rate of spontaneous mutagenesis observed in a *tif* mutant grown at 42°C (47) or in cells containing pKM101 (42), have raised the possibility that error-prone repair can, at least to some limited extent, introduce incorrect nucleotides in the absence of any known lesion. A simplistic prediction of this model is that *mutH*, *mutL*, *mutS*, and *uvrD* mutations would increase susceptibility to all *recA*$^+$-dependent mutagens, including NQO and UV; as described above, this is not the case. However, one possible explanation for this result is that the ratio of targeted mutagenesis to untargeted mutagenesis (as defined above) could vary widely among *recA*$^+$-dependent mutagens. If this ratio is high for NQO and UV, then *mutH*, *mutL*, *mutS*, and *uvrD* mutations would not be predicted to have detectable effects on the total number of mutants caused by these treatments. Both the chemical nature of the lesions introduced by a particular mutagen and the extent to which the mutagen induces error-prone repair could influence this ratio.

Evaluation. We are currently attempting to test some of the predictions made by these specific models. For example, according to model I, *mutH*, *mutL*, *mutS*, and *uvrD* strains should be defective in the removal of MNNG-induced DNA damage, but according to models II and III they should not be. Also, it should be noted that the models are not necessarily mutually exclusive. For example, the enhancement of MMS mutagenesis observed in *mutH*, *mutL*, *mutS*, and *uvrD* strains could result from increases in both untargeted mutagenesis (model III) and targeted mutagenesis (model I or II). None of the models provides a straightforward explanation for the observation that the mutator strains containing pKM101 are less mutable with MMS than the wild-type strain containing pKM101, although this result does suggest the possibility that both *mutH*$^+$ *mutL*$^+$ *mutS*$^+$ *uvrD*$^+$-dependent repair processes and error-prone repair either compete for the same lesions or share some common intermediate(s) or both. Finally, it should be kept in mind that we have confined our discussion to models in which the roles of the *mutH*, *mutL*, *mutS*, and *uvrD* products resemble their apparent roles in methyl-instructed mismatch repair.

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