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Isolation and Characterization of *Escherichia coli* K-12 Mutants Unable to Induce the Adaptive Response to Simple Alkylating Agents

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When *Esherichia coli* cells are exposed to a low level of simple alkylating agents, they induce the adaptive response which renders them more resistant to the killing and the mutagenic effects of the same or other alkylating agents. This paper describes the isolation of one strain that was deficient in mutagenic adaptation and five that were deficient in both mutagenic and killing adaptation, confirming previous suggestions that killing and mutagenic adaptation are, at least to some extent, separable. These six strains have been called Ada mutants. They were more sensitive to the killing and mutagenic effects of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) than the unadapted Ada$^+$ parent. Thus, the adaptation pathway is responsible for circumventing some alkylation-induced damage even in cells that are not preinduced. The increase in mutation frequency seen in Ada cells treated with MNNG was the same whether the cells were *lexA*$^+$ or *lexA*, showing that the extra mutations found in Ada$^-$ strains do not depend upon the SOS pathway. Ada strains accumulated more O^6-methyl guanine lesions than the Ada$^+$ parent on prolonged exposure to MNNG, and this supports the idea that O^6-methyl guanine is the most important lesion for MNNG-induced mutagenesis. The *ada* mutations have been shown to map in the 47 to 53-min region of the *E. coli* chromosome.

When *Escherichia coli* cells are grown in low levels of certain alkylating agents they induce a repair system which renders them resistant to both the mutagenic and the killing effects of a subsequent encounter with the same or another alkylating agent (3, 4, 13, 14). This error-free repair system appears to be due, at least in part, to the induction of enzymes affecting the level of O^6-alkyl guanine (15). A variety of DNA repair mutants have been analyzed for their ability to acquire resistance to the mutagenic effects of alkylating agents by prior adaptation (3). *polA* strains were unable to induce resistance to the lethality of alkylating agents, but they were able to acquire mutagenic resistance (4); this suggested that mutagenic adaptation and killing adaptation may, at least to some extent, be distinct pathways. However, all other mutant strains so far examined have been proficient in both aspects of adaptation. To gain a further understanding of adaptation, it was clearly necessary to isolate mutants either deficient or constitutive in the response. This paper describes the isolation and characterization of some mutants unable to induce mutagenic adaptation.

The method depended upon screening colonies from a mutagenized population for those that were easily mutagenized by low levels of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). Similar techniques have previously been used to isolate colonies with an elevated spontaneous mutation frequency (1a, 5). In the present study an amino acid auxotroph was grown in the presence of MNNG under limiting conditions to produce a micro-colony in which revertant papillae could develop. Bacteria that adapt to the ambient level of MNNG tend to produce colonies without papillae. Mutants that cannot adapt produce colonies with numerous papillae and therefore can be identified.

MATERIALS AND METHODS

Chemical reagents. Ethyl methane sulfonate (EMS) and MNNG were obtained from Sigma Chemical Co. Methyl methane sulfonate (MMS) was from the Aldrich Chemical Co.

Strains. The *E. coli* K-12 strains used are listed in Table 1. The *lexA ada-5* double mutant was constructed as follows: initially, a Rif^r Met$^-$ Ada5 strain was constructed by using P1 grown on Gr2R (Rif^r *metA*); *lexA* was then introduced by screening for UV-sensitive Met$^+$ transductants, using P1 grown on

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TABLE 1. *Bacterial strains*

Strain	Genotype	Source	Reference
AB1157	F⁻ *thr-1 leu-6 proA2 his-4 thi-1 argE lacY1 galK ara-14 xyl-15 mtl-1 tsx-33 rpsL sup-37*	Mount	Howard-Flanders (2)
AB1157 *ada*	As AB1157 *ada*	Present study	
AB1157 *ada-5 lexA*	As AB1157 *ada-5 lexA*	Present study	
Gr2R	*rpoB metA*	Schendel	
AB2494	As AB1157 *arg*⁺ *met*⁻ *lexA1*	Schendel	Howard-Flanders (2)
KL16	Hfr *thi-1 rel-1*	Schendel	Low (9)
AT2444	Hfr (Hayes) *thi-1 rel*	Schendel	Bachmann (1)
KL96	Hfr *thi-1 rel-1*	Lloyd	Low (9)
KL983	Hfr *xyl-7 lacY1* or *ZAmgl*P1λ	Lloyd	Low (9)
PK191	Hfr Δ(*proB-lac*)XIII *sup-56 thi-1*	Lloyd	Low (9)
KLF43/KL259	F'143 *lysA tyrA/thi-1 tyrA2 pyrD34 his-68 trp-45 thyA33 recA1 mtl-2 xyl-7 malA1 galK35 rpsL118* λ^r λ	Lloyd	Low (8)
KLF42/KL253	F'142 *tyr* Δ(*pts-1*) *supN/thi-1 tyrA2 pyrD34 his-68 trp-45 recA1 mtl-2 xyl-7 malA1 galK35 rpsL118* λ^r λ	Lloyd	Low (8)
KLF29/JC1553	F'129 *dsdA his/argG6 metB1 his-1 leu-6 recA1 mtl-2 xyl-7 malA1 gal-6 lacY1 rpsL104 tonA2 tsx-1* λ^r λ⁻ *supE44*	Lloyd	Low (8)
CGSC 4281	F'15 *lysA fuc/argG6 metB1 his-1 leu-6 thyA23 recA1 lacY1 gal-6 malA1 mtl-2 xyl-7 rpsL104 tonA2 tsx-1* λ^r λ⁻ *supE44*	Lloyd	Low (8)
G43442	F⁻ *thi-1 lysA22 thyA61 argA21 lysD31 cysC43 pheA97 malA1*	Lloyd	Morand et al. (12)
AT2681	F⁻ *thi-1 argHl purFl his-1 glyA6 mtl-2 xyl-7 malA1 rpsL8* or *rpsL9* λ^r *supE44*	Lloyd	Taylor (16)

AB2494. Thy⁻ derivatives of AB1157 and the AB1157 Ada⁻ isolates were made by using the trimethoprim method described by Miller (11).

Media and plating conditions. Media used for the growth of bacterial strains were supplemented with salts solutions as described by Miller (11). All methods for bacterial growth and plating conditions were followed as previously described (3). Plates containing MNNG were made with 10× M9 salt solution, which was prepared as follows: 65.5 g of $NaH_2PO_4 \cdot 7H_2O$; 30 g of KH_2PO_4; 5 g of NaCl; 10 g of NH_4Cl to 1 liter with water and adjusted to the required pH (normally pH 5.0) with NaOH. MNNG plates were routinely prepared on the day of plating and dried for 15 min at 37°C.

Isolation procedure for *ada* mutants. MNNG was shown to be unstable in plates by examining the survival of AB1157 and *polA* strains on plates containing varying concentrations of MNNG which had been freshly made or stored for 24 h at 37°C. Its stability could be increased by making bacterial plates with 10× M9 salts solution at pH 5.0; under these conditions, its half-life is more than 24 h. Unfortunately, this alteration ruled out the use of the common "sectored colony" techniques involving a color indicator because they are highly pH dependent (1a, 5). Therefore, the following procedure was used. AB1157 is an arginine-requiring auxotroph and this marker is readily revertible by MNNG. Under conditions of limiting arginine (1 μg/ml) microcolonies will form, and in the presence of MNNG *arg*⁺ papillae can be detected by microscopic examination after several days of growth. The fraction of colonies that show papillae is determined (i) by the amount of arginine, because this controls the final colony size, and (ii) by the amount of MNNG in the plates. Plates containing 0.1 μg of MNNG and 0.075 μg of arginine per ml balanced these two factors so that less than 4% of the colonies formed by AB1157 showed *arg*⁺ papillae.

Mutagenized stock. A culture of AB1157 was grown in supplemented minimal medium to 2×10^8 cells per ml, treated with MNNG (10 μg/ml) for 10 min, and then filtered, washed, and suspended in 1 ml. A 0.1-ml amount of this mutagenized culture was diluted into 20 ml of supplemented minimal medium and left overnight on the bench to reach saturation. A 0.2-ml portion of this overnight culture was diluted into 10 ml of medium and grown at 37°C to 2×10^8 cells per ml. The number of Val^r mutants in this mutagenized stock was estimated. The stock was kept frozen in 10% dimethyl sulfoxide. When required it was thawed, diluted 1/50 into supplemented minimal medium, grown to 2×10^8 cells per ml, and plated on selective plates.

Treatments with MNNG. All treatments with MNNG and adaptation analyses were as previously described (3). Killing adaptation was analyzed by exposure to a given concentration of MNNG, and the survival was measured at 6-min intervals under nonadaptive or potentially adapting conditions. In experiments involving long exposures to MNNG (>60 min), the cultures were kept in exponential phase by regular

dilution with prewarmed medium and MNNG was added at each dilution to maintain the required concentration. This also served, therefore, to maintain fresh MNNG in the medium.

Cross-reactivity. The adaptative procedure in MMS involved 60 min of growth in 0.002% MMS (preadaptation) followed by 90 min of growth in 0.02% MMS. Challenge concentrations of MMS and EMS were 0.5 and 2%, respectively. UV survival curves were performed by irradiating bacteria suspended in top agar on supplemented minimal plates. For analysis of mutagenesis, cells (at 2×10^8 per ml) were irradiated for various times in 5 ml of supplemented minimal medium in 9-cm-diameter petri dishes (liquid < 1 mm thick), then incubated with aeration at 37°C for 1 h before being filtered, resuspended in 1 ml of M9 salts solution, and plated as previously described (3) to determine *arg*$^+$ mutants and survivors. The UV source was an unfiltered model 12 Hanovia UV bactericidal lamp.

Spontaneous mutation frequency. The procedure used to determine spontaneous mutation frequency was based on the fluctuation test of Luria and Delbrück (10). Overnight cultures of AB1157 and Ada5 were diluted 1/100 and grown to 2×10^8 cells per ml in L broth. From these cultures, ten 2-ml cultures of each were set up containing approximately 200 cells per ml on L broth and shaken for 18 h at 37°C. The cultures were then appropriately diluted and plated for survivors on tryptone-yeast extract plates and for rifampin-resistant mutations on supplemented minimal plates containing 100 μg of rifampin per ml.

Analysis of alkylated purines. All procedures used for analysis of alkylated purines were those described by Schendel and Robins (15).

Bacterial matings. All mating were performed in liquid culture as described by Miller (11), with the ratio of donor to recipient cells being 1:5.

Rapid analysis of mutagenesis by MNNG. Overnight cultures, 1 ml, were diluted (50 μl into 1 ml) and grown to approximately 5×10^7 cells per ml. A 100-μl amount was plated for valine-resistant mutants (untreated control), and 0.1 μg of MNNG was added for 90 min, followed by 0.5 μg of MNNG for 30 min, after which suitable platings were made to estimate the frequency of valine-resistant mutations.

RESULTS

Isolation procedure. The basis of the isolation procedure is outlined in Materials and Methods. The sequence of steps used for identifying the adaptation-deficient mutants in a mutagenized culture is as follows.

(Step i) A mutagenized stock was plated to give approximately 200 colonies per plate onto low-pH plates containing 0.075 or 0.1 μg of MNNG and 0.075 μg of arginine per ml, plus all the other amino acid requirements of AB1157.

(Step ii) After 2 and 3 days of incubation, all colonies having *arg*$^+$ papillae were streaked onto plates containing 0.1 μg of arginine per ml (and all other required amino acids) to give single colonies. Under these conditions *arg*$^+$ and *arg* colonies could be distinguished after 2 to 3 days of growth.

(Step iii) One arginine-requiring colony from each isolate was spread with a toothpick onto sectors of two low-pH MNNG (0.075 μg/ml) plates, one arginine limiting and the other threonine limiting (0.075 μg/ml).

In essence, the procedure involved picking colonies which contained *arg*$^+$ papillae, reisolating an *arg* cell from the colony, and then retesting on MNNG plates for both *arg*$^+$ and *thr*$^+$ reversion rates. Of the original papillated colonies about 5% showed a consistently high mutation rate. An additional test was designed specifically to pick up adaptation-deficient mutants (as opposed to mutants that simply were more easily mutated by MNNG). It involved first plating the cells on low-pH plates containing 0.05 μg of MNNG per ml and then respreading the colonies on fresh arginine-limited, high-pH plates containing 5 μg of MNNG per ml. The presence of multiple revertant colonies on the second plate only occurred if the cells had not become adapted on the first plate. This test was not used routinely, however.

In all, six adaptation-deficient mutants were isolated, one from one mutagenized culture and five from another. Their frequency seemed to be about 10^{-3}, which was about the same as the frequency of valine-resistant mutants. Each of these mutants could be readily distinguished from the parent AB1157 by the various plate tests described above.

Examination of these isolates for their ability to induce mutagenic adaptation. The first step in the investigation of these MNNG-sensitive isolates was to study their accumulation of mutations when growing in 0.5 μg of MNNG per ml (a concentration in which the parent strain will grow and adapt). The results are shown in Fig. 1 for the six isolates. All show a higher mutation frequency than AB1157 and continue to accumulate mutations for at least 40 min. The isolates were also tested directly for mutagenic adaptation by being grown in 0.1 μg of MNNG per ml for 90 min before transfer to 0.5 μg/ml. Such prior treatment of the parent strain AB1157 prevents almost all of the mutation induction by 0.5 μg/ml. In contrast, prior treatment of the isolates, far from lowering the mutation rate, actually raised it slightly. The result for one of the isolates is shown in Fig. 2. The six strains are therefore adaptation deficient and will henceforth be called Ada (a seventh MNNG-sensitive strain had been selected, but it proved on this test to be capable of adaptation

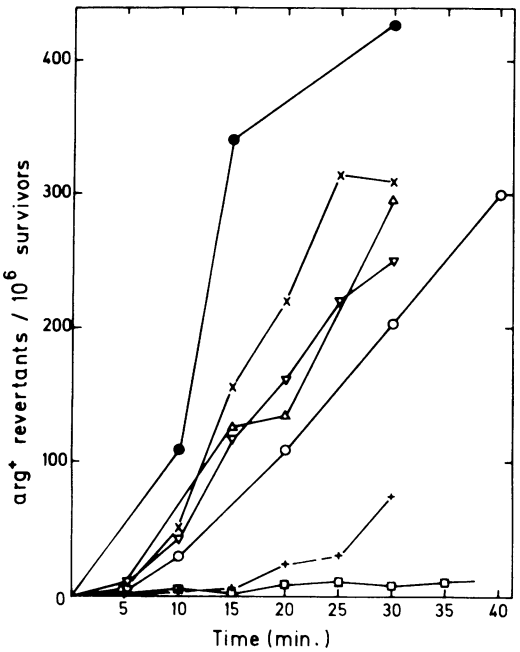

FIG. 1. *Frequency of arg⁺ revertants accumulated by adaptation-deficient strains growing in 0.5 μg of MNNG per ml. Symbols: ×, Ada1; ▽, Ada2; △, Ada3; +, Ada4; ○, Ada5; ●, Ada6; □, AB1157(Ada⁺).*

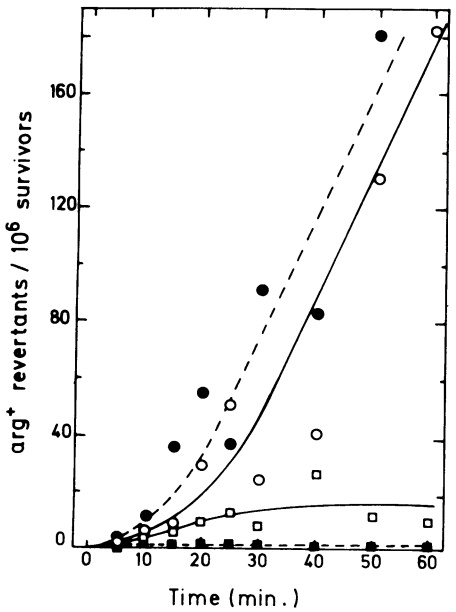

FIG. 2. *Frequency of arg⁺ revertants accumulated by AB1157 (□, ■) and Ada5 (○, ●) in 0.5 μg of MNNG per ml with (closed symbols) or without (open symbols) prior exposure to 0.1 μg of MNNG per ml for 90 min.*

and so it is not discussed any further).

Examination of Ada strains for their ability to induce killing adaptation. To examine whether these isolates were proficient in killing adaptation, their survival in the presence of a challenge dose of MNNG was measured with or without prior adaptation. The potentially adapting concentration was 0.1 μg/ml, since all the isolates seemed able to grow well at this concentration, and in addition the parent strain, AB1157, was able to acquire killing adaptation at this concentration. Figure 3 shows that all the isolates were more sensitive to the killing effects of 5 μg of MNNG per ml in the unadapted state than unadapted AB1157. Only one (Ada3) was able to induce some level of killing adaptation; for the other isolates prior growth in 0.1 μg of MNNG per ml did not enhance survival to the challenge dose (Fig. 4).

Response of Ada strains to other concentrations of MNNG. Figure 1 showed that all the Ada strains continue to accumulate mutations in 0.5 μg/ml for 30 to 40 min. On further incubation, however, the mutation frequency reached a plateau. Figure 5 shows the mutation frequency of two Ada strains during prolonged exposure to 0.1 μg of MNNG per ml. It can be seen that here too mutations are produced linearly but eventually reach a plateau, even though the population, in this case, continued to grow. At a still lower concentration (0.02 μg/ml) Ada5 was found to accumulate only a low level of mutations even after 7 h of growth. This

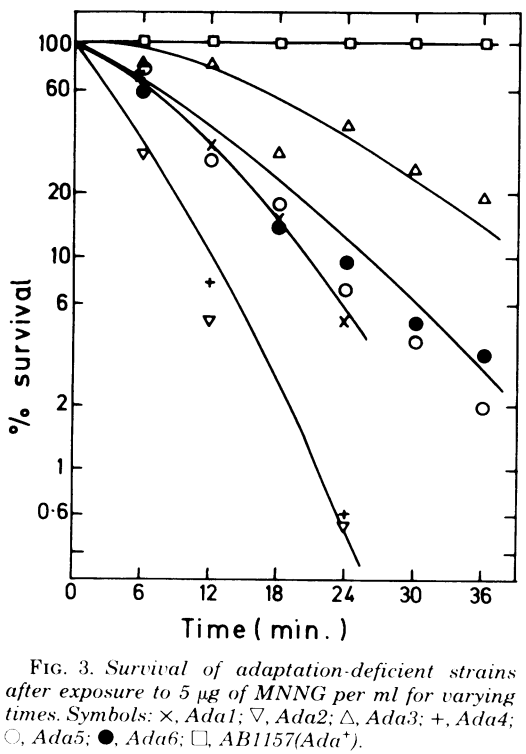

FIG. 3. *Survival of adaptation-deficient strains after exposure to 5 μg of MNNG per ml for varying times. Symbols: ×, Ada1; ▽, Ada2; △, Ada3; +, Ada4; ○, Ada5; ●, Ada6; □, AB1157(Ada⁺).*

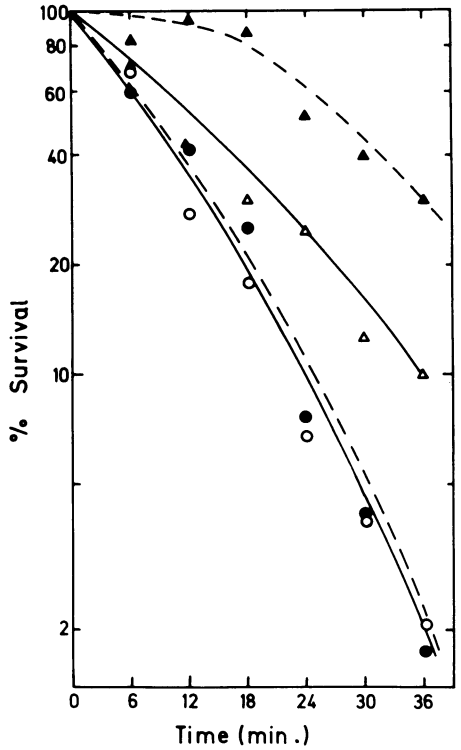

FIG. 4. *Survival of Ada3 and Ada5 after exposure of 5 μg of MNNG per ml for varying times under nonadaptive and potentially adapting conditions. Symbols: △, nonadapted Ada3; ▲, adapted Ada3; ○, nonadapted Ada5; ●, adapted Ada5.*

suggests that Ada5 may retain some ability to handle low levels of alkylation. It is not clear, however, whether this represents yet another repair pathway (as, for example, that postulated by Schendel et al. [14]) or some leakiness of the adaptation pathway in this strain.

Sensitivity of adaptation-deficient strains to other mutagenic agents. The adaptation response has previously been shown to be induced by and produce resistance to other alkylating agents. In contrast, it cannot be induced by, or produce resistance to, UV lesions. Figure 6 shows the response of Ada5 and its parent AB1157 to EMS and MMS when nonadapted or after growth in the presence of low levels of MMS. (Prior exposure to a low dose of EMS was not investigated because this agent has been observed to be a poor inducer of the adaptation response; prior exposure to a low dose of MNNG was not examined because the large number of mutations introduced by the low-dose treatment would have swamped the mutations introduced by the challenge with MMS or EMS.) Clearly, treatment with a low dose of MMS, like treatment with MNNG, does not induce the adaptation response in Ada5, and, in addition, this strain accumulates more mutations when challenged with MMS than unadapted AB1157. In general, the relative mutagenic sensitivity of the various adaptation-deficient strains to MMS follows closely their mutagenic sensitivity to MNNG (see Fig. 1). In contrast, the adaptation-deficient strains were not more mutagenized by

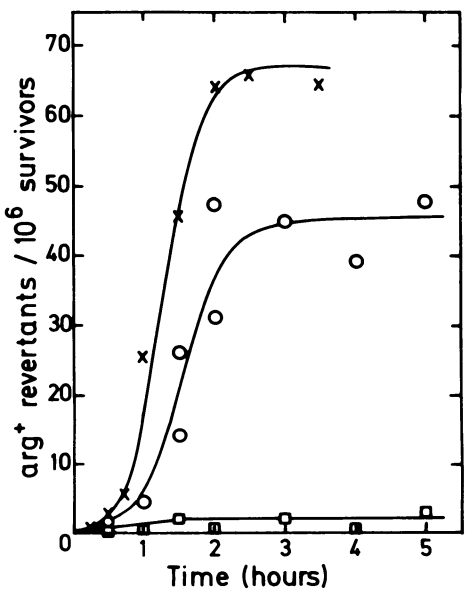

FIG. 5. *Frequency of arg+ revertants accumulated by Ada1 (×), Ada5 (○), and AB1157 (□) during growth in 0.1 μg of MNNG per ml.*

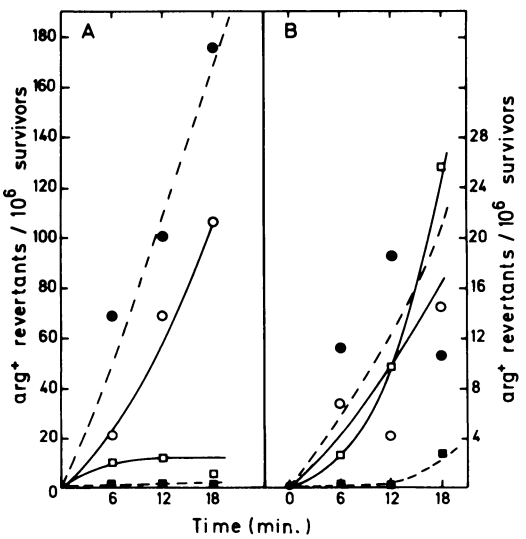

FIG. 6. *Frequency of arg+ revertants accumulated by Ada5 (○, ●) and AB1157 (□, ■) after exposure to 0.5% MMS (A) and 2% EMS (B) for varying times with (closed symbols) or without (open symbols) prior exposure to 0.02% MMS for 90 min.*

EMS than their parent, AB1157. The significance of these results will be dealt with in the Discussion.

The Ada strains were also investigated for their killing and mutagenic sensitivity to UV irradiation. All the strains were as resistant to the lethal effects of UV irradiation as their parent, AB1157 (data not shown). Figure 7 shows that some of the strains are slightly more readily mutagenized by UV irradiation than the parent strain.

Spontaneous mutation frequency of Ada strains. The spontaneous mutation frequency was determined by measuring the frequency of rifampin-resistant mutants in 10 parallel cultures of Ada5 and AB1157 grown in rich medium. The median mutation frequencies for AB1157 and Ada5 were estimated to be 2.8 and 3.7 Rif^r mutants per 10^8 survivors, respectively. This difference was not considered to be significant.

Examination of a *lexA ada-5* double mutant. To determine whether the additional mutations arising in Ada strains are independent of SOS repair (i.e., are like the mutations produced in wild-type cells by low levels of MNNG [14]), a *lexA* mutation was introduced into the Ada5 strain by P1 transduction. Figure 8 compares the response of *ada-5* and a *lexA ada-5* double mutant to 0.5 and 5 μg of MNNG per ml and demonstrates that the mutation frequency of this Ada strain is not affected by the *lexA* mutation.

Accumulation of O^6-methyl guanine by *ada-5*. In a previous publication (15) it has been shown that the decreased mutagenesis observed in adapted bacteria when challenged with MNNG is closely correlated with a decreased accumulation of O^6-methyl guanine in the DNA. One would therefore expect to find that Ada strains accumulate more O^6-methyl guanines in their DNA after long periods of exposure to MNNG than wild-type cells. To investigate this, conditions were chosen which had been shown to produce the maximum difference in mutation frequency between the Ada strain and its Ada^+ parent, namely, growth for 90 min in 0.1 μg of MNNG per ml (potentially adapting conditions) and then exposure to 0.5 μg of [^{3}H]MNNG per ml for varying times. Table 2 shows that Ada5 accumulated roughly one O^6-methyl guanine for every 10 N^7-methyl guanine, whereas its adapted parent did not accumulate significant levels of O^6-methyl guanine after this level of challenge.

Genetic location of *ada*. Crosses between *ada* recipients and various ada^+ Hfr strains (as shown in Table 3) suggested that *ada* lies between 51 and 61 min; for example, 42% of the his^+ exconjugants produced by crossing Hfr KL16 with *ada-5* were Ada^+, whereas no Ada^+ His^+ exconjugants were obtained when Hfr

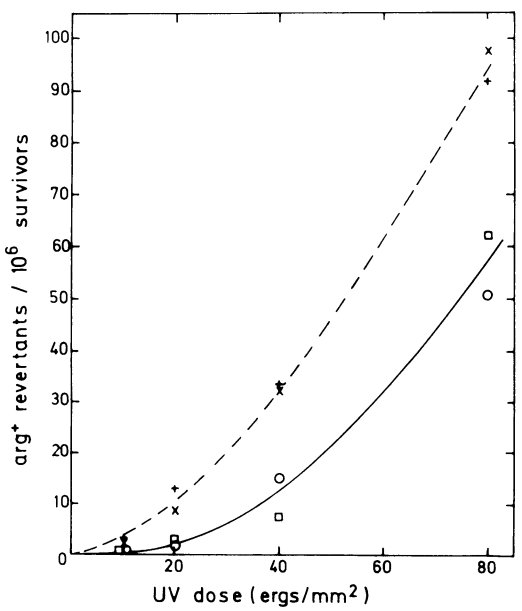

FIG. 7. *Frequency of arg^+ revertants accumulated by Ada1 (×), Ada4 (+), Ada5 (○), and AB1157 (□) after varying doses of UV.*

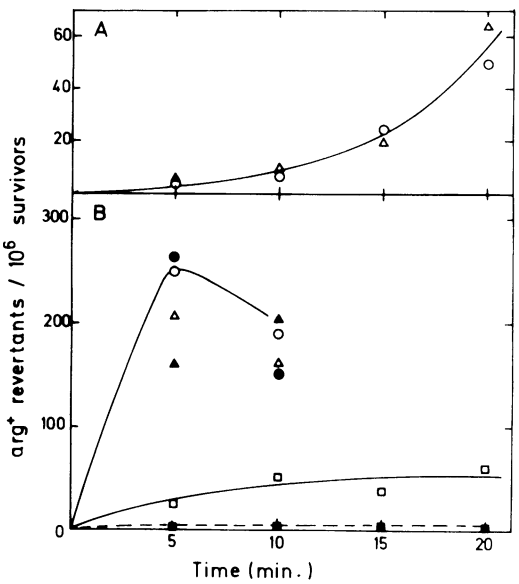

FIG. 8. *Frequency of arg^+ revertants accumulated by ada-5 (○, ●), ada-5 lexA (△, ▲), and AB1157 (□, ■) after exposure to (A) 0.5 μg of MNNG per ml and (B) 5 μg of MNNG per ml with (closed symbols) or without (open symbols) prior exposure to 0.1 μg of MNNG per ml for 90 min.*

KL983 was used. The origin of the Hfr strains, their direction of transfer, and all relevant markers are shown in Fig. 9. The same Hfr crosses were performed with all the other *ada* mutants, and the results suggested that all mapped in approximately the same genetic region.

In an attempt to locate *ada* more precisely a *cysC ada-5* strain was constructed and crossed with KL16 (Table 4). The 15% co-conjugation of *ada* with *cysC* suggested *ada* lay to the right of *cysC*. To use additional markers, an *ada* Hfr strain was constructed with an origin and direction of transfer like that of KL16 by crossing F'15 with AB1157 *thyA ada-5* and selecting *thy*$^+$ exconjugants. Two further crosses (shown in Table 4) were performed using this *ada* Hfr. Unfortunately, the various F' strains with which this Hfr was to be crossed could not grow on pH 5.0 plates and so special plate tests had to be devised for each recipient strain. From these crosses *ada* was shown to be 80% co-conjugatable with *purF*.

In an attempt to examine the dominance or recessivity of the *ada-5* mutation, a variety of F' plasmids were introduced into AB1157 *ada-5*. Merodiploids were selected using F'142 and F'129; none proved to be Ada$^+$. Unfortunately, F'142 has a small deletion in its episome, and thus the region between 51 and 65 min was not completely covered. It was possible, therefore, that either *ada* happened to fall in that deleted region or *ada* was dominant.

In conclusion, therefore, it has only been shown that *ada* maps within approximately 3 min of *purF*. P1 cotransduction of *purF* with *ada-5* could not be observed out of 200 *purF*$^+$ transductants analyzed. More precise mapping of *ada* requires its transfer to other suitably

TABLE 2. *Accumulation of O^6-methyl guanine (O^6) and N^7-methyl guanine (N^7) by Ada5 and AB1157 during growth in 0.5 μg of [^{3}H]MNNG per ml*[a]

Accumulation by:	Total cpm in fractions			
	30 min		60 min	
	N^7	O^6	N^7	O^6
AB1157	328	5	1,096	5
Ada5	1,349	173	3,564	404

[a] Ada5 and AB1157 were first grown for 90 min in 0.1 μg of MNNG per ml (potentially adapting conditions) and then challenged with 0.5 μg of [^{3}H]MNNG per ml; samples were taken after 30 and 60 min. Total counts per minute for samples of approximately 3 × 10^{10} bacteria are given.

TABLE 3. *Crosses between various Hfr strains and ada-5*

Hfr donor	Recipient	Marker selected	No. analyzed	% Prototrophs Ada$^+$
KL16	AB1157 *ada-5*	His$^+$	117	42
AT2444	AB1157 *ada-5*	Pro$^+$	120	0
KL96	AB1157 *ada-5*	His$^+$	80	0
KL983	AB1157 *ada-5*	His$^+$	100	0
PK191	AB1157 Thy *ada-5*	Thy$^+$	58	52
PK191	AB1157 Thy *ada-5*	His$^+$	62	55
KL99	AB1157 Thy *ada-5*	His$^+$	77	53

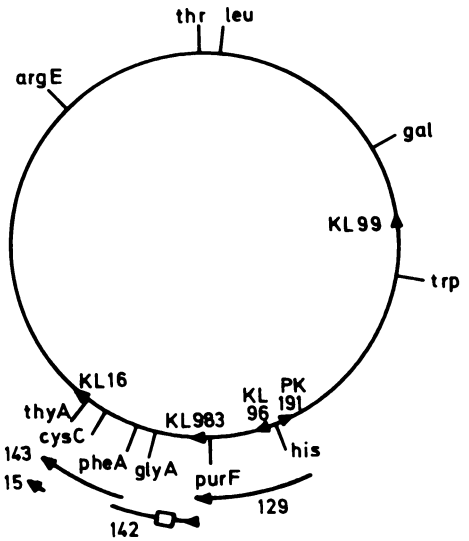

FIG. 9. *Genetic linkage map of E. coli K-12 modified from Taylor and Trotter (16). Hfr origins and directions of transfer are indicated by arrowheads.*

TABLE 4. *Other crosses with ada-5 strains*

Donor	Recipient	Selected marker	No. analyzed	Subsequent analysis (%)
KL16 (*ada*$^+$ *cys*$^+$)	*ada cys*	Cys$^+$	100	Ada$^-$, 15
F15/1157 *thy ada-5*	GY3442 *cys phe*	Cys$^+$	120	Ada$^-$, 7
		Phe$^+$	143	Ada$^-$, 6
F15/1157 *thy ada-5*	AT2681 *gly purF*	Gly$^+$	181	Ada$^-$, 49; PurF$^+$, 52
		PurF$^+$	140	Ada$^-$, 76; Gly$^+$, 87
		Gly$^+$ PurF$^+$	132	Ada$^-$, 80

marked strains, and each new strain must be investigated for a suitable plate test. No mutants, deficient in any form of DNA repair, have been previously reported to map in this area. This suggests that *ada* is indeed a mutation in a new gene involved in DNA repair.

DISCUSSION

The adaptive repair system has been shown previously to have two, somewhat separable components: mutagenic adaptation, namely, an inducible resistance to the mutagenic effects of MNNG, and killing adaptation, the inducible resistance to the killing effects of MNNG. For example, the kinetics of mutagenic adaptation are quite distinct from that of killing adaptation, and *polA* strains are unable to induce killing adaptation even though they are proficient in mutagenic adaptation. Five of the Ada isolates were unable to induce either killing adaptation or mutagenic adaptation, whereas a sixth isolate, Ada3, was proficient in killing adaptation. The order of sensitivity to the mutagenic effects of MNNG by the Ada strains was Ada6 > Ada1 > Ada2, Ada3 > Ada5 > Ada4; their order of sensitivity to the lethal effects was Ada4 > Ada2 > Ada1 > Ada6, Ada5 > Ada3. The lack of correlation between these two is further evidence that killing adaptation and mutagenic adaptation may be partially distinct. Nevertheless, since five of the isolates are deficient in both processes it is likely that the mechanisms have gene products in common, although proof that these two deficiencies result from a single gene mutation will have to await more detailed genetic studies.

Adaptation-deficient strains accumulate more mutations than their Ada$^+$ parent, even in the unadapted state and even after short exposure (e.g., 5 min) to MNNG. This demonstrates that when unadapted wild-type bacteria are exposed to even a short pulse of MNNG the adaptation response is operating to prevent some potential mutations from arising. However, it is not possible, from such experiments, to determine whether the adaptive response is partly constitutive or is being induced by the short pulse of mutagen and is able to handle some of the lesions after the pulse is finished.

It has been shown previously (14) that *lexA* strains accumulate fewer mutations when treated with MNNG than *lexA*$^+$ strains, especially at higher MNNG doses. This had led to the suggestion that most mutations arise by *lex*-independent pathways at low doses (probably by replication errors) and by induction of the *lexA*$^+$-dependent SOS pathway at high doses. Adaptation is believed to prevent mutations arising in part by inhibiting replication errors and in part by preventing mutations arising by the SOS pathway. However, an *ada-5 lexA* double mutant was no more mutagenized by high or low doses of MNNG than the *ada-5* strain alone, showing that all the extra mutations caused by the presence of the *ada* mutation are *lexA* independent. This suggests that these extra mutations arise by replication errors and thus mask any mutations arising by the SOS error-prone pathway.

It has been shown previously that the adaptive response can be induced by other alkylating agents, including MMS. In the present study, MMS, like MNNG, was unable to induce mutagenic adaptation in the Ada strains. It was most interesting that the Ada strains were more mutagenized than their unadapted Ada$^+$ parent by MMS but not by EMS. It has already been suggested in this paper that the adaptation pathway can act to reduce the mutation frequency from short pulses of mutagen even when the pathway has not been previously induced. This could result from a constitutive level of adaptive repair or its subsequent induction after the short pulse of mutagen. EMS, unlike MNNG and MMS, is a very poor inducer of adaptation in AB1157 strains. Therefore, the adaptation pathway probably does not become induced in unadapted bacteria to decrease their mutagenesis by EMS; (although if adaptation is induced by MNNG, it can act on EMS-induced lesions). Since Ada and unadapted Ada$^+$ strains were equally mutagenized by EMS, this suggests that there is not a constitutive level of the adaptive pathway but that its effect on short exposures to mutagens like MMS and MNNG is due to its subsequent induction.

None of the Ada strains was sensitive to the killing effects of UV, but their response to its mutagenic effects was surprising. Several of the isolates (but not Ada5) were more mutagenized by UV, but the increase in UV-induced mutagenesis in Ada strains was not a big effect in comparison to their heightened MNNG-induced mutagenesis rate. In this respect Ada strains are not behaving like *tif* strains, which are characteristically more mutagenized by UV irradiation only at low UV doses, suggesting that their sensitivity to UV-induced mutagenesis cannot simply be explained by their increased ability to induce SOS functions. One explanation might be that UV irradiation, in addition to producing pyrimidine dimers, produces some other minor lesion which may be repaired by the adaptation pathway.

It has been shown that Ada5 accumulates an excess number of O^6-methyl guanine bases

their DNA in comparison to Ada$^+$ bacteria upon prolonged exposure to a low concentration of [^{3}H]MNNG. Under these same conditions extra mutations also arise in this strain, supporting the theory (6, 7, 15) that O^6 methylation of guanine accounts for most MNNG-induced mutagenesis. It should be noted, however, that though an excess number of O^6-methyl guanine bases are observed in Ada5 after prolonged exposure to MNNG, this result does not show whether more O^6-methyl guanine lesions are actually produced in Ada5 or fewer lesions are quickly repaired.

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