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Genetics

Induction of specific *Escherichia coli* genes by sublethal treatments with alkylating agents

(adaptive response/*aid* mutation/operon fusion/alkylation damage/DNA repair)

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ABSTRACT **Fusions of the *lac* operon to genes induced by treatment with sublethal levels of alkylating agents have been selected from random insertions of the Mu-dl(Ap^R*lac*) phage by screening for induction of β-galactosidase activity in the presence of methyl methanesulfonate. Genetic analysis reveals that these fusions resulted from insertion of Mu-dl(Ap^R*lac*) into two regions of the chromosome. One region (*aidA*) is near *his* and, based on phenotypic effects, appears to represent insertion into the *alkA* gene. The other region (*aidB*) is in the 92.3- to 98-min region, which harbors no previously identified genes involved in repair of alkylation damage. The *aidB* fusions caused increased resistance to alkylating agents and caused little or no change in the biological effects of adaptation to alkylating agents. Unlike the *aidA* fusions, *aidB* fusions showed increased β-galactosidase activity in untreated cells in a growth phase-dependent fashion. The *ada-5* mutation, which blocks expression of the adaptive response, decreased induction of β-galactosidase activity in both *aidA* and *aidB* fusions after alkylation treatments. Thus, both *aidA* and *aidB* share with adaptive response a common regulatory mechanism involving the *ada* gene. The growth phase-dependent control of the *aidB* fusions, however, is unaffected by *ada*, suggesting that a second regulatory mechanism exists that controls only *aidB*.**

When cells of *Escherichia coli* are treated with sublethal levels of alkylating agents for several generations, the mutagenic and lethal effects of subsequent high-level treatments are decreased (1, 2). This induced resistance to the deleterious effects of alkylating agents is termed the adaptive response. Based on genetic and physiological studies, the adaptive response is thought to be due to the induction of specific gene products that act to repair alkylation-induced lesions in DNA (1).

Subsequent biochemical studies have confirmed that at least two gene products are induced by such adaptive treatments (3–6). Both of these products act to repair specific lesions produced in DNA by alkylating agents. One of the induced enzymes is a glycosylase, the product of the *alkA* gene, which removes N^3-methyladenine, N^3-methylguanine, and N^7-methylguanine (5, 6). The second induced gene is a methyltransferase that removes the methyl group from O^6-methylguanine (3, 4).

The adaptive response is controlled by the *ada* gene (7, 8). The molecular mechanism(s) by which *ada* functions is not known, although recent evidence suggests it may be a positive regulator (ref. 9; P. LeMotte and G. Walker, personal communication). Different isolates of *ada* mutants show different effects on the protection against lethality and mutagenesis resulting from adaptation. Some *ada* mutants block one induced repair response more than the other, and no correlation exists between protection against killing and mutagenesis (7). This is consistent with the hypothesis that at least two different products are induced by adaptive treatments—one that repairs lesions that are primarily lethal, and one that repairs lesions that are primarily mutagenic.

To expand our understanding of the adaptive response and to identify genes induced specifically by alkylation treatments, we have begun to search for mutants in this process by using the phage Mu-dl(Ap^R*lac*) as a mutagen (10). This phage inserts into the *E. coli* chromosome in an apparently random fashion (10). The *lac* gene carried by this phage lacks a promoter and is, therefore, not expressed. When insertion of this phage occurs next to a promoter and in the proper orientation, β-galactosidase transcription can occur (10). In addition, since β-galactosidase is synthesized only from the external promoter, it is subject to the regulatory mechanisms that control this promoter. Moreover, because the largest target for insertion is generally the protein-coding sequence of the gene, mutant phenotypes frequently result from insertion. Thus, mutant phenotypes can provide insights into the functions of the afflicted genes.

In this study, we selected insertions of Mu-dl(Ap^R*lac*) that place β-galactosidase synthesis under the regulation of promoters of genes induced by treatments with methyl methanesulfonate (MeSO). We describe the isolation of such mutants, the genetic loci, their response to MeSO and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), and the effect of *ada-5* on their regulation.

MATERIALS AND METHODS

Bacterial Strains. Strains MV1561, MV1563, MV1564, MV1565, and MV1571 and their respective *ada-5 gyrA* derivatives MV1574, MV1575, MV1576, MV1577, and MV1578, are all derivatives of MV1161 that contain *aid*::Mu-dl(Ap^R-*lac*) insertions. MV1161 is a φX174-sensitive S13-sensitive strain, which was derived from AB1157 (11) by screening isolates resistant to phage HK19 for sensitivity to φX174 and S13 (12). Thus, it carries the marker *rfa-550*. Therefore, strains MV1161-MV1578 are all *argE3 his-4 leu-6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 rpsL31 supE44 tsx-33 rfa-550*. In addition to these markers, the *gyrA ada-5* derivatives were constructed by P1-transduction, using P1 grown on strain BS24 (*gyrA ada-5*) (8), selecting for nalidixic acid-resistant transductants and screening for the increased MNNG mutability and sensitivity indicative of the *ada-5* mutation (7, 8).

JC13068 was obtained from A. J. Clark (University of California, Berkeley) and carries all of the markers of MV1161 except *leu-6* and *rfa-550*; in addition, it carries *recB21 recC22 sbcB15 sfiB103* and *malE*::Tn*10*. The Hfr strains used have been described by Bachmann and Low (13).

Mutant Isolation. The method for construction of operon

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Abbreviations: MeSO, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; Ap^R, ampicillin resistant.

fusions was adapted from the methods described by Casadaban and Cohen (10). Cells were infected with the Mu-dl(Ap^R-*lac*) phage at low multiplicities, centrifuged, and resuspended in a 10-fold greater volume of L broth (14). The incubation step for expression of ampicillin resistance (Ap^R) was eliminated and the cells were plated immediately on L agar plates containing ampicillin (25 μg/ml). Although this considerably decreased the number of transductants, it ensured that each Ap^R colony was of independent origin. In later screenings, the transduction frequency was increased by adding a 10-ml layer of L agar to the surface of an L ampicillin plate just before use. This allowed time for expression of the Ap^R phenotype before ampicillin diffusion began to kill ampicillin-sensitive colonies.

Ap^R colonies were then replica-plated onto two lactose indicator plates [X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside), 40 μg/ml; E salts; glucose, 0.4%; Bacto Casamino acids, 0.2%; thiamine, 0.2 μg/ml], one of which also contained 0.02% MeSO, and the plates were incubated overnight at 30°C. Because of instability of MeSO in solution, plates were poured, cooled for 20 min at room temperature, dried for 1 hr at 37°C, cooled again for 20 min at room temperature, and then used immediately. Colonies that showed different color responses on the two indicator plates were tested further in liquid medium by treating logarithmic phase cells with 0.05% MeSO and assaying β-galactosidase activity in crude extracts at various times after treatment. β-Galactosidase activities were assayed essentially as described by Miller (15), using the chloroform lysis method. In addition, an A_{600} measurement always accompanied the production of each crude extract in order to normalize to cell density.

Genetic Methods. P1-transductions were carried out by the method of Willets *et al.* (14). Matings were done by mixing donors and recipients (5:1) in wells of a microtiter plate and incubating for 2 hr at 30°C. After incubation, the microtiter plates were placed on ice. The cultures were then diluted, mixed in a Vortex to break up mating pairs, and plated on selective media. Either Arg$^+$ Sm^R or Thr$^+$ Leu$^+$ Sm^R recombinants were selected and tested for recombination between the *aid*$^+$ and *aid*::Mu-dl(Ap^R*lac*) by screening for loss of the Ap^R phenotype. In each cross, at least 100 recombinants of each type were selected and tested for loss of the Ap^R marker.

Adaptation. Cells were grown overnight in minimal medium (E salts; glucose, 0.4%; Bacto Casamino acids, 0.2%; thiamine, 0.2 μg/ml), diluted 1:25, and regrown to ≈10^8 cells per ml. MNNG was added at the inducing concentration, and the cells were grown for another 2 hr to elicit the adaptive response. Cell concentrations were then readjusted to ≈10^8 cells per ml, and the cultures were treated with several challenge doses of MNNG for 30 min at 30°C. The inducing doses were the highest that showed no effect on growth or mutagenesis. For strains MV1161, MV1561, MV1563, and MV1564, the inducing dose of MNNG was 1 μg/ml and the challenge doses of MNNG routinely used were 5, 10, 20, 40, 60, 90, and 120 μg/ml. For strains MV1565 and MV1571, the inducing dose was 0.1 μg/ml and the challenge doses of MNNG usually used were 1, 2.5, 5, 10, 20, and 40 μg/ml.

To determine survival after challenge, cells were immediately diluted 1:100 and chilled, then diluted further and plated on DSEM plates (16) [a semi-enriched medium containing Davis salts/0.4% glucose/Arg, Leu, Pro, Thr (each at 100 μg/ml)/0.2 μg of thiamine per ml/0.004% Bacto Casamino acids]. To determine mutagenesis after challenge, cells were centrifuged for 2 min in a microfuge, washed once with E salts containing 4% $Na_2S_2O_3$ to inactivate residual MNNG, and resuspended in E salts. ($Na_2S_2O_3$ itself affected neither survival nor mutagenesis.) Undiluted cells were then plated on DSEM plates. In addition, total viable cell titers were again determined in order to assess the fraction of cells lost during the post-treatment processing (10%–50%). All data presented in the figures are the averages of two or more independent experiments.

RESULTS

Isolation of Mu-dl(Ap^R*lac*) Insertion Mutants. Our screening procedure yielded 10 mutants (from ≈120,000 Ap^R colonies screened) that exhibited increased levels of β-galactosidase activity after treatment with MeSO. Since MeSO induces both the adaptive response and the SOS response (17, 18), all Mu-dl(Ap^R*lac*) insertion mutants were first tested for β-galactosidase induction by UV. Of the 10 mutants, 5 were UV-inducible and were assumed to be insertions in genes of the SOS system similar to those isolated by Kenyon and Walker (19). The other 5 were inducible by alkylating agents but not by UV. These were termed *a*lkylating-agent *in*ducible (*aid*) mutants.

Genetic Mapping of the *aid*::Mu-dl(Ap^R*lac*) Insertions. The *aid*::Mu-dl(Ap^R*lac*) insertions were assigned to approximate genetic loci either by P1-transduction or by mating with several Hfr strains. In both cases, recombinants were selected that had lost specific auxotrophic markers and were then tested for concomitant loss of the Ap^R marker. The approximate map positions of all *aid*::Mu-dl(Ap^R*lac*) mutations are shown in Fig. 1, together with the origins of transfer of the relevant Hfr strains.

To test for possible insertion into *alkA*, all strains were tested for linkage of *aid*::Mu-dl(Ap^R*lac*) insertions to the *his* operon by P1-mediated transduction using P1·KL14(*his*$^+$ *aid*$^+$). The *aid*::Mu-dl(Ap^R*lac*) mutations in strains MV1565 and MV1571 were found to be linked to the *his* operon with cotransduction frequencies of 20% and 18%, respectively.

The insertions in strains MV1561, MV1563, and MV1564 were assigned to the 91- to 98-min region on the basis of the following results. Ampicillin-sensitive recombinants were recovered at a frequency of 31%–50% among Thr$^+$ Leu$^+$ Sm^R recombinants from crosses of each of these strains with Hfr donor strains KL14 and Ra2, and at a frequency of 31%–62% among Arg$^+$ Sm^R recombinants from crosses of each of these strains with Hfr strains P801, BW113, and KL226. In contrast, no ampicillin-sensitive recombinants were recovered among 100 Arg$^+$ Sm^R recombinants from each cross of MV1561, MV1563, and MV1564 with Hfr KL209, or among 100 Thr$^+$ Leu$^+$ Sm^R recombinants from each cross of these three recipients with HfrH. Thus, the Mu-dl(Ap^R*lac*) insertions in these three strains lie in the region transferred by Hfr strains KL14, Ra2, P801, BW113, and KL226, and must lie between the origins of transfer of Hfr strains KL209 and HfrH.

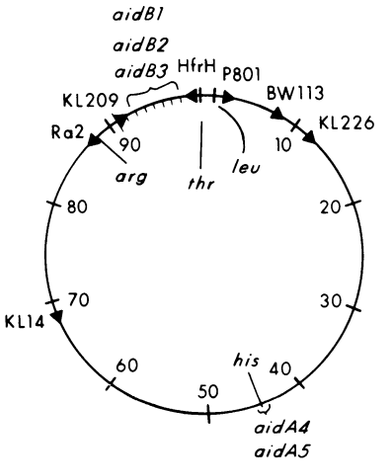

FIG. 1. Genetic loci of *aid*::Mu-dl(Ap^R*lac*) insertion mutations, transfer origins of Hfr strains and their direction of transfer.

The 91- to 92.3-min region was eliminated as a possible site of insertion because of the lack of linkage between the *malE* gene (91 min) and the *aidB*$^+$ allele when P1 phage grown on strain JC13068 (*malE*::Tn*10*) were used to transduce the *aidB* mutants. Among the *malE*::Tn*10* transductants tested (70, 77, and 67 for MV1561, MV1563, and MV1564, respectively) none had lost the *aid*::Mu-dl(Ap^R*lac*) allele. Using the formula of Wu (20) to convert cotransduction frequency to map distance and taking into account the 9.3-kilobase inclusion of Tn*10* in the P1 donor, each of the *aid*::Mu-dl(Ap^R*lac*) insertions lies more than 1.3 min from *malE*. Thus, the *aidB* gene(s) resides in the 92.3- to 98-min region of the *E. coli* chromosome.

Induction of β-Galactosidase Activity by DNA Damaging Agents. β-Galactosidase induction kinetics of representative *aid*::Mu-dl(Ap^R*lac*) mutations are shown in Fig. 2. Based on the phenotypic and genetic characteristics, there appear to be two *aid* mutations: *aidA*, which is present in strains MV1565 and MV1571 and *aidB*, which is present in strains MV1561, MV1563, and MV1564. Minor phenotypic variations are seen when individuals of either the *aidA* or *aidB* groups are compared with one another; it is not clear whether these variations are due to insertion into different genes or allelic variation resulting from insertion into different sites within a single gene. Further study of the individuals within a group will be required to answer this question.

All strains were compared for induction of β-galactosidase activity by MeSO and MNNG at two doses—the optimal dose for expression of the adaptive response, and the dose that yields maximal expression of β-galactosidase activity, which is a reflection of the optimal expression of the *aid* genes (aid response). In wild-type AB1157 derivatives, the optimal dose for expression of the adaptive response by MeSO is 0.01% (18). Fig. 2 shows that both *aidA* and *aidB* fusions show β-galactosidase induction by MeSO at this concentration. The maximum induction of β-galactosidase activity was attained after treatment with 0.05% MeSO in *aidB* and 0.025% MeSO in *aidA*. *aidA* reached a level of β-galactosidase activity approximately twice that seen in the *aidB* strains.

The *aidA* and *aidB* strains differ greatly in their response to MNNG. Both the optimal inducing doses for adaptive response and the dose for maximal induction of β-galactosidase activity are 10-fold greater in *aidB* than in *aidA*. For wild type and *aidB*, the optimal dose for expression of the adaptive response by MNNG was determined to be 1 μg/ml; for *aidA*, it was 0.1 μg/ml (see section on effects of *aid*::Mu-dl(Ap^R*lac*) insertions on the adaptive response). When MNNG is used as the inducing agent at the optimal dose for expression of the adaptive response, only *aidA* shows induction of β-galactosidase activity (Fig. 2*B*). Only when higher doses of MNNG (10 μg/ml) are used is β-galactosidase induction seen in *aidB* (Fig. 2*B*). Thus, MNNG induces *aidB* strains but not when used at levels that induce the adaptive response in these strains (see below).

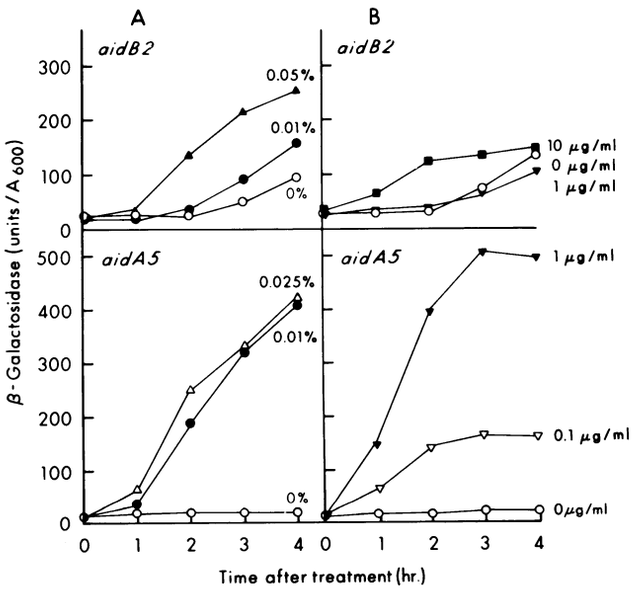

FIG. 2. Expression of β-galactosidase activity in untreated and alkylated cells. (*A*) Untreated and MeSO-induced cells; (*B*) untreated and MNNG-treated cells. Untreated controls shown in *A* are the average values of all experiments; controls in *B* are from the same experiments in which MNNG treatment (1 μg/ml) was performed.

Maximal induction of β-galactosidase activity by MNNG is attained in *aidB* at 10 μg/ml and in *aidA* at 1 μg/ml (Fig. 2*B*). When induction of β-galactosidase activity by the two agents, MeSO and MNNG, is compared, MNNG is a better inducing agent than MeSO in *aidA*. In *aidB*, the opposite is true and MeSO is a better inducing agent.

Effect of *ada*-5 on Induction of β-Galactosidase Activity. The *ada-5* mutation was introduced into each of the *aid* mutants. The optimal dose for induction of β-galactosidase activity was decreased by *ada-5* in the *aidB* mutants, but not in the *aidA* mutants. When β-galactosidase activity was compared under optimal β-galactosidase-inducing conditions for each strain, both *aidA* and *aidB* showed decreased β-galactosidase induction when the *ada-5* mutation was present (Table 1). Thus, both *aidA* and *aidB* are influenced by *ada* and appear to be under its regulatory control.

Constitutive Expression of β-Galactosidase Activity. The *aidB* strains showed a small increase in β-galactosidase activity even in untreated cells (Fig. 2*A*). To further examine this effect, the assay was extended. The results are shown in Fig. 3, together with growth curves monitored by absorbance at 600 nm. The *aidB* strains showed increasing β-galactosidase activities in untreated cells. This increase began during the late logarithmic phase of the growth cycle. When the cells reached stationary phase, the β-galactosidase activity stabilized at a level 5–10 times higher than that seen in logarithmically growing cells. This is not a general feature of *aid* insertions, because *aidA* strains did not show this derepression (Fig. 3). Thus, the increase in β-galactosidase activity in these strains is indicative of specific derepression of *aidB* fusions. Unlike the induction seen in response to alkylation treatment, the induction in undamaged cells is unaffected when *ada-5* is introduced into *aidB* strains (Fig. 3). Therefore, this type of regulation appears not to be controlled by the *ada* gene.

Table 1. β-galactosidase induction in *ada*$^+$ and *ada-5* derivatives of *aid* mutants

Strain	Genotype *aid*	Genotype *ada*	MNNG, μg/ml	MNNG-induced β-galactosidase activity, units/A_{600}
MV1561	*B1*	+	10	103.7
MV1574	*B1*	*5*	5	30.3
MV1563	*B2*	+	10	139.6
MV1575	*B2*	*5*	5	47.7
MV1564	*B3*	+	10	120.3
MV1576	*B3*	*5*	5	41.6
MV1565	*A4*	+	1	383.6
MV1577	*A4*	*5*	1	82.5
MV1571	*A5*	+	1	341.9
MV1578	*A5*	*5*	1	90.1

MNNG doses used were those determined to produce the maximum induction of β-galactosidase activity in each strain. MNNG-induced β-galactosidase activity equals total activity in treated cells minus activity in untreated cells 2 hr after the addition of MNNG.

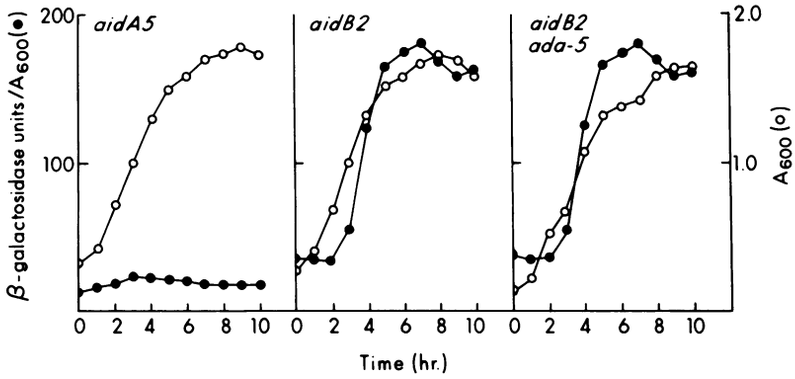

FIG. 3. Growth-phase-dependent expression of β-galactosidase activity in cells of *aidA5* (MV1571), *aidB2* (MV1563), and *aidB2 ada-5* (MV1575). ●, β-Galactosidase activity; ○, cell density (A_{600}).

Effects of Mu-dl(Ap^R*lac*) Insertions on Killing. Since expression of β-galactosidase activity requires insertion of Mu-dl(Ap^R*lac*) into a gene, disruption of that gene's protein-coding sequence is a frequent outcome (10). Thus, the function of an *aid* gene may be surmised by examining mutant phenotypes.

The *aidA* mutants exhibit a distinct phenotype: they are sensitive to several alkylating agents. Fig. 4 compares the survival of all mutants after treatment with MNNG. The *aidA* strains are considerably more sensitive than the *aid*$^+$ parental strain. These two mutants are also more sensitive to MeSO and to the ethylating agent ethyl methanesulfonate (data not shown). Although the survival data show some variability, the *aidB* strains, especially *aidB1* and *aidB3*, consistently showed a higher MNNG survival than did the wild-type strain. The *aidB2* strain showed only a slightly higher survival than wild type, and because of variability in these experiments, it is not clear whether this represents a real increase in survival.

None of the *aid* mutations affected survival or mutagenesis after UV treatment (data not shown), indicating that their repair defects are specific for lesions induced by alkylating agents.

Effects of aid::Mu-dl(Ap^R*lac*) Mutations on the Adaptive Response. MNNG is the most effective inducer of the adaptive response in *E. coli* K-12 strains (2). Under our conditions, adaptation in the parental strain was attained by first incubating the cells until they were growing logarithmically and then treating with the highest concentration that affects neither growth nor mutagenesis (1 μg/ml for all strains except MV1565 and MV1571, which were adapted with 0.1 μg/ml).

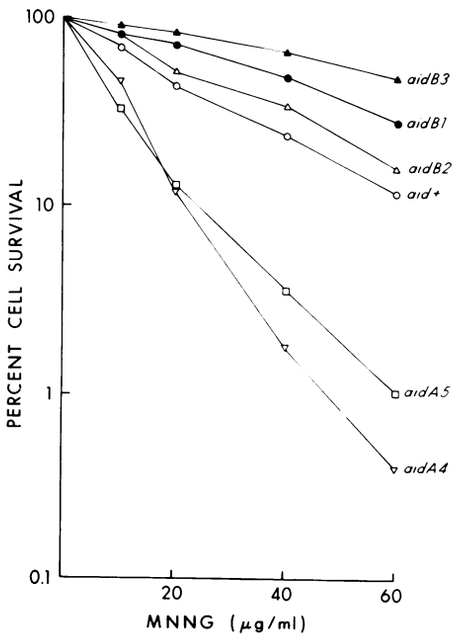

FIG. 4. Cell survival after treatment with different concentrations of MNNG for 30 min at 30°C.

Table 2 shows the effect of the *aid* mutations on both lethality and mutagenesis in unadapted and adapted cells. For each strain, the challenge dose shown in the table was the dose at which the greatest difference was seen between unadapted and adapted cells. The dose used to induce the adaptive response and the challenge doses used for the *aid-4* and *aid-5* mutants are lower, presumably due to their greater sensitivity to MNNG. All strains show adaptation when mutagenesis is measured. However, neither of the *aidA* mutants showed any significant difference in survival between unadapted and adapted cells. This lack of effect on lethality in *aidA* is seen with MNNG challenges ranging from 1 μg/ml to 120 μg/ml (data not shown).

Based on the ability of MNNG at 0.1 μg/ml to decrease the mutagenicity of the challenge dose in the *aidA* strains, adaptive response was clearly expressed under these conditions. Therefore, the *aidA* insertions have not only caused sensitivity to alkylating agents, but have also blocked expression of adaptation to the lethal effects of MNNG.

DISCUSSION

The initial screening of strains containing random insertions of the phage Mu-dl(Ap^R*lac*) yielded five fusions of the *lac* operon to promoters of genes that are induced by sublethal treatments with MeSO. These fusions represent a unique set, because UV treatment caused no increase in β-galactosidase activity (data not shown). Therefore, none of the *aid*::Mu-dl(Ap^R*lac*) insertions were in genes associated with the SOS response and, therefore, differ from the *din* insertions isolated by Kenyon and Walker (19).

In this study, we use the term adaptive response to refer to the biological effects of sublethal treatments with alkylating agents (i.e., decreased lethality and mutagenesis after subsequent challenge treatments). The term aid response refers to the induction of genes by alkylation treatments. We make this distinction because the involvement of *aid* genes in the adaptive response is not clear, nor will all *aid* mutations necessarily share the same regulatory elements with the genes of the adaptive response.

The *aidA* insertion mutants contained Mu-dl(Ap^R*lac*) insertions that mapped near the *his* operon, increased sensitivity to alkylating agents, and blocked adaptation to the lethal effects but not the mutagenic effects of alkylation damage. The similarity of the phenotype of *aidA* to that of an *alkA* mutant and their similar linkage to the *his* operon (6, 21) suggests that these mutants may have resulted from insertions into the *alkA* gene. This hypothesis is confirmed by the result that the *aidA* mutants are deficient in the inducible 3-methyl guanine glycosylase activity of the *alkA* gene product (P. Karran, personal communication).

An unusual phenotype emerged from investigations of the

Table 2. Effect of *aid*::Mu-dl(Ap^R*lac*) mutations on adaptive response

Strain	*aid* genotype	MNNG challenge dose, μg/ml	% cell survival: Unadapted cells	% cell survival: Adapted cells	MNNG challenge dose, μg/ml	Mutation frequency: Unadapted cells	Mutation frequency: Adapted cells
MV1161	+	120	2.0	10.8	10	834	14.4
MV1561	*B1*	120	9.8	35.4	10	939	6.9
MV1563	*B2*	120	4.7	31.5	10	990	4.4
MV1564	*B3*	120	14.5	62.0	10	654	0
MV1565	*A4*	40	1.8	4.6	5	506	140
MV1571	*A5*	40	4.9	3.3	5	497	131

Mutation frequency is expressed as number of induced mutants per surviving cell plated ($\times 10^{-7}$).

characteristics of the *aidB* mutants. Although *aidB* is similar to wild type with respect to adaptation, a consistent several-fold increase in resistance to killing by MNNG is seen, especially in *aidB1* and *aidB3*, which suggests that the loss of their wild-type functions improves tolerance to alkylating agents.

Several possible explanations can account for this increased MNNG resistance. The *aidB* cells could be less permeable to MNNG than wild type, or, because decreased thiol levels reduce the metabolic conversion of MNNG to the proximal mutagen methylnitrosamine (22), *aidB* strains may contain decreased levels of thiols. Alternatively, the product of the *aidB* gene or genes may affect cell survival in some as yet undetermined fashion similar to the increase in UV survival that results from *sfi* mutations in *E. coli* B strains induced for the SOS response (23).

Whatever the function of the wild-type gene(s) corresponding to *aidB*, it is clear that the aid response induced by MeSO includes genes that have no apparent role in the adaptive response. For example, *aidB* insertions affect neither adaptation to mutagenesis nor killing in any detectable fashion, but are induced when *aidB* mutants are treated with levels of MeSO that elicit the adaptive response.

The link between the adaptive response and the induction of *aid* mutations is indicated by their common regulation by the *ada* gene. The *ada-5* mutation decreases expression of both the adaptive response (7) and the induction of β-galactosidase activity in all *aid*::Mu-dl(Ap^R*lac*) fusion strains. Thus, both the aid response and the adaptive response are controlled by the *ada* gene.

Although all of the *aid* genes require *ada*$^+$ for induction by alkylation treatment, an additional regulatory mechanism must also control *aidB*, based on the lack of effect of *ada-5* on the induction of β-galactosidase in undamaged cells. The induction of *aidB* occurs late in the logarithmic phase of growth and stabilizes at a high level during the stationary phase. Since this induction occurs in the absence of alkylation damage and is unaffected by *ada*, and *aidB* promoter must respond to inducing signals other than those provided by alkylation damage to DNA, and it must also be controlled by another regulatory mechanism in addition to that defined by the *ada* gene.

A key difference between the aid response and the adaptive response is evident when the induction of *aid* fusions is examined at concentrations of MNNG that elicit the adaptive response. The adaptive response is effectively induced when *aidB* strains are exposed to a MNNG concentration of 1 μg/ml. However, this concentration of MNNG does not cause induction of β-galactosidase activity in *aidB*. Only when higher concentrations of MNNG are used is *aidB* induced.

The reason for these differences may simply be that levels of alkylating agent that are suboptimal for induction of *aid* gene expression must be used to see the adaptive response. Levels of MNNG that are optimal for *aid* gene induction in either *aidA* or *aidB* cause sufficient mutagenesis to mask the antimutagenic effects of subsequent MNNG challenge treatments. According to this view, the genes responsible for the adaptive response are a subset of the genes of the aid response. The treatment conditions that are optimal for measuring the adaptive response are too low to induce all of the *aid* genes, and only those *aid* genes that are most easily induced will be expressed under these conditions. Further work with *aid* mutants should clarify the regulatory response of *E. coli* to alkylating agents, which is more extensive and more complex than initially revealed in the adaptive response.

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