ESCHERICHIA COLI GENE INDUCTION BY ALKYLATION TREATMENT

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ABSTRACT

Searches for alkylation-inducible (*aid*) genes of *Escherichia coli* have been conducted by screening random fusions of the Mu-dl(Ap^R *lac*) phage for fusions showing increased β-galactosidase activity after treatment with methylating agents, but not after treatments with UV-irradiation. In this report we describe gene fusions that are specifically induced by alkylation treatments. Nine new mutants are described, and their properties are compared with the five mutants described previously. The total of 14 fusion mutants map at five distinct genetic loci. They can be further subdivided on the basis of their induction by methyl methanesulfonate (MMS) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). *alkA*, *aidB* and *aidD* are induced by both agents and appear to be regulated by *ada*. Neither *aidC* nor *aidI* is regulated by *ada*. Moreover, since *aidC* is induced only by MNNG and *aidI* is induced only by MMS, these two genes are likely to be individually regulated. Thus, there appear to be at least three different regulatory mechanisms controlling *aid* genes.

TREATMENT of *E. coli* with sublethal levels of alkylating agents for several generations causes the induction of a number of genes that function in the repair of alkylation damage to DNA (SCHENDEL and ROBINS 1978; KARRAN, LINDAHL and GRIFFIN 1979; KARRAN, HJELMGREN and LINDAHL 1982; EVENSEN and SEEBERG 1982). Phenotypic characterizations linked the induction of these gene products to a reduction in both the lethal and mutagenic effects of subsequent high-level treatments with alkylating agents (SAMSON and CAIRNS 1977; JEGGO *et al.* 1977). This induced resistance to alkylating agents has been called the adaptive response (SAMSON and CAIRNS 1977) and is regulated by the *ada* gene product (JEGGO 1979; SEDGWICK 1982).

In a previous report (VOLKERT and NGUYEN 1984), we described the isolation and initial characterization of fusions of the *lac* operon to promoters of genes induced in response to methyl methanesulfonate (MMS) treatment. These fusions were constructed by using the Mu-dl(Ap^R *lac*) phage (CASADABAN and COHEN 1979). We showed that the two alkylation inducible (*aid*)

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genes, *alkA*, (formerly called *aidA*) and *aidB*, shared with the adaptive response a common regulatory pathway involving the *ada* gene.

In this study, additional screenings for *aid* mutants were conducted using both *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and MMS as selective agents. The concentrations of MNNG and MMS that allowed identification of both *alkA*::Mu-dl(Ap^R *lac*) and *aidB*::Mu-dl(Ap^R *lac*) on lactose indicator plates were first determined and used in these screens. These screens combined with the previous screen resulted in the isolation of fusions mapping at five different loci. *alkA* and *aidD* are fusions to two genes that have previously been identified and have been shown to be involved in the adaptive response. *alkA* codes for the inducible glycosylase that removes *N*-3-methyladenine, *N*-3-methylguanine and *N*-7-methylguanine (EVENSEN and SEEBERG 1982; KARRAN, HJELMGREN and LINDAHL 1982; NAKABEPPU *et al.* 1984). *aidD* is a fusion to the *ada-alkB* operon that has apparently inactivated the *alkB* gene (JEGGO 1979; SEDGWICK 1982, 1983; KATAOKA, YAMAMOTO and SEKIGUCHI 1983). The remaining fusions—*aidB*, *aidC* and *aidI*—represent new alkylation-inducible genes.

MATERIALS AND METHODS

Strains: The bacterial and phage strains used in this study are listed in Table 1.

Mutant isolation: The method for constructing fusions was adapted from CASADABAN and COHEN (1979) essentially as described by VOLKERT and NGUYEN (1984). However, in the current isolation, MNNG (0.5 μg/ml) and/or MMS (0.05%) were used as selective agents for alkylation-inducible fusions of Mu-dl(Ap^R *lac*). These concentrations were high enough to cause color development in *aidB* colonies during overnight exposure and were sufficiently low so as not to kill *alkA* fusion mutants. These concentrations are considerably lower than those needed for optimal *aid* gene induction in liquid media (see below) because the exposure to alkylating agents is considerably longer on plates and because colony development and cell survival are necessary in the screenings but are not necessary when enzyme levels are being determined. All solid media containing MNNG were adjusted to pH 6 to increase MNNG stability. Colonies that showed different color responses on the lactose indicator plates, with and without the alkylating agent, were tested further.

Construction of λpSG1 or λpl(209) derivatives: Replacement of Mu-dl(Ap^R *lac*) by λpl(209) was carried out as described by KOMEDA and IINO (1979). A transductional method for production of λpSG1 derivatives was devised. λpSG1 is a derivative of λpl(209) containing the transposon Tn*9* in the *lacY* gene (SILHAVY, BERMAN and ENQUIST 1984). *aid*::Mu-dl(Ap^R *lac*) fusion strains were infected by λpSG1 at a multiplicity of infection of 0.001 and were incubated at 30° for 20 min to allow phage adsorption. Cell/phage complexes were then plated on L plates containing 25 μg/ml chloramphenicol (Cam) to select for λpSG1 lysogens. At least ten such lysogens were then picked and inoculated into a single tube containing L Cam broth. This mixture was then incubated at 42° to cause loss of the original Mu-dl(Ap^R *lac*) phage. P1 grown on this mixture was then used to transduce MV1161 to Cam^R. The Cam^R transductants were then screened for Amp^S and ability to induce β-galactosidase after MMS or MNNG treatments.

Several of the Mu-dl(Ap^R *lac*) fusion strains contained more than one insertion. This transductional step allowed the production of λpSG1 single fusion mutants even from strains containing multiple Mu-dl(Ap^R *lac*) fusions or rearrangements, providing the insertions were unlinked. The requirement for cotransduction of β-galactosidase inducibility with Cam^R essentially assured that λpSG1 was fused to the promoter of interest, since all other Cam^R transductants would not show β-galactosidase induction.

TABLE 1

Bacterial and bacteriophage strains

Strain	Genotype[a] *alk/aid*	Genotype[a] *ada*	Derivation or reference
A. MV strains			
MV1161	+	+	VOLKERT and NGUYEN (1984)
MV1174	+	+	His^+MeSO^s(*alkA1*) transductant of MV1161(P1·MS20(*his*$^+$*alkA1*)
MV1185[b]	+	+	VOLKERT and HARTKE (1984)
MV1196	+	+	Tet^R(*zee-976*::Tn*10*) transductant of MV1161 (P1·SK2651(*zee-976*::Tn*10 his*$^+$)) screened for retention of *his-4* allele
MV1561	*B1*	+	VOLKERT and NGUYEN (1984)
MV1563	*B2*	+	VOLKERT and NGUYEN (1984)
MV1564	*B3*	+	VOLKERT and NGUYEN (1984)
MV1565	*A50*	+	VOLKERT and NGUYEN (1984). Now called *alkA50*::Mu-dl(Ap^R*lac*)
MV1571	*A51*	+	VOLKERT and NGUYEN (1984). Now called *alkA51*::Mu-dl(Ap^R*lac*)
MV1601	*D6*	+	Mu-dl(Ap^R *lac*) insertion mutant of MV1161
MV1608	*C8*	+	Mu-dl(Ap^R *lac*) insertion mutant of MV1161
MV1616	*-9*	+	Mu-dl(Ap^R *lac*) insertion mutant of MV1161
MV1621	*C10*	+	Mu-dl(Ap^R *lac*) insertion mutant of MV1161
MV1624	*B11*	+	Mu-dl(Ap^R *lac*) insertion mutant of MV1161
MV1638	*B12*	+	Mu-dl(Ap^R *lac*) insertion mutant of MV1161
MV1648	*B13*	+	Mu-dl(Ap^R *lac*) insertion mutant of MV1161
MV1649	*B14*	+	Mu-dl(Ap^R *lac*) insertion mutant of MV1161
MV1652	*115*	+	Mu-dl(Ap^R *lac*) insertion mutant of MV1161
MV1700	*B1*	*10*::Tn*10*	Tet^RAda^- transductant of MV1561
MV1701	*B2*	*10*::Tn*10*	Tet^RAda^- transductant of MV1563
MV1702	*B3*	*10*::Tn*10*	Tet^RAda^- transductant of MV1564
MV1703	*A50*	*10*::Tn*10*	Tet^RAda^- transductant of MV1565
MV1704	*A51*	*10*::Tn*10*	Tet^RAda^- transductant of MV1571
MV1707	*C8*	*10*::Tn*10*	Tet^RAda^- transductant of MV1608
MV1708	*-9*	*10*::Tn*10*	Tet^RAda^- transductant of MV1616
MV1709	*C10*	*10*::Tn*10*	Tet^RAda^- transductant of MV1621
MV1751	*B11*	*10*::Tn*10*	Tet^RAda^- transductant of MV1624
MV1752	*B12*	*10*::Tn*10*	Tet^RAda^- transductant of MV1638
MV1753	*B13*	*10*::Tn*10*	Tet^RAda^- transductant of MV1648
MV1754	*B14*	*10*::Tn*10*	Tet^RAda^- transductant of MV1649
MV1756	*115*	*10*::Tn*10*	Tet^RAda^- transductant of MV1652
MV1900	*A104*::λpSG1	+	λpSG1 replacement of *alkA50*::Mu-dl(Ap^R*lac*)
MV1902	*A105*::λpSG1	+	λpSG1 replacement of *alkA50*::Mu-dl(Ap^R*lac*)
MV1904	*B101*::λpSG1	+	λpSG1 replacement of *aidB1*::Mu-dl(Ap^R*lac*)
MV1906	*B102*::λpSG1	+	λpSG1 replacement of *aidB2*::Mu-dl(Ap^R*lac*)
MV1908	*B103*::λpSG1	+	λpSG1 replacement of *aidB3*::Mu-dl(Ap^R*lac*)
MV1910	*C108*::λpSG1	+	λpSG1 replacement of *aidC8*::Mu-dl(Ap^R*lac*)
MV1912	*C110*::λpSG1	+	λpSG1 replacement of *aidC10*::Mu-dl(Ap^R*lac*)
MV1914	*D106*::λpSG1	+	λpSG1 replacement of *aidD6*::Mu-dl(Ap^R*lac*)
MV1916	*-109*::λpSG1	+	λpSG1 replacement of *aid-9*::Mu-dl(Ap^R*lac*)
MV1918	*B111*::λpSG1	+	λpSG1 replacement of *aidB11*::Mu-dl(Ap^R*lac*)
MV1920	*B112*::λpSG1	+	λpSG1 replacement of *aidB12*::Mu-dl(Ap^R*lac*)
MV1922	*B113*::λpSG1	+	λpSG1 replacement of *aidB13*::Mu-dl(Ap^R*lac*)
MV1924	*B114*::λpSG1	+	λpSG1 replacement of *aidB14*::Mu-dl(Ap^R*lac*)
MV1929	*1115*::λpSG1	+	λpSG1 replacement of *aidI15*::Mu-dl(Ap^R*lac*)

TABLE 1—Continued

Strain	Relevant genotype	Derivation or reference	Source
B. Miscellaneous strains			
CS1110	Tn*10* insertion near *ser*U(43 min)		C. SCHAITMAN
GW5352	*ada10*::Tn*10*	LEMOTTE *and* WALKER (1985)	P. LEMOTTE
Hfr Broda 8	Hfr PO118 *his*$^+$	BACHMANN and LOW (1980)	B. BACHMANN
KD1092L	*mutL218*::Tn*10*	*mutL218*::Tn*10* (SIEGEL *et al.* 1982) transductant of KD1088 (DEGNEN and COX 1974)	R. FOWLER
KL99	Hfr PO42 *his*$^+$	BACHMANN and LOW (1980)	B. BACHMANN
KL208	Hfr PO43 *pro*$^+$	BACHMANN and LOW (1980)	B. BACHMANN
KL226	Hfr PO2A *pro*$^+$	BACHMANN and LOW (1980)	B. BACHMANN
Ma1103	Mu-dl(Ap^R*lac*) *araB*::Mu-c^{ts}	CASADABAN and COHEN (1979)	M. CASADABAN
MS20	*alkA1 his*$^+$	YAMAMOTO *et al.* (1978)	M. SEIKIGUCHI
SK2651	*zee-976*::Tn*10 his*$^+$		S. KUSHNER
C. Bacteriophage strains			
Mu-dl(Ap^R *lac*)		CASADABAN and COHEN (1979)	M. CASADABAN
λpl(209)	Mu *lac* hybrid	CASADABAN (1976)	G. WEINSTOCK
λpSG1	*lacY*::Tn*9*	Tn*9* insertion mutant of λpl(209)	G. WEINSTOCK
P1 *virA*			A. J. CLARK

[a] All strains in section A, except MV1185, are derivatives of MV1161, which is a φX174, S13 sensitive derivative of AB1157, and, unless indicated otherwise, carry the following additional mutations: *argE3, his-4, leu-6, proA2, thr-1, ara-14, galK2, lacY1, mtl-1, xyl-5, thi-1, rpsL31, supE44, tsx-33, rfa-550.*

[b] MV1185 is *arg*$^+$ *rfa*$^+$ and carries the following mutations in addition to those listed in footnote a: *uvrA6 malE*::Tn*10.*

RESULTS

Isolation of Mu-dl(Ap^R *lac*) insertion mutants: In this screen, 55 MNNG- or MMS-inducible fusion mutants were isolated from approximately 370,000 random insertion mutants that were constructed using the phage Mu-dl(Ap^R *lac*). Of these fusion mutants, 46 were also UV-inducible and were assumed to contain fusions to promoters of *din* genes of the SOS response (KENYON and WALKER 1980). The remaining nine mutants were inducible only by MNNG, MMS or both, but not by UV. These nine strains contained fusions that were specifically induced by an alkylating agent and, therefore, met our criteria for alkylation-inducible (*aid*) genes. These nine mutants combined with the five described previously were mapped to five distinct genetic loci.

Genetic loci of *aid*::Mu-dl(Ap^R *lac*) fusions: The genetic loci of all five classes of *aid* fusions are shown in Figure 1. All *aid* mutations except *aidI* have been linked to known markers by P1 transduction. These linkages are shown in Table 2. *aidA* has been described previously. It is an insertion into *alkA*

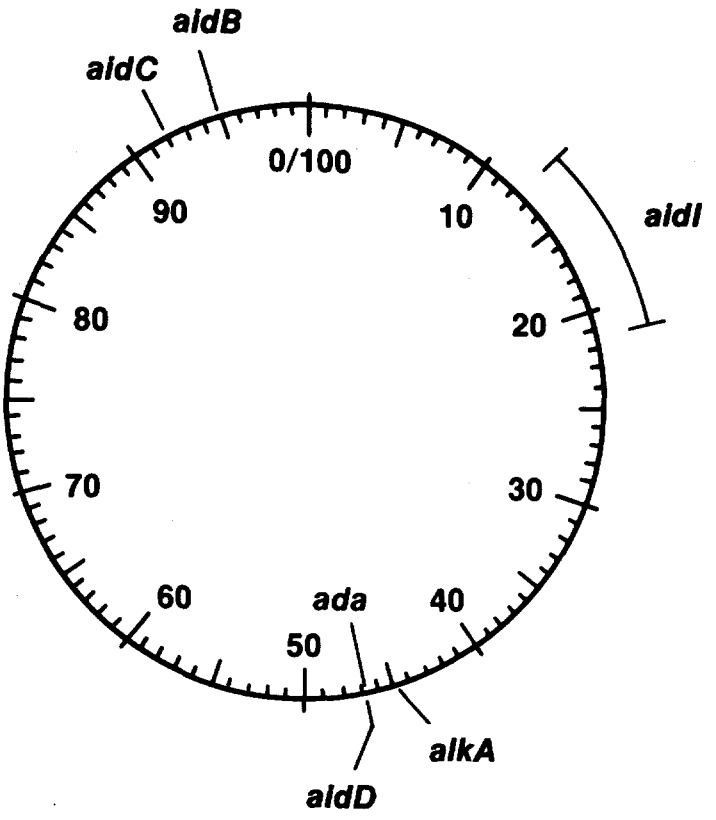

FIGURE 1.—Genetic loci of *aid* alleles and genes associated with the adaptive response.

TABLE 2

Linkages of *aid* mutations

Recipient allele	Strain	Selected marker	Cotransduction frequency[a] (%)	Total transductants tested	Approximate distance from selected marker (min)[b]
alkA50	MV1565	*zee-976*::Tn*10*	22	96	0.79
alkA51	MV1571	*zee-976*::Tn*10*	26	96	0.72
aid-9	MV1624	*zee-976*::Tn*10*	26	96	0.72
aidB1	MV1561	*mutL218*::Tn*10*	68	128	0.24
aidB2	MV1563	*mutL218*::Tn*10*	62	144	0.29
aidB3	MV1564	*mutL218*::Tn*10*	63	128	0.29
aidB111	MV1918	*mutL218*::Tn*10*	63	48	0.29
aidB12	MV1638	*mutL218*::Tn*10*	67	48	0.25
aidB13	MV1648	*mutL218*::Tn*10*	58	48	0.33
aidB14	MV1649	*mutL218*::Tn*10*	56	48	0.35
aidC8	MV1608	*malE*::Tn*10*	18	281	0.87
aidD6	MV1601	*ada10*::Tn*10*	100	50	<0.01

[a] Percentage loss of Ap^R or Cam^R.
[b] Calculated according to the formula of WU (1966).

TABLE 3

Genetic mapping of the *alkA* locus

Strain	Genotype	Percentage of transductants exhibiting donor phenotype[a]		
		His	AlkA	His and AlkA
MV1900	*his-4 alkA104*::λpSG1	48	22	2
MV1902	*his-4 alkA105*::λpSG1	48	26	5
MV1916	*his-4 aid-9*::λpSG1	49	26	4
MV1174	*his*$^+$ *alkA1*	62	18	6

[a] In crosses with MV1900, MV1902, MV1016, the donor strain was P1·SK2651 (*alk*$^+$ *his*$^+$ *zee-976*::Tn*10*), and 96 recipients from each cross were tested for His$^+$ and loss of the *alkA*::λpSG1 fusion. In the crosses with MV1174, the donor strain was P1·MV1196 (*alk*$^+$ *his-4 zee-976*::Tn*10*), and the Tet^R transductants were tested for His$^-$ and MMS^S phenotypes.

(VOLKERT and NGUYEN 1984), a gene that codes for an alkylation inducible glycosylase that removes several different alkylated bases from DNA (KARRAN, HJELMGREN and LINDAHL 1982; EVENSEN and SEEBERG 1982; MCCARTHY, KARRAN and LINDAHL 1984; NAKABEPPU *et al.* 1984).

alkA has been tentatively assigned a locus between *serU* (*supD*) and *his* (BACHMANN 1983) (43.3 min). Our results show that *alkA1* and *alkA*::λpSG1 fusions map on the opposite side of *his* from *serU*, near 45 min.

This location for *alkA* is based on two crosses. Strain CS1110 contains a Tn*10* insertion that is 80% cotransducible with *serU* (*supD*) (43 min) (C. SCHNAITMAN, personal communication). When this strain is used as a donor in a P_1 transductional cross, the *his*$^+$ marker (44 min) of the donor was cotransduced with Tet^R at a frequency of 8–9%. *alk*$^+$ was not cotransducible with this Tn*10* element among a total of 192 Tet^R transductants tested. This indicates that *alkA*::λpSG1 does not lie between this Tn*10* insertion and the *his* marker.

In the second cross, P1 grown on SK2651 (*zee-976*::Tn*10 his*$^+$) was used to transduce *alkA*::λpSG1 fusion strains. *zee-976*::Tn*10* lies on the opposite side of *his* from *serU*, at approximately 44.5 min (S. KUSHER, personal communication). The donor *his*$^+$ marker yielded a cotransduction frequency of 48–49% with *zee-976*::Tn*10* and the donor *alkA*$^+$ marker was cotransduced with a frequency of 22–26% in crosses using strains MV1900, MV1902 and MV1916 as recipients (Table 3). Since both the donor *his*$^+$ and *alk*$^+$ markers were coinherited with *zee-976*::Tn*10* at a frequency of only 2–5%, *zee-976*::Tn*10* must lie between *his* and *alkA*::λpSG1 fusions. These results, like those from crosses with P1·CS1110, place *alkA*::λpSG1 on the opposite side of *his* from *serU*. In addition, the crosses with P1·SK2651 place *alkA* approximately 1–1.5 min from *his*, at 45–45.5 min. Similar linkages were also obtained for *alkA*1 and *his* in a cross of P1·MV1196 (*his-4 alkA*$^+$) and MV1174 (*his*$^+$ *alkA1*) (Table 3).

aid-9::Mu-dl(Ap^R *lac*) maps at a position near *alkA* and shares many phenotypic characteristics with *alkA* fusions (see below). Although it shows different

TABLE 4

Genetic mapping of the *aidC* locus

	Tet^R recombinants exhibiting unselected phenotypes (%)[a]	
	UV^R	UV^S
Ap^R	67	15
Ap^S	0.7	17

[a] Total Tet^R transductants tested was 281, donor was MV1185, recipient was MV1608 *aidC8*::Mu-dl(Ap^R *lac*).

β-galactosidase induction characteristics from other *alkA* fusions (see below), it is likely to be another independent fusion to *alkA* exhibiting altered expression of β-galactosidase (CASADABAN 1976).

aidB is linked to *mutL218*::Tn*10* with a cotransduction frequency of 62–68%, placing it approximately 0.24 min from *mutL* (Table 2).

The *aidB* fusions define a gene or genes linked to, but separable from, *mutL*, *aidB* mutants have also been tested for induction at high temperatures to determine if this fusion may lie in the nearby *mop* (*groEL*) locus that codes for a heat-shock gene. No induction was seen either at 42° or after UV treatments (data not shown), two treatments that are capable of inducing the heat-shock response (NEIDTHARDT *et al.* 1981; NEIDTHARDT, VAN BOGELEN and LAU 1983; KRUEGER and WALKER 1984). Thus, *aidB* is not an allele of this damage-inducible operon, but represents a fusion to another alkylation-inducible gene in this region.

The *aidC* locus is linked to *malE*::Tn*10* (Table 2). It is unlikely to be an insertion into the *uvrA* gene, which is also linked to *malE*, since *aidC* mutants do not show induction of β-galactosidase activity after UV treatment, nor are they UV-sensitive (data not shown) (KENYON and WALKER 1980, 1981).

To establish the order of *malE*, *uvrA* and *aidC*, the following cross was performed. P_1 phage was grown on a *malE*::Tn*10* *uvrA6* *aid*$^+$ strain, MV1185 (*i.e.*, Tet^R UV^S Ap^S), and was to transduce a *mal*$^+$ *uvr*$^+$ *aidC8*::Mu-dl(Ap^R *lac*) strain MV1608 (*i.e.*, Tet^S UV^R Ap^R). This allowed inheritance of the donor *uvrA6* and *aid*$^+$ alleles to be determined by coinheritance of UV sensitivity and ampicillin sensitivity among tetracycline-resistant transductants. These results are shown in Table 4. A gene order of *malE*—*uvrA*—*aidC* can be inferred from the relative frequencies of the multiple crossover events. The relative distances of *uvrA* and *aidC* from *malE* are 0.63 and 0.87 min, respectively, based on their cotransduction frequencies of 32% and 18%. Taking into account the increased distance between *malE* and *uvrA*, due to the inclusion of 9300 base pairs of Tn*10* DNA in the donor strain (FOSTER *et al.* 1981), *uvrA* is linked to *malE* with the expected frequency (MOUNT, LOW and EDMISTON 1972). This places *aidC* very near *uvrA*.

aidD::Mu-dl(Ap^R *lac*) is lost whenever *ada10*::Tn*10* from the P_1 donor strain GW5352 is incorporated by recombination (Table 2). Thus, *aidD*::Mu-dl (Ap^R *lac*) is very tightly linked to *ada*, lying less than 0.01 min from *ada10*::Tn*10*.

It is likely to be a fusion to the *ada* promoter. If *aidD* represents an *ada*::Mu-dl (Ap^R *lac*) fusion, then it must be a fusion that has not destroyed the coding capacity for the regulatory component of the *ada* gene, because some *ada* function appears to be required for its own induction (SEDGWICK 1983), although truncated forms of *ada* are sufficient for autoregulation (LEMOTTE and WALKER 1985). *aidD* is likely to be an insertion into the *alkB* gene, which lies in the *ada* operon just downstream from *ada* (SEDGWICK 1983). This position for *aidD* is consistent with this mutant's ability to express adaptation toward the mutagenic effects of MNNG (see below).

aidI has been mapped by Hfr crosses. Crosses with Hfr's KL226 and KL99, whose points of origin lie at 13 and 23 min, respectively, do not result in loss of Mu-dl(Ap^R *lac*). Two other Hfr's, Broda 8 and KL208, both of which transfer the region that lies between 13 and 23 min, caused a loss of Ap^R. Hfr Broda 8 caused an 11% loss among His^+ recombinants and Hfr KL208 caused a 22% loss of Ap^R among Pro^+ recombinants. This placed *aidI*::Mu-dl(Ap^R *lac*) between the points of origin of these two Hfr strains excluding those portions of this region that are transferred by KL226 or KL99. These results placed *aidI* between 13 and 23 min.

MNNG sensitivity: Relative sensitivities to MNNG are shown in Figure 2. *alkA, aidB* and *aidD* affect survival from MNNG treatment, whereas *aidC* and *aidI* do not. *alkA* and *aid-9* both caused sensitivity to MNNG to essentially the same degree (a three-fold change in slope compared to wild type). This is consistent with the suggestion that both are insertions into the same gene or into two genes affecting the same repair pathway. *aidD* causes sensitivity to MNNG that is intermediate between *aidD* and aid^+, about a 1.5-fold change in slope. The threefold increased resistance resulting from the *aidB*::Mu-dl(Ap^R *lac*) fusion is shown for comparison and has been described elsewhere (VOLKERT and NGUYEN 1984). Of the seven independently isolated insertions mapping in the *aidB* region, only three showed a definite increase in MNNG resistance (MV1561, MV1564, MV1648), indicating that these seven insertions are either linked to different promoters or affect different components of the same operon.

Effect of *aid*::Mu-dl (Ap^R *lac*) mutations on the adaptive response: Table 5 shows the effect of pretreatment with low levels of MNNG on survival and mutagenesis after MNNG challenge. The inducing doses for expression of the adaptive response and the challenge doses used for each of the strains listed were those that yielded the greatest difference between unadapted and adapted cells.

All strains show at least some ability to adapt toward the mutagenic effects of MNNG, based on the reduction in mutation frequency when adapted cells were compared with unadapted cells. When adaptation toward the lethal effects of MNNG were measured, aid^+, *aidB, aidC, aidD* and *aidI* showed adaptation, whereas *alkA* and *aid-9* did not. Therefore, *alkA* and *aid-9* block the cell's ability to adapt to the lethal effects of MNNG, which is consistent with inactivation of *alkA* (KARRAN, HJELMGREN and LINDAHL 1982; EVENSEN and SEEBERG 1982; NAKABEPPU *et al.* 1984).

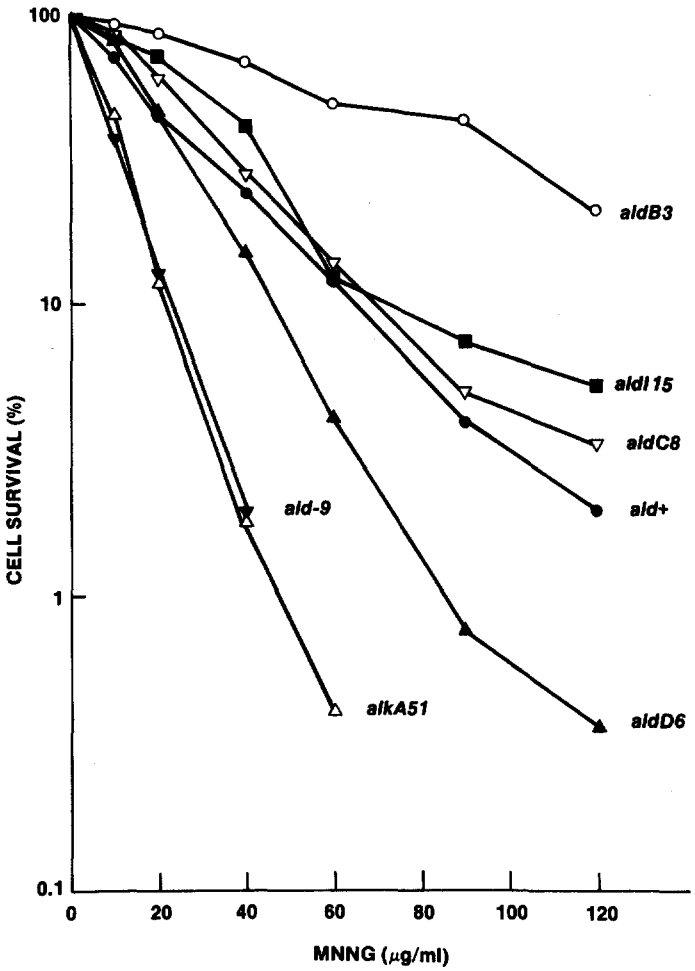

FIGURE 2.—Survival of *aid* mutants and their *aid*$^+$ parent strain after 30-min exposures to various concentrations of MNNG. MV1161, *aid*$^+$ (●); MV1564, *aidB3* (○); MV1571, *alkA51* (△); MV1601, *aidD6* (▲); MV1608, *aidC8* (▽); MV1616, *aid-9* (▼); MV1652 (*aidI15*) (■).

Alkylation induction of gene expression in *aid*::Mu-dl(Ap^R *lac*) fusions: Figure 3 shows the induction of β-galactosidase activity by MNNG treatment. MNNG doses used for each strain were the optimum doses for β-galactosidase induction determined beforehand for each strain (data not shown). Excluding *aid-9*, independent isolates mapping at a particular locus are quite similar in their induction characteristics (data not shown). Thus, the differences in β-galactosidase expression upon alkylation treatment generally reflect relative differences in *aid* gene expression. The one exception is *aid-9*, a putative *alkA* fusion, which differs from other *alkA* fusions, showing about a twofold greater level of β-galactosidase expression than *alkA* and a higher optimum induction dose (Figure 3). *aidI* differs from other *aid* fusions in that it is not induced by MNNG treatment (Figure 4).

TABLE 5

Effect of *aid*::Mu-dl(Ap^R *lac*) mutations on the adaptive response to alkylating agents

Allele	MNNG challenge dose (μg/ml)	Cell survival (%)		MNNG challenge dose (μg/ml)	Mutation frequency[a]	
		Unadapted cells	Adapted cells		Unadapted cells	Adapted cells
aid$^+$	120	2.0	10.8	10	834	14.4
alkA51	40	4.9	3.3	5	497	131
aidB3	120	14.5	62.0	10	990	4.4
aidC8	120	2.4	13.6	5	699	6.0
aidD6	60	3.3	12.0	5	750	80
aidI15	120	5.3	24.6	5	1676	0.0

[a] Mutation frequency equals His$^+$ revertants per 10^7 surviving cells plated.

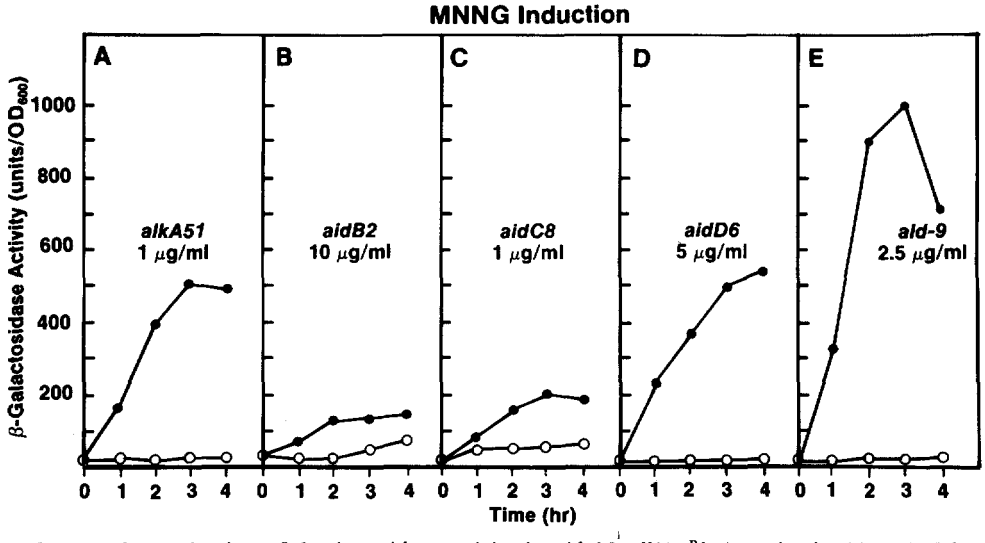

FIGURE 3.—Induction of β-galactosidase activity in *aid*::Mu-dl(Ap^R*lac*) strains by MNNG. The optimal MNNG concentration, in terms of β-galactosidase activity, was first determined for each strain and then used to obtain the results shown above. The MNNG concentration used is listed in each panel; the open circles (○) indicate untreated controls, the closed circles (●) indicate MNNG-treated cells. A, MV1571 *alkA51*; B, MV1563 *aidB2*; C, MV1608 *aidC8*; D, MV1601 *aidD6*; E, MV1616 *aid-9*.

MMS induction is shown in Figure 5. Again, the optimal dose for β-galactosidase induction was first determined for each strain (data not shown). *aidC* is not shown in this figure since both *aidC* fusion strains were induced by MNNG and not by MMS. Figure 6 shows the level of β-galactosidase activity reached in an *aidC* strain 3 hr after beginning MMS treatment. No increase above the untreated control was seen at any MNNG dose tested. Thus, treatments with these two methylating agents result in the induction of a common set of genes (*alkA*, *aidB* and *aidD*). In addition, each agent also caused induction of unique *aid* genes; *aidC* was induced only by MNNG, and *aidI* was

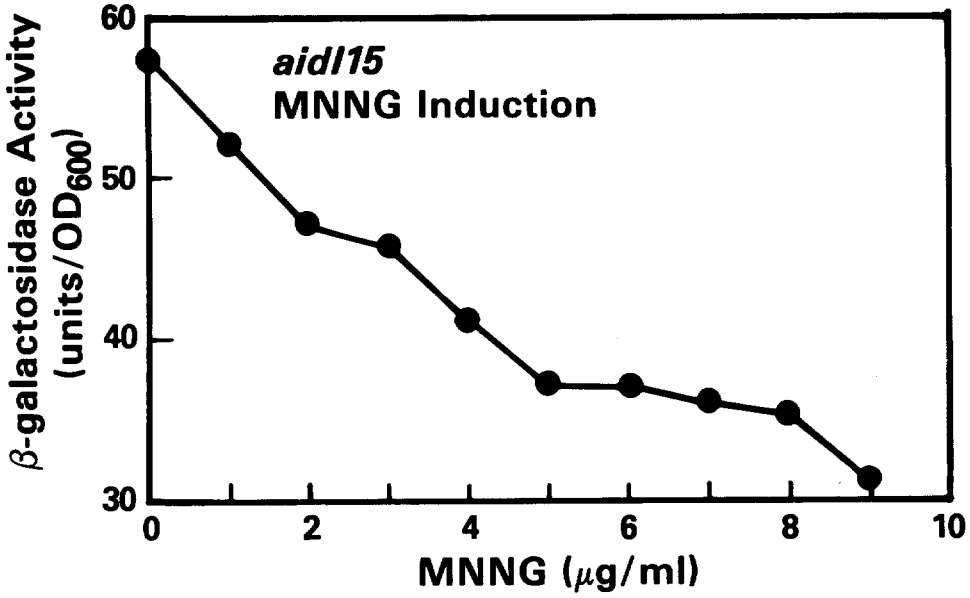

FIGURE 4.—β-Galactosidase activity in an MNNG-treated *aidI*::Mu-dl(Ap^R *lac*) strain (MV1652). Cells were exposed to various concentrations of MNNG for 2 hr at 30° and were assayed for β-galactosidase activity.

induced only by MMS. These differences in induction by MNNG *vs.* MMS suggest that there are multiple regulatory mechanisms controlling the different types of *aid* genes.

Effect of *ada10*::Tn*10* on *aid*::Mu-dl(Ap^R *lac*) induction: To investigate the effect of an *ada* mutation on *aid* gene induction, the *ada10*::Tn*10* mutation (LEMOTTE and WALKER 1985) was introduced into all *aid* strains except *aidD*. These *ada10*::Tn*10* transductants were then tested for ability to induce β-galactosidase activity after MNNG or MMS treatment. These results are shown in Figure 7. *aidD* was not included because this fusion mapped at *ada* (Table 2), and the Mu-dl(Ap^R *lac*) fusion was lost whenever *ada10*::Tn*10* was incorporated by recombination. The *ada* gene product appears to be a positive regulatory element of the adaptive response (SEDGWICK 1983; LEMOTTE and WALKER 1985). This *ada10*::Tn*10* insertion mutation blocks the cell's ability to adapt (P. LEMOTTE and G. C. WALKER, personal communication) and, like the *ada-5* mutation, affects *alkA* and *aidB* induction by MNNG (Figure 7 and VOLKERT and NGUYEN 1984). This mutation differs from *ada-5* in that its effects are more severe: *ada-5* merely reduced β-galactosidase induction of *alkA* and *aidB*, whereas the *ada10*::Tn*10* mutation completely blocked their induction, as well as that of *aid-9*. However, *ada10*::Tn*10* had no effect on induction of either *aidC* or *aidI*, indicating that these two genes are not subject to *ada* control.

DISCUSSION

The use of Mu-dl(AP^R *lac*) provides a convenient tool for the construction of operon fusions to genes known to be induced under specific conditions even

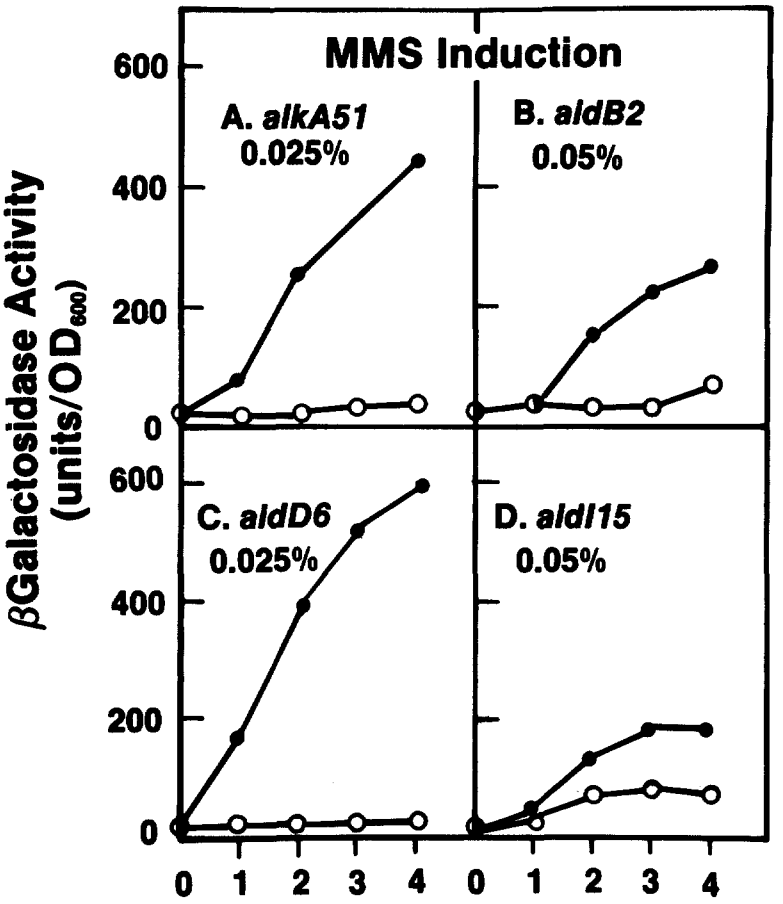

FIGURE 5.—Induction of β-galactosidase activity in *aid*::Mu-dl(AP^R*lac*) strains by MMS. The optimal concentration, in terms of β-galactosidase activity, was first determined for each strain and then used to obtain the results shown here. The open circles (○) indicate untreated controls, the closed circles (●) indicate MMS-treated cells. A, MV1571 (*alkA51*); B, MV1563 (*aidB2*); C, MV1601 (*aidD6*); D, MV1652 (*aidI15*).

when their genetic loci, function or phenotypic effects are unknown. In this report, and the previous report (VOLKERT and NGUYEN 1984), we selected for fusions to genes induced specifically in response to treatments with methylating agents. Combining the fusion mutants isolated from the two sets of screenings, a total of 65 independent MNNG- or MMS-inducible fusion mutants were isolated. Most of these 65 mutants were also inducible by UV treatment. Only 14 were specifically induced by alkylation treatments and not by UV treatment. These 14 mutants mapped at five distinct loci and represent fusions to at least five different genes.

The fusions mapping at these five loci can be further subdivided on the basis of the their induction by the two methylating agents used. *alkA, aidB* and *aidD* were inducible by both MNNG and MMS. *alkA, aidB* and, presumably,

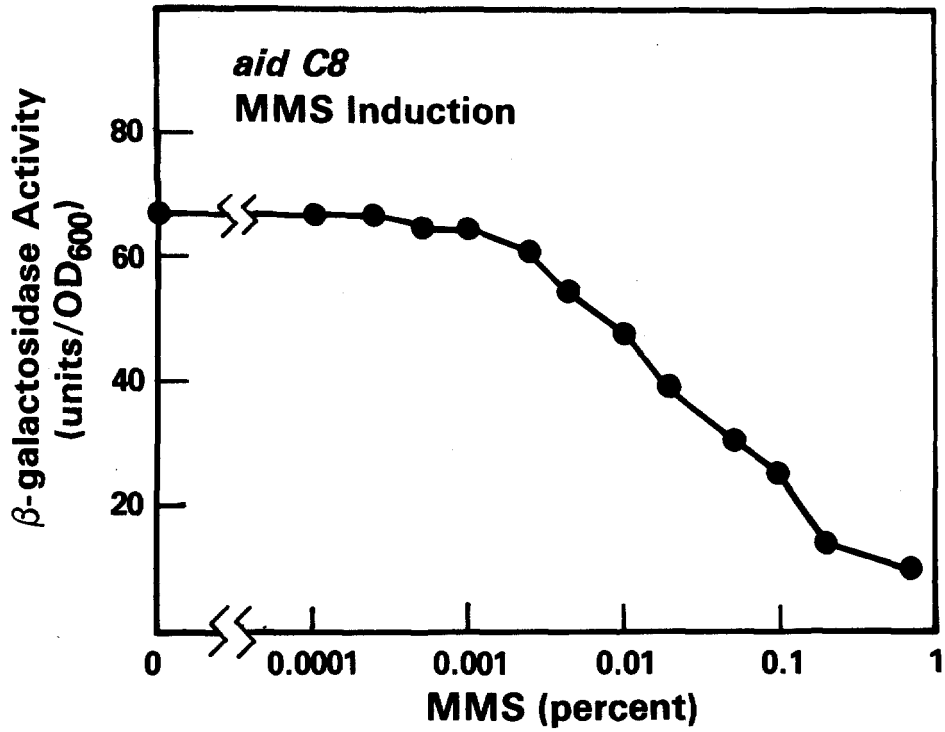

FIGURE 6.—β-Galactosidase activity in an MMS-treated *aidC* strain, MV1608, *aidC8*. Cells were exposed to MMS at various concentrations. Three hours after exposure began, cell extracts were prepared and assayed for β-galactosidase activity.

also *aidD* are controlled by *ada*. *aidC* and *aidI* differ in that *aidC* is induced only after MNNG treatment and *aidI* is induced only after MMS treatment. Neither is controlled by *ada*, and their differential induction suggests that they are also likely to be under individual regulatory control.

The different patterns of gene induction seen when these two simple methylating agents are compared shows that the cellular response to alkylating agents is not a simple, constant response to a variety of alkylation treatments. Instead, there are multiple alkylation-induced responses that differ depending on the agent used to elicit the response.

These differences in gene induction patterns suggest differences in induction signals produced by MNNG and MMS that, in turn, presumably reflect differences in the reaction products produced, or metabolism of these agents by the cell. In the case of the *aidC* gene, its induction by only MNNG could simply be due to alkylation at reactive oxygens in the DNA, because these types of lesions are efficiently produced by MNNG, but are much more rare after MMS treatment (SINGER 1982). Such explanations cannot readily account for the MMS-specific induction of *aidI*, because MMS is not known to produce any lesions that are not also produced by MNNG. Therefore, MMS either produces some as yet unrecognized lesions in DNA that are not produced by MNNG,

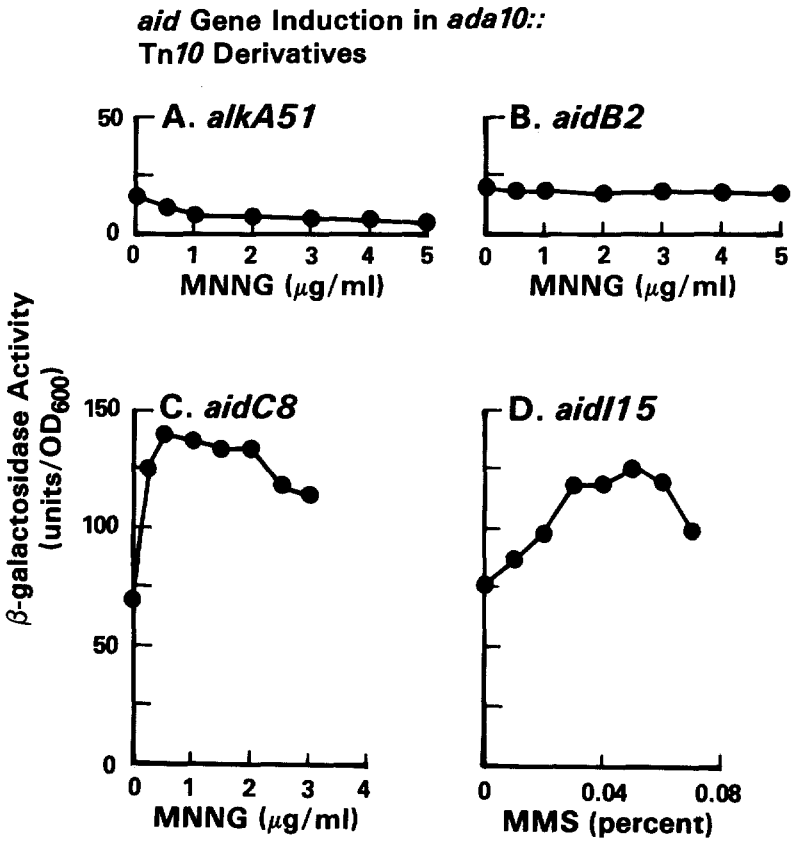

FIGURE 7.—β-Galactosidase activity in *ada10*::Tn*10* derivatives of *aid*::Mu-dl(Ap^R *lac*) strains. Cells were exposed to MNNG at various concentrations. After 2 hr, cell extracts were prepared and assayed for β-galactosidase activity. A, MV1704 (*ada10*::Tn*10 alkA51*); B, MV1701 (*ada10*::Tn*10 aidB2*); C, MV1707 (*ada10*::Tn*10 aidC8*); D, MV1756 (*ada10*::Tn*10 aidI15*).

or are produced inefficiently, or *aidI* responds to effects of MMS on cellular components other than DNA.

Based on these results and studies of the adaptive response (JEGGO *et al.* 1977; SEDGWICK 1982, 1983), all alkylation treatments appear to cause the induction of the *ada*-controlled set of genes, which are presumed to be largely responsible for the reduction in lethality and mutagenesis associated with the adaptive response. In addition, there are also agent-specific responses that vary depending on the agent used. It remains to be determined how much variability in gene induction occurs when a wider variety of alkylating agents are used.

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