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Induction and Autoregulation of *ada*, a Positively Acting Element Regulating the Response of *Escherichia coli* K-12 to Methylating Agents

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The *ada* gene of *Escherichia coli* K-12, the regulatory locus for the adaptive response to methylating agents, coded for a 39,000-dalton protein. An adjacent gene coding for a 27,000-dalton protein was coregulated with *ada*. The Ada protein was strongly induced upon exposure of cells to methylating agents such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. An analysis of *ada* regulation with an *ada-lacZ* operon fusion showed that *ada*$^+$ function was required for induction of *ada* transcription. Derivatives of the *ada* gene truncated from the 3' end produced proteins which were more potent in stimulating transcription than the product of the intact *ada* gene, indicating that the transcription-activating function of the Ada protein resided in its amino terminus. The sequence of the *ada*-regulatory region and the identification of the start site of *ada* transcription are also presented.

Exposure of DNA to methylating and ethylating agents results in the production of a variety of DNA lesions. Alkylation at the O^6 position of guanine is particularly detrimental because this modified base preferentially pairs with thymine, producing a high mutation frequency after replication of the DNA (25). A common mechanism for the repair of this type of damage has been found in a number of organisms, including *Escherichia coli*, *Bacillus subtilis*, rodents, and humans (1, 7, 9, 12, 18, 19). A protein termed O^6-methylguanine-DNA methyltransferase transfers the methyl group to a cysteine residue on itself, thereby inactivating itself (19). In *E. coli*, this enzyme also acts on O^4-methylthymine (16). Exposure of *E. coli* to an alkylating agent such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) induces resistance to the lethal and mutagenic effects of subsequent exposures to methylating and ethylating agents (21). This has been termed the adaptive response and involves induction of O^6-methylguanine-DNA methyltransferase, 3-methyladenine-DNA glycosylase II, and the product of the *aidB* gene (5, 13, 18, 27). The *E. coli* enzyme 3-methyladenine-DNA glycosylase II, the product of the *alkA* gene, is active in the repair of 3-methyladenine, 3-methylguanine, 7-methylguanine, O^2-methylcytosine, and O^2-methylthymine (13, 16).

The gene which controls the adaptive response has been called *ada*. *ada* mutants are hypersensitive to both mutagenesis and killing by MNNG and cannot induce the synthesis of either O^6-methylguanine-DNA methyltransferase or 3-methyladenine-DNA glycosylase II (5, 18). In this paper we report the identification of the *ada* gene product and an analysis of *ada* regulation with an *ada-lacZ* operon fusion. We found that *ada* was autoregulatory and that transcription of the *ada* gene was induced to very high levels by exposure of cells to MNNG. Our evidence is consistent with the hypothesis that the *ada* gene product or a modified version of the *ada* gene product acts positively to stimulate transcription of the *ada* gene.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* K-12 strains used were: AB1157 (4); its *ada* derivatives, PJ1, PJ3, PJ5, and PJ6, which are the same as AB1157 but also, respectively, *ada-1*, *ada-3*, *ada-5*, and *ada-6* (11); GW5352, the same as AB1157 but also *ada-10*::Tn*10 del-16 del-17* (see below); GW5353, the same as AB1157 but also *zeh-790*::Tn*5* (see below), GW5354, the same as AB1157 but also φ(*ada'-lacZ*$^+$)*1* (see below); BW140 *lacU169 rpsL* (from B. Wanner Emory University).

The plasmids used were: pSE101 (4), a kanamycin-resistant derivative of pSC101; pGS100 (G. De Vos, E. R. Signer, and G. C. Walker, unpublished data), a pBR322 derivative with a *lacZ* operon fusion fragment from pMC903 (2) and kanamycin and spectinomycin drug resistance genes contained on a *Bam*HI fragment. The following plasmids were as described below: pGW2620, an *ada*$^+$ derivative of pSE101; pGW2622, which contains an *ada'-lacZ*$^+$ fusion derived by cloning the *Bam*HI fragment of pGS100 into an *Sau*3A1 site of pGW2620; pGW2607, an *ada*$^+$ derivative of pBR322; pGW2609, an *Sma-Pvu*II deletion derivative of pGW2607; pGW2610, an *Hae*II deletion derivative of pGW2607; pGW2611, an *Acc*I deletion derivative of pGW2607; pGW2612, an *Eco*RI deletion derivative of pGW2607.

Genetic procedures and recombinant DNA techniques. P1 transductions were performed as described by Miller (17). Transpositions of Tn*5* were performed as described by Krueger and Walker (14). Transpositions of Tn*10 del-16 del-17* were performed in the same way as Tn*5* transpositions except that the strain being used had plasmid pNK290 which contains an HH104 mutant of IS*10*$_R$ to complement the transposition-defective Tn*10 del-16 del-17* (6). The *ada'-lacZ*$^+$ fusion on plasmid pGW2622 was crossed into the chromosome by transforming linearized plasmid into strain JC7623 by the method described by Winans and Walker (28). The *ada'-lacZ*$^+$ fusion was then transduced into strain AB1157 to make the strain GW5354.

A quick screen for the mutability and sensitivity of strains was performed by patching cells in sectors on a minimal plate containing a limiting amount of arginine and applying MNNG to a filter disk in the center of the plate. After 2 to 3

days, the number of Arg^+ revertants could be scored, and the killing radius due to the MNNG treatment could be determined. More detailed mutagenesis and killing assays were performed in liquid culture. Cells grown in supplemented M9/glucose medium were adapted for 1 h in 0.1 μg of MNNG per ml and then challenged with doses of MNNG from 0 to 5 μg/ml at a cell density of approximately 3×10^8 cells per ml. Mutagenesis was measured by plating cells on supplemented M9/glucose plates containing a limiting amount of arginine. Survival determinations were done in parallel.

Recombinant DNA techniques were as described previously (4). Plaque hybridization for the detection of *ada* clones in λSE4 was performed as described previously (4).

Protein labeling and electrophoresis. Maxicell procedures were performed for the labeling of plasmid-encoded proteins and were carried out with the strain RB901 as described by Elledge and Walker (4). In certain experiments, cells were treated with 2 μg of MNNG per ml 20 min before labeling with [^{35}S]methionine to examine induction of the Ada protein by MNNG. For whole cell labeling, cells were grown to an optical density at 600 nm of 0.5, treated with MNNG, labeled for 5 min with [^{35}S]cysteine 20 min later, and chased with cold cysteine for 1 min. Electrophoresis of proteins on sodium dodecyl sulfate-polyacrylamide gels was performed as described previously (4).

DNA sequence determination. DNA sequence determination of M13 clones by the dideoxy-chain termination procedure was performed as described by Sanger et al. (22).

Mung bean nuclease mapping. RNA was isolated from strain BW140 containing *ada*$^+$ plasmid pGW2607 by the method of Salser et al. (20). A ^{32}P-labeled single-stranded DNA probe was synthesized with a single-stranded DNA template from a *Hin*dIII-*Acc*I clone of the 5′ end of the *ada* gene in M13mp11 (New England BioLabs, Inc.). Synthesis was carried out by extension of the M13 13-mer primer (New England BioLabs) in the presence of ^{32}P-labeled dATP and unlabeled dGTP, dCTP, and dTTP. The product was cleaved with *Sal*I (which cleaves the *Acc*I site) and electrophoresed on a denaturing urea-polyacrylamide gel. The small radioactive band corresponding to the 370-base fragment extending from the primer to the *Sal*I site was eluted from the gel, and 50 ng of the DNA was hybridized at 68°C for 1 h to 50 μg of mRNA from BW140(pGW2607). This was digested with 1 U of mung bean nuclease (P-L Biochemicals, Inc.) at 37°C for 30 min. The size of the protected DNA fragment was determined by electrophoresis on a urea-polyacrylamide gel with ^{32}P-end-labeled *Hae*III-digested φX174 DNA as a standard.

β-Galactosidase assays. β-Galactosidase assays were performed as described by Miller (17). Cells were grown in supplemented M9/glucose medium, and treatments were done at an optical density at 600 nm of 0.1 to 0.2. Induction by MNNG was at a concentration of 2 μg/ml. MNNG was added at 0 h and remained in the samples throughout the experiment.

RESULTS

Isolation of an *ada*::Tn*10 del-16 del-17* mutation. At the time we began to clone the *ada* locus there was some doubt as to whether *ada* mutants were dominant or recessive since there had been a report that an F′ factor covering the *ada* region failed to complement *ada* mutants; however, the particular F′ factor used carried a deletion near *ada* (11). We therefore attempted to isolate an insertion mutation at the *ada* locus. First, we isolated a number of Tn*5* insertions that

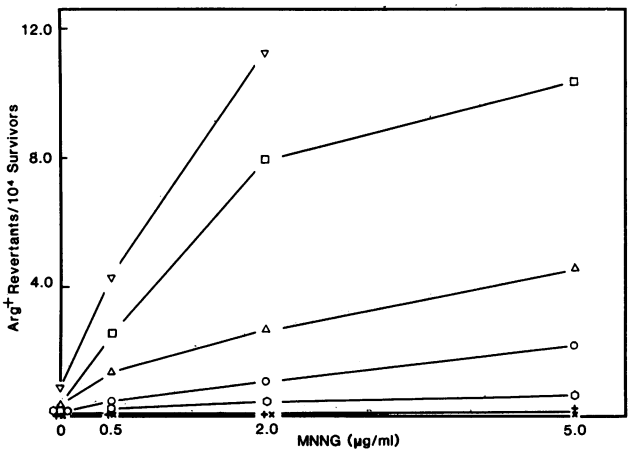

FIG. 1. Mutagenesis by MNNG. Cells were adapted with 0.1 μg of MNNG per ml and then challenged with different concentrations of MNNG. The reversion frequency of Arg^- to Arg^+ was scored. Symbols: ○, *ada*$^+$; □, *ada-5*; ▽, *ada-10*::Tn*10*; △, *ada-10*::Tn*10* plus λ *ada*$^+$; +, *ada-10*::Tn*10* plus pGW2620; ×, *ada-10*::Tn*10* plus pGW2607; ⬡, *ada-10*::Tn*10* plus pGW2609.

were cotransducible with *gyrA*, a marker known to be 25% linked to the *ada* locus (23), and chose one (*zeh-790*::Tn*5*) that was 17% cotransducible with known *ada* mutations. We then isolated approximately 120 mini-Tn*10* (Tn*10 del-16 del-17*; [6]) insertions that were cotransducible with the *zeh*::Tn*5* insertion and screened these for sensitivity to mutagenesis and killing by MNNG. One of these mini-Tn*10* insertion mutants was hypermutable with MNNG (Fig. 1) and hypersensitive to killing by MNNG (data not shown). In this strain, tetracycline resistance was 17% cotransducible with *zeh-790*::Tn*5* and 25% cotransducible with *gyrA*, suggesting that the mini-Tn*10* had inserted into the *ada* locus. The fact that an *ada* mutation could be generated by an insertion suggested that the *ada* gene is not essential for viability and that the *ada* gene product plays a positive role in the regulation of the adaptive response.

Cloning of the *ada* locus. We used the *ada* insertion mutant to generate a probe to the *ada* locus. Chromosomal DNA from the *ada*::Tn*10 del-16 del-17* mutant (designated *ada-10*::Tn*10*) was partially digested with *Sau*3A1 and ligated with *Bam*HI-cleaved pSE101 (4), and a recombinant plasmid was isolated that carried the tetracycline-resistance gene of Tn*10 del-16 del-17* and a piece of the adjacent chromosomal DNA. A 3.0-kilobase *Sal*I-*Hin*dIII fragment of this adjacent chromosomal DNA was nick translated and used as a probe for plaque hybridization of a library of *E. coli* K-12 DNA in the vector λSE4 (S. J. Elledge and G. C. Walker, manuscript in preparation). Recombinant phage that hybridized to the probe were lysogenized into an Ind^- lysogenic *ada-10*::Tn*10* strain and the double lysogens were screened for their susceptibility to mutagenesis and killing by MNNG. Of the eight phage isolates tested, seven were found to complement the *ada-10*::Tn*10* mutant (Fig. 1) and thus were presumed to carry the *ada*$^+$ gene.

We then subcloned a 4.5-kilobase *Bgl*II-*Bam*HI fragment into the *Bam*HI site of the relatively low-copy-number vector pSE101 (4) to give plasmid pGW2620 and then further subcloned a 2.3-kilobase *Hin*dIII-*Bam*HI fragment into the *Hin*dIII-*Bam*HI sites of the higher-copy-number vector pBR322 to give plasmid pGW2607 (Fig. 2). Both of these plasmids made *ada* mutants even more resistant to MNNG

mutagenesis than were *ada*$^+$ cells (Fig. 1). In maxicells, pGW2607 directed the synthesis of 39,000- and 27,000-dalton proteins in addition to the β-lactamase protein encoded by the vector (Fig. 3). This observation was similar to that reported by Sedgwick (24) for a pAT153 derivative carrying the *ada* locus of *E. coli* B. However, in contrast to the observations of Sedgwick, these two proteins were only weakly expressed. We found that the addition of MNNG to the maxicells 20 min before labeling greatly increased the synthesis of both the 39,000- and the 27,000-dalton proteins (Fig. 3). These observations suggested that either the synthesis of both proteins was being induced by MNNG or the synthesis of only the 39,000-dalton protein was being induced and the 27,000-dalton protein was a degradation product. The increased synthesis with MNNG in maxicells was only observed with the pBR322 ada$^+$ derivative and not with the pSE101 *ada*$^+$ derivative, suggesting that either the *ada* gene had to be present in sufficient copy number for this induction to occur or that some negative regulatory element was encoded in the 2.2-kilobase *Bgl*II-*Hin*dIII fragment of the pSE101 derivative.

Identification of the *ada* gene product. A series of deletion derivatives of plasmid pGW2607 was constructed (Fig. 2), and their protein products were examined in maxicells (Fig. 3). Plasmid pGW2609 directed the synthesis of a protein which, on some gels, appeared to migrate just slightly faster than the 39,000-dalton protein, whereas plasmid pGW2612 directed the low-level synthesis of the 27,000-dalton protein. We could not detect the synthesis of either protein or of truncated products with the other deletion derivatives. These results suggested that the 39,000-dalton protein was encoded in the left portion of the *Hin*dIII-*Bam*HI fragment of pGW2607, and the 27,000-dalton protein was encoded in the right portion (Fig. 2). The facts that the 27,000-dalton protein was inducible with MNNG and that it was synthesized at a low level from plasmid pGW2612 suggested either that the gene for the 27,000-dalton protein was located in an operon downstream of the 39,000-dalton protein or that synthesis of the 39,000-dalton protein was required for efficient synthesis of the 27,000-dalton protein.

The ability of these deletion derivatives to complement the hypermutability of various *ada* mutants was then examined. Plasmid pGW2609, which appears to encode a slightly truncated form of the 39,000-dalton protein, complemented all the *ada* mutants tested, although it did not make cells as exceptionally resistant to MNNG mutagenesis as did parental plasmid pGW2607 (Fig. 1). In contrast, plasmid pGW2612, which only encodes the 27,000-dalton protein, failed to

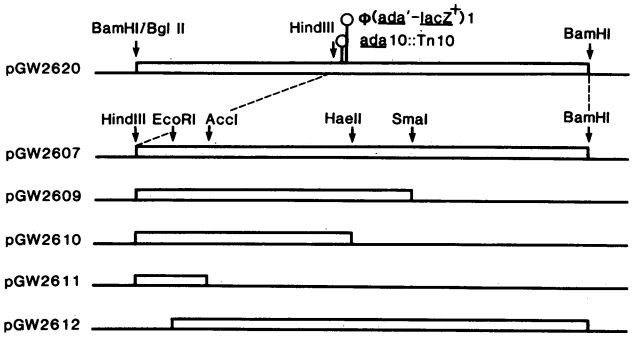

FIG. 2. Maps of plasmids containing *ada* DNA. The position is indicated of the insertion of *lacZ* to form ϕ(*ada'-lacZ*$^+$)*1*, making plasmid pGW2622, and also of *ada-10*::Tn*10*.

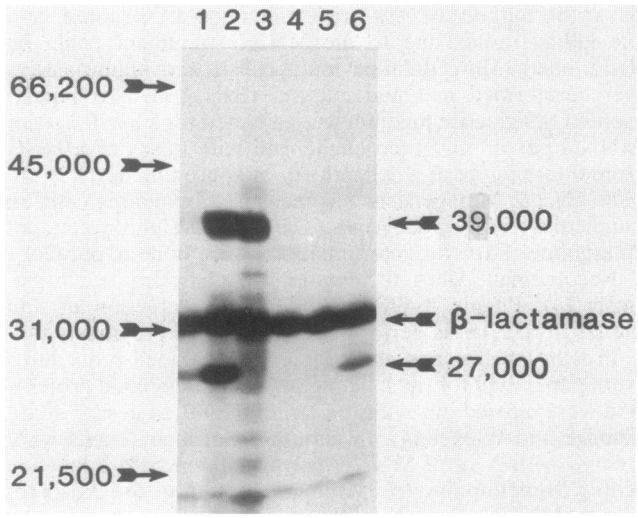

FIG. 3. Sodium dodecyl sulfate-polyacrylamide electrophoresis of [^{35}S]methionine-labeled maxicells of *ada*-derivative plasmids. Lane 1, no MNNG treatment; lanes 2 through 6, cells were treated with 2 μg of MNNG per ml 20 min before labeling. Cells contained the following plasmids: Lanes 1 and 2, pGW2607; lane 3, pGW2609; lane 4, pGW2610; lane 5, pGW2611; lane 6, pGW2612. Values are molecular weights.

complement any of the *ada* mutants. These results indicated that the 39,000-dalton protein is the *ada* gene product. Interestingly, plasmid pGW2610, which has even more DNA deleted than pGW2609 and would be expected to produce an Ada protein truncated by 20 to 30%, was able to complement *ada-3* and *ada-6* mutants but not *ada-1*, *ada-5*, and *ada-10*::Tn*10* mutants. The *ada-3* and *ada-6* mutants are somewhat less sensitive to killing than the other *ada* mutants (11) and thus may retain partial function. Presumably, plasmid pGW2610 is encoding the synthesis of a truncated Ada protein which is able to complement these *ada* mutants but not the others. Our failure to detect a protein product from pGW2610 in maxicells could be due to the instability of the truncated protein or to some more complex reason.

When we exposed *ada*$^+$ cells to MNNG (2 μg/ml) and then labeled them with a 5-min pulse with [^{35}S]cysteine, we were unable to detect the synthesis of a 39,000-dalton protein on a one-dimensional sodium dodecyl sulfate-polyacrylamide gel (Fig. 4). When a similar experiment was carried out with cells containing the *ada*$^+$ pBR322 derivative, pGW2607, a very substantial fraction of the protein synthesis of the cells was devoted to the synthesis of the 39,000-dalton protein (Fig. 4). We were also able to see a faint band corresponding to the 27,000-dalton protein (Fig. 4). Since cells containing the *ada*$^+$ pBR322 derivative are so resistant to MNNG mutagenesis (Fig. 1), we looked for the production of the 19,000-dalton O^6-methylguanine-DNA methyltransferase (3; T. Lindahl, personal communication). On a one-dimensional gel, we saw only a very faint band which may correspond to this protein. At a higher dose (50 μg/ml) of MNNG, we were able to see the induction of proteins with mobilities corresponding to those of the *groEL* and *dnaK* proteins. These two heat-shock proteins can be induced, in an *htpR*$^+$-dependent fashion, by exposure to nalidixic acid or high doses of UV (15), and it appears that 50 μg of MNNG per ml causes a similar induction.

Construction of an *ada'-lacZ*$^+$ operon fusion. To facilitate the analysis of *ada* regulation, we constructed an *ada'-lacZ*$^+$

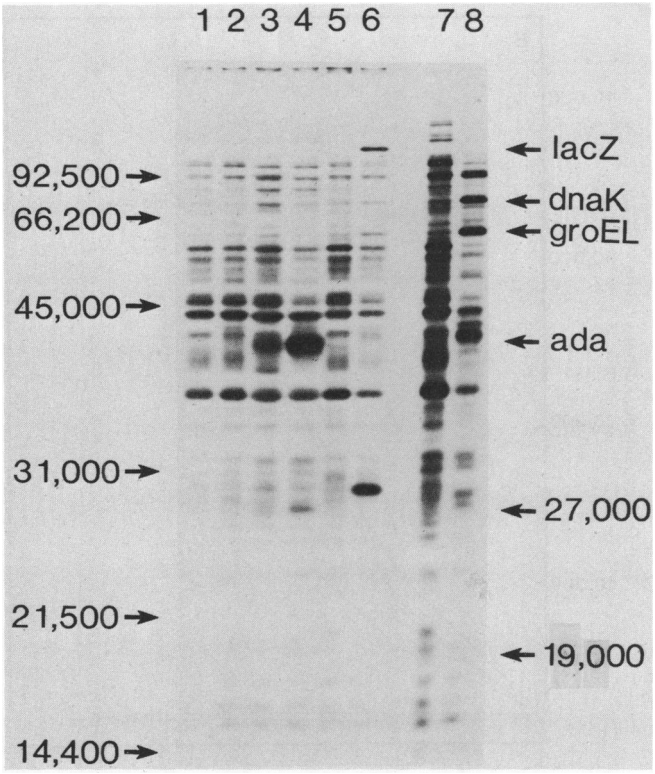

FIG. 4. Induction of proteins in whole cells by exposure to MNNG. Lanes 1, 3, 5, and 7, no MNNG exposure; lanes 2, 4, and 6, exposure to 2 μg of MNNG per ml 20 min before labeling; lane 8, exposure to 50 μg of MNNG per ml 20 min before labeling. Lanes 1 through 6, cells were labeled with [^{35}S]cysteine; lanes 7 and 8, cells were labeled with [^{35}S]methionine. Lanes 1 and 2, *ada*$^+$ strain; lanes 3 and 4, *ada*$^+$ strain containing *ada*$^+$ plasmid pGW2607; lanes 5 and 6, *ada*$^+$ strain containing *ada'-lacZ*$^+$ fusion plasmid pGW2622; lanes 7 and 8, *ada*$^+$ strain containing pGW2607. Values are molecular weights.

operon fusion. Plasmid pGW2620 linearized with *Sau*3A1 was isolated from a gel and was ligated with a *Bam*HI fragment from pGS100 containing the *lacZ* gene without its promoter and also genes coding for kanamycin and spectinomycin resistance (G. De Vos, E. R. Signer, and G. C. Walker, unpublished data). The resulting plasmids were screened for their ability to complement an *ada* mutant. One plasmid, pGW2622, was obtained that had arisen by a simple insertion of the *Bam*HI fragment containing *lacZ* into a *Sau*3A1 site in the *ada* locus of pGW2620. Since this plasmid failed to complement all *ada* mutants tested, including *ada-10*::Tn*10*, the insertion must have disrupted the *ada* gene; as shown below, this insertion also created an operon fusion of the *lacZ* gene to the promoter of the *ada* gene. The fusion was then subcloned into pBR322 for more accurate mapping. The position of the *lacZ* insertion is indicated in Fig. 2; by restriction mapping, the position of the fusion was extremely close to the position of the *ada-10*::Tn*10* insertion. Since this fusion was flanked on both sides by *ada* DNA, we were able to cross the fusion into the *E. coli* chromosome by transforming linearized pGW2622 DNA into a *recB recC sbcB* mutant (28). The fusion was then transduced into our standard strain AB1157 and the resulting mutant GW5354 was shown to have an Ada$^-$ phenotype.

Effect of *ada* mutations and *ada* deletions on *ada* expression. When *ada*$^+$ cells carrying plasmid pGW2622 were exposed to MNNG, an induction of β-galactosidase as high as 40,000 U per optical density at 600 nm was observed (Fig. 5A); plasmid pGW2622 used the replication system of pSC101 which has a copy number of 4 to 6. When the cells were labeled with [^{35}S]cysteine after exposure to MNNG, a band corresponding in size to β-galactosidase was seen on a sodium dodecyl sulfate-polyacrylamide gel (Fig. 4). These observations indicated that pGW2622 carried an operon fusion of *lacZ* to the promoter of the *ada* gene and confirmed our earlier conclusion that expression of the *ada* gene is strongly induced by MNNG. It also confirmed our conclusion that the *ada* gene is transcribed from left to right on our map (Fig. 2).

When the pGW2622-containing cells carried the *ada-10*::Tn*10* mutation, they expressed a basal level of β-galactosidase but showed no induction upon exposure to MNNG (Fig. 5B). Thus, the induction of *ada* by MNNG requires *ada*$^+$ function, and so *ada* is autoregulatory. Plasmid pGW2622 was also introduced into various *ada* mutants. When these derivatives were exposed to MNNG, they all exhibited induction of β-galactosidase to some extent (Fig. 5B). The *ada-6* mutant seemed the most deficient, whereas the other *ada* mutants showed almost the same degree of induction as an *ada*$^+$ strain. We also made the observation that introducing the compatible high-copy-number *ada*$^+$ plasmid pGW2607 led to a high constitutive level of expression of the *ada'-lacZ*$^+$ fusion that was not increased by exposure to MNNG (Fig. 5A).

A strain containing a single copy of the *ada'-lacZ*$^+$ fusion in its chromosome expressed a low level of β-galactosidase and, as expected, showed no induction upon exposure to MNNG, since it was an *ada* mutant (Fig. 6A). Introduction of a single copy of the *ada*$^+$ gene by lysogenizing with one of the *ada*$^+$ λSE4 derivatives had no effect on the basal level of β-galactosidase, but strong induction of β-galactosidase was observed upon exposure to MNNG (Fig. 6A). When the *ada*$^+$ pBR322 derivative was introduced into the strain carrying the *ada'-lacZ*$^+$ fusion in the chromosome, a higher basal level of expression was observed (1,000 U per optical density at 600 nm), and exposure to MNNG led to some increase in the synthesis of β-galactosidase (Fig. 6B). Most interestingly, we observed that introduction of the plasmids pGW2609 and pGW2610 (Fig. 2) increased the basal levels of β-galactosidase expression to 5,000 and 22,000 U per optical density at 600 nm, respectively, and that these levels were not increased by exposure to MNNG (Fig. 6B). Since both of these plasmids appear to direct the synthesis of truncated derivatives of the Ada protein, it appears that it is the amino terminus of this protein which is responsible for the positive activation of *ada* and that a truncated product of Ada is a considerably more potent transcription activator than the intact Ada protein.

Mapping and sequence of the *ada* regulatory region. The experiments described above indicated that the regulatory region of *ada* must lie between the *Hin*dIII site of the DNA in pGW2622 and the site of the *lacZ* fusion (Fig. 2). We therefore determined the DNA sequence from the *ada*$^+$ plasmid in this region (Fig. 7). To locate the *ada* promoter, we isolated mRNA from *ada*$^+$ cells containing plasmid pGW2607; the presence of pGW2607 in a cell leads to a high basal level of *ada* expression (Fig. 5 and 6, Table 1). Mung bean nuclease was used to map the start point of transcription (Fig. 8) to approximately 281 bases upstream of the *Acc*I site, which corresponds to base pair 94 in Fig. 7. Inspection of the sequence immediately upstream of the transcription

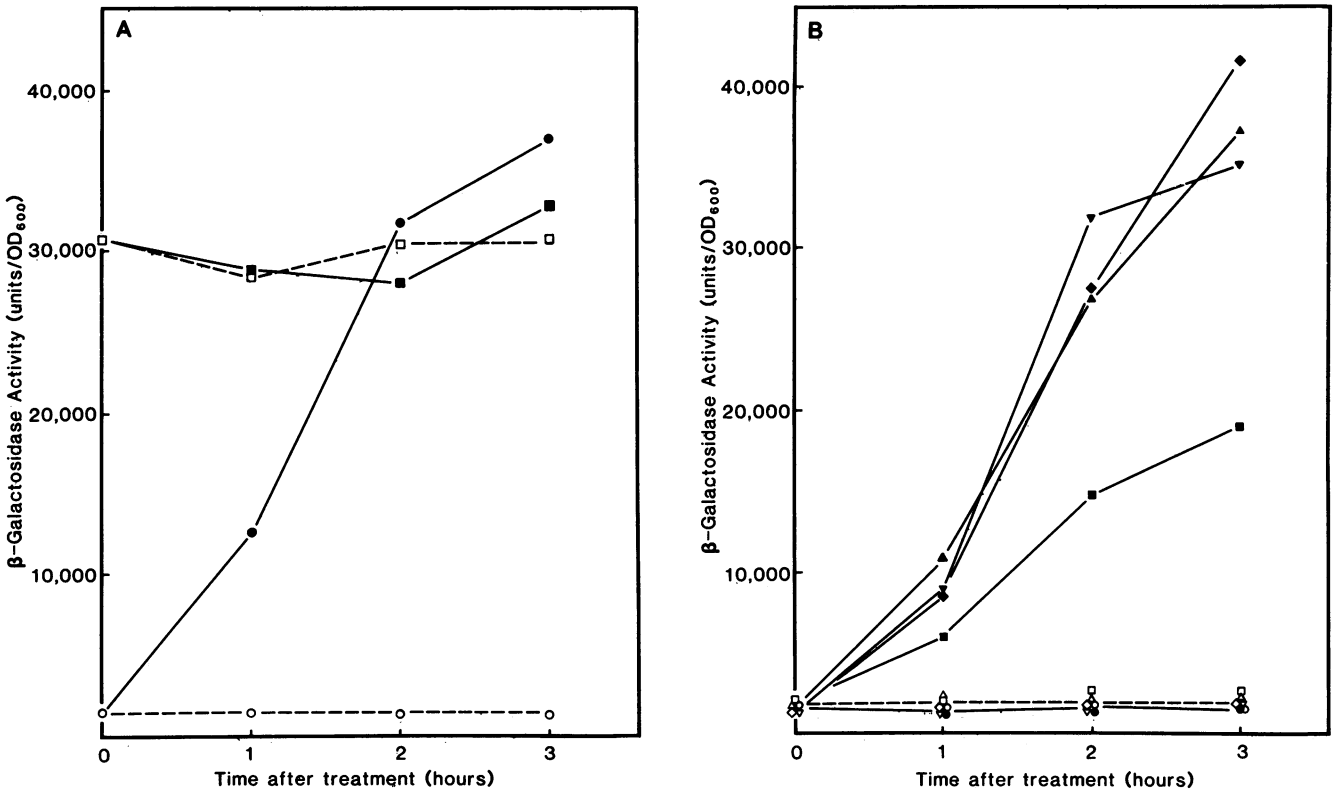

FIG. 5. Induction of β-galactosidase in strains containing *ada'-lacZ*$^+$ fusion plasmid. A. *ada*$^+$ strain containing plasmid pGW2622 (○, ●). *ada*$^+$ strain containing plasmids pGW2622 and pGW2607 (□, ■). B. Induction of β-galactosidase in different *ada* mutants containing plasmid pGW2622. Symbols: ◇, ◆, *ada-1*; △, ▲, *ada-3*; ▽, ▼, *ada-5*; □, ■, *ada-6*; ○, ●, *ada-10*::Tn*10*. For A and B: Open symbols, no MNNG; closed symbols, 2 μg of MNNG per ml.

start point revealed a sequence with a significant homology to the −10 region of many *E. coli* promoters (10). If the optimum spacing of 17 base pairs was used, the corresponding −35 region had only limited homology to the −35 consensus sequence for *E. coli* promoters. A spacing of 15 base pairs allows better homology to the −35 consensus sequence, but this spacing is considered to be poor for the functioning of *E. coli* promoters (10).

We also determined the sequence of the beginning of the *ada'-lacZ*$^+$ fusion and found that the *lacZ* fusion occurred at the GATC site at position 128. We have not yet determined the sequence of the *ada-10*::Tn*10* insertion but have determined that it lies extremely close to the position of the *lacZ* fusion by restriction mapping. There is a sequence beginning at position 117 that has near-perfect homology to sequences that serve as hotspots for Tn*10* insertion (8). This would place the Tn*10* insertion just 7 base pairs upstream of the position of the *lacZ* fusion.

Two possible initiation codons for the Ada protein are at bases 102 and 166. The first of these has a good potential Shine-Dalgarno sequence preceding it, whereas the second does not. This would be consistent with the idea that the ATG at 102 is the first codon of the Ada protein. We have, however, been able to see a small amount of a 39,000-dalton protein in maxicells of plasmids containing the *ada-lacZ* fusion. This could be a fusion protein that was created by the insertion of the fragment carrying *lacZ* into the *ada* gene. However, at the present time we cannot rule out the possibility that the first codon of the Ada protein is at base pair 166.

DISCUSSION

The results described in this paper indicate that the *ada* gene of *E. coli* codes for a 39,000-dalton protein. The Ada protein or some modified or processed form of it is required for the adaptive response of *E. coli* to methylating and ethylating agents, a response that includes the induction of O^6-methylguanine-DNA methyltransferase and 3-methyladenine-DNA glycosylase II. We showed that synthesis of the *ada* gene product is induced by exposure to the methylating agent MNNG and that the Ada protein or some derivative of it acts positively in trans to increase expression of an *ada-lacZ* operon fusion, suggesting a stimulation of transcription of the *ada* gene. The high levels of Ada protein synthesized in induced cells reflect the high levels of *ada* transcription that we were able to observe with the *ada-lacZ* fusion. The Ada protein or some derivative of it also acts to increase the transcription of an *alkA*::Mu d(Ap *lac*) fusion (27; LeMotte and Walker, unpublished data) and of other loci in the *E. coli* chromosome (27).

Recent results of Teo et al. (26) suggest that the 19,000-dalton O^6-methylguanine-DNA methyltransferase that has been so well characterized (3, 16, 19; T. Lindahl, personal communication) is a processed product of the Ada protein that is produced during the biochemical purification procedure. This 19,000-dalton protein has methyltransferase activities for O^6-methylguanine and O^4-methylthymine (16). Our results suggest that the ability of the Ada protein to function as a positive regulator can be mutationally separated, at least in part, from its ability to affect mutagenesis

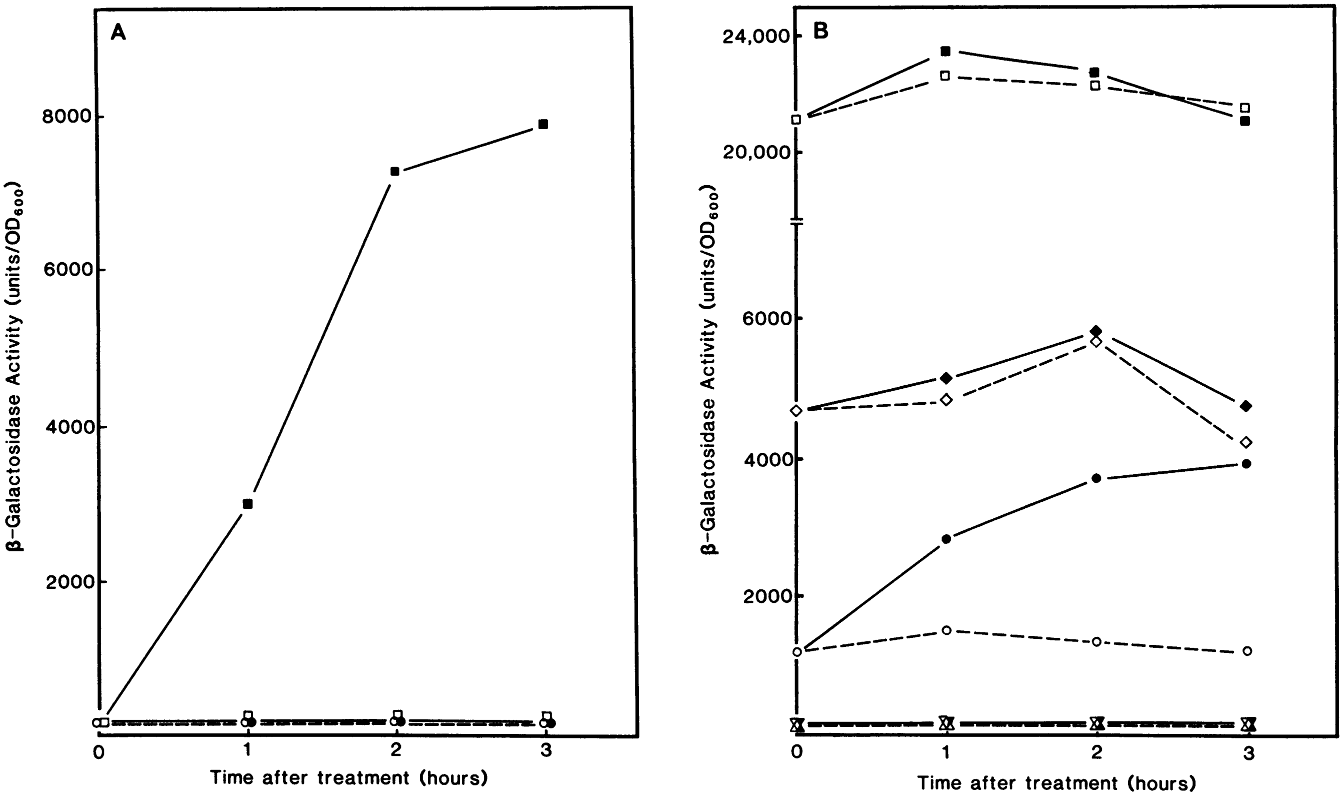

FIG. 6. Induction of β-galactosidase in strains containing a chromosomal *ada'-lacZ*$^+$ fusion. A. Symbols: ○, ●, φ(*ada'-lacZ*$^+$)*l*; □, ■, (*ada'-lacZ*$^+$)*l* plus λ *ada*$^+$. B. Induction of β-galactosidase in strains containing a chromosomal *ada'-lacZ*$^+$ fusion and different *ada*-derivative plasmids. Symbols: △, ▲, pGW2611; ▽, ▼, pGW2612; ○, ●, pGW2607; ◇, ◆, pGW2609; □, ■, pGW2610. For A and B: Open symbols, no MNNG; closed symbols, 2 μg of MNNG per ml.

and cell survival after exposure to methylating and ethylating agents. For example, cells carrying the *ada-1* and *ada-5* alleles are very sensitive to mutagenesis and killing by MNNG, yet the Ada proteins from these mutants are apparently able to increase the transcription of the *ada* gene in

```
HindIII
AAGCTTCCTT GTCAGCGAAA AAAATTAAAG CGCAAGATTG TTGGTTTTTG    50

   -35                          -10
CGTGATGGTG ACCGGGCAGC CTAAAGGCTA TCCTTAACCA GGGAGCTGAT   100

 ?                      ada-lacZ
TATGAAAAAA GCCACATGCT TAACTGACGA TCAACGCTGG CAATCTGTCT   150

                 ?         EcoRI
TAGCCCGCGA CCCGAATGCC GACGGCGAAT TCGTTTTCGC CGTGCGTACC   200

ACAGGCATCT TTTGCCGTCC GTCTTGCCGC GCCAGACATG CTTTGCGGGA   250
```

FIG. 7. Sequence of the 5' regulatory region of the *ada*$^+$ gene. The −10 and −35 regions of the *ada* promoter are shown in bold type. The arrow indicates the approximate start of transcription. Two possible ATG initiation codons and the position of insertion of *lacZ* in φ(*ada'-lacZ*$^+$)*l* are also indicated. Upstream of the *ada* promoter, the dotted lines indicate the area with homology to cII-activated promoters.

response to MNNG, at least when the *ada* gene is present on a plasmid (Fig. 5B). The *ada-10*::Tn*10* insertion mutation we isolated and the *ada-lacZ* fusion which we constructed and then crossed into the chromosome, each of which maps very early in the *ada* transcriptional unit, appear to be completely deficient in *ada* function. Strains carrying either of these mutations have no ability to induce an *ada-lacZ* fusion and are at least as sensitive, if not more sensitive, to mutagenesis and killing by MNNG as are all previously isolated *ada* mutants that we tested.

We found that simply increasing the dosage of the *ada*$^+$ gene leads to an increased basal level of expression of the *ada-lacZ* fusion. This finding is consistent with the observation of Sedgwick (24) that a multicopy plasmid carrying the *ada* gene and the linked gene for the 27,000-dalton protein increased the levels of O^6-methylguanine-DNA methyltransferase and 3-methyladenine-DNA glycosylase II. However, in these experiments one is not able to distinguish between the Ada protein itself acting as the positively acting element and some derivative of the Ada protein produced in vivo acting as the functional regulator. One particular observation suggests that the actual positively acting element is a derivative of the Ada protein. Restriction mapping indicates that plasmid pGW2610 must code for a truncated form of the Ada protein. To date, we have not been able to detect this putative truncated Ada protein in maxicells. However, this plasmid is able to reduce the mutability of *ada-3* and *ada-6* cells, and its presence leads to very high basal levels of synthesis of the *ada-lacZ* fusion (Table 1). These results suggest that a truncated Ada product is able to increase the transcription of the *ada* gene much more than an unmodified

TABLE 1. Summary of *ada*-derivative plasmids and phage

Plasmid or phage	Mol wt of protein(s) produced from plasmid	Complementation of *ada* mutants	Effect of plasmid on *ada-lacZ* expression[a]	
			Without MNNG	With MNNG
pGW2620	39,000 27,000	All[b]	NT[c]	NT
pGW2607	39,000 27,000	All	1,200	4,000
pGW2609	39,000	All	5,000	5,000
pGW2610	Truncated 39,000[d]	*ada-3 ada-6*	22,000	22,000
pGW2611	None	None	160	160
pGW2612	27,000	None	160	160
No plasmid			160	160
λ *ada*$^+$	NT	All	160	8,000

[a] The plasmid was introduced into a strain with *ada-lacZ* in the chromosome. These values are from Fig. 6.
[b] *ada-1*, *-3*, *-5*, *-6*, and *-10* were tested.
[c] NT, Not tested.
[d] Presumably codes for a truncated form of the 39,000-dalton protein, although this has not been identified.

Ada product and also that the functions for positive regulation are not contained in the carboxy terminus of the protein.

If the actual positive regulator is indeed a derivative of the Ada protein, it could either be an intact Ada protein that has been modified in some way and undergone a conformational change or a smaller polypeptide arising from a proteolytic cleavage(s) of the Ada product. An obvious modification would be the methylation of the protein resulting from action of its methyl-accepting activity(s) (26). It is even possible that the Ada protein becomes methylated and then is cleaved to generate the active positive regulator.

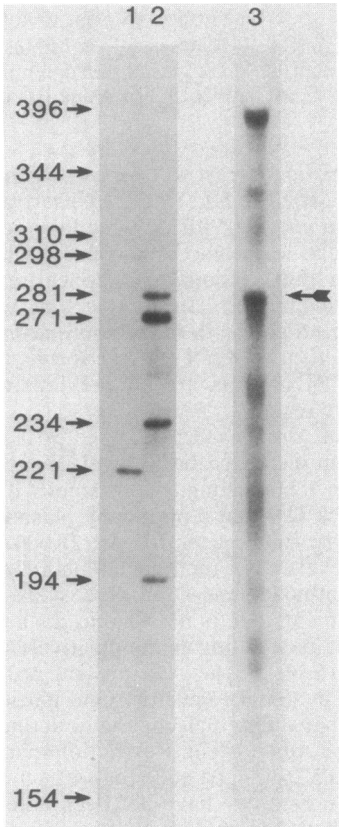

FIG. 8. Mung bean nuclease mapping of the start of *ada* transcription. Lane 1, pBR322 *Eco*RI-*Hin*fI-size standard; lane 2, φX174 *Hae*III-size standard; lane 3, mung bean nuclease digest of the *Hin*dIII-*Acc*I fragment of *ada* DNA hybridized to mRNA from *ada*$^+$ cells containing pGW2607. The arrow on the right indicates the protected DNA fragment of approximately 281 bases in length.

The fact that the potential −35 region of the *ada* promoter has poor homology with the −35 consensus sequence is consistent with the idea that Ada or an Ada-derived product acts as a positively acting factor by stimulating the binding of RNA polymerase to the promoter. The sequence upstream of the −35 region is characterized by a T-rich region of 10 to 15 bases preceded by an A-rich region. This could allow the formation of secondary structure which could be involved in *ada* regulation. Another interesting feature is that just 10 base pairs and 20 base pairs upstream of the −35 region are the sequences TTGC and TTGT, respectively. This would place these sequences on the same side of the helix as the −35 region where interaction of RNA polymerase and the DNA occurs. In the case of the *c*II-activated promoters p_I and p_{RE}, the repeated sequence TTGC also occurs on two consecutive turns of the helix, in this case bracketing the −35 region. It may be that these two proteins, cII and Ada, have some similarities in the mechanism of transcriptional activation.

A possible model for the regulation of the adaptive response of *E. coli* is diagrammed in Fig. 9. The cell produces low levels of Ada protein constitutively. Exposure of a cell to a methylating or ethylating agent leads to alkylation of the DNA, generating various lesions including O^6-methylguanine, O^4-methylthymine, and phosphotriesters. A methyltransferase activity of the Ada protein leads to methylation of the Ada protein. This methylation may cause a conformational change in the protein, causing it to become active in stimulating transcription. Truncation of the Ada protein in

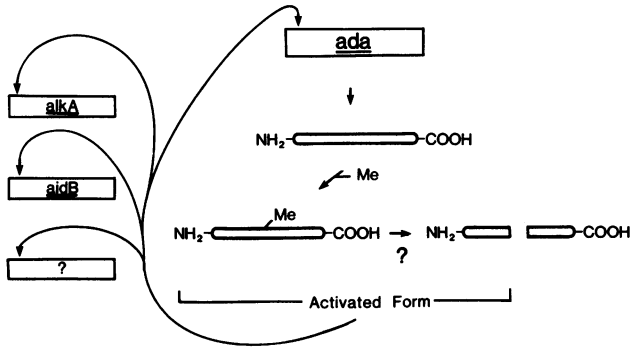

FIG. 9. Model of *ada* regulation. Me, Methyl.

our deletion derivatives may mimic this conformational change. Another possibility is that processing of the Ada protein may occur, generating an amino terminal fragment which then serves as the positive activator for *ada* transcription, as well as for transcription of other *ada*-regulated loci. The extreme susceptibility of the 39,000-dalton Ada protein to degradation to a 19,000-dalton protein during purification (26) suggests that it might be susceptible to some small amount of proteolytic processing or autodigestion in vivo that is sufficient to generate a positive transcriptional activator.

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