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Preferential DNA damage prevention by the *E. coli AidB* gene: a new mechanism for protection of specific genes

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Abstract

aidB is one of four genes of *E. coli* that is induced by alkylating agents and regulated by Ada protein. Three genes (*ada*, *alkA*, and *alkB*) encode DNA repair proteins that remove or repair alkylated bases. However, the role of AidB remains unclear despite extensive efforts to determine its function in cells exposed to alkylating agents. The *E. coli* AidB protein was identified as a component of the protein complex that assembles at strong promoters. We demonstrate that AidB protein preferentially binds to UP elements, AT rich transcription enhancer sequences found upstream of many highly expressed genes, several DNA repair genes, and housekeeping genes. AidB allows efficient transcription from promoters containing an UP element upon exposure to a DNA methylating agent and protects downstream genes from DNA damage. The DNA binding domain is required to target AidB to specific genes preferentially protecting them from alkylation damage. However, deletion of AidB's DNA binding domain does not prevent its antimutagenic activity, instead this deletion appears to allow AidB to function as a cytoplasmic alkylation resistance protein. Our studies identify the role of AidB in alkylating agent exposed cells and suggest a new cellular strategy in which a subset of the genome is preferentially protected from damage by alkylating agents.

Keywords

AidB protein; alkylating agents; DNA protection; UP element

Introduction

The *E. coli aidB* gene is one of four genes of the adaptive response to alkylation damage and is regulated by Ada protein (For review see: [1,2]). Ada protein is a methyltransferase that functions as a transcriptional activator after transfer of a methyl group from DNA to a

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cysteine residue in its amino terminal domain. The alkylation of Ada is stable and activates it to function as a transcriptional activator that induces expression of the *ada-alkB* operon, the *alkA* and *aidB* genes. Ada, AlkA and AlkB are enzymes that repair different alkyl lesions in DNA. Ada removes alkyl groups from O^6alkylguanine, O^4 alkylthymine by transferring them to a cysteine residue in its C-terminal domain [3]. Its amino terminal domain is also a methyltransferase that repairs one stereoisomer of alkylated phosphates by transferring them to a cysteine residue in its N-terminal domain [4,5]. AlkA is a glycosylase that removes 6 different types of alkylated bases from DNA [6] and AlkB is an a-ketoglutarate-Fe(II)-dependent DNA dioxygenase that repairs 1-alkyladenine and 3-alkylcytosine lesions by oxidizing the alkyl groups to unstable derivatives that spontaneously decay restoring the bases to their original state [7,8].

The role for AidB in alkylated cells has remained an unsolved problem. AidB has similarity to the acyl-CoA dehydrogenase family of metabolic enzymes and has weak isovaleryl CoA-dehydrogenase activity [9,10]. AidB was also shown to be a flavoprotein that binds nonspecifically to double stranded DNA. This observation led to the suggestion that it might be a repair enzyme [9]. The recent crystal structure of AidB revealed that its flavin binding site lies within an interior channel, while its DNA binding site is accessible only from the exterior of the protein and is spatially distant from its flavin binding region. Based on these observations, it was suggested that AidB might instead bind and protect DNA by inactivating alkylators before they are able to react with DNA.

In this study we demonstrate that AidB has sequence specific DNA binding activity that targets AidB to UP element-containing genes. We propose the gene specific targeting of AidB protein to be a new cellular strategy that results in preferential protection from alkylation damage and counteracts transcription inhibition by alkylating agents at a subset of the genome, i.e., at genes controlled by promoters with UP elements.

Materials and Methods

Bacterial strains and plasmids

The bacterial strains and plasmids used in this work are listed in Table 1.

Cloning of the *aidB* gene

The *E. coli aidB* gene was amplified from the bacterial chromosome by PCR using the primers listed in Table 2. The amplification product was digested with *Nde*I and HindIII (underlined in Table 2) and cloned into the pET22b (+) vector (Novagen) creating the plasmid pET22b-*aidB*. The resulting expression vector contains a 6X histidine tag to allow protein purification by Ni^{2+} affinity chromatography. Plasmid construction was verified by automated DNA sequencing. The recombinant AidB protein was produced and purified as described previously [11].

Electrophoretic mobility shift assay (EMSA)

EMSA experiments were performed using *rrnB* $P1_{wt}$ as the biotin-labeled DNA probe. Sense and antisense oligonucleotides (Table 2) were annealed by incubation at 95°C for 5 min and successive gradual cooling to room temperature. Purified recombinant AidB was incubated with 20 ng of biotinylated DNA *rrnB* $P1_{wt}$ for 20 min at room temperature in 20 μl of buffer Z (25 mM HEPES pH 7.6, 50 mM KCl, 12.5 mM $MgCl_2$, 1 mM DTT, 20% glycerol, 0.1% triton). Protein-DNA complexes were separated on 5% native polyacrylamide gel (29:1 cross-linking ratio) in 0.5x TBE (45 mM Tris pH 8.0, 45 mM boric acid, 1 mM EDTA) at 200 V (20 V/cm) at room temperature. Afterwards, electrophoretic transfer to a nylon membrane was carried out in 0.5× TBE at 380 mA for 45 min, and the

transferred DNA was cross-linked to the membrane with UV light. After incubation in blocking buffer for 1 h at room temperature, the membrane was incubated with streptavidin-HRP conjugate (Sigma) for 30 min at room temperature. The membrane was washed and visualized with SuperSignal chemiluminescence reagent (Pierce).

Competition experiments were performed using increasing quantities (100×–500×) of either unlabelled *rrnB* $P1_{wt}$, which contains its UP element used as a specific competitor or *rrnB* $P1_{\Delta UP}$ is used as a non-specific competitor.

Construction of fusion plasmids for transcription assays

The *lacZ* gene was amplified from genomic DNA of *E. coli* by PCR using the primers listed in Table 2. The amplification product was digested with *Hind*III and *Xho*I (underlined in Table 2) and cloned into the pET22b (+) vector (Novagen) generating the plasmid pET22b-*lacZ*. The *rrnB* P1 promoter with (*rrnB* $P1_{wt}$) and without its UP element (*rrnB* $P1_{\Delta UP}$), P*leuA* and P*ompF* were amplified by PCR, digested with *Sph*I and *Hind*III and inserted into pET22b-*lacZ* linearized with the same restriction enzymes. The resulting plasmids, designated as listed in Table 1, were verified by automated DNA sequencing.

In vivo transcription assays

MG1655 and MV5924 *E. coli* strains were individually transformed with pET22b-*lacZ*, pET22b-P *rrnB* $P1_{wt}$-*lacZ*, pET22b-P *rrnB* $P1_{\Delta UP}$-*lacZ*, pET22b-P*leuA*-*lacZ* and pET22b-P*ompF*-*lacZ* plasmids. These bacterial cultures grown overnight in LB medium at 30°C, were diluted 1:100 in fresh medium. At an $A_{600\ nm}$ of 0.4, the cultures were divided in four aliquots: one was not supplemented and the other three aliquots were supplemented with MNNG (5μg/ml), ENNG (5μg/ml), MMS 0.04%, respectively. Cellular pellets were collected during the exponential growth phase. β-galactosidase activity from the promoters-*lacZ* fusions was determined by measuring ONPG-hydrolysis, as described by Miller [12] and was compared to the activity obtained using a promoterless *lacZ* gene.

Isolation of plasmid DNA and damage assay

The MG1655 and MV5924 *E. coli* strains bearing pET22b-*lacZ* were grown overnight in LB medium at 30°C; these bacterial cultures were then diluted 1:100 in fresh medium. At an $A_{600\ nm}$ of 0.4, the cultures were divided in four aliquots: one was not supplemented and the other three aliquots were supplemented with MNNG (5μg/ml), ENNG (5μg/ml), MMS 0.04%, respectively. After addition of alkylating agent, the bacterial cells were allowed to grow for 3 h; the plasmid DNA was isolated and served as a probe for the estimation of alkylated bases. The plasmids were divided into 2 aliquots, one of which was treated with the *E. coli* AlkA (a kind gift from Patrick J. O'Brien) and AP Endo (NEB); the other aliquot did not receive further treatment (control). Treatment with AlkA was performed in 70 mM MOPS, pH 7.5, 1 mM EDTA, 1mM DTT, 5% glycerol for 30 min at 37°C, followed by treatment with AP Endo for 1 h at 37°C. Then the samples were subjected to electrophoresis in 0.8% agarose gel for ~1 h at 80 V using 40 mM Tris, pH 7.8, 1 mM EDTA buffer.

Determination of DNA damage in the *lacZ* gene

MG1655 and MV5924 *E. coli* strains were individually transformed with pET22b-*lacZ*, pET22b-P *rrnB* $P1_{wt}$-*lacZ*, pET22b-P *rrnB* $P1_{\Delta UP}$-*lacZ*. These bacterial cultures grown overnight in LB medium at 30°C, were diluted 1:100 in fresh medium. At an $A_{600\ nm}$ of 0.4, the cultures were divided in two aliquots, and one was supplemented with 0.04% MMS to activate the adaptive response. The bacterial cells were allowed to grow for 3h. Then, the plasmids under study were isolated from these bacterial cells and were digested with *Hind*III and *Xho*I to release the *lacZ* fragment. To estimate the presence of alkyl lesions, the DNA

fragments were treated or not with the AlkA and AP Endo proteins. The samples were then subjected to electrophoresis on alkaline agarose gels in 30 mM NaOH, 1 mM EDTA, pH 8 buffer, at 60 V for 3 h at 25°C. The gel was neutralized by soaking in a solution containing 1.5 M NaCl and 1 M Tris-HCl, pH 7.6 for 1 h. Finally, the gel was stained in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) containing SYBR® Gold for 30 min at 25°C and the samples were then analysed for single-strand DNA breaks.

Cell survival and mutagenesis

Cell survival was tested by growing cells to a density of 1–3 × 10^8 cells/ml, treating with MNNG for 30 min, then diluting cells in phosphate buffered saline containing 4% $Na_2S_2O_3$ to inactivate residual MNNG [13], then plating cells on LB plates [13]. Mutation frequencies were determined using DSEM plates [13]. Cultures grown to approximately 3 × 10^8 cells per ml then spread on alkylating agent containing plates and incubated for 3 days at 37°C and Arg^+ mutant colonies counted. Since alkylating agents are relatively unstable, plates containing mutagens were made by first adding alkylators at volumes needed to attain the specified final concentration, then adding 25 ml cooled (50°C) DSEM medium. The plates were cooled for 20 min, then dried by incubation at 37°C for 20 min with covers removed and immediately inoculated. All mutagenesis measurements were made at sub-lethal doses of alkylators using strains deficient in most alkylation specific DNA repair mechanisms *ada-alkB*Δ25:: CmR *alkA1 tag-1 aidBΔ35*::TetR) carrying either the vector pTrc99A, or pTrc99A derivatives that express *aidB* alleles.

Results

To identify proteins that bind the upstream regions of strong promoters, we investigated the protein complex that assembles at the upstream elements of the *rrnB* P1 promoter (see Supplemental Data and Supplemental FigureS1) by comparing proteins that bind to a sequence containing the −35 region and the UP element, an A/T rich enhancer sequence that constitutes the upstream element of many genes [14–16],, but not to a similar sequence lacking the UP element. The presence of the *E. coli* AidB protein among these proteins was unexpected, and suggested a possible regulatory role for AidB in transcription.

AidB preferentially binds DNA containing UP elements

Figure 1 shows that AidB protein binds to DNA containing the *rrnB* $P1_{wt}$ promoter retarding the fragment in an electrophoretic mobility shift experiment (EMSA). When *rrnB* $P1_{wt}$ DNA is used as competitor, there is a rapid loss of binding to the labeled DNA. However, when the rrnB $P1_{\Delta UP}$ promoter lacking the UP element is used as competitor, no inhibition of binding to the labeled *rrnB* $P1_{wt}$ sequence is seen even when it is added at a 500-fold excess. This indicates that AidB protein preferentially binds to rrnB P1 promoter only when the UP element is present. Similar results were also seen when random DNA containing the same base pair composition was used as competitor (see supplemental data). The preferential binding is not restricted to the *rrnB* $P1_{wt}$ promoter, since binding of AidB to its own promoter also requires the presence of the UP element [11].

Functional analysis of AidB during transcription

In order to determine whether the presence of AidB at the *rrnB* P1 promoter might be of biological significance, we tested its effect on transcription from this promoter by *in vivo* transcription assays. In addition, we tested other promoters that differ with respect to presence or absence of an UP element, namely: 1) the *rrnB* p1 promoter with its UP element ($rrnB_{WT}$), 2) the rrnB promoter deleted of its UP element ($rrnB_{\Delta UP}$), 3) P_{leuA}, which lacks an UP element and 4) P_{ompF}, which has an UP element. All promoters were individually fused to a promoterless *lacZ* gene contained in the reporter plasmid pET22b-*lacZ*. Both

MG1655 (wild type) and MV5924 (Δ*aidB*) *E. coli* strains were then transformed with the fusion plasmids and grown in LB medium, either in the absence or in the presence of alkylating agents (MMS, MNNG, ENNG). After 2 hours incubation in the presence or absence of the alkylating agent, β-galactosidase activity was measured during the exponential growth phase. As shown in Fig. 2A, wild type and *aidB* mutant strains not exposed to alkylators showed identical levels of β-galactosidase activity, indicating that the presence of AidB has no effect on transcription in untreated cells experiencing normal growth.

When MG1655 cells are treated with MMS, MNNG, or ENNG, transcription is reduced by roughly 2-fold for all promoters tested. In contrast, the *aidB* mutant showed a much more severe reduction in transcription, especially at the $rrnB_{WT}$ and P_{ompF}, the two UP element containing promoters (Fig. 2). This suggests that the interaction of AidB protein with this class of promoters is of functional significance and that AidB prevents transcription block by alkylation stress. The smaller effect of the *aidB* deletion on the two promoters lacking UP elements is consistent with preferential binding of AidB to this region (Fig. 1). Taken together, these data strongly suggest that AidB is required for high levels of transcription during alkylation stress and that it has a more pronounced effect on transcription from promoters containing an upstream UP element sequence. While it is formally possible that AidB is a transcriptional regulator of UP element containing genes when alkylating agents are present, a more likely explanation, based on its role as part of an alkylation inducible DNA repair response, is that AidB prevents or repairs DNA damage in specific regions of the genome, preferentially preserving the coding capacity of genes transcribed from UP element containing promoters.

AidB reduces the level of alkylation damage in DNA

To test directly if AidB might be able to prevent or repair alkylation damage to DNA the pET22b-*lacZ* plasmid was isolated from wild type and *aidB* mutant cells grown either in the absence or in the presence of alkylators (MMS, MNNG, ENNG) and served as a probe for the estimation of alkylated bases in DNA. The plasmids were divided into 2 aliquots, one was treated with *E. coli* AlkA (a gift from Patrick J. O'Brien) and AP Endonuclease IV (AP Endo) (New England Biolabs); the other aliquot did not receive further treatment and served as a control. The AlkA glycosylase recognizes and removes a wide variety of alkylated bases converting them to abasic sites [6] and AP Endo is an apurinic/apyrimidinic (AP) endonuclease that converts the abasic sites to nicks [17,18]. The combined action of these two enzymes on a damage1d plasmid results in the conversion of the covalently closed circular (supercoiled) DNA to open circular and, if lesions are closely spaced, linear forms. AlkA treated and untreated plasmids were then subjected to electrophoresis on agarose gels and tested for conversion of the supercoiled form to open circular and linear forms. As shown in Fig. 3A, alkyl lesions were not detected in plasmids isolated from bacteria grown in LB medium without the addition of alkylating agents, indicating there is no detectable endogenous damage or non-specific cleavage by these enzymes *in vitro*. When plasmid DNA isolated from wild type cells exposed to alkylating agents was analyzed (Fig. 3B–D), treatment with AlkA and AP Endo did not result in nicking (Lane 2) indicating a lack of DNA damage, but when DNA isolated from the alkylating agent treated *aidB* mutant was analyzed, the supercoiled fraction was completely absent after AlkA/AP Endo treatment and there was an increase in both open circular and linear forms (Lanes 4). These results indicate that the presence of AidB reduces the level of alkylation damage in plasmid DNA. Moreover, AidB protects DNA from all three alkylating agents tested, although they differ in the nature of DNA lesions they produce. Indeed, MNNG methylates and ENNG ethylates DNA more effectively at O^6-G than MMS. In contrast MMS methylates double stranded

DNA primarily at N^7-G and N^3-A sites and in single stranded DNA regions it also methylates N^1-A and N^3-C more efficiently than MNNG [19].

AidB preferentially protects DNA regions downstream of an UP element

Since AidB appears to protect DNA from alkylating agents very effectively (Figure 3), the lack of an alkylation sensitivity phenotype of *aidB* mutants remains a puzzle (see Figure 5 and [13,20]). The observation that AidB protein allows more effective transcription of genes with UP element promoters in the presence of an alkylating agent (Fig. 2) and has a higher affinity for UP element containing promoters [11], suggests that AidB may not protect the entire genome equally and may show a preference for UP element containing regions of DNA. To test this possibility, we analyzed the effect of AidB on alkylation damage *in vivo* in the *lacZ* fragment. In this experiment, we used three plasmids, each carrying the *lacZ* gene fused to three different upstream sequences: the $rrnB_{WT}$ promoter, the $rrnB_{\Delta UP}$ promoter, and a third plasmid carrying a promoterless *lacZ* gene. We investigated whether the presence of AidB might affect the content of alkyl lesions within the *lacZ* sequences. Wild type and *aidB* mutant cells containing these plasmids were first treated with MMS. After isolation, the plasmids were digested with restriction enzymes to release the *lacZ* fragment. The *lacZ* fragment was then purified by agarose gel electrophoresis and divided into two aliquots. One aliquot was treated with the AlkA/AP Endo to nick the DNA at the lesion sites. The samples were then subjected to electrophoresis on alkaline agarose gel to denature DNA and to separate nicked from full-length ssDNA fragments. Since only undamaged strands will run as full-length molecules, the fraction of strands containing lesions can be estimated by comparing the AlkA/AP Endo treated samples with controls not treated with AlkA/AP Endo. Figure 4 shows that the *aidB* mutant cells are not able to protect the *lacZ* gene regardless of the upstream sequence present and all DNA samples are equally sensitive to AlkA/AP Endo treatment (Lanes 8, 10,12). In Wild type cells, essentially all DNA from the $rrnB_{WT}$ bearing plasmid exposed to AlkA/AP Endo remains as full length (Fig. 4, compare Lanes 3 and 4). When *lacZ* is fused to the $rrnB_{\Delta UP}$ promoter, the sample treated with AlkA/AP Endo (Lane 6) shows a clear decrease in the amount of full-length fragments compared with the control DNA not treated with AlkA/AP Endo (Lane 5). Treatment of *lacZ* from the promoterless plasmid with AlkA/AP Endo resulted in an almost complete loss of full-length DNA fragments indicating a higher level of damage (Compare Lanes 1 and 2), thus confirming that the presence of AidB is required for the protection against alkyl damage. It also suggests that transcription itself cannot be solely responsible for damage prevention, since transcription at the onset of damage is identical in wild type and the *aidB* mutant (Fig. 2). Additionally, there is no detectable difference in damage levels when DNA samples isolated from the *aidB* mutant are compared with one another despite the markedly higher level of transcription of the *lacZ* gene transcribed from the *rrnB* $P1_{wt}$ element versus the promoterless *lacZ*. By contrast, plasmids from MMS treated wild type cells show a clear difference in their levels of protection from alkylation damage. Figure 4 shows that *lacZ* fused to the UP element containing the *rrnB* $P1_{wt}$ promoter is well protected from MMS exposure when compared, either to the samples from the *aidB* mutant, or *lacZ* fused to the *rrnB* promoter that lacks the UP element, or has no promoter.

These results demonstrate that AidB preferentially protects the DNA of genes transcribed from UP element-containing promoters to a greater extent than DNA fragments bearing promoters lacking an UP element.

AidB does not confer cellular resistance to alkylation damage

The *aidB* mutant strain MV5924was tested for its sensitivity to MNNG damage. Figure 5 shows that even a complete deletion of *aidB* results in little or no sensitivity to MNNG when compared with its isogenic wild type. The *aidB* mutation also does not affect MMS

sensitivity (Figure S3, supplementary material). These results are consistent with previous observations that mutants carrying insertions in the aidB gene show no increase in sensitivity to alkylating agents [13]. A lack of alkylation sensitivity of the *aidB* deletion mutant is inconsistent with a general DNA damage prevention mechanism, since the ability to prevent damage throughout the genome should result in increased resistance. However, protection of only some DNA regions would prevent damage to only those genes that are targets for AidB protein and is unlikely to have a major effect on overall survival.

Based on the 3-dimensional structure of AidB protein, it has been suggested that AidB is unlikely to function as a DNA repair protein. Instead it may bind DNA and enzymatically inactivate alkylators as they approach the DNA. This notion is based on the observation that the dehydrogenase active site of AidB is spatially distant from its DNA binding face and accessible only from the exterior of the protein [23]. Since a DNA repair protein predicts that the DNA binding domain will be required for activity, we constructed an AidB mutant that lacks the entire DNA binding domain (*aidBΔ440–541*). This mutant has previously been shown to have IVD activity identical to the wild type, but no detectable DNA binding activity [11]. Since wild type AidB protein functions as an antimutator when cells are grown in the presence of MNNG, we tested if the DNA binding deficient AidB mutant protein retains the antimutator activity. Figure 6 shows that the *aidBΔ440–541* mutant allele is as active as the wild type allele in the antimutator assay, indicating that DNA binding is not required for the alkylation resistance function of AidB and suggesting DNA binding instead serves to target AidB to specific genes.

Discussion

The biological role of AidB has long been uncertain. Our data demonstrate that AidB prevents DNA damage by alkylating agents and counteracts the block to transcription that results upon exposure to alkylating agents, especially in genes that are transcribed from promoters containing UP elements. These effects are seen after treatment with MMS, MNNG and ENNG, three alkylating agents that produce different DNA lesions or damage spectra [24]. The result that ENNG damage is also prevented is especially interesting since ENNG lesions are repaired not only the by adaptive response repair system, but also by nucleotide excision repair in *E. coli* [25].

The result that AidB can prevent DNA damage seems inconsistent with the result that loss of AidB function by a complete deletion has little or no effect on MMS or MNNG sensitivity of the mutant strain (Figure 5 and S3). However, effects of an *aidB* mutation on DNA damage and mutagenesis were seen at sublethal doses of alkylating agents (Figures 3–6). Since Ada dependent *aidB* induction is relatively weak compared to that of other adaptive response genes [13,20], it is possible that AidB protein levels are too low to provide adequate protection against lethal doses of alkylating agents. The primary function of AidB may be to protect DNA from the low levels of alkylators that are produced as by-products of stationary phase metabolism [26–29], a possibility that is consistent with the observation that *aidB* is induced and expressed at elevated levels in stationary phase [10,30,31].

The result that the AidB protein specifically binds to DNA sequences that include the UP element [11] (see also Fig. 1), suggests that the lack of increased sensitivity to high levels of alkylating agents in the *aidB* mutant (Fig. 5) may also be due to the fact that AidB only protects a subset of the genome, leaving other genes, including essential ones, exposed to DNA damage. The *aidB* mutant phenotype is consistent with targeted repair or damage prevention and is analogous to the effect seen in strains that lack the ability to carry out transcription-coupled repair (TCR) of UV damage, the only other gene specific repair or damage prevention system currently known. A TCR deficient *mfd* mutant shows only a

modest decrease in cellular resistance to UV, but a dramatic reduction in the rate at which repair of active genes occurs [32–34]. Thus, the AidB prevention mechanism appears to be a cellular strategy to preferentially protect a subset of genes. In this case the genes include ones important for basic metabolic processes and key DNA repair genes. AidB is targeted towards genes whose promoters have upstream UP elements. This includes genes such as most of the ribosomal RNA genes and many tRNA genes as well as several key DNA repair genes required for recovery from alkylation damage such as *recA, polA, sulA, recN* the *ada-alkB* operon, and *aidB* itself [14,35,36].

The presence of a functional *aidB* gene protects UP element genes from alkylation damage and results in more efficient transcription in the presence of alkylating agents. *lacZ* fused to the two UP element containing *rrn* and *ompF* promoters are transcribed 10- and 6- fold more efficiently in the presence of an alkylating agent than *lacZ* fused to an *rrn* promoter whose UP element has been deleted, or the *leuA* promoter, which has no UP element. Although it is possible that AidB has regulatory effects on these genes, a lower level of template damage should clearly contribute to the transcription efficiency.

Promoters lacking an UP element, and thus not efficiently bound by AidB protein still show a slightly higher level of transcription in wild type versus *aidB* mutants upon alkylation (2.2 and 2-fold enhancement for $rrnB_{\Delta UP}$ and P_{leu}, Fig. 2). It is unclear if this *aidB*-dependent enhancement of transcription in the presence of an alkylating agent represents some direct protection by *aidB*, or is an indirect effect of the elevated levels of ribosomes, tRNAs and possibly other components of the translational machinery that are transcribed at a higher levels in the *aidB*+ strain under these conditions. The observation that the protection of *lacZ* fused to the *rrn* promoter lacking an UP element and the observation that plasmid DNA shows better protection in wild type than in an *aidB* mutant strain (Fig. 4), suggests that there may be some general protection resulting from the presence of *aidB*, especially when it is highly expressed, or induced for a long period of time as in these experiments. Under these conditions AidB may initially protect the genes preferentially targeted, followed by other parts of the genome if AidB protein accumulates to sufficiently high levels. The precise mechanism of action of AidB remains to be determined, though it is possible that it provides protection of DNA adjacent to its preferred binding site, either by simply inactivating alkylating agents and reducing the local concentration, or by polymerizing into multimers that extend from the initial binding site. In the latter case, it is likely to protect both by shielding the DNA and by inactivating alkylators.

However, the MNNG resistance resulting from expression of the DNA binding deficient *aidB* mutant protein, *AidB(Δ440–541)* indicates that the mutant lacking DNA binding activity still functions to prevent alkylation mutagenesis. This observation makes it unlikely that AidB functions by simply binding and coating the DNA, thus preventing access by alkylators. The ability of the DNA binding defective AidB protein to prevent mutagenesis suggests that AidB is not a DNA repair protein, since DNA repair would be inhibited by lack of DNA binding activity. Instead, AidB is more likely to function to prevent damage by detoxifying alkylating agents, which could reduce DNA alkylation even in the absence of DNA binding activity by reducing the intracellular concentration of active alkylators. A role for AidB in alkylating agent detoxification is also consistent with earlier work on AidB and analysis of the structural features of the protein [10,23]. Determination of the precise mechanism by which AidB may inactivate alkylating agents requires further work to examine the chemistry of the hypothetical process.

It is unclear how widespread preferential damage prevention mechanisms such as AidB are, if other prokaryotes and eukaryotes have similar damage prevention proteins, or if the strategy of preferential DNA protection extends to mechanisms that prevent damage by

other agents. In *E. coli* the *dps* gene is highly expressed in stationary phase and prevents oxidative DNA damage. Unlike AidB, however, this protein is produced at very high levels and appears to function as a genome wide protective protein. It is unclear if it may also have a preference for specific sequences when it is expressed at lower levels [37].

Highlights

In this manuscript we demonstrate that aidB binds preferentially to UP element containing genes and preferentially protects them from alkylation damage. Additionally, the reduction in transcription normally seen in the presence of alkylators is much greater in the aidB mutant than in wild type, indicating that transcription capacity is preserved when aidB is functional. We further demonstrate that aidB overexpression reduces mutagenesis and that this does not require the DNA binding domain, suggesting that it does not repair DNA, but prevents damage from occurring.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Volkert MR, Landini P. Transcriptional responses to DNA damage. Curr. Opin. Microbiol. 2001; 4:178–185. [PubMed: 11282474]
2. Landini P, Volkert MR. Regulatory responses of the adaptive response to alkylation damage: a simple regulon with complex regulatory features. J. Bacteriol. 2000; 182:6543–6549. [PubMed: 11073893]
3. McCarthy TV, Karran P, Lindahl T. Inducible repair of O-alkylated DNA pyrimidines in *Escherichia coli*. EMBO J. 1984; 3:545–550. [PubMed: 6370685]
4. Teo I, Sedgwick B, Kilpatrick MW, McCarthy TV, Lindahl T. The intracellular signal for induction of resistance to alkylating agents in *E. coli*. Cell. 1986; 45:315–324. [PubMed: 3009022]
5. Nakabeppu Y, Sekiguchi M. Regulatory mechanisms for induction of synthesis of repair enzymes in response to alkylating agents: Ada protein acts as a transcriptional regulator. Proc. Natl. Acad. Sci. USA. 1986; 83:6297–6301. [PubMed: 3529081]
6. O'Brien PJ, Ellenberger T. The *Escherichia coli* 3-methyladenine DNA glycosylase AlkA has a remarkably versatile active site. J Biol Chem. 2004; 279:26876–26884. [PubMed: 15126496]
7. Trewick SC, Henshaw TF, Hausinger RP, Lindahl T, Sedgwick B. Oxidative demethylation by *Escherichia coli* AlkB directly reverts DNA base damage. Nature. 2002; 419:174–178. [PubMed: 12226667]
8. Falnes PO, Johansen RF, Seeberg E. AlkB-mediated oxidative demethylation reverses DNA damage in Escherichia coli. Nature. 2002; 419:178–182. [PubMed: 12226668]
9. Rohankhedkar MS, Mulrooney SB, Wedemeyer WJ, Hausinger RP. The AidB component of the Escherichia coli adaptive response to alkylating agents is a flavin-containing, DNA-binding protein. J Bacteriol. 2006; 188:223–230. [PubMed: 16352838]
10. Landini P, Hajec LI, Volkert MR. Structure and transcriptional regulation of the Escherichia coli adaptive response gene aidB. J. Bacteriol. 1994; 176:6583–6589. [PubMed: 7961409]
11. Rippa V, Amoresano A, Esposito C, Landini P, Volkert M, Duilio A. Specific DNA binding and regulation of its own expression by the AidB protein in Escherichia coli. J Bacteriol. 2010; 192:6136–6142. [PubMed: 20889740]

12. Miller, JH. Experiments in Molecular Genetics. Cold Spring Harbor Laboratories, Cold Spring Harbor; 1972.
13. Volkert MR, Nguyen DC. Induction of specific *Escherichia coli* genes by sublethal treatments with alkylating agents. Proc. Natl. Acad. Sci. USA. 1984; 81:4110–4114. [PubMed: 6330740]
14. Gourse RL, Ross W, Gaal T. UPs and downs in bacterial transcription initiation: the role of the alpha subunit of RNA polymerase in promoter recognition. Mol Microbiol. 2000; 37:687–695. [PubMed: 10972792]
15. Estrem ST, Gaal T, Ross W, Gourse RL. Identification of an UP element consensus sequence for bacterial promoters. Proc. Natl. Acad. Sci. USA. 1998; 95:9761–9766. [PubMed: 9707549]
16. Ross W, Gosink KK, Salomon J, Igarashi K, Zou C, Ishihama A, Severinov K, Gourse RL. A third recognition element in bacterial promoters: DNA binding by the a subunit of RNA polymerase. Science. 1993; 262:1407–1413. [PubMed: 8248780]
17. Ljungquist S, Lindahl T. Relation between Escherichia coli endonucleases specific for apurinic sites in DNA and exonuclease III. Nucleic Acids Res. 1977; 4:2871–2879. [PubMed: 333397]
18. Ljungquist S. A new endonuclease from Escherichia coli acting at apurinic sites in DNA. J Biol Chem. 1977; 252:2808–2814. [PubMed: 16002]
19. Singer B. The chemical effects of nucleic acid alkylation and their relation to mutagenesis and carcinogenesis. Prog Nucleic Acid Res Mol Biol. 1975; 15:219–284. [PubMed: 237307]
20. Volkert MR, Nguyen DC, Beard KC. *Escherichia coli* gene induction by alkylation treatment. Genetics. 1986; 112:11–26. [PubMed: 3080354]
21. Volkert MR. Adaptive response of *Escherichia coli* to alkylation damage. Environ. Molec. Mutagen. 1988; 11:241–255. [PubMed: 3278898]
22. Lindahl T, Sedgwick B, Sekiguchi M, Nakabeppu Y. Regulation and expression of the adaptive response to alkylating agents. Annu. Rev. Biochem. 1988; 57:133–157. [PubMed: 3052269]
23. Bowles T, Metz AH, O'Quin J, Wawrzak Z, Eichman BF. Structure and DNA binding of alkylation response protein AidB. Proc Natl Acad Sci U S A. 2008; 105:15299–15304. [PubMed: 18829440]
24. Singer, B.; Grunberger, D. Molecular Biology of Mutagens and Carcinogens. New York: Plenum Press; 1983.
25. Bonanno K, Wyrzykowski J, Chong W, Matijasevic Z, Volkert MR. Alkylation resistance of E. coli cells expressing different isoforms of human alkyladenine DNA glycosylase (hAAG). DNA Rep. 2002; 1:507–517.
26. Rebeck GW, Samson L. Increased spontaneous mutation and alkylation sensitivity of Escherichia coli strains lacking the ogt O6-methylguanine DNA repair methyltransferase. J. Bacteriol. 1991; 173:2068–2076. [PubMed: 2002008]
27. Sedgwick B. Nitrosated peptides and polyamines as endogenous mutagens in O6-alkylguanine-DNA alkyltransferase deficient cells. Carcinogenesis. 1997; 18:1561–1567. [PubMed: 9276631]
28. Taverna P, Sedgwick B. Generation of edogenous methylating agents by nitrosation in *Escherichia coli*. J. Bacteriol. 1996; 178:5105–5111. [PubMed: 8752326]
29. Sedgwick B. Oxidation of methylhydrazines to mutagenic methylating derivatives and inducers of the adaptive response of Escherichia coli to alkylation damage. Cancer Res. 1992; 52:2693–2697.
30. Landini P, Hajec LI, Nguyen LH, Burgess RR, Volkert MR. The leucine-responsive protein (Lrp) acts as a specific repressor for s^S-dependent transcription of the *Escherichia coli aidB* gene. Molec. Microbiol. 1996; 20:947–955. [PubMed: 8809748]
31. Volkert MR, Hajec LI, Matijasevic Z, Fang F, Prince R. Induction of the *Escherichia coli aidB* gene under oxygen limiting conditions requires a functional *rpoS (katF)* gene. J. Bacteriol. 1994; 176:7638–7645. [PubMed: 8002588]
32. Selby CP, Sancar A. Mechanisms of transcription-repair coupling and mutation frequency decline. Microbiol Rev. 1994; 58:317–329. [PubMed: 7968917]
33. Selby CP, Sancar A. Molecular Mechanism of Transcription-Repair Coupling. Science. 1993; 260:53–58. [PubMed: 8465200]
34. Selby CP, Witkin E, Sancar A. *Escherichia coli mfd* mutant deficient in "mutation frequency decline" lacks strand-specific repair: *in vitro* complementation with purified coupling factor. Proc. Natl. Acad. Sci. USA. 1991; 88 11574-11478.

35. Ross W, Aiyar SE, Salomon J, Gourse RL. Escherichia coli promoters with UP elements of different strengths: modular structure of bacterial promoters. J Bacteriol. 1998; 180:5375–5383. [PubMed: 9765569]

36. Landini P, Volkert MR. RNA polymerase a subunit binding site in positively controlled promoters: a new model for RNA polymerase/promoter interaction and transcriptional activation in the E. coli ada and aidB genes. EMBO J. 1995; 14:4329–4335. [PubMed: 7556074]

37. Martinez A, Kolter R. Protection of DNA during oxidative stress by the nonspecific DNA-binding protein Dps. J. Bacteriol. 1997; 179:5188–5194. [PubMed: 9260963]

38. Jensen KF. The Escherichia coli K-12 "wild types" W3110 and MG1655 have an rph frameshift mutation that leads to pyrimidine starvation due to low pyrE expression levels. J Bacteriol. 1993; 175:3401–3407. [PubMed: 8501045]

39. Murphy KC, Campellone KG. Lambda Red-mediated recombinogenic engineering of enterohemorrhagic and enteropathogenic E. coli. BMC Mol Biol. 2003; 4:11. [PubMed: 14672541]

40. Volkert MR. Altered induction of the adaptive response to alkylation damage in *Escherichia coli recF* mutants. J. Bacteriol. 1989; 171:99–103. [PubMed: 2536670]

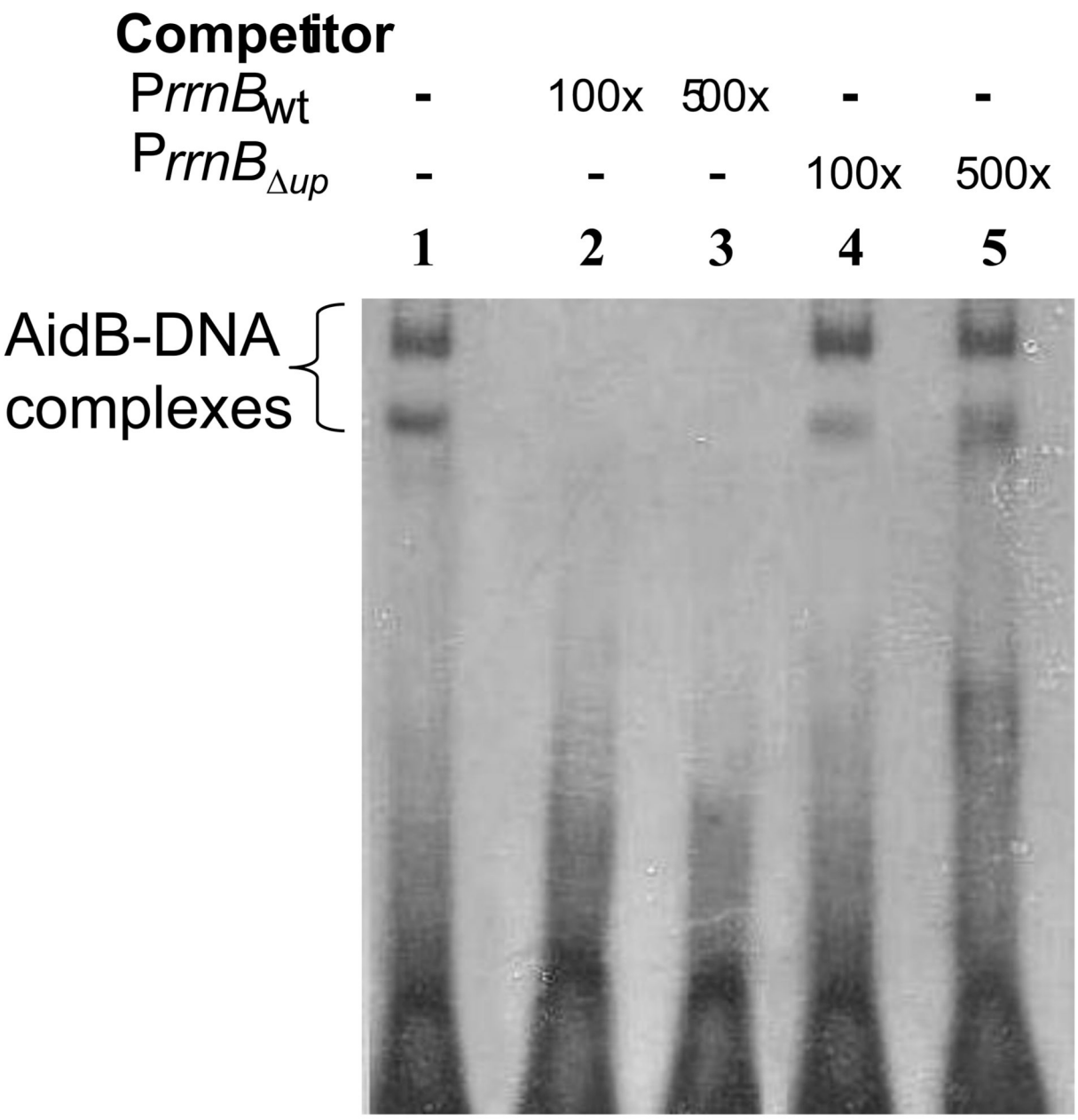

Figure 1. Gel retardation

Experiments were performed by incubating the AidB protein with *rrnB* $P1_{wt}$; competitors were included as indicated. Lane 1: AidB protein incubated with *rrnB* $P1_{wt}$. Lanes 2–3: Competition assay with *rrnB* $P1_{wt}$ (100×–500×) as specific competitor. Lanes 4–5: Competition assay with *rrnB* $P1_{\Delta UP}$ as non specific competitor (100×–500×).

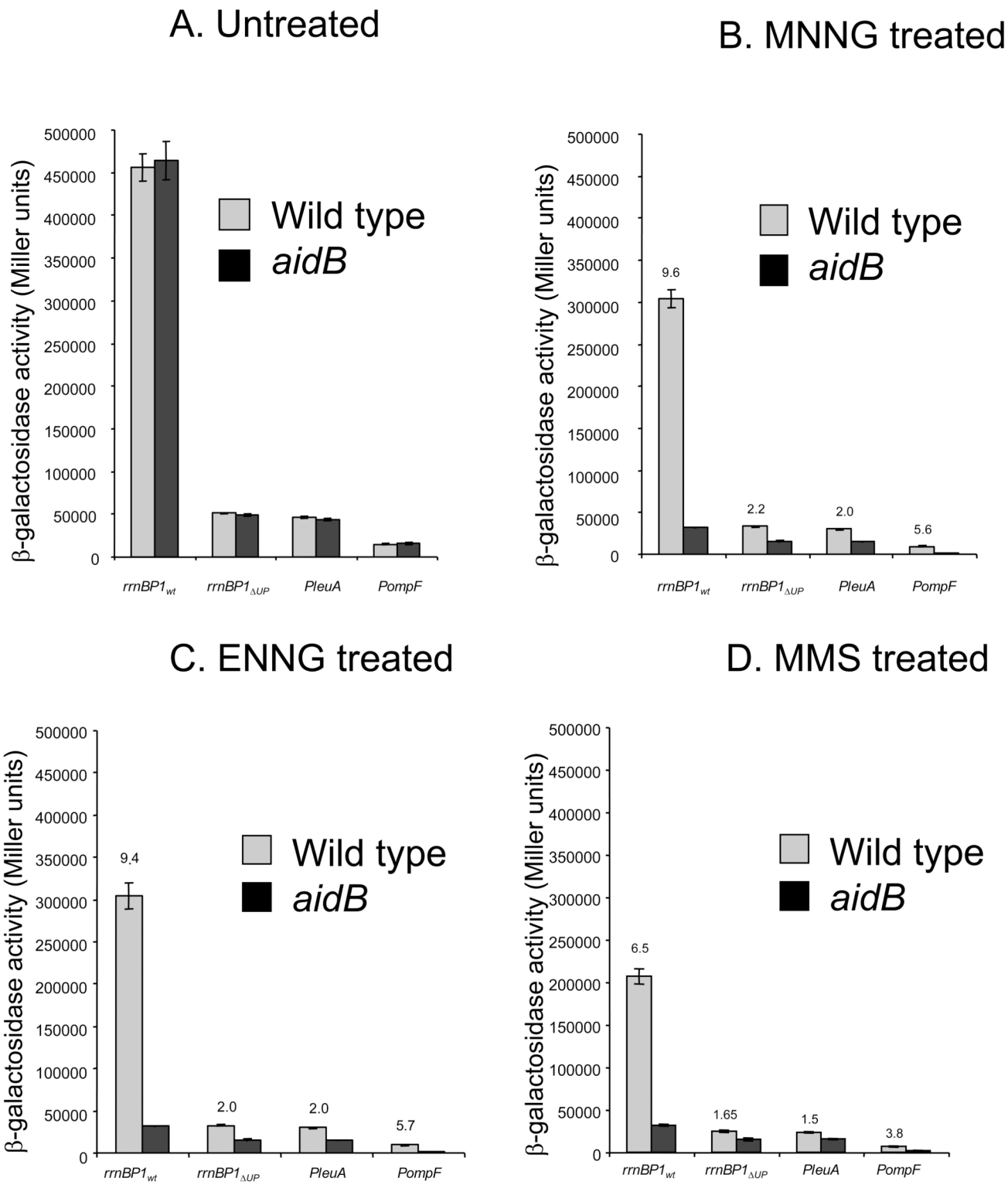

Figure 2. *In vivo* transcription of *lacZ* fused to different promoters

The pET22b-P *rrnB* $P1_{wt}$-*lacZ*, pET22b-P *rrnB* $P1_{\Delta UP}$-*lacZ*, pET22b-P*leu*A-*lacZ* and pET22b-P*omp*F-*lacZ* plasmids were individually introduced into MG1655 (wild type) and

MV5924 (Δ*aidB*) *E. coli* strains and the specific activity of β-galactosidase was determined in the absence (A) and in the presence of MNNG (5μg/ml) (B), ENNG (5μg/ml) (C), MMS 0.04% (D. The activities of promoters are reported in Miller units; the activity obtained using a promoterless *lacZ* gene was subtracted. Numbers above bars refer to the ratio of the β-galactosidase activity of the promoter measured in the wild type cells to the activity of that same promoter in the *aidB* mutant strain. Means and standard deviations have been calculated from four independent assays.

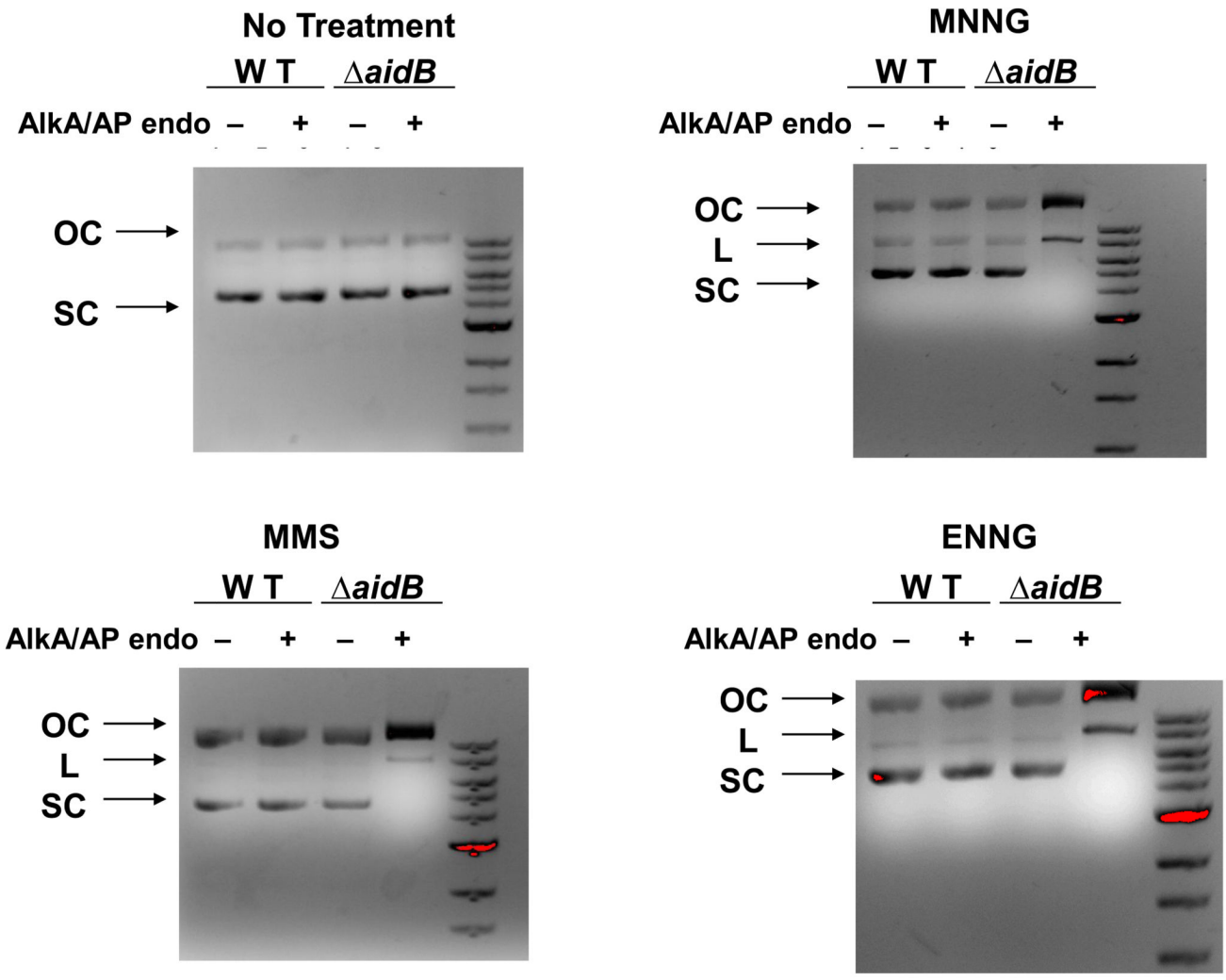

Figure 3. Plasmid damage assay

The pET22b-*lacZ* DNA was isolated from wild type (Lanes 1–2) and Δ*aidB* (Lanes 3–4) *E. coli* strains grown in the absence (A) or in the presence of MNNG (5μg/ml) (B), MMS 0.04% (C), ENNG (5μg/ml) (D), digested (Lanes 2, 4) or not (Lanes 1, 3) with AlkA and AP Endo and subjected to agarose gel electrophoresis. Lane 5, 1 Kb DNA marker (NEB). OC: open circular; L: linear; SC: supercoiled.

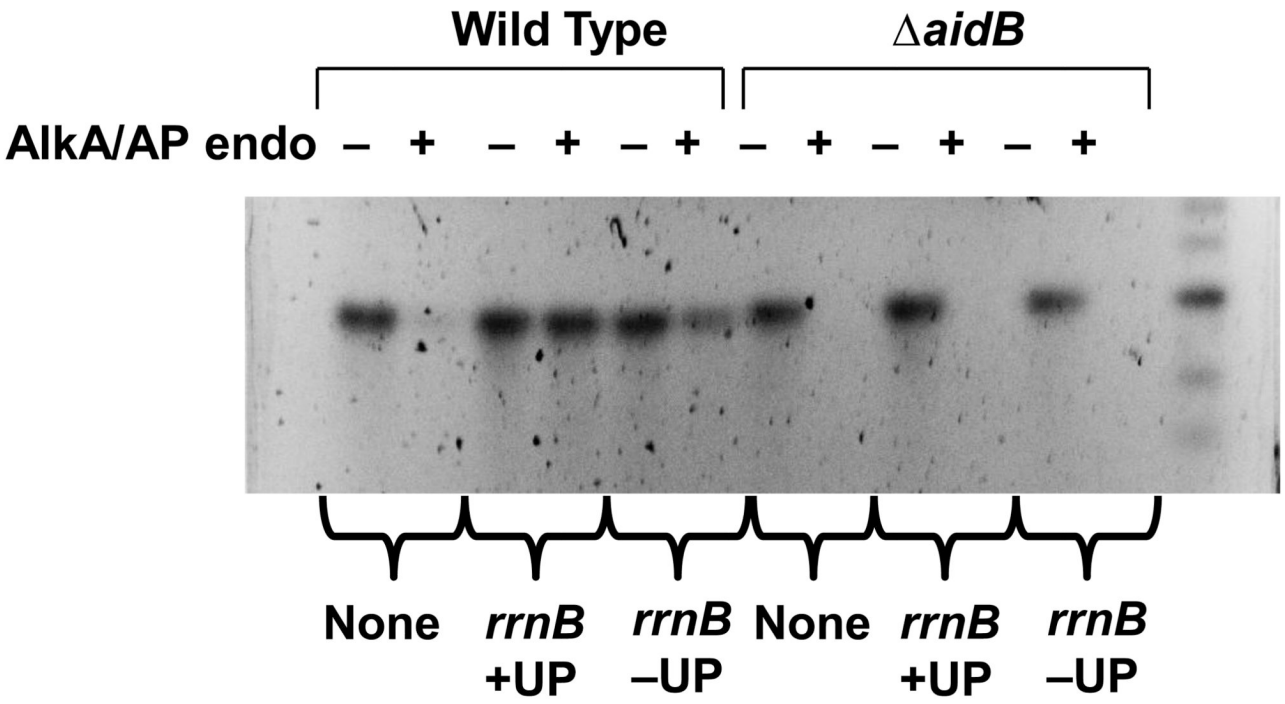

Figure 4. AidB preferentially protects DNA regions containing an UP element

The pET22b-*lacZ*, pET22b-P *rrnB* $P1_{wt}$-*lacZ* and pET22b-P *rrnB* $P1_{\Delta UP}$-*lacZ* plasmids were isolated from wild type (Lanes 1–6) and Δ*aidB* (Lanes 7–12) *E. coli* strains grown in the presence of MMS 0.04% and digested to release and purify the *lacZ* fragment. The lacZ containing DNA fragments were untreated (Lanes 1, 3, 5, 7, 9, 11) or treated (Lanes 2, 4, 6, 8, 10, 12) with AlkA and AP Endo and subjected to electrophoresis on alkaline agarose gel. Lanes 1, 2, 7, 8: *lacZ* lacking a promoter; Lanes 3, 4, 9, 10: *lacZ* fused to the *rrnB* $P1_{wt}$ promoter with its UP element; Lanes 5, 6, 11, 12: *lacZ* fused to the *rrnB* $P1_{\Delta UP}$ promoter without its UP element; Lane 13: 1Kb DNA marker (NEB).

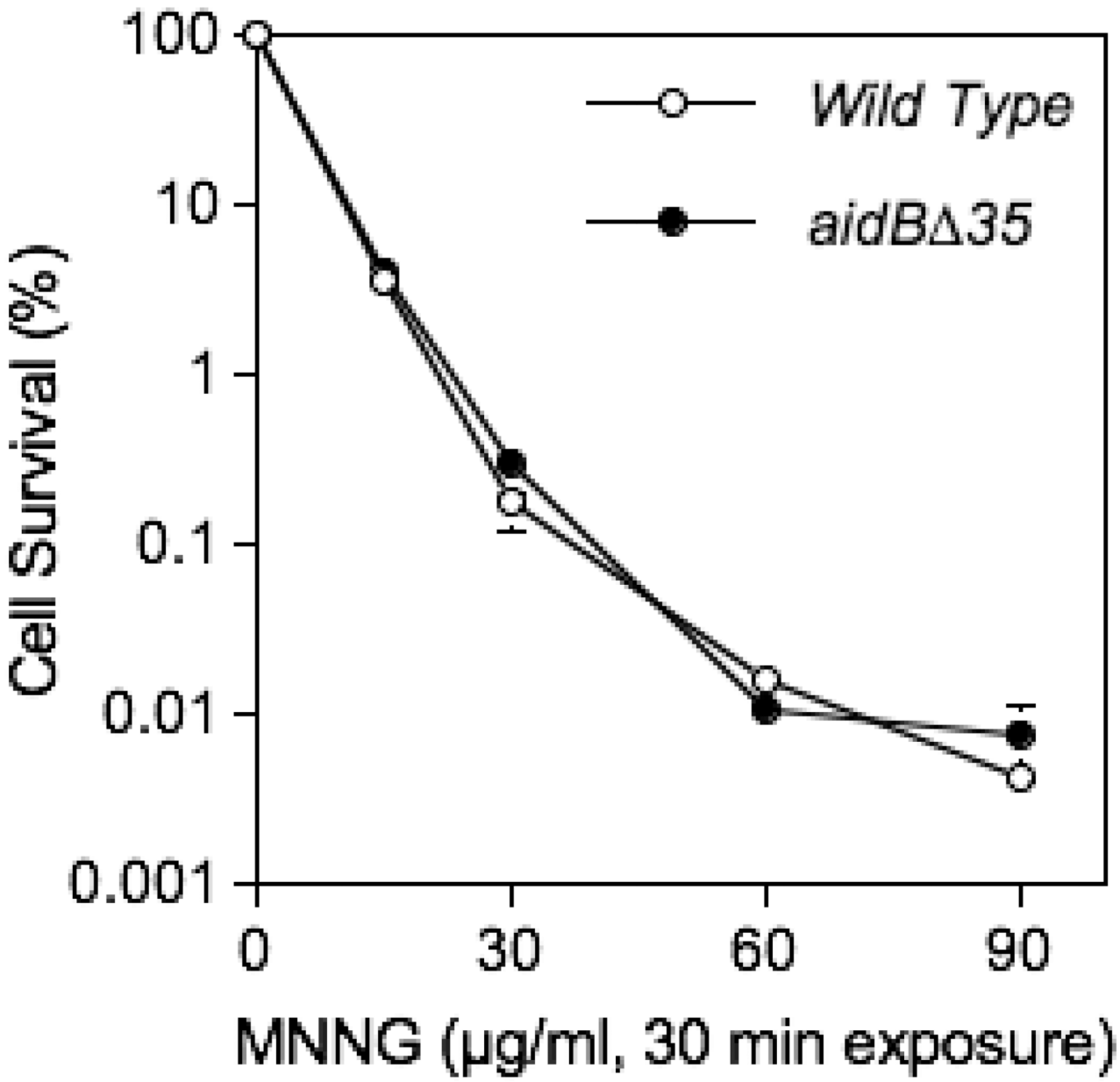

Figure 5. Alkylating agent sensitivity of strains deficient in adaptive response genes

Cells were grown to a density of approximately 1–3 × 10^8 cells/ml and treated with MNNG as indicated. Wild type (MG1655), *aidBΔ35::TetR* (MV5924). Each data point represents 3 (15, 90 μg/ml) or 6 repetitions (0, 30 and 60 μg/ml). Standard errors of the mean are shown where visible beyond the data point.

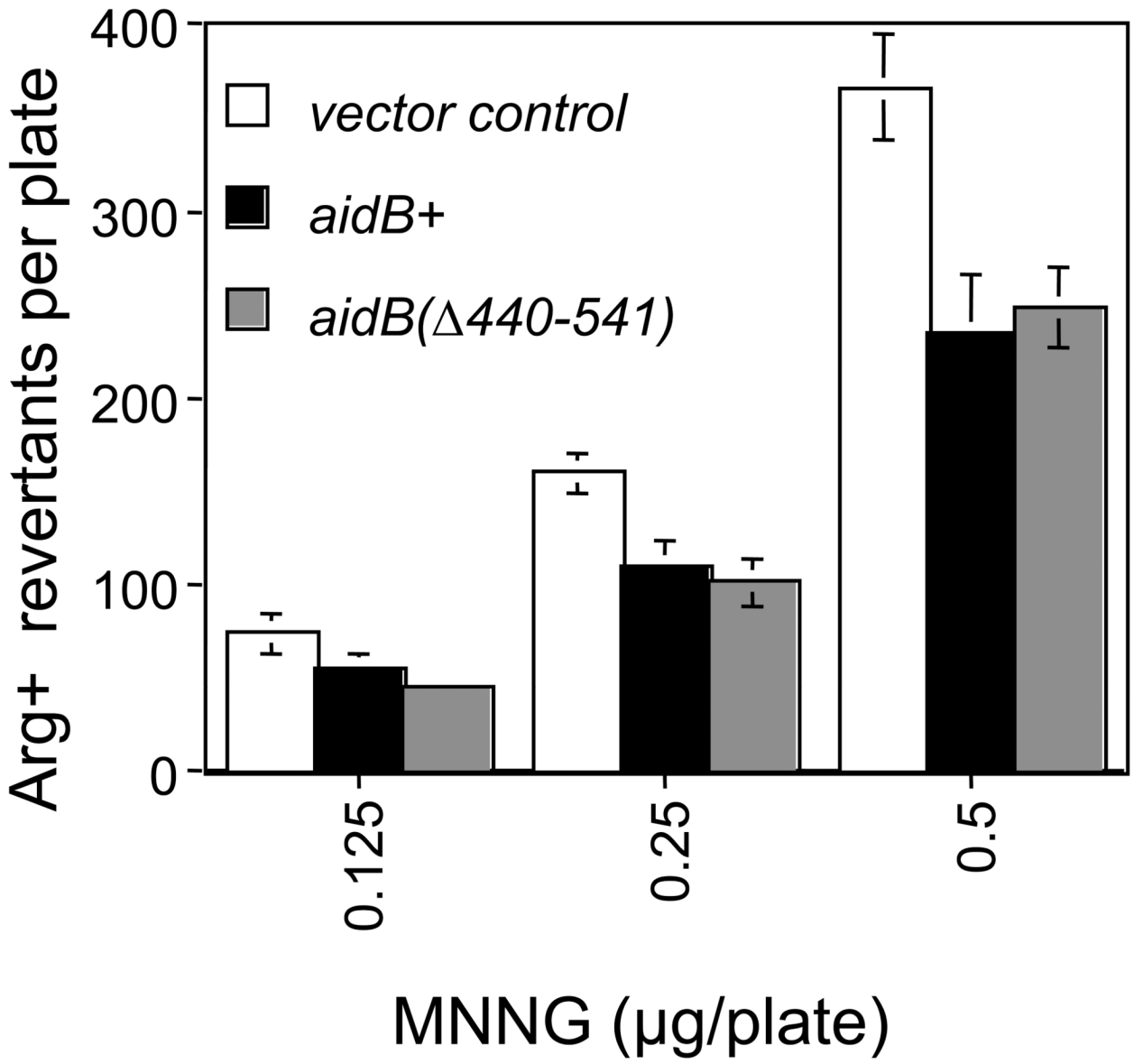

Figure 6. Antimutagenic activity of wild type and AidB(Δ440–541)

Cells were grown to a density of approximately 3×10^8 cells per ml and undiluted cultures were spread on DSEM plates ± alkylating agents and mutation frequencies determined. All mutagenesis experiments were conducted at sublethal doses of alkylating agents using strains deficient in most alkylation specific DNA repair mechanisms: *aidB*$^+$ (MV6782, *ada-alkB*Δ25:: CmR *alkA1 tag-1 aidBΔ35*::TetR/pTrc99A-AidB$^+$); *aidBΔ440–541* (MV6790, *ada-alkB*Δ25::CmR *alkA1 tag-1 aidBΔ35*::TetR/pTrc99A-AidBΔ440–451); vector control (MV6780, *ada-alkB*Δ25::CmR *alkA1 tag-1 aidBΔ35*::TetR/pTrc99A).

Table 1

Bacterial strains and plasmids

Strains/plasmids	Description	Reference or source
Strains		
MG1655	wild-type; F^- λ^- *ilvG rfb50 rph1*	[38]
MV5924	*aidBΔ::TetR* derivative of MG1655 in which the *aidB* gene is replaced by a tetracycline resistance cassette using the methods of Murphy and Campellone [39]	[11]
MV6774	*ada-alkB*Δ25::CmR *alkA1 tag-1 aidBΔ35*::TetR derivative of MV1161 [13]	This study
MV6780	*ada-alkB*Δ25::CmR *alkA1 tag-1 aidBΔ35*::TetR/pTrc99A)	This study
MV6782	*ada-alkB*Δ25::CmR *alkA1 tag-1 aidBΔ35*::TetR/pMV435 (pTrc99A-AidB$^+$)	This study
MV6790	*ada-alkB*Δ25::CmR *alkA1 tag-1 aidBΔ35*::TetR/pMV1526 (pTrc99A-AidBΔ440– 451)	This study
Plasmids		
pET22b(+)	carries an N-terminal *pelB* signal sequence for potential periplasmic localization, plus an optional C-terminal His-tag sequence	This study
pET22b-*aidB*	pET22bΔ(*Nde*I-*Hind*III)Ω(*aidB* gene)	This study
pET22b-*lacZ*	pET22bΔ(*Hind*III-*Xho*I)Ω(*lacZ* gene)	This study
pET22b-P*rrnB*(+UP)-*lacZ*	pET22b-*lacZΔ*(*Sph*I-*Hind*III)ΩP*rrnB*(+UP)	This study
pET22b-P*rrnB*(−UP)-*lacZ*	pET22b-*lacZΔ*(*Sph*I-*Hind*III)ΩP*rrnB*(−UP)	This study
pET22b-P*leuA*-*lacZ*	pET22b-*lacZΔ*(*Sph*I-*Hind*III)ΩP*leuA*	This study
pET22b-P*ompF*-*lacZ*	pET22b-*lacZΔ*(*Sph*I-*Hind*III)ΩP*ompF*	This study
pTrc99A	E. coli expression vector	[10]
pMV435	pTrc99A-AidB$^+$	[10]
pMV1526	pTrc99A-AidBΔ440–451	This study

Table 2

Oligonucleotides

aidB Fw	5'-ATACATATGGTGCACTGGCAAACTCA-3'
aidB Rv	5'-ATAAAGCTTTAACACACACACTCCCC-3'
lacZ Fw	5'-TGTAAGCTTATAACAATTTCACACAGGAA-3'
lacZ Rv	5'-CGGCTCGAGTTATTTTTGACACCAGAC-3'
rrnB P1(+UP) Fw	5'-TAAAGCATGCTCAGAAAATTATTTTAAATTTC-3'
rrnB P1Rv	5'-ATTAAGCTTAGGAGAACCCCGCTGA-3'
rrnB P1(−UP) Fw	5'-ATTTGCATGCCCTCTTGTCAGGCC-3'
P*leuA* Fw	5'-ATAAGCATGCGGGACGTTTTTATTGCG-3'
P*leuA* Rv	5'-AAGAAGCTTGATAAAGCGAACGATGTG-3'
P*ompF* Fw	5'-ATTTGCATGCACAAAGTTCCTTAAATTTTA-3'
P*ompF* Rv	5'-TAAAAGCTTAATAAAAATTTACGGAACTATTG-3'
rrnB P1(+UP) Fw bio (emsa)	5'-bio-AGAAAATTATTTTAAATTTCCTCTTGTCAGGCCGGAATAAC TC CCTATAAT-3'
rrnB P1(+UP) Rv (emsa)	5'-ATTATAGGGAGTTATTCCGGCCTGACAAGAGGAAATTTAA AATAA TTTTCT-3'
rrnB P1(+UP) Fw (emsa)	5'-AGAAAATTATTTTAAATTTCCTCTTGTCAGGCCGGAATAAC TCCCT ATAAT-3'
rrnB P1(−UP) Fw (emsa)	5'-CCTCTTGTCAGGCCGGAATAACTCCCTATAAT-3'
rrnB P1(−UP) Rv (emsa)	5'-ATTATAGGGAGTTATTCCGGCCTGACAAGAGG-3'