A region of the Ada DNA-repair protein required for the activation of *ada* transcription is not necessary for activation of *alkA*

(DNA methyltransferase/adaptive response/transcriptional activation)

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ABSTRACT   The adaptive response of *Escherichia coli* protects cells against the mutagenic and toxic effects of alkylating agents. This response is controlled by the Ada protein, which not only functions as the transcriptional activator of the *ada* and *alkA* genes but also possesses two DNA methyltransferase activities. Ada is converted into an efficient transcriptional activator by transferring a methyl group from a DNA methylphosphotriester to its own Cys-69 residue and then binds to a DNA sequence (the Ada box) present in both the *ada* and *alkA* promoters. Although the Ada protein initially appeared to regulate the *ada* and *alkA* genes in a similar fashion, our studies show that the wild-type Ada protein and its truncated derivatives can differentially regulate *ada* and *alkA* transcription. *In vivo*, lower levels of wild-type methylated Ada are needed to activate *ada* transcription than *alkA* transcription. In cells exposed to alkylating agents, the N-terminal half of Ada, which contains the DNA-binding domain, is sufficient for efficient activation of *alkA*, but not *ada*, transcription. Moreover, truncated derivatives containing 80–90% of Ada are extremely strong constitutive activators of *ada* but are only inducible activators of *alkA* transcription. These results suggest that the mechanism by which Ada activates *ada* transcription differs from that by which it activates *alkA* transcription.

The adaptive response of *Escherichia coli* (1), an induced resistance to mutagenesis and killing by alkylating agents such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), is regulated by the Ada protein. Ada is a remarkable 39-kDa protein that not only functions as a transcriptional activator (for reviews, see refs. 2 and 3) but also has two DNA-repair activities. One activity resides in the C-terminal domain of Ada and irreversibly transfers a methyl group from the premutagenic lesions O^6-methylguanine or O^4-methylthymine to the Cys-321 residue in Ada (4–6). The other activity resides in the N-terminal domain of Ada and removes a methyl group from one of the diastereoisomers of DNA methylphosphotriesters and irreversibly transfers it to the Cys-69 residue within Ada (7–9).

In vitro experiments suggest that the transfer of a methyl group to Cys-69 of Ada during the latter type of repair converts Ada into an efficient transcriptional activator of the *ada alkB* operon and of the *alkA* gene (11, 12). The *alkA* gene encodes a glycosylase that removes the cytotoxic lesion 3-methyladenine and certain other lesions (10). The methylated Ada protein, in contrast to the unmethylated protein, binds efficiently to an asymmetric sequence, termed the Ada box, that appears upstream of the *ada* and *alkA* promoters (12). *In vitro*, RNA polymerase can bind to the *ada* and *alkA* promoters only when the Ada protein is present.

Thus, Ada appears to be a one-component system that is covalently modified in response to an environmental signal to become a transcriptional activator. However, we recently reported results suggesting that there may be an additional level of complexity to this system. In that study, we showed that alterations of the C-terminal domain of the Ada protein influenced its inducibility, specificity, and strength as a transcriptional activator (13). In particular, the experiments suggested that Ada might be capable of differentially regulating the *ada alkB* operon and the *alkA* gene. In this study, we have explored the issue of the differential activation of *ada* and *alkA* by Ada and have determined whether deletions of portions of the Ada C-terminal domain can alter its properties as a transcriptional activator. Our results indicate that the mechanism by which Ada activates *ada* transcription differs in some significant respect from the way in which Ada activates *alkA* transcription.

MATERIALS AND METHODS

Oligonucleotide-Directed Mutagenesis and Plasmid Constructions. Oligonucleotide-directed *in vitro* mutagenesis was done by using the Amersham system. Mutagenized *ada* fragments were sequenced to ensure that no other mutations were introduced. pGW2607, carrying the *ada alkB* operon, was digested with *Bam*HI and *Sma* I, treated with the Klenow fragment of DNA polymerase I, and religated to generate pDS400. An oligonucleotide (5'-TGTGGCTTTTT-TCATAGCTGTTTCCTG-3') was prepared that directs deletion of the *ada* promoter while placing the *ada* gene under control of the *lac* promoter from M13mp11. The 5' end of the gene, now under *lac* control, was then isolated on a *Pvu* II–*Mlu* I fragment and cloned into a blunted *Eco*RI and a *Mlu* I site of pDS400, to yield the plasmid pDS411. An oligonucleotide (5'-CATTATTTAGCCGTCATGCCCAGCG-3') was designed that places a termination codon after the codon for Lys-178. After mutagenesis, a fragment containing the mutation was isolated on a *Bss*HII-blunted *Sal* I fragment and cloned into a *Bss*HII and a blunted *Sph* I site of pDS411 to yield the plasmid, pDS415. The oligonucleotide 5'-GG-ATCCTCGTATGCGTCAGCTGAGCTCTCGACAA-GCTTTAACTAACTAA-3' and its complement were hybridized and inserted downstream of the *ada* gene in pDS411 to generate a plasmid, pDS416. Then 3'-deletion mutagenesis of pDS416 was done by using the Stratagene exonuclease III/mung bean nuclease kit (Stratagene) or using an exonuclease III/exonuclease VII protocol (BRL/Life Technologies). Some plasmids generated by this exonuclease III deletion protocol are listed in Table 3.

Screen for Constitutive Activators of *ada* and *alkA* Transcription. The screen for constitutive activators of *ada* and

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alkA transcription was done on minimal glucose 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) plates containing 0.04 mM isopropyl β-D-thiogalactopyranoside (IPTG).

RESULTS

Regulation of *ada–lacZ* and *alkA–lacZ* in Cells Carrying P$_{lac}$-*ada*. Our previous genetic analyses of *ada* were complicated by the fact that the Ada protein is autoregulatory. Thus, mutations that converted Ada into a constitutive transcriptional activator caused high levels of expression of the altered Ada protein, which proved deleterious to the cells. Therefore, the *ada* promoter was deleted, and the *ada* gene was placed under control of the *lac* promoter by oligonucleotide-directed deletion mutagenesis. This altered *ada* gene was then cloned into a pBR322 derivative to yield the plasmid pDS411. In a *lacI^q* strain carrying an *ada*-null allele and the plasmid pDS411, the level of transcription of *ada–lacZ* and *alkA–lacZ* fusions is influenced by (*i*) the level of IPTG, which controls the levels of Ada protein, and (*ii*) alkylating agents, such as MNNG, which can lead to the posttranslational activation of the Ada protein as a positively acting transcription factor. The increase in transcription of *ada–lacZ* and *alkA–lacZ* fusions in *ada*-null mutants carrying pDS411 due to growth of the cells in IPTG will be termed IPTG induction. At a given IPTG concentration, the increase in the ability of Ada and Ada derivatives to activate transcription of *ada–lacZ* and *alkA–lacZ* fusions due to exposure of cells to MNNG will be referred to as MNNG induction.

The Ada Protein Can Differentially Regulate *ada–lacZ* and *alkA–lacZ* Expression. When *lacI^q* strains carrying an *ada*-null mutation and the plasmid pDS411 were grown without IPTG and MNNG, there was no increase in expression of a chromosomal *ada–lacZ* or *alkA–lacZ* operon fusion compared with that seen in the same strain containing pBR322 (Table 1). Nevertheless, despite the absence of IPTG, a small amount of Ada protein must be produced in cells carrying pDS411 because transcription of the *ada–lacZ* fusion is induced 7-fold upon exposure of cells to MNNG (Table 1). No such MNNG-induced increase of the *ada–lacZ* fusion was seen for cells carrying the vector, pBR322. Surprisingly, no MNNG induction of the *alkA–lacZ* fusion was seen. These results suggest that higher levels of methylated Ada protein are needed to activate *alkA* transcription than *ada* transcription.

When *lacI^q* strains carrying an *ada*-null mutation and the plasmid pDS411 were grown in minimal glucose medium containing a low level of IPTG (0.02 mM, which induces low-level Ada synthesis), the chromosomal *ada–lacZ* and *alkA–lacZ* operon fusions were slightly or moderately activated and could be induced further by adding MNNG (Table 1). These results are consistent with other *in vivo* studies of the induction of *ada* and *alkA* genes by the wild-type protein (13, 14, 18). Therefore, these growth conditions for liquid culture were chosen for analyzing the *ada* derivatives described below.

A second example of the differential regulation of *ada* and *alkA* by Ada was encountered when *lacI^q* strains with an *ada*-null mutation and pDS411 were grown in medium containing 1 mM IPTG, causing overproduction of the Ada protein. When no MNNG was present, strong constitutive activation of *ada–lacZ* and *alkA–lacZ* operon fusions was seen, indicating that the unmethylated Ada protein, when present at high levels, can effectively activate *ada* and *alkA* transcription. After MNNG induction of the *ada–lacZ* fusion in cells overexpressing Ada protein (1.0 mM IPTG), β-galactosidase units were 50% less than that seen for cells producing low levels of Ada protein (0.02 mM IPTG) and then treated with MNNG (Table 1). In contrast, after MNNG

Table 1. Regulation of *ada* and *alkA* transcription in cells containing pDS411

| Plasmid | Relative levels of β-galactosidase activity | | | |
| | *ada–lacZ* | | *alkA–lacZ* | |
	− MNNG	+ MNNG	− MNNG	+ MNNG
No IPTG				
pBR322	1.0 (60)	1.0 (60)	1.0 (7)	1.0 (7)
pDS411	1.1 (63)	8.2 (491)	0.9 (6)	1.0 (7)
IPTG at 0.02 mM				
pBR322	1.0 (58)	1.1 (62)	1.0 (6)	1.0 (6)
pDS411	3.0 (175)	28.4 (1650)	9.8 (59)	36.7 (220)
IPTG at 1.0 mM				
pBR322	1.0 (48)	1.0 (48)	1.0 (7)	1.0 (7)
pDS411	10.8 (520)	13.5 (650)	22.9 (160)	32.9 (230)

β-Galactosidase assays were done essentially as described (15), except that after reaching OD$_{600}$ of 0.2–0.3, cells were incubated with various levels of IPTG for 1 hr before MNNG addition. Cells were then grown for another hour with or without MNNG (1 μg/ml) addition. GW7105 is a Δ*lacU169 pro$^+$* derivative of AB1157 (16) with an *ada–lacZ* operon fusion and an F'[*proA$^+$B$^+$ lacI^q lacZΔM15* Tn*10* (Tc^R)]. GW7106, a Δ*ada-25* Δ*lacU169 pro$^+$* derivative of MV1902, also contains an F'[*proA$^+$B$^+$ lacI^q lacZ*::Tn*5* (Kan^R)]. MV1902 is a *alkA104*::λpSG1 *rfa-550* derivative of AB1157 (17). Both the *ada–lacZ* (GW7105) and *alkA–lacZ* (GW7106) strains are *ada*-null mutants. All strains are *E. coli* K-12. For each IPTG concentration, β-galactosidase activity (units per OD$_{600}$) of each plasmid-containing strain (number in parentheses) was normalized to the activity seen with the same uninduced (−MNNG) strain containing pBR322 (left-hand number).

induction of the *alkA–lacZ* fusion in strains overproducing Ada protein (1.0 mM IPTG), the number of β-galactosidase units was 90% the number of β-galactosidase units seen for cells producing low levels of Ada (0.02 mM IPTG) and then induced with MNNG (Table 1). Although these examples of differential regulation of *ada* and *alkA* described here could, in principle, be explained by different binding affinities of Ada for the two promoters, experiments described below suggest that the mechanism of activation may differ for the two genes.

A C-Terminal Region of Ada Is Required for Activation of *ada* but Not *alkA* Transcription. *In vitro*, Ada can be cleaved by the OmpT protein in a hinge region at position Lys-178–Gln-179 to yield N- and C-terminal domain fragments that each retain their respective DNA methyltransferase activities. Therefore, to determine how a deletion of the C-terminal domain would alter the transcriptional activation properties of Ada we constructed an *ada* derivative encoding only the first 178 amino acids of Ada (Ada178).

The N-terminal domain of Ada, when produced at low levels (0.02 mM IPTG), cannot activate *ada* transcription under MNNG-induced or uninduced conditions (Table 2). Even when expression of the N-terminal derivative is increased by growing cells in 1 mM IPTG, no observable induction of the *ada* gene occurs whether or not the cells are exposed to MNNG (data not shown). Immunoblots of extracts from cells producing the N-terminal domain at a low level suggest that the steady-state levels of this derivative are somewhat lower than that of wild-type protein (Fig. 1). However, a lower steady-state level of Ada178 compared with wild-type protein cannot account for its inability to activate *ada* expression because, even at this level, Ada178 still functions as an MNNG-inducible activator of *alkA* transcription similar to the wild-type Ada protein (Table 2).

The N-terminal domain of Ada (induced with as much as 1 mM IPTG) could not protect the cells against the mutagenic and toxic effects of MNNG in a patch mutagenesis assay (13) because this derivative lacks the C-terminal domain that

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Table 2. Effects of low levels of expression of Ada derivatives on *ada* and *alkA* transcription

		β-Galactosidase activity, units per OD_{600}					
		ada–lacZ		Fold induction	*alkA–lacZ*		Fold induction
Plasmid	Ada derivative	− MNNG	+ MNNG		− MNNG	+ MNNG	
pBR322	None	58	62	1.1	6	6	1.0
pDS411	Wild type	175	1650	9.2	59	220	3.7
pDS417	Ada285	4100	5300	1.3	11	110	10.0
pDS418	Ada287	5500	5600	1.0	18	160	8.9
pDS419	Ada288	3700	5300	1.4	17	140	8.2
pDS420	Ada290	3400	5000	1.5	11	99	9.0
pDS421	Ada309	3100	5000	1.6	12	100	8.3
pDS422	Ada314	2900	5200	1.8	11	78	7.1
pDS423	Ada315	4300	4900	1.1	14	120	8.6
pDS415	Ada178	66	82	1.2	23	147	6.4

β-Galactosidase assays of cells pretreated with 0.02 mM IPTG were done as described for Table 1. Both *ada–lacZ* (GW7105) and *alkA–lacZ* (GW7106) strains are *ada*-null mutants.

possesses the O^6-methylguanine DNA methyltransferase activity (data not shown).

Isolation of Ada Deletion Derivatives That Constitutively Activate *ada* Transcription. To further localize the region of Ada required for activation of *ada* transcription, we analyzed truncated Ada derivatives. To facilitate the construction of random 3'-unidirectional deletions of *ada*, an oligonucleotide was inserted downstream of the *ada* gene in pDS411 to yield the plasmid pDS416. This oligonucleotide contained (*i*) a
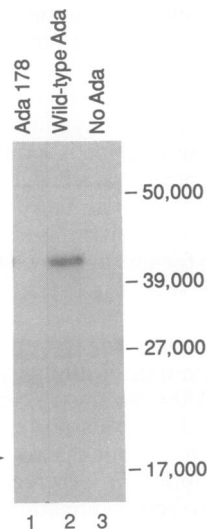

FIG. 1. Immunoblot of Ada178. Cells were grown in minimal glucose medium to an OD_{600} of 0.2–0.3, diluted 1:1 in fresh medium containing 0.04 mM IPTG (0.02 mM IPTG final concentration), and then grown for 2 more hours before harvesting. Equal amounts of cells from each culture, boiled in lysis buffer, were analyzed by electrophoresis of proteins on SDS/polyacrylamide gels, as described (16) and subsequent immunoblotting. The *lacI^q* strain GW7105 contained an *ada–lacZ* operon fusion at the *ada* locus and one of the following plasmids: pDS415 (lane 1), pDS411 (lane 2), and pBR322 (lane 3). Immunoblots were done as described (19), except as noted. Addition of dye in the prestained molecular-weight markers (Bio-Rad) used in this immunoblot alters protein-standard mobilities. These standards, therefore, allow only a rough estimate of the molecular weights of blotted proteins. Rabbit polyclonal antibodies were raised against intact Ada protein. In other experiments, identical SDS/polyacrylamide gels of Ada derivatives and wild-type protein were stained with Coomassie blue or were transferred to a membrane and probed with α-Ada antibodies. These control experiments showed that the relative levels of wild-type and Ada derivative proteins seen in immunoblots were similar to those observed by Coomassie blue staining of SDS/PAGE gels (data not shown).

series of translational terminators in all three reading frames to prevent fusion of the truncated Ada protein to vector-encoded amino acids, (*ii*) unique 5'- and 3'-overhang restriction sites for construction of exonuclease III-generated deletions of *ada*, and (*iii*) a unique *Hind*III site placed between the 5'- and 3'-overhang restriction sites and the translational stop codons to determine whether the plasmids retained the translational terminators after exonuclease III treatment.

To screen for constitutive activation of *ada* transcription, pDS416 derivatives carrying 3' deletions of *ada* from different exonuclease III time points were transformed into a *lacI^q* strain containing a chromosomal *ada–lacZ* operon fusion. The transformed cells were plated on minimal glucose plates containing X-Gal and 0.04 mM IPTG. Under these conditions, colonies carrying the original plasmid, pDS416, were light blue. In one experiment, 31 dark-blue colonies were detected among 15,000 colonies screened, and these occurred at a particularly high frequency in the plasmid pool that had been digested with exonuclease III for 5 min. Twenty-one of the 31 plasmids isolated retained the *Hind*III site and contained a mutation resulting in the constitutive activation of the *ada–lacZ* chromosomal fusion.

Restriction endonuclease analysis of plasmids that constitutively activated the *ada–lacZ* fusion showed that they all contained a deletion in the same region of the *ada* gene. To be certain that the exonuclease III deletion protocol functioned properly, plasmids from randomly selected colonies from each time point were analyzed by restriction endonuclease analysis and were found to contain deletion endpoints throughout the *ada* gene, indicating that the procedure worked as expected. The wild-type Ada protein contains 354 amino acids. Eleven of the plasmids carrying *ada* deletions that encoded constitutive activators of *ada* transcription were sequenced and shown to encode gene products containing 285–315 Ada amino acids—i.e., 80–90% of the intact protein. These derivatives also contained at the C-terminal end of the protein 4, 6, or 7 additional amino acids derived from the oligonucleotide. The sequences of seven of these Ada derivatives chosen for further characterization are listed in Table 3. All these deletion derivatives have lost the Cys-321 residue that accepts a methyl group from the premutagenic lesions O^6-methylguanine or O^4-methylthymine and were unable to complement an *ada*-null mutation in a patch mutagenesis assay (data not shown).

Failure to Isolate Ada Deletion Derivatives That Constitutively Activate *alkA* Transcription. None of the Ada deletion derivatives isolated as strong constitutive activators of *ada* transcription were strong constitutive activators of *alkA* transcription. Therefore, a parallel screen to look directly for truncated Ada derivatives that could constitutively activate a

chromosomal *alkA–lacZ* fusion was done. To ensure that the exonuclease III procedure and the screen itself were functioning properly, an *ada–lacZ* strain was transformed at the same time as the *alkA–lacZ* strain with a sample of the digested pDS416 DNA. Although we could isolate additional constitutive activators of *ada* transcription, no constitutive activators of *alkA* transcription were detected of 66,000 colonies screened. Other studies of the *ada* gene demonstrated that in the screen for constitutive activators of *alkA*, we can easily detect Ada derivatives that activate *alkA* transcription to levels 5 times higher than that seen with the wild-type protein (unpublished data). Therefore, the data suggest that it may not be possible to obtain truncated Ada derivatives that constitutively activate *alkA* transcription to levels at least 5-fold higher than that of the wild type.

C-Terminal Truncations of Ada Differentially Activate *ada* and *alkA* Transcription. In cells grown in 0.02 mM IPTG (but without MNNG), the Ada deletion derivatives containing 80–90% of the intact protein increased *ada* expression to levels as much as 31-fold higher than that seen with wild-type Ada protein (Table 2). These levels were 3-fold higher than that seen with MNNG-activated wild-type protein. Also the ability of these Ada deletion derivatives to activate *ada* transcription was not significantly increased after exposure of cells to MNNG. When expressed at low levels (0.02 mM IPTG), the Ada deletion derivatives that were strong constitutive activators of an *ada–lacZ* fusion were weak constitutive activators of an *alkA–lacZ* fusion. However, these derivatives, like the wild-type protein, were good MNNG-inducible activators of the *alkA–lacZ* fusion (Table 2). Taken together with the other observations, these results indicate that some region located between amino acids 178 and 285 is necessary for Ada to activate *ada* transcription.

Steady-State Levels of the Strong Constitutive Activators of *ada*. Several results indicate that the ability of the truncated Ada derivatives to constitutively activate *ada* transcription is not simply due to higher steady-state levels of the mutant proteins. (*i*) Overproduction of the wild-type protein resulted in the constitutive activation of both *ada* and *alkA* (Table 1). In contrast, the Ada derivatives that were strong constitutive activators of *ada* expression are only weak constitutive activators of *alkA* (Table 2). (*ii*) Furthermore, immunoblots of cell extracts suggest that the amounts of these truncated Ada proteins were lower than that of the wild-type protein (Fig. 2).

DISCUSSION

The experiments described here suggest that a higher level of the methylated Ada protein appears to be required for

Table 3. Amino acid sequence of Ada derivatives with a truncated C-terminal domain

Plasmid	Ada derivative	Ada amino acids	C-terminal amino acids from vector
pBR322	None	None	
pDS416	Wild type	354	
pDS417	Ada285	285	ARQALTN
pDS418	Ada287	287	LDKL
pDS419	Ada288	288	SSTSFN
pDS420	Ada290	290	PRQALTN
pDS421	Ada309	309	LDKL
pDS422	Ada314	314	RRQALTN
pDS423	Ada315	315	LDKL

DNA sequencing was done as described (13), except that reactions were done with Sequenase (United States Biochemical). The truncated Ada derivatives are referred to by the number of Ada amino acids they retain; for example, protein Ada285 contains 285 Ada-encoded amino acids. The one-letter symbols for amino acids are used.
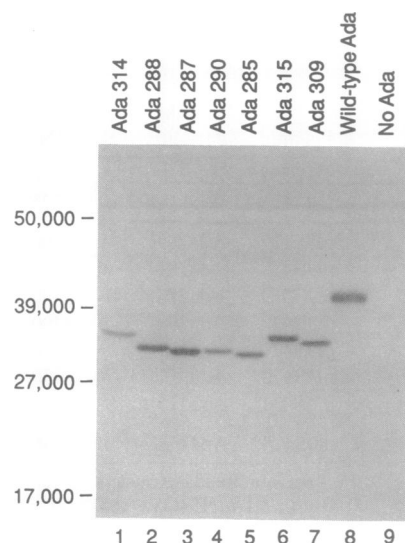

FIG. 2. Immunoblot of Ada deletion derivatives. The experiment was done as described in Fig. 1. Lanes: 1, pDS422; 2, pDS419; 3, pDS418; 4, pDS420; 5, pDS417; 6, pDS423; 7, pDS421; 8, pDS411; and 9, pBR322.

activation of *alkA* transcription than for *ada* transcription. Also, studies of cells producing a low level of Ada protein indicate that unmethylated Ada protein can activate both *ada* and *alkA* transcription but that the methylated form of the Ada protein is more efficient at stimulating transcription at both promoters. These studies, thus, resolve a question raised by previous conflicting *in vitro* results (11, 12) as to whether or not *alkA* transcription could be activated equally well by methylated or unmethylated Ada protein. The observation that *ada* transcription is induced less efficiently by MNNG when Ada is present at a high level (Table 1, compare lines 4 and 6) suggests the possibility that excess unmethylated Ada protein can inhibit transcription of *ada*. Such a potential mechanism for shutting down the adaptive response is attractive because Ada is irreversibly activated by methylation at Cys-69.

In addition, our results suggest that the mechanism by which the Ada protein transcriptionally activates *ada* differs from that of *alkA*. Although the 178-amino acid N-terminal domain (containing 50% of Ada protein) is sufficient for the MNNG-inducible transcriptional activation of *alkA*, it cannot activate *ada* transcription *in vivo* regardless of MNNG induction. These *in vivo* results are consistent with those from an *in vitro* transcription system containing fragments of the Ada protein generated by *in vitro* cleavage of the intact protein by a cellular protease (20).

The amino acids in the C-terminal domain, between ≈178 and 285, in addition to the N-terminal 178 amino acids, appear to be required for Ada to activate *ada* transcription. In the absence of MNNG, Ada derivatives containing 80–90% of the wild-type protein are strong activators of *ada* transcription but poor activators of *alkA* expression. Thus, the C-terminal 10–20% of Ada makes the ability of the protein to function as a transcriptional activator of *ada* conditionally dependent on the methylation state of Cys-69. In contrast, the ability of Ada to activate *alkA* transcription depends on methylation of Cys-69, irrespective of whether C-terminal residues are present or not. Taken together, these results and the fact that amino acid substitutions (unpublished results; refs. 21 and 22) can convert the unmethylated Ada protein into a constitutive activator suggest that a major role of the methylation of Cys-69 is to cause a specific conformational change of Ada protein that influences both its N-terminal and C-terminal domains.

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Ada is known to bind to similar DNA sequences in the *ada* and *alkA* promoters (11, 12). DNase I footprinting studies have shown that this DNA-binding capacity resides in the methylated N-terminal domain of Ada (9). Therefore, with respect to the *ada* gene, the N-terminal domain of Ada protein (Ada178) appears to function as a protein defective in positive control; it is unable to activate *ada* transcription whether or not it is MNNG-induced, but, when methylated, it can bind specifically to the *ada* promoter.

There are several differences between the *ada* and *alkA* promoters that could contribute to the differential regulation of the genes. The amount of spacing between the Ada box and the proposed −35 site in each promoter differ. The Ada promoter contains the Ada box at position −58 to −46, 7 base pairs (bp) away from the start of the most recently proposed −35 region (−39 to −34) (23). In contrast, the *alkA* promoter contains the Ada box at position −42 to −30, which almost completely overlaps the proposed −35 site (−33 to −28). This 16-bp difference in linear spacing of the promoters results in a difference in rotational orientation as well. Also, two nucleotides within the Ada box, as well as the sequences of the promoter regions outside of the Ada box, including the −35 and −10 regions, differ. In particular, a region of dyad symmetry exists upstream of the *ada* promoter and partially overlaps the asymmetric Ada box but does not occur in the sequence upstream of the *alkA* promoter.

These differences in the *ada* and *alkA* promoters and the results presented here suggest a model in which the same elements in the N-terminal domain of Ada would be involved in binding to the Ada boxes of *ada* and *alkA*. However, due to the difference in spacing between the Ada box and the −35 region, the portion of Ada that facilitates successful interaction of RNA polymerase with the *ada* promoter to increase transcription, would differ from the portion of Ada that performs similarly at the *alkA* promoter. The elements of Ada necessary and sufficient for the stimulation of the *alkA* promoter would be located in the N-terminal domain of the protein. In contrast, elements located in the C-terminal domain, between amino acids 178 and 285, must be necessary for activation of *ada* transcription. It is possible that elements located in the N-terminal domain of Ada also specifically participate in activation of *ada* transcription. This model would also suggest that the methylated N-terminal domain of Ada can bind to the Ada box upstream of the *ada* promoter but cannot activate transcription because it is too small and too distant from the −35 site to facilitate the appropriate interactions of RNA polymerase with the *ada* promoter. Consistent with this model, a 3-bp deletion and 1- and 5-bp insertions between the Ada box and the −35 region either drastically lowered or abolished induction of *ada*, suggesting that the spacing between these two regions is critical (23). On the other hand, the small N-terminal domain can facilitate successful RNA polymerase binding and transcriptional activation of the *alkA* promoter because the Ada box overlaps the −35 site at which RNA polymerase binds. It is also conceivable that Ada could perform several functions as a transcriptional activator, not all of which would be required at every promoter regulated by Ada.

Certain inducible eukaryotic transcriptional activators, such as the GAL4 protein from yeast (24, 25) and the gluticorticoid receptor from mammalian cells (26), can also be converted into constitutive activators by truncation of part of their C-terminal domain. Also, truncation of the N-terminal domain of AraC converts it into a constitutive activator of *ara*

genes. However, in contrast to Ada, which is normally activated by a covalent modification in the N-terminal domain of the protein, GAL4 and the gluticorticoid receptor interact with an effector molecule at their C-terminal domains. Furthermore, indirect evidence suggests that the AraC protein interacts with arabinose in the N-terminal domain (27). There is presently no evidence that the conversion of Ada into an efficient transcriptional activator involves the interaction of the C-terminal region of Ada with another protein. The induction of *ada* transcription seen upon exposure of cells to MNNG can be reproduced *in vitro* by comparing activation of *ada* transcription in a reaction containing methylated (at Cys-69) or unmethylated Ada protein and RNA polymerase (12).

1. Samson, L. & Cairns, J. (1977) *Nature (London)* **267**, 281–283.
2. Lindahl, T., Sedgwick, B., Sekiguchi, M. & Nakabeppu, Y. (1988) *Annu. Rev. Biochem.* **57**, 133–157.
3. Shevell, D. E., Friedman, B. M. & Walker, G. C. (1990) *Mutat. Res.* **233**, 53–72.
4. Demple, B., Jacobsson, A., Olsson, M., Robins, P. & Lindahl, T. (1982) *J. Biol. Chem.* **257**, 13776–13780.
5. Teo, I., Sedgwick, B., Demple, B., Li, B. & Lindahl, T. (1984) *EMBO J.* **3**, 2151–2157.
6. Demple, B., Sedgwick, B., Robins, P., Totty, N., Waterfield, M. D. & Lindahl, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2688–2692.
7. McCarthy, T. V. & Lindahl, T. (1985) *Nucleic Acids Res.* **13**, 2683–2698.
8. Sedgwick, B., Robins, P., Totty, N. & Lindahl, T. (1988) *J. Biol. Chem.* **263**, 4430–4433.
9. Sedgwick, B. & Hughes, S. (1988) in *Mechanisms and Consequences of DNA Damage Processing*, eds. Friedberg, E. & Hanawalt, P. (Liss, New York), pp. 127–132.
10. McCarthy, T. V., Karran, P. & Lindahl, T. (1984) *EMBO J.* **3**, 545–550.
11. Nakabeppu, Y. & Sekiguchi, M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6297–6301.
12. Teo, I., Sedgwick, B., Kilpatrick, M. W., McCarthy, T. V. & Lindahl, T. (1986) *Cell* **45**, 315–324.
13. Shevell, D. E., LeMotte, P. K. & Walker, G. C. (1988) *J. Bacteriol.* **170**, 5263–5271.
14. LeMotte, P. K. & Walker, G. C. (1985) *J. Bacteriol.* **161**, 888–895.
15. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
16. Elledge, S. J. & Walker, G. C. (1983) *J. Mol. Biol.* **164**, 175–192.
17. Volkert, M. R., Nguyen, D. C. & Beard, K. C. (1986) *Genetics* **112**, 11–26.
18. Sedgwick, B. (1983) *Mol. Gen. Genet.* **191**, 466–472.
19. Long, S., McCune, S. & Walker, G. C. (1988) *J. Bacteriol.* **170**, 4257–4265.
20. Yoshikai, T., Nakabeppu, Y. & Sekiguchi, M. (1988) *J. Biol. Chem.* **263**, 19174–19180.
21. Hughes, S. J. & Sedgwick, B. (1989) *J. Biol. Chem.* **264**, 21369–21375.
22. Takano, K., Nakabeppu, Y. & Sekiguchi, M. (1988) *J. Mol. Biol.* **201**, 261–271.
23. Nakamura, T., Tokumoto, Y., Sakumi, K., Koike, G., Nakabeppu, Y. & Sekiguchi, M. (1988) *J. Mol. Biol.* **202**, 483–494.
24. Johnston, S. A., Salmeron, J. M., Jr., & Dincher, S. S. (1987) *Cell* **50**, 143–146.
25. Ma, J. & Ptashne, M. (1987) *Cell* **50**, 137–142.
26. Godowski, P. J., Rusconi, S., Miesfeld, R. & Yamamoto, K. R. (1987) *Nature (London)* **325**, 365–368.
27. Menon, K. P. & Lee, N. L. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3708–3712.