

# The *Escherichia coli* Ada Protein Can Interact with Two Distinct Determinants in the $\sigma^{70}$ Subunit of RNA Polymerase According to Promoter Architecture: Identification of the Target of Ada Activation at the *alkA* Promoter

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**The methylated form of the Ada protein (<sup>m</sup>Ada) activates transcription from the *Escherichia coli* *ada*, *aidB*, and *alkA* promoters with different mechanisms. In this study we identify amino acid substitutions in region 4 of the RNA polymerase subunit  $\sigma^{70}$  that affect Ada-activated transcription at *alkA*. Substitution to alanine of residues K593, K597, and R603 in  $\sigma^{70}$  region 4 results in decreased Ada-dependent binding of RNA polymerase to the *alkA* promoter in vitro and impairs *alkA* transcription both in vivo and in vitro, suggesting that these residues define a determinant for <sup>m</sup>Ada- $\sigma^{70}$  interaction. In a previous study (P. Landini, J. A. Bown, M. R. Volkert, and S. J. W. Busby, *J. Biol. Chem.* 273:13307–13312, 1998), we showed that a set of negatively charged amino acids in  $\sigma^{70}$  region 4 is involved in <sup>m</sup>Ada- $\sigma^{70}$  interaction at the *ada* and *aidB* promoters. However, the alanine substitutions of positively charged residues K593, K597, and R603 do not affect <sup>m</sup>Ada-dependent transcription at *ada* and *aidB*. Unlike the  $\sigma^{70}$  amino acids involved in the interaction with <sup>m</sup>Ada at the *ada* and *aidB* promoters, K593, K597, and R603 are not conserved in  $\sigma^S$ , an alternative  $\sigma$  subunit of RNA polymerase mainly expressed during the stationary phase of growth. While <sup>m</sup>Ada is able to promote transcription by the  $\sigma^S$  form of RNA polymerase ( $E\sigma^S$ ) at *ada* and *aidB*, it fails to do so at *alkA*. We propose that <sup>m</sup>Ada can activate transcription at different promoters by contacting distinct determinants in  $\sigma^{70}$  region 4 in a manner dependent on the location of the Ada binding site.**

Exposure of *Escherichia coli* cells to sublethal concentrations of DNA-methylating agents such as methyl methanesulfonate (MMS) triggers the expression of a set of genes which allows *E. coli* to tolerate the toxic and mutagenic effects of such agents. This process is called the adaptive response (10, 29). The Ada protein plays a dual role in the adaptive response, being both a DNA repair protein and a transcription activator. Ada is a 354-amino-acid methyltransferase able to remove methyl groups from both the DNA strand and damaged nucleotides. Methyl groups are transferred to two cysteine residues in the protein itself, at positions 69 and 321 (7). Methylation of cysteine at position 69 triggers a conformational change in the Ada protein; cysteine-69-methylated Ada (<sup>m</sup>Ada) is a specific DNA binding protein able to promote transcription of its own gene, *ada*, and of the *alkB* gene, whose transcription is also driven by the *ada* promoter (25, 32). In addition, <sup>m</sup>Ada activates transcription from the promoters of at least two more genes, *alkA* and *aidB* (14, 25, 32). The AlkA protein is a DNA repair protein able to excise methylated bases from DNA (32), while AidB appears to be involved in metabolic detoxification of some alkylating agents, such as MNNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) (13). AlkB is a membrane protein, and its function is unknown (34). All Ada-dependent promoters display a common sequence, AAT(N)<sub>6</sub>GCAA, thought to be the recognition sequence for <sup>m</sup>Ada (14).

A number of observations show that the mechanism of transcription activation by Ada at the *alkA* promoter differs from that at the *ada* and *aidB* promoters. Both the unmethylated

and the methylated forms of the Ada protein can activate *alkA* transcription in vivo and in vitro (9, 27). In contrast, the unmethylated Ada protein activates very poorly, if at all, transcription from the *ada* and *aidB* promoters, and it has been shown to be inhibitory for the *ada* promoter at high concentrations (26). Moreover, Ada mutants capable of activating *alkA* but not *ada*, and vice versa, have been isolated (1, 27, 30). These observations strongly suggest that different determinants in the Ada protein are involved in the interaction with RNA polymerase at different adaptive response promoters.

The *alkA* promoter also differs from the *ada* and *aidB* promoters in its architecture and in the location of the Ada binding site relative to the promoter elements. In *ada* and *aidB*, the Ada binding site is, respectively, 5 and 7 bp upstream of a fairly well conserved  $-35$  sequence (14, 28); at the *alkA* promoter, the Ada binding site overlaps a  $-35$  hexamer which bears no similarity to the consensus sequence (1, 9). The adaptive response promoters also differ in their interaction with RNA polymerase: at *ada* and *aidB*, RNA polymerase binds the promoters in the absence of Ada, mostly through  $\alpha$  subunit/UP-like element contacts, to form an unusual binary complex which is poorly active in transcription initiation (15). Binding of <sup>m</sup>Ada results in the formation of a transcription initiation-competent ternary complex. In a recent study (18), we showed that the target for activation by <sup>m</sup>Ada is in region 4 of the  $\sigma^{70}$  subunit of RNA polymerase (amino acids 574 to 613); several negatively charged amino acids in this region (E574, E575, E591, E605, and D612, as well as hydrophobic residue I590) appear to be involved in <sup>m</sup>Ada- $\sigma^{70}$  interaction. At *alkA*, no RNA polymerase-promoter binary complex in the absence of Ada is detectable, and both forms of the Ada protein appear to increase RNA polymerase binding affinity to the promoter region (17). Finally, <sup>m</sup>Ada is able to activate transcription by

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RNA polymerase containing  $\sigma^S$  ( $E\sigma^S$ ) at *ada* and *aidB* (16, 31) while no such evidence has been shown for *alkA*.  $\sigma^S$  is the main  $\sigma$  subunit during stationary phase (19).

In this report, we present evidence that region 4 of  $\sigma^{70}$  is also the target of activation by the Ada protein at the *alkA* promoter; however, different determinants are involved in Ada- $\sigma^{70}$  interaction. Three positively charged amino acids in  $\sigma^{70}$  region 4 (K593, K597, and R603) play a major role in Ada-dependent transcription at *alkA*. Moreover, the Ada protein is unable to activate transcription by  $E\sigma^S$  at the *alkA* promoter, consistent with the fact that the K593, K597, and R603 residues are not conserved in  $\sigma^S$ . Based on these observations, we conclude that  $^{me}Ada$  can activate transcription via interaction with distinct determinants in  $\sigma^{70}$  region 4, depending on the location of its binding site.

#### MATERIALS AND METHODS

**In vivo transcription from the *alkA* promoter.** A plasmid library carrying mutations resulting in single alanine substitutions at 17 amino acids of  $\sigma^{70}$  region 4 (obtained from C. Gross, University of California—San Francisco; plasmids are derivatives of pGEX-2T $\sigma^{70}$  [8]) was used to transform strain MV3764. MV3764 carries a *lacZ* fusion within the chromosomal *alkA* gene. The strains obtained from transformation of MV3764 were tested for  $\beta$ -galactosidase activity to quantify the effects of the alanine substitutions in  $\sigma^{70}$  region 4 on *alkA* transcription. Cells were grown overnight in Luria broth supplemented with 20  $\mu$ g of tetracycline per ml, 25  $\mu$ g of chloramphenicol per ml, and 80  $\mu$ g of ampicillin per ml, diluted 1:100 in fresh medium, and grown to an optical density at 600 nm of 0.1. Ada-dependent transcription of *alkA* was induced by the addition of 0.02% MMS. Samples were taken after 2 h, and specific  $\beta$ -galactosidase activity was determined as described in reference 23.

**In vitro transcription.** Mutated  $\sigma^{70}$  subunits affecting *alkA* transcription in vivo, as well as wild type  $\sigma^{70}$ , were purified as described previously (8).  $\sigma^S$  (obtained from A. Kolb, Institut Pasteur) was purified as described previously (6). RNA polymerase holoenzymes were reconstituted by adding the purified  $\sigma$  subunits to RNA polymerase core enzyme (Epicentre Technology) at a 5:1 ratio (except for  $\sigma^{70}_{RA603}$ , which was added at a 20:1 ratio because of its lower affinity for core RNA polymerase [21]). Core enzyme and the required  $\sigma$  factor were incubated for 20 minutes at 37°C in a solution of 50 mM Tris-HCl (pH 7.6), 100 mM KCl, and 50% glycerol; the reconstituted RNA polymerase holoenzymes were then used for in vitro transcription. Reconstituted RNA polymerases were incubated for 15 min at 37°C in a solution of 40 mM Tris-HCl (pH 8.0), 7.5% (wt/vol) glycerol, 30 mM KCl, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, and 20  $\mu$ g of bovine serum albumin per ml in the presence or absence of either form of the Ada protein. Transcription reactions were started by the addition of 0.1 mM (each) GTP, ATP, and CTP, 0.002 mM UTP, 10  $\mu$ Ci of [ $\alpha$ -<sup>32</sup>P]UTP, and 250  $\mu$ g of heparin per ml. The reaction was stopped after 5 min at 37°C, and samples were analyzed on a denaturing polyacrylamide gel. The DNA fragments used as templates were a 205-bp *EcoRI* DNA fragment from pYN3066 (25) carrying the *lacUV5* promoter (used as an internal control) and a 166-bp *HindIII/EcoRI* fragment from pMV466 carrying the *alkA* promoter (17). The two fragments were obtained by PCR amplification with *Pfu* enzyme. PCR products were separated by electrophoresis in 1% agarose and recovered with the GeneClean kit (Bio 101). The amount of transcription was quantified after normalization to the *lacUV5* transcript by a PhosphorImager (Molecular Dynamics). In the experimental results shown in Fig. 2, three Ada-dependent transcripts from the *alkA* promoter can be detected; the presence of more than one transcript is likely to be due to the production of different-length DNA fragments in the PCR amplification of the *alkA* promoter region rather than to the presence of alternative transcription start points in the *alkA* promoter (cf. reference 17).

**Gel retardation assays.** The 166-bp *HindIII/EcoRI* fragment carrying the *alkA* promoter was also used for gel retardation. The fragment was labeled with [ $\alpha$ -<sup>32</sup>P]dATP (3,000 Ci/mmol) (Amersham) by filling in with DNA polymerase I Klenow fragment (Pharmacia). A 10,000-cpm sample (corresponding to ca. 5 fmol) of labeled fragment was used in a 20- $\mu$ l final volume of reaction buffer (50 mM Tris-HCl [pH 7.6], 50 mM NaCl, 2.5 mM dithiothreitol, 6.25% glycerol, 25  $\mu$ g of herring sperm DNA per ml). When necessary,  $^{me}Ada$  was present at 0.2  $\mu$ M. Reconstituted RNA polymerase was added to a final concentration of 50 nM, and samples were incubated for 30 min at 37°C prior to the addition of 250  $\mu$ g of heparin per ml. After 5 min of incubation, samples were loaded onto a 4% native polyacrylamide gel. Gels were run at 10 V/cm in 0.25 $\times$  TBE (22.5 mM Tris-borate, 0.5 mM EDTA)–1.25% glycerol, and bands were visualized by autoradiography. Quantification of retarded bands was performed with a PhosphorImager.

## RESULTS

### Effects of mutations in *rpoD* on *alkA* transcription in vivo.

We transformed strain MV3764 (*alkA::lacZ*) with a set of plasmids carrying *rpoD* alleles with single alanine substitutions at 17 amino acids of  $\sigma^{70}$  region 4.  $\beta$ -Galactosidase assays were performed to quantify the effects of the alanine substitutions, as well as the effect of the EV575 substitution, which we had found to affect transcription from the *ada* promoter (18). In strain MV3764, wild-type  $\sigma^{70}$  from the chromosomal *rpoD* gene is expressed at normal levels; since in the absence of IPTG (isopropyl- $\beta$ -D-thiogalactopyranoside) the mutated *rpoD* alleles from the plasmid are expressed at a low level and mutated  $\sigma$  subunits are present in only a slight excess to wild-type  $\sigma^{70}$  (data not shown), a reduction of *alkA* expression to below 80% of the wild type was considered significant (Fig. 1). Expression of four mutant *rpoD* alleles resulted in lower levels of in vivo transcription at the *alkA* promoter: EV575 (down to 67.7% of the level observed with wild-type  $\sigma^{70}$ -encoding plasmid), KA593 (76.1%), KA597 (76.3%), and RA603 (69.6%). No mutations resulted in significantly increased levels of *alkA* transcription (more than 120% of the wild type [Fig. 1]), nor was *alkA* transcription in the absence of MMS significantly altered by any *rpoD* allele tested (data not shown). In a previous study, we had found that a set of negatively charged residues in  $\sigma^{70}$  (E574, E575, E591, E605, and D612) negatively affects transcription of the *ada* promoter in vivo (18); of these substitutions, only EV575 significantly affected *alkA* promoter activity (Fig. 1). Expression of the KA593, KA597, and RA603 mutated  $\sigma$  subunits did not result in decreased transcription from the *ada* promoter in vivo (18).

**Mutant  $\sigma^{70}$  subunits affect *alkA* transcription in vitro.** Purified wild-type and mutant  $\sigma$  subunits EV575, KA593, KA597, and RA603 were used for reconstitution of RNA polymerase and in vitro transcription (Fig. 2 and 3). The  $E\sigma^{70}_{EV575}$ ,  $E\sigma^{70}_{KA593}$ ,  $E\sigma^{70}_{KA597}$ , and  $E\sigma^{70}_{RA603}$  forms of RNA polymerase were all able to carry out transcription from the *lacUV5* promoter with roughly the same efficiency as the wild type, suggesting that these mutant forms of  $\sigma^{70}$  are not affected in factor-independent transcription. In contrast, transcription from the *alkA* promoter by  $E\sigma^{70}_{KA593}$  (Fig. 2, lanes 4 to 6)  $E\sigma^{70}_{KA597}$  (lanes 7 to 9), and  $E\sigma^{70}_{RA603}$  (lanes 10 to 12), in the presence of either Ada or  $^{me}Ada$ , was only 28 to 45% of the transcription obtained with wild-type  $\sigma^{70}$  (Fig. 3). These results suggest that these mutant  $\sigma$  subunits are indeed defective in their interaction with the Ada protein and that both methylated and unmethylated Ada activate transcription by contacting the same determinants in  $\sigma^{70}$ . Surprisingly, Ada-dependent transcription of *alkA* by  $E\sigma^{70}_{EV575}$  was not impaired (Fig. 3) despite the fact that expression of  $\sigma^{70}_{EV575}$  had the strongest effect on *alkA* expression in vivo. A possible reason for this discrepancy is the very strong negative effect of  $\sigma^{70}_{EV575}$  overexpression on transcription of *ada* in vivo (18), which results in lower intracellular concentrations of the Ada protein; this, in turn, would result in suboptimal activation of the *alkA* promoter.

**Gel retardation assays.** In the presence of either form of Ada, a stable ternary complex, ( $^{me}Ada$ )-RNA polymerase-*alkA*, which is resistant to challenge with heparin is formed and can be detected in gel retardation assays (Fig. 4). In order to investigate whether the KA593, KA597, and RA603 substitutions in  $\sigma^{70}$  region 4 affect the formation of the ternary complex, gel retardation assays were performed with RNA polymerase reconstituted with mutated  $\sigma$  subunits. The results in Fig. 4 show that  $E\sigma^{70}_{KA593}$ ,  $E\sigma^{70}_{KA597}$ , and  $E\sigma^{70}_{RA603}$  are all impaired in formation of the ternary complex with the *alkA*

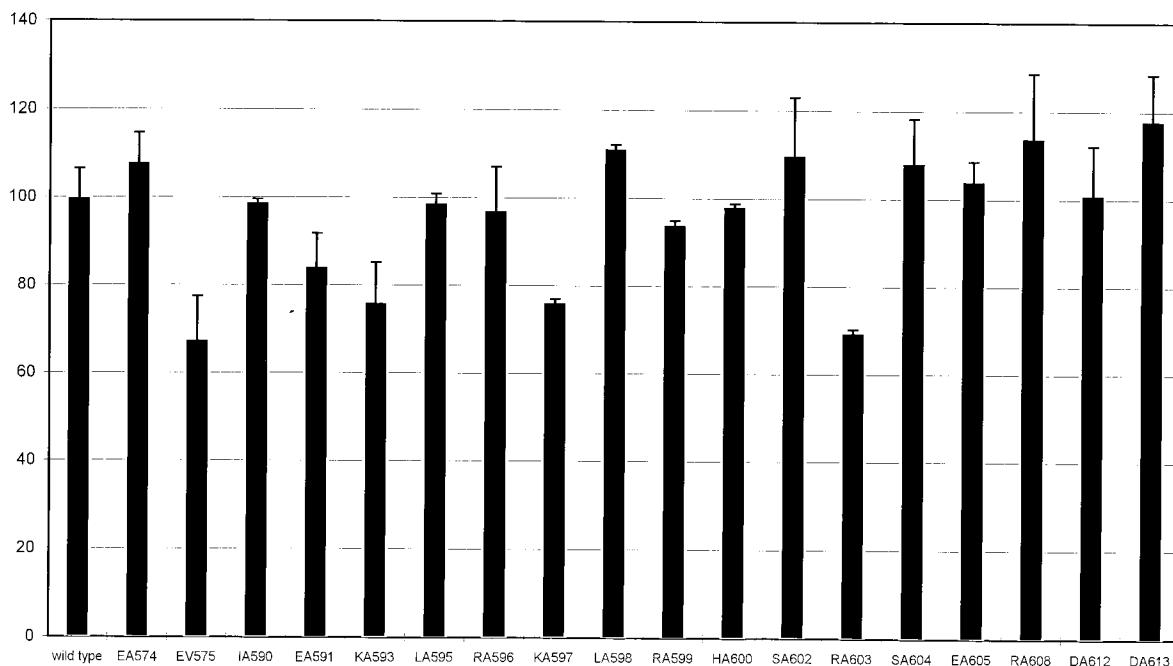


FIG. 1. In vivo transcription from the *alkA* promoter. The effects of the substitutions in  $\sigma^{70}$  region 4 on *alkA* expression are shown as percentages of *alkA* expression with wild-type  $\sigma^{70}$ . Values ( $\pm$  standard deviations) are averages of four independent experiments. The average value for the wild type was 342 Miller units.

promoter and  $^{m}Ada$ . However, the defect was more dramatic for  $E\sigma^{70}_{KA593}$  (Fig. 4, lanes 5 and 6) and  $E\sigma^{70}_{KA597}$  (lanes 7 and 8) than for  $E\sigma^{70}_{RA603}$ , which was still able to promote formation of the ternary complex at about 50% of wild-type levels (lanes 9 and 10). This result was unexpected, since the KA593, KA597, and RA603 substitutions have very similar effects on *alkA* transcription both in vitro and in vivo (Fig. 1 to 3). It is possible that in addition to interaction with Ada,  $E\sigma^{70}_{RA603}$  is also impaired in transcription initiation at *alkA* at a later step, such as promoter clearance, which is likely to be Ada-independent. Interestingly,  $E\sigma^{70}_{RA603}$  has been shown to

be defective in transcription initiation at the factor-independent promoter RNA-I (21).

**$E\sigma^{70}$ - and  $E\sigma^S$ -dependent transcription in vitro.** Although interaction with  $\sigma^{70}$  region 4 is the principal mechanism of transcription activation by  $^{m}Ada$ , the results of the in vitro transcription experiments strongly suggest that the target of its activation at *alkA* differs from that at *ada* and *aidB* at the amino acid level. At the *ada* and *aidB* promoters, the E574, E575, I590, E591, E605, and D612 side chains are important for activation by  $^{m}Ada$  (18). With the sole exception of D612, these residues are conserved in  $\sigma^S$ , consistent with the ability of  $^{m}Ada$  to promote transcription by  $E\sigma^S$  at both *ada* and *aidB* (16, 31). In contrast, the K593, K597, and R603 residues, which appear to be the target amino acids for activation by Ada at the *alkA* promoter, are not conserved in  $\sigma^S$ . Thus, we tested  $E\sigma^S$  for Ada-dependent transcription at *alkA*. The results of this experiment are shown in Fig. 5. While either form of the Ada protein clearly activated transcription by  $E\sigma^{70}$  (lanes 1 to 3), no stimulation of transcription by  $E\sigma^S$  was detected (lanes 4 to 6); in fact,  $^{m}Ada$  displayed a weak negative effect on *alkA* transcription by  $E\sigma^S$  (compare lane 4 to lane 6). We also tested  $E\sigma^S$  in gel retardation assays: as expected from the results of the in vitro transcription experiments,  $^{m}Ada$  failed to promote the formation of a stable heparin-resistant ternary complex with  $E\sigma^S$  and the *alkA* promoter (Fig. 6).

## DISCUSSION

The results presented in this report show that substitution to alanine of amino acids K593, K597, and R603 in region 4 of the  $\sigma^{70}$  subunit of RNA polymerase disrupts Ada-dependent transcription from the *alkA* promoter (Fig. 1 to 3). Thus, we propose that Ada activates *alkA* transcription via direct interaction with  $\sigma^{70}$  region 4. The target for activation by Ada is located immediately downstream of the helix-turn-helix DNA binding motif, which is responsible for recognition of the  $-35$  pro-

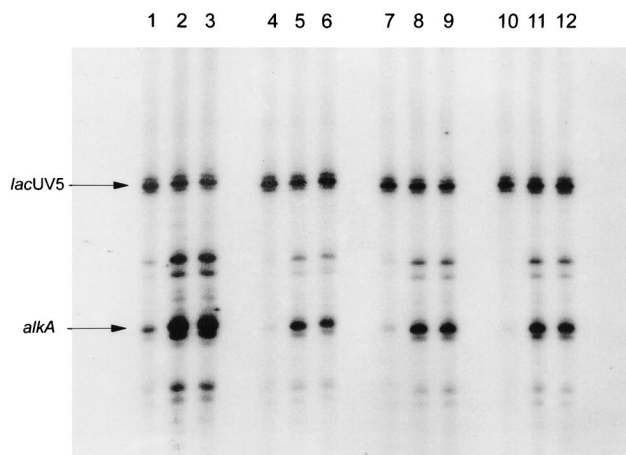


FIG. 2. In vitro transcription with reconstituted RNA polymerases (50 nM). Lanes 1 to 3, wild-type  $E\sigma^{70}$ ; lanes 4 to 6,  $E\sigma^{70}_{KA593}$ ; lanes 7 to 9,  $E\sigma^{70}_{KA597}$ ; lanes 10 to 12,  $E\sigma^{70}_{RA603}$ . Lanes 1, 4, 7, and 10, no Ada protein; lanes 2, 5, 8, and 11, 0.2  $\mu$ M unmethylated Ada; lanes 3, 6, 9, and 12, 0.2  $\mu$ M methylated Ada. The positions of the main *lacUV5* and *alkA* transcripts are indicated.

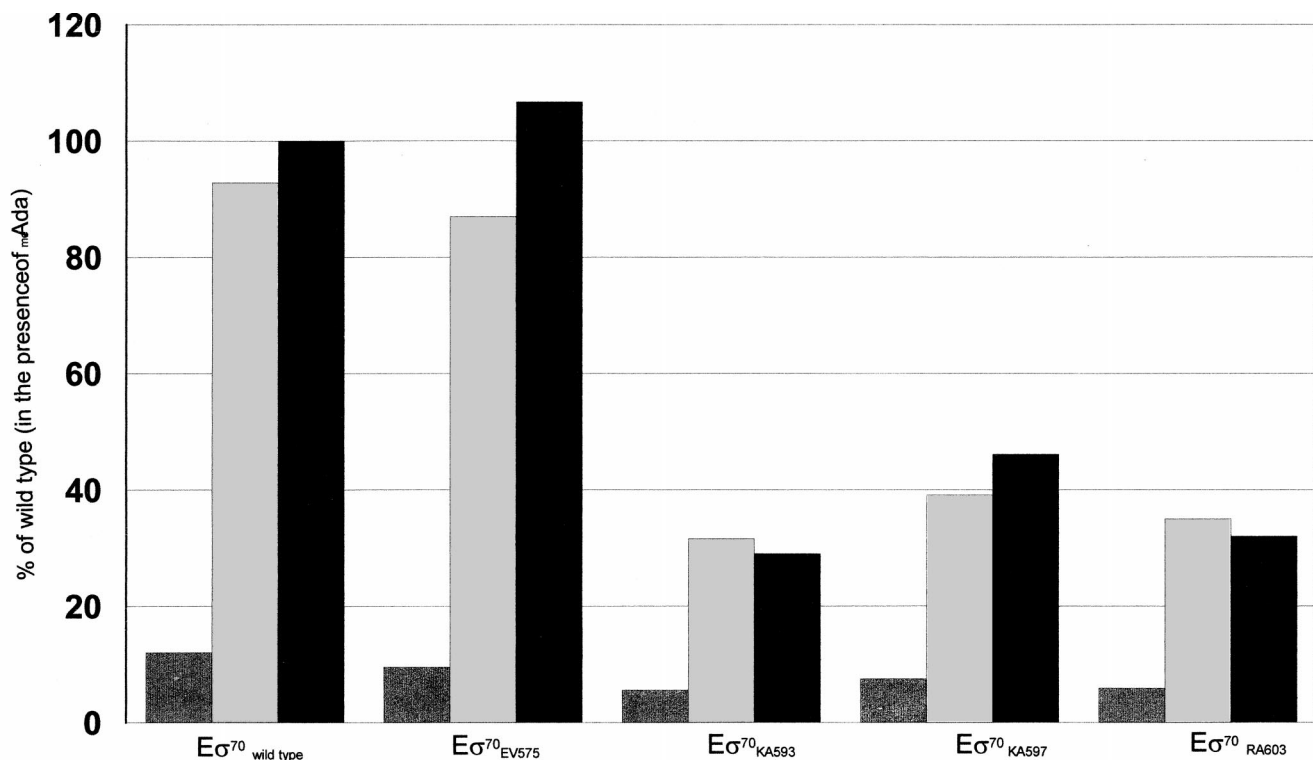


FIG. 3. Ratio of *alkA/lacUV5* transcripts. Values are percentages of transcription by wild-type  $E\sigma^{70}$  in the presence of  $m^e$ Ada and are averages of three independent experiments. Dark grey bars, transcription levels in the absence of Ada; light grey bars, transcription levels in the presence of unmethylated Ada; black bars, transcription levels in the presence of methylated Ada. Standard deviations were less than 15%.

moter element (20) and is the target of several transcription activators (12, 21, 22). Interestingly, although  $\sigma^{70}$  region 4 is also the target for Ada activation at the *ada* and *aidB* promoters, two different sets of amino acids are involved. A striking difference between the two sets is the nature of the amino acids involved: all except one of the  $\sigma^{70}$  residues necessary for  $m^e$ Ada-dependent transcription at *ada* and *aidB* are negatively charged (18), in contrast to the positive charges of K593, K597, and R603.

It is noteworthy that K593, K597, and R603 are also targets for other activator proteins: substitution to alanine of any of these residues severely affects transcription activation by FNR and by cyclic AMP receptor protein (CRP), respectively, at the *dmsA* and *melR(Con)* promoters (21). Although the three-dimensional structure of  $\sigma^{70}$  has not yet been solved, Lonetto et al. (21) recently proposed two alternative model structures based on the highly similar DNA binding regions of the NarL and Cro proteins (3, 24). According to both models, residues K593 and K597 belong to a surface-exposed patch, thus providing a target for interaction with activator proteins such as

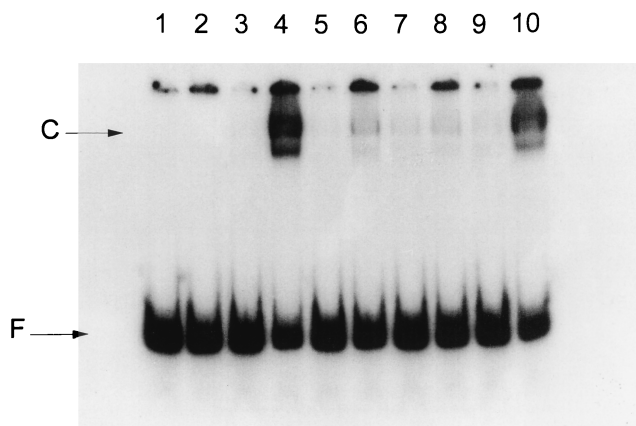


FIG. 4. Gel retardation assays performed with reconstituted RNA polymerase. Odd-numbered lanes, no  $m^e$ Ada; even-numbered lanes,  $0.2 \mu M$   $m^e$ Ada. Lanes 1 and 2, no RNA polymerase; lanes 3 and 4, wild-type  $E\sigma^{70}$ ; lanes 5 and 6,  $E\sigma^{70}_{KA593}$ ; lanes 7 and 8,  $E\sigma^{70}_{KA597}$ ; lanes 9 and 10,  $E\sigma^{70}_{RA603}$ . F, unbound *alkA* promoter DNA; C, RNA polymerase-*alkA* complex.

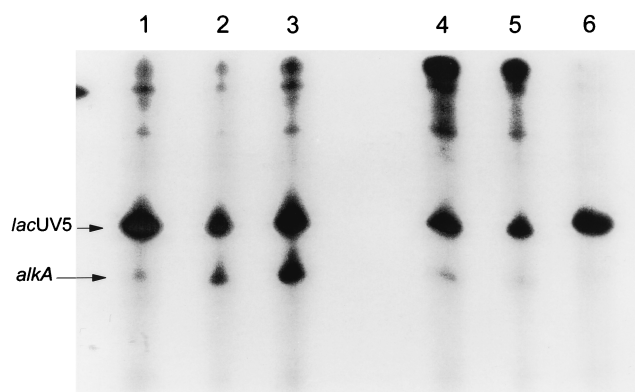


FIG. 5. In vitro transcription with  $E\sigma^{70}$  (lanes 1 to 3) and  $E\sigma^S$  (lanes 4 to 6) forms of RNA polymerase. Lanes 1 and 4, no Ada protein; lanes 2 and 5,  $0.2 \mu M$  Ada; lanes 3 and 6,  $0.2 \mu M$   $m^e$ Ada. The positions of the main *lacUV5* and *alkA* transcripts are indicated.

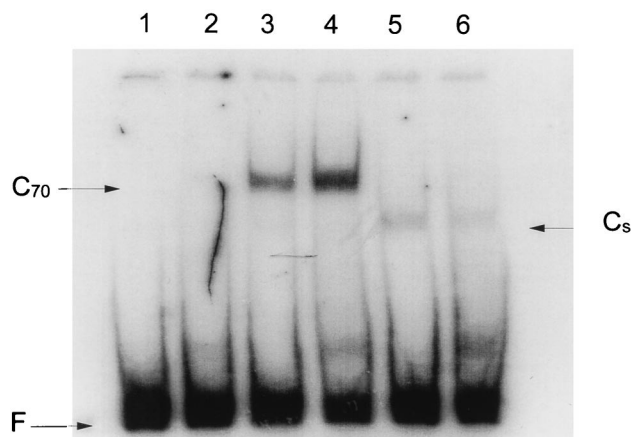


FIG. 6. Gel retardation assays performed with  $E\sigma^{70}$  and  $E\sigma^S$  forms of RNA polymerase. Odd-numbered lanes, no  $^{m^c}$ Ada; even-numbered lanes,  $0.2 \mu\text{M}$   $^{m^c}$ Ada. Lanes 1 and 2, no RNA polymerase; lanes 3 and 4,  $E\sigma^{70}$ ; lanes 5 and 6,  $E\sigma^S$ . F, unbound *alkA* promoter DNA;  $C_{70}$  and  $C_S$ ,  $E\sigma^{70}$ -*alkA* and  $E\sigma^S$ -*alkA* complexes, respectively.

Ada, while R603 is removed from the protein surface and in closer contact with the helix-turn-helix DNA binding motif (21). The location of the R603 residue would suggest that the RA603 substitution can affect *alkA* transcription by altering the general conformation of region 4, consistent with the effects of the RA603 mutation on factor-independent transcription at some promoters (21) and with the results of gel retardation experiments at the *alkA* promoter (Fig. 4).

To our knowledge, the Ada protein is the first example of a transcription activator able to contact two distinct determinants in the  $\sigma^{70}$  subunit of RNA polymerase in a promoter-specific fashion. The use of different targets in region 4 of  $\sigma^{70}$  appears to depend upon the location of the Ada binding site, which in the *alkA* promoter is positioned between  $-47$  and  $-35$ , i.e., one helical turn downstream compared to the Ada binding site in *ada* and *aidB* (1, 9, 14, 25). Additional contributions to transcription activation of the *alkA* promoter are provided by interactions between the  $\alpha$  subunit of RNA polymerase and the Ada protein (17). It is significant that other transcription activators, such as the Mor protein of bacteriophage Mu and CRP, also display the ability to interact with both  $\alpha$  and  $\sigma^{70}$  subunits, suggesting that this might be a common feature for activators whose binding site overlaps the  $-35$  sequence (2, 5, 11).

The presence of two distinct activation targets in  $\sigma^{70}$  determines the ability by Ada to activate transcription by RNA polymerase containing  $\sigma^S$  ( $E\sigma^S$ ).  $\sigma^S$  is the main  $\sigma$  subunit of RNA polymerase during stationary phase (4, 19). The methylated form of the Ada protein is able to activate transcription by  $E\sigma^S$  as well as  $E\sigma^{70}$  at both the *ada* and *aidB* promoters (16, 31). These observations are consistent with the fact that the  $\sigma^{70}$  amino acids important for activation by  $^{m^c}$ Ada are also conserved in  $\sigma^S$  (18). In contrast, we have shown in this study that  $^{m^c}$ Ada is unable to recruit  $E\sigma^S$  to the *alkA* promoter (Fig. 6), thus failing to stimulate *alkA* transcription by  $E\sigma^S$  (Fig. 5), consistent with the lack of conservation of K593, K597, and R603 in  $\sigma^S$ .

What is the physiological reason for the lack of *alkA* transcription by  $E\sigma^S$ ? It has been proposed recently that low levels of endogenous methylation damage of DNA take place during stationary phase, possibly through amino acid nitrosation (31), resulting in weak induction of *aidB* and *ada*, but not of *alkA*, at

the onset of stationary phase (12a, 31, 33). In stationary phase, it might be more efficient for *E. coli* to prevent the accumulation of endogenously produced methylating agents than to repair DNA damage by expression of the *alkA* gene. Thus, *aidB*, which is responsible for detoxification of some methylating agents (13), and AlkB, a membrane protein cotranscribed with *ada* and possibly involved in their excretion (34), would be preferentially expressed during stationary phase.

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