Expression of the Escherichia coli Ada Regulon in Stationary Phase: Evidence for rpoS-Dependent Negative Regulation of alkA Transcription

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The Escherichia coli Ada protein activates σ70-dependent transcription at three different promoters (ada, aidB, and alkA) in response to alkylation damage of DNA. During stationary phase, however, the methylated form of Ada shuts off expression of alkA; this repression is specific for σ5-dependent transcription. Thus, at the alkA promoter, the Ada protein can act as both a positive and negative modulator of the adaptive response to alkylation damage, depending on the cell’s physiological state.

The adaptive response of Escherichia coli to alkylation damage of DNA is regulated by the Ada protein. This response protects cells from the mutagenic and cytotoxic effects of alkylating agents present in the environment and from reactive endogenous metabolites (9, 12, 13). The Ada protein is both a DNA repair protein and a transcriptional regulator. Ada is able to transfer methyl groups from DNA to two of its own cysteine residues (10). Upon self-methylation, Ada stimulates transcription of four genes, ada, aidB, alkA, and alkB (whose transcription is directed by the ada promoter) (10, 15). The target site for Ada activation on RNA polymerase is the σ subunit. However, the determinants in both the Ada protein and RNA polymerase that are required for transcription activation at the ada and aidB promoters differ from the determinants required at alkA. A negatively charged set of amino acids in σ70 is the target for Ada activation at ada and aidB, while a positively charged cluster interacts with Ada at the alkA promoter (6, 7). At the ada and aidB promoters, the methylated form of Ada (meAda) is essential for transcription activation, whereas transcription from the alkA promoter is activated by both the unmethylated and methylated forms (4, 10). Finally, the C-terminal domain of Ada, which is necessary for activation at the ada and aidB promoters, is dispensable at alkA (1). Thus, the Ada protein activates different promoters by different mechanisms.

Previous work on Ada-dependent regulation had focused on exponentially growing cells in which σ50 is the major functional σ factor. We have now investigated the regulation of Ada-dependent promoters in stationary-phase cells. During stationary phase, the levels of an alternative σ factor, σ5 (8), rise to about 30 to 40% of σ70 levels. In addition, several changes in the cellular environments, such as increased concentrations of glutamate and polyphosphate, a decrease of DNA superhelicity, and increased levels of Rsd, an anti-σ factor specific for σ70, contribute to preferential utilization of σ5 (3). Previous in vitro studies had shown that meAda can activate σ5-dependent transcription at ada and aidB but not at alkA (5, 7, 13). To measure Ada-activated, σ5-dependent transcription in vivo, we used either an rpoS-deficient strain (MV2792 [16]) or the corresponding wild type (MV1161) transformed with derivatives of the low-copy-number plasmid pRS1274 carrying the ada, aidB, or alkA promoter regions as previously described (4, 7). The rpoS mutation does not cause detectable variations in pRS1274 copy number in stationary phase nor does it affect the in vivo expression of the Ada-independent lacUV5 promoter (data not shown). Cells were grown in LB medium, and 0.04% of the alkylating agent methyl methanesulfonate (MMS) was added at an optical density at 600 nm (OD600) of 0.04 (to study exponential phase) or 1.0 (to study stationary phase) (Fig. 1).

In exponential phase, MMS clearly induces expression from the ada, aidB, and alkA promoters (Fig. 1A, C, and E). Expression from the ada and alkA promoters was not affected by the rpoS mutation, while aidB expression was reduced, consistent with previous observations (5, 16). It is important to note that, when added to exponentially growing cells, 0.04% MMS result in only a slight reduction in the growth rate (to about 75%), which is insufficient to cause a sharp increase in rpoS expression. However, a relatively small increase of σ5 might be sufficient for full activation of aidB transcription, due to the strong affinity of RNA polymerase containing σ5 (Eσ5) for the aidB promoter (5).

A more complex pattern emerged when cells were treated with MMS at the onset of stationary phase. In the absence of MMS induction, levels of Ada-independent expression were two- to threefold higher in the wild type than in rpoS strains. This effect is independent of the presence of a functional ada gene (data not shown). These results are in agreement with previous observations that Eσ5 is able to carry out transcription from the ada and aidB promoters even in the absence of meAda (5, 13) and suggests a similar situation for alkA. MMS treatment had very different effects on transcription at different promoters. With the aidB promoter, clear induction that is strongly dependent on a functional rpoS gene is observed, mirroring the behavior observed in exponential phase. With the ada promoter, induction was found in both the wild-type and rpoS strains, again similar to the situation in exponential phase. In sharp contrast, treatment with MMS failed to induce expression of alkA in the wild-type strain, while in the rpoS strain alkA expression was increased by ca. 7.5-fold (Fig. 1F).

The negative effect of the rpoS gene on Ada-dependent transcription of alkA was surprising. Although the Ada protein is known to be unable to stimulate Eσ5-dependent transcription from the alkA promoter in vitro (7), we expected some induction in vivo, since Eσ50 is also available. We performed in
vivo transcription experiments to compare Eσ^70 and Eσ^S, using the conditions described previously (7). The results summarized in Fig. 2 show that Eσ^S is more efficient than Eσ^70 in carrying out transcription from the alkA promoter in the absence of Ada and that the methylated form of the Ada protein represses Eσ^S-dependent transcription approximately three-fold. Confirmation that m^6Ada acts as a specific repressor for Eσ^S came from gel retardation experiments with Eσ^70 and Eσ^S forms of RNA polymerase. The results in Fig. 3 show that no interaction between Eσ^70 and the alkA promoter was detected in the absence of Ada (lane 4). In contrast, incubation with Eσ^S resulted in an almost complete retardation of the promoter fragment (Fig. 3, lane 7); several bands are detectable, possibly a consequence of partial dissociation of the binary complex during electrophoresis or formation of higher orders of Eσ^S-alkA complexes. Addition of either form of the Ada protein stimulated binding of Eσ^70 to alkA (Fig. 3, lanes 5 and 6). In contrast, addition of m^6Ada, but not of the unmethylated form of the protein, resulted in the dissociation of the Eσ^S-alkA binary complex (Fig. 3, lanes 8 and 9).

Our previous study (7) showed that the activation of the alkA promoter is dependent on the interaction between Ada and a positively charged cluster in σ^70. The fact that this cluster is not conserved in σ^S neatly explains why Ada is unable to activate transcription by Eσ^S at the alkA promoter. Our present results show that m^6Ada negatively regulates Eσ^S-dependent transcription of alkA, and this mechanism is used by E. coli to down-regulate alkA. Since methylation of Ada is irreversible, cells have the problem of shutting off the adaptive response once alkylation damage has been removed. Two different mechanisms have already been proposed: proteolytic cleavage of m^6Ada in the hinge region linking the N-terminal to the C-terminal domain of the protein (1), and negative regulation of the ada gene by the unmethylated form of the Ada protein (11). However, since both the N-terminal fragment of m^6Ada and the unmethylated form of the protein can activate transcription at alkA, it was postulated that the simple dilution of the Ada protein over several growth cycles must eventually result in the return to basal levels of expression from alkA (1, 11). The function of m^6Ada as a negative regulator of Eσ^S-dependent transcription suggests an alternative mechanism: alkA expression is activated as long as transcription is mostly σ^70 dependent. When cells reach stationary phase, concentrations of Eσ^S increase. Eσ^S can bind and carry out transcription.

FIG. 1. In vivo expression of the adaptive response genes. (A and B) ada; (C and D) aidB; (E and F) alkA. (A, C, and E) cultures in exponential phase at time 0 (OD_{600}, 0.04); (B, D, and F) cultures in stationary phase at time 0 (OD_{600}, 1.0). Symbols: diamonds, wild-type strain; squares, rpoS strain; open symbols, no MMS added (Ada-independent transcription); closed symbols, 0.04% MMS added at time zero. Data shown are from a typical experiment.
from the \( \text{alkA} \) promoter (Fig. 2 and 3); however, \( ^{\text{m}} \text{Ada} \) prevents \( \text{Er}^{\text{S}} \) from binding to \( \text{alkA} \) (Fig. 3), thus reducing the amount of RNA polymerase available for \( \text{alkA} \) transcription. This results in low levels of \( \text{alkA} \) transcription in stationary phase even upon \( \text{MMS} \) treatment. Negative regulation by a functional \( \text{rpoS} \) gene has been observed for another \( \sigma^{70} \)-dependent gene, \( \text{uspA} \) (2), suggesting that \( \sigma \) factors might indeed compete for a limiting amount of RNA polymerase during stationary phase. The need to express \( \text{alkA} \) only in the exponential phase of growth might be due to the role of the \( \text{AlkA} \) protein, whose main function is to remove 3-methyladenine DNA glycosylase, encoded by the constitutive gene \( \text{tag} \), might be sufficient for the repair of this lesion, making high-level expression of the \( \text{alkA} \) gene no longer necessary.

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**REFERENCES**


