A Mutation in the *rpoA* Gene Encoding the α Subunit of RNA Polymerase That Affects *metE-metR* Transcription in *Escherichia coli*

SAMINA JAFRI,^{1,2} MARK L. URBANOWSKI,¹ AND GEORGE V. STAUFFER^{1,2*}

Department of Microbiology¹ and Genetics Ph.D. Program,² The University of Iowa, Iowa City, Iowa 52242

Received 22 August 1994/Accepted 19 November 1994

The DNA-binding protein MetR belongs to the LysR family of transcriptional activators and is required for expression of the *metE* and *metH* promoters in *Escherichia coli*. However, it is not known if this activation is mediated by a direct interaction of MetR with RNA polymerase. In a search for RNA polymerase mutants defective in MetR-mediated activation of the *metE* gene, we isolated a mutation in the α subunit of RNA polymerase that decreases *metE* expression independently of the MetR protein. The mutation does not affect expression from the *metH* promoter, suggesting that the α subunit of RNA polymerase interacts differently at these two promoters. The mutation was mapped to codon 261 of the *rpoA* gene, resulting in a change from a glutamic acid residue to a lysine residue. Growth of the mutant is severely impaired in minimal medium even when supplemented with methionine and related amino acids, indicating a pleiotropic effect on gene expression. This *rpoA* mutation may identify either a site of contact with an as yet unidentified activator protein for *metE* expression or a site of involvement by the α subunit in sequence-specific recognition of the *metE* promoter.

Methionine biosynthesis in *Escherichia coli* and *Salmonella typhimurium* involves both positive and negative transcriptional control. Positive control is mediated by the *metR* gene product, a DNA-binding protein that belongs to the LysR family of bacterial activator proteins (10). MetR acts in *trans* to activate *metE* and *metH* gene expression (5, 20, 41). Homocysteine, an intermediate in the methionine pathway, functions as a coregulator, increasing expression of *metE* and decreasing expression of *metH* (6, 39). The MetR binding sites for *metE* and *metH* have been defined genetically and biochemically; a single binding site lies adjacent to the RNA polymerase binding site for the *metH* gene (4), and two adjacent binding sites overlap the -35 promoter region for the *metE* gene (40, 45). It is possible that the MetR-mediated activation of the *metE* and *metH* genes involves a direct interaction of MetR with RNA polymerase, although there is no genetic or biochemical evidence to support this model.

Negative control is mediated by the *metJ* gene product, with S-adenosylmethionine as the corepressor (27). MetJ represses most of the *met* genes, including *metE* and *metR* (27, 44), but does not have a direct effect on *metH* expression (38).

The *metE* and *metR* promoters overlap and are divergently transcribed from a common regulatory region (23). It has been shown that the *metR* gene is negatively regulated by its own gene product (37), and genetic experiments suggest that the mechanism of this repression may be by steric hindrance of the *metR* promoter by MetR-RNA polymerase bound to the *metE* promoter (40). Thus, MetR may play only an indirect role in autoregulation, its main function being to facilitate transcription by RNA polymerase at the *metE* promoter.

For several bacterial activators, studies suggest that the site of interaction of RNA polymerase is localized in the α subunit of the RNA polymerase complex, which is encoded by the *rpoA*

gene. Studies indicate that the amino-terminal two-thirds of the α subunit is sufficient for the formation of active enzyme molecules (9, 12, 13). The carboxy-terminal region has been proposed to interact with a number of transcriptional activators that bind upstream of the promoter region (14-17). CRP, the cyclic AMP receptor protein that regulates the expression of a number of catabolite-sensitive operons, is known to activate the *lac* operon by direct interaction with the α subunit of the RNA polymerase complex (15, 46). Genetic evidence suggests that there is a direct interaction between the C-terminal region of the α subunit of RNA polymerase and the bacteriophage P2 *ogr* gene product, a positive regulator of transcription from phage P2 and P4 late promoters (1). Mutations that specifically affect transcriptional control by OmpR and EnvZ, a two-component regulatory system that controls porin gene expression in *E. coli*, have also been isolated and lie in the same region of *rpoA* (28, 30). There is evidence for a similar interaction between the α subunit of RNA polymerase and FNR, a transcriptional activator that controls the expression of certain anaerobically induced genes in *S. typhimurium* (18). These and other results suggest that the C-terminal domain of the α subunit is involved in the positive control of transcription by directly interacting with transcriptional activators. A set of activators that make contacts with this region of the α subunit have been termed class I transcription factors, and in most cases they have been shown to bind just upstream of the promoter -35 region (17). More recently there have been reports of the α subunit making direct contact with DNA (3, 26). An AT-rich sequence located upstream of the -35 region of the *E. coli* rRNA promoter *rmB* P1 stimulates transcription in the absence of any accessory proteins, and mutations in the carboxy-terminal region of the α subunit prevent this stimulation (26). Since mutations that block activator interactions as well as mutations that inhibit stimulation by upstream elements fall in the same region of the α subunit, the two mechanisms may be related.

In this study, we describe a mutation in the α subunit of

* Corresponding author. Phone: (319) 335-7791. Fax: (319) 335-9006.

TABLE 1. *E. coli* strains used

Strain	Relevant genotype ^a	Source
GS162	Wild type	This laboratory
GS722	<i>metJ97 metB1 ΔmetF::Mu</i>	This laboratory
GS244	<i>ΔmetR::Mu</i>	This laboratory
GS597	<i>metJ97</i>	This laboratory
GS748	<i>metJ97 ΔmetR::Mu</i>	This laboratory
GS1040	<i>rpoA(E261K)</i>	This laboratory
GS1041	<i>metJ97 metB1 ΔmetF::Mu rpoA(E261K) λmetR-lacZ</i>	This laboratory
SP312	<i>Δ(gal-bio)76 relA1 spoT1 thi-1 deoC76 Δ(deoD-trpR)251</i>	R. L. Somerville
JMS4540	<i>F⁻ Δ(argF-lac)U169 rpsL150 relA flb5301 ptsF25 deoC1 φ(ompF'-lacZ⁺)16-13 zhc-3::Tn10</i>	T. J. Silhavy
SA2600	<i>F⁻ his rpsL relA</i>	S. Adhya
SA2777	<i>F⁻ his rpsL relA Δcrp::Cm</i>	S. Adhya

^a All GS strains also carry the *thi pheA905 ΔlacU169 araD129*, and *rpsL150* mutations.

RNA polymerase that affects *metE-metR* transcription in *E. coli*. The effects are not due to the inability of the α subunit to interact with the MetR protein. Possible models for altered regulation are discussed.

MATERIALS AND METHODS

Bacterial strains. *E. coli* strains used in this study are described in Table 1.

Media and growth conditions. The complex media used were Luria broth (LB) and tryptone broth (21). The minimal medium used was Vogel and Bonner minimal salts (42) supplemented with 0.4% glucose (GM). GM was always supplemented with phenylalanine and thiamine, since most strains carry the *pheA905* and *thi* markers. Supplements and antibiotics were added where indicated at the following concentrations: L-methionine, 50 μ g/ml; phenylalanine, 50 μ g/ml; thiamine, 1 μ g/ml; Casamino Acids, 0.1%; tetracycline, 10 μ g/ml; chloramphenicol, 30 μ g/ml; ampicillin, 100 μ g/ml; streptomycin, 25 μ g/ml; and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal), 25 μ g/ml. Growth rates were determined by monitoring the optical density at 600 nm of aerobically growing cultures.

Construction of λ lysogens. Bacterial strains were lysogenized with a *λmetE-lacZ*·*metR-uid* fusion as previously described (36). This phage, hereafter referred to as *λmetE-lacZ*, is isogenic to the *λmetE-lacZ*·*metR-galK* fusion described previously (40) except that it carries the promoterless *uidA* reporter gene of *E. coli* instead of the *galK* reporter gene (11). Strains were also lysogenized with *λmetR-lacZ* (37), *λmetH-lacZ* (38), *λmetB-lacZ* (36), *λmetA-lacZ* (19), *λmetC-lacZ* (22), *λmetF-lacZ* (31), *λglyA-lacZ* (33), or *λgcvT-lacZ* (32) bacteriophage. All λ lysogens carry the c1857 mutation resulting in a temperature-sensitive repressor and were grown at 30°C. After single-colony purification, lysogens were tested for the presence of a single copy of λ phage by testing their ability to support lytic infection by λ c190c17 (29).

P1 transductions. Standard methods were used for P1 transduction experiments (21).

Nitrosoguanidine mutagenesis. Cultures of *E. coli* JMS4540 carrying Tn10 inserted near *rpoA* were mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine as described previously (21).

β -Galactosidase enzyme assay. β -Galactosidase enzyme activity was assayed from log-phase cultures harvested at an optical density of approximately 0.5 as described by Miller (21), using the chloroform-sodium dodecyl sulfate lysis procedure. All results are averages from at least two separate assays in which the activity of each sample was determined in triplicate. Standard deviations between assays varied by less than 10% of the reported means.

PCR amplification and DNA sequence analysis. Chromosomal DNA was isolated from 100 μ l of *E. coli* cells grown in LB overnight. The cell pellet was resuspended in 100 μ l of digestion buffer (50 mM Tris-HCl [pH 8.5], 1 mM EDTA, 5% Tween 20, 200 μ g of proteinase K per ml) and incubated for 3 h at 55°C. The mixture was heated to 95°C for 10 min to inactivate the proteinase K. Two microliters of the supernatant was used for amplification of the *rpoA* gene by PCR. The primers used were based on the sequence of the *E. coli* genes flanking *rpoA*. Primer A (5'-CCGGATCCACCTGATCGTCGAGCTTTAC TCC-3') originated from the 3' prime end of the *rpsD* gene and carries a *Bam*HI site near the 5' end. Primer B (5'-GGCCCCGAGCCACTCTTACGATGGCGC ATGACC-3') originated from the 5' end of the *rplQ* gene and carries an *Ava*I site of pBR322 near the 5' end. Amplifications were carried out in a total volume of 100 μ l, containing 2 μ l of chromosomal DNA, under standard conditions

(Perkin-Elmer Cetus, Norwalk, Conn.). DNA was denatured for 1 min at 95°C, hybridized for 1 min at 55°C, and extended for 2 min at 72°C for 30 cycles. Each amplification reaction yielded a prominent 1-kb product which was purified on a 1% low-melting-temperature agarose gel. DNA fragments were directly sequenced by using an automated DNA sequencer (model 373A DNA sequencer; Applied Biosystems, Foster City, Calif.) at the University of Iowa Core Facility. The entire *rpoA* gene was sequenced by using primers A and B and an internal primer (5'-GAATAAATCTGGCATTGGCC-3') beginning at base 306 from the 5' end of the *rpoA* gene.

RESULTS

Mutant selection. For several bacterial activators, it has been suggested that the site of interaction of the activator with RNA polymerase is localized in the α subunit in RNA polymerase (14, 16, 17). Hence, we targeted the *rpoA* gene, which encodes the α subunit, in a search for mutants defective in MetR-mediated activation. The selection for *rpoA* mutations took advantage of the fact that activation of *metE* by the MetR protein results in a reciprocal repression from the divergently transcribed *metR* promoter (40). Strain GS722 is a *metJ metB metF* mutant. The *metJ* mutation prevents methionine-mediated repression, and the *metF* mutation results in the accumulation of homocysteine when cultures are supplemented with methionine (this combination results in maximal expression of the *metE* promoter). This strain also carries a deletion of the chromosomal *lacZ* gene. Strain GS722 was lysogenized with a λ phage carrying a *metR-lacZ* gene fusion, in which *lacZ* expression is under the control of the *metR* regulatory region. This lysogen gives light blue colonies on L-agar plates supplemented with X-Gal because the MetR protein activates the *metE* promoter and represses the *metR-lacZ* fusion. Strain JMS4540 contains the *zhc-3::Tn10* insertion that is approximately 65% linked to *rpoA* by P1 transduction. JMS4540 was mutagenized with nitrosoguanidine, a P1 *cml clr-100* phage lysate was prepared on the mutagenized cells, and this lysate was then used to transduce GS722 lysogenized with the *λmetR-lacZ* fusion phage. Tetracycline-resistant (*Tc*^r) transductants were selected on L agar supplemented with tetracycline and X-Gal. Approximately 92,000 *Tc*^r transductants were screened, and one dark blue mutant with increased β -galactosidase activity was isolated. The mutant was designated GS1041.

Strain GS1041 was initially tested for growth on GM supplemented with methionine, since we intended to grow strains in this medium for β -galactosidase assays. However, the mutant failed to grow on GM plus methionine. Even supplementation of GM with Casamino Acids resulted in only poor growth, suggesting that the mutation is pleiotropic. To confirm that the mutation had increased *metR-lacZ* expression, the mutant and parent lysogens were grown in tryptone broth, and β -galactosidase levels were determined. *metR-lacZ* expression was increased about fourfold, from 256 U of activity in the parental lysogen to 969 U in the mutant.

Mapping the mutation by P1 transduction. An initial transduction experiment was carried out to see if the mutation responsible for the inability of GS1041 to grow on GM supplemented with methionine or Casamino Acids is cotransducible with the *zhc-3::Tn10* element. A P1 *cml clr-100* phage lysate was prepared on lysogen GS1041 and used to transduce strain GS162. *Tc*^r transductants were selected on L agar containing tetracycline and scored on GM. About 65% (127 of 200) of the *Tc*^r transductants failed to grow on GM plates (i.e., were GM⁻), suggesting that the mutation responsible for the inability to grow on GM is cotransducible with the *zhc-3::Tn10* element with the same frequency reported for the *rpoA* gene (30). One *Tc*^r GM⁻ transductant was saved and designated GS1040.

To establish that the mutation maps in the *rpoA* gene, we did

TABLE 2. Effect of the *rpoA*(E261K) mutation on *metE* expression

Strain ^a	<i>met</i> genotype	β -Galactosidase activity (Miller units)
GS162	Wild type	43
GS162 <i>rpoA</i> (E261K)		5
GS597	<i>metJ</i>	9,071
GS597 <i>rpoA</i> (E261K)		1,806
GS748	<i>metJ metR</i>	431
GS748 <i>rpoA</i> (E261K)		49

^a Cells were grown in tryptone broth; all carried a λ *metE-lacZ* fusion.

a three-factor cross using P1 *cml clr-100* phage grown on GS1040 (*rpsL* [streptomycin resistant {Str^r}], *zhc-3::Tn10* GM⁻) as a donor and SP312 (Tc^s Str^s) as the recipient. Transductants were selected on L agar supplemented with tetracycline and then scored for Str^r and the ability to grow on GM plates supplemented with methionine. The gene order at min 72 to 74 of the *E. coli* chromosome is known to be *rpoA zhc-3::Tn10 rpsL* (2, 30). Of 160 Tc^r transductants scored, 104 failed to grow on GM plates supplemented with methionine. Of the 104 Tc^r transductants that failed to grow on GM plus methionine (and thus had acquired the new mutation), 96 were Str^r and 8 were Str^s. The *zhc-3::Tn10* and *rpsL* mutations are 87% co-transducible. If the new mutation was on the opposite side of Tn10 relative to *rpoA*, four crossovers would be required to generate a GM⁻ Tc^r Str^s transductant. Since 8% of the GM⁻ Tc^r transductants are Str^s, the result suggests that the mutation lies in or near *rpoA*.

Nucleotide sequence of the *rpoA* mutation. To verify that the mutation lies in the *rpoA* gene and to identify the change in the DNA sequence responsible for the mutant phenotype, the *rpoA* genes from the wild type and the mutant were amplified by PCR and the products were sequenced (see Materials and Methods). A single G-to-A transition was identified in the mutant at the first nucleotide of codon 261, which results in a change from a glutamic acid residue (E) to a lysine residue (K) at amino acid 261 of the α subunit of RNA polymerase and was thus designated *rpoA*(E261K). To eliminate the possibility that the mutation was introduced in *rpoA* by PCR amplification, the gene from the mutant was independently amplified a second time, and the identical change was identified. This amino acid change lies in a small region of approximately 10 amino acids that has been shown to interact with a number of activator proteins at different promoters (17).

Effect of the *rpoA* mutation is MetR independent. To determine the effects of the mutation on *metE-lacZ* expression in a wild-type background, we tried to lysogenize strain GS1040 with a λ *metE-lacZ* fusion phage. However, GS1040 was unable to be lysogenized with this phage or with any other λ *metE-lacZ* fusion phage tested. Therefore, the P1 *cml clr-100* lysate prepared on GS1040 was used to transduce strain GS162 that had been lysogenized with the λ *metE-lacZ* phage. Tc^r transductants were selected on L agar supplemented with tetracycline and X-Gal. Tc^r blue transductants (indicating that the λ *metE-lacZ* phage was still present) were then scored on GM. One Tc^r GM⁻ blue transductant was saved for further analysis.

The *rpoA*(E261K) mutation decreased *metE-lacZ* expression in the GS162 lysogen about ninefold (Table 2). To test whether the decrease in *metE-lacZ* expression was due to the inability of the mutant RNA polymerase to be activated by MetR, we transduced the *rpoA*(E261K) mutation into strains GS597 and GS748, a *metJ* and a *metJ metR* strain, respectively. The *metJ* mutation, which inactivates the *metJ* repressor, increases the β -galactosidase levels from the *metE-lacZ* fusion sufficiently

high in the absence of the MetR activator protein to be accurately measured. The *rpoA*(E261K) mutation had a fivefold down effect on *metE-lacZ* expression in a *metJ* strain (Table 2). In lysogen GS748 λ *metE-lacZ*, however, the *rpoA*(E261K) mutation resulted in a ninefold decrease in β -galactosidase activity compared with the control lysogen (Table 2). This ninefold decrease in *metE-lacZ* expression caused by the *rpoA*(E261K) mutation is independent of the MetR protein. The results suggest that the decrease in *metE-lacZ* expression is also independent of the MetJ protein.

Phenotype of the mutant. During these studies, we observed that both the original mutant lysogen GS1041 and the Tc^r transductant GS1040 formed smaller colonies on L agar after overnight growth compared with the parental strain GS722 λ *metR-lacZ* or the wild-type strain GS162. Therefore, growth rates were determined for the parental strain GS162 and the Tc^r transductant GS1040 grown in L broth at 30°C. The *rpoA*(E261K) mutation in GS1040 resulted in a reduced growth rate, from a doubling time of 40 min for the isogenic wild-type strain GS162 to 59 min for the mutant. In addition, the mutation in GS1040 resulted in an inability to grow on L-agar plates at 42°C when the NaCl concentration was reduced from 1 to 0.5%.

We also noted several additional altered phenotypes associated with the *rpoA*(E261K) mutation. (i) growth of phage P4 is affected by this mutation (34). This effect is different from the previously reported *rpoA* mutations that block phage P2 and P4 growth, because the *rpoA*(E261K) allele preferentially blocks phage P4 growth but does not affect phage P2 (1, 7). (ii) Wild-type λ phage makes clear plaques on the *rpoA*(E261K) mutant strains. Since the *rpoA* mutation could be readily transduced into a strain lysogenized with a λ *metE-lacZ* phage, the results suggest that λ cI repressor production from the λ *p_{RM}* promoter is sufficient to maintain lysogeny and that possibly the *rpoA*(E261K) allele results in an inability to produce sufficient cI repressor for the establishment of lysogeny. A similar phenotype has been reported previously for another *rpoA* mutation, *phs*, which is a single nucleotide substitution resulting in a change from a lysine residue to a glutamic acid residue at amino acid 271 of the α subunit (43). (iii) The transformation frequency of strains carrying the *rpoA*(E261K) mutation is decreased 100-fold. (iv) Mutant strains have acquired resistance to 5 μ g of ampicillin per ml. (v) Mutant cells have a distinct colony morphology under a dissecting microscope; single colonies on Luria agar appear granular, and the edges are undulate.

The mutant phenotype is due to the *rpoA*(E261K) allele. The *rpoA*(E261K) allele was generated by nitrosoguanidine mutagenesis. Nitrosoguanidine is an alkylating agent that acts preferentially at DNA-replicating forks and is known to produce clusters of closely linked mutations in DNA (8). Since the *rpoA* gene is flanked by a number of ribosomal genes, it is possible that the resultant phenotype observed is due to a secondary mutation and not due to the *rpoA*(E261K) allele. To show that the mutant phenotype is associated with the *rpoA*(E261K) allele, we selected possible revertants of lysogen GS162 λ *metE-lacZ* carrying the *rpoA*(E261K) allele that were able to grow on GM. A GM⁺ colony in which the *rpoA*(E261K) allele could not be separated by P1 transduction from the new mutation that allows growth on GM was chosen for further analysis. This strain showed a normal colony morphology on L agar and a normal generation time in tryptone broth relative to an isogenic control lysogen, the transformation frequency had been restored, and phage λ was able to lysogenize the strain.

The *rpoA* gene from this strain was amplified by PCR (see Materials and Methods), and the amplified product was se-

TABLE 3. Effect of the *rpoA(E261K)* mutation on expression of *met* genes

Strain ^a	Gene fusion	<i>met</i> genotype	β-Galactosidase activity (Miller units)
GS162	<i>λmetA-lacZ</i>	Wild type	11
GS162 <i>rpoA(E261K)</i>	<i>λmetA-lacZ</i>		42
GS162	<i>λmetB-lacZ</i>	Wild type	87
GS162 <i>rpoA(E261K)</i>	<i>λmetB-lacZ</i>		167
GS162	<i>λmetC-lacZ</i>	Wild type	12
GS162 <i>rpoA(E261K)</i>	<i>λmetC-lacZ</i>		40
GS162	<i>λmetF-lacZ</i>	Wild type	31
GS162 <i>rpoA(E261K)</i>	<i>λmetF-lacZ</i>		23
GS162	<i>λmetH-lacZ</i>	Wild type	43
GS162 <i>rpoA(E261K)</i>	<i>λmetH-lacZ</i>		68
GS244	<i>λmetH-lacZ</i>	<i>metR</i>	21
GS244 <i>rpoA(E261K)</i>	<i>λmetH-lacZ</i>		24

^a Cells were grown in tryptone broth.

quenced. A single A-to-C transversion occurred again at the first nucleotide of codon 261, resulting in a change from the lysine codon at position 261 to a glutamine codon. Although this is not a true revertant, it is unlikely that in this strain there was a secondary mutation responsible for the mutant phenotype and linked to the *rpoA* allele that also reverted simultaneously. Hence, the phenotype of the *rpoA(E261K)* mutant strain is likely due to the *rpoA(E261K)* allele.

The *rpoA(E261K)* mutation is not a general down mutation. To test whether the *rpoA(E261K)* allele was a general down mutation for transcription, we tested its effects on other *met* genes by transducing the mutation into strain GS162 lysogenized with different *met-lacZ* fusion phage. All of the *met-lacZ* fusions tested, except the *metF-lacZ* fusion, showed increased β-galactosidase levels ranging from 1.5- to 4-fold (Table 3) compared with control lysogens. In strains carrying the *rpoA(E261K)* mutation, increased expression of the *metH-lacZ* gene fusion is possibly due to increased levels of MetR protein, since the *rpoA(E261K)* allele also results in a fourfold increase in expression of the *metR-lacZ* fusion. We tested this hypothesis by examining the effect of the *rpoA* mutation in a *metR* background. In the absence of MetR activator (GS244 *λmetH-lacZ*), the *rpoA* mutation had no stimulatory effect (Table 3), indicating that the 1.6-fold increase seen in the *metR*⁺ lysogen GS162 *λmetH-lacZ* in the presence of the *rpoA(E261K)* mutation may be due to an increase in MetR levels. This result shows that the *rpoA(E261K)* mutation is not a down mutation for transcription in general. We are attempting to determine why *metA-lacZ*, *metB-lacZ*, and *metC-lacZ* expression is elevated in the GS162 *rpoA(E261K)* strain, since the increased expression of the *metA-lacZ* gene fusion in the mutant was found to be independent of the MetR protein (data not shown), and the *metB* and the *metC* genes are not known to be regulated by MetR.

Effects of the *rpoA(E261K)* mutation on *glyA-lacZ* and *gcvT-lacZ* expression. We determined the effects of the *rpoA(E261K)* mutation on the regulation of two other genes involved in one-carbon metabolism. The *rpoA(E261K)* mutation was transduced into wild-type GS162 lysogenized with either a *glyA-lacZ* fusion, monitoring expression of *glyA* (encoding serine hydroxymethyltransferase), or a *gcvT-lacZ* fusion, monitoring expression of *gcv* (encoding the glycine cleavage enzyme system). In the GS162 lysogen, the *rpoA(E261K)* mutation resulted in a 1.4-fold increase in the level of expression from the *glyA-lacZ* fusion (Table 4). It is known that MetR is

TABLE 4. Effect of the *rpoA(E261K)* mutation on *glyA* and *gcvT* expression

Strain ^a	Gene fusion	<i>met</i> genotype	β-Galactosidase activity (Miller units)
GS162	<i>λglyA-lacZ</i>	Wild type	1,827
GS162 <i>rpoA(E261K)</i>	<i>λglyA-lacZ</i>		2,473
GS244	<i>λglyA-lacZ</i>	<i>metR</i>	1,619
GS244 <i>rpoA(E261K)</i>	<i>λglyA-lacZ</i>		1,690
GS162	<i>λgcvT-lacZ</i>	Wild type	528
GS162 <i>rpoA(E261K)</i>	<i>λgcvT-lacZ</i>		1,203
GS244	<i>λgcvT-lacZ</i>	<i>metR</i>	595
GS244 <i>rpoA(E261K)</i>	<i>λgcvT-lacZ</i>		475

^a Cells were grown in tryptone broth.

required for full expression of the *glyA* gene (24). To determine if the increased expression is due to overproduction of MetR due to the *rpoA(E261K)* mutation, we also transduced the *rpoA* mutation into the *metR* strain GS244 lysogenized with the *glyA-lacZ* fusion. Expression of the *glyA-lacZ* fusion was nearly identical in the GS244 and GS244 *rpoA(E261K)* lysogens (Table 4).

A role for MetR in *gcv* expression has not been demonstrated (24). Consistent with this earlier result, *gcvT-lacZ* expression is nearly identical in GS162 and GS244 lysogens (Table 4). However in the GS162 lysogen, the *rpoA* mutation resulted in a 2.3-fold increase in *gcvT-lacZ* expression that was reduced in the GS244 *rpoA(E261K)* lysogen (Table 4). We do not know if the MetR-dependent increase in *gcvT-lacZ* expression in the presence of the *rpoA(E261K)* mutation is direct or indirect.

DISCUSSION

We have isolated a mutation in the *rpoA* gene, encoding the α subunit of RNA polymerase, that decreases *metE-lacZ* expression. The mutation, designated *rpoA(E261K)*, is a G-to-A transversion at the first nucleotide of codon 261, changing a glutamic acid residue to a lysine residue at amino acid 261 in the C-terminal domain of the α subunit of RNA polymerase. The *rpoA(E261K)* mutation is pleiotropic; strains carrying the *rpoA(E261K)* mutation fail to grow on GM medium and are defective in λ lysogenization, phage P4 growth, and transformation. Mutant strains also show altered colony morphology and are sensitive to low salt concentrations at 42°C. However, the *rpoA(E261K)* mutation is not a general down mutation for all promoters. A glutamine residue at amino acid 261 reverts most of the phenotypes back to wild type even though *metE* expression is still fivefold below normal (data not shown).

It has been reported that CRP interacts with amino acid 261 of the α subunit of RNA polymerase at the *lac* promoter. Among the mutations isolated from a screen of plasmid-encoded *rpoA* mutants defective in catabolite gene activator protein-dependent activation of the *lac* promoter, the *rpoA(E261K)* allele had the most significant down effect, and showed a threefold decrease in *lac* operon expression (35). We have assayed β-galactosidase activity in *lac*⁺ strains carrying the *rpoA(E261K)* mutation on the chromosome and confirmed a two- to threefold decrease in *lac* operon expression compared with the isogenic control strain. However, in an *rpoA(E261K)* Δ*crp*::Cm double mutant, the *rpoA(E261K)* mutation increased *lac* operon expression fourfold compared with the Δ*crp*::Cm strain (data not shown). Thus, we were unable to show whether the two- to threefold effect seen in the CRP⁺

strain was mediated by CRP. The *rpoA* mutation did not have a significant effect on the expression of the CRP-dependent *gal* and *mal* promoters, as judged by the ability of *rpoA*(E261K) mutant strains to utilize these sugars on MacConkey agar plates. These results are consistent with earlier observations that C-terminal deletions in the α subunit of RNA polymerase did not affect the ability of CRP to activate the *gal* P1 promoter (25).

The *rpoA*(E261K) allele resulted in a ninefold decrease in *metE-lacZ* expression in the wild-type strain GS162 and in the *metJ metR* lysogen GS748 (Table 2). These results suggest that the decrease in *metE-lacZ* expression caused by the *rpoA*(E261K) mutation is independent of the MetR protein, as well as the *metJ* protein, since the same decrease in *metE-lacZ* expression is seen in the absence of both of these *trans*-acting factors. Interestingly, the mutation resulted in only a fivefold decrease in *metE-lacZ* expression in the *metJ* strain; it is possible that the severity of the *rpoA*(E261K) mutation is dependent in part on the strength of the promoter. Nevertheless, the fact that *metE-lacZ* expression is reduced 37-fold in a *metJ metR rpoA*(E261K) strain compared with the *metJ rpoA*(E261K) strain shows that the MetR protein is required for *metE-lacZ* expression even in the presence of the *rpoA*(E261K) mutation and that the *rpoA*(E261K) effect on *metE* regulation is by an independent mechanism. MetR is the only known activator protein that regulates expression of the *metE* promoter. However, we have not eliminated the possibility of an unidentified activator protein required for *metE* expression that might be affected by the *rpoA*(E261K) mutation. Since, at least for the *lac* promoter, *rpoA*(E261K) reportedly disrupts CRP-mediated activation, we tested whether CRP might be an unidentified activator of *metE*. However, we found no stimulatory effect of CRP on *metE-lacZ* expression; in fact, a *crp* deletion mutation in GS162 results in a threefold increase in *metE-lacZ* expression compared with the *crp*⁺ lysogen (data not shown).

An alternative possibility is that the *rpoA*(E261K) mutation affects *metE* expression by disrupting direct protein-DNA contacts between the α subunit of RNA polymerase and the *metE* promoter DNA. Ross et al. (26) have shown that an upstream sequence rich in AT content increases promoter activity at the *E. coli* *rmB* P1 promoter and that this activation is due to direct interaction of the α subunit and this region of the DNA. An AT-rich sequence does occur just upstream of the *metE* -35 promoter region (23). Characterization of mutations in this region will be necessary to determine if this sequence affects *metE* transcription in a *metR* background.

We have recently isolated suppressor mutations that map in *rpoA* and allow growth on GM plus methionine but not GM alone. Thus, the general growth requirement seen with mutants carrying the *rpoA*(E261K) allele has been separated from the decreased *metE* expression. These suppressor strains will be used to select for possible *cis*- or *trans*-acting mutations that revert the *metE*-down phenotype and may provide evidence in support of one of the models cited above.

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