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The Glutamic Acid Residue at Amino Acid 261 of the α Subunit Is a Determinant of the Intrinsic Efficiency of RNA Polymerase at the *metE* Core Promoter in *Escherichia coli*

SAMINA JAFRI,[1,2]† MARK L. URBANOWSKI,[1] AND GEORGE V. STAUFFER[1,2]*

Department of Microbiology[1] and Genetics Ph.D. Program,[2] University of Iowa, Iowa City, Iowa 52242

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A mutation in the *rpoA* gene (which encodes the α subunit of RNA polymerase) that changed the glutamic acid codon at position 261 to a lysine codon decreased the level of expression of a *metE-lacZ* fusion 10-fold; this decrease was independent of the MetR-mediated activation of *metE-lacZ*. Glutamine and alanine substitutions at this position are also *metE-lacZ* down mutations, suggesting that the glutamic acid residue at position 261 is essential for *metE* expression. In vitro transcription assays with RNA polymerase carrying the lysine residue at codon 261 indicated that the decreased level of *metE-lacZ* expression was not due to a failure of the mutant polymerase to respond to any other *trans*-acting factors, and a deletion analysis using a λ*metE-lacZ* gene fusion suggested that there is no specific *cis*-acting sequence upstream of the −35 region of the *metE* promoter that interacts with the α subunit. Our data indicate that the glutamic acid at position 261 in the α subunit of RNA polymerase influences the intrinsic ability of the enzyme to transcribe the *metE* core promoter.

The efficiency with which transcription is initiated by RNA polymerase depends on the intrinsic strength of the promoter as well as on the influence of *trans*-acting regulatory factors. Positive control during transcription initiation is a common mechanism for the regulation of gene expression (23, 28). Although a large number of *trans*-acting regulatory proteins and their binding sites on DNA have been identified (25, 28), relatively little is known about their mechanisms of action. The involvement of direct protein-protein contacts between RNA polymerase and transcription factors has been proposed to explain transcriptional activation (27, 29), though we have limited knowledge of the mechanism involved or the sites of contact, especially in RNA polymerase. Recently, several groups have reported that the sites of interaction of a number of activators with RNA polymerase are localized in its α subunit, which is encoded by the *rpoA* gene, specifically in the carboxy-terminal region (13–16, 32, 34, 43). Studies indicate that the amino-terminal two-thirds of the α subunit is sufficient for the formation of active enzyme molecules (9, 11, 12). More recently there have been reports that the α subunit may also make contacts with DNA to activate transcription (1, 30). An AT-rich sequence located upstream of the −35 region of the *Escherichia coli* rRNA promoter *rrnB* P1 stimulates transcription in the absence of any accessory proteins, and mutations in the carboxy-terminal region of the α subunit prevent this stimulation (30).

The DNA-binding protein MetR belongs to the LysR family of bacterial activator proteins (10, 31) and is required for the activation of a number of methionine biosynthetic genes in *E. coli* and *Salmonella typhimurium* (4, 8, 21, 22, 39). For two of the genes, *metE* and *metH*, the MetR binding sites required for activation were defined genetically and biochemically and lie just upstream of the RNA polymerase binding site (3, 22, 38, 42). 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.

Previously, in a search for mutants that might delineate an interaction of the MetR protein with the α subunit of RNA polymerase, we isolated an *rpoA* mutant having a ninefold lower level of *metE-lacZ* expression than that of the wild type, even in a *metR* background (17). This mutation was shown to change a glutamic acid residue at amino acid 261 to lysine (E261K) in the C-terminal domain of the α subunit of RNA polymerase. This mutation, here designated *rpoA*(E261K), also affects the expression of other genes in the methionine and glycine cleavage pathways, though it is not a general down mutation for all promoters since regulation of a number of other genes, including *metH*, is not affected. The mutant strain fails to grow on glucose minimal medium (GM) plus methionine and results in a reduced growth rate in Luria broth (L broth), from a doubling time of 40 min for the isogenic wild-type strain GS162 to 59 min for the mutant. It also results in an inability to grow on Luria agar (L agar) plates at 42°C when the NaCl concentration is reduced from 1 to 0.5%. Growth of phage P4 is affected by this mutation, wild-type λ phage makes clear plaques on mutant strains, and transformation frequencies of strains carrying this mutation are decreased 100-fold. Mutant strains are resistant to 5 μg of ampicillin per ml and have a distinct colony morphology under a dissecting microscope (single colonies on L agar appear granular and the edges are undulate).

It has been reported that cyclic AMP receptor protein interacts with amino acid 261 of the α subunit of RNA polymerase at the *lac* promoter (36). We have, however, excluded a possible role of cyclic AMP receptor protein in mediating either a direct or indirect down regulation at the *metE* promoter (17). Both in vivo and in vitro experiments described in this paper demonstrate that the *rpoA*(E261K) mutation does not identify a site of contact with any other unidentified activator protein at the *metE* promoter, nor is it involved in an interaction with a possible DNA element upstream of the *metE* −35 promoter sequence. Our data suggest that the glutamic acid residue at amino acid 261 of the α subunit is a determi-

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

† Present address: Section of Microbiology, Cornell University, Ithaca, NY 14853.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype[a]	Source
GS162	Wild type	This laboratory
GS748	*metJ97* Δ*metR*::Mu	This laboratory
GS1040	*rpoA*(E261K)	This laboratory
GS1070	*rpoA*(E261Q)	This laboratory
GS1071	*rpoA*(E261K, V306E)	This laboratory
pHTf1α	Ap^r, ori-pBR322, ori-f1, *lppP-'lacPUV5-rpoA*	R. Ebright
pHTf1α261A	Ap^r, ori-pBR322, ori-f1, *lppP-'lacPUV5-rpoA261A*	R. Ebright
pRLG593	Ap^r, ori-pBR322, *lacPUV5*	R. Gourse

[a] All strains also carry the *thi*, *pheA905*, Δ*lacU169*, *araD129*, and *rpsL150* mutations. Ap^r, ampicillin resistance.

nant of the intrinsic efficiency of RNA polymerase at the *metE* promoter.

MATERIALS AND METHODS

Bacterial strains. All strains used in this study were derivatives of *E. coli* K-12 and are described in Table 1.

Media and growth conditions. L broth and tryptone broth have been described previously (24). The minimal medium used was Vogel and Bonner minimal salts (40) supplemented with 0.4% glucose (GM) or with lactose. GM or the similar lactose medium was supplemented with phenylalanine and thiamine, since all strains carry the *pheA905* and *thi* markers. Agar was added at 1.5% to make solid media. Supplements and antibiotics were added as follows: L-methionine, 50 μg/ml; phenylalanine, 50 μg/ml; thiamine, 1 μg/ml; Casamino Acids, 0.1%; tetracycline, 10 μg/ml; chloramphenicol, 30 μg/ml; ampicillin, 100 μg/ml; and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), 50 μg/ml.

Construction of λ lysogens. Phage λ*metE-lacZ* · *metR-uidA* is isogenic to the λ*metE-lacZ* · *metR-galK* fusion (38) except that it carries the promoterless *uidA* reporter gene of *E. coli* instead of the *galK* reporter gene (18) and was constructed as follows. Plasmid pElac · Ruid was constructed by replacing the *galK* gene in plasmid pElac · Rgal with the *uidA*-coding region from plasmid pRAJ275 (Clonetech, Palo Alto, Calif.). First, the unique *Nco*I site of pRAJ275 was filled in with the large fragment of DNA polymerase I and a 10-mer *Nhe*I linker was added. The 1.8-kb *Nhe*I-*Eco*RI DNA fragment carrying the *uidA* gene was then isolated. This fragment was ligated into the *Nhe*I and *Eco*RI sites downstream of the *metR* control region in plasmid pElac · Rgal (38). The approximately 7,700-bp fragment carrying both the *metE-lacZYA* fusion and the *metR-uidA* fusion was isolated from plasmid pElac · Ruid and cloned into the temperature-sensitive phage λgt2 by a previously described method (38). The resulting λ*metE-lacZ* · *metR-uid* fusion phage, here designated λ*metE-lacZ*, was used to lysogenize *E. coli* strains of interest as previously described (37).

Two deletion mutants, λ*metE-lacZ*Δ1 and λ*metE-lacZ*Δ2, were generated by making internal primers corresponding to two different regions upstream of the *metE* promoter. Both primers had an *Eco*RI site at their 5′ ends. These primers were used in two independent PCRs in conjunction with an internal *lacZ* primer, with plasmid pElac · Ruid as a template, to amplify approximately 300-bp DNA fragments containing the *metE-metR* regulatory regions. Each amplified product was digested with *Eco*RI and *Bam*HI, and the DNA fragment carrying the truncated *metE* gene from the original plasmid, pElac · Ruid, was isolated. This fragment was ligated into the original *lacZ* fusion vector, pMC1403 (5), at the unique *Eco*RI and *Bam*HI sites, creating in-frame fusions of the 22nd codon of *metE* with the 8th codon of *lacZ*. Each DNA fragment containing the *metE-lacZ* gene fusion and *lacYA* genes was isolated from this intermediate plasmid and cloned into the unique *Eco*RI site in the temperature-sensitive phage λgt2 as described previously (38). The resulting phages, λ*metE-lacZ*Δ1 and λ*metE-lacZ*Δ2, have deletions that start 25 bp upstream and 2 bp upstream, respectively, of the proposed −35 region of the *metE* promoter.

Two *metE* promoter mutants were constructed by megaprimer site-directed mutagenesis of the *metE-metR* control region with outside primers that included *Eco*RI and *Bam*HI restriction sites at the same locations as in the original *metE-lacZ* fusion. The mutated control regions were then cloned into the *Eco*RI and *Bam*HI sites of pMC1403 and cloned into λgt2 as described above.

All λ lysogens carry the *c*I857 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 for their ability to support lytic infection by λ *c*I90*c*17 (33).

P1 transductions. Standard methods were used for bacteriophage P1 transduction experiments (24).

Bacterial transformation. Transformation of *E. coli* was as described previously (7).

β-Galactosidase enzyme assay. β-Galactosidase enzyme activities were assayed from log-phase cultures harvested at an optical density of approximately 0.5 according to the method of Miller (24) by a 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 12% of the reported means.

PCR amplification. Chromosomal DNA was isolated from *E. coli* cells, and the *rpoA* gene was amplified by PCR as described previously (17). The primers used were based on the sequence of the *E. coli* genes flanking *rpoA*. Primer A, 5′-CCGGATCCCACCTGATCGTCGAGCTTTACTCC-3′, originated from the 3′ end of the *rpsD* gene and carries a *Bam*HI site (underlined) near the 5′ end. Primer B, 5′-GGCCCGAGCCACTCTTACGATGGCGCATGACC-3′, originated from the 5′ end of the *rplQ* gene and carries an *Ava*I site (underlined) of pBR322 near the 5′ end.

DNA sequence analysis. DNA fragments were directly sequenced with an automated DNA sequencer (model 373A; Applied Biosystems, Foster City, Calif.) at the University of Iowa Core Facility. The entire *rpoA* gene was sequenced with the above-described primers, A and B, and an internal primer, 5′-GAATAAATCTGGCATTGGCC-3′, beginning at base 306 from the 5′ end of the *rpoA* gene. The inserts in the λ fusion were sequenced with the *lacZ* primer described above.

Purification of the *rpoA*(E261K) mutant RNA polymerase. The mutant RNA polymerase holoenzyme was purified from strain GS1040 according to the procedure of Chamberlin et al. (6). The holoenzyme was incubated with a twofold excess of purified σ subunit (obtained from R. Landick) to ensure that it was σ saturated.

In vitro transcriptions. Runoff transcription assays were performed as described by Wu et al. (42). In Fig. 2, the template was a 480-bp *Bam*HI-*Mfe*I fragment from plasmid pElac · Ruid containing the *metE-metR* regulatory region cloned between two transcription terminators (the phage T7 early gene transcription terminator and the phage P22 *ant* gene transcription terminator) with the *Bgl*II and *Eco*RI sites in plasmid pSW305 (41). The resulting plasmid was cleaved at flanking *Pvu*II and *Sma*I sites to yield a 1,300-bp template. The lengths of the terminated transcripts originating from the promoters on this template were 546 and 544 bases for *metE* and 395 and 392 bases for *metR*. A 300-bp *Eco*RI-*Sst*II fragment from plasmid pGS201 (3) containing the *metH* regulatory region was cloned into the *Eco*RI-*Bpm*I site of pSW305. This plasmid was cut with *Pvu*II and *Sma*I to yield a 1,200-bp template for the synthesis of a 502-nucleotide, truncated *metH* transcript. In Fig. 3, the templates were 400-bp *Eco*RI-*Bam*HI fragments containing the *metE-metR* control region from either the mutant *−7A* promoter or the mutant *−29C* promoter. The lengths of the runoff transcripts from the promoters on these fragments were 235 and 233 bases for *metE* and 112 and 109 bases for *metR*.

Template DNA was preincubated in buffer (10 mM Tris HCl [pH 7.4], 50 mM KCl, 8 mM $MgCl_2$, 125 μg of bovine serum albumin per ml) at 37°C for 5 min. Where indicated in Fig. 2, MetR protein was added and incubated with template DNA in the presence of 2 mM D,L-homocysteine, its coactivator, for 10 min. Wild-type RNA polymerase holoenzyme (final concentration, 10 nM; Epicentre, Madison, Wis.) or the mutant RNA polymerase holoenzyme was then added as indicated in Fig. 2 and incubated for an additional 30 min to allow the formation of open complexes. Nucleotide triphosphates (final concentrations, 200 μM for ATP, GTP, and CTP and 40 μM for [^{32}P]UTP) were then added in the presence of heparin (final concentration, 50 μg/ml) and incubated for 15 min to allow one round of transcription. All reactions were carried out at 37°C. Samples were analyzed by electrophoresis on a 5% denaturing polyacrylamide gel (20). The gel was dried, and the bands corresponding to the *metE* and *metH* transcripts were analyzed with an Ambis 4000 β scanner. The amount of mutant RNA polymerase added was adjusted to give a level of transcription of the *metH* template equal to that seen with the wild-type enzyme; this amount of mutant enzyme was also shown to give a level of transcription equal to that of the wild-type enzyme with the RNA1 promoter and the *lacUV5* promoter on plasmid pRLG593 as the template.

RESULTS

Suppressor mutations in the *rpoA* gene. We previously reported that the *rpoA*(E261K) mutation results in at least six phenotypic changes from the wild-type phenotype (Table 2), including an inability to grow on L agar plates at 42°C when the NaCl concentration was reduced from 1 to 0.5% (17). In a search for mutants that could suppress the various phenotypes, a mutant that was no longer sensitive to low salt concentrations at high temperature was isolated. Two additional phenotypes, poor transformation efficiency and an inability to grow in defined media, were also suppressed in this new strain and proved to be useful in the analysis of the original *rpoA*(E261K) mutation. The new mutation could not be separated from the original *rpoA*(E261K) allele by P1 transduction, implying that

TABLE 2. Phenotypes associated with the *rpoA* alleles

Property tested[a]	Wild type	*rpoA* allele		
		E261K	E261K, V306	E261Q
λ lysogenization	+	−	−	+
Transformation	+	−	+	+
Growth on GM	+	−	−	+[c]
Growth on GM plus methionine	+	−	+	+
Growth on low salt[b] at 42°C	+	−	+	+
Undulate colonies	−	+	−	−

[a] Properties were tested with GS162 and the wild type or *rpoA* with substitutions at amino acid 261 of the α subunit.
[b] Low salt was L agar plates with 0.5% NaCl.
[c] The *rpoA*(E261Q) allele is a weak (twofold) suppressor of the *metE-lacZ* phenotype (17). Presumably this increase in the level of *metE* expression is sufficient to allow growth on GM without methionine.

the suppressor mutation might be intragenic. The *rpoA* gene from this strain was amplified by PCR (see Materials and Methods), and the amplified product was sequenced. The original G-to-A transition at the first nucleotide of codon 261, which resulted in a change from a glutamic acid residue to a lysine residue at amino acid 261 of the α subunit of RNA polymerase, was still present. In addition, a T-to-A transversion was identified in the second nucleotide of codon 306, which resulted in a change from a valine residue to a glutamic acid residue. This double-mutant allele was designated *rpoA* (E261K, V306E). β-Galactosidase levels in a λ*metE-lacZ* lysogen carrying the new mutation were about ninefold lower than in a wild-type strain (Table 3), indicating that the *metE* down phenotype associated with the original *rpoA*(E261K) mutation was still present. The ability of the new strain to be transformed allowed us to test complementation of the original *rpoA*(E261K) allele by another *rpoA* allele at codon 261 (specifying alanine) that was carried on a plasmid.

Alanine substitution at amino acid 261 of the α subunit. It is possible that in a wild-type strain the side chain of glutamic acid 261 is required for activating *metE* expression and that lysine at position 261 in the mutant has disrupted this function; alternatively, glutamic acid may not be required, but when it was replaced with a lysine, the bulk or the opposite charge of this substituted residue did not fit in. To distinguish between these two possibilities, we tested the effect of an alanine substitution at position 261; alanine substitution eliminated side-chain atoms beyond the β carbon and hence eliminated interactions made by the side-chain atoms. If glutamic acid is important at position 261 for *metE-lacZ* expression, then replacement by alanine should not restore full promoter function. Plasmids pHTf1α and pHTf1α261A (36), which overexpress either the wild-type or a mutant α subunit with an alanine substitution at amino acid 261, respectively, were used to test the effects on *metE-lacZ* expression. The *rpoA*(E261K, V306E) mutations were first transduced into wild-type strain GS162 carrying a λ*metE-lacZ* fusion. The *rpoA*(E261K, V306E) mutations on the chromosomal copy of the *rpoA* gene resulted in a ninefold decrease in *metE-lacZ* expression (Table 3), similar to the decrease seen with the original *rpoA*(E261K) mutation. There was an increase in *metE-lacZ* expression in both GS162 and GS162 *rpoA*(E261K, V306E) when these strains were transformed with plasmid pHTf1α, which produces the wild-type α factor. However, the level of *metE-lacZ* expression in GS162 *rpoA*(E261K, V306E) remained 3.4-fold lower than in the GS162 lysogen, indicating that the plasmid-encoded wild-type α was not expressed at a level sufficient to drive formation of predominantly wild-type α-containing RNA polymerase. When the mutant strain with GS162 *rpoA*(E261K, V306E) was transformed with the mutant plasmid pHTf1α261A, the β-galactosidase activity remained at 24 Miller units, the same level observed in the nontransformed GS162 *rpoA*(E261K, V306E) lysogen, indicating that alanine cannot substitute for glutamic acid at position 261. Furthermore, expression of the *metE-lacZ* fusion decreased when the wild-type strain GS162 was transformed with plasmid pHTf1α261A, consistent with glutamic acid being required at position 261. The inability of pHTf1α261A to decrease β-galactosidase levels to approximately 24 Miller units was likely due to incomplete replacement of the wild-type α by αA261.

Effect of *metE* promoter deletion mutants. The α subunits of RNA polymerase have been proposed to interact with some promoter DNAs within sequence-specific UP-element regions upstream of the −35 region, and purified α has been shown to bind to these elements (30). We tested whether the *rpoA* (E261K)-dependent decrease in the level of *metE-lacZ* expression involves specific DNA sequences upstream of the −35 region of the *metE* promoter. Two fusions were constructed in which all *metE* promoter sequences were deleted upstream of base pair −35 (λ*metE-lacZ*Δ2) and base pair −58 (λ*metE-lacZ*Δ1) (see Materials and Methods) (Fig. 1). The effect of the E261K mutation was then assayed in deletion mutants in the absence of the two known regulatory proteins, MetR and MetJ. When this mutation was transduced into each of these fusion strains, the level of *metE-lacZ* expression was still reduced five- to sevenfold, similar to the fivefold effect seen in the original lysogen (Table 4). This analysis suggests that there is no specific base pair sequence upstream of the *metE* promoter DNA involved in an interaction with the α subunit.

Effect of the α E261K RNA polymerase in vitro. Although the in vivo analyses indicate that the down effect of the E261K allele is independent of the two known regulatory proteins for *metE*, it is possible that the E261K mutation has interfered with the interaction of some other hypothetical factor and α (or another subunit of RNA polymerase whose interaction is relayed through amino acid 261 in α). The deletion analysis indicates that the *metE* promoter region upstream of the −35 region cannot be a binding site for such a hypothetical factor but does not exclude other promoter regions as binding sites and does not exclude RNA polymerase-interacting factors that do not bind DNA. We addressed this problem using an in vitro transcription assay employing purified wild-type RNA polymerase or the mutant polymerase with the α E261K substitution. Two DNA fragments were used as templates, one carry-

TABLE 3. Alanine substitution at amino acid 261 of the α subunit and its effect on *metE-lacZ* expression

Strain[a]	α-producing plasmid	β-Galactosidase activity[b]
GS162		238
GS162 *rpoA*(E261K, V306E)		25
GS162	pHTf1α	512
GS162 *rpoA*(E261K, V306E)	pHTf1α	152
GS162	pHTf1α261A	137
GS162 *rpoA*(E261K, V306E)	pHTf1α261A	24

[a] Cells were grown in GM plus methionine. All strains carry a λ*metE-lacZ* fusion.
[b] Units of activity are Miller units (24).

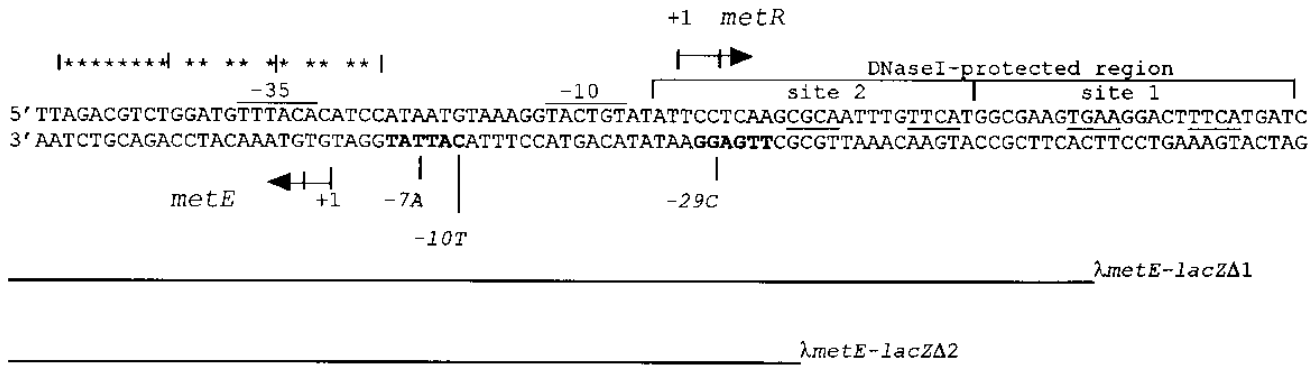

FIG. 1. Promoter mutations in the *metE-metR* control region. The designation of the *metE* and *metR* transcription start sites is from the work of Plamann and Stauffer (26). For *metR*, the −10 and −35 promoter elements are indicated above the top strand; for *metE*, these elements are in boldface type on the lower strand. MetR activator binding sites 1 and 2 are indicated within the DNase I-protected region (42). The three tandem repeats of the MetJ binding sequence (5′-AGACG TCT-3′) are shown above the DNA sequence, and the bases identical to the bases of the consensus sequence are indicated by asterisks. Point mutations in the −10 and −35 regions of the mutant *metE* promoters are shown below the wild-type sequence. The *metE* promoter regions that are present in the λ*metE-lacZ*Δ1 and λ*metE-lacZ*Δ2 fusions are indicated below the DNA sequence by a bar.

ing the divergent *metE-metR* promoters and the other carrying the *metH* promoter. The *metH* promoter has been shown in vivo to be unaffected by the *rpoA*(E261K) mutation (17); thus, levels of *metH* transcription were equalized for both polymerases as a control for differences in specific activities of the preparations. We confirmed that the mutant and wild-type specific activities were equivalent by showing that under these assay conditions, both enzymes had equal abilities to transcribe the ColE1 replicon RNA1 promoter and the *lacUV5* promoter (Fig. 2B), which have been shown in vitro to be insensitive to deletion of the C terminus of α beyond amino acid 235 (30) and, in the case of *lacUV5*, insensitive to α E261K (36).

Transcription from the *metE*, *metR*, and *metH* promoters was assayed in the presence or absence of the MetR protein. Although in the absence of the MetR activator protein, the level of transcription of the *metE* promoter with wild-type polymerase was too low to demonstrate the down effect of the mutant polymerase (Fig. 2A, lanes 1 and 3), we have shown previously that the defect in transcription in vivo by the mutant occurs even in *metR* deletion strains. Addition of MetR protein increased the level of transcription of *metE* and *metH* for both the wild-type and the mutant polymerase (Fig. 2A, lanes 2 and 4). However, the ratio of *metE* transcription compared with that of *metH* transcription was much greater for the wild-type polymerase than for the mutant polymerase. Since MetR activation in vivo is independent of the E261K effect, this experiment demonstrated that the decreased level of *metE-lacZ* expression was not due to the disruption of a site of contact with any hypothetical activator protein, since the activator protein would have to have been present as a contaminant of the highly purified wild-type RNA polymerase. Another possibility is that there was an inhibitor of *metE* transcription in the mutant extract. However, transcription from the *metE* promoter in the presence of a mixture of the wild-type and the mutant polymerases was additive (Fig. 2A, lane 5), indicating that there was no *metE*-specific repressor in the mutant extract.

It should be possible to compare the abilities of the mutant and wild-type polymerases to transcribe the *metE* promoter in vitro even in the absence of MetR activator if mutant derivatives of the promoter having higher basal levels of transcription that is still sensitive to α E261K can be used as templates. Two mutant promoters were constructed in vitro in the *metE-lacZ* fusion by making a single-base-pair change towards the consensus in either the −10 or the −35 promoter element at position −7 or at position −29, respectively (Fig. 1). The resulting fusion phages, λ*metE-lacZ-7A* and λ*metE-lacZ-29C*, were used to lysogenize the *metR metJ* mutant strain GS748 (in this strain, the absence of both regulatory proteins best approximates the in vitro transcription assay having only RNA polymerase). β-Galactosidase levels in lysogens 748λ*metE-lacZ-7A* and 748λ*metE-lacZ-29C* were 9-fold and 26-fold higher, respectively, than in the parent lysogen 748λ*metE-lacZ* (Table 5). When the *rpoA*(E261K) mutation was transduced into these lysogens, the fivefold decrease in the level of *metE-lacZ* expression seen with the wild-type fusion was also observed with the promoter mutants. Thus, although these stronger promoter elements resulted in higher basal levels of expression, they did not overcome the effect of the E261K mutation and could be used as templates in the in vitro transcription assay in the absence of MetR activator. In vitro transcription assays using the *−7A* and *−29C* promoter templates were performed as described above except that MetR protein was omitted. As shown in Fig. 3, the mutant polymerase gave very low levels of transcription of both the *−7A* and *−29C* templates compared with those of wild-type polymerase, consistent with the effects seen in vivo with these templates.

Expression from the *metR* promoter in the in vitro transcription assays was seen to be reciprocally related to expression from the *metE* promoter, whether the negatively autoregulating MetR protein was present in the assay or not (Fig. 2A and 3). This is consistent with a previously proposed model for *metR* autoregulation involving steric competition of polymer-

TABLE 4. Effect of the *rpoA*(E261K) mutation on the λ*metE-lacZ* deletions in a *metJ metR* mutant strain

Strain	Gene fusion	β-Galactosidase activity[a]
GS748	λ*metE-lacZ*	194
GS748 *rpoA*(E261K)	λ*metE-lacZ*	37
GS748	λ*metE-lacZ*Δ1	162
GS748 *rpoA*(E261K)	λ*metE-lacZ*Δ1	30
GS748	λ*metE-lacZ*Δ2	211
GS748 *rpoA*(E261K)	λ*metE-lacZ*Δ2	28

[a] Cells were grown in tryptone broth. Units of activity are Miller units (24).

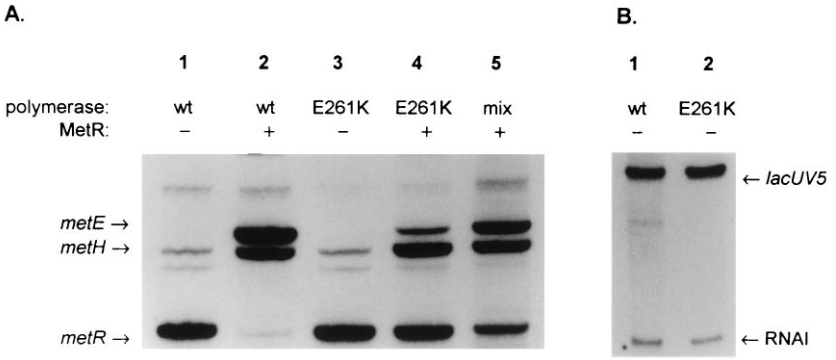

FIG. 2. In vitro transcription of the *metE* promoter by the α E261K polymerase. (A) DNA fragments (2 to 3 nM) containing the *metE-metR* and the *metH* promoters were used as templates in transcriptions with wild-type (wt) polymerase (lanes 1 and 2), α E261K polymerase (lanes 3 and 4), or a mixture of both polymerases (lane 5). MetR protein was added, where indicated (+), to a final concentration of 100 nM. Wild-type RNA polymerase was present at a concentration of 10 nM. The mutant α E261K polymerase was titered to give a level of *metH* transcription similar to that of the wild-type RNA polymerase and was then used for the assay. In vivo experiments show that *metH* transcription is not affected by the α E261K mutation (17). (B) Supercoiled plasmid pRLG593 was used as the template (0.5 nM) and carries the ColE1 replicon RNA1 promoter and the *lacUV5* promoter. Wild-type (lane 1) and mutant (lane 2) polymerases were added to the same final concentrations as in panel A.

ase bound to the *metE* or *metR* promoters, with MetR protein playing an indirect role in autoregulation by facilitating binding of polymerase at the *metE* promoter (38). This model is based on genetic analysis of *metE* promoter down mutations which did not affect the binding sites for MetR but still showed derepression of the *metR* promoter.

DISCUSSION

Studies of a number of positively regulated systems suggest that the RNA polymerase complex is positioned on a promoter such that the C-terminal 85 amino acids of the α subunit (αCTD) is capable of interacting with either sequence-specific or nonspecific DNA upstream of the −35 region or activator proteins that bind in this region (2). Recent nuclear magnetic resonance data indicate that the αCTD contains four helices and two long arms at the terminals of the domains (19). The arms are proposed to enclose the hydrophobic core of αCTD. These studies indicate that residue 261 is located at the start of helix 1. Chemical-shift perturbation experiments to probe the binding site in αCTD at the *rrnB* P1 promoter DNA indicate that helix 1 and the surrounding region are involved in the interaction with DNA. In addition, the COOH-terminal region of the α subunit appears to be the contact site for class I transcription factors (16) and residue 261 has been proposed to be a critical determinant for protein-protein interaction with the cyclic AMP receptor protein at the *lac* promoter (36). Thus, both nuclear magnetic resonance and genetic data suggest that amino acid 261 is located at a site that might allow it to make protein-DNA contacts with the promoter or to make protein-protein contacts with an activator, affording a role for this amino acid in α as a receiver of input signals from the promoter and/or bound factors. Experiments in this study demonstrate that the decrease in the level of *metE-lacZ* expression caused by the *rpoA*(E261K) mutation is independent of the MetR protein or another unidentified activator protein. In addition, deletion of sequences upstream of the *metE* −35 promoter region did not result in a significant decrease in the level of *metE-lacZ* activity that could be related to the *rpoA*(E261K) effect (Table 4), suggesting that the decrease in the level of *metE-lacZ* expression in the *rpoA*(E261K) mutant strain is not due to a disruption of a protein-DNA contact between the α subunit of RNA polymerase and a specific

TABLE 5. Effect of α E261K polymerase on *metE* promoter up mutants

Strain	Gene fusion	β-Galactosidase activity[a]
GS748	λ*metE-lacZ*	194
GS748 *rpoA*(E261K)	λ*metE-lacZ*	37 (5.2)
GS748	λ*metE-lacZ-7A*	1,778
GS748 *rpoA*(E261K)	λ*metE-lacZ-7A*	383 (4.6)
GS748	λ*metE-lacZ-29C*	4,782
GS748 *rpoA*(E261K)	λ*metE-lacZ-29C*	957 (5.0)
GS748	λ*metE-lacZ-10T*	4,776
GS748 *rpoA*(E261K)	λ*metE-lacZ-10T*	1,396 (3.4)

[a] Cells were grown in tryptone broth. Units of activity are Miller units (24). Numbers in parentheses indicate the fold decrease from the GS748 level to the GS748 *rpoA*(E261K) level.

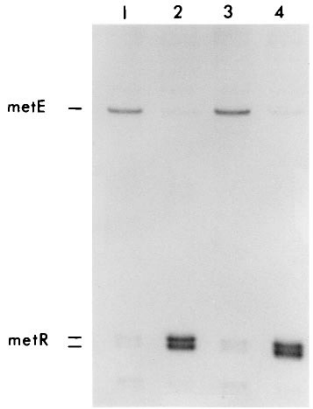

FIG. 3. In vitro transcription of mutant *metE* promoters by the α E261K polymerase. DNA fragments containing the *metE-metR* control regions of the mutant promoter *metE-29C* (1 nM) or *metE-7A* (3 nM) were used as templates for in vitro runoff transcriptions with either the wild-type or mutant polymerases at the same polymerase concentrations noted in the legend to Fig. 2. Lane 1, *−29C*, wild-type polymerase; lane 2, *−29C*, E261K polymerase; lane 3, *−7A*, wild-type polymerase; lane 4, *−7A*, E261K polymerase.

sequence, such as a UP element in the upstream region of *metE* promoter DNA.

There are at least two models consistent with our data of how the E261K amino acid change in the αCTD, which is presumably positioned upstream of the *metE* promoter, affects *metE-lacZ* expression. (i) The glutamic acid residue at position 261 might be required for a nonspecific interaction with DNA in the promoter region; however, the function of such a nonspecific interaction would probably not be simply to overcome the limitations of weak −10 and −35 promoter elements since the *−7A* and *−29C metE* promoter mutants, although 9-fold and 26-fold stronger than the wild-type promoter, nonetheless are decreased proportionately in the *rpoA*(E261K) mutants the same fold as the wild-type promoter (Table 5). It should be noted that in an in vivo genetic selection, we have isolated a *metE* promoter up mutant (unpublished result) having a towards-consensus base pair change at position −10 (λ*metE-lacZ-10T*) that in strain GS748 shows a small suppressing effect of the E261K mutation (Table 5 and Fig. 1). (ii) Alternatively, the change at position 261 might alter the structure of α or have a direct effect on the overall RNA polymerase structure, such that an essential function of some other region of RNA polymerase at the *metE* promoter is disrupted. Regardless of whether the amino acid itself interacts with promoter DNA or influences the way other domains of RNA polymerase interact at the promoter, the *rpoA*(E261K) mutation is irrelevant in how RNA polymerase interacts at the *metH* promoter, which is regulated normally in the presence of the *rpoA*(E261K) allele (17). We envision the structure of RNA polymerase bound to these two promoters as being different, with amino acid 261 of the α subunit being positioned to play some critical role in *metE* promoter activity but serving no essential function for the *metH* promoter. Such a structural difference could result from the promoter DNA sequence itself inducing a change in the bound RNA polymerase, similar to other systems in which it has been shown that DNA plays a role in inducing a conformational change in DNA-binding proteins (35). Such a model would predict that it should be possible to isolate compensatory mutations in other RNA polymerase subunits that would increase the level of *metE-lacZ* expression. We have devised a genetic selection and will attempt to isolate such mutations, assuming they are not lethal.

A suppressor mutation of *rpoA*(E261K) that maps in the *rpoA* gene itself (V306E) allows growth on GM plus methionine but not on GM alone, and the level of *metE-lacZ* expression is still 10-fold lower than that of the wild type. We found that this general growth requirement of the original mutant could also be suppressed by an alanine residue (which does not have a side chain beyond the β carbon) at amino acid 261. Thus, the general growth requirement seen with the original mutant carrying the *rpoA*(E261K) allele can be separated from the decreased level of *metE-lacZ* expression. In addition, different chromosomal alleles of the *rpoA* gene have different phenotypes for some of the other properties that we tested (Table 2). Thus, although the *rpoA*(E261K) allele affects the expression of other genes, it probably does so in different ways.

We have demonstrated here that a mutation in the αCTD may directly affect the intrinsic efficiency of RNA polymerase at certain promoters, independent of activator proteins or UP elements. We do not know the exact step(s) in transcription initiation inhibited by the original *rpoA*(E261K) allele. Additional experiments to study the kinetics of the reaction at the *metE* promoter will provide more information. As the *rpoA*(E261K) mutation is pleiotrophic, it will be interesting to determine if other promoters are regulated similarly.

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