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Functional Equivalence of *Escherichia coli* σ^E and *Pseudomonas aeruginosa* AlgU: *E. coli rpoE* Restores Mucoidy and Reduces Sensitivity to Reactive Oxygen Intermediates in *algU* Mutants of *P. aeruginosa*

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Mucoid colony morphology is the result of the overproduction of the exopolysaccharide alginate and is considered to be a major pathogenic determinant expressed by *Pseudomonas aeruginosa* during chronic respiratory infections in cystic fibrosis. Conversion to mucoidy can be caused by mutations in the second or third gene of the stress-responsive system *algU mucA mucB*. AlgU is 66% identical to the alternative sigma factor RpoE (σ^E) from *Escherichia coli* and *Salmonella typhimurium* and directs transcription of several critical alginate biosynthetic and regulatory genes. AlgU is also required for the full resistance of *P. aeruginosa* to reactive oxygen intermediates and heat killing. In this work, we report that *E. coli* σ^E can complement phenotypic defects of *algU* inactivation in *P. aeruginosa*: (i) the *rpoE* gene from *E. coli* complemented an *algU* null mutant of *P. aeruginosa* to mucoidy; (ii) the presence of the *E. coli rpoE* gene in *P. aeruginosa* induced alginate production in the standard genetic nonmucoid strain PAO1; (iii) the plasmid-borne *E. coli rpoE* gene induced transcription of *algD*, a critical *algU*-dependent alginate biosynthetic gene; and (iv) when present in *algU*::Tc^r mutants, *E. coli rpoE* partially restored resistance to paraquat, a redox cycling compound that increases intracellular levels of superoxide radicals. A new gene, *mclA*, encoding a polypeptide with an apparent molecular mass of 27.7 kDa was identified immediately downstream of *rpoE* in *E. coli*. The predicted product of this gene is 28% identical (72% similar) to MucA, a negative regulator of AlgU activity in *P. aeruginosa*. The results reported in this study demonstrate that RpoE and AlgU are functionally interchangeable in *P. aeruginosa* and suggest that elements showing sequence similarity to those known to regulate AlgU activity in *P. aeruginosa* are also present in other bacteria.

Chronic respiratory infections with *Pseudomonas aeruginosa* are the leading cause of high mortality and morbidity in patients with cystic fibrosis (CF) (6). Alginate overproduction in *P. aeruginosa* is a major virulence factor expressed by this bacterium in the CF-affected lung (15, 27). The pathogenic effects of alginate in CF are believed to represent the cumulative result of a multitude of proposed functions which include adherence (37, 49), recalcitrance to antibiotic treatments (3), evasion of phagocytosis and resistance to killing by polymorphonuclear leukocytes and macrophages (7, 47, 54), quenching of reactive oxygen intermediates (e.g., superoxide radicals) (55), and scavenging of oxidants such as hypochlorite (32).

Alginate synthesis is dependent upon expression of several genes within the alginate biosynthetic gene cluster at 34 min of the *P. aeruginosa* chromosome (11). At the beginning of this cluster is the *algD* gene, which encodes GDPmannose dehydrogenase (GMDH) (14). GMDH converts GDPmannose into GDPmannuronate, a direct precursor for alginate polymerization (14). The biosynthetic steps preceding GDPmannuronate are shared with lipopolysaccharide biosynthetic pathways and, although necessary for alginate synthesis, are not entirely alginate specific (10, 26). In keeping with the pivotal role of GDPmannose dehydrogenase, transcriptional activation of *algD* is always associated with the mucoid status of *P. aeruginosa* and represents a committing step towards increased alginate synthesis. Activation of *algD* requires participation of several regulatory genes (15), including signal transduction elements (13, 24, 61) and, most importantly, the recently identified putative alternative sigma factor AlgU (38, 41).

The *algU* gene is the first gene that has been characterized (38) within the genetic locus (Fig. 1) known to be allelic with a subset of classical *muc* mutations. The *muc* loci were first described by Fyfe and Govan (22) as a major site of genetic alterations governing conversion to mucoidy. Others have also studied this locus (21, 36), but the molecular characterization of individual genes has been achieved only recently (15, 25, 38–41, 52). The *algU* gene (38), also known as *algT* (16, 62), is followed by two accessory genes, *mucA* (39, 40) and *mucB* (*algN*) (25, 39). AlgU on one side and MucA and MucB on the other have antagonistic roles in the control of *algD*. They also provide the genetic framework for mutations which determine the mucoid or nonmucoid status of *P. aeruginosa* (40). AlgU plays a positive regulatory role in alginate biosynthesis. Insertional inactivation of *algU* on the chromosome of mucoid *P. aeruginosa* mutants abrogates mucoid phenotype, alginate production, and *algD* transcription (38). Under normal circumstances MucA and MucB counteract AlgU activity and suppress mucoidy in wild-type *P. aeruginosa*. Inactivation of these negative regulators increases *algD* transcription and results in a mucoid phenotype (39, 40). Moreover, mutations in the *mucA* gene are responsible for the conversion to mucoidy in a significant number of CF isolates (40). It is believed that MucA, and possibly indirectly MucB, controls AlgU activity

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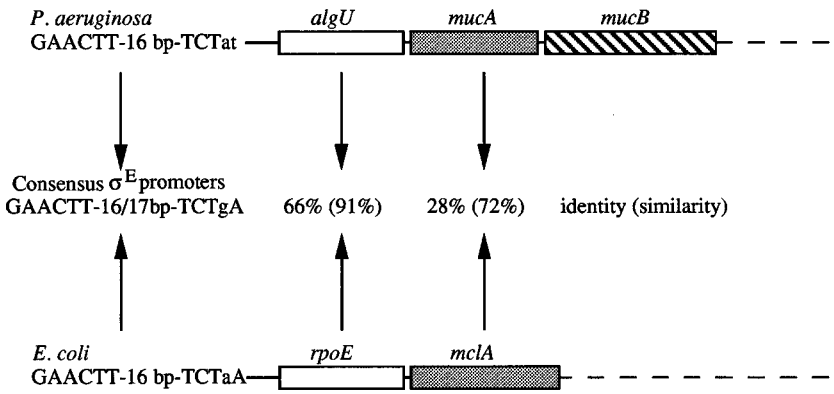

FIG. 1. Summary of the organization of the *algU mucA mucB* gene cluster controlling conversion to mucoidy in *P. aeruginosa* and its relationship to *E. coli rpoE* and *mclA*. The gene products of *algU* and *rpoE* are 66% identical (91% overall similarity). The predicted gene product of *mclA* is 28% identical to MucA (72% total similarity). The putative third gene in *E. coli* corresponding to *mucB* has not as yet been identified. The *algU* gene is necessary for alginate synthesis and mucoid phenotype in *P. aeruginosa* (15, 38). It is also required for full resistance of *P. aeruginosa* to reactive oxygen intermediates and heat killing (41). The *E. coli rpoE* gene (15, 35, 41) encodes the biochemically characterized alternative sigma factor σ^E (18, 60) known to direct transcription of the P3 promoter of the major heat shock sigma factor gene *rpoH* and *htrA* (18), a gene required for survival under extreme heat shock in *E. coli* (33) and exposure to reactive oxygen intermediates in *S. typhimurium* (30). Mutations in the second gene of the *algU mucA mucB* gene cluster are responsible for conversion to mucoidy in a significant number of CF isolates (40). Insertional inactivation of *mucB* has similar phenotypic consequences (39). The promoters transcribed by σ^E or dependent on AlgU display a distinct canonical promoter sequence (GAACTT at −35 separated by 16 or 17 bp from TCTgA at −10). The σ^E (AlgU)-dependent promoters of *P. aeruginosa algU* and *E. coli rpoE* are shown.

and prevents it from activating the subordinate promoters such as *algD* (15). In support of this notion are the findings that spontaneous second-site suppressor mutations which result in pseudoreversion from mucoidy to nonmucoidy sometimes occur in *algU*, suggesting its epistatic relationship to *mucA* (16, 52). Furthermore, while the presence of a functional *algU* gene induces *algD* transcription in a heterologous system, this activity can be suppressed by supplying the negative regulators *mucA* and *mucB* (52).

The first indications that AlgU may function as a sigma factor came from the initial report (38) of its similarity to the alternative sigma factor Spo0H, which controls sporulation and other post-exponential-phase processes in *Bacillus* spp. Intriguingly, the reported similarities between Spo0H and AlgU, although statistically significant, did not extend to the rest of the superfamily of σ^{70} proteins (34) at a sufficiently high level to be directly detected in global homology searches (38). However, subsequent sequence similarity analyses in conjunction with the substantial growth of sequence databases have explained this apparent paradox (15, 35, 41). On the basis of these more recent comparisons, AlgU is one of the founding members of a novel family of alternative sigma factors which are only distantly related to the σ^{70} class of sigma factors. Spo0H shares similarities with both groups of proteins and is placed in between these two superfamilies (35). More importantly, these recent developments have uncovered the presence of close homologs of AlgU in *Escherichia coli* and *Salmonella typhimurium* (66% identity and 91% overall similarity) (35, 41). Several independent observations (references 15, 35, and 41 and annotation to GenBank entry D13169) have indicated that the *E. coli* homolog of AlgU is the alternative sigma factor σ^E which has been previously characterized at the biochemical level (18, 60). The *E. coli* and *S. typhimurium* genes encoding σ^E have been named *rpoE* (15, 35, 41). Although no mutations in *rpoE* have been reported in *E. coli* and *S. typhimurium*, it is possible to predict at least some phenotypic consequences of *rpoE* inactivation. σ^E has been shown to transcribe the major heat shock sigma factor gene *rpoH* in *E. coli* from its P3 promoter under conditions of extreme heat shock (18). It is also the only sigma factor transcribing *htrA* (*degP*), a gene required for resistance to heat killing in *E. coli* (33) and for resistance to reactive oxygen intermediates and full virulence of *S. typhimurium* (30). Congruent with these functions of σ^E, inactivation of *algU* decreases the *P. aeruginosa* survival rate upon exposure to high temperatures and diminishes its resistance to superoxide-generating redox cycling compounds (41). This effect is independent of the mucoid or nonmucoid status of the parental strain in which *algU* has been inactivated (41). Furthermore, *algU* transcription is induced by environmental stress such as heat shock (53). These observations suggest that *algU* plays a physiological role similar to that of *rpoE* in enteric bacteria and that alginate production is only a subset of systems regulated by *algU* in *P. aeruginosa*. In order to address the hypothesis of functional equivalence between *algU* and *rpoE*, in this work we used the *E. coli rpoE* gene for cross-complementation of *algU* mutants in *P. aeruginosa*. We show that the *rpoE* gene can substitute *algU* in *P. aeruginosa* and also report the presence of a gene (*mclA*; for *mucA* like) located downstream of *rpoE* which shows sequence similarity of its predicted gene product to MucA, a negative regulator of AlgU.

MATERIALS AND METHODS

Media and growth conditions. *P. aeruginosa* was grown on *Pseudomonas* isolation agar (Difco) supplemented with 150 μg of gentamicin per ml, 300 μg of carbenicillin per ml, or 300 μg of tetracycline per ml when required. *E. coli* was grown on Luria broth supplemented with 100 μg of ampicillin per ml, 30 μg of kanamycin per ml, 13 μg of gentamicin per ml, or 12 μg of tetracycline per ml when required. All incubations were carried out at 37°C.

Bacterial strains and plasmids. Bacterial strains and plasmids used in this study are shown in Table 1. *P. aeruginosa* PAO6853 was constructed by integrating the plasmid pHYDX on the chromosome of PAO1 via a single crossover recombinational event. Plasmid pHYDX was generated by cloning the 9.5-kb *Hin*dIII fragment carrying an *algD-xylE* fusion and several genes located downstream of *algD* into pCMobB (45). Integration on the chromosome was confirmed by Southern blot hybridization.

Plasmids pHY17EC, pHYAG, and pHYEG were constructed by inserting a 1.7-kb PCR-generated fragment (or its modifications) into the broad-host-range plasmid vector pVDtac39 in the following series of steps. The 1.7-kb fragment corresponding to the *E. coli rpoE* and *mclAΔ8* genes was generated by PCR amplification of the corresponding chromosomal region of *E. coli* K-12 with the following primers: IMO2 (5′CCGGCTGCGCCGCTACCG3′) and ECA1 (5′GA ATTCCTGGCACCTGTAC3′). The 1.7-kb amplification product carried the 5′ end of the *E. coli nadB* gene (which is divergently transcribed from *rpoE*), the intergenic region containing the *nadB* and *rpoE* promoters, and a truncated *mclA* gene (*mclAΔ8*). The 1.7-kb PCR-generated fragment was cloned into pCRII with a TA Cloning kit (Invitrogen) according to the procedures supplied by the

TABLE 1. Bacterial strains and plasmids

Bacterial species and strain or plasmid	Relevant properties[a]	Reference or source
P. aeruginosa		
PAO1	Prototroph Alg$^+$ (*algU*$^+$ *mucA*$^+$ *mucB*$^+$)	28
PAO6852	PAO1 Alg$^-$ (*algU*::Tc^r)	41
PAO6853	PAO1 *algD*$^+$ *algD-xylE* Cb^r Tc^r	This work
PAO381	FP2$^+$ *leu-38* Alg$^+$ (*algU*$^+$ *mucA*$^+$ *mucB*$^+$)	22
PAO568	PAO381 Alg(Con) (*algU*$^+$ *mucA2* *mucB*$^+$)	22
PAO670	PAO568 Alg$^-$ (*algU*::Tc^r)	38
E. coli		
K-12	Wild type	G. Storz
DH5α	*lacZΔM15 recA1*	Bethesda Research Laboratories
Plasmids		
pCRII	3.9-kb Ap^r Km^r ColE1 *lacZ*α	Invitrogen
pKI11G	pUC18 1.8-kb *Bam*HI Gm^r cassette	59
pT7-6	ColE1 ϕ10 promoter Ap^r	S. Tabor
pGP1-2	Ori(p15) P_LT7 gene 1 Km^r	57
pVDtac39	IncQ/P4 *mob tra tac lacZ′ lacI*^{q} Ap^r	12
pRK2013	ColE1 *mob tra* (RK2) Km^r	20
pHY17EC	pVDtac39 *rpoE*$^+$ *mclAΔ8*	This work
pHYAG	pVDtac39 *rpoE*$^+$ *mclA*::Gm^r	This work
pHYEG	pVDtac39 *rpoE*::Gm^r *mclAΔ8*	This work
pTE17	pT7-6 *rpoE*$^+$ *mclAΔ8*	This work
pTAG	pT7-6 *rpoE*$^+$ *mclAΔ8*::Gm^r	This work
pTEG	pT7-6 *rpoE*::Gm^r *mclAΔ8*	This work
pmclAΔ8	pT7-6 *mclAΔ8*	This work
pmclA	pT7-6 *mclA*$^+$	This work
pHYDX	pCMobB *algD-xylE mob* Ap^r	This work

[a] Alg$^+$, wild-type phenotype; Alg$^-$, inability to produce alginate due to *algU* mutation; Alg(Con), constitutive production of alginate (mucoid phenotype).

manufacturer. The DNA sequence of the cloned insert was verified, and one of the positive clones, termed pCR17EA, was used for further manipulations. The 1.7-kb insert from pCR17EA was cloned as an *Eco*RI fragment into pVDtac39 (12) to generate pHY17EC. For the construction of pHYEG and pHYAG, the 1.7-kb insert was first cloned into a modified pUC12 vector (pUC12H) which had its *Hin*dIII site eliminated (by cutting pUC12 with *Hin*dIII, filling in the ends, and blunt end ligation), and the resulting plasmid was termed p12HEA. To generate pHYEG, p12HEA was digested with *Hin*dIII, the ends were filled in with Klenow DNA polymerase I fragment, and a modified Gm^r cassette from pKI11G (59) (originally derived from the broad-host-range plasmid pPH1JI [51]) was inserted into this site located 155 bp downstream of the *rpoE* initiation codon. The resulting plasmid (pUCEG) was digested with *Eco*RI, and the 3.5-kb insert (the 1.7-kb fragment plus the Gm^r cassette inserted into *rpoE*) was cloned into pVDtac39. To generate pHYAG, p12HEA was digested with *Eco*RV (located 162 bp downstream of the initiation codon of *mclA*) and the modified Gm^r cassette was inserted into this site. The resulting plasmid, pUCAG, was digested with *Eco*RI, and the 3.5-kb fragment (the original 1.7-kb fragment plus the Gm^r cassette inserted in *mclA*) was excised and cloned into pVDtac39. To generate the T7 expression clones pTE17, pTAG, and pTEG, the *Eco*RI inserts from pCR17EA, pUCAG, and pUCEG, respectively, were excised and cloned into the *Eco*RI site of pT7-6.

PCR procedures. Conditions for PCR amplification of chromosomal sequences were as previously described (41). For inverse PCR, a modified protocol of Ochman et al. (46) was used. To amplify the complete *mclA* gene, *E. coli* K-12 chromosomal DNA was digested with *Hin*dIII and ligated and a 1:10-diluted mixture containing ligation products was subjected to PCR amplification with the following primers: (i) IMI1 (5′ATAGCTTCCCTCGCTCGG3′; corresponding to the sequence beginning at position 693 and ending at position 710 of GenBank entry D13169) and ECA2 (5′CCGTGACTCAATGCGGGG3′; corresponding to positions 510 to 492 of D13169) and (ii) IMI1 and ECA3 (5′GCCCGGTAAGC CTGGG3′; positions 189 to 174 of D13169). PCR products of 2.0 kb (IMI1-ECA2) and 1.7 kb (IMI1-ECA3) were obtained, subjected to direct PCR sequencing, and cloned into pCRII (yielding plasmids pHY1861 and pHY1865, respectively). In each case sequence analysis confirmed that the 3′ end of the *mclA* gene was cloned. Based on this sequence, a new primer, ECA4 (5′AAG GTTCGGGAGTGGCCGAGGCG3′), located 58 bp downstream of the 3′ end of *mclA* was designed. ECA4 in combination with IM12 (5′CGGACCAGGTCC TGGTTG3′), located 16 bp downstream of the initiation codon of *rpoE*, permitted amplification of the complete *mclA* gene by conventional nested PCR. The resulting 1.3-kb fragment also carried an incomplete *rpoE* gene truncated at its 5′ end.

Enzyme and alginate assays. Catechol 2,3-dioxygenase (CDO) was assayed in sonic extracts as previously described (39). Cells were suspended in 5 ml of sterile saline and spun in an SM24 rotor. Cell pellets were suspended in 5 ml of 50 mM potassium phosphate buffer (pH 7.5)–10% acetone. Sonication was on ice for 1 min, followed by centrifugation in an SM24 rotor at 10,000 rpm. The CDO assay was carried out in 3 ml (total volume) of 50 mM potassium phosphate buffer (pH 7.5)–0.33 mM catechol by monitoring the increase of A_{375} in a Shimadzu UV160 spectrophotometer. The molar extinction coefficient of the reaction product, 2-hydroxymuconic semialdehyde, is 4.4×10^4. One unit of CDO is defined as the amount of enzyme oxidizing 1 μmol of catechol per min at room temperature. Alginate production was assayed as previously described (31).

DNA sequencing, recombinant DNA methods, and genetic manipulations. DNA sequencing was carried out as previously described (41). All recombinant DNA procedures were done according to standard methods (4). Plasmids were transferred to *P. aeruginosa* by triparental conjugations as previously described (38).

Visualization of gene products by using the T7 RNA polymerase-promoter system. Polypeptides encoded by cloned genes were visualized by expression in *E. coli* by a temperature-inducible T7 expression system (plasmid vectors pT7-6 and T7 RNA polymerase encoded by pGPI-2) (57) and protein labeling with [^{35}S]methionine and [^{35}S]cysteine (Expre^{35}S^{35}S protein labeling mix; 1,000 Ci/mmol; DuPont NEN) with previously described modifications (38). Proteins were separated on sodium dodecyl sulfate (SDS)–12% polyacrylamide gels. ^{14}C-labeled methylated proteins (Amersham) were used as M_r standards. The gels were fixed in 10% acetic acid, washed with H_2O, and impregnated with 1 M salicylic acid, and bands representing radiolabeled polypeptides were detected by autofluorography at −70°C.

Susceptibility to killing by reactive oxygen intermediates. Sensitivity to paraquat was determined by measuring the diameter of the zone of killing surrounding disks (6 mm; BBL) soaked with 10 μl of 1.9% paraquat and placed on a 2-ml soft agar (0.6%) layer (containing 100 μl of *P. aeruginosa* overnight cultures) solidified on top of 25 ml of 1.5% agar in Luria broth. Measurements were done after overnight incubation.

Nucleotide sequence accession numbers. The sequence of the *E. coli mclA* gene has been submitted to GenBank (accession number U10148). The translated sequence of the *E. coli rpoE* has been deposited in the SWISS-PROT database (accession number P34086) and is encoded by the DNA region (accession number D13169) upstream of the *nadB* gene (divergently transcribed).

RESULTS

***E. coli rpoE* restores the mucoid phenotype in *P. aeruginosa* with insertionally inactivated *algU*.** In order to test whether *rpoE* and *algU* are functionally equivalent, we cloned the *rpoE* gene from *E. coli* K-12 on the basis of the nucleotide sequence in GenBank entry D13169. The cloning of *algU* in *P. aeruginosa* was greatly facilitated by inclusion of downstream sequences which later proved to contain negative regulators of this putative sigma factor and thus reduced the toxicity of plasmid-borne *algU*. Following this rationale, a 1.7-kb PCR fragment carrying *rpoE*, 466 bp upstream of *rpoE*, and the entire 658-bp region downstream of *rpoE* based on the available information in D13169 (35, 41) was cloned into the broad-host-range vector pVDtac39. The resulting plasmid, pHY17EC, was conjugated into the nonmucoid *algU*::Tc^r strain PAO670 (38). PAO670 is derived from the mucoid strain PAO568, which carries the characterized *mucA2* mutation responsible for its mucoid phenotype (40). All PAO670 exconjugants harboring pHY17EC displayed the mucoid phenotype (Fig. 2, left panel, PAO670) and produced large amounts of alginate (76.4 μg/mg [wet weight] of cells) similar to those observed in the parental mucoid strain PAO568. The complementation effect was independent of isopropyl-β-D-thiogalactopyranoside (IPTG) addition to the medium, suggesting that the basal level of transcription of the cloned *rpoE* gene was sufficient to restore the mucoid phenotype. In contrast, PAO670 harboring the vector only displayed nonmucoid colony morphology and production

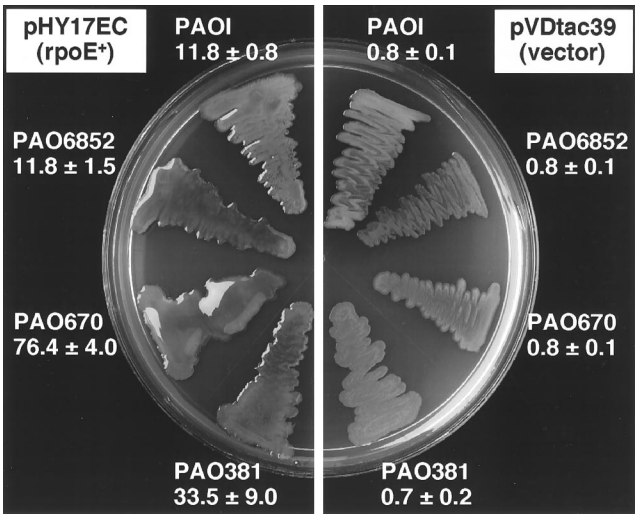

FIG. 2. Complementation of *P. aeruginosa algU* mutants to mucoid phenotype and induction of alginate production in *P. aeruginosa* by the 1.7-kb DNA fragment carrying *E. coli rpoE*. (Left) Four different *P. aeruginosa* strains harboring plasmid pHY17EC carrying the 1.7-kb fragment with the *rpoE* gene from *E. coli*; (right) same strains harboring the vector pVDtac39 used to construct pHY17EC. *P. aeruginosa* strains: PAO1 (nonmucoid, wild type), PAO6852 (PAO1 *algU*::Tc^r), PAO670 (*algU*::Tc^r derivative of the mucoid strain PAO568), and PAO381 (nonmucoid strain parental to PAO568; it carries the wild-type *algU mucA mucB* gene cluster). Numbers indicate alginate production expressed in micrograms per milligram (wet weight) of cells.

of uronic acids that was at or below the level of detection (Fig. 2, right panel, PAO670). These results suggest that *rpoE* from *E. coli* can complement the *algU* mutation in *P. aeruginosa* PAO670 and can restore the high-level production of alginate and mucoidy in this strain.

The cloned DNA fragment used in complementation experiments contained additional sequences upstream and downstream of *rpoE*. The upstream region carried the putative promoter(s) of *rpoE* and the beginning of the divergently transcribed *nadB* gene. However, the region located downstream of *rpoE* appeared to encode an additional polypeptide (see below). To rule out the possibility that the complementation observed in PAO670 was due to the gene located downstream of *rpoE*, two derivatives of pHY17EC were constructed. Plasmid pHYEG carried insertionally inactivated *rpoE* (the Gm^r cassette was inserted into the *Hin*dIII site of *rpoE*), while plasmid pHYAG carried active *rpoE* and an insertionally inactivated downstream gene (the Gm^r cassette was inserted into the *Eco*RV site downstream of *rpoE*). When PAO670 harbored pHYAG, the production of alginate and mucoidy were restored to levels similar to those observed with PAO670 harboring pHY17EC (56.4 ± 5.0 μg of alginate per mg [wet weight] of cells). In contrast, when PAO670 carried pHYEG (*rpoE*::Gm^r), the levels of alginate production (0.8 ± 0.1 μg/mg [wet weight] of cells) were equivalent to the background detected in PAO670 (Fig. 2). These experiments established that the *E. coli rpoE* gene complemented the *algU*::Tc^r mutation in PAO670 and restored the mucoid phenotype in this strain of *P. aeruginosa*.

***E. coli rpoE* partially complements sensitivity to reactive oxygen intermediates in *algU* mutants of *P. aeruginosa*.** Inactivation of *algU* in *P. aeruginosa* confers other phenotypic consequences in addition to the loss of alginate production. One of the prominent defects is the increased sensitivity to paraquat (41), a redox cycling compound which increases intracellular levels of superoxide radicals (19). In order to examine the possibility that *rpoE* also complements this defect in *P. aeruginosa*, strain PAO670 (*algU*::Tc^r) harboring different plasmid constructs was tested for sensitivity to paraquat. When PAO670 contained pHYAG, which carried the functional *rpoE* gene, the susceptibility to killing by paraquat was significantly reduced. The detected zones of growth inhibition were intermediate between the sensitivities of the *algU*::Tc^r strain PAO670 and the *algU*$^+$ strain PAO568 parental to PAO670 (Table 2). Similar results were obtained with pHY17EC (data not shown). When the *rpoE* gene was inactivated, the presence of the resulting construct, pHYEG, in PAO670 did not improve its resistance to killing by paraquat.

The increased sensitivity of *algU*::Tc^r *P. aeruginosa* to paraquat is known to be irrespective of the mucoid or nonmucoid character of the parental *algU*$^+$ strain (41). For example, inactivation of *algU* in the standard genetic strain PAO1 (nonmucoid) which carries the wild-type *algU mucA mucB* gene cluster results in increased inhibition of growth by paraquat (41) similar to that observed in PAO670. In order to test whether *E. coli rpoE* can also complement paraquat sensitivity in the PAO1 background, pHYEG and pHYAG were introduced into the *algU*::Tc^r mutant strain PAO6852, derived from PAO1. The results of these experiments (Table 2) showed a pattern similar to that observed with PAO568 and PAO670: the plasmid carrying *rpoE*$^+$ (pHYAG) was able to partially restore resistance to paraquat to intermediate levels between the sensitivities of PAO1 (*algU*$^+$) and PAO6852 (*algU*::Tc^r). Inactivation of *rpoE* (plasmid pHYEG) abrogated the ability to

TABLE 2. Paraquat sensitivity in *P. aeruginosa algU* mutants harboring *E. coli rpoE*

Strain (genotype)[a]	Plasmid (genes)[b]	Growth inhibition zone diam (mm ± SE)[c]
PAO1 (*algU*$^+$)	None	17.0 ± 0.3
PAO6852 (PAO1 *algU*::Tc^r)	None	22.1 ± 0.4
	pHYAG (*rpoE*$^+$ *mclAΔ8*::Gm^r)	20.1 ± 0.2
	pHYEG (*rpoE*::Gm^r *mclAΔ8*)	22.7 ± 0.2
PAO568 (*algU*$^+$ *mucA2*)	None	16.3 ± 0.3
PAO670 (PAO568 *algU*::Tc^r)	None	23.0 ± 0.6
	pHYAG (*rpoE*$^+$ *mclAΔ8*::Gm^r)	17.5 ± 0.2
	pHYEG (*rpoE*::Gm^r *mclAΔ8*)	22.0 ± 0.3

[a] Two pairs of isogenic *algU*$^+$ and *algU*::Tc^r *P. aeruginosa* strains (PAO1 with PAO6852 and PAO568 with PAO670) were used. PAO1 is the standard genetic strain carrying the wild-type *algU mucA mucB* gene cluster and is phenotypically nonmucoid. PAO568 carries the *mucA2* mutation and has the mucoid phenotype.

[b] pHYAG and pHYEG are derivatives of pHY17EC (see Fig. 2) with the gentamicin resistance (Gm^r) cassette inserted into *mclAΔ8* and *rpoE*, respectively.

[c] Sensitivity to killing by paraquat is expressed as the diameter of growth inhibition zone surrounding filter disks impregnated with 10 μl of 1.9% paraquat (see Materials and Methods). *P* values (*t* test) were 2.2×10^{-4} for PAO6852(pHYAG) and PAO6852(pHYEG) and 6.2×10^{-5} for PAO670(pHYAG) and PAO670(pHYEG).

improve resistance of PAO6852 to paraquat. The results of these experiments further confirmed the functional similarity of *rpoE* and *algU* and their interchangeability in *P. aeruginosa*, extending it to the partial complementation of the paraquat sensitivity phenotype in *algU* mutants of this organism.

Plasmid-borne *E. coli rpoE* induces alginate production in wild-type nonmucoid *P. aeruginosa*. Mutations in *mucA* and *mucB* are believed to relieve AlgU from inhibition by negative regulators (39, 40). In addition, a study carried out with plasmids (25), which are now known to have contained a functional *algU* gene with both downstream negative regulators inactivated, has indicated that plasmid-borne *algU* can induce mucoidy in *P. aeruginosa*. It appeared possible, on the basis of the presumed equivalence of *rpoE* and *algU*, that the presence of *rpoE* on a plasmid in wild-type nonmucoid *P. aeruginosa* induces alginate production and causes a mucoid phenotype. These predictions were borne out: when plasmid pHY17EC (*rpoE*$^+$) was introduced in PAO1 or PAO381, which both have the wild-type *algU mucA mucB* gene cluster, it induced moderate to high-level alginate production and caused mucoid colony morphology on *Pseudomonas* isolation agar plates (Fig. 2). The plasmid-borne *rpoE* was not acting through *algU*, since pHY17EC also induced mucoidy in PAO6852 (an *algU*::Tc^r derivative of PAO1) to levels comparable to those seen in PAO1 harboring the same plasmid (Fig. 2). When the plasmid construct pHYEG with inactivated *rpoE* was used in these experiments, no induction of alginate production was observed in PAO6852 (detectable uronic acid per mg [wet weight] of cells was 0.8 ± 0.1 μg). In contrast, plasmid pHYAG, which had the Gm^r cassette inserted downstream of *rpoE*, induced alginate production in PAO6852 (10.8 ± 0.5 μg/mg [wet weight] of cells) comparable to the effect seen with pHY17EC (Fig. 2). These experiments further substantiated the functional similarity of *algU* and *rpoE*. The presented results also illustrate the striking finding that an *E. coli* gene can induce mucoidy and alginate production in wild-type nonmucoid *P. aeruginosa.*

Plasmid-borne *rpoE* induces *algD* transcription in *P. aeruginosa*. The basis for *rpoE*-dependent induction of mucoidy in *P. aeruginosa* was further investigated. AlgU is a positive regulator of *algD* transcription in *P. aeruginosa* (38). Inactivation of *algU* abrogates *algD* expression (38). In addition, two other promoters, the proximal promoters of *algR* and *algU*, are known to absolutely depend on *algU* for expression (41). All three promoters contain the highly conserved −35 and −10 σ^E canonical promoter sequences (15, 41). Since *algU* and *algR* are transcribed from multiple promoters (some of which do not depend on AlgU) while the only known promoter of *algD* is AlgU dependent, we chose to investigate effects of *rpoE* on *algD* transcription. In order to perform such analyses, a derivative of the standard genetic *P. aeruginosa* strain PAO1 that carried an *algD-xylE* transcriptional fusion on the chromosome was constructed (see Materials and Methods). The resulting strain, PAO6853, carried a full complement of alginate biosynthetic genes, including a functional copy of *algD* in addition to the chromosomally integrated *algD-xylE* fusion (see Materials and Methods), but was nonmucoid since it carried the wild-type *algU mucA mucB* cluster. Introduction of the plasmid pHYAG (*rpoE*$^+$) into PAO6853 induced alginate production (Table 3) and caused mucoid colony morphology, albeit at a relatively low level compared to the fully mucoid strains such as those carrying *muc* mutations. When *algD* expression was examined by monitoring the activity of the reporter gene product, there was a threefold induction of *algD* transcription on the chromosome of PAO6853. Although modest, this elevation of *algD* transcription was commensurate with the phenotypic effects observed in this strain (slightly mucoid phenotype and a moderate level of detectable alginate [Table 3]). The induction of *algD* expression was *rpoE* dependent, since inactivation of *rpoE* on the plasmid abrogated effects on *algD* transcription and alginate production (Table 3, plasmid pHYEG) in comparison to the insertion of the Gm^r cassette downstream of *rpoE*, which had no apparent effects.

TABLE 3. Activation of *algD* transcription in *P. aeruginosa* PAO6853[a] by *E. coli rpoE*

Plasmid[b] (gene)	Alginate production (μg of uronic acid/mg [wet wt] of cells ± SE)[c]	*algD-xylE* activity (CDO U/mg ± SE)[d]
None	1.00 ± 0.03	1.95 ± 0.10
pHYAG (*rpoE*$^+$)	9.42 ± 0.46	6.25 ± 0.57
pHYEG (*rpoE*::Gm^r)	1.00 ± 0.05	2.10 ± 0.10

[a] PAO6853 is a derivative of the standard genetic strain PAO1 that carries the wild-type *algU mucA mucB* cluster and a chromosomally integrated *algD-xylE* fusion (see Materials and Methods). This strain is merodiploid for *algD* (*algD*$^+$ *algD-xylE*) and has a functional complement of all alginate biosynthetic genes. All strains were grown on *Pseudomonas* isolation agar plates for 48 h at 37°C before harvesting. *Pseudomonas* isolation agar was supplemented with 150 μg of gentamicin per ml for strains harboring plasmids.

[b] Plasmids pHYAG and pHYEG are as described in Table 2.

[c] *P* value (*t* test) for PAO6853(pHYEG) and PAO6853(pHYAG), 4×10^{-4}.

[d] Transcription of *algD* was monitored by determining the activity of CDO (the gene product of *xylE*) in cell extracts. Assay conditions and units are defined in Materials and Methods. *P* value (*t* test) for PAO6853(pHYEG) and PAO6853 (pHYAG), 7×10^{-3}.

Detection of the polypeptides encoded by *rpoE* and a gene located downstream of *rpoE*. In order to further characterize the *rpoE* clone used in our experiments, we examined protein products specified by the inserts of pHY17EC, pHYEG, and pHYAG in a T7 expression system. The results of these experiments are shown in Fig. 3. When the 1.7-kb insert of pHY17EC was placed behind the T7 promoter on pT7-6 in the direction of *rpoE* transcription, two ^{35}S-labeled polypeptides with apparent molecular masses of 25 (P25) and 27 (P27) kDa were observed (Fig. 3, lane 2). Reversal of the insert orientation so that the T7 promoter was in the opposite direction of *rpoE* transcription resulted in no detectable ^{35}S-labeled polypeptides (Fig. 3, lane 5). In order to determine which polypeptide(s) was responsible for the effects observed in genetic complementation studies, the inserts of pHYEG and pHYAG were also cloned into pT7-6 in the orientation which permitted transcription of *rpoE* from the T7 promoter. Insertion of the Gm^r cassette into the *Eco*RV site located 162 bp downstream of the *rpoE* coding region (Fig. 3, construct 3; equivalent to the insert of pHYAG) resulted in the disappearance of P27, but the P25 band was unaffected (Fig. 3, lane 3). The presence of the Gm^r cassette in the *Hin*dIII site 155 bp downstream of the *rpoE* ATG codon (Fig. 3, construct 4; equivalent to the insert of pHYEG) resulted in a loss of both bands specified by the 1.7-kb insert of pHY17EC. These findings are consistent with P25 being the gene product of *rpoE*. Moreover, since the same DNA inserts used in T7 expression analyses were also examined in cross-complementation studies described in the previous sections, it was possible to conclude that the complementation of the *algU* defects in *P. aeruginosa* was due to the gene encoding P25 (*rpoE*) and not due to the gene encoding P27. Furthermore, the apparent molecular mass of the *rpoE* gene product based on its electrophoretic mobility was close to the previously reported molecular mass of purified σ^E (24 kDa) (18, 60). Both the previously reported molecular mass and the observed molecular mass in this study are some-

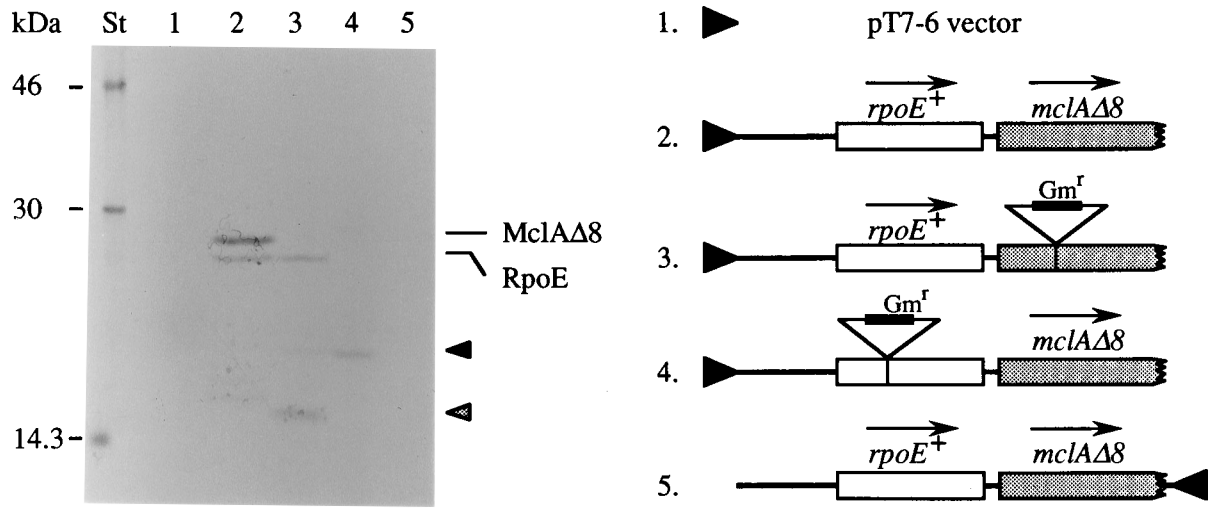

FIG. 3. T7 expression analysis of the polypeptides encoded by the 1.7-kb fragment carrying *E. coli rpoE* and a truncated *mclA* gene. (Left) Autoradiogram of [^{35}S]methionine- and [^{35}S]cysteine-labeled polypeptides separated by SDS-PAGE. Lanes: St, ^{14}C-radiolabeled molecular mass standards; 1, pT7-6 (no insert); 2, pT7-6 with a 1.7-kb insert carrying *rpoE* (wild type) and *mclAΔ8* (a truncated version of *mclA* missing 8 C-terminal amino acids; see text) cloned in the direction of T7 transcription; 3, same plasmid as in lane 2 with the Gm^r cassette inserted into *mclAΔ8*; 4, same plasmid as in lane 2 with the Gm^r cassette inserted in the *rpoE* gene; 5, same insert as in lane 2 cloned in the opposite orientation in pT7-6. Bars indicate mass standards, MclAΔ8, or RpoE. The filled triangle denotes gentamicin acetyltransferase (17.5 kDa) encoded by the Gm^r cassette. The band visible in lane 3 (stippled arrowhead) is a fortuitous fusion product of the truncated *mclA* gene and the sequences within the Gm^r cassette. (Right) Schematic representation of inserts used for expression analysis in the left panel. Large triangles represent the T7 promoter, while overhead arrows indicate direction of transcription of *rpoE* and *mclA*. Lane numbers in the left panel correspond to construct numbers on the right.

what higher than the molecular mass predicted from the sequence (21,694 kDa). This can be ascribed to the anomalous electrophoretic mobility of sigma factors, which is their almost ubiquitous property (44) linked to the unusually high negative charge (the predicted pI of *E. coli* σ^E is 5.2).

Sequence analysis of *E. coli mclA*. On the basis of T7 expression analyses of the 1.7-kb fragment containing the *rpoE* gene, it appeared likely that there was another gene located immediately downstream of *rpoE*. To investigate this possibility, we examined whether an open reading frame with a coding capacity for P27 could be found downstream of *rpoE*. The available DNA sequence from GenBank entry D13169, which contains the originally deposited nucleotide sequence subsequently assigned to *E. coli rpoE* (references 15, 35, and 41 and annotation to D13169), also contains a 658-bp region downstream of *rpoE*. By analyzing this sequence, we could not find a contiguous open reading frame downstream of *rpoE* matching the coding capacity required for P27. Intriguingly, a homology search indicated that this DNA region had the capacity to encode a conceptual gene product which appeared truncated at its N-terminal end but displayed statistically significant similarity with *P. aeruginosa* MucA. One explanation for these observations was that the reported DNA sequence deposited under GenBank accession number D13169 contained a mistake in this region. To test this possibility, we sequenced the relevant region of the cloned 1.7-kb fragment and found four T residues at position 368 where the sequence from entry D13169 contains five Ts. This was further confirmed by direct sequencing of the PCR products obtained with chromosomal DNA from two *E. coli* strains (K-12 and VD1870 [52]). When the corrected sequence was examined, this indicated the presence of a contiguous open reading frame with translational initiation signals. However, these studies also revealed that GenBank entry D13169 and the cloned 1.7-kb fragment did not include the stop codon of the gene downstream of *rpoE*. To clone the 3′ end of this gene, an inverse PCR strategy was applied. Two combinations of primers were used (see Materials and Methods) to amplify *Hin*dIII-digested and ligated *E. coli* K-12 chromosomal DNA. As predicted, two PCR products of 1.7 and 2.0 kb were generated by this procedure. Direct analysis of the PCR products confirmed that they carried the desired chromosomal sequences and that the 3′ end of the gene downstream of *rpoE* as well as an additional 1.2-kb region had been cloned.

The complete nucleotide sequence of the gene downstream of *rpoE* is shown in Fig. 4. The open reading frame corresponding to this gene has a coding capacity for a polypeptide displaying significant similarity to MucA from *P. aeruginosa* (Fig. 5) (28% identity and 72% overall similarity). The predicted gene product of *mclA* has an M_r of 24,320 calculated from its primary structure. This is considerably less than the apparent M_r of the polypeptide product determined from its electrophoretic mobility (see below) but could perhaps be attributed to its low pI (4.95). The predicted gene product of *mclA* is also bigger than MucA (Fig. 5), and a relatively large insertion (20 amino acids located between residues 72 and 93) is predicted in MclA relative to MucA on the basis of homology programs used to align the sequences.

The polypeptide detected in our initial T7 expression analysis using the 1.7-kb fragment displayed an apparent molecular mass of 27 kDa (Fig. 3). This polypeptide was most likely a hybrid product of the truncated *mclA* gene (lacking the last eight codons and the termination signal) and a fortuitous in-frame fusion with the vector sequences (CGATGA) that supplied one additional amino acid and a stop codon as verified by sequencing the 3′ end junction. Since the nucleotide sequence of the full-size *mclA* gene was determined, it was possible to clone the complete *mclA* gene behind the T7 promoter and attempt to detect its gene product. A new set of primers was used to amplify chromosomal sequences and clone the complete *mclA* gene on a contiguous DNA fragment (see Materials and Methods). The resulting 1.3-kb PCR fragment, encompassing a portion of *rpoE* (truncated at its 5′ end), the coding region of *mclA*, and a 75-bp region downstream of *mclA*, was

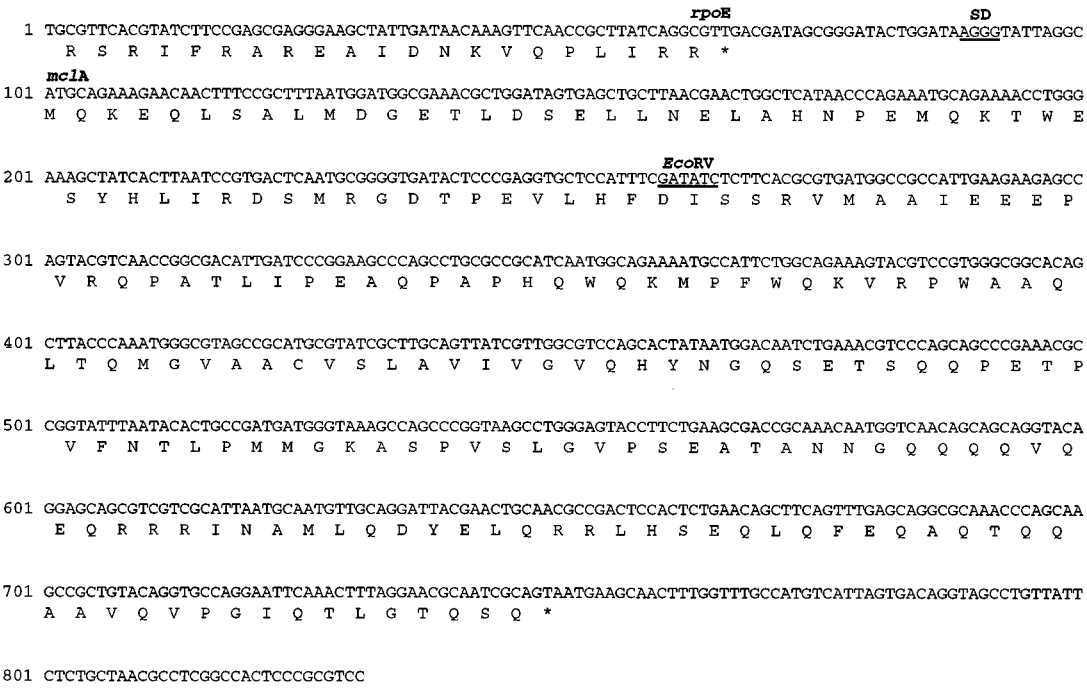

FIG. 4. Complete nucleotide sequence of the *E. coli mclA* gene. The 3′ end of the *rpoE* gene is shown. The *Eco*RV site used for insertional inactivation of *mclA* on plasmids is shown. Asterisks, stop codons; SD, putative Shine-Delgarno sequence (ribosomal binding site).

cloned into pT7-6. The resulting plasmid (pmclA) carried the complete *mclA* gene cloned in the direction of T7 transcription (Fig. 6, insert 3) and *rpoE* truncated at its 5′ end (*Hin*dIII site at position 155 of the *rpoE* coding region). As a control, a similar cloning strategy (see Materials and Methods for details) was applied to subclone the corresponding region of the 1.7-kb fragment used in the initial analyses. The resulting plasmid, pmclAΔ8, had the 5′ end of the insert located at the same *Hin*dIII site within the *rpoE* gene as in pmclA but had its 3′ end coinciding with the 3′ end of the 1.7-kb fragment carrying the truncated *mclA* gene (lacking the last eight codons and the stop signal) (Fig. 6, insert 2). When the radiolabeled polypeptide products of pmclA (Fig. 6, lane 3 and insert 3) and pmclAΔ8 (Fig. 6, lane 2 and insert 2) were examined by SDS-polyacrylamide gel electrophoresis (PAGE) and autoradiography, an increase in apparent M_r of MclA vis-à-vis that of MclAΔ8 compatible with the predicted increase in M_r of the gene product expected from the full-size *mclA* gene was observed. These experiments supported the assigned open reading frames and provided detection of the gene product of *mclA*. Collectively, these findings and similarity comparisons indicate that *E. coli* has a gene that encodes a polypeptide with a predicted primary structure similar to that of MucA and that the arrangement of *rpoE* and *mclA* closely resembles that of *algU* and *mucA* in *P. aeruginosa.*

DISCUSSION

In this work the interchangeability of *rpoE* from *E. coli* and *algU* from *P. aeruginosa* has been demonstrated. This finding, along with the strong sequence conservation of AlgU and RpoE (15, 41), suggests that *algU* encodes the σ^E equivalent of

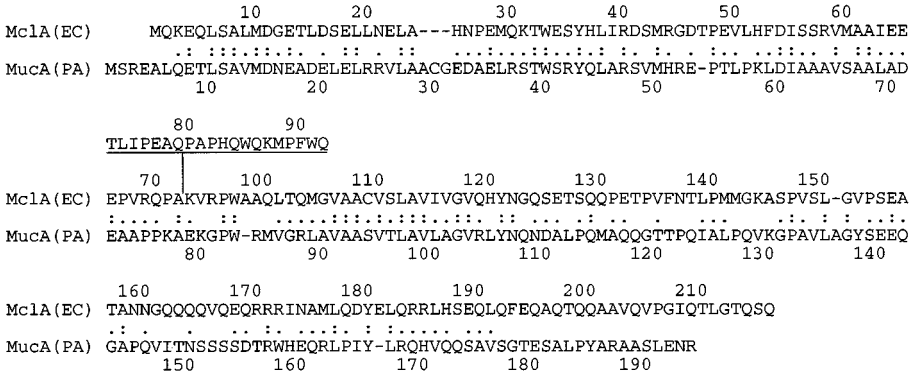

FIG. 5. Sequence alignment of the predicted gene products of MucA and MclA. The sequence from positions 73 to 92 of MclA (underlined) is shown as an insertion in MclA to permit optimal alignment and appears to have no counterpart in MucA. Double dots, identities; single dots, conservative amino acid changes. MucA and MclA display 28% identity and 72% overall similarity.

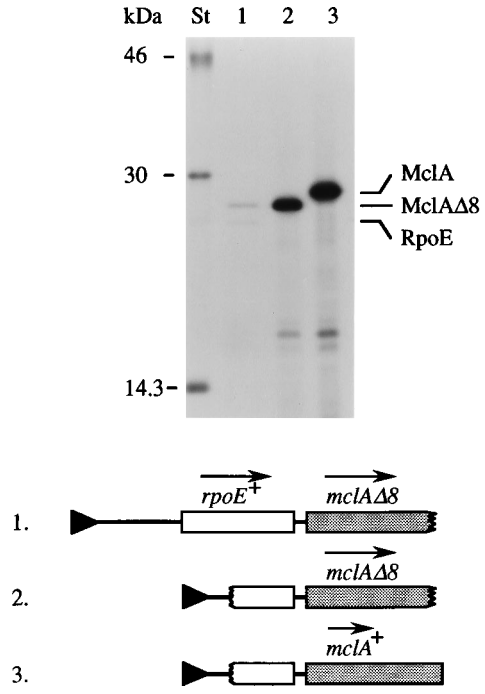

FIG. 6. Detection of the full-size *mclA* gene product by T7 expression analysis. The *mclA* gene was cloned by inverse PCR as described in Results and Materials and Methods and placed in pT7-6. (Top) Autoradiogram of radiolabeled gene products. Lanes: St, molecular mass standards; 1, insert containing the 1.7-kb DNA fragment with *rpoE* and *mclAΔ8* cloned in pT7-6 in the direction of T7 promoter transcription (same as lane 2, Fig. 3); 2, insert carrying a truncated *rpoE* (at its 5′ end) and *mclAΔ8*; 3, insert carrying truncated *rpoE* (as in lane 2) but containing the full-size *mclA* gene from *E. coli*. (Bottom) Schematic representation of constructs used in T7 expression analysis. Construct numbers correspond to lane numbers in the top panel. Other markings are as described in the Fig. 3 legend.

P. aeruginosa. In further support of this conclusion are the similarities of the canonical sequences of *rpoE*- and *algU*-dependent promoters (15, 41) and the phenotypic consequences of *algU* inactivation (41). Since σ^E has been biochemically characterized as an alternative sigma subunit of RNA polymerase in *E. coli* (18, 60), it is possible to conclude that AlgU is the analogous sigma factor of *P. aeruginosa.*

The findings reported in this work also demonstrate cross-complementation of a lesion in a sigma factor gene between two relatively distant species. Other examples of interspecies complementation using sigma factor genes are available. *Shigella flexneri rpoS* can complement the acid-sensitive phenotype of *E. coli* HB101 (56). Also, *S. typhimurium rpoS* has been cloned by complementing acid susceptibility of an *rpoS* mutant *E. coli* strain (48). In addition to these examples of complementation between closely related species, it is also known that the *Bacillus subtilis* σ^D gene can restore motility in *E. coli* with an inactivated flagellar sigma factor gene (9). Thus, although limited, precedents of cross-complementation using sigma factor genes are known. It is nevertheless of interest that the functions of σ^E and AlgU in *E. coli* and *P. aeruginosa* are sufficiently conserved to permit complementation of alginate production which appears to be the exclusive property of the latter organism.

The observed complementation of the *algU* mutation in *P. aeruginosa* with *E. coli rpoE* is full with regard to the effects on alginate production and restoration of the mucoid phenotype. Moreover, plasmid-borne *rpoE* can induce mucoidy in nonmucoid wild-type *P. aeruginosa* and activate chromosomally encoded *algD*. The latter observation is in keeping with the report of Goldberg et al. (25), who noticed that a plasmid carrying active *algU* (also termed *algT*) but inactive *mucA* and *mucB* (*algN*) induces mucoidy in nonmucoid *Pseudomonas* strains. Since none of the plasmids with *rpoE* used in the present study contained a complete *mclA* gene, this can help explain the observed results. Moreover, other studies (39, 52) indicate that both negative regulators MucA and MucB may be required for efficient suppression of AlgU activity. Since a putative gene corresponding to *mucB* was not present on the plasmid constructs with *E. coli rpoE*, σ^E was most likely fully active in our experiments. It is also possible that apart from the copy number effect, *E. coli* σ^E does not efficiently interact with *P. aeruginosa* MucA and MucB, which may be a contributing factor to the observed strong complementation of mucoidy. In contrast to alginate production, the other phenotype tested in *P. aeruginosa*, i.e., sensitivity to the redox cycling compound paraquat, was only partially complemented by *E. coli rpoE*. It should be mentioned that plasmid-borne *algU* can fully complement the paraquat sensitivity phenotype of *algU*::Tc^r *P. aeruginosa* (data not shown). The reasons underlying the observed differential complementation are not understood. It is possible that this phenomenon reflects some differences in AlgU and σ^E stability under the conditions used or specificities of their interactions with some target promoters or transcriptional factors at such sites.

Since AlgU and σ^E are functionally interchangeable in *P. aeruginosa*, as has been demonstrated in this work, the accumulated information on σ^E biochemistry on one side (18, 60) and the genetics of the *algU mucA mucB* system on the other (16, 25, 38–41, 52) may be applicable to both systems. For example, although a formal and direct biochemical demonstration that AlgU is a sigma factor still remains to be reported, the extensive biochemical characterization of σ^E as an RNA polymerase subunit which directs transcription of σ^E-dependent promoters (18, 60) may be transferable in its first approximation to AlgU. Conversely, although σ^E has been well characterized at the biochemical level, the gene for this sigma factor was not identified until recently (15, 35, 41). The recognition of the *rpoE* gene in *E. coli* has been facilitated by the strong sequence conservation of its predicted gene product with AlgU (15, 35, 41). Likewise, the negative regulators of AlgU in *P. aeruginosa*, MucA and MucB (38–40), may assist future analysis of the control of σ^E in *E. coli*, *S. typhimurium*, and possibly other bacteria. A new *E. coli* gene located downstream of *rpoE* has been identified in this work. This gene, *mclA*, encodes a polypeptide that, on the basis of its predicted amino acid sequence, shows significant similarity to MucA. The primary goal of the present study was to investigate the functional similarity of *rpoE* and *algU* as it pertains to *P. aeruginosa* physiology and was not directed towards studying the role of the putative regulators of *rpoE* in *E. coli*. With this regard, although the DNA fragment cloned by inverse PCR (plasmids pHY1861 and pHY1865; see Materials and Methods) may contain the putative *mucB* homolog, its characterization was not pursued. However, since an *E. coli* strain carrying the *algD-lacZ* fusion was available (52), it was of interest in the context of *algD* expression to test whether a mutation in *mclA* would increase *algD* transcription, analogous to the effects of *mucA* mutations (40, 52). Our attempts to insertionally inactivate *mclA* on the chromosome of this strain of *E. coli* were unsuccessful. It is perhaps of interest to note that *mucA* mutants are difficult to generate in *P. aeruginosa*. Although specific *mucA* alleles with point mutations can be exchanged on the chromosome of *P. aeruginosa*, an insertional inactivation of *mucA* has not been achieved, in contrast to the ease in obtaining insertionally

inactivated *mucB* (39). While no mutations are currently available in *mclA*, the previously published data on *algD* expression in *E. coli* support the notion that there are negative regulators of σ^E activity in this organism (52). However, the function of *mclA* remains to be directly investigated, and no definitive conclusions about its activity can be drawn on the basis of the experiments presented in this work.

The analyses of AlgU and its negative regulators MucA and MucB were at first undertaken solely in order to understand the genetic and physiological processes governing conversion to mucoidy and alginate production in *P. aeruginosa*. However, it appears now that the information gained from *P. aeruginosa* will have a more general significance for the studies of adaptation processes under stress conditions in gram-negative bacteria. The nature of the systems induced by σ^E (AlgU) (18, 30, 33) suggests that this sigma factor contributes to bacterial defense against extreme environmental stress. A recent study has demonstrated that transcription of *algU* from its AlgU-dependent proximal promoter can be induced by heat shock (53). Moreover, another studied AlgU-dependent promoter (the proximal promoter of *algR*) is inducible by osmolarity changes in addition to heat shock (53). These findings are in keeping with the report that mutations affecting outer membrane proteins in *E. coli*, normally regulated by osmolarity changes, affect σ^E activity (43). While these independent observations appear to be in agreement, it remains to be analyzed how AlgU and its negative regulators, MucA and MucB, and by extension σ^E and its putative controlling elements such as MclA interact. It will also be of interest to study how the external stimuli (e.g., heat shock, osmolarity changes, the presence of reactive oxygen species or other oxidants, and possibly other denaturing agents) are recognized and transduced to permit activation of AlgU- and σ^E-dependent genes.

Although the precise mechanism of the negative regulation of AlgU by MucA and MucB is not known, we have proposed that MucA may act as an anti-sigma factor (39, 40), as has been shown to be a mode of regulation with several other sigma factors (1, 5, 23, 29). To actually demonstrate interactions of such elements, it will be necessary to study the purified AlgU, MucA, and MucB proteins. In addition, future analysis of σ^E and its putative regulators in *E. coli*, facilitated by the observations reported here, may provide comparative information regarding further similarities or differences between the two systems. Moreover, it appears that the homologs of AlgU (σ^E) are widespread among gram-negative organisms. For example, we have recently characterized a cluster of genes in *Azotobacter vinelandii* that is highly homologous to the genes for AlgU, MucA, and MucB (42). Thus, the genetic and biochemical investigations of the mode of action of the negative regulators of AlgU (σ^E) will have an impact on our understanding of the recognition of environmental stress and how such signals are transduced into the process of gene activation in bacteria.

The system which governs conversion to mucoidy in *P. aeruginosa* may be of general significance for pathogenic bacteria. Understanding how AlgU functions and what other genes are controlled by this sigma factor may uncover important aspects of pathogenesis in organisms such as *Salmonella* spp., in which one of the known genes (*htrA*) regulated by σ^E is required for survival in macrophages and virulence in mice (30). Interestingly, an *htrA* homolog in *Brucella abortus* is a major immunoreactive protein during infection (17, 58). Moreover, the *htrA* gene of *B. abortus* has a recognizable σ^E promoter and is inducible by heat shock (50). In addition, a *Rochalimaea henselae* antigen that shares similarities with *htrA* may be of significance in disease caused by this pathogen (2), while a putative serine protease showing homology to HtrA is an immunoreactive protein in *Mycobacterium avium* (8). Since several traits controlled by σ^E (AlgU) have already been defined as virulence or persistence factors (15, 27, 30), it is likely that further investigations of the σ^E (AlgU) regulon will uncover additional determinants, thus providing more examples of the role that stress-responsive systems play in bacterial pathogenesis. Such analyses may be facilitated by the recent characterization (35, 38, 41) of the genes encoding σ^E and AlgU and by the availability of *P. aeruginosa* mutants (38–40, 52) with inactivated *algU*.

ACKNOWLEDGMENTS

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