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Pseudomonas aeruginosa infection in cystic fibrosis: nucleotide sequence and transcriptional regulation of the *algD* gene

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ABSTRACT

Pulmonary infection by mucoid, alginate producing, Pseudomonas aeruginosa is a major complication in patients suffering from cystic fibrosis (CF). To analyze the mechanisms leading to the emergence of mucoid P. aeruginosa in CF lungs, control of the algD gene coding for GDPmannose dehydrogenase was studied. Transcriptional activation of algD was shown to be necessary for alginate production. Sequencing of algD and its promoter revealed multiple direct repeats upstream of the transcription start and throughout the promoter region. Using the algD-xylE transcriptional fusion the algD promoter was demonstrated to be under positive control by the algR gene. This gene has previously been shown to undergo antibiotic promoted chromosomal amplification resulting in the emergence of the mucoid phenotype. These findings provide a basis for better understanding the control of mucoidy in P. aeruginosa.

INTRODUCTION

The major factor contributing to the high morbidity and mortality in cystic fibrosis (CF) is chronic pulmonary infection by mucoid (alginate producing) Pseudomonas aeruginosa (1). Alginate is a slimy exopolysaccharide composed of O-acetylated, β(1-4) linked D-mannuronic and L-guluronic acids (2), which, when combined with the abnormally viscous CF lung mucus, causes respiratory duct obstruction. Alginate is also believed to facilitate bacterial colonization and to play a role in protection of infecting cells from the host defense mechanisms and antibiotic therapy (1). It is interesting that mucoid forms of P. aeruginosa are rarely encountered in nature with their appearance being restricted to a very limited ecological niche present in CF patients' lungs (1). It has been shown that in the course of prolonged antibiotic treatment of pulmonary infections, the initially infecting, nonmucoid P. aeruginosa characteristicly shifts into the mucoid phenotype (3,4). However, the factors responsible for the emergence of mucoid P. aeruginosa in CF lungs are still eluding satisfactory definition.

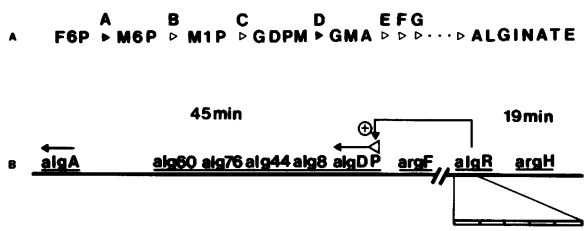


Fig. 1.
Summary of alginate biochemistry, genetic organization and regulation. (A) Alginate biosynthetic pathway. Intermediates: F6P, fructose 6-phosphate; M6P, mannose 6-phosphate; M1P, mannose 1-phosphate; GDPM, GDPmannose; GMA, GDPmannuronic acid. Enzymes: A, phosphomannose isomerase (PMI); B, phosphomannomutase; C, GDPmannose pyrophosphorylase; D, GDPmannose dehydrogenase (GMD); steps E, F and G are putative enzymatic activities involved in alginate polymerization, epimerization and export through the cell membrane. Filled triangles denote enzymes and corresponding genes presently known (6-8). Open triangles represent steps for which genes and enzymes have not been characterized. (B) Genetic organization of alginate genes. Genes algA and algD code for PMI and GMD, respectively (7,8). The algR is a positive regulatory gene necessary for algD transcription (see results). It is also part of a chromosomal amplification at 19 min of P. aeruginosa chromosomal map (schematically represented by tandem open rectangles) inducing alginate production in rec-2 P. aeruginosa grown on kanamycin supplemented media (43). Mutations alg-60, alg-76, alg-44 and alg-8 are the representatives of yet uncharacterized alginate genes belonging to separate genetic complementation groups (10). These mutations together with algA and algD gene form a cluster at 45 min on the P. aeruginosa chromosomal map (10).

The alginate biosynthetic pathway (5-8) and the organization of alginate specific genes in P. aeruginosa (9,10) have been elucidated in part (Fig. 1). We have recently shown that, in contrast to other known alginate specific chromosomal regions, gene algD coding for the GDPmannose dehydrogenase (GMD) undergoes strong transcriptional activation in mucoid cells, suggesting that the oxidation of GDPmannose to the alginate precursor GDPmannuronate, catalyzed by GMD plays a major role in the commitment of cells to alginate production (8). Here we report molecular details concerning positive regulation of the algD gene, the DNA sequences of the algD structural gene and its promoter region, and evidence that the algR gene is involved in the positive control of algD transcription.

MATERIALS AND METHODS

Materials

Reverse transcriptase and S1 nuclease were purchased from Boehringer Mannheim. The DNA sequencing kit, restriction endonucleases and other DNA

modification enzymes were from either Bethesda Research Laboratories or New England Biolabs, Inc. 2'-Deoxyinosine 5'-triphosphate was purchased from Pharmacia. The Cyclone subcloning system and computer software for DNA and protein sequence analysis were obtained from International Biotechnologies, Inc. The primer oligonucleotide GGAATCCGATTATTCG was synthesized using Applied Biosystems 380A DNA synthesizer and the phosphoramidite method (11).

Bacterial strains, plasmids, and phages.

P. aeruginosa strains used in this study were described previously (9,10). Escherichia coli strains were: JM83 (12), JM103 (13), and HB101 (14). Constructions of plasmids pVD2X (8) and pRK2013 (15) were reported previously. Bacteriophage vectors used were M13mp18 and M13mp19 (16).

Conjugal plasmid transfer and enzyme assays.

Triparental bacterial conjugation and assay of catechol 2,3 dioxygenase were described previously (8). Glucose 6-phosphate dehydrogenase was assayed according to the published procedure (17).

Recombinant DNA techniques and RNA isolation.

All techniques for cloning and handling DNA were according to the published procedures (14). Total cellular RNA was isolated by the modification of CsCl method adapted for bacterial samples and was described previously (8).

DNA sequence determination and analysis.

After initial cloning of DNA fragments in M13mp18 and M13mp19 bacteriophages, a sequential series of deletions (18) were made to produce overlapping clones for DNA sequence analysis by the chain termination method (19). To avoid "compression" problems due to the high GC content, a twenty-fold molar excess of 2'-deoxyinosine triphosphate over 2'-deoxyguanosine triphosphate was added to the deoxy/dideoxy nucleotide reaction mixtures as described (20). Polymerization reactions were carried out in a volume of 5 µl for 15 min at 42°C and for an additional 15 min at 42°C with 0.2 mM dCTP (final concentration). Deionized formamide (10 µl) was added and samples were boiled for 3 min and electrophoresed on 7 M urea, 7.5% acrylamide gels in TBE buffer (100 mM Tris/100 mM boric acid/2 mM EDTA, pH 8.3). Before autoradiography, gels were fixed in 10% acetic acid, washed in water for 30 min, transferred to Whatman 3MM paper and dried. DNA sequence data were handled and analysed using DNA database manager (21) and DNA/protein sequence analysis software (22).

S1 nuclease protection analysis.

Radiolabeled single stranded DNA probes were prepared using recombinant M13 bacteriophage templates and universal or specific oligonucleotide primers, by minor modifications of a published method (23). The polymerization reaction was carried out in two steps: first 20 min at 42°C with [α-^{32}P] dCTP (800 Ci/mmol) and then for additional 20 min at 42°C with 0.2 mM dCTP (final concentration). After heating for 5 min at 65°C, DNA was digested with an appropriate restriction endonuclease, denatured by boiling for 3 min in 70% formamide/10 mM EDTA, and electrophoresed on a 5% polyacrylamide (1:50, N,N'-methylene-bis acrylamide:acrylamide) gel in TBE buffer. After visualization of radioactive bands by autoradiography, the labeled probe was electroeluted and ethanol precipitated, with the addition of 10 µg of tRNA, 0.1 volume of 3 M sodium acetate and 2.5 volumes of ethanol for 10 min in dry ice. The DNA probe was dissolved in 10 mM PIPES (pH6.5)/0.1% SDS and mixed with RNA dissolved in water. NaCl and PIPES (pH 6.5) were added to 0.5 M and 25 mM (final concentration), respectively, and volume adjusted to 25 µl. After hybridization for 1 h at 67°C, 300 µl of ice cold S1 nuclease buffer (280 mM NaCl/50 mM sodium acetate/4.5 mM $ZnSO_4$/20 µg/ml single stranded DNA, pH 4.6) and 1000 units of S1 nuclease were added. Digestion was performed for 30 min at 37°C and stopped by phenol extraction and ethanol precipitation. The precipitate was disolved in deionized formamide and loaded on a sequencing gel.

Reverse transcription.

Reverse transcription was carried out using the following conditions. RNA (3-5 µl) was mixed with 2 µl of gel purified end labeled oligonucleotide (2 ng) dissolved in 10 mM Tris.HCl (pH 7.5)/1 mM EDTA, and sequentially heated at 85°C for 3 min, 65°C for 3 min, and 42°C for 5 min. Final volume of the reaction mixture was adjusted to 10 µl to give 40 mM Tris.HCl (pH 8.3)/40 mM KCl/8 mM $MgCl_2$/0.1 mM DTT/0.5 mM each of dATP, dTTP, dCTP, and dGTP. The reaction was initiated by the addition of 20 units of reverse transcriptase, incubated for 30 min at 42°C, and then reaction was stopped by the addition of H_2O to 300 µl followed by phenol extraction and ethanol precipitation. The products were dissolved in deionized formamide and used as described for S1 nuclease protection analysis.

RESULTS

Promoter region of the algD gene contains multiple direct repeats

In order to study molecular details of algD control, we first determined the complete nucleotide sequence for the corresponding DNA region (schematically represented in Fig. 2C and displayed in Fig. 3). To localize the algD promoter region, a putative site of the transcriptional control, the mRNA start was determined by S1 nuclease protection analysis and reverse transcription using a synthetic oligonucleotide. Initially, the approximate location of mRNA initiation site was determined using uniformly labeled single stranded DNA probes. These were made using templates from clones of DNA fragments in M13 bacteriophages and M13 universal sequencing primer (16). When these probes were hybridized to the total cellular RNA from mucoid and nonmucoid cells, a pattern of protection from digestion by S1 nuclease was obtained as summarized in Fig. 2D. These results confirmed the previously predicted direction of algD transcription based on orientation dependent, enzymological and genetic complementation studies with the cloned algD gene (8). However, we found that the use of M13 universal sequencing primer was not suitable to precisely pinpoint the mRNA initiation site. This was due to the mRNA start deduction, which included addition of a number of nucleotides to the observed size of a protected probe. This number was equal to the primer sequence plus the M13 sequence overhang that was not present in mRNA and was therefore exposed to the S1 nuclease attack. Since the sequencing ladder shows variability in the distances for consecutive bases, this introduces an error of usually 4 to 20 bp, depending on the size of a probe and the DNA region. For this reason,the precise location of the algD mRNA start was determined by reverse transcription and S1 nuclease protection (Fig. 4) using a probe produced by extension of a 16-mer oligonucleotide starting 61 bp downstream of the actual mRNA start fixed to G at the position 170 (see Fig. 3). As seen in Fig. 4, mRNA initiation sites matched perfectly using these two independent analyses.

The striking characteristic of the sequence upstream from the mRNA start was the presence of multiple direct repeats as depicted in Fig. 5. Inspection of the region upstream of the putative RNA polymerase binding site (-50 to -110) revealed the most interesting features (Fig. 5). A set of tandem 47 bp and 8 bp inverted repeats found in this region would not

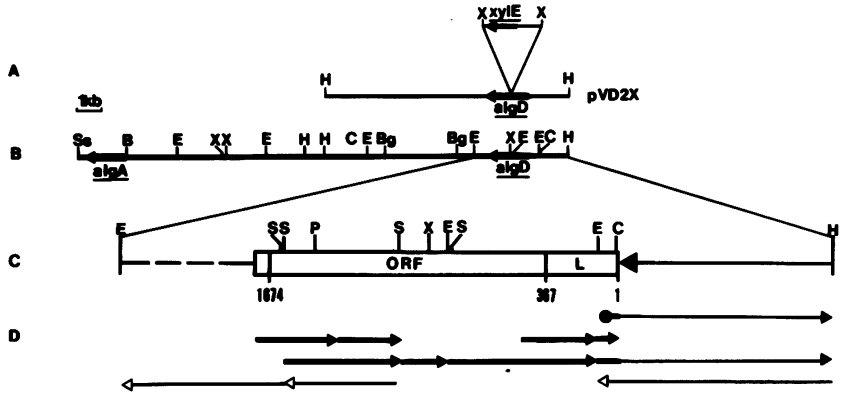


Fig. 2.
Summary of the DNA sequence and S1 nuclease protection analyses and the relative position of algD-xylE transcriptional fusion. (A) Transcriptional fusion of algD and xylE genes. Plasmid pVD2X (8) carries a 2.2 kb XhoI insert carrying xylE gene (44) in the direction of algD transcription. The original 9.5kb HindIII fragment carrying algD gene was cloned in broad host range vector pVDZ'2 (8). (B) Restriction map of the alginate gene cluster at 45 min and location of algD gene. Restriction endonuclease sites: B, BamHI; Bg, BglII; C, ClaI; E, EcoRI; H, HindIII; Ss, SstI; X, XhoI. (C) Summary of algD gene DNA sequence analysis. Various DNA fragments between HindIII and the leftmost EcoRI site on the scheme were cloned in M13mp18 and M13mp19 bacteriophages (16) and a sequential series of deletions (18) were made to produce overlapping clones for DNA sequence analysis by the chain termination method (19). Each strand was sequenced at least twice in different overlapping clones. Large triangle denotes promoter region and the orientation of transcription. Rectangles represent the DNA region transcribed in mucoid cells. L, leader mRNA; ORF, open reading frame. Restriction sites as in (B) with the addition of P, PvuII and S, SalI. Numbering starts from mRNA start (Position 170 in Fig. 3). (D) Summary of S1 protection analysis of algD gene region with total RNA from mucoid cells. Arrows represent uniformly labeled single stranded probes made using a universal 17 bp sequencing primer (16) or oligonucleotide complementary to the positions from 46 to 61 downstream of the mRNA start (filled circle) and phage templates from original or sequential deletion product clones as described in Materials and methods. Arrows indicate orientation of the probes. Thick and thin lines indicate protection and no protection, respectively, upon hybridization of the probes to mRNA from mucoid cells and digestion with S1 nuclease. Open arrows indicate probes running in the same orientation as the mRNA and therefore were not protected. Each probe was also assayed with the RNA from nonmucoid cells but in all cases no protection was observed (data not displayed).

be unexpected because regions with dyad symmetry have been found in protein - DNA recognition sites (24). However, in the case of algD the same DNA region has a 46 bp region with a hyphenated direct repeat (Fig. 5, repeats w to z). This direct repeat consists of segments (w, x, y and z)

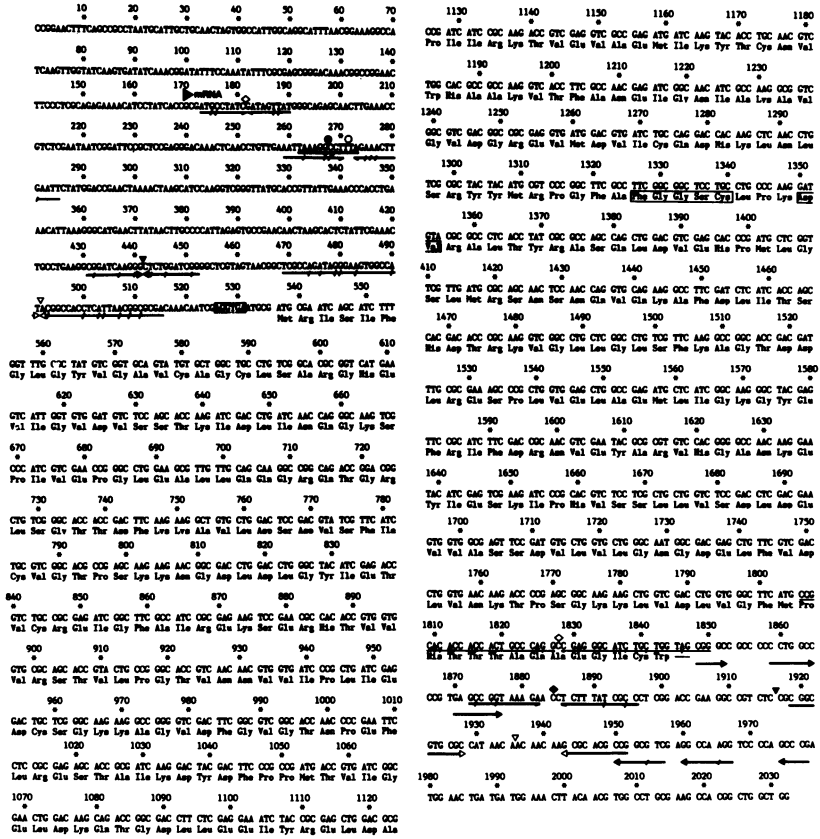
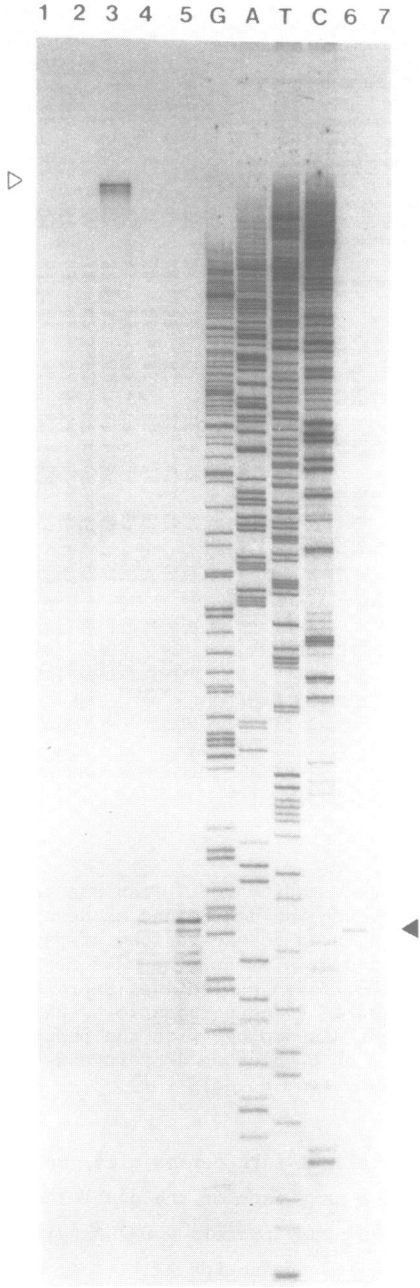


Fig. 3.
DNA sequence of the algD gene coding for GDPmannose dehydrogenase and flanking regions. DNA sequence was determined as described in the legend for Fig. 2. Underlined sequences denote regions of perfect or imperfect dyad symmetries as indicated by slashes. The mRNA start is indicated by triangle above position 170. Boxed nucleotides represent a region complementary to the 3' end of P. aeruginosa 16 S rRNA. Boxed amino acid sequences represent regions of homology with the peptides containing the catalytic site thiol group of UDPglucose dehydrogenase from bovine liver and histidinol dehydrogenase from E. coli (31).

that are repeated with a helical turn periodicity. Such spacing brings homologous sequences to the same face of the double helix, and might play a role in the putative regulatory protein - DNA binding.

Open reading frame of the algD transcript

The nucleotide sequence of the algD gene region (Fig. 3) revealed a single open reading frame (ORF) with a coding capacity for a polypeptide


1 2 3 4 5 G A T C 6 7

of 47.6 kD running in the direction of algD transcription. This is in good agreement with the M_r of the algD gene product determined to be 48 kD by SDS-PAGE (8). The ORF has a XhoI site 560 bp downstream of the ATG codon. We have shown previously that the insertion of a DNA fragment in this site causes inactivation of the algD gene (8). Preceeding the ATG codon, a 5 bp sequence complementary to the 3' end of P. aeruginosa rRNA was found possibly representing a ribosomal binding site (RBS) (25). High preference (90%) for G and C in the wobble positions of codons in the algD gene has also been reported for other sequenced chromosomal P. aeruginosa genes in our laboratory or elsewhere (26-29; I. Crawford, personal communication) . This bias is possibly related to the high GC content of Pseudomonas (30) but was suprisingly absent in the case of glutamic acid codons. This phenomenon could also be observed in other P. aeruginosa genes (26-29). Among 9 cysteines in the translated sequence of algD, the amino acid sequence around Cys residue 268 showed similarity to peptides containing active site cysteines from bovine liver UDPglucose dehydrogenase and E. coli histidinol dehydrogenase, other known pyridine nucleotide - linked, 4 electron transfer dehydrogenases (30) (Fig. 3).

Following the algD coding region several regions were found with the potential for producing stem-loop structures (Fig. 3). However, none of these were followed by the runs of T residues, typical of rho-independent

Fig. 4.
Transcription start of algD mRNA and comparison of cellular mRNA contents in mucoid and nonmucoid P. aeruginosa. A single stranded DNA probe was produced using virion DNA of the rightmost HindIII-EcoRI fragment cloned in M13mp19 as a template and hexadecanucleotide primer as described in Fig. 2 legend. Reverse transcription was performed with the same oligonucleotide labeled at the 5' end with ^{32}P, using the RNA preparations used for S1 nuclease protection studies. Reaction products were electrophoresed through 7M urea/7% acrylamide sequencing gels in lanes adjacent to the sequencing ladder (GATC, antisense strand) produced using the same primer and template as for S1 nuclease mapping. S1 nuclease mapping, lanes: 1) no RNA; 2) RNA from nonmucoid strain 8822 harboring plasmid pAD2 containing the 9.5 kb HindIII-HindIII insert (9) with the algD gene (8), 10 µg; 3) DNA probe (indicated by open arrowhead), 4) RNA from mucoid strain 8821, 10 µg, and 5) RNA from mucoid strain 8821 harboring plasmid pAD2. Reverse transcription of 10 µg of RNA from: 6) mucoid strain 8821, and 7) nonmucoid strain 8822, both harboring plasmid pAD2. Since the primer was kinased for reverse transcription it had an apparent electrophoretic mobility 0.5 bp higher than the corresponding chain terminated product from the sequencing ladder (38).

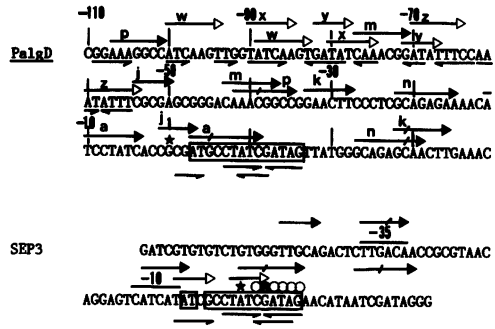


Fig. 5.
Structural features of the algD promoter: the presence of multiple direct repeats. Numbering begins with the mRNA start indicated by the star (position 170 in Fig. 3) and distances of 10 bp are indicated by vertical bars. Regions with dyad symmetry are underlined and oriented. Arrows above the sequence indicate direct repeats. Slashes indicate mismatches or extra bases. Corresponding repeats are indicated by the same letter. Repeats w, x, y and z, form a 36 bp long hyphenated partially overlapping direct repeat, that also overlaps with a 55 bp long region of dyad symmetry. The algD promoter (PalgD) is compared with Streptomyces lividans SEP3 promoter (38). Regions of homology are boxed. Circles indicate bases shared among SEP2, SEP3 and SEP6 promoters (38).

terminators. It is not known at present whether transcription might be modulated or terminated at these sites. The ORF coding for GMD is preceeded by a 367 nucleotides long leader mRNA with a distinctly low GC content (49%) as compared to the coding region (63%). Despite the presence of multiple ATG codons, no significant ORFs were detected within this sequence. However, the presence of inverted repeats (Fig. 3) might play some regulatory role.

S1 nuclease protection and reverse transcription studies of algD control

We have previously compared the algD transcription in mucoid and nonmucoid P. aeruginosa cells by RNA-DNA dot blot and Northern hybridizations, and shown that the algD gene undergoes transcriptional activation in mucoid cells (8). Here we used more sensitive techniques to determine the levels of algD transcription in nonmucoid P. aeruginosa. Results of these studies are displayed in Fig. 4, showing that the transcription of algD was absent in nonmucoid cells as judged by both S1 nuclease protection and reverse transcription. No protection from S1 nuclease was observed with RNA from nonmucoid cells even in heavily overloaded gels (data not shown). Even more, the algD mRNA was not detectable either when plasmid

TABLE 1

Gene *algD* is under positive control by the *algR* gene.

Strain	Genotype or phenotype	Specific activities CDO	G6PD
8821	*his-1* *alg*$^+$	11800 ±1170	22±3
8822	*his-1* *alg-1*	0 ± 180	28±5
8830	*his-1* Alg$^+$	14300 ±1710	25±3
8835	*his-1* *algD5*	13800 ±2480	32±4
8838	*his-1* *alg-8*	13300 ±2260	20±3
8874	*his-1* *alg-44*	13500 ±1620	24±4
8897	*his-1* *alg-76*	11300 ±1810	28±3
8887	*his-1* *alg-60*	12700 ±1270	27±4
8873	*his-1* *algA43*	6200 ± 930	28±5
8852	*his-1* *algR22*	0 ± 130	28±5

The *algD-xylE* transcription fusion plasmid pVD2X was introduced into different *P. aeruginosa* strains by triparental conjugation. Strain 8821 is mucoid CF isolate, 8822 is its spontaneous nonmucoid revertant, while strain 8830 has a stable mucoid phenotype and was obtained by EMS mutagenesis of strain 8822. All other strains are the EMS induced mutant strains derived from strain 8830 (see map in Fig. 1B) (9,10). Cultures were grown on *Pseudomonas isolation* agar for 16 h at 37°C. Catechol 2,3 dioxygenase (CDO) activity was determined in at least four independent preparations. A basal activity (1500 mU/mg), detected when the same bacterial strains harbored vector with only the promoterless *xylE* gene as an insert, was subtracted to obtain the displayed values. Glucose 6-phosphate dehydrogenase (G6PD) was determined in the same extracts as a control activity. Plasmid copy number was constant among the strains, as judged by plasmid isolation in CsCl gradients. Specific activities are expressed as mU per mg of protein. One U is defined as the amount of enzyme converting 1 µmole of substrate per min at 25°C.

pAD2 (9), carrying *algD* sequences from mucoid strain 8830 (8) and replicating with a copy number of 4-6, was present in nonmucoid strain 8822 (Fig. 4, lane 2), or when *algD* was present on a high copy number plasmid, as studied by transcriptional fusion analysis (unpublished results). In contrast, the presence of plasmid pAD2 in mucoid strain 8821 increased the mRNA level by approximately 5-fold (Fig. 4, lanes 4 and 5). In addition, the mRNA start sites matched for both chromosomal and plasmid borne

transcripts. The absence of any observable repressor titration effect with the algD promoter present on a plasmid in nonmucoid cells, was an indication that activation process might be involved.

Gene algR positively regulates algD transcription

Transcriptional fusions are exploited in many systems where genetic or environmental factors influencing promoter activities have been studied (32). Pseudomonas genetics has suffered from the lack of a system comparable to the lac fusions in E. coli or cat fusions in mammalian cells. However, recently we have used xylE gene, previously shown to be a potential promoter probe (33), to construct a transcription fusion in a heterologous Pseudomonas system (8). The xylE gene codes for catechol 2,3 dioxygenase (CDO), an easily assayable enzyme that is normally lacking in P. aeruginosa. We have previously used the algD-xylE transcription fusion to confirm transcriptional activation of the algD gene in mucoid cells (8). Here we used this fusion (pVD2X, Fig. 2A) to screen available alg mutations (9,10) for the presence of a putative positive regulatory gene. Table 1 summarizes these results. Only one mutation, alg-22 (9) (here denoted as algR22, Table 1) mapping apart from the alginate gene cluster, completely abolished transcription of algD. This suggests that the corresponding gene, algR, codes for a factor required for algD transcription.

DISCUSSION

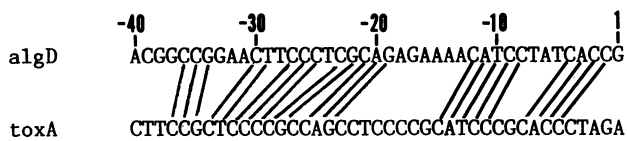
The algD promoter structure

The algD promoter area is rich in AT bases (46% GC) relative to the overall high GC content of Pseudomonas DNA (60-69% GC) (30) and the algD coding region (63% GC, Fig. 3). This is also true for Pseudomonas nah and sal operons (34). Enrichment in AT content of promoter areas has been noted in strong E. coli promoters (35,36). It is known that RNA polymerase preferentially binds to the AT rich DNA regions (37). Thus, high AT content might also be a characteristic of strong promoters in Pseudomonas. Low GC content of the leader mRNA could also contribute to the strength of the algD promoter by providing less resistance to the DNA unwinding.

The most remarkable feature of algD promoter is the presence of direct repeats. The presence of direct repeats, although less extensive than in the algD promoter, has also been indicated for a recently described set of Streptomyces lividans promoters (SEP) active in Escherichia coli (38). Another interesting finding was the sequence similarity around the algD and SEP3 mRNA starts (Fig. 5). In addition, the existence of two partially

overlapping palindromes around both the SEP3 and algD mRNA starts, might have significance for destabilization of the double helix in the process of transcription initiation. However, SEP promoters have canonical E. coli -10 and -35 regions while algD showed homology only at -10. A sequence CATCCT, located 7 bp upstream from the algD mRNA start, resembles the canonical TATAAT -10 hexamer of E. coli, sharing the most conserved positions (39) and deviating from it no more than some other E. coli promoters (40).

We have discussed elsewhere (41) the features of known Pseudomonas promoters that show similarities to either σ^{70} (rpoD) or σ^{60} (ntrA, rpoN) recognized promoter sequences in E. coli. However, algD and another P. aeruginosa chromosomally encoded gene, toxA (42), have promoters that show very little homology to either groups of promoters (5, and 4 matches, respectively, with σ^{70} recognized consensus seqence of E. coli). In contrast, the algD and toxA promoters are almost 50% homologous, with conserved regions clustered around -10 and -20 to -35 as indicated with bars between the sequences:



The significance of these findings must await comparison to other chromosomal promoters of P. aeruginosa as they become available, particularly those regulating transcription of other alginate genes.

Control of alginate production in mucoid P. aeruginosa.

Evidence presented here and elsewhere (8) strongly suggest that the transcriptional activation of at least one alginate gene (algD) is a mechanism by which P. aeruginosa becomes mucoid. In this work we have determined the essential role of algR gene in the activation of algD promoter (see Table 1 and Fig. 1B). We have previously shown that a chromosomal region of at least 16.8 kb spanning algR can induce alginate production when amplified in an originally nonmucoid rec-2 P. aeruginosa (43). It may be of particular interest that amplification of algR is induced by the growth of rec-2 P. aeruginosa in the presence of kanamycin. This might be pertinent to the situation in CF lungs, since the antibiotic treatment of recurrent pulmonary infections in CF patients often involves aminoglycosides.

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