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Cloning and Characterization of the *Pseudomonas aeruginosa lasR* Gene, a Transcriptional Activator of Elastase Expression

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We report the discovery of the *lasR* gene, which positively regulates elastase expression in *Pseudomonas aeruginosa* PAO1. The *lasR* gene was cloned by its ability to restore a positive elastase phenotype in strain PA103, a strain which possesses the elastase structural gene (*lasB*) but fails to synthesize the enzyme. Nucleotide sequence analysis revealed an open reading frame of 716 nucleotides encoding a protein of approximately 27 kDa. A labeled LasR protein of 27 kDa was detected in *Escherichia coli* by using a T7 RNA polymerase expression system. A chromosomal deletion mutant of the *lasR* gene was constructed in PAO1 by gene replacement. This mutant (PAO-R1) is devoid of elastolytic activity and elastase antigen. The deduced amino acid sequence of LasR is 27% homologous to the positive activator LuxR of *Vibrio fischeri* and the suspected activator 28K-UvrC of *E. coli*. Northern (RNA) analysis of total cellular RNA from PAO1, PAO-R1, and PAO-R1 containing the *lasR* gene on a multicopy plasmid (pMG1.7) revealed that a functional *lasR* gene is required for transcription of the elastase structural gene (*lasB*).

Pseudomonas aeruginosa produces an extracellular elastase which contributes to its pathogenicity. The enzyme is a metalloprotease with a broad substrate specificity encompassing biologically important host molecules such as elastin, collagen, transferrin, immunoglobins, and some complement components. The synthesis of elastase is regulated such that maximal enzyme production occurs during the late-logarithmic and stationary growth phases. Several environmental factors such as high concentrations of iron, ammonium chloride, glucose, and some antibiotics even at 10% of the MIC inhibit elastase synthesis (24).

The kinetics of elastase expression are analogous to those of exotoxin A (18, 24). Enzyme production is biphasic; the initial iron-independent phase is followed by an iron-repressible late logarithmic phase. This biphasic pattern of exotoxin A expression is controlled, at least in part, by the *regA* gene, a positive regulator of exotoxin A expression (18). A comparison of the levels of β-galactosidase produced by *Escherichia coli* and *P. aeruginosa* containing a β-galactosidase protein fusion encompassing the promoter region of the *lasB* gene indicates that the *lasB* promoter is approximately 0.1% as active in *E. coli* as in *P. aeruginosa* (40). A possible explanation for this is that *E. coli* may lack a factor(s) necessary for optimal *lasB* promoter function. The parallels between elastase and exotoxinA expression, coupled with the weak *lasB* promoter activity in *E. coli*, suggest the existence in *P. aeruginosa* of a positive regulator of elastase expression.

We attempted to clone a positive regulator by taking advantage of the naturally occurring strain PA103 (29). This hypertoxigenic sputum isolate produces no detectable extracellular or intracellular elastase and only small amounts of extracellular alkaline protease (23). Southern analysis indicated that PA103 possesses the *lasB* gene; however, further experimentation revealed that the *lasB* gene is poorly transcribed in PA103. It was reasoned that the elastase-negative phenotype of PA103 might be due to the absence of a positive activator.

In this paper, we report the cloning, sequencing, and characterization of a positive transcriptional regulator of the *lasB* gene of *P. aeruginosa*, which we have named *lasR* (the regulator of *lasB*). The deduced amino acid sequence of the LasR protein exhibits homology to a group of procaryotic transcriptional activators. In this regard, we show that a functional *lasR* gene is required for transcription of the *lasB* gene.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. Bacterial strains and plasmids are listed in Table 1. *P. aeruginosa* was grown in peptone trypticase soy broth (PTSB) unless otherwise indicated (35). The other rich media were 2 × YT for single-strand phage rescue and Luria-Bertani broth (30). Vogel Bonner minimal medium (VBMM), M9 minimal medium, 1.5% skim milk, and elastin overlay agar plates were described previously (30, 46, 55). When appropriate, the following antibiotic concentrations were used. For *E. coli*, 20 μg of tetracycline (TC) per ml, 100 μg of ampicillin (AP) per ml, 50 μg of kanamycin (KN) per ml, and 200 μg of rifampin (RF) per ml were used. For *P. aeruginosa*, 100 μg of TC per ml, 200 μg of carbenicillin (CB), and 500 μg of streptomycin (SM) per ml were used.

Mobilization of the PAO1 gene bank into PA103-AP2. A pLAFR-PAO1 gene bank for *E. coli* HB101 was transferred to PA103-AP2 with the help of the conjugative properties of MM294(pRK2013) (16). Transconjugants were initially selected on VBMM–100 μg of TC per ml. After 24 to 48 h, transconjugants were then picked onto skim milk plates to assay for extracellular protease production.

DNA biochemistry. Recombinant DNA techniques were performed according to well-established procedures (6, 30, 53). Plasmids were transformed into *P. aeruginosa* by a combination of $CaCl_2$ and $RbCl_2$ treatments as previously described (3).

Western blot (immunoblot) analysis of lysates and superna-

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TABLE 1. Strains and plasmids

Strain or plasmid	Description	Source or reference
Strains		
P. aeruginosa		
PAO1	Prototroph, elastase positive	22
PAO1-SR	Sm^r, PAO1, elastase positive	This study
PAO-R1	Sm^r Δ*lasR*::*tet*	This study
PA103	Elastase negative, alkaline protease positive	29
PA103-AP2	EMS mutant of PA103, elastase negative, alkaline protease negative	23
E. coli		
HB101	Sm^r *pro leu thi lacY hsd-20 endA recA ara-14 galK xyl-5 mtl-1 supE44*	7
K38	Host for pT7 expression system, HfrCλ	39
MM294	*endA hsdR thi pro*	2
XL1-Blue	Tc^r *recA1 lac endA1 gyrA96 thi hsdR17 supE44 relA1* (F′ *proAB* $lacI^q$ *lacZΔM15* Tn*10*)	Stratagene
SM10	Kn^r *thi thr leu tonA lacY supE recA*:RP-2-Tc::Mu	44
Plasmids		
pCP13	Tc^r Km^r IncP λcos$^+$, broad-host-range cloning vector	10
pLAFR1	Tc^r IncP λcos$^+$, broad-host-range cloning vector	19
pRK2013	Km^r ColE1 Tra $(RK2)^+$	16
pRO1614	Ap^r, source of 1.8-kb stabilizing fragment	36
pRTP1	Ap^r *rpsL oriT cos*	48
pUC18	Ap^r, general cloning vector	31
pSW200	pUC18 containing 1.8-kb *Pst*I stabilizing fragment from pRO1614	This laboratory
pT7-6	Ap^r ColE1, contains T7 promoter	50
pGP1-2	Km^r P15A *cI857*, T7 polymerase gene	50
pKS	High-copy-number cloning and sequencing vector	Stratagene
pSK	pKS with polylinker in opposite orientation	Stratagene
pMJG1	pLAFR1 containing ca. 20-kb *Eco*RI fragment from PAO1	This study
pMJG2	pLAFR1 containing ca. 20-kb *Eco*RI fragment from PAO1	This study
pMG11	pSW200 with 11-kb *Eco*RI fragment from pMJG2	This study
pMG3.9	pSW200 with 3.9-kb *Kpn*I-*Eco*RI fragment from pMG11	This study
pMG319	pRTP1 with *lasR* deletion tagged with Tc^r	This study
pMG1.7	pSW200 with 1.7-kb *Sac*II-*Eco*RI fragment from pMG3.9	This study
pMG131.7	pCP13 with 1.7-kb *Sac*II-*Cla*I fragment from pMG1.7	This study
pT7-61.7	pT7-6 containing a 1.7-kb *Sac*I-*Eco*RI fragment	This study

tants. Supernatants were isolated from 18-h cultures grown in PTSB at 32°C. Lysates were prepared from 10-ml cultures of late-logarithmic-phase cells grown under identical conditions. Cells were pelleted, washed with phosphate-buffered saline (PBS) and resuspended in 2 ml of PBS containing 20 mM EDTA. Cells were then broken in a French pressure cell. Samples (40-μl lysates and 10-μl supernatants) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Laemmli (28). Separated proteins were transferred to nitrocellulose as described by Towbin et al. (52). The blot was blocked with 5% Blotto (5% nonfat dry milk in Towbin saline [TS; 150 mM NaCl, 50 mM Tris-HCl, pH 7.5]) and incubated with polyclonal rabbit antielastase antiserum (5) at a 1:1,000 dilution. The blots were washed with TS, incubated with 1 μCi of ^{125}I-staphylococcal protein A (Dupont, NEN) in TS for 1 h at 37°C, washed, dried, and exposed to X-ray film (Kodak XAR) at −70°C with an intensifying screen. Polyclonal rabbit antielastase antiserum was raised in female New Zealand White rabbits by using commercially available *Pseudomonas* elastase (Nagase Biochemicals). Protein concentrations were determined by the BCA method (Pierce Chemical Corp.).

DNA sequencing. The 1.7-kb *Sac*II-*Eco*RI fragment was subcloned into the sequencing vectors pKS and pSK. A number of subclones were constructed on the basis of convenient enzyme sites. When no sites were present, 20-mer oligonucleotides were synthesized on a 380B Applied Biosystems DNA synthesizer and used as primers. DNA was sequenced with the Sequenase kit (U.S. Biochemicals Corp.), which is based upon the dideoxy-chain termination method (41). Single-stranded template was produced as described by Stratagene. Double-strand sequencing was also done by the method of Kraft et al. (25). Stretches of high GC content, notorious for compressions, were resequenced by using 7-deaza-dGTP to resolve ambiguities (4, 33). Sequence data were analyzed by using IBI DNA/protein sequence analysis software and the University of Wisconsin Genetics Computer Group Software packages (13, 37).

Assay for elastolytic activity. Elastolytic activity was detected by two assays. Bacteria were plated on nutrient agar coated with 5 ml of top agar containing insoluble bovine neck ligament elastin (Sigma) (35). The disappearance of elastin beneath and around the colonies after 24 to 48 h of incubation at 37°C, resulting in a clear zone, is indicative of elastase production. Elastase activity of supernatants was assayed by using elastin Congo red (ECR; Sigma) as a substrate (35). Samples of 50 μl of supernatant from 18-h cultures were added to tubes containing 20 mg of ECR and 1 ml of 10 mM Na_2HPO_4, pH 7.0, and incubated with agitation for 4 h at 37°C. Insoluble ECR was removed by centrifugation, and the absorbance at an optical density of 495 nm of the supernatants was determined.

Selective labeling of the LasR protein. The LasR protein

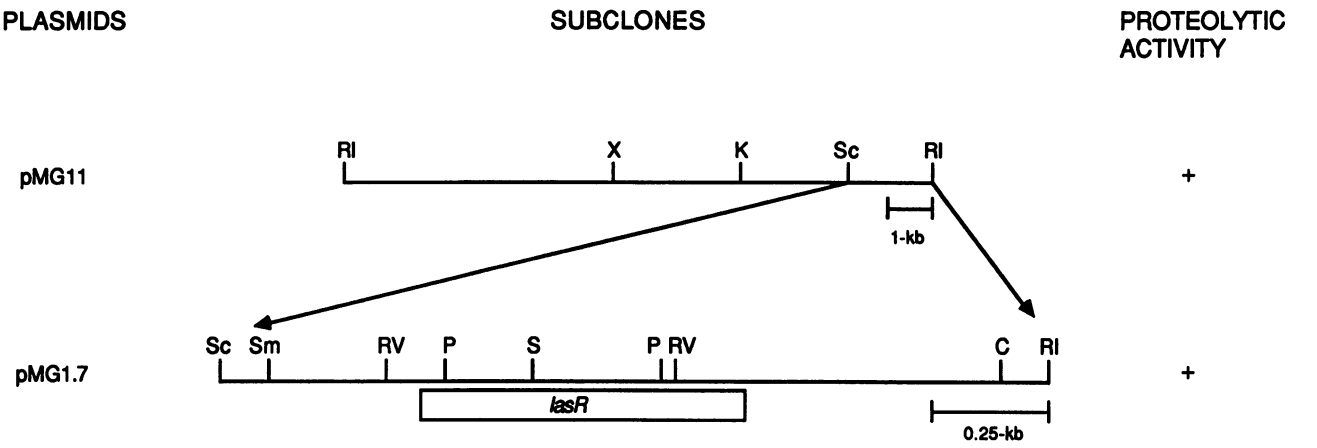


FIG. 1. Localization of proteolytic activity to a 1.7-kb *Sac*II-*Eco*RI fragment. Biologic activity was assayed by transforming PA103-AP2 with a particular subclone, plating on 1.5% skim milk agar, and scoring for proteolysis (zones of clearing). C, *Cla*I; K, *Kpn*I; RI, *Eco*RI; RV, *Eco*RV; P, *Pst*I; S, *Sal*I; Sc, *Sac*II; Sm, *Sma*I; X, *Xho*I.

was selectively labeled by a previously described method (50). *E. coli* K38(pGP1-2) cells harboring recombinant plasmids were grown in Luria-Bertani medium at 30°C to mid-log phase (optical density at 595 nm, ~0.4). One milliliter of cells was removed, washed with M9 medium, resuspended in M9 medium plus 0.01% 19 amino acids minus methionine, and incubated for 60 min at 30°C (50). Cells were shifted to 42°C for 20 min, RF was added to 200 μg/ml, and cells were incubated for an additional 10 min at 42°C. Cells were then shifted to 30°C for 30 min. Samples of 0.5 ml were then pulsed with 10 μCi of [^{35}S]methionine for 5 min at 30°C. Pellets were resuspended in 150 μl of loading buffer (60 mM Tris-HCl, 2% SDS, 5% β-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue) and boiled for 5 min, and 40 μl of each was run on a 10% SDS-PAGE gel. The gel was soaked in Amplify (Amersham) for 30 min, dried, and exposed to X-ray film at −70°C.

Gene replacement. A PAO1 deletion mutant of the *lasR* open reading frame was created by using the plasmid cloning vector pRTP1, a plasmid originally constructed for gene replacement in *Bordetella pertussis* (48). The Tc cartridge was isolated from pBR322, and its ends were made blunt with T4 polymerase (Bethesda Research Laboratories, Inc.). The blunt-end Tc gene (1.4 kb) was then cloned into a 649-bp *Eco*RV deletion of the 3.9-kb subclone (3.25 kb) containing *lasR*. This recombinant 4.65-kb *Hin*dIII-*Eco*RI fragment was then cloned into pRTP1 to generate a gene replacement plasmid, pMG319. This plasmid was mobilized into a spontaneous Sm-resistant PAO1 (PAO-SR) by a biparental plate mating with SM10(pMG319). Transconjugants were plated onto VBMM–100 μg of TC per ml to select for single crossover events. Tc^r transconjugants were then picked onto plates with VBMM–500 μg of SM per ml–100 μg of TC per ml to select for loss of the vector sequence containing the Sm^s allele.

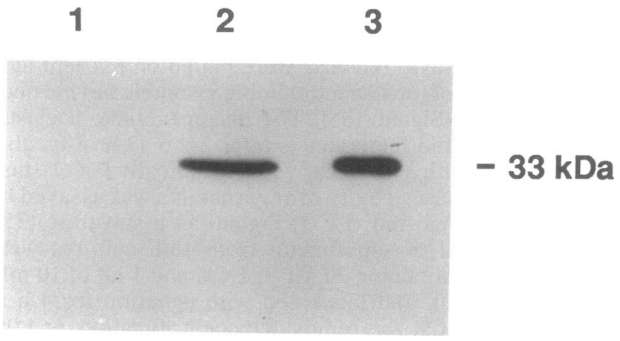


FIG. 2. Autoradiograph of immunoblot of supernatants probed with rabbit antielastase antiserum. Lanes: 1, PA103(pLAFR); 2, PA103(pMG131.7); 3, purified elastase.

Southern blot analysis of deletion mutants. Chromosomal DNA was prepared from four putative deletion mutants and PAO1 (53). Approximately 5 μg of each sample was digested with *Pst*I, separated on a 0.7% agarose gel, transferred to nylon (Amersham), and hybridized to a ^{32}P-labeled, nick-translated 1.3-kb *Pst*I probe according to the manufacturer's specifications. After stringent washes, the blot was exposed to X-ray film.

RNA biochemistry and analysis. Total RNA was prepared from cultures grown at 37°C in PTSB without antibiotic pressure to an optical density at 540 nm of ~1.4. RNA was isolated by centrifugation through a 5.7 M CsCl cushion essentially as previously described (12). The cold buffer wash contained 20 mM aurin tricarboxylic acid, and the lysis buffer contained 10 mM vanadyl ribonucleoside complexes and 0.05% diethyl pyrocarbonate (12, 38). Pelleted RNA was resuspended in RNase-free water, precipitated with ethanol, and stored as an ethanol precipitate. When needed, aliquots were precipitated, extracted once with phenol to remove residual vanadyl complexes, and reprecipitated. Northern blot analysis was performed by the method of Fourney et al. (17). Seven micrograms of RNA from each sample was fractionated on a 0.66 M formaldehyde–1.2% agarose gel, transferred to nylon, and hybridized to a ^{32}P-labeled 1.27-kb *Sca*I internal fragment of the *lasB* gene. After stringent washes, the nylon was exposed to Kodak XAR film at −70°C with an intensifying screen. The RNA samples were prestained with ethidium bromide before fractionation. This allowed visualization of the RNA before the transfer to nylon. The 23S and 16S bands were intense and sharp; no degradation was apparent in any lane.

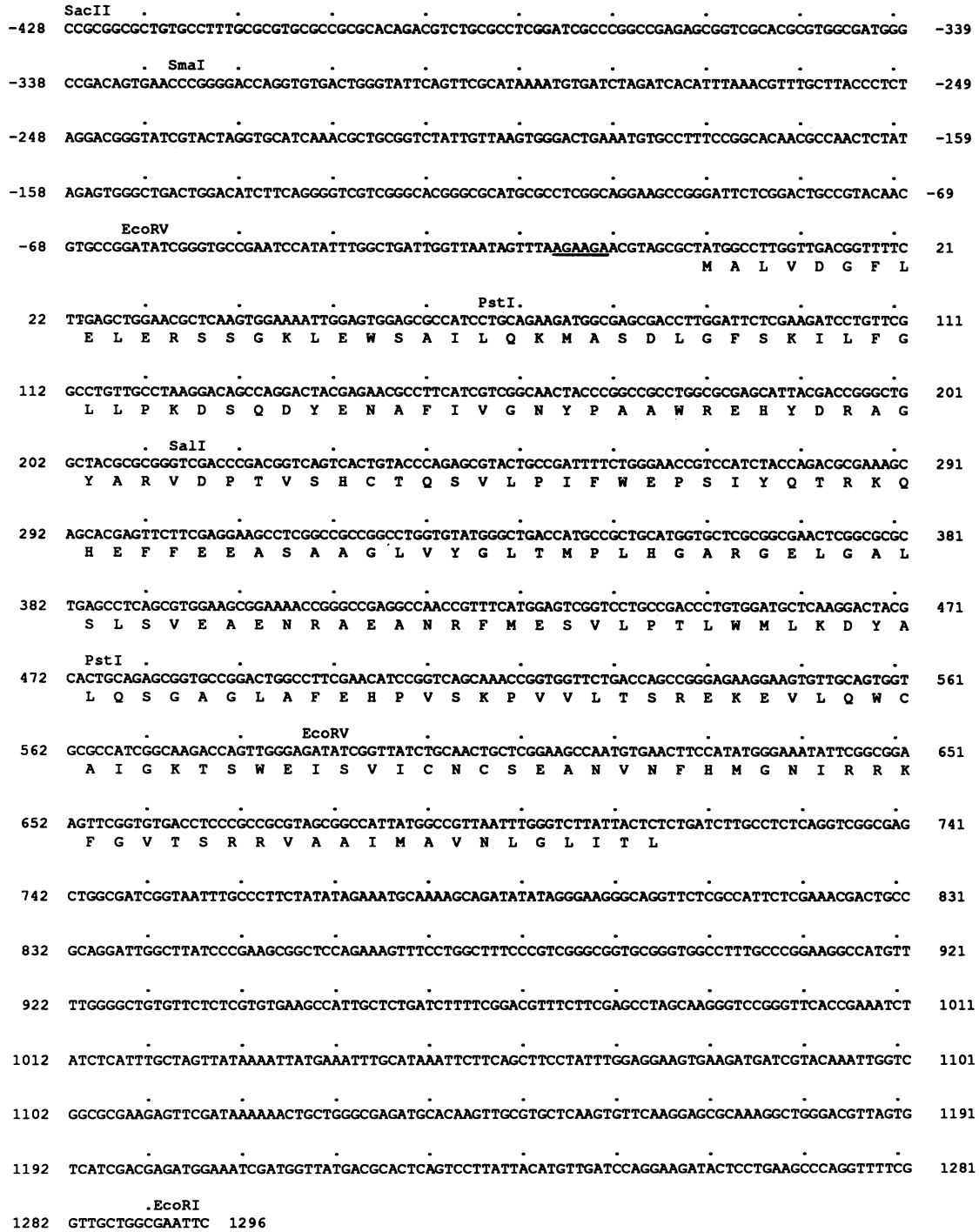


FIG. 3. Nucleotide sequence of the 1,725-bp *Sac*II-*Eco*RI fragment containing the *lasR* open reading frame. The deduced amino acid sequence (single-letter code) is shown below the nucleotide sequence. A putative ribosomal binding site is underlined.

Nucleotide sequence accession number. The nucleotide sequence of the 1.7-kb *Sac*II-*Eco*RI fragment has been submitted to GenBank and was assigned accession no. M59425.

RESULTS

Cloning of the *lasR* gene from *P. aeruginosa*. The skim milk plate assay facilitates the detection of extracellular elastase. Wild-type PA103 produces small zones of clearing on these plates primarily due to alkaline protease. To avoid any background problems during the initial cloning and subcloning procedures, a well-characterized EMS mutant, PA103-AP2, was used. PA103-AP2 produces no extracellular proteases, and therefore no zones of clearing are apparent on skim milk plates (23). It was anticipated that the successful cloning of a positive regulator would result in large zones of clearing.

A pLAFR-PAO1 gene bank in HB101 was mobilized into PA103-AP2 with the help of pRK2013. Transconjugants were initially selected on plates with VBMM–100 μg of TC per ml and then picked onto plates with skim milk–100 μg of TC per ml. Of 2,000 colonies assayed, two positive clones displayed approximately 2-mm zones around the colonies at 24 h. Cosmids pMJG1 and pMJG2 were isolated from these clones and transferred to HB101 to facilitate DNA biochemistry. Restriction of both recombinant plasmids with *Eco*RI yielded a common 11-kb fragment which was subcloned into pSW200 to yield pMG11. The proteolytic phenotype was associated with this 11-kb fragment. Elastin Congo red assays and Western blot analysis on the supernatants of PA103-AP2(pMG11) cultures revealed that the zones of clearing on skim milk were due primarily to elastase activity (data not shown).

Figure 1 illustrates the localization of biologic activity to a 1.7-kb *Sac*II-*Eco*RI piece of DNA. When *Eco*RI-digested PAO1 and PA103 genomic DNAs were probed with a ^{32}P-labeled 1.7-kb *Sac*II-*Eco*RI fragment, bands of 11 and 15 kb, respectively, hybridized with the probe (data not shown). This indicates that at least part of the *lasR* sequence is present on the chromosome of PA103 but on a different restriction fragment from that in PAO1. The subcloning scheme made use of PA103-AP2 and a multicopy stabilized pUC vector. Further studies were done with PA103 and the low-copy-number, broad-host-range vector pLAFR and/or pCP13.

Detection of elastase in the supernatant of PA103 (pMG131.7). The elastin plate assay and the ECR analysis detected substantial elastolytic activity in PA103(pMG131.7) compared with that in the vector control (data not shown). Ten microliters of supernatant was subject to 10% SDS–PAGE, transferred to nitrocellulose, and probed with rabbit antielastase antiserum at a 1:1,000 dilution. As demonstrated by the autoradiograph in Fig. 2, elastase antigen was synthesized and secreted into the medium of PA103(pMG131.7). No elastase antigen was detected in the PA103(pLAFR) supernatant (Fig. 2, lane 1) or in cell extracts of PA103 (pLAFR) (data not shown).

DNA sequence analysis and expression in *E. coli*. The nucleotide sequence of the 1.7-kb *Sac*II-*Eco*RI fragment was determined by the Sanger dideoxy method (Fig. 3). Computer analysis revealed an open reading frame consisting of 716 bases which we designated *lasR*. A potential ribosomal binding site was found at 10 to 11 nucleotides upstream from the putative start of translation. The frequency of *P. aeruginosa* codon usage and the GC content (59%) are high throughout the *lasR* open reading frame, good indicators that *lasR* represents an actual *P. aeruginosa* gene (22, 56). The translated protein has a deduced molecular mass of 26,618 Da. The percentage of hydrophobic residues (leucine, isoleucine, methionine, and valine) predicts a relatively hydrophilic protein. No likely transmembrane regions were detected by a Kyte and Doolittle hydropathy analysis (27).

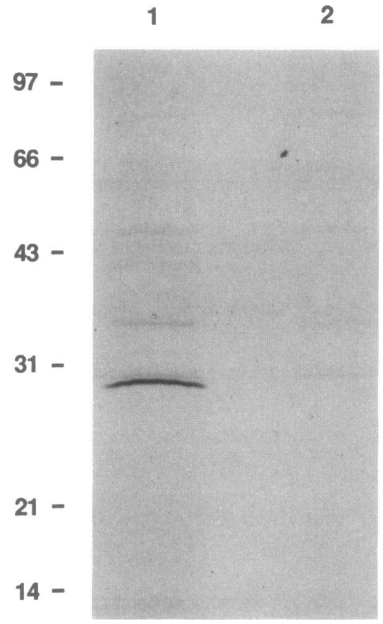
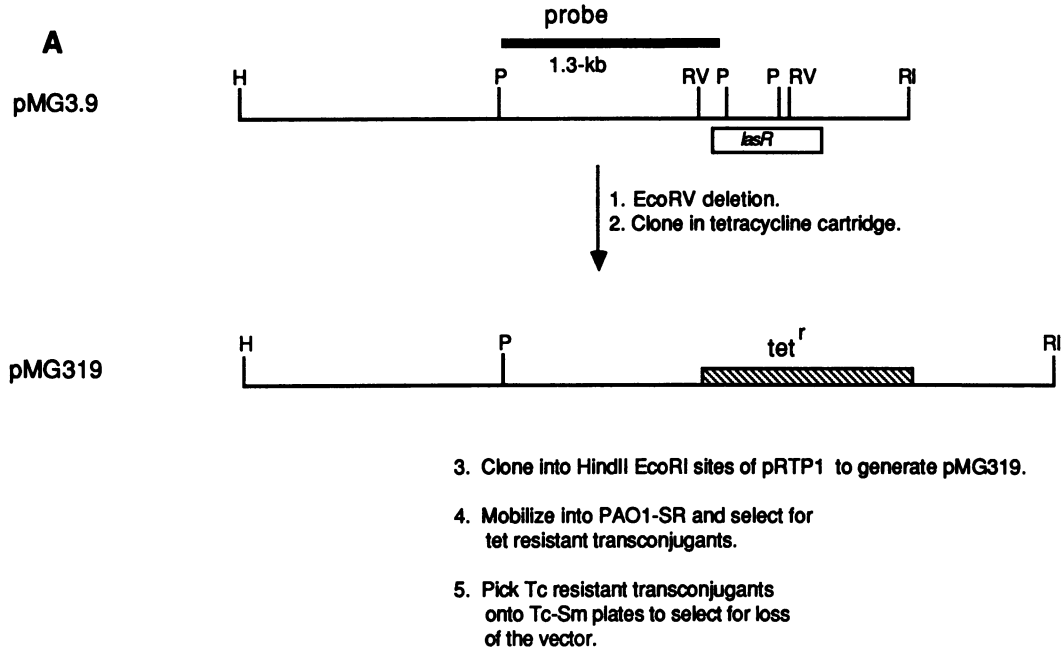


FIG. 4. Autoradiograph illustrating the selective labeling of the LasR protein with [^{35}S]methionine. Lanes: 1, K38(pGP1-2)(pT7-61.7); 2, K38(pGP1-2)(pT7-6) (vector control). Molecular mass markers (in kilodaltons) are indicated at the left.

To show that the *lasR* gene was translated, we tested for expression in *E. coli*. The *lasR* gene was placed under control of the T7 promoter, and its translation product was labeled as previously described (50). Figure 4 demonstrates the selective labeling of a protein of ca. 27 kDa in the extract of K38(pGP1-2)(pT7-61.7) (Fig. 4, lane 1), in good agreement with the deduced molecular mass of 26,600 Da for the LasR protein. No labeled protein was seen in the vector control extract (Fig. 4, lane 2).

Construction and characterization of *lasR* deletion mutant in PAO1. We were able to isolate the *lasR* gene from PAO1 by its ability to restore a positive elastase phenotype in strain PA103; however, the genetic background of PA103 is not as well characterized as that of PAO1. To further our studies of *lasR* and assess its function, we constructed a chromosomal deletion mutation of *lasR* in PAO1. A 649-bp *Eco*RV fragment, encompassing 590 bp of the *lasR* sequence, was deleted from pMG3.9. The deletion was tagged with the Tc gene and cloned into the gene replacement vector pRTP1 to generate pMG319 (Fig. 5A). This plasmid contains a vegetative origin of replication derived from ColE1 and hence cannot stably replicate in *P. aeruginosa*. After transfer of pMG319 via conjugation to PAO-SR, transconjugants were plated onto VBMM–100 μg of TC per ml to select for those organisms in which pMG319 stably integrated into the chromosome at areas of homology to the cloned sequence. Selection for the loss of the vector sequence containing the dominant Sm^s allele identified Tc^r and Sm^r transconjugants which should represent those strains in which allelic ex-



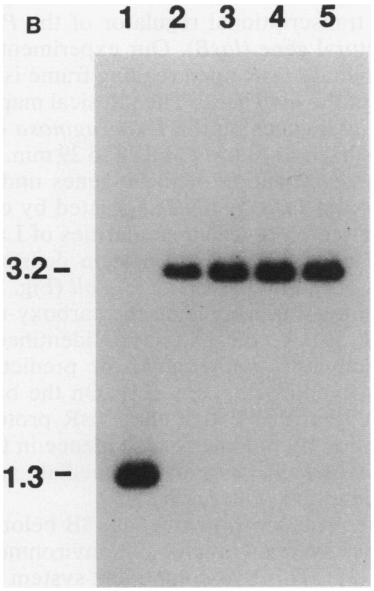


FIG. 5. (A) Cloning strategy for the construction of pMG319, the plasmid used to replace the *lasR* gene with a deletion construct on the PAO1 chromosome. H, *Hin*dIII; P, *Pst*I; RV, *Eco*RV; RI, *Eco*RI. (B) Genomic Southern hybridization analysis illustrating the replacement of the *lasR* open reading frame with a Tc-marked *lasR* deletion. All samples were digested to completion with *Pst*I and hybridized to the ^{32}P-labeled 1.3-kb *Pst*I fragment shown in panel A. Lanes: 1, PAO1; 2 to 5, four PAO-R1 clones. The molecular sizes of the bands (kilobases) are indicated at the left.

change had occurred. If gene replacement had occurred, the two *Pst*I sites contained within the *Eco*RV deletion would have been lost. Digestion of the wild-type PAO1 chromosome with *Pst*I yields a 1.3-kb fragment which was used as the probe in the Southern blot analysis. For a strain in which gene replacement had occurred, a *Pst*I digestion would yield a fragment of at least 3.2 kb, depending on how far from the *Eco*RI site the next chromosomal *Pst*I site maps. Southern analysis demonstrated a shift in the hybridization pattern from the 1.3-kb wild type to 3.2 kb for four PAO1 Sm^r Tc^r isolates (Fig. 5B). These results indicated that in all four PAO1 Sm^r Tc^r strains, the *lasR* gene was replaced with the Tc-marked deletion. One such mutant (PAO-R1) was chosen for further characterization.

No elastase activity was detected in PAO-R1 by the elastin plate or ECR assay (data not shown). Immunoblot analysis demonstrated no intracellular or extracellular elastase antigen in PAO-R1 (Fig. 6, lanes 2 and 4), whereas elastase antigen was readily detected in the parent PAO1 positive control (Fig. 6, lanes 1 and 3). Moreover, PAO-R1 was complemented in *trans* with pMG1.7, a multicopy plasmid containing the *lasR* gene (Fig. 6, lane 5). These observations are consistent with our hypothesis that *lasR* encodes a positive regulator of the *lasB* gene.

The *lasR* gene affects *lasB* expression at the transcriptional level. Many positive activators exert their effect at the transcriptional level. To assess whether the *lasR* gene affects *lasB* transcription, we prepared total RNA from PAO1, PAO-R1, PAO-R1(pMG1.7), and PAO-R1(pSW200) and probed each sample for the presence of the *lasB* message.

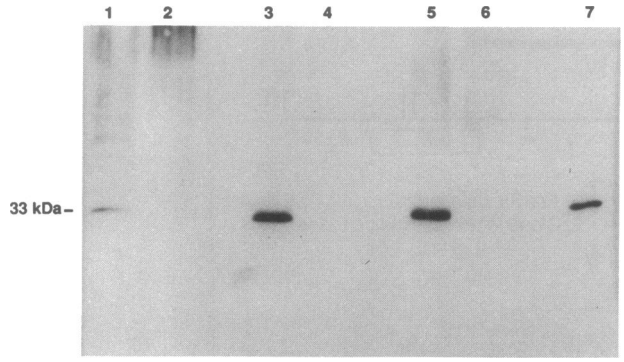


FIG. 6. Autoradiograph of an immunoblot illustrating the intracellular and extracellular elastase null phenotype of PAO-R1 and the complementation of this mutant when the *lasR* gene is supplied in *trans*. Lanes: 1, PAO1 cell lysate; 2, PAO-R1 cell lysate; 3 to 6, supernatants; 3, PAO1; 4, PAO-R1; 5, PAO-R1(pMG1.7); 6, PAO-R1(pSW200); 7, purified elastase.

RNA isolated from PAO1 hybridized to the *lasB* probe at ca. 1.8 kb, a transcript size consistent with the initial 54-kDa translation product of *lasB* (Fig. 7, lane 1) (5). No *lasB*-specific mRNA was detected in PAO-R1 (Fig. 7, lane 2). The presence of the *lasR* gene in *trans* in PAO-R1(pMG1.7) restored the presence of *lasB* mRNA and gave a hybridization pattern indistinguishable from that of PAO1 (Fig. 7, lanes 3 and 4).

Comparison of LasR with other regulatory proteins. The program TFASTA provided by the Genetics Computer Group Software package was run with the predicted amino acid sequence of LasR. Similarity was found to two proteins: the 28-kDa protein encoded by ORF1 in the upstream regulatory region of the *uvrC* gene and the LuxR protein of *Vibrio fischeri* (14, 15, 26). The 28-kDa ORF1 protein is a suspected positive regulator of *uvrC*. LuxR is a known activator of the bioluminescence operon of *V. fischeri*. The homology is scattered throughout the three proteins; however, there is a preponderance of conserved sequences in the carboxy terminus (Fig. 8A). In order to refine our search, we performed a profile search by using the 60 amino acids from the carboxy termini which exhibit the highest degree of identity (Fig. 8B). The profile search yielded a group of regulatory proteins, some belonging to two-component procaryotic regulatory systems such as the *narL narX* nitrate reductase system of *E. coli* or the *fixL fixJ* nitrogen regulation system of *Rhizobium meliloti* (11, 47). By using weighted matrices, Henikoff et al. (21) previously identified the primary sequence similarities between LuxR, 28K-UvrC, and the group of more distantly related sensor-regulator proteins. Henikoff also identified TrpO, as shown Fig. 8B.

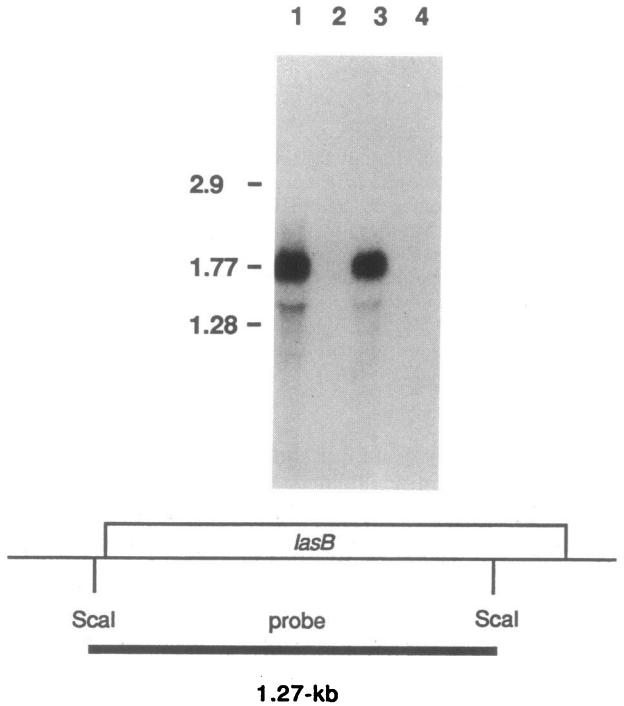


FIG. 7. Northern blot analysis of total cellular RNA hybridized to a ^{32}P-labeled 1.27-kb *ScaI* fragment internal to the *lasB* gene. Lanes: 1, PAO1; 2, PAO-R1; 3, PAO-R1(pMG1.7); 4, PAO-R1(pSW200). The molecular size standards (in kilobases) are indicated to the left.

DISCUSSION

We have identified the *lasR* gene of *P. aeruginosa* PAO1 as a positive transcriptional regulator of the *Pseudomonas* elastase structural gene (*lasB*). Our experiments have illustrated that an intact *lasR* open reading frame is required for transcription of the *lasB* gene. The physical map positions of the *lasR* and *lasB* genes on the *P. aeruginosa* chromosome have been localized to 13 to 14 and 28 to 29 min, respectively (54). The physical location of these genes underscores the *trans* activation of *lasB* by *lasR* suggested by our data.

A computer search revealed similarities of LasR to LuxR of *V. fischeri* and a 28K-UvrC protein defined in the upstream region of the *uvrC* gene of *E. coli* (Fig. 8A). A more refined homology search utilizing the carboxy-terminal segment of LasR, LuxR, and 28K-UvrC identified a group of procaryotic activators with known or predicted carboxy-terminal DNA-binding domains (21). On the basis of these similarities, it is probable that the LasR protein activates *lasB* transcription by binding to a sequence in the upstream region of the *lasB* gene or to an intermediate gene which in turn directly interacts with *lasB*.

Many of the proteins shown in Fig. 8B belong to sensor-regulator kinase systems involved in environmental sensing and response (32). This two-component system is a common theme of regulation among procaryotes, especially of virulence determinants (32, 49). A conserved N-terminal domain is characteristic of the various subclasses of response regulators and reflects a conserved phosphotransfer enzymology (49). The N termini of LasR, LuxR, and 28K-UvrC share homology; however, no similarities to amino termini of any family of response regulators have been detected. The sensor-regulator motif of LuxR appears to be contained within one molecule. It may be that LasR, LuxR, and perhaps 28K-UvrC constitute a family of response regulators which are actually one-molecule sensor-regulator systems. The N terminus would function as sensor and transmit a particular signal to the DNA-binding carboxy terminus.

The environmental or intracellular signals which regulate elastase production are not understood. Enzyme expression is tightly coupled to the growth cycle. Bioluminescence in *V. fischeri* is also cell cycle dependent; the LuxR protein apparently responds to cell density by sensing increasing

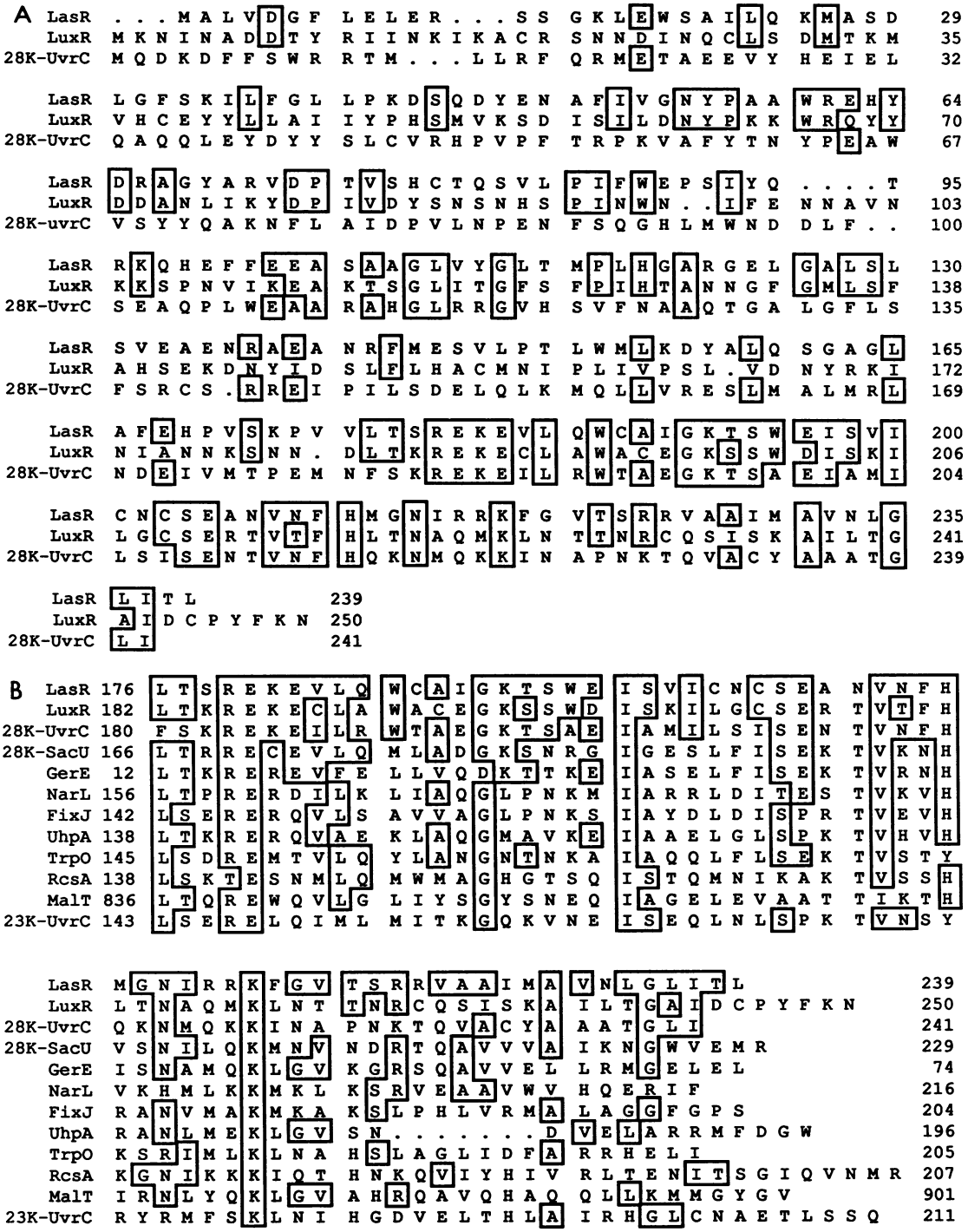


FIG. 8. (A) Comparison of predicted sequences of *P. aeruginosa* LasR, *V. fischeri* LuxR, and *E. coli* 28K-UvrC. Areas of identity are boxed (14, 15, 21, 43). (B) Comparison of predicted carboxy-terminal sequences of a group of procaryotic activators with LasR. *B. subtilis* 28K-SacU and GerE, *E. coli* NarL, *R. meliloti* FixJ, *E. coli* UhpA, *P. aeruginosa* TrpO, *Klebsiella pneumoniae* RcsA, and *E. coli* MalT and 23K-UvrC (1, 8, 9, 11, 20, 21, 26, 43, 47, 51) are shown.

concentrations of an autoinducer metabolite. Mutational analysis suggests that the amino terminus of LuxR is the autoinducer binding domain (42, 45). The amino terminal similarities between LasR and LuxR and the cell density dependence of elastase synthesis and bioluminescence raise the possibility that LasR, like LuxR, also functions in a cell-density-dependent manner (42). At late stages of cell growth, nutrients become limiting. If the function of elastase is to provide the organism with nourishment in the form of small peptides or free amino acids, then perhaps LasR activates *lasB* transcription in response to carbon or nitrogen deprivation by sensing a starvation-induced metabolite. The

sacU locus which regulates degradative enzyme production in *Bacillus subtilis* has been proposed to respond in a similar manner (34). The contribution of elastase to virulence may simply be a consequence of nutrient limitation. The *lasR* gene may also be involved in the iron regulation of *lasB* expression similar to the function of *regA* with regard to *toxA* transcription (18). Further studies will be required to determine those stimuli which affect *lasB* expression through the regulation of LasR synthesis.

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