JOURNAL OF BACTERIOLOGY, July 2002, p. 3765–3773 Vol. 184, No. 14
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.14.3765–3773.2002

Functional Analysis and Regulation of the Divergent *spuABCDEFGH-spuI* Operons for Polyamine Uptake and Utilization in *Pseudomonas aeruginosa* PAO1

Chung-Dar Lu,[1]* Yoshifumi Itoh,[2] Yuji Nakada,[2] and Ying Jiang[1]

Department of Biology, Georgia State University, Atlanta, Georgia 30303,[1] and National Food Research Institute, Kannondai 2-1-12, Tsukuba, Ibaraki 305-8642, Japan[2]

Received 29 January 2002/Accepted 24 April 2002

A multiple-gene locus for polyamine uptake and utilization was discovered in *Pseudomonas aeruginosa* PAO1. This locus contained nine genes designated *spuABCDEFGHI* (*spu* for spermidine and putrescine utilization). The physiological functions of the *spu* genes in utilization of two polyamines (putrescine and spermidine) were analyzed by using Tn*5* transposon-mediated *spu* knockout mutants. Growth and uptake experiments support that the *spuDEFGH* genes specify components of a major ABC-type transport system for spermidine uptake, and enzymatic measurements indicated that *spuC* encodes putrescine aminotransferase with pyruvate as the amino group receptor. Although *spuA* and *spuB* mutants showed an apparent defect in spermidine utilization, the biochemical functions of the gene products have yet to be elucidated. Assays of *lacZ* fusions demonstrated the presence of agmatine-, putrescine-, and spermidine-inducible promoters for the *spuABCDEFGH* operon and the divergently transcribed *spuI* gene of unknown function. Since the observed induction effect of agmatine was abolished in an *aguA* mutant where conversion of agmatine into putrescine was blocked, putrescine or spermidine, but not agmatine, serves as the inducer molecule of the *spuA-spuI* divergent promoters. S1 nuclease mappings confirmed further the induction effects of the polyamines on transcription of the divergent promoters and localized the transcription initiation sites. Gel retardation assays with extracts from the cells grown on putrescine or spermidine demonstrated the presence of a polyamine-responsive regulatory protein interacting with the divergent promoter region. Finally, the absence of the putrescine-inducible *spuA* expression and putrescine aminotransferase (*spuC*) formation in the *cbrB* mutant indicated that the *spu* operons are regulated by the global CbrAB two-component system perhaps via the putative polyamine-responsive transcriptional activator.

Polyamines (including putrescine and spermidine) are a group of ubiquitous polycations necessary for cell growth and also serve as precursors for acetyl- and S-adenosyl-polyamines of important physiological functions in microorganisms (31, 36). Biosynthesis of putrescine and spermidine has been extensively studied in *Escherichia coli*, and the cognate enzymes and structural genes have been established (Fig. 1). In *Pseudomonas aeruginosa*, the presence of all homologues of the *E. coli* putrescine and spermidine biosynthetic *speABCDE* genes (8) in the PAO1 genome (www.pseudomonas.com) suggests common pathways in these two microorganisms.

In *P. aeruginosa*, putrescine can be synthesized from arginine through the catabolic arginine decarboxylase (ADC) pathway (22). The ADC pathway is one of four catabolic pathways for arginine utilization in pseudomonads (11). In this pathway, exogenous arginine is converted sequentially into agmatine, putrescine, 4-aminobutyrate, and succinate before it is channeled into the tricarboxylic acid cycle (Fig. 1). Recently, we characterized the *aguBA* operon of *P. aeruginosa* PAO1 (25) that encodes agmatine deiminase (*aguA*) and *N*-carbamoylputrescine amidinohydrolase (*aguB*) converting agmatine into putrescine in this pathway (13) (Fig. 1). While exogenous arginine can induce the synthesis of ADC catalyzing the first step of the pathway, agmatine and *N*-carbamoylputrescine are responsible for the subsequent induction of the *aguBA* operon regulated by AguR repressor (25). Thus, the regulatory mechanism of the ADC pathway is significantly different from those of other two arginine catabolic pathways, the arginine succinyltransferase (16, 18, 38) and the arginine deiminase pathways (6, 20, 23), in which exogenous arginine exerts its induction effect by the presence of the arginine-responsive ArgR regulator (27, 29, 30).

The catabolic route of putrescine is part of the ADC pathway. Conversion of putrescine into 4-aminobutyrate requires two enzymes: putrescine aminotransferase and 4-aminobutyraldehyde dehydrogenase (Fig. 1). While genetic evidence has indicated that the *kauB* gene encodes a bifunctional 4-aminobutyraldehyde/4-guanidinobutyraldehyde dehydrogenase (17), putrescine aminotransferase has not been characterized, and the corresponding gene remains to be identified in *P. aeruginosa*.

In the present study, we report the characterization of the divergent *spu* operons of nine genes which are located adjacent to the *aguRBA* genes and are responsible for polyamine utilization. Among these genes, components of a major spermidine transport system and the putrescine aminotransferase of the ADC pathway were identified. We also demonstrated the induction effect of putrescine and spermidine on the expression of these divergent operons and a putative polyamine-respon-

* Corresponding author. Mailing address: Department of Biology, Georgia State University, P.O. Box 4010, Atlanta, GA 30302-4010. Phone: (404) 651-2531. Fax: (404) 651-2509. E-mail: biocdl@panther.gsu.edu.

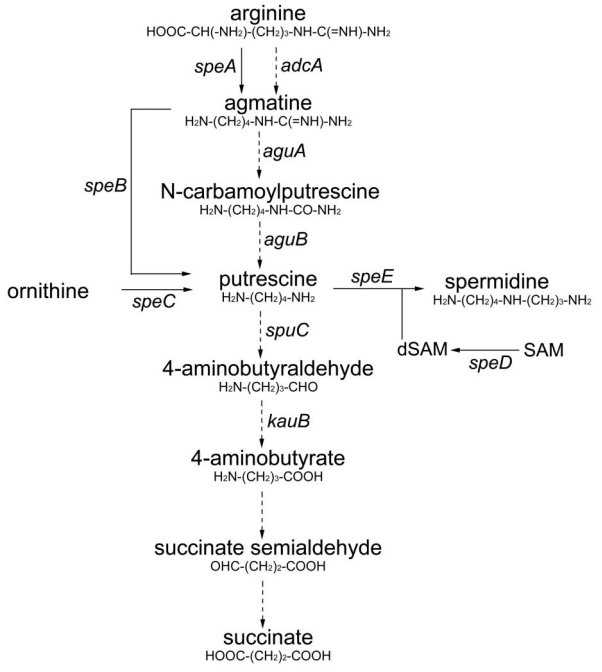


FIG. 1. Schematic presentation of the ADC pathway and polyamine biosynthesis. Solid and broken arrows represent biosynthetic and catabolic pathways, respectively. SAM, *S*-adenosyl methionine; dSAM, decarboxylated SAM. The *speABCDE* genes, which encode biosynthetic ADC, agmatine ureidohydrolase, ornithine decarboxylase, SAM decarboxylase, and spermidine synthase, respectively (8), have been characterized in *E. coli*. The *aguAB* genes encode agmatine deiminase and *N*-carbamoylputrescine aminotransferase as characterized recently (25). The *spuC* gene as reported in this study encodes putrescine-pyruvate aminotransferase. The *kauB* and *adcA* genes represent the bifunctional 4-aminobutyraldehyde/guanidiobutyraldehyde dehydrogenase (17) and the arginine-inducible ADC (22).

sive *trans*-acting factor interacting with the regulatory region of the divergent promoters.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. Bacterial strains and plasmids used in this study are listed in Table 1. The Luria-Bertani (LB) enriched medium or nutrient yeast broth (21) was used for strain construction with the following supplements as required: ampicillin, 50 μg/ml (for *E. coli*); carbenicillin, 100 μg/ml (for *P. aeruginosa*); streptomycin, 500 μg/ml; and tetracycline, 50 μg/ml. The minimal medium P (MMP) (12) was used for the growth of *P. aeruginosa* with supplements of carbon and nitrogen sources at 20 mM as indicated.

Construction of mutant strains. The EZ::TET-1 insertion system (Epicentre) based on the in vitro Tn*5* transposition system (9) was used for generation of knockout mutations. Two *Eco*RI fragments covering the entire *agu* and *spu* loci were purified from cosmid pGU2 and subcloned separately into the *Eco*RI site of the conjugation vector pRTP1. Each resulting target plasmid DNA was incubated with the transposase and the transposon with a tetracycline resistance marker, and the in vitro transposon insertion reaction was carried out under the conditions recommended by the manufacturer. After the reaction, the reaction mixture was used to transform *E. coli* DH5α, and transformants were selected on LB plates with tetracycline. The insertion sites of mutant clones were mapped by *Nco*I restriction endonuclease digestion and subsequently by nucleotide sequencing with a transposon-specific flanking primer. For gene replacement, the resulting transposon insertion plasmids were first introduced into *E. coli* SM10 and then mobilized into the spontaneous streptomycin-resistant *P. aeruginosa* strain PAO1-Sm by biparental plate mating (7). After incubation at 37°C overnight, transconjugants were selected on LB plates supplemented with tetracycline and streptomycin. Knockout mutants of *spuC* were also constructed by using an Flp recombinase target (FRT) sequence and an Ωsp/sm cassette. The 3.3-kb *Eco*RI-*Xho*I fragment carrying *spuC* was first subcloned from cosmid pGU2 into suicide plasmid pEX18Ap between the *Eco*RI and *Sal*I sites (14) to yield plasmid pYJ96. The Ωsp/sm and Gm-GFP-FRT cassettes were excised as *Sma*I fragments from plasmids pHP45 Ωsp/sm (4) and pSP858 (14), respectively, and subsequently inserted into the blunt-ended *Not*I site of *spuC* on pYJ96. The resultant plasmids were then mobilized into strain PAO1, as described above, to obtain strains PAO4479 (*spuC*::Ωsp/sm) and PAO4480 (*spuC*::Gm-GFP-FRT). The Gm-GFP sequence in the cassette on the *spuC* gene was then removed by introducing plasmid pFLP2 (carrying the Flp recombinase gene) into strain PAO4480 as described previously (14), yielding strain PAO4486 (*spuC*::FRT).

Construction of *lacZ* fusions. Plasmid pQF52, a broad-host-range *lacZ* translational fusion vector (30), was used in the construction of promoter fusions. For construction of pGU101 (P_{spuI}::*lacZ*) and pGU102 (P_{spuA}::*lacZ*), a DNA fragment containing the *spuI-spuA* intergenic region was amplified from pGU2 by PCR with two oligonucleotide primers: oligo-1 (5′-GCCGGTGATATCGAGG GCGAA-3′) and oligo-2 (5′-GAGACCGCCAGCGCCGTTGAC-3′). The PCR fragment was cloned to the *Sma*I site of pQF52 so that the 68th codon of *spuI* and the 39th codon of *spuA* were fused in-frame to *lacZ* of the vector in the resulting plasmids. The orientation of the insert on the plasmids was confirmed by nucleotide sequencing.

Transformation of *E. coli* and *P. aeruginosa* by plasmid DNA was performed as described by Chung et al. (2) with magnesium ion for one-step preparation of competent cells. For determination of *lacZ* expression, the levels of β-galactosidase activity in logarithmically growing cells were measured by using ONPG (*o*-nitrophenyl-β-D-galactopyranoside) as the substrate (24) with cell extract prepared by passing cells through a French pressure cell at 8,000 lb/in^2. Protein concentration was determined by the method of Bradford (1).

S1 nuclease mappings. RNA samples were prepared from *P. aeruginosa* PAO1 grown in MMP to an optical density at 600 nm (OD_{600}) of 0.5 to 0.6. A 30-ml portion of the culture was collected by centrifugation at 12,000 × *g* at 4°C for 5 min, and RNA was purified from the suspended cell pellet by a previously described protocol (27).

Procedures for hybridization and S1 nuclease digestion were followed as described by Greene and Struhl (10). For characterization of *spuI* and *spuA* promoters, a double-stranded DNA fragment covering the 363-bp *spuI-spuA* intergenic region (Fig. 4b) was amplified by PCR from pGU101 with a pair of oligonucleotide primers: oligo-5 (5′-AACGGCACGCTGGGGTAC-3′) and oligo-6 (5′-GGGATGAAGACCGATCTGC-3′). One of these two primers was end labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase before PCR, and the resulting PCR product was purified after agarose gel electrophoresis on a 1% agarose gel. For each reaction, 50 μg of RNA was hybridized with the radioactive probe, and experiments were performed quantitatively to permit comparison of levels of transcripts under different growth conditions. The sizes of these transcripts were determined against a nucleotide sequencing ladder of the probe DNA.

Spermidine uptake experiments. Cultures were grown in glutamate-MMP in the absence or presence of 20 mM spermidine. Cells were harvested during logarithmic growth, washed twice with MMP, suspended at a concentration of ca. 10^8 cells/ml (OD_{600} = 0.1) in MMP containing chloramphenicol (250 μg/ml). After incubation of the cell suspension for 5 min at 37°C, ^{3}H-labeled spermidine (New England Nuclear) was added to a final concentration of 20 μM (44.5 mCi/mmol), and samples (0.5 ml) were withdrawn at various time intervals. Cells were collected on a cellulose membrane filter (0.22 μm [pore size], type GS; Millipore) and washed with 5 ml of MMP. Incorporated radioactivity was measured in a liquid scintillation spectrometer (Beckman). In competition experiments, cold agmatine, putrescine, or spermidine (final concentration of 2 mM) was mixed with the ^{3}H-labeled spermidine before being added to the cell suspension.

Gel retardation assays. A 0.5-kb DNA fragment containing the *spuI-spuA* divergent promoter region was purified from pGU101 after digestion with *Hin*dIII and *Bam*HI restriction enzymes. The DNA probe was prepared by labeling with [α-^{32}P]dATP by using the Klenow fragment. The radioactively labeled DNA probe (0.1 nM) was allowed to interact with crude cell extracts (2 μg) in 20 μl of a mixture containing 50 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM EDTA, 5% (vol/vol) glycerol, and 200 ng of nonspecific sheared calf thymus DNA. Reaction mixtures were incubated for 10 min at room temperature and applied to a 5% polyacrylamide gel in Tris-acetate-EDTA running buffer. After being dried, the gel was autoradiographed by exposure to a phosphorimager plate (Fuji).

Measurements of putrescine aminotransferase activity. Cultures were grown to an OD_{600} nm of 0.5 in MMP supplemented with the indicated carbon and nitrogen sources at 20 mM and harvested by centrifugation. Cell extracts were prepared by passing cells through a French pressure cell at 8,000 lb/in^2. The assay

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics[a]	Reference or source
Strains		
P. aeruginosa		
PAO1	Wild type	12, 27
PAO1-Sm	Spontaneous Sm^r of PAO1	29
PAO4455	*cbrB*::ΩKm	26
PAO4479	*spuC*::Ωsp/sm	This study
PAO4480	*spuC*::Gm-GFP-FRT	This study
PAO4486	*spuC*::FRT	This study
PAO4505	*aguBA*::Gm	25
PAO5001	*aguA*::Tc	25
PAO5003	*aguR*::Tc	25
PAO5004	PA0295::Tc	This study
PAO5005	*spuI*::Tc	This study
PAO5006	*spuA*::Tc	This study
PAO5007	*spuB*::Tc	This study
PAO5008	*spuC*::Tc	This study
PAO5009	*spuD*::Tc	This study
PAO5010	*spuE*::Tc	This study
PAO5011	*spuF*::Tc	This study
PAO5012	*spuG*::Tc	This study
PAO5013	*spuH*::Tc	This study
PAO5100	*cbrAB*::Gm	This study
E. coli		
DH5α	F$^-$ *endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1 recA1 gyrA relA1* Δ*(lacIZYA-argF)U169 deoR* [Φ80d*lac*Δ*(lacZ)*M15]	Bethesda Research Laboratories
HB101	*supE44 hsdS20*(rB-mB-) *recA13 ara-14 proA2 lacY galK2 rpsL20 xyl-5 mtl-1*	21
S17-1	*pro thi hsdR* Tp^r Sm^r: chromosome::RP4-2 Rc::Mu-Km::Tn7	34
SM10	*thi-1 thr leu tonA lacY supE recA*::RP4-2-Tc::Mu (Km^r)	14
Plasmids		
pEX18Ap	Ap^r, ColE1 replicon, *oriT sacB*	14
pFLP2	Ap^r, pRO1600 replicon, *oriT sacB flp c*I857	14
pGU2	Ap^r, a cosmid clone carrying the *agu* locus	25
pGU101	Ap^r, pQF52 derivative carrying the P_{spuI}-*lacZ* fusion	This study
pGU102	Ap^r, pQF52 derivative carrying the P_{spuA}-*lacZ* fusion	This study
pHP45ΩSp/Sm	Ap^r, pBR322 derivative, Ωsp/sm	4
pNIC6011	Ap^r, pACYC177 and pVS1 replicons	26
pQF52	Ap^r, *lacZ* translational fusion vector	30
pRK2013	Km^r, *tra* (RK2)	3
pRTP1	Ap^r, conjugation vector	35
pSP858	Ap^r, pBR322 derivative carrying the Gm-GFP-FRT cassette	14
pYJ96	Ap^r, pEX18Ap derivative carrying the 3.3-kb *Eco*R-*Xho*I *spuC* fragment	This study

[a] Antibiotic resistance: Ap^r, ampicillin for *E. coli* and carbenicillin for *P. aeruginosa*; Gm^r, gentamicin; Km^r, kanamycin; Sm^r, streptomycin; Sp^r, spectinomycin, Tc^r, tetracycline.

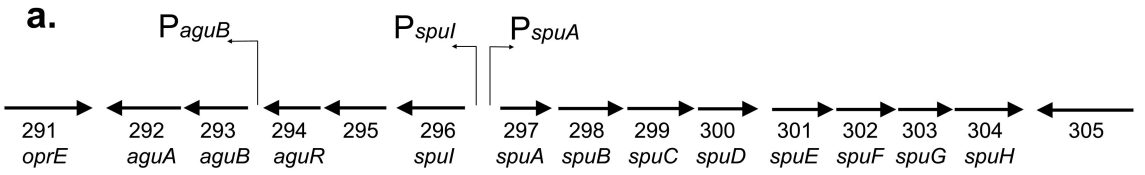
mixture (0.8 ml) contained 125 mM Tris-HCl (pH 10), 6.25 mM putrescine, 6.25 mM pyruvate, 125 μM pyridoxal phosphate, 1.25 mM o-aminobenzaldehyde, and cell extract (ca. 50 μg). After 20 min of incubation at 37°C, 0.2 ml of 10% (wt/vol) trichloroacetic acid was added. The protein precipitates were removed by centrifugation, and the absorbance at 435 nm was measured. The amounts of 4-aminobutyraldehyde formed were calculated from the molar extinction coefficient ($A_{435} = 0.17 \times 10^3$/M/cm) of Δ^1-pyrroline (the cyclic form of 4-aminobutyraldhyde) after reaction with o-aminobenzaldehyde. One unit of enzyme activity was defied as the amount of the enzyme that yielded 1 μmol of product per min.

RESULTS

Construction and growth phenotype analysis of knockout mutants in the *spu* locus. In our previous report on characterization of the *agu* genes for agmatine utilization, the upstream genes (Fig. 2) with potential biochemical functions in polyamine transport and metabolism have caught our attention. These genes encode components for a putative ABC transporter of polyamines (PA0295 and PA0300 to PA0304) and enzymes of unknown functions (PA0296 to PA0299). To investigate the possible involvement of these genes in polyamine uptake and metabolism, knockout mutants of these genes were generated by in vitro transposon mutagenesis, followed by biparental conjugation for homologous recombination as described in Materials and Methods. The artificial transposon cassette, EZ::TET-1, used here does not have a polar effect on expression of polycistronic transcripts since no transcriptional terminator sequence is present in the insertion cassette and as has been verified from a previous study (25). Utilization of agmatine, putrescine, and spermidine by these mutants as the sole source of carbon and nitrogen was examined in MMP, and the results of observed growth phenotypes are summarized in Fig. 2b.

In comparison to the wild-type strain PAO1, mutant strains PAO5006 to PAO5013 were defective in the utilization of

a.



b.

Strain	Genotype	Glu	Agm	Put	Spd
PAO1	wild type	+++	+++	+++	+++
PAO4479	*spuC*::Ωsp/sm	+++	−	−	−
PAO4486	*spuC*::FRT	+++	+	−	++
PAO5001	*aguA*::Tc	+++	−	+++	+++
PAO5003	*aguR*::Tc	+++	+++	+++	+++
PAO5004	PA0295::Tc	+++	+++	+++	++
PAO5005	*spuI*::Tc	+++	+++	+++	++
PAO5006	*spuA*::Tc	+++	+++	+++	+
PAO5007	*spuB*::Tc	+++	+++	+++	−
PAO5008	*spuC*::Tc	+++	+	−	+
PAO5009	*spuD*::Tc	+++	+++	+++	−
PAO5010	*spuE*::Tc	+++	+++	+++	−
PAO5011	*spuF*::Tc	+++	+++	+++	−
PAO5012	*spuG*::Tc	+++	+++	+++	−
PAO5013	*spuH*::Tc	+++	+++	+++	−

FIG. 2. (a) Organization of the *agu* and *spu* loci. Relative location and transcriptional orientation of genes in these loci are represented by arrows. Each gene is marked with the PA gene numbers assigned by the *P. aeruginosa* genome annotation project (www.pseudomonas.com) and the corresponding gene designation. P_{aguB}, P_{spuI}, and P_{spuA} represent the locations of characterized promoters in the *agu-spu* loci. (b) Utilization of polyamines by *P. aeruginosa* PAO1 and mutants in the *agu-spu* loci. Cell growth was tested on MMP plates with 20 mM concentrations of the following supplements as indicated: Glu, glutamate; Agm, agmatine; Put, putrescine; and Spd, spermidine. +++, Growth in 16 h; ++, growth in 24 h; +, growth in 48 h; −, no growth or very poor growth in 48 h. It was observed that suppressor mutants of *spuC* rise spontaneously at high frequency on putrescine and agmatine plates with a much larger colony size than the parent strains.

putrescine and/or spermidine, and the affected genes of these mutants were identified in a putative operon of eight open reading frames (PA0297 to PA0304; Fig. 2). Accordingly, these genes were designated *spuABCDEFGH* for spermidine and putrescine utilization. Although spermidine utilization was affected to different extents by any of the *spu* mutations, strain PAO5008 (*spuC*::Tc) was defective completely on putrescine and partially on agmatine utilization. The knockout mutants of the PA0295 and PA0296 genes, which are divergently transcribed from the *spuABCDEFGH* genes, did not show any apparent defect on the utilization of spermidine, putrescine, and agmatine.

Additionally, two *spuC* mutants were constructed by inserting either an FRT sequence (PAO4486) or a streptomycin Ω-loop cassette (PAO4479). Like strain PAO5008, strain PAO4486 exhibited a growth defect on both putrescine and agmatine. In contrast, strain PAO4479 showed no growth on agmatine, putrescine, and spermidine. Since the *spuC* function is dispensable for spermidine utilization in PAO4486 and PAO5008, the observed growth defect of PAO4479 on spermidine appeared to be due to an expected polar effect of the Ω cassette in *spuC* on expression of the downstream *spuDEFGH* genes and thus supports the expression of these transport genes (see below) from a promoter upstream of *spuC*.

SpuDEFGH are components of a major uptake system of spermidine in *P. aeruginosa*. In accordance with the growth phenotype analysis (Fig. 2), the primary amino acid sequences of these Spu proteins exhibit the highest similarities to those of the Pot transport systems of *E. coli* for putrescine and spermidine uptake. Sequence analysis indicated that SpuD and SpuE are the periplasmic binding protein components, SpuF is the ATPase component, and SpuG and SpuH are the inner membrane permease components for an ABC-type transport system.

To substantiate the proposed function of Spu proteins, spermidine uptake experiments were conducted in the wild-type strain PAO1 and two *spu* mutants (*spuD* and *spuE*) grown in glutamate minimal medium in the presence or absence of spermidine. As shown in Fig. 3a, spermidine uptake in the wild-type strain PAO1 was induced ca. 10-fold by the presence of spermidine in the growth medium. Although the induction effect of spermidine persisted in the *spuD* and *spuE* mutants, the induction levels were significantly reduced in these mutants. The initial rate of spermidine uptake in both mutants

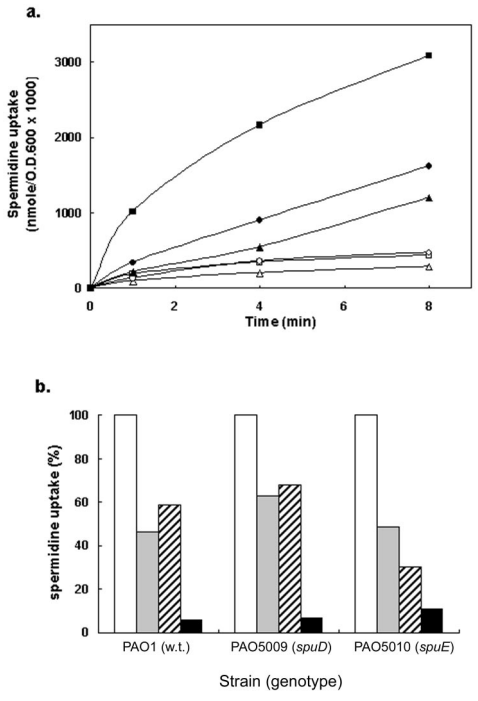


FIG. 3. (a) Induction of spermidine uptake by exogenous spermidine in *P. aeruginosa* PAO1 (squares) and its *spuD* (circles) and *spuE* (triangles) mutants. Cultures grown in glutamate-MMP in the absence (open symbols) or in the presence (filled symbols) of spermidine were harvested in the early log phase and used for spermidine transport assays as described in Materials and Methods. (b) Competition tests of spermidine uptake in wild-type PAO1, PAO5009 (*spuD*), and PAO5010 (*spuE*). Cells were grown in MMP with glutamate and spermidine, and spermidine transport assays were done in the absence (□) and in the presence of putrescine (■), cold agmatine (▨), and spermidine (■) in a 100-fold molar excess of [^{3}H]spermidine. All datum points represent the average of two measurements with standard errors of <10%.

was ca. 20% of the wild-type level. These results indicate that both SpuD and SpuE are components of an inducible spermidine transport system that accounts for most of the spermidine uptake activities in strain PAO1 growing on spermidine.

The ligand specificity of the Spu transport system was analyzed by competition tests, and the results are shown in Fig. 3b. When challenged with a 100-fold molar excess of cold spermidine, putrescine, and agmatine, the spermidine uptake in the wild-type strain PAO1 as measured by uptake of radioactive spermidine was reduced to 6, 46, and 58%, respectively. Similar levels of reduction were also observed in the *spuD* and *spuE* mutants. These results indicate that spermidine is the preferred ligand molecule of the SpuDEFGH transport system.

Abolishment of the putrescine aminotransferase activity in the *spuC* mutant. As shown in Fig. 2, the *spuC* knockout mutants (PAO4479, PAO4486, and PAO5008) lose the ability to utilize putrescine as the sole source of carbon and nitrogen. A homology search against the protein database revealed that the SpuC protein exhibits strong sequence similarities to the class III enzymes of the pyridoxal-dependent aminotransferase family, with the highest similarity (53% similarity) to an omega amino acid-pyruvate aminotransferase of *P. putida* (PDB accession number P28269). Accordingly, the possibility of *spuC* encoding a putrescine-pyruvate aminotransferase (PATase) was investigated. With pyruvate rather than α-ketoglutarate as the amino group receptor, the results of PATase measurements in the wild-type strain PAO1 revealed that the putrescine- or agmatine-grown cells exhibited a fivefold-higher level of PATase activity than that of the glutamate-grown cells (Table 2). The induction effects of putrescine and agmatine were reduced to 2.5-fold by the presence of glutamate in the growth medium. In contrast, in the *spuC* mutant, the level of PATase activity was greatly diminished and no longer inducible by putrescine. These results demonstrated that the *spuC* gene encodes PATase for putrescine catabolism.

TABLE 2. Putrescine aminotransferase and agmatine deiminase activities in PAO1 and its derivatives deficient in *spuC* or *cbrB*

Strain (genotype)	Supplement(s)[a]	Sp act (U/mg of protein)[b] of:	
		Putrescine aminotransferase	Agmatine deiminase
PAO1 (wild type)	Glu	2.0	5.0
	Glu+Put	4.9	ND
	Put	10.4	ND
	Glu+Agm	4.7	52.0
	Agm	10.1	ND
PAO4486 (*spuC*)	Glu	0.8	ND
	Glu+Put	0.2	ND
PAO4455 (*cbrB*)	Glu	0.2	2.0
	Glu+Put	0.2	ND
	Glu+Agm	ND	42.0

[a] Cells were grown in MMP with supplements as indicated at 20 mM. Glu, glutamate; Put, putrescine; Agm, agmatine.
[b] ND, not determined. Values are the averages of two measurements, with the standard errors (not shown) all below 10% of the corresponding averages.

Transcriptional induction of the *spuA* promoter by polyamines. Based on the gene organization and the results of functional analysis as described above, we hypothesized that the *spuABCDEFGH* genes constitute an operon which is subjected to regulation by exogenous polyamines. To test this hypothesis, the *PA0296-spuA* intergenic region was cloned into a *lacZ* fusion vector, pQF52, and the resulting *spuA*::*lacZ* fusion plasmid, pGU102, was introduced into the wild-type strain PAO1.

The recombinant PAO1 harboring pGU102 was grown in the glutamate minimal medium in the absence or presence of agmatine, putrescine, or spermidine. As measured by β-galactosidase activity, expression of the *spuA*::*lacZ* fusion was increased 11-, 8-, and 14-fold by exogenous agmatine, putrescine, and spermidine, respectively (Table 3). These results indicate the presence of a polyamine-responsive *spuA* promoter in the intergenic region.

Since agmatine can serve as the precursor for polyamines synthesis via the ADC pathway (Fig. 1), the induction effect of agmatine could be due to the elevated intracellular pools of putrescine and/or spermidine. Along this line, a genetic block on conversion of agmatine to putrescine is expected to abolish the induction effect of agmatine. To test this hypothesis, plasmid pGU102 was introduced into an *aguA* mutant PAO5001, and the expression profile of the *spuA*::*lacZ* fusion was determined. As shown in Table 3, the induction effect of agmatine

TABLE 3. Measurements of the β-galactosidase activity in strains of *P. aeruginosa* harboring either pGU101 or pGU102

Plasmid (promoter)	Host strain (genotype)	β-Galactosidase sp act[a] (nmol/min/mg)			
		Glu	Glu+Agm	Glu+Put	Glu+Spd
pGU102(P_{spuA})	PAO1 (wild type)	17	190	143	238
	PAO5001 (*aguA*)	30	30	229	324
	PAO5003 (*aguR*)	29	290	210	339
	PAO5100 (*cbrAB*)	32	30	37	ND
pGU101(P_{spuI})	PAO1 (wild type)	110	860	733	500
	PAO5001 (*aguA*)	166	144	1062	727
	PAO5003 (*aguR*)	224	1034	1047	644

[a] Cells were grown in MMP with supplements as indicated at 20 mM. Glu, glutamate; Agm, agmatine; Put, putrescine; Spd, spermidine. ND, not determined. Values are the averages of two measurements, with the standard errors (not shown) all below 5% of the corresponding average.

on the *spuA* promoter activity was completely diminished in the *aguA* mutant, whereas exogenous putrescine and spermidine still exerted induction effects of 7.6- and 10.8-fold, respectively.

The *spuI* gene is also subjected to transcriptional induction by polyamines. As shown in Fig. 1, sequence analysis predicted that the PA0296 gene is divergently transcribed from the *spuABCDEFGH* genes. The amino acid sequences of PA0296 and SpuB possess 34% identity to each other and exhibit 26 and 28% identity, respectively, to the amino acid sequence of glutamine synthetase (GlnA) of *P. aeruginosa*. However, while the *spuB* gene is required for spermidine utilization, the knockout mutant of the PA0296 gene, as in strain PAO5005 (Fig. 2), did not show any growth defect on agmatine, putrescine, and spermidine. Nevertheless, the results as described below indicate that the expression of PA0296 is inducible by exogenous polyamines. Therefore, the PA0296 gene was tentatively designated *spuI*.

The effects of agmatine, putrescine, and spermidine on the *spuI* promoter activity were analyzed by measurements of β-galactosidase activity from the *spuI*::*lacZ* fusion on plasmid pGU101, which contains the same *spuI-spuA* intergenic region as pGU102 but in a reverse orientation on the vector pQF52. As shown in Table 3, exogenous agmatine, putrescine and spermidine exerted eight-, seven-, and fivefold induction effects, respectively, on the *spuI* expression in the wild-type strain PAO1. In the *aguA* mutant, an induction effect by agmatine was abolished, whereas the effects of putrescine and spermidine persisted. These results indicate that the *spuI* promoter is also inducible by polyamines, a finding similar to that seen with the divergently transcribed *spuA* promoter.

Nucleotide sequence analysis revealed the presence of a strong transcriptional terminator structure at the end of the *spuI* coding sequence. In fact, this transcriptional terminator abolished the extension process by *Taq* DNA polymerase during cycle sequencing reactions at 55°C (data not shown). Therefore, the *spuI* gene likely stands as a single-gene operon.

S1 nuclease mappings of the divergent *spuA* and *spuI* promoters. The expression patterns of *spuA* and *spuI* promoters in the wild-type strain of *P. aeruginosa* PAO1 were analyzed by S1 nuclease mappings. As shown in Fig. 4a, polyamine-inducible transcripts were detected for the divergent *spuA* and *spuI* pro-

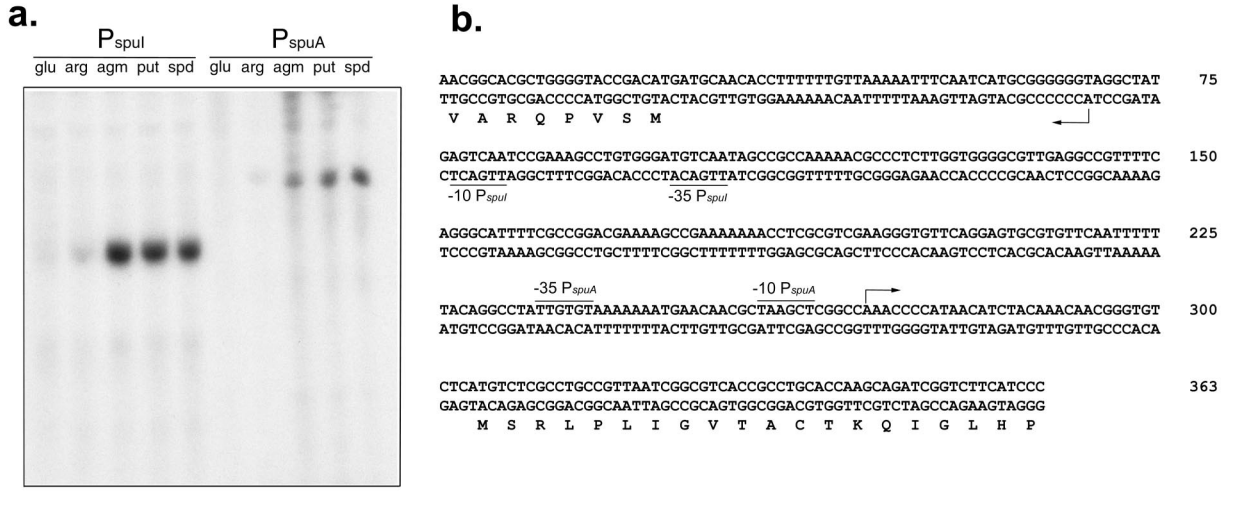


FIG. 4. (a) S1 nuclease mapping of P_{spuI} and P_{spuA} transcripts. RNA samples and the radioactive probe for S1 nuclease mapping were prepared as described in Materials and Methods. RNA samples were purified from cells grown in MMP with 20 mM concentrations of the supplements as indicated: glu, glutamate; arg, arginine; agm, agmatine; put, putrescine; and spd, spermidine. (b) Nucleotide sequence of the *spuI-spuA* divergent promoter region. The 5′ ends of the *spuI* and *spuA* transcripts are indicated as arrows, and the possible −10 and −35 sequences for the corresponding promoters are underlined.

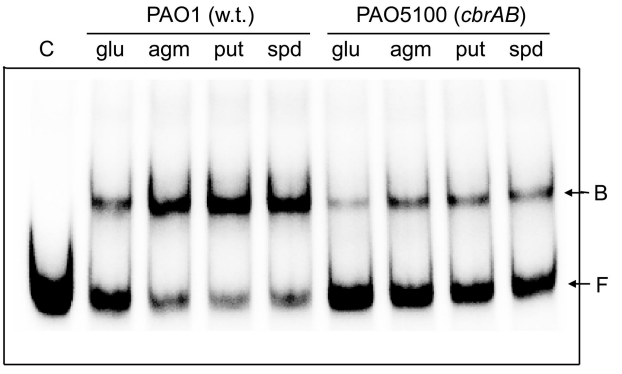


FIG. 5. Gel retardation assays of a polyamine-inducible *trans*-acting factor and the *spuI-spuA* regulatory region. The crude cell extracts were prepared from cells grown in MMP containing 20 mM glutamate (glu), agmatine (agm), putrescine (put), or spermidine (spd). In the binding reactions, the radioactive DNA probe was incubated with the crude extract as indicated. Lane C is a control without cell extract. F, free probe; B, bound probe.

moters, a finding consistent with the results of the *lacZ* fusion studies as described above. By running against a nucleotide sequencing ladder (data not shown), the 5′ ends of the transcripts from these two promoters were determined to be 45 and 34 bp upstream from the proposed ATG initiation codons of *spuI* and *spuA*, respectively. Sequences resembling the consensus −35 and −10 regions of σ^{70} promoters were found in the appropriate distance from the determined 5′ end of each transcript (Fig. 4b). Since all S1 mappings were done with the same amount of total RNA, higher transcript levels of *spuI* than *spuA* suggested a stronger promoter activity of *spuI*. These results are consistent with the measurements of *lacZ* fusions (Table 3).

Evidence for the presence of *trans*-acting factors in control of the divergent *spuA* and *spuI* promoters. The *aguR* gene immediately upstream of the *spu* genes (Fig. 2a) regulates the *aguBA* operon for agmatine catabolism (25). To analyze possible control of the *spu* expression by *aguR*, the *spuA* and *spuI* expression profiles were determined in the *aguR* mutant PAO5003. As shown in Table 3, both *spuA* and *spuI* promoters remained inducible by polyamines in the *aguR* mutant. In fact, the induced levels of *spuA* and *spuI* promoters in the *aguR* mutant were ca. 50% higher than those in the wild-type PAO1. However, the AguR protein did not interact with the *spuA-spuI* intergenic region by gel retardation assays (data not shown), excluding the possibility of AguR as the transcriptional regulator of the *spuA* and *spuI* promoters.

By gel retardation assays with cell extracts of the wild-type strain PAO1, we could detect the presence of a DNA-binding protein interacting specifically with the *spuA-spuI* intergenic region. As shown in Fig. 5, the formation of a DNA-protein complex was increased in the crude extracts of cells grown in the presence of agmatine, putrescine, or spermidine. These results suggest a possible role of this putative DNA-binding protein as a *trans*-acting factor in the regulation of the divergent *spuA* and *spuI* promoters.

Another possible candidate for the transcription regulator of *spu* genes could be the CbrAB two-component system (26). The *cbrAB* mutants are unable to utilize agmatine, putrescine, and spermidine, as well as a variety of other compounds (26). In the *cbrB* mutant PAO4455, the activity of agmatine deiminase (encoded by *aguA*) remained inducible by exogenous agmatine to the levels comparable to that of the wild-type strain (Table 2). Therefore, it is conceivable that the defect of agmatine utilization in PAO4455 is the result of a block on putrescine catabolism. To investigate whether the *spuABCDEFGH* operon is controlled by *cbrB*, we measured putrescine aminotransferase activity (encoded by *spuC*) and β-galactosidase activity from the *spuA*::*lacZ* fusion in the *cbr* mutants. When grown in the glutamate minimal medium, the level of PATase in PAO4455 (*cbrB*) was significantly lower than that of the wild-type strain PAO1 (Table 2). Furthermore, this enzyme was no longer induced by exogenous putrescine. Consistent with these results, the *spuA*::*lacZ* expression of pGU102 in the *cbrAB* mutant (PAO5100) also became noninducible by agmatine and putrescine (Table 3).

Gel retardation assays were performed to analyze the effect of *cbrAB* on the expression of the putative polyamine-responsive regulatory protein for the *spuI-spuA* divergent promoters. As shown in Fig. 5, in comparison to the wild-type PAO1, the induction effect of agmatine, putrescine, and spermidine on formation of the putative polyamine-responsive regulatory protein was apparently reduced in the *cbrAB* deletion mutant PAO5100.

DISCUSSION

In the present study, we have identified and characterized the divergent *spuI-spuABCDEFGH* operons for spermidine uptake and spermidine-putrescine utilization. Several lines of evidence supported that the ABC transporter system encoded by the *spuDEFGH* genes is the major spermidine uptake system in *P. aeruginosa*. First, knockout mutants of any of these genes abolish specifically spermidine utilization as the sole source of carbon and nitrogen in *P. aeruginosa* (Fig. 2). Second, spermidine uptake is inducible in spermidine-grown cells and is greatly reduced in either *spuD* or *spuE* mutant (Fig. 3a). Third, spermidine, rather than putrescine (or agmatine), is the preferred ligand molecule for SpuD and SpuE (Fig. 3b). The primary amino acid sequences of SpuD and SpuE are 57% identical to each other, and both exhibit an average of 54% identity to PotF and 35% identity to PotD of *E. coli*. The PotF and PotD proteins of *E. coli* are the preferential periplasmic binding proteins for putrescine and spermidine, respectively (15, 19). Contrary to such sequence similarities, SpuD and SpuE prefer spermidine rather than putrescine as the transport ligand as determined empirically in this report. Furthermore, unlike the *E. coli* Pot transport systems in which a single binding protein is sufficient for the transport activity, both SpuD and SpuE are required for the optimal uptake of spermidine by the SpuDEFGH system. As shown in Fig. 2a, residual spermidine uptake can still be observed in either *spuD* or the *spuE* mutant. The possibility of another polyamine uptake system contributing to spermidine uptake in *P. aeruginosa* cannot be ruled out from this study.

Four putative enzymes are encoded by the *spuABC* and *spuI* genes. While mutations on *spuI* did not show any growth defect on agmatine and polyamines, the contiguous *spuABC* genes are all related to polyamine catabolism. Except for SpuC, the

biochemical functions of the other enzymes remain to be elucidated. Based on the results of genetic and biochemical studies, we conclude that the *spuC* gene encodes the PATase, catalyzing the conversion of putrescine into 4-aminobutyraldehyde. Unlike the PATases of *E. coli* and *Klebsiella pneumoniae* that use α-ketoglutarate as the amino acceptor (5, 32, 33), pyruvate serves as the amino receptor for the PAO1 PATase. Based on this new assignment of *spuC* in the ADC pathway, one would expect a growth defect of *spuC* mutants on agmatine. Indeed, the *spuC* mutants grow poorly on agmatine. Similar basal growth on agmatine has been observed with the *aguA* and *aguB* mutants, presumably due to the presence of a second route for agmatine utilization, as has been reported for *P. cepacia* (37); in this organism, agmatine can be converted by agmatine dehydrogenase into guanidinobutyraldehyde, a precursor of guanidinobutyrate in the arginine dehydrogenase pathway.

From the results of *lacZ* fusion assays and S1 nuclease mappings, we demonstrated that the divergent *spuA* and *spuI* promoters are inducible by exogenous agmatine, putrescine, and spermidine. The abolishment of agmatine effect in the *aguA* mutant supports the notion that putrescine and/or spermidine is the inducer molecule(s) for activation of these promoters. We were not able to differentiate further the effects of putrescine and spermidine due to the fact that putrescine is the precursor as well as the catabolic product of spermidine and that mutants defective in spermidine synthetase and spermidine catabolic enzyme were not available. Little is known about the catabolism of spermidine in pseudomonads. It has been reported that exogenous spermidine can be cleaved by spermidine dehydrogenase into putrescine and 3-aminopropionaldehyde in a strain of *Pseudomonas* sp. (28).

The *aguR* gene, which is located immediately upstream of the *spu* genes and encodes a repressor protein in regulation of the *aguBA* operon (25), was excluded from the present study as the regulatory gene of *spu* operons. In search of a *trans*-acting factor in control of the *spu* promoters, the results of gel retardation assays (Fig. 5) revealed the presence of a polyamine-responsive DNA-binding protein forming a nucleoprotein complex with the *spuA-spuI* regulatory region. With respect to the control by polyamines, the patterns of this nucleoprotein complex formation correlate well with the induction profiles of the *spuA* and *spuI* promoters, suggesting a transcriptional activator feature of this DNA-binding protein that has yet to be identified.

Another regulatory element in control of the *spu* operons is the CbrAB two-component system of *P. aeruginosa*. Although the physiological signal activating the CbrAB system is not known, the importance of this system in a global control of catabolic pathways in *P. aeruginosa* has been reported (26). The present results indicate that CbrB participates in polyamine induction of the *spuA* promoter (Table 3) and thereby the *spuC* gene encoding PATase (Table 2) but plays no role in the agmatine-inducible expression of *aguA* encoding agmatine deiminase (Table 2). These results led us to conclude that the previously reported growth defects of *cbrAB* mutants on agmatine, putrescine, and spermidine are a consequence of abolished induction of *spu* genes by these compounds. While CbrB, an NtrC-type transcriptional activator, would be involved in activation of the σ^{54} promoters, the *spuA* promoter appears to have the consensus −10 and −35 sequences for σ^{70}-RNA polymerase holoenzyme (Fig. 4b). The CbrB response regulator might regulate the expression of the putative polyamine-inducible transcription factor gene as described above.

One interesting feature of these *spu* genes is the presence of two putative glutamine synthetase homologues encoded by *spuB* and *spuI*. The SpuB and SpuI proteins exhibit significant identity (42 and 35%, respectively) to another glutamine synthetase homologue encoded by the *ycjK* gene of unknown function in *E. coli*. The *ycjK* gene of *E. coli* is also divergently transcribed from an operon of five genes: *ycjL-ycjC-aldH-ordL-goaG*. Interestingly, YcjL and GoaG show 44 and 30% identity, respectively, to SpuA and SpuC at the amino acid sequences. While SpuC was identified in this report as putrescine or pyruvate aminotransferase, the results of sequence comparison suggested an amidotransferase activity to SpuA. The intriguing similarities of these operons in gene arrangement and protein sequences between *E. coli* and *P. aeruginosa* suggest that they might be evolutionarily conserved due to a common or related physiological function in polyamine metabolism that has yet to be elucidated.

ACKNOWLEDGMENTS

We thank T. Nishijyo for help with construction of the PAO1 mutants.

This work was supported in part by research grant MCB9985660 from the National Science Foundation to C.-D.L.

REFERENCES

1. **Bradford, M.** 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
2. **Chung, C. T., S. L. Niemela, and R. H. Miller.** 1989. One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. Proc. Natl. Acad. Sci. USA **86:**2172–2175.
3. **Comai, L., C. Schilling-Cordaro, A. Mergia, and C. M. Houck.** 1983. A new technique for genetic engineering of *Agrobacterium* Ti plasmid. Plasmid **10:**21–30.
4. **Fellay, R., J. Frey, and H. Krisch.** 1987. Interposon mutagenesis of soil and water bacteria: a family of DNA fragments designed for in vivo insertional mutagenesis of gram-negative bacteria. Gene **52:**147–154.
5. **Friedrich, B., and B. Magasanik.** 1979. Enzymes of agmatine degradation and the control of their synthesis in *Klebsiella aerogenes*. J. Bacteriol. **137:** 1127–1133.
6. **Galimand, M., M. Gamper, A. Zimmermann, and D. Haas.** 1991. Positive FNR-like control of anaerobic arginine degradation and nitrate respiration in *Pseudomonas aeruginosa*. J. Bacteriol. **173:**1598–1606**.**
7. **Gambello, J. R., and B. H. Iglewski.** 1991. Cloning and characterization of the *Pseudomonas aeruginosa lasR* gene, a transcriptional activator of elastase expression. J. Bacteriol. **173:**3000–3009.
8. **Glansdorff, N.** 1996. Biosynthesis of arginine and polyamines, p. 408–433. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
9. **Goryshin, I. Y., and W. S. Reznikoff.** 1998. Tn*5* in vitro transposition. J. Biol. Chem. **273:**7367–7374.
10. **Greene, J. M., and K. Struhl.** 1993. S1 analysis of mRNA using M13 template, p. 4.6.2–4.6.13. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. John Wiley & Sons, New York, N.Y.
11. **Haas, D., M. Galimands, M. Gamper, and A. Zimmermann.** 1990. Arginine network of *Pseudomonas aeruginosa*: specific and global controls, p. 303–316. *In* S. Silver, A.-M. Chakrabarty, B. Iglewski, and S. Kaplan (ed.), *Pseudomonas*: biotransformations, pathogenesis, and evolving biotechnology. ASM Press, Washington, D.C.
12. **Haas, D., B. W. Holloway, A. Schambock, and T. Leisinger.** 1977. The genetic organization of arginine biosynthesis in *Pseudomonas aeruginosa.* Mol. Gen. Genet. **154:**7–22.
13. **Haas, D., H. Matsumoto, P. Moretti, V. Stalon, and A. Mercenier.** 1984. Arginine degradation in *Pseudomonas aeruginosa* mutants blocked in two arginine catabolic pathways. Mol. Gen. Genet. **193:**437–444.

14. **Hoang, T. T., R. R. Karhoff-Schweizer, A. J. Kutchma, and H. P. Schweizer.** 1998. A broad-host-range Flp-*FRT* recombination system for site-specific excision of chromosomally located DNA sequence: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. Gene **212:**77–86.
15. **Igarashi, K., and K. Kashiwagi.** 1999. Polyamine transport in bacteria and yeast. Biochem. J. **344:**633–642.
16. **Itoh, Y.** 1997. Cloning and characterization of the *aru* genes encoding enzymes of the catabolic arginine succinyltransferase pathway in *Pseudomonas aeruginosa*. J. Bacteriol. **179:**7280–7290.
17. **Jann, A., H. Matsumoto, and D. Haas.** 1988. The fourth arginine catabolic pathway of *Pseudomonas aeruginosa*. J. Gen. Microbiol. **134:**1043–1053.
18. **Jann, A., V. Stalon, C. Vander Wauven, T. Leisinger, and D. Haas.** 1986. N2-succinylated intermediates in an arginine catabolic pathway of *Pseudomonas aeruginosa.* Proc. Natl. Acad. Sci. USA **83:**4937–4941.
19. **Kashiwagi, K., R. Pistocchi, S. Shibuya, S. Sugiyama, K. Morikawa, and K. Igarashi.** 1996. Spermidine-preferential uptake system in *Escherichia coli*. J. Biol. Chem. **271:**12205–12208.
20. **Lu, C.-D., H. Winteler, A. Abdelal, and D. Haas.** 1999. The ArgR regulatory protein, a helper to the anaerobic regulator ANR during transcriptional activation of the *arcD* promoter in *Pseudomonas aeruginosa*. J. Bacteriol. **181:**2459–2464.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
22. **Mercenier, A., J. P. Simon, D. Haas, and V. Stalon.** 1980. Catabolism of L-arginine by *Pseudomonas aeruginosa*. J. Gen. Microbiol. **116:**381–389.
23. **Mercenier, A., J. P. Simon, C. Vander Wauven, D. Haas, and V. Stalon.** 1980. Regulation of enzyme synthesis in the arginine deiminase pathway of *Pseudomonas aeruginosa.* J. Bacteriol. **144:**159–163.
24. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Nakada, Y., Y. Jiang, T. Nishijyo, Y. Itoh, and C.-D. Lu.** 2001. Molecular characterization and regulation of the *aguBA* operon, responsible for agmatine utilization in *Pseudomonas aeruginosa*. PAO1 J. Bacteriol. **183:**6517–6524.
26. **Nishijyo, T., D. Haas, and Y. Itoh.** 2001. The *cbrA-cbrB* two-component regulatory system controls the utilization of multiple carbon and nitrogen sources in *Pseudomonas aeruginosa*. Mol. Microbiol. **40:**917–931.
27. **Nishijyo, T., S. M. Park, C. D. Lu, Y. Itoh, and A. T. Abdelal.** 1998. Molecular characterization and regulation of an operon encoding a system for transport of arginine and ornithine and the ArgR regulatory protein in *Pseudomonas aeruginosa*. J. Bacteriol. **180:**5559–5566.
28. **Padmanabhan, R., and K. Kim.** 1965. Oxidation of spermidine by a *Pseudomonas*. Biochem. Biophys. Res. Commun. **19:**1–5.
29. **Park, S.-M., C.-D. Lu, and A. T. Abdelal.** 1997. Cloning and characterization of *argR*, a gene that participates in regulation of arginine biosynthesis and catabolism in *Pseudomonas aeruginosa* PAO1. J. Bacteriol. **179:**5300–5308.
30. **Park, S.-M., C.-D. Lu, and A. T. Abdelal.** 1997. Purification and characterization of an arginine regulatory protein, ArgR, from *Pseudomonas aeruginosa* and its interactions with the control regions for the *car*, *argF*, and *aru* operons. J. Bacteriol. **179:**5309–5317.
31. **Pegg, A. E.** 1986. Recent advances in the biochemistry of polyamines in eukaryotes. Biochem. J. **234:**249–262.
32. **Prieto-Santos, M. I., J. Martin-Checa, R. Balana-Fouce, and A. Garrido-Pertierra.** 1986. A pathway for putrescine catabolism in *Escherichia coli*. Biochim. Biophys. Acta **880:**242–244.
33. **Shaibe, E., E. Metzer, and Y. S. Halpern.** 1985. Metabolic pathway for the utilization of L-arginine, L-ornithine, agmatine, and putrescine as nitrogen sources in *Escherichia coli* K-12. J. Bacteriol. **163:**933–937.
34. **Simon, R., U. Priefer, and A. Puhler.** 1983. A broad-host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram-negative bacteria. Bio/Technology **1:**784–790.
35. **Stibitz, S., W. Black, and S. Falkow.** 1986. The construction of a cloning vector designed for gene replacement in *Bordetella pertussis*. Gene **50:**133–140.
36. **Tabor, C. W., and H. Tabor.** 1984. Polyamines in microorganisms. Microbiol. Rev. **49:**81–99.
37. **Tricot, C., A. Pierard, and V. Stalon.** 1990. Comparative studies on the degradation of guanidino and ureido compounds by *Pseudomonas.* J. Gen. Microbiol. **136:**2307–2317.
38. **Vander Wauven, C., and V. Stalon.** 1985. Occurrence of succinyl derivatives in the catabolism of arginine in *Pseudomonas cepacia.* J. Bacteriol. **164:**882–886.