

Roles of LysP and CadC in Mediating the Lysine Requirement for Acid Induction of the *Escherichia coli cad* Operon

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Expression of the *Escherichia coli cadBA* operon, encoding functions required for the conversion of lysine to cadaverine and for cadaverine excretion, requires at least two extracellular signals: low pH and a high concentration of lysine. To better understand the nature of the lysine-dependent signal, mutants were isolated which expressed a *cadA-lacZ* transcription fusion in the absence of lysine while retaining pH regulation. The responsible mutation in one of these isolates (EP310) was in *cadC*, a gene encoding a function necessary for transcriptional activation of *cadBA*. This mutation (*cadC310*) is in a part of the gene encoding the periplasmic domain of CadC and results in an Arg-to-Cys change at position 265, indicating that this part of the protein is involved in responding to the presence of lysine. Three other mutants had mutations mapping in or near *lysP* (*cadR*), a gene encoding a lysine transport protein that has previously been shown to regulate *cadA* expression. One of these mutations is an insertion in the *lysP* coding region. Thus, in the absence of exogenous lysine, LysP is a negative regulator of *cadBA* expression. Negative regulation by LysP was further demonstrated by showing that *lysP* expression from a high-copy-number plasmid rendered *cadA-lacZ* uninducible. Expression of *cadA-lacZ* in a strain carrying the *cadC310* allele, however, was not affected by the plasmid-expressed *lysP*. Cadaverine was shown to inhibit expression of the *cadA-lacZ* fusion in *cadC*⁺ cells but not in a *cadC310* background.

Bacteria respond to changes in external pH by altering their pattern of gene expression as well as other physiological processes (1, 3, 9, 14, 15, 18–20, 28–30, 39, 41). In many cases the responsible pH change does not alter internal pH, suggesting that bacteria possess transmembrane signaling systems which ultimately influence the transcription and translation machineries. Despite the important role that this physicochemical parameter plays in cell physiology, the components and operation of these signaling systems are poorly understood. One of the first observations concerning the alteration of enzymatic functions in bacteria as a consequence of low external pH was of increased levels of amino acid decarboxylases (13). These decarboxylation reactions result in excretion of the decarboxylated amino acid, release of CO₂, and an increase in external pH. The exact physiological role of each of these reactants and products in growth and survival of the cell at low pH has not been thoroughly examined. An adaptation response occurs when *Escherichia coli* and *Salmonella typhimurium* are exposed to moderate acid conditions, which serves to protect them from a more severe drop in external pH (8–12, 33, 36). *E. coli* mutants defective in lysine decarboxylase carry out a normal adaptation response, suggesting that these systems for responding to low external pH are distinct (35).

To better understand how bacteria sense and respond to changes in external pH, we have been studying the genetic elements involved in acid induction of the *E. coli* operon encoding lysine decarboxylase. This operon (*cadBA*) is located at min 93.5 and encodes lysine decarboxylase (*cadA*) and a hydrophobic protein which serves as a lysine-cadaverine exchanger (*cadB*) (1, 23, 24, 46). Located immediately upstream of *cadBA*, but transcribed separately, is *cadC*, a gene encoding a protein required for *cadBA* transcription. On the basis of its predicted amino acid sequence, a model of how CadC func-

tions in *cadBA* activation has been proposed (46). In short, CadC is predicted to be a cytoplasmic membrane protein with its amino terminus located in the cytoplasm. This intracellular domain has strong sequence similarity to the DNA-binding domains of the RO_{II} subclass of bacterial response regulators (31), although it has not been directly demonstrated that CadC binds to the *cadBA* promoter region. The extracellular domain is predicted to be involved in sensing a signal generated under conditions of external acidification. In addition to CadC, the *hns* gene product has been implicated as playing a role in repressing *cadBA* at neutral external pH, perhaps through its influence on DNA topology (39). There are other examples in which bacterial membrane proteins that are involved in regulating gene expression are thought to transmit pH signals. The VirA protein of *Agrobacterium tumefaciens* (47) is a membrane-bound histidine kinase which responds to several different signals, including pH, to trigger expression of genes involved in the bacterium-host interaction. The ToxR protein of *Vibrio cholerae* (6) shares with CadC the presence of a DNA-binding domain at the amino terminus, followed by transmembrane and periplasmic domains. Like CadC, ToxR is thought to directly activate promoters upon receiving external signals, among them an acidic environment.

In addition to low external pH, the presence of exogenous lysine and low O₂ tension are required to achieve maximal synthesis of lysine decarboxylase (37). As with pH, the gene products and associated factors required for sensing and responding to these signals have not been fully elucidated. It is clear, however, that all of these signals activate *cadBA* expression through a *cadC*-dependent pathway (46). Mutational analysis has implicated a locus at min 46.5 (*cadR* and *lysP* alleles) which when mutated results in increased levels of lysine decarboxylase in the absence of lysine (32, 44). Some of these mutations also confer two additional phenotypes, a defect in lysine-specific transport and resistance to thiosine (Ts^r), a toxic lysine analog. Analysis of a transposon mutant which was both Ts^r and defective in lysine transport led to the identification of an open reading frame defined as *lysP* (43) which, on the basis

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TABLE 1. Bacteria and plasmids

<i>E. coli</i> strain or plasmid	Genotype or relevant characteristics	Source or reference
Strains		
W3110	F ⁻ IN(<i>rmD-rmE</i>)	F. Neidhardt, University of Michigan
W3210	Δ(<i>lacIOPZYA</i>) <i>proC</i> ::Tn5	F. Neidhardt, University of Michigan
JLS8602	F ⁻ <i>araD139</i> Δ(<i>ara-leu</i>)7697 Δ(<i>proAB-argF-lacIPOZYA</i>)XIII <i>rpsL</i> Nal ^r <i>exa-1</i> ::Mu dII734(Km <i>lac</i>)	42
CAG12098	MG1655 <i>zeg-722</i> ::Tn10	40
GNB8385K	<i>araD139</i> Δ(<i>argF-lac</i>)U169 <i>rpsL150 relA1 ffbB5301 deoC1 ptsF25 rbsR cadB</i> ::Mu dII734(Km <i>lac</i>)	24
E2088	JLS8602 <i>cadC1</i> ::Tn10	46
E608	<i>lac</i> F' <i>lacI^a cam</i>	46
E2230	JLS8602 F' <i>lacI^a cam</i>	46
EP240	W3110 Δ(<i>lacIOPZYA</i>) <i>proC</i> ::Tn5 [W3110 × P1(W3210)]	This work
EP242	W3110 Δ(<i>lacIOPZYA</i>) [EP240 × P1(W3110)]	This work
EP243	EP242 <i>exa-1</i> ::Mu dII734(Km <i>lac</i>) ^a [EP242 × P1(JLS8602)]	This work
EP247	W3110 <i>cadC1</i> ::Tn10 [W3110 × P1(E2088)]	This work
EP302	EP243 EMS mutant (see text)	This work
EP310	EP243 EMS mutant (see text)	This work
EP314	EP243 <i>cadC1</i> ::Tn10 [EP243 × P1(E2088)]	This work
EP517	EP243 <i>lysP1</i> ::Tn10 <i>cam</i>	This work
EP527	EP243 EMS mutant (see text)	This work
EP542	EP243 F' <i>lacI^a cam</i> (EP243 × E608 ^b)	This work
EP544	EP542 <i>cadC310</i>	This work
EP609	EP243 <i>cadB</i> ::Mu dII734(Km <i>lac</i>) [EP243 × P1(GNB8385K)]	This work
Plasmids		
pUC19	Cloning vector, Ap ^r	25
pGB2	pSC101-based cloning vector, Sp ^r /Sm ^r	4
pPH2200	pBR322-based plasmid expressing <i>cadC</i> , Ap ^r	46
pGCadC-1	pGB2-based vector expressing <i>cadC</i> , Sp ^r /Sm ^r	This work
pLysP	pUC19-based plasmid expressing <i>lysP</i> , Ap ^r	43
pPH489	pGCadC-1 derivative with <i>cadC</i> from EP310 (PCR of EP310 DNA with DA15 and DA68)	This work
pPH490	Same as pPH489 but from an independent PCR	This work
pPH528	pGCadC-1 derivative with carboxyl end of <i>cadC</i> from EP310 (PCR of EP310 DNA with DA15 and DA43)	This work

^a The *exa-1*::Mu dII734 allele is an insertion of Mu dII734 in *cadA*, resulting in a *cadA-lacZ* operon fusion. This insertion also encodes kanamycin resistance.

^b Matings were done by mixing log-phase cultures of donor and recipient (5:1) in LB, incubating for 30 min at 32°C, and selecting for exconjugants on LB-kanamycin-chloramphenicol plates.

of the predicted amino acid sequence, is similar to a family of amino acid permeases containing multiple transmembrane segments. The previously isolated *cadR* mutants which affect lysine decarboxylase synthesis most likely contain mutations either in *lysP* or in an unidentified element affecting LysP synthesis or function, leading to the suggestion that LysP itself, or a lysine-dependent signal, plays a role in *cad* operon regulation. The role that this permease plays in *cadBA* regulation needs to be evaluated in light of the recent identification of the role of CadC in activating the operon.

Here we describe studies to identify those elements involved in the lysine-mediated component of *cadBA* expression. Evidence is presented which demonstrates that both CadC and LysP are involved in sensing the lysine-generated signal. In addition, we show that cadaverine, the product of lysine decarboxylation, acts as a negative effector of *cadBA* expression. This negative effect is mediated through CadC, but is independent of LysP.

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MATERIALS AND METHODS

Strains and growth conditions. The *E. coli* K-12 strains used and their relevant characteristics are shown in Table 1. LB was as described previously (26). Medium was solidified with agar

at a final concentration of 1.3%. Phage P1vir was used to transduce genetic loci between strains as described previously (26). MOPS [3-(*N*-morpholino)propanesulfonic acid]-based media were MOPS-glucose-minimal (MGM) (27) and MOPS-glucose-rich (MGR) media; MGR contained 19 of the 20 common L-amino acids (no L-lysine), as described previously (45). MGM and MGR either were pH adjusted with NaOH or HCl to pH 7.6 (MGM-7.6 and MGR-7.6) or contained 100 mM MES [2-(*N*-morpholino)ethanesulfonic acid] and were pH adjusted to pH 5.8 (MGM-5.8 and MGR-5.8). LB-7.6 and LB-5.8 were buffered with 100 mM MOPS and 100 mM MES, respectively, and pH adjusted with either NaOH or HCl. The external pH was maintained within 0.2 pH unit during cell growth in these media throughout the course of the experiments. The cadaverine used was in the free-base form (C₅H₁₄N₂). Antibiotics were used in plate and liquid media at the following concentrations: ampicillin sodium salt, kanamycin sulfate, and spectinomycin, 50 μg/ml; tetracycline, 10 μg/ml; streptomycin, 100 μg/ml; and chloramphenicol, 35 μg/ml. X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) was used at 40 μg/ml in solid media. S-(β-Aminoethyl)-L-cysteine (thiosine) was used in MGR at 100 μg/ml. Unless indicated otherwise, lysine refers to L-lysine.

Isolation of lysine-independent mutants. EP243 was grown at 32°C in LB to an optical density at 420 nm (OD₄₂₀) of 0.5 and then centrifuged at 6,000 × *g*. The cells were suspended in an equal volume of TBS (50 mM Tris-HCl [pH 7.4 at 25°C],

150 mM NaCl), collected again by centrifugation, and resuspended in one-half volume of TBS. Methanesulfonic acid ethyl ester (EMS) was added to the cells to a final concentration of 1.5%, and the mixture was shaken at 32°C for 30 min. Sodium thiosulfate was added to a final concentration of 4% to stop the reaction; this was followed by three successive centrifugations and washes with TBS. The cells were then suspended in liquid MGR-7.6 to an OD₄₂₀ of 0.05 and incubated overnight at 32°C. Dilutions of the cultures were then plated onto MGR-5.8 plates containing 40 µg of X-Gal per ml and incubated overnight at 32°C. Blue colonies were collected from independent mutagenesis treatments. Mini-Tn10cam mutants were isolated by infecting EP243 with λNK1324 as described previously (17). The infected cells were then plated as described for the EMS mutagenesis except that the plates contained chloramphenicol.

Molecular biology methodology. Ligations were carried out by using T4 DNA ligase (BRL-Gibco) for 16 h at 15°C. *E. coli* was made competent for transformation by using CaCl₂ and was used as described previously (22). Plasmid DNA was isolated by an alkaline lysis procedure (2) or with plasmid isolation kits supplied by QIAGEN Inc. (Chatsworth, Calif.). DNA fragments were analyzed by agarose or acrylamide gel electrophoresis followed by staining with ethidium bromide. DNA fragments were purified from agarose gels by using the GENECLAN II kit (BIO 101 Inc., La Jolla, Calif.) as described by the manufacturer. pGCadC-1 was constructed by isolating the *EcoRI*-*Bam*HI *cadC* fragment from plasmid pPH2200 (46) and inserting it into *EcoRI*-*Bam*HI-digested pGB2. The *cadC* allele from EP310 was amplified from chromosomal DNA by PCR (38) as modified for use with bacterial cultures (16) in 100-µl reaction mixtures containing 5 µl of an overnight culture grown in LB-7.6–67 mM Tris-HCl (pH 8.8 at 25°C)–16.6 mM ammonium sulfate–5 mM magnesium acetate–1.0 mM each deoxyribonucleoside triphosphate (dNTP)–1.0 µM each primer–1 U of *Taq* polymerase–100 µl of mineral oil overlay. Twenty-five cycles were done in a Perkin-Elmer Cetus (Norwalk, Conn.) DNA Thermal Cycler 480 as follows: 1 min at 95°C, 1 min at 50°C, and 1 min at 72°C. These cycles were followed by 1 min at 95°C, 1 min at 50°C, and 10 min at 72°C. Three independent reactions were carried out from independent cultures of EP310. The entire *cadC* sequence (46) was amplified by using primers DA68 (5'-CTCTAAGCTTAATATTACTTCTGGTTC) and DA15 (5'-TAGTGGATCCTTATTCTGAAGCAAGAAA). The resulting products were digested with *Hind*III and *Bam*HI and used to replace wild-type *cadC* in plasmid pGCadC-1 which had previously been digested with *Hind*III and *Bam*HI. The carboxyl half of the *cadC* coding region from EP310 was amplified in the same manner by using DA15 and DA43 (5'-TTCTCCACCTTTATGGTG). The resulting PCR products were digested with *Bst*XI and *Bam*HI, purified, and used to replace the analogous wild-type fragment in pGCadC-1. Primers used in the PCR analysis to determine the location of Tn10cam were DA84 (5'-GGTTTCCGAAACTAAAACC) and DA85 (5'-CTTATCGTTCTGCGGGAAC), corresponding to the amino and carboxyl ends, respectively, of *lysP* (43), and DA86 (5'-CGGCAAAGCACCGCCG) and DA87 (5'-CTGCCTCCAGAGCCTG), corresponding to unique sequences near the ends of mini-Tn10cam (17). PCRs were in a 100-µl final volume consisting of 6 ng of purified genomic DNA, 5 mM magnesium chloride, 200 µM each dNTP, 1 µM each primer, 1 U of *Taq* polymerase, and 100 µl of mineral oil overlay. Thirty cycles were carried out as described above for the *cadC* PCRs. DNA sequencing was done by using the Sequenase 2.0 kit (U.S. Biochemicals, Cleveland, Ohio) with

double-stranded plasmid DNA and primers (not shown) corresponding to *cadC* sequences and vector sequences flanking *cadC* in pGCadC-1. The sequences of both strands of the indicated regions of *cadC* were determined.

β-Galactosidase assays. Cells were grown to saturation in MGR-7.6 (minus lysine) containing the appropriate antibiotic (if the strain contained a plasmid) at 32°C. Five milliliters of the indicated media, in 50-ml culture flasks, was inoculated with 0.02 ml of the saturated culture, and the cells were grown for 3 to 5 h at 32°C while shaking at 200 rpm. The level of β-galactosidase was determined essentially as described previously (26), using cells which were made permeable with sodium dodecyl sulfate and chloroform, with the following variations made in the measurements and calculation. Units were calculated as follows: units = 400 OD₄₀₅/tvOD₅₉₅, where *t* is time (in minutes), *v* is volume (in milliliters) of culture assayed, and OD₄₀₅ and OD₅₉₅ measurements were made by placing 0.1 ml of the reaction mix or culture, respectively, into microtiter plates (Costar, Cambridge, Mass.) and reading the absorbance in a Thermomax plate reader (Molecular Devices, Menlo Park, Calif.). These units closely approximate Miller units. The data presented in the text and the tables are from one experiment. All of the values (except for those in Table 5, for which two independent experiments were done) have been determined three or more times from independent experiments, and the values reported here are within ±25% of the mean from the other experiments.

RESULTS

Lysine effect on *cadBA* expression. Maximal synthesis of lysine decarboxylase in *E. coli* B had previously been shown to occur when cells were grown under O₂-limiting conditions in low-pH medium (pH 5.5) supplemented with 0.8% lysine (37). To further define and understand the signals needed for expression of the *cadBA* operon in *E. coli* K-12, we examined the expression of a *cadA-lacZ* transcription fusion in defined media. Expression was assessed by assaying for β-galactosidase activity following growth of EP243 (*cadA-lacZ*) in either defined minimal media or defined rich media. The data for expression in rich media (MGR based) are shown in Table 2. In minimal media, β-galactosidase levels were ≤5 U when EP243 was grown in either MGM-7.6, MGM-7.6 supplemented with lysine to 10 mM, or MGM-5.8. The level of β-galactosidase when EP243 was grown in MGM-5.8 supplemented with lysine to 10 mM, however, was 567 U, showing that of the 20 common amino acids, only exogenously added lysine is required for expression. To demonstrate that lysine-dependent expression required *cadC*, an EP243 derivative containing a Tn10 insertion in *cadC* (EP314) was shown not to express the fusion under any condition. Synthesis of β-galactosidase in a strain containing a chromosomal *cadB-lacZ* transcription fusion was also found to be dependent on growth in the presence of lysine (reference 24 and data not shown). Taken together, these results suggest that neither the *cadA* nor *cadB* gene product is involved in the mechanism responsible for repression in the absence of lysine.

To determine which loci are involved in mediating the lysine requirement, mutants of EP243 were isolated in which *cadA-lacZ* was expressed in the absence of exogenously added lysine. Cells were mutagenized either chemically with EMS or with the transposon mini-Tn10cam, and mutants were isolated on the basis of their ability to produce blue colonies on MGR-5.8-X-Gal plates in the absence of lysine. The levels of β-galactosidase produced by one Tn10cam mutant (EP517) and three independent EMS-generated mutants (EP302,

TABLE 2. Expression of *cadA-lacZ* in *cadC* and *lysP* variants

Strain ^a	β-Galactosidase (U) ^b			
	pH 7.6		pH 5.8	
	- Lys	+ Lys	- Lys	+ Lys
EP243 (<i>cadC</i> ⁺)	≤5	≤5	≤5	515
EP314 (<i>cadC1::Tn10</i>)	6	≤5	7	9
EP302 (<i>lysP302</i>)	16	16	271	743
EP310 (<i>cadC310</i>)	7	26	645	1,018
EP517 (<i>lysP1::Tn10cam</i>)	26	19	526	857
EP527 (<i>lysP527</i>)	63	46	289	950

^a All strains contained the *cadA-lacZ* fusion from JLS8602.

^b Cells were grown overnight in MGR-7.6, diluted 250-fold into the indicated medium, grown for 3 h, and assayed as described in Materials and Methods. pH 7.6 and pH 5.8 indicate the pH of the MGR medium. + Lys indicates that the medium was supplemented with L-lysine to 10 mM, and - Lys indicates that no exogenous lysine was added.

EP310, and EP527) are shown in Table 2. Compared with the wild-type level, all mutants exhibited increased levels of β-galactosidase in the absence of lysine.

The *cadR* (*lysP*) locus near min 46.5 was defined by mutants with one or more of the following three phenotypes: increased lysine decarboxylase synthesis, thiosine resistance (Ts^r), or defective lysine transport (32, 43, 44). Although a defined insertion in *lysP* has been shown to confer the latter two phenotypes (43), the relationships between Ts^r, *cadA* expression, and the *lysP* open reading frame have not been clearly defined. To determine if the responsible mutations in any of our mutants were in this region, each was transduced to Tet^r with P1 grown on CAG12098, a strain with a *Tn10* near min 46.5. Tet^r recombinants displaying lysine-dependent (wild-type) expression of *cadA-lacZ* were obtained with EP302, EP527, and EP517 at frequencies of 10⁻¹. To show that these mutations were sufficient to cause lysine-independent expression, Tet^r transductants displaying the mutant phenotype (from the previous transduction) were used as donors in P1 transduction experiments with EP243 as the recipient. In all three cases Tet^r transductants displaying the mutant phenotype were obtained. The mutants were checked for Ts^r on MGR-7.6 plates containing 100 μg of thiosine per ml. EP302, EP527, and EP517 formed colonies, whereas EP243 (wild type) and EP310 did not. The lysine-independent *cadA* expression and Ts^r phenotypes could not be separated by P1-mediated recombination. Together these data suggested that EP302, EP517, and EP527 contain lesions in, or near, *lysP*.

The exact location of *Tn10cam* in EP517 was determined with a PCR protocol. Primers homologous to the ends of the transposon, and oriented in a manner such that extension from the 3' ends of the primers would result in the synthesis of flanking chromosomal sequences (outward facing, DA86 and DA87), and primers homologous to the ends of *lysP*, and oriented in a manner such that extension would result in *lysP* DNA being synthesized (inward facing, DA84 and DA85), were used in combination (one transposon primer and one *lysP* primer per reaction) in PCRs with DNA from EP243 (wild type) and EP517 as the template. If the transposon was within the *lysP* coding region in EP517, two of the four combinations of *lysP* and *Tn10cam* primers should result in products whose sizes add up to approximately the size of the *lysP* coding region (i.e., similar to the product derived by using EP243 template and the two *lysP* primers). On the basis of the results from this experiment (data not shown), the *Tn10cam* was localized to the carboxyl quartile of *lysP*. The exact locations of the

EMS-generated mutations in EP527 and EP302 were not determined. Thus, a defined insertion in *lysP* conferred on the cell lysine-independent *cadA* expression.

A mutation in *cadC* confers lysine-independent *cadA* expression. Since EP310 did not contain a *lysP* mutation, we checked to see if it had a *cad*-linked lesion. The *cadA::Mu d(Km lac)* allele was transduced from EP310 to the *cad*⁺ strain EP242, and the resulting Km^r transductants were screened on MGR-5.8-X-Gal plates for their ability to express β-galactosidase in the absence of lysine. Transductants displaying the mutant phenotype were obtained at a frequency of 6 × 10⁻¹, showing that the mutation was linked to *cadA*. Logical candidates for the location of this mutation were the elements already identified as regulating expression, namely, *cadC* and the *cadBA* promoter region. The following experiments showed that the responsible mutation was in *cadC*. A region extending from 180 bp upstream of the *cadC* start codon to the *cadC* stop codon was amplified by PCR with chromosomal DNA from EP310 and primers DA15 and DA68 (Fig. 1). The resulting *cadC* DNA contained *Hind*III and *Bam*HI sites on the ends (encoded in the primer sequences) that were used for subsequent replacement of wild-type *cadC* in plasmid pGCadC-1. Two of the resulting plasmids (pPH489 and pPH490), derived from independent PCRs, were introduced by transformation into E2230, a strain containing the *cadC1::Tn10* and *cadA::Mu d(Km lac)* alleles. The resulting transformants expressed increased levels of β-galactosidase, compared with cells containing pGCadC-1, when grown in medium lacking lysine (Table 3). To further localize the mutation, a portion of the mutant *cadC*, corresponding to the carboxyl 274 codons of *cadC*, was amplified by using EP310 chromosomal DNA as a template and primers DA15 and DA43 (Fig. 1). The resulting fragments were digested with *Bst*XI and *Bam*HI and used to replace the homologous region in pGCadC-1. Again, two independently derived plasmids conferred the mutant phenotype when introduced into E2230. The levels of β-galactosidase from E2230 containing one of these plasmids, pPH528, as well as pPH489, are shown in Table 3. The DNA sequence of *cadC* corresponding to the carboxyl 274 amino acids from pPH489 and pPH490 was determined. Both had a C·G-to-T·A change at the first position of codon 265 of *cadC*, which would be predicted to result in an Arg-to-Cys change in CadC. In addition, pPH490 had a C·G-to-T·A change in the third position of codon Asn-466; however, no amino acid change in CadC is predicted from this mutation.

Increased *lysP* expression inhibits induction of *cadBA*. The results described above showed that in the absence of lysine, the LysP permease negatively regulates CadC activity. From this one might expect that increasing the level of LysP would inhibit the ability of wild-type levels of CadC to activate *cadBA* transcription. This hypothesis was tested by introducing a *lysP* expression plasmid, pLysP, into EP542, an EP243 derivative carrying an F' *lacI^q cam* plasmid, and measuring β-galactosidase levels. As shown in Table 4, the presence of pLysP prevented *cadA-lacZ* expression even in medium containing 10 mM lysine. The presence of pLysP did not prevent expression when the strain carried the mutant *cadC310* allele (Table 4). Thus, LysP-mediated inhibition, like the lack of expression observed in the absence of exogenously added lysine, was overcome by a single amino acid change in the periplasmic domain of CadC.

Cadaverine acts as a negative regulator of *cadA-lacZ* expression. Expression of the *cadBA* operon is associated with an increase in the pH of the growth medium and production and excretion of cadaverine and CO₂. Of these three possible regulators (pH, cadaverine, and CO₂), only pH was evaluated

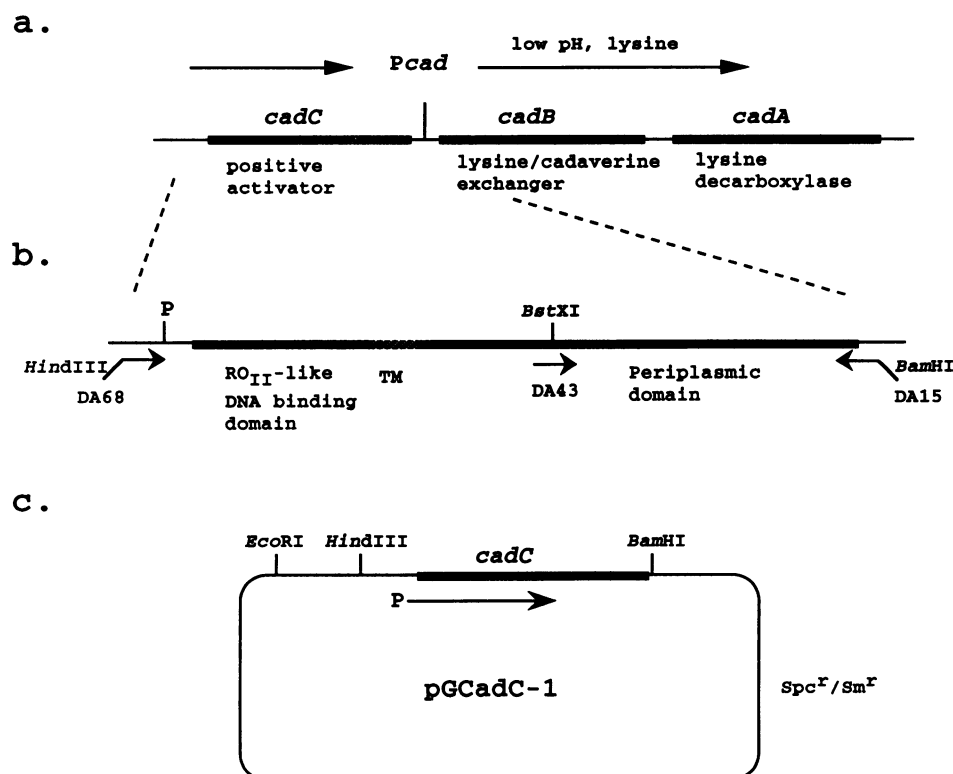


FIG. 1. Relevant details of the *E. coli* *cad* operon and plasmid pGCadC-1. (a) *Pcad* is the promoter responsible for pH- and lysine-regulated *cadBA* expression. CadC positively activates *cadBA* expression. The arrows above the genes indicate the direction of transcription. (b) Details of *cadC* and the coding regions for the different domains of CadC. TM, transmembrane segment. DA15, DA43, DA68, and the accompanying arrows indicate the primers used in the PCRs described in the text. (c) pGCadC-1 was constructed as described in Materials and Methods. P refers to a promoter located within the *E. coli* chromosomal *cadC* fragment that was used to construct pGCadC-1. The *lac* promoter in pGCadC-1 is located between the *EcoRI* and *HindIII* sites and oriented in the same direction as P.

in the experiments described above, since the insertion in *cadA* resulted in no lysine decarboxylase synthesis (thus, no CO₂ or cadaverine is produced via this pathway). To determine if cadaverine itself plays a regulatory role, independent of an increase in extracellular pH, the levels of β -galactosidase in EP243 were determined following growth in media buffered at pH 5.8 containing exogenous lysine and cadaverine at various concentrations. As shown in Fig. 2, cadaverine, at concentrations 10- to 100-fold lower than that of lysine, inhibited expression of *cadA-lacZ*. Other lysine analogs and polyamines were checked for their ability to inhibit expression. These included the products of arginine decarboxylation (agmatine) and ornithine decarboxylation (putrescine) as well as spermi-

dine, methyl and ethyl esters of L-lysine, and D-lysine. As shown in Table 5, at high concentrations (5 to 10 mM) several of these compounds also inhibited expression. However, only cadaverine could completely inhibit at a concentration of 0.5 mM. In addition, none of them could substitute for L-lysine in inducing expression (Table 5).

Mutations in *cadC* and *lysP* that altered the regulatory response to the lysine signal were tested for their effects on cadaverine-mediated repression. EP302 (*lysP302*), EP517 (*lysP1::Tn10cam*), and EP310 (*cadC310*) were assayed for β -galactosidase following growth with lysine and cadaverine. As shown in Table 6, only EP310 displayed high-level β -galactosidase expression in the presence of cadaverine. Thus, the Arg-to-Cys codon change found in *cadC310*, but not the *lysP* mutations, conferred both substantial lysine-independent and cadaverine-resistant *cadA-lacZ* expression. From these studies we conclude that both LysP and CadC are involved in responding to lysine, while of the two, CadC mediates the responses to pH and cadaverine. Although CadB is thought to be a lysine-cadaverine exchanger, under these conditions its activity was not required for the cadaverine effect (Table 6).

DISCUSSION

The pattern of lysine decarboxylase synthesis is not unlike those of enzymes in other catabolic pathways; the presence of the substrate, L-lysine, induces synthesis, while the product, cadaverine, represses synthesis. Investigation of this pathway,

TABLE 3. Expression of *cadA-lacZ* in strains containing wild-type and mutant *cadC* alleles on plasmids

Plasmid ^a	β -Galactosidase (U) ^b	
	- Lys	+ Lys
pGB2	9	≤ 5
pGCadC-1	≤ 5	2,429
pPH489	1,950	1,773
pPH528	2,026	2,182

^a E2230 was transformed with the indicated plasmid.

^b Cells were grown overnight in MGR-7.6-spectinomycin, diluted 250-fold into MGR-5.8 with (+ Lys) or without (- Lys) 10 mM L-lysine, and grown for 3 h prior to assay.

TABLE 4. *lysP* expressed from a multicopy plasmid inhibits *cadA-lacZ* expression

Strain ^a	β-Galactosidase (U) ^b	
	- Lys	+ Lys
EP542/pUC19	≤5	451
EP542/pLysP	≤5	32
EP544/pUC19	470	641
EP544/pLysP	225	435

^a EP542 and EP544 both contain the *cadA-lacZ* fusion, F' *lacI*^q. EP542 is *cadC*⁺, and EP544 is *cadC310*.

^b Cells were scraped from MGR-7.6-ampicillin plates, suspended in MGR-5.8-ampicillin with (+ Lys) or without (- Lys) 10 mM L-lysine to an OD₄₂₀ of 0.05, and grown for 3 h prior to assay.

however, offers the opportunity to explore two basic mechanistic aspects of bacterial gene regulation for which well-described paradigms are lacking: (i) control of gene expression by a signaling system that senses and responds to external pH and (ii) transcriptional activation by a membrane-bound protein. This work describes a genetic approach to better understand the mechanism involved in mediating the lysine signal required for CadC activation of *cadBA* transcription. By isolating and characterizing mutants which expressed a *cadA-lacZ* transcription fusion in the absence of exogenously added lysine, we hoped to identify the genes involved in mediating lysine induction as well as to understand how these gene products affect lysine-mediated signal transduction. Not surprisingly, mutations in *cadC* and *lysP* were identified. Most importantly, one of the *lysP* alleles contained an insertion in the *lysP* coding region. These results are consistent with an interpretation suggesting that in the absence of lysine the LysP permease is a negative regulator of CadC activity.

We consider two general mechanisms by which the function of LysP could be linked to the ability of CadC to activate *cadA-lacZ* expression. The first possibility is that the level of either external or internal lysine determines the activity of CadC and that LysP involvement in the process is to regulate the concentration of lysine in these two environments. The second possibility is that LysP itself regulates CadC activity and

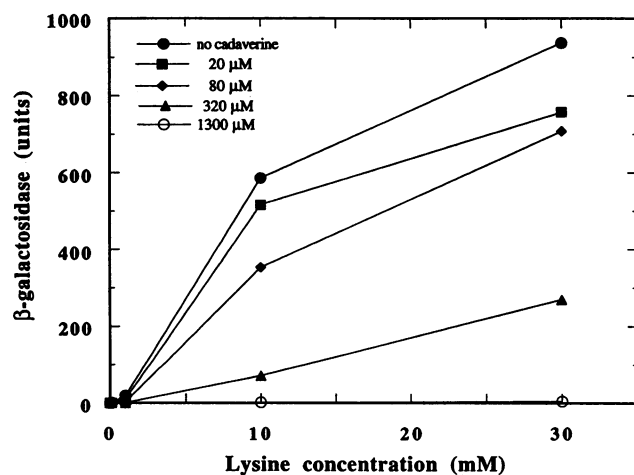


FIG. 2. Expression of *cadA-lacZ* fusion as a function of lysine and cadaverine concentrations. EP243 was grown to saturation in MGR-7.6, diluted 250-fold into MGR-5.8 containing lysine and/or cadaverine at the indicated concentrations, and grown for 3 h prior to being assayed for β-galactosidase as described in Materials and Methods.

TABLE 5. Inhibition of *cadA-lacZ* expression by lysine analogs and polyamines^a

Addition	Concn (mM)	β-Galactosidase (U)	
		+ Lys	- Lys
None		342	≤5
Cadaverine	0.5	≤5	NT ^b
	5.0	≤5	NT
	10.0	≤5	≤5
Spermidine	0.5	395	NT
	5.0	220	NT
	10.0	226	≤5
Putrescine	0.5	286	NT
	5.0	26	NT
	10.0	9	6
Agmatine	0.5	198	NT
	5.0	9	NT
	10.0	≤5	7
L-Lysine ethyl ester	0.5	190	NT
	5.0	≤5	NT
	10.0	≤5	≤5
L-Lysine methyl ester	0.5	204	NT
	5.0	26	NT
	10.0	6	≤5
D-Lysine	10.0	351	≤5

^a EP243 was grown overnight in MGR-7.6 and diluted 250-fold into MGR-5.8 with (+ Lys) or without (- Lys) L-lysine at a 10 mM final concentration and, where indicated, with lysine analogs at the concentrations shown. The cultures were grown for 3 h prior to assay. The pH of the cultures did not change by more than 0.1 pH unit during the course of the experiment.

^b NT, not tested.

that the role of lysine is to regulate the activity of LysP. The observation that an insertion in *lysP* confers lysine-independent *cadA-lacZ* expression can be accommodated by either of these models. With the first model one could propose that CadC needs to interact with lysine in order to be active and that LysP competes for the available lysine. Eliminating LysP, then, eliminates the need for exogenous lysine. If this model is correct, one has to assume that in the absence of LysP there is sufficient lysine available from normal metabolic processes to activate CadC. With the second model one would propose that

TABLE 6. Effect of cadaverine on *cadA-lacZ* expression in *cadC* and *lysP* mutants

Strain ^a	β-Galactosidase (U) ^b	
	- Cadaverine	+ Cadaverine
EP243 (<i>cadC</i> ⁺)	515	≤5
EP302 (<i>lysP302</i>)	743	14
EP310 (<i>cadC310</i>)	1,018	698
EP517 (<i>lysP1::Tn10cam</i>)	857	38
EP527 (<i>lysP527</i>)	950	92
EP609 (<i>cadC</i> ⁺ <i>cadB-lacZ</i>)	857	23

^a All but EP609 contain the *cadA-lacZ* fusion. EP609 contains a *cadB-lacZ* fusion.

^b Cells were grown overnight in MGR-7.6 and diluted 250-fold into MGR-5.8 supplemented with 10 mM L-lysine with (+ cadaverine) or without (- cadaverine) 1.3 mM cadaverine. Cells were grown for 3 h prior to assay. Except for EP609, the data for expression without cadaverine are from Table 2.

in the absence of exogenous lysine, LysP interacts with CadC to keep it in an inactive state. Lysine would then relieve the negative effect of this interaction by binding to LysP and/or CadC. Thus, eliminating LysP would eliminate the lysine requirement. The major feature distinguishing these models is that the roles of lysine and LysP are reversed with respect to which is the primary CadC effector and which serves to modulate the primary activity. None of the data described here clearly differentiate between these models. The lysine-independent expression phenotype of the *cadC310* mutant can be explained by each model. If CadC and LysP compete for lysine (first model), one would argue that the Arg-to-Cys change in CadC confers an increased affinity for lysine or the ability to become activated in the absence of lysine. If the second model (LysP-CadC interaction) is correct, the *cadC310* mutation would be predicted to alter CadC such that it is no longer a target for LysP-mediated inhibition.

Perhaps most striking about the data presented here is that they indicate an apparent lack of overlap in the mechanisms involved in transmitting the lysine and pH signals. Both the *cadC310* and *lysP* mutants, while displaying lysine-independent *cadA-lacZ* expression, were regulated almost normally by pH. Although these mechanisms appear to be distinct, both responses are mediated through CadC. Moreover, the region of the *cadBA* promoter identified as the target for mediating the pH response was indistinguishable from the site mediating the lysine response (24). There also seem to be different mechanisms involved in sensing lysine and cadaverine, since the *lysP::Tn10cam* mutant was altered for lysine signaling yet still exhibited substantial cadaverine-mediated repression. The fact that the *cadC310* allele ameliorates the cadaverine effect raises the possibility that CadC may interact directly with cadaverine. As with any of the signals, we cannot rule out the possibility that cadaverine interacts with one or more unidentified factors which, in turn, inhibit CadC function. Further evidence supporting the notion that separate mechanisms are involved in sensing lysine and cadaverine comes from the observation that other lysine analogs and other polyamines prevented expression when at the same concentration as lysine (10 mM), yet none served as lysine substitutes for induction (Table 5). The lysine recognition system utilized by the inducing system appears to be fairly strict with respect to what is recognized, while that used by the repressing system is apparently less restricted.

At present there are no biochemical data to suggest what the active and inactive states of CadC are or how these states might be controlled by the different signals (pH, lysine, and cadaverine). The steps in *Pcad* transcriptional activation that are controlled by these signals are also not known. Since CadC appears to be a DNA-binding protein (5, 46), the most obvious control point would be binding of CadC to the promoter region. DNA sequences upstream of the promoter, when placed on a multicopy plasmid, have been shown to compete *in vivo* with *Pcad* on the chromosome for a factor required for activation (24, 46). It has not been shown, however, that CadC is the limiting factor. Within the *Pcad* region is a short inverted repeat which is a good candidate for such a CadC-binding site.

Similar to the observation that overexpression of *lysP* prevents CadC-mediated induction of *cadBA*, high levels of the MalK component of the *E. coli* maltose transport system result in failure of the activator, MalT, to induce transcription of the *mal* operon (34). The analogy between the two systems can be extended further by noting that *cadC* and *malT* mutants can be isolated which are no longer sensitive to increased levels of LysP or MalK, respectively (Table 2) (21). Mutants lacking MalK show increased *mal* gene expression in the absence of

exogenously added maltose or maltodextrins, an observation that parallels the finding that strains lacking LysP express *cadBA* in the absence of lysine. Mutational analysis suggested that MalK controls the level of an intracellular inducer required for MalT activation (7, 21). This model is similar, in principle, to one of the models described above, which states that LysP controls CadC activity by titrating out lysine. In both cases the transporter controls expression by controlling inducer availability. The mechanisms by which they accomplish this, however, could be quite different. In the Cad system, if external lysine is the important signal, then the inducer may be removed merely by interacting with another membrane protein (LysP). In the Mal system, MalK has been proposed to directly destroy the inducer.

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