

# Identification of ArgP and Lrp as Transcriptional Regulators of *lysP*, the Gene Encoding the Specific Lysine Permease of *Escherichia coli*<sup>∇†</sup>

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Received 12 July 2010/Accepted 14 March 2011

**Expression of *lysP*, which encodes the lysine-specific transporter LysP in *Escherichia coli*, is regulated by the concentration of exogenous available lysine. In this study, the LysR-type transcriptional regulator ArgP was identified as the activator of *lysP* expression. At lysine concentrations higher than 25  $\mu$ M, *lysP* expression was shut off and phenocopied an *argP* deletion mutant. Purified ArgP-His<sub>6</sub> bound to the *lysP* promoter/control region at a sequence containing a conserved T-N<sub>11</sub>-A motif. Its affinity increased in the presence of lysine but not in the presence of the other known coeffector, arginine. *In vivo* data suggest that lysine-loaded ArgP and arginine-loaded ArgP compete at the *lysP* promoter. We propose that lysine-loaded ArgP prevents *lysP* transcription at the promoter clearance step, as described for the lysine-dependent regulation of *argO* (R. S. Laishram and J. Gowrishankar, *Genes Dev.* 21:1258-1272, 2007). The global regulator Lrp also bound to the *lysP* promoter/control region. An *lrp* mutant exhibited reduced *lysP* expression in the absence of external lysine. These results indicate that ArgP is a major regulator of *lysP* expression but that Lrp modulates *lysP* transcription under lysine-limiting conditions.**

Amino acid transporters play several important roles in bacteria. Besides their function in nutrient supply, these systems are also involved in osmoregulation, pH homeostasis, signal transduction, and detoxification. More than one uptake system normally exists for the transport of a single amino acid, allowing bacteria to adapt to different environmental conditions. *Escherichia coli* has three different transport systems for the uptake of the amino acid L-lysine that differ in transport mechanism, substrate specificity, apparent Michaelis constant ( $K_m$ ), and regulation of their synthesis. The lysine-arginine-ornithine (LAO) system is encoded by *argT-hisJQMP*. HisQMP<sub>2</sub> forms the ABC-transporter; HisJ and ArgT are periplasmic binding proteins that are specific for histidine and lysine, arginine, or ornithine, respectively (40). The cadaverine-lysine antiporter CadB imports lysine and excretes cadaverine (48) but is produced only under conditions of low pH. Last, but not least, LysP is a specific transporter for L-lysine that belongs to the amino acid, polyamine, and organocation (APC) transporter family (8, 49). Considering the important role of lysine and LysP in amino acid metabolism and pH homeostasis in *E. coli*, the aim of this work was to investigate the factors and mechanisms involved in the transcriptional regulation of *lysP*.

The *lysP* gene was originally named *cadR* because its mu-

tants exhibit a pleiotropic phenotype including derepressed levels of lysine decarboxylase CadA, in addition to a reduction in lysine transport (37). The Cad system, which plays a role in pH homeostasis in *Enterobacteria*, comprises the membrane-integrated transcriptional activator CadC and the *cadBA* operon, encoding the lysine decarboxylase CadA and the lysine-cadaverine antiporter CadB (26, 27, 58). This system is induced under conditions of low external pH and the simultaneous presence of exogenous lysine. As a result of the lysine decarboxylation reaction, which consumes one cytoplasmic H<sup>+</sup>, cadaverine is produced and subsequently excreted, leading to an increase of the external pH. For a long time it was unclear how the function of LysP was linked to the regulation of the Cad system. Tetsch et al. (2008) demonstrated that LysP is able to modulate the activity of the membrane-integrated protein CadC (55). According to the proposed model, LysP and CadC interact via their transmembrane domains in the absence of lysine. This interaction blocks CadC-dependent expression of the *cadBA* operon. In the presence of lysine, LysP releases CadC and CadC becomes susceptible to activation by low pH. These findings suggest that LysP has an additional regulatory function, which is typical for so-called trigger transporters (54). Thus, in addition to transport activity, LysP senses lysine availability and transduces the signal to CadC.

Neely and Olson (1996) demonstrated that a high external lysine concentration downregulates *lysP* expression (32). Expression of most of the genes belonging to the lysine biosynthesis pathway in *E. coli* is repressed by lysine, but there are multiple modes of regulation known (2, 34, 50). In *E. coli* and *Bacillus subtilis*, expression of *lysC*, which encodes one of the isoenzymes that catalyze the first step in the lysine biosynthesis pathway, is controlled by direct binding of lysine to a conserved leader sequence in its mRNA (34, 52). Orthologs of *lysP* in Gram-positive bacteria are controlled by lysine-dependent

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† Supplemental material for this article may be found at <http://jbb.asm.org/>.

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<sup>∇</sup>Published ahead of print on 25 March 2011.

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant genotype or description	Reference or source
<i>E. coli</i> strains		
MG1655	F <sup>-</sup> λ <sup>-</sup> <i>ilvG rfb50 rph-1</i>	1
MG1655-Δ <i>lacZ</i>	MG1655 Δ <i>lacZ</i> ::Tet <sup>r</sup>	K. Jahreis (personal gift)
MC4100	F <sup>-</sup> <i>araD139 Δ(argF-lacZ)U169 rpsL150 relA flb-530</i> Str <sup>r</sup>	7
MG16R	MG1655 Δ <i>lacZ</i> ::Tet <sup>r</sup> <i>rpsL150</i> Str <sup>r</sup>	This work
MG16R4	MG1655 Δ <i>lacZ</i> ::Tet <sup>r</sup> <i>rpsL150 ΔlysP::rpsL-neo</i> Km <sup>r</sup> Str <sup>s</sup>	This work
MG-LR	MG1655 Δ <i>lacZ</i> ::Tet <sup>r</sup> <i>rpsL150 ΔlysP P<sub>lysP</sub>::lacZ</i> Str <sup>r</sup>	This work
MG-LR9	MG-LR Δ <i>lysR</i> ::Km <sup>r</sup> Str <sup>r</sup>	This work
MG-LR10	MG-LR Δ <i>yeiE</i> ::Km <sup>r</sup> Str <sup>r</sup>	This work
JCP95	pop3125 ( <i>dapBp</i> [-118/+35]- <i>lacZ</i> ) Δ <i>argP</i> ::Cam <sup>r</sup>	2
MG-LR17	MG-LR Δ <i>argP</i> ::Cam <sup>r</sup> Str <sup>r</sup>	This work
MG-LR15	MG-LR Δ <i>lrp</i> ::Km <sup>r</sup> Str <sup>r</sup>	This work
MG-LR4	MG-LR P <sub>lysPΔTN11A</sub> :: <i>lacZ</i> (deletion from position -83 to position -52 in the <i>lysP</i> promoter)	This work
MG16R12	MG1655 Δ <i>lacZ</i> ::Tet <sup>r</sup> <i>rpsL150 ΔcadBA::rpsL-neo</i> Km <sup>r</sup> Str <sup>s</sup>	S. Ude (unpublished results)
MG-CR	MG1655 Δ <i>lacZ</i> ::Tet <sup>r</sup> <i>rpsL150 ΔcadBA P<sub>cadBA</sub>::lacZ</i> Str <sup>r</sup>	S. Ude (unpublished results)
MG-CR15	MG-CR Δ <i>lrp</i> ::Km <sup>r</sup> Str <sup>r</sup>	This work
BL21(DE3) pLysS	F <sup>-</sup> <i>ompT</i> r <sup>-</sup> m <sup>-</sup> B	51
Plasmids		
pBAD33	Arabinose-inducible P <sub>BAD</sub> promoter; pACYC184 ori; Amp <sup>r</sup>	18
pBAD <i>lysP</i>	<i>lysP</i> in pBAD33; Amp <sup>r</sup>	55
pBAD24	Arabinose-inducible P <sub>BAD</sub> promoter, pBR322 ori; Amp <sup>r</sup>	18
pBAD <i>argP</i>	<i>argP</i> cloned in the EcoRI and HindIII sites of pBAD24; Amp <sup>r</sup>	This work
pBAD <i>lrp</i>	<i>lrp</i> cloned in the EcoRI and HindIII sites of pBAD24; Amp <sup>r</sup>	This work
pET21a	T7 promoter based expression vector with His tag; Amp <sup>r</sup>	Novagen
pET21 <i>argP</i>	<i>argP</i> cloned in the NdeI and XhoI sites of pET21a; Amp <sup>r</sup>	This work
pET16b	T7 promoter based expression vector with His tag; Amp <sup>r</sup>	Novagen
pET16 <i>lrp</i>	<i>lrp</i> cloned in the NdeI and BamHI sites of pET16b; Amp <sup>r</sup>	This work
pRS415	Operon fusion vector	47
pRS <i>lysP</i>	pRS415:: <i>lysP</i> promoter (positions -218 to +28)	This work
pRS <i>lysP0</i>	pRS415:: <i>lysP</i> promoter with a deletion from position -88 to position -53	This work
pRS <i>lysP1</i>	pRS415:: <i>lysP</i> promoter with replacement of A/C at position -53	This work
pRS <i>lysP2</i>	pRS415:: <i>lysP</i> promoter with replacement of T/G at position -65	This work
pRS <i>lysP3</i>	pRS415:: <i>lysP</i> promoter with replacements of A/C and T/G	This work

riboswitches, named LYS or L-box elements (43). However, lysine riboswitches are not found in the 5' untranslated region of *lysP* mRNA of Gram-negative bacteria (43). Here, we show that *lysP* transcription is subject to two different types of control. The LysR-type transcriptional regulator (LTTR) ArgP was identified as a major regulator of *lysP* transcription. In addition, *lysP* expression is fine-tuned by the global regulator Lrp (leucine-responsive protein). Lrp was also found to be involved in the regulation of *cadBA* expression.

#### MATERIALS AND METHODS

**Bacterial strains and growth conditions.** The strains used in this study are listed in Table 1. *E. coli* JM109 (60) was used as a carrier for all plasmids. For the construction of the reporter strains, MG-LR and MG-CR, a method based on *rpsL* counterselection in combination with the Red/ET recombination system was employed (19) according to the protocol recommended by the technical manual of the Quick and Easy *E. coli* deletion kit (Gene Bridges). Briefly, the coding sequence of the target gene (*lysP* or *cadBA*) was replaced by an *rpsL-neo* cassette (Gene Bridges) by Red/ET recombination in strain MG16R (Table 1) to give strains MG16R4 and MG16R12. Afterwards, the *rpsL-neo* cassette was replaced by promoterless *lacZ* using the Red/ET recombination technique, according to the following procedure. Strain MG16R4 or MG16R12 carrying plasmid pRedET (Gene Bridges) was transformed with a linear DNA fragment comprising the promoterless *lacZ* gene flanked with homology sequences for the target genes, and subsequently, clones of interest were selected on LB agar plates (P<sub>lysP</sub>::*lacZ* fusion) or LB (pH 5.8) agar plates (P<sub>cadBA</sub>::*lacZ* fusion) containing 50 μg ml<sup>-1</sup> 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and 50 μg ml<sup>-1</sup> streptomycin. Blue colonies were tested for kanamycin sensitivity. The Km<sup>r</sup> clones were verified by colony PCR followed by DNA sequencing.

*E. coli* strains MG-LR4, MG-LR9, MG-LR10, and MG-LR15 were constructed using the Quick and Easy *E. coli* deletion kit (Gene Bridges) according to the instructions of the manufacturer. Introduction of the *argP*::Cam<sup>r</sup> allele into MG-LR and *lrp*::Km<sup>r</sup> into MG-CR was performed by P1vir-mediated phage transduction (56), using JCP95 and MG-LR15, respectively, as donor strains. Strain BL21(DE3)pLysS was cultivated in tryptone-phosphate medium (29) and used as a host for pET plasmids (Novagen) for protein overproduction. For determination of *lysP* expression levels, cells were grown in minimal medium (15) supplemented with glucose or fructose at a final concentration of 0.4% (wt/vol). For determination of *cadBA* expression levels, cells were cultivated in glucose minimal medium; the phosphate buffer of the medium was adjusted to either pH 5.8 or pH 7.6. Antibiotics were used at the following concentrations: ampicillin, 100 μg ml<sup>-1</sup>; kanamycin, 50 μg ml<sup>-1</sup>; chloramphenicol, 34 μg ml<sup>-1</sup>; tetracycline, 12.5 μg ml<sup>-1</sup>; and streptomycin, 50 μg ml<sup>-1</sup>.

**Construction and analysis of *lysP* promoter variants.** A sequence of 246 bp encompassing the whole *lysP* promoter/control region (positions -218 to +28) (see Fig. 2) was amplified by PCR with primers lysPupEcoRI (5' GAA TTC CGC TTT CTG GAC TAT TGC GAT C 3') and lysPprBamHI (5' CGG GAT CCA CAA AAA TGC TAT CCA TCT TAA 3') and cloned upstream of the promoterless *lacZ* gene in vector pRS415 (47). Introduction of deletions or point mutations of the conserved T-N<sub>11</sub>-A motif was achieved by purchasing the corresponding synthetic DNA fragments (Mr. Gene, Regensburg, Germany), which were subcloned into the EcoRI and BamHI sites of pRS415. *E. coli* strain MG1655-Δ*lacZ* was transformed with the resulting plasmids to test *lysP* expression as described below.

**In vivo *lysP* and *cadBA* expression studies.** Expression of *lysP* and *cadBA* *in vivo* was determined by means of β-galactosidase assays. For the analysis of P<sub>lysP</sub>::*lacZ* expression, cells of an overnight culture grown in minimal medium were inoculated into fresh medium (supplemented with amino acids where indicated), resulting in an optical density at 600 nm (OD<sub>600</sub>) of 0.05. Cultures were grown aerobically in Erlenmeyer flasks at 37°C. To determine the expression of

*P<sub>cadB</sub>::lacZ*, cells were precultured in minimal medium at pH 7.6 and then inoculated into fresh minimal medium at pH 5.8 or pH 7.6, supplemented with lysine and/or arginine where indicated. Cultures were incubated under microaerobic conditions at 37°C to mid-logarithmic growth phase.  $\beta$ -Galactosidase activity measurements were performed as previously described (55) for at least three independent experiments. Values are given in Miller units (MU), which were calculated according to Miller (28).

**Molecular biology techniques.** Plasmid DNA and genomic DNA were isolated by using a HiYield plasmid minikit (Sued-Laborbedarf Gauting) and a DNeasy blood and tissue kit (Qiagen), respectively. DNA fragments were purified from agarose gels using a Hi-Yield PCR cleanup and gel extraction kit (Sued-Laborbedarf Gauting). Phusion high-fidelity DNA polymerase or Phire hot-start DNA polymerase (Finnzymes) was used according to the supplier's instructions. Restriction enzymes were purchased from New England Biolabs and used according to the manufacturer's directions.

**Purification of Lrp and ArgP.** *E. coli* BL21(DE3)pLysS harboring plasmid pET16*lrp* or pET21*argP* (Table 1) was grown to exponential phase at 30°C, and expression of genes encoding N-terminally His-tagged Lrp (His<sub>6</sub>-Lrp) or C-terminally His-tagged ArgP (ArgP-His<sub>6</sub>) was induced with 0.5 mM isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG). After 3 h of induction, cells were harvested and washed with 100 mM Na-K-phosphate buffer (pH 7.5) at 4°C. The cell pellet was frozen in liquid nitrogen and stored at -80°C until use. Cells were lysed by passage through a high-pressure cell disrupter (Constant Systems). After centrifugation of the disrupted cells, the supernatant containing the His<sub>6</sub> protein was incubated with Ni<sup>2+</sup>-nitrilotriacetic acid (NTA) resin (Qiagen) preequilibrated with lysis buffer (20 mM imidazole, 50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 10% [vol/vol] glycerol, pH 8.0). After 1 h of incubation, the protein-resin complex was washed twice with washing buffer (50 mM imidazole, 50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 10% [vol/vol] glycerol, pH 8.0). Finally, the His-tagged protein was eluted in several fractions with buffer containing 250 mM imidazole, 50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, and 10% (vol/vol) glycerol, pH 8.0. His<sub>6</sub>-Lrp was dialyzed against Lrp binding buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM dithiothreitol [DTT], 0.1 mM EDTA, 10% [vol/vol] glycerol) and ArgP-His<sub>6</sub> against ArgP-binding buffer (24) at 4°C. The purified proteins were stored in the corresponding binding buffers in the presence of 1 mM phenylmethylsulfonyl fluoride (PMSF) at 4°C and used within 1 week or stored at -20°C. Protein concentration was determined according to Bradford (3).

**EMSAs.** Probes for the electrophoretic mobility shift assay (EMSA) were amplified by PCR using primers labeled at their 5' ends with the 6-isomer of carboxyfluorescein (6-FAM) and genomic DNA from *E. coli* MG1655 as a template unless indicated otherwise. To analyze the binding of ArgP-His<sub>6</sub> to the *lysP* promoter/control region, three different fragments were used: (i) a fragment of 276 bp comprising the sequence from position -218 to position +58 (*P<sub>lysP</sub>* fragment) (see Fig. 2), which was obtained by PCR using primers *lysPup* (5'-CGCTTCTGGACTATTGCGATC-3') and *lysPprlow* (5'-CGCTTCTGTGGTTT TAGTTTCG-3'); (ii) a fragment of 142 bp comprising the sequence from position -84 to position +58 (T-N<sub>11</sub>-A fragment), which was amplified with primers TN11A (5'-TATAATCCCTGGGCGATCATG-3') and *lysPprlow*; and (iii) a fragment of 93 bp (-35-10 fragment, comprising positions -35 to +58) obtained by amplification with primers -35 (5'-CGGAAGGATTGCCAATCGT-3') and *lysPprlow*. To evaluate the binding of His<sub>6</sub>-Lrp to the *lysP* promoter/control region, only the *P<sub>lysP</sub>* fragment was used. To determine the binding of His<sub>6</sub>-Lrp to the *cadB4* promoter/control region, a fragment comprising positions -150 to +72 upstream of the *cadB* gene was cloned into the EcoRI and BamHI sites of the pUC19 plasmid (60) and amplified with primers 6-FAM uni-24 (5'-ACGACGTTGTAACACGACGGCCAG-3') and rev-24 (5'-TTCACACAGGAAACAGCTATGACC-3'). As a control for nonspecific binding, a DNA fragment of 258 bp within the *lysP* coding sequence (control fragment) obtained by amplification with *lysPcup* (5'-ACATCAGCGTTAGTCCGT-3') and *lysPclow* (5'-ATGGAGGTCAGGAAGCACA-3') was used. After PCR amplification, the obtained DNA fragments were purified by 7% (wt/vol) polyacrylamide gel electrophoresis according to the protocol of the GenElute gel extraction kit (Sigma). ArgP-DNA binding assays were performed by incubating 30 fmol of a DNA fragment with increasing concentrations of ArgP-His<sub>6</sub> in 25  $\mu$ l ArgP binding buffer supplemented with 12  $\mu$ g ml<sup>-1</sup> sonicated salmon sperm DNA as a nonspecific competitor, 50  $\mu$ g ml<sup>-1</sup> bovine serum albumin, and, where indicated, 0.1 mM lysine or arginine. Binding of His<sub>6</sub>-Lrp to DNA was performed with 30 fmol of a DNA fragment and increasing concentrations of His<sub>6</sub>-Lrp in 25  $\mu$ l Lrp binding buffer supplemented with 20  $\mu$ g ml<sup>-1</sup> salmon sperm DNA and 0.1 mM lysine where indicated. After incubation at 25°C for 30 min, complexes were resolved by electrophoresis in a 6.5% (wt/vol) polyacrylamide gel under a constant voltage of 10 V/cm at room temperature for 1.5 h. Gels were scanned with a Typhoon Trio imager (Amersham Biosciences) under an excitation wavelength

of 488 nm. Quantification of free DNA and protein-bound DNA was performed by densitometry using the ImageQuant 5.0 analysis software program (Molecular Dynamics). The quantified data were plotted versus the protein concentration to obtain the binding profile. The apparent dissociation constants ( $K_D$ ) of ArgP-DNA binding assays were determined to be the protein concentration at which the fraction of bound DNA equals 0.5. The binding profiles obtained from the Lrp binding assays were fitted to the Hill equation to determine the  $K_D$  value and the Hill coefficient ( $n$ ).

**DNase I footprinting assays.** DNase I footprinting analysis was performed according to the method described by Sandaltzopoulos and Becker (44). One hundred fifty nanograms of a *P<sub>lysP</sub>* DNA fragment (bases -218 to +58) labeled at its 5' end with 6-FAM as described above was incubated with different concentrations of ArgP-His<sub>6</sub> in ArgP binding buffer supplemented with 12  $\mu$ g ml<sup>-1</sup> sonicated salmon sperm DNA as a nonspecific competitor, 50  $\mu$ g ml<sup>-1</sup> bovine serum albumin, and, where indicated, 0.1 mM lysine in a final volume of 50  $\mu$ l. After incubation at 25°C for 30 min, 50  $\mu$ l of a 5 mM CaCl<sub>2</sub> solution was added, and incubation was prolonged for 1 min. Subsequently, 0.25 U of DNase I was added, and after 5 min, the reaction was stopped by adding 0.5 ml DF buffer from the Hi-Yield PCR cleanup and gel extraction kit (Sued-Laborbedarf Gauting). After purification, the DNA fragments were analyzed with an ABI PRISM 377 DNA sequencer, and the data were evaluated with the Peak Scanner software program (Applied Biosystems).

**DNA affinity purification assay for the identification of DNA-binding proteins.** To isolate putative transcriptional regulators of *lysP*, a DNA affinity purification protocol was applied (17). For this purpose, a biotinylated *P<sub>lysP</sub>* fragment was generated by PCR using primers *lysPup*, labeled with biotin at the 5' end, and *lysPprlow*. As a control, a biotinylated DNA fragment located within the *lysP* coding sequence (obtained by amplification with biotin-labeled *lysPcup* and *lysPclow*) was used. About 600 pmol of the biotin-labeled DNA fragments was immobilized with streptavidin-coated magnetic particles (Chemagen Biopolymer-Technologies) according to the manufacturer's instructions. For the preparation of the cytoplasmic protein extract, *E. coli* MG1655 was cultivated in 800 ml of glucose minimal medium to an OD<sub>600</sub> of 0.8. Cells were harvested at 4°C, washed with cold protein binding buffer B (PBB) (41), resuspended in 8 ml of the same buffer, and broken with a French press. After centrifugation to remove the cellular debris, the supernatant extract was incubated with DNA-coated magnetic beads (previously equilibrated with PBB) at room temperature for 30 min. Washing to remove unspecific bound proteins and elution of tightly bound proteins was performed as described by Rey et al. (41). Eluted fractions were collected, subjected to sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) (23), and stained with Coomassie blue. Proteins were identified by peptide fingerprint analysis (20) using a matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) system (Voyager DE STR; Applied Biosystems). Samples were prepared and analyzed as described previously (59).

## RESULTS

***lysP* transcription is negatively regulated by lysine.** Previous work showed that *lysP* mRNA declines within 4 min after addition of 10 mM lysine (32). To analyze the concentration-dependent effect of external lysine on *lysP* transcription in more detail, *E. coli* strain MG-LR, an MG1655 derivative that carries a *P<sub>lysP</sub>::lacZ* fusion, was constructed. In this strain, the coding sequence of the *lysP* gene was replaced by the reporter gene *lacZ*, so that the *lacZ* gene is located exactly in the same genetic context as the *lysP* gene. Since the MG-LR strain is  $\Delta$ *lysP*, an expression plasmid carrying the *lysP* gene (*pBAD<sub>lysP</sub>*) (Table 1) was introduced into this strain to determine whether the expression from the *lysP* promoter (*P<sub>lysP</sub>*) was affected by the presence of LysP.  $\beta$ -Galactosidase activities indicated that *lysP* expression was completely repressed by external lysine concentrations of 50  $\mu$ M and higher (Fig. 1). The availability of LysP did not alter the lysine-mediated repression (Fig. 1).

**Identification of ArgP as a regulator of *lysP* expression.** Analysis of the *lysP* promoter revealed a conserved T-N<sub>11</sub>-A motif, a typical binding site of LysR-type transcriptional regu-



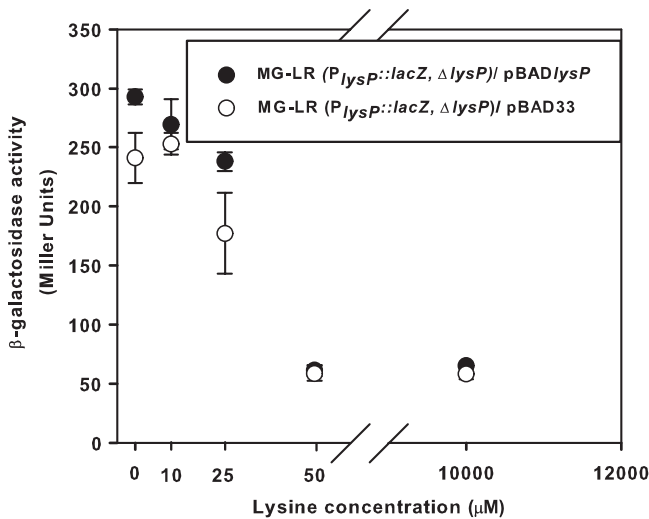


FIG. 1. Effect of the external lysine concentration and LysP on *lysP* expression. Cultures of *E. coli* strain MG-LR (*P<sub>lysP</sub>::lacZ ΔlysP*) with plasmid pBAD33 or pBAD*lysP* were grown in fructose minimal medium supplemented with different concentrations of external lysine and 0.006% (wt/vol) arabinose to induce the expression of the *lysP* gene cloned in the pBAD33 plasmid. When cultures reached an OD<sub>600</sub> of ~0.5, samples were collected and β-galactosidase activities were determined. The experiment was performed in triplicate, and error bars indicate standard deviations from the means.

lators (LTTRs) that is in close proximity to a sequence with strong similarity to the consensus sequence for σ<sup>70</sup>-dependent *E. coli* promoters (Fig. 2). Based on this motif and previous data from the literature (2, 8, 16, 50), three LTTRs were selected as putative transcriptional regulators of *lysP*: LysR, YeiE, and ArgP. LysR is the activator protein required for expression of *lysA*, which encodes the enzyme that catalyzes the last step in lysine biosynthesis, the decarboxylation of diaminopimelate (DAP), into lysine (50). LysR is responsive to the intracellular concentration of DAP and lysine. YeiE is encoded by a gene located immediately upstream of *lysP*, and the induction of *yeiE* expression increased the expression of *lysP* (16). Unexpectedly, under the conditions tested (minimal medium at pH 7.6 and 5.8 with or without the addition of external lysine and rich medium), neither a deletion of *lysR* nor a deletion of *yeiE* affected the expression of *lysP* (data not shown). It has been reported that mutations in *argP* affect the uptake of arginine, ornithine, and lysine (8). However, the direct implication of ArgP in the regulation of genes encoding the corresponding transport proteins responsible for the uptake of these amino acids has never been analyzed.

To evaluate whether ArgP was involved in the transcriptional regulation of *lysP*, a nonfunctional *argP* allele

(Δ*argP*::Cam<sup>r</sup>) was transduced into strain MG-LR carrying the chromosomal *P<sub>lysP</sub>::lacZ* fusion. The resulting mutant, named MG-LR17, and the *argP*<sup>+</sup> parent strain were grown in glucose minimal medium with or without the addition of 0.1 mM lysine and analyzed for β-galactosidase activities (Fig. 3A). The results clearly showed that expression of *lysP* did not occur in the *argP* mutant, either in the absence or in the presence of lysine.

To confirm the role of ArgP in the regulation of *lysP* transcription, the *argP* gene was cloned into plasmid pBAD24 under the control of the arabinose-inducible promoter (18). The *argP* mutant MG-LR17 was transformed with the resulting plasmid, named pBAD*argP* (Table 1). Expression of *lysP* was monitored in cells of the *argP*<sup>+</sup> strain (MG-LR) bearing the pBAD24 vector and the *argP* mutant with pBAD24 or pBAD*argP* grown in glucose minimal medium plus arabinose, with and without the addition of lysine. As shown in Fig. 3B, plasmid-carried *argP* fully restored the *lysP* expression pattern in the MG-LR17 mutant. In the absence of lysine, transcription of *lysP* in strain MG-LR increased immediately. In the presence of lysine, induction was prevented within the first hours of growth and slowly increased after prolonged growth, presumably due to lysine limitation. In the *argP* mutant, induction of *lysP* did not occur. However, plasmid-carried *argP* rescued *lysP* expression in mutant MG-LR17. In the absence of lysine, induction of *lysP* occurred after *argP* expression was induced (2.5 h). In the presence of lysine, the expression pattern of this complemented mutant was dependent on the growth phase and probably on lysine availability. These results indicated that ArgP is responsible for the transcriptional activation of *lysP* in the absence of lysine.

Previously, ArgP was identified as a lysine-dependent regulator of *argO*, which encodes an arginine exporter (24, 30), and *dapB*, which encodes an enzyme of the lysine biosynthesis pathway (2). Transcription of these genes was also affected by the presence of arginine in the culture medium, indicating that both lysine and arginine are cofactors of ArgP (2, 24). Therefore, the effect of arginine on the expression of the *P<sub>lysP</sub>::lacZ* fusion in strains MG-LR and MG-LR17 was tested. Figure 3C shows the β-galactosidase activities of cells cultivated in glucose minimal medium without amino acids or supplemented with lysine, arginine, or lysine plus arginine. When arginine was added to the growth medium, *lysP* expression in the MG-LR strain was induced to the same extent as in cells that were grown in the absence of amino acids. Importantly, the presence of arginine partially relieved the repressive effect of lysine, as indicated by the >3-fold-higher β-galactosidase activities (251 ± 41.1 Miller units [MU]) of cells that were cultivated in arginine plus lysine medium than of those that were grown in lysine medium (β-galactosidase activity, 67 ± 6.3



FIG. 2. Nucleotide sequence of the *lysP* regulatory region (positions -218 to +58). Predicted -35 and -10 promoter motifs (BProm; <http://linux1.softberry.com/berry.phtml?topic=bprom&group=help&subgroup=gfindb>) and the start of transcription (position +1) previously identified by primer extension analysis (32) are indicated. Gray-shaded nucleotides show the potential LTTR binding site identified *in silico* with the conserved T-N<sub>11</sub>-A motif. Zones protected against DNase I digestion are boxed (see Fig. 5). The start codon is marked in bold letters.

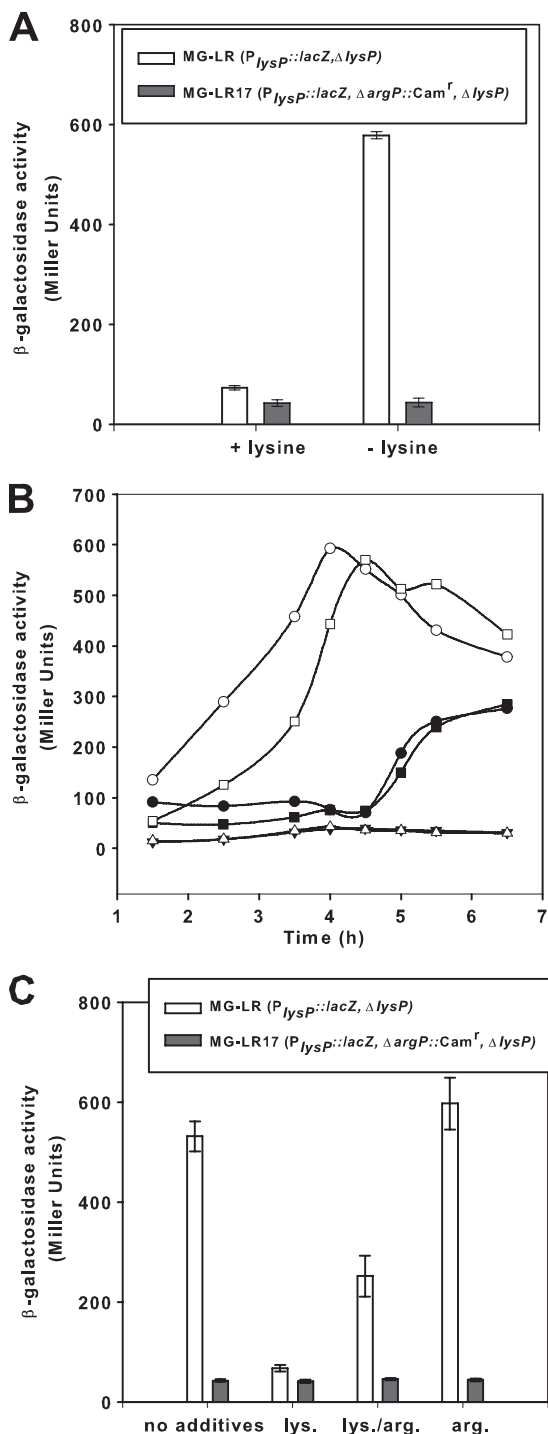


FIG. 3. Regulation of *lysP* expression by lysine, arginine, and ArgP. (A) Effect of the *argP* deletion on *lysP* expression. Parent strain MG-LR ( $P_{lysP}::lacZ, \Delta lysP$ ) and the *argP* mutant MG-LR17 ( $P_{lysP}::lacZ, \Delta argP::Cam^r, \Delta lysP$ ) were grown aerobically in glucose minimal medium with or without the addition of 0.1 mM lysine. When cultures reached an  $OD_{600}$  of  $\sim 0.8$ , samples were analyzed for  $\beta$ -galactosidase activity. (B) Complementation of the *argP* mutant with pBAD $_{argP}$ . Strains MG-LR/pBAD24 ( $\circ, \bullet$ ), MG-LR17/pBAD24 ( $\triangle, \blacktriangledown$ ), and MG-LR17/pBAD $_{argP}$  ( $\square, \blacksquare$ ) were grown in glucose minimal medium without lysine (open symbols) or with the addition of 0.1 mM lysine (closed symbols). Arabinose at a final concentration of 0.2% (wt/vol) was added to all cultures after 2.5 h of growth.  $\beta$ -Galactosidase activities were determined at different time points during growth. (C) Effect

of basic amino acids on *lysP* expression. Strains MG-LR and MG-LR17 were cultivated as described for panel A in glucose minimal medium without supplementation or supplemented with 10 mM lysine (lys.) and/or arginine (arg.) for determination of  $\beta$ -galactosidase activity. All experiments were performed at least three times, and where indicated, error bars represent standard deviations from the means.

MU). As expected, there was no *lysP* expression in the  $\Delta argP$  mutant under all tested conditions. **ArgP binds to the *lysP* promoter/control region at a T-N<sub>11</sub>-A motif in the presence and absence of lysine.** To determine whether ArgP directly regulates *lysP* transcription, we tested binding of ArgP to the *lysP* promoter/control region. For this purpose, ArgP with a C-terminal hexahistidine tag (ArgP-His<sub>6</sub>) was purified. In a control experiment, this ArgP derivative complemented strain MG-LR17, indicating that the His<sub>6</sub> tag did not alter the function of ArgP (data not shown). A fluorescently labeled DNA fragment encompassing the *lysP* promoter/control region from position  $-218$  to position  $+58$  ( $P_{lysP}$  fragment) was incubated with increasing concentrations of purified ArgP-His<sub>6</sub> in the presence of salmon sperm DNA as a nonspecific competitor (Fig. 4A). A DNA fragment of similar size within the *lysP* coding sequence was used as a control for nonspecific binding (Fig. 4B). ArgP-His<sub>6</sub> specifically bound to the  $P_{lysP}$  fragment with an apparent  $K_D$  of  $125 \pm 13$  nM (Fig. 4A and F).

To determine whether lysine or arginine affected binding of ArgP to  $P_{lysP}$ , these amino acids were added to the binding assay at a final concentration of 0.1 mM (Fig. 4C and D). Whereas arginine did not affect the binding of ArgP to the *lysP* promoter/control region, the presence of lysine increased the binding affinity approximately 2-fold ( $K_D$ ,  $63 \pm 9$  nM) (Fig. 4C and F). This differential effect of lysine and arginine on DNA affinity of ArgP has already been reported for the *argO* control region (24).

As already mentioned, a potential ArgP-binding site, ATG AAGGTGTCTTAT, is centered at position  $-59$  in the *lysP* promoter/control region (Fig. 2). To evaluate the importance of this sequence for binding of ArgP to the *lysP* control region, a DNA fragment from position  $-84$  to position  $+58$  containing the T-N<sub>11</sub>-A conserved motif (T-N<sub>11</sub>-A fragment) and another, from position  $-35$  to position  $+58$ , without this T-N<sub>11</sub>-A sequence ( $-35$ -10 fragment) were incubated with ArgP in the presence and absence of lysine (Fig. 4E). A retarded band was apparent when ArgP was incubated with the T-N<sub>11</sub>-A fragment, whereas in the presence of the DNA fragment without the T-N<sub>11</sub>-A motif ( $-35$ -10 fragment), only a faint retarded band was seen, indicating that the T-N<sub>11</sub>-A sequence is required for proper binding of ArgP to the *lysP* promoter/control region. Moreover, the affinity of ArgP for the T-N<sub>11</sub>-A fragment was found to be slightly higher in the presence of lysine (Fig. 4E).

To study the ArgP-binding site in the *lysP* promoter/control region in more detail, DNase I footprinting analysis was performed in the absence and presence of lysine (Fig. 5). ArgP-His<sub>6</sub> protected the stretch from position  $-91$  to position  $-47$ , with an intervening unprotected region between positions  $-76$  and  $-71$  (Fig. 2 and 5). Importantly, ArgP bound to the same

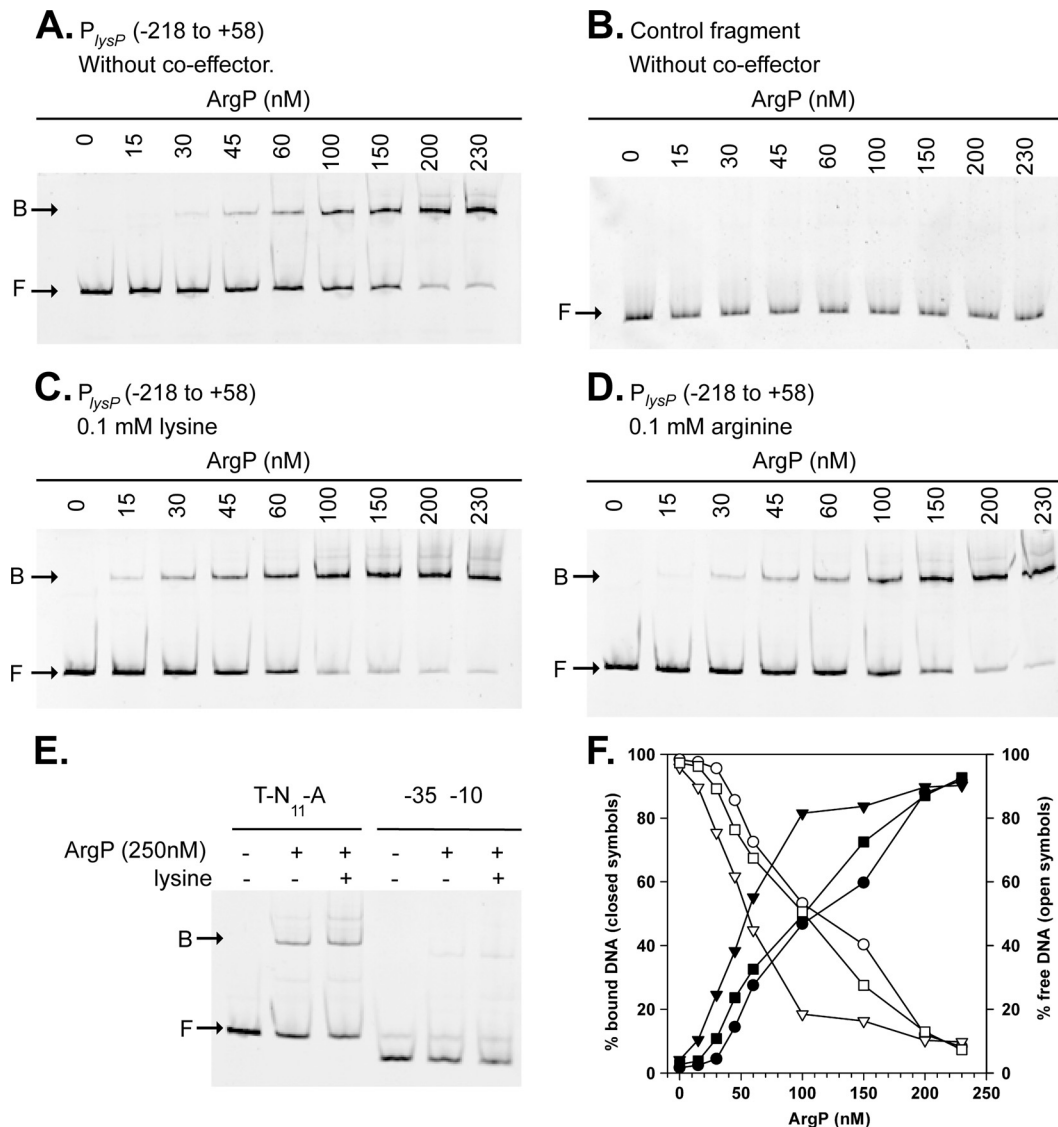


FIG. 4. Binding of ArgP to the *lysP* control region. (A, C, and D) Electrophoretic mobility shift assays (EMSAs) of a fluorescently labeled DNA fragment from bp -218 to bp +58 encompassing the *lysP* promoter/control region ( $P_{lysP}$ ) with increasing concentrations of purified ArgP-His<sub>6</sub> in the absence of coeffector (A) or in the presence of 0.1 mM lysine (C) or 0.1 mM arginine (D). The positions of free DNA (F) and ArgP-DNA complexes (B) are marked with arrows. (B) A DNA fragment within the *lysP* coding sequence was used as a control for unspecific binding. (E) Binding of ArgP-His<sub>6</sub> to a fragment from position -84 to position +58 bearing the potential ArgP-binding site (T-N<sub>11</sub>-A) and to a fragment from position -35 to position +58 (-35-10) in the presence or absence of lysine. (F) Binding curves obtained after the quantification of free DNA (open symbols) and ArgP-bound DNA (closed symbols) in EMSA gels without coeffector (○, ●) in the presence of lysine (▽, ▼) or arginine (□, ■).

sites in the presence and absence of its coeffector lysine (Fig. 5B and C).

**Elimination or modification of the T-N<sub>11</sub>-A motif affected *lysP* expression *in vivo*.** To evaluate the importance of the T-N<sub>11</sub>-A motif within the *lysP* promoter/control region for *in vivo* expression of *lysP*, a fragment encompassing this sequence (nucleotides -83 to -52) (Fig. 2) was deleted in the MG-LR strain, resulting in strain MG-LR4. Elimination of this motif completely abolished *lysP* expression under all conditions tested (Fig. 6), indicating its importance for ArgP-mediated transcriptional regulation. To analyze the effect of point mutations within this motif, the whole *lysP* promoter/control region was fused to a promoterless *lacZ* gene in vector pRS415

(Table 2). The deletion of the motif (pRS*lysP0*) or the replacement of both conserved T and A nucleotides (pRS*lysP3*) prevented *lysP* induction under lysine-limiting conditions. The replacements of A (pRS*lysP1*) or T (pRS*lysP2*) did not abolish *lysP* induction but significantly reduced it (Table 2).

**Isolation and identification of other proteins that specifically bind to the *lysP* promoter/control region.** So far, our results demonstrate that ArgP directly binds to the *lysP* promoter/control region and that the T-N<sub>11</sub>-A sequence located close to the RNA polymerase binding site is important for binding and crucial for *lysP* transcription. According to the *in vivo* analyses, ArgP is a transcriptional activator of *lysP* in the absence of lysine. The *in vitro* experiments demonstrated that

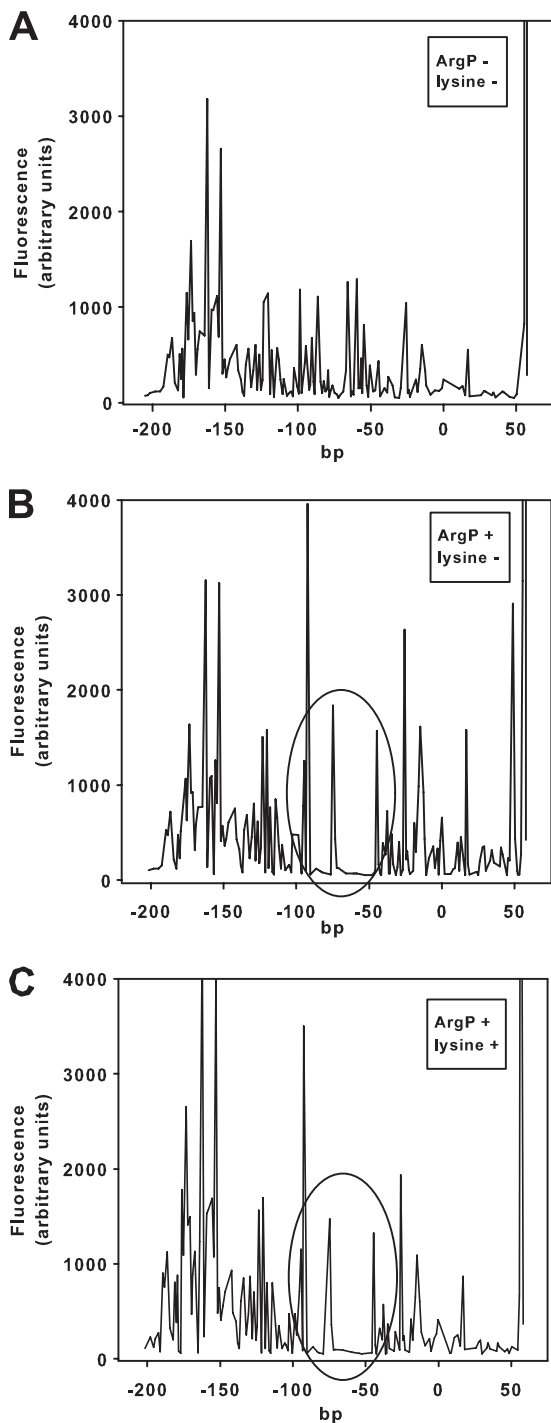


FIG. 5. Determination of the ArgP-binding site within the *lysP* control region. DNase I digestion patterns were determined for a DNA fragment from position  $-218$  to position  $+58$  of the *lysP* control region labeled with fluorescein at the 5' end of the top strand. Panel A shows the restriction pattern obtained in the absence of purified ArgP-His<sub>6</sub>, and panels B and C show the pattern obtained in the presence of 4.3  $\mu$ M purified protein in the absence (B) or presence (C) of 0.1 mM lysine. Regions protected by ArgP (position  $-91$  to  $-77$  and  $-70$  to  $-47$ ) are encircled.

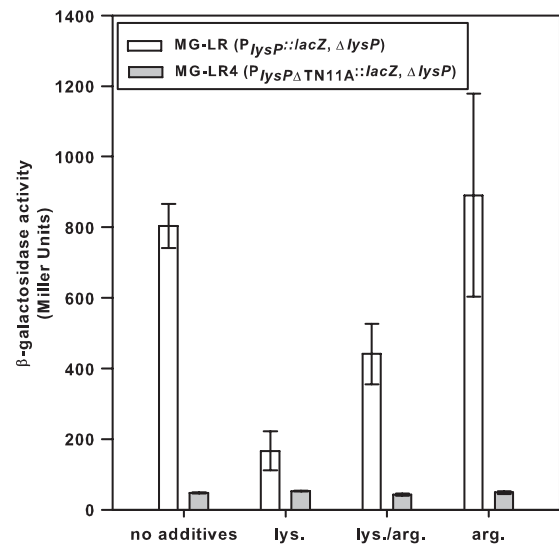


FIG. 6. Effect of elimination of the T-N<sub>11</sub>-A motif in the *lysP* control region on *lysP* expression. Parent strain MG-LR ( $P_{lysP}::lacZ \Delta lysP$ ) and strain MG-LR4 ( $P_{lysP\Delta TN11A}::lacZ \Delta lysP$ ) were grown aerobically to an OD<sub>600</sub> of  $\sim 0.8$  in glucose minimal medium without supplementation or supplemented with 10 mM lysine (lys.) and/or arginine (arg.) for determination of  $\beta$ -galactosidase activity. All experiments were performed at least three times. Error bars represent standard deviations from the means.

ArgP binds more avidly to the *lysP* control region in the presence of lysine, which might underlie the lysine-mediated prevention of *lysP* expression similarly to the previously described shutoff of *argO* expression by lysine-loaded ArgP (24). Alternatively, another protein might coregulate *lysP* repression. This scenario is plausible, because ArgP and Lrp competitively activate *argO* (35). To search for other proteins that might bind

TABLE 2. Effects of modifications within the *lysP* promoter/control region on *lysP* expression<sup>a</sup>

<i>E. coli</i> strain or strain carrying plasmid	Description of modification within the <i>lysP</i> promoter/control region	Induction of <i>lysP</i> upon lysine limitation ( $lysP_{no\ lysine}/lysP_{10\ mM\ lysine}$ ) <sup>b</sup>
MG-LR	None	7.90
MG1655- $\Delta lacZ$ /pRS $lysP$	None	3.43
MG-LR4	Deletion of T-N <sub>11</sub> -A motif (nucleotides $-83$ to $-52$ )	1.03
MG1655- $\Delta lacZ$ /pRS $lysP0$	Deletion of T-N <sub>11</sub> -A motif (nucleotides $-88$ to $-53$ )	1.28
MG1655- $\Delta lacZ$ /pRS $lysP1$	Replacement of A/C at position $-53$	2.77
MG1655- $\Delta lacZ$ /pRS $lysP2$	Replacement of T/G at position $-65$	1.85
MG1655- $\Delta lacZ$ /pRS $lysP3$	Replacement of A/C (position $-53$ ) and T/G (position $-65$ )	1.24

<sup>a</sup> Strains were grown aerobically in glucose minimal medium in the absence or presence of 10 mM lysine to an OD<sub>600</sub> of  $\sim 0.8$ .  $\beta$ -Galactosidase activity was determined and served as a measurement for *lysP* expression.

<sup>b</sup> The ratio between *lysP* expression levels in the absence and presence of lysine indicates the inducibility of the *lysP* promoter. Data were obtained from at least three independent experiments, and average values (the standard deviation was about 15%) were used for calculating the ratios.



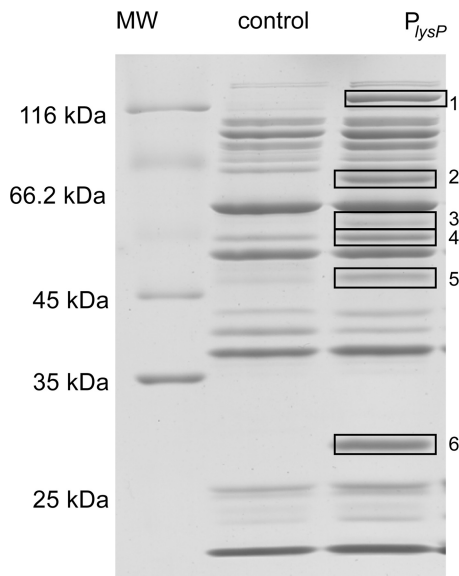


FIG. 7. SDS-PAGE of proteins after DNA affinity purification. A biotin-labeled DNA fragment from position -218 to position +58 encompassing the *lysP* promoter/control region ( $P_{lysP}$ ) and a DNA fragment within the *lysP* coding region (control) were bound to streptavidin-coated magnetic beads and subsequently incubated with a soluble extract of *E. coli* MG1655 grown in glucose minimal medium. Tightly bound proteins were eluted with a high-ionic-strength buffer and separated by SDS-PAGE. Boxed bands correspond to proteins that specifically bind to the *lysP* promoter/control region (Table 3). MW, molecular mass marker.

to the *lysP* promoter, a DNA affinity purification approach was used. The 276-bp fragment encompassing the *lysP* promoter/control region (positions -218 to +58) was biotinylated, linked to streptavidin-coated magnetic beads, and incubated with a concentrated soluble protein extract from *E. coli* MG1655 grown in glucose minimal medium. The same was done with a fragment of 258 bp within the *lysP* coding sequence, which served as a control. Proteins tightly bound to the DNA fragments were eluted as described in Materials and Methods and analyzed by SDS-PAGE. As shown in Fig. 7, several proteins that specifically bound to the *lysP* promoter/control region and not to the control fragment were detected.

The binding proteins were identified by MALDI-TOF mass spectrometry. With the exception of one, we were able to identify the eluted proteins (Table 3). All of them turned out to be DNA-binding proteins. The most abundant protein was the leucine-responsive-protein, Lrp. As Lrp is a global transcriptional regulator that controls the expression of numerous genes in response to the availability of amino acids and nitro-

gen bases (6, 9, 33), the role of this protein in *lysP* expression was analyzed in more detail.

**Lrp stimulates transcription of *lysP* by direct binding to its control region.** To determine whether Lrp influences *lysP* transcription, the *lrp* gene was inactivated in *E. coli* MG-LR, resulting in strain MG-LR15 ( $\Delta lrp::Km^r$ ). Expression of the  $P_{lysP}::lacZ$  fusion in strains MG-LR and MG-LR15 was monitored during growth in glucose minimal medium with and without the addition of lysine. Figure 8A shows that the  $P_{lysP}$  activity of the  $\Delta lrp::Km^r$  mutant in the absence of lysine is lower than that of the parent strain. At the late exponential growth phase (optical density at 600 nm [OD<sub>600</sub>] of about 1.0), the expression level of *lysP* in the MG-LR15 strain was about 50% lower than that in the parent strain MG-LR. The presence of 0.1 mM lysine reduced *lysP* expression in both strains. Therefore, Lrp seems to be an activator of *lysP* transcription in the absence of lysine.

The regulatory effect of Lrp is sometimes modulated by the effector molecule L-leucine (6, 33). Because a high leucine concentration in the culture medium decreases the growth rate due to isoleucine restriction (39), the effect of leucine on *lysP* expression was analyzed by comparing the  $\beta$ -galactosidase activity of bacteria cultivated in glucose minimal medium supplemented with isoleucine and valine to that of cells grown under the same conditions but in the presence of isoleucine, valine, and leucine. We found that L-leucine had no effect on  $P_{lysP}$  activity in the  $\Delta lrp::Km^r$  mutant or in the *lrp*<sup>+</sup> strain (Fig. 8B). To confirm the activator role of Lrp in *lysP* transcription, the pBAD*lrp* plasmid (in which the *lrp* gene was cloned under the control of the arabinose promoter) (Table 1) was introduced into strain MG-LR15, and *lysP* expression levels were monitored before and after the addition of L-arabinose to the culture medium. Figure 8C clearly shows that the induction of *lrp* expression stimulated *lysP* transcription. Together, these results suggest that Lrp potentiates the activator effect of ArgP on *lysP* transcription in the absence of lysine.

Among the regulatory targets of Lrp, there are many transcription factors that participate in the regulation of amino acid metabolism and molecule transport (9). Therefore, it was important to determine whether the activator role of Lrp in *lysP* expression was direct or indirect. Peeters et al. have recently demonstrated that *argP* expression was not regulated by Lrp (35). Thus, EMSAs were performed in the presence of an excess of nonspecific competitor to assess the binding of purified His<sub>6</sub>-Lrp to the *lysP* promoter/control region (Fig. 9). One to four different retarded complexes were observed when various concentrations of Lrp were incubated with the 276-bp fragment encompassing the *lysP* promoter/control region ( $P_{lysP}$ ) (Fig. 9A). The apparent  $K_D$  obtained for the binding

TABLE 3. Identification of proteins after DNA affinity purification by peptide fingerprint analysis

Band <sup>a</sup>	Identified protein
1.....	HsdR; host restriction endonuclease R. Subunit of EcoKI restriction-modification system
2.....	HsdM; modification methyltransferase component of the EcoKI restriction-modification system
3.....	HsdS; specificity-determinant component of EcoKI restriction-modification system
4.....	NadR; transcriptional regulator
5.....	Nonidentified protein
6.....	Lrp; leucine-responsive protein

<sup>a</sup> The band numbers correspond to those shown in Fig. 7.



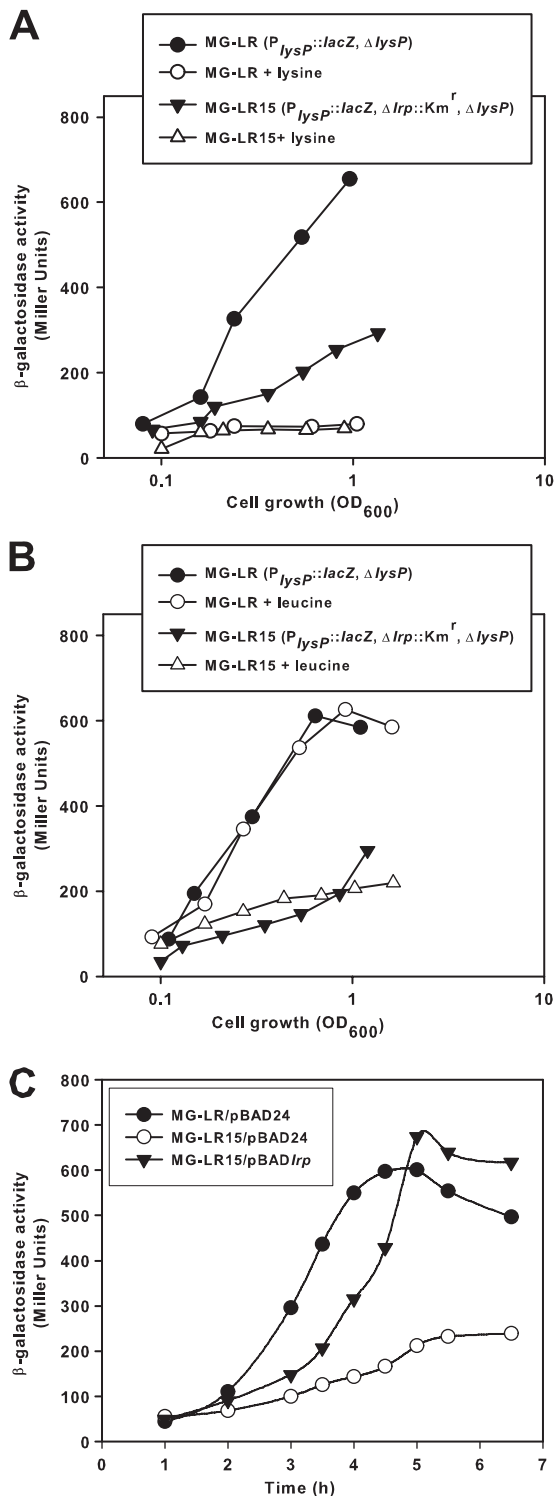


FIG. 8. Effect of *lrp* deletion on *lysP* expression. *E. coli* strains MG-LR ( $P_{lysP}::lacZ, \Delta lysP$ ) and MG-LR15 ( $P_{lysP}::lacZ, \Delta lysP, \Delta lrp::Km^r$ ) were grown aerobically in glucose minimal medium with or without the addition of 0.1 mM lysine (A). To test the effect of leucine on *lysP* expression, the growth medium was supplemented with 0.6 mM valine and 0.4 mM isoleucine, with or without the addition of 10 mM leucine (B). At different times during growth samples were analyzed for  $\beta$ -galactosidase activity. (C) Complementation of the MG-LR15 ( $P_{lysP}::lacZ, \Delta lysP, \Delta lrp::Km^r$ ) mutant with the pBAD*lrp* plasmid. Strains MG-LR/pBAD24, MG-LR15/pBAD24 and MG-LR15/pBAD*lrp* were

reaction was  $610 \pm 44$  nM (Fig. 9D), and the Hill coefficient was 3.

As previously reported for several Lrp-regulated genes, the concentration-dependent binding of Lrp to *lysP* suggests cooperative binding of Lrp dimers to multiple binding sites resulting in complexes with different stoichiometries. Since the *in vivo* experiments demonstrated that the presence of lysine affected *lysP* expression levels (Fig. 8A), we evaluated whether lysine affects the binding of Lrp to the *lysP* control region *in vitro*. As shown in Fig. 9C and D, the addition of L-lysine to the binding assay neither changed the Lrp affinity nor changed the binding pattern. When His<sub>6</sub>-Lrp was incubated with a DNA fragment encompassing the *lysP* coding sequence (control fragment), no retarded bands were observed (Fig. 9B), confirming the specific binding of Lrp to the *lysP* control region.

**Lrp modulates expression of the *cadBA* operon.** The Lrp regulon comprises several genes involved in amino acid synthesis and degradation. For example, expression of *ldcC*, coding for the constitutive lysine decarboxylase of *E. coli*, is down-regulated by Lrp (53). Considering the important role of LysP in regulating the expression of *cadA* (55), which encodes the inducible lysine decarboxylase, we analyzed the effect of Lrp on transcription of the *cadBA* operon, which encodes the inducible lysine decarboxylase CadA and the lysine/cadaverine antiporter CadB. For this purpose, two strains that carry chromosomal promoter-*lacZ* fusions ( $P_{cadBA}::lacZ$ ) and are either *lrp*<sup>+</sup> (MG-CR) or *lrp* null ( $\Delta lrp::Km^r$ , MG-CR15) were constructed. Cells were cultivated in glucose minimal medium at physiological (pH 7.6) or low (pH 5.8) pH, with or without the addition of 5 mM lysine, and  $\beta$ -galactosidase activities were determined. As expected, the  $P_{cadBA}$  promoter was active only in cells that were exposed to low external pH in the presence of lysine (Fig. 10A), a condition that is known to induce the Cad system (27). However, under inducing conditions, the  $P_{cadBA}$  activity in the  $\Delta lrp::Km^r$  mutant strain was >2-fold lower than that in the parent strain, suggesting that Lrp stimulates expression of *cadBA*.

To corroborate these results, the pET16*lrp* plasmid (Table 1) was introduced into the  $\Delta lrp::Km^r$  mutant strain and the  $P_{cadBA}$  activity in strains MG-CR/pET16b, MG-CR15/pET16b, and MG-CR15/pET16*lrp* was determined. The pET16b plasmid was chosen as an expression vector in this case, because the T7-driven promoter ensures very low expression levels in strain MG1655. The results presented in Fig. 10B reveal that the reintroduction of *lrp* into the  $\Delta lrp::Km^r$  mutant increases the  $P_{cadBA}$  activity to levels similar to those for the parent strain. It has been demonstrated that Lrp does not alter intracellular levels of CadC, the transcriptional activator of the *cadBA* operon (42). Therefore, the possibility of an indirect effect of Lrp via CadC can be discarded.

To evaluate the capacity of Lrp to bind to the  $P_{cadBA}$  pro-

grown in glucose minimal medium, and after 2.5 h 0.2% arabinose (wt/vol) was added to induce the expression of the *lrp* gene cloned in pBAD24.  $\beta$ -Galactosidase activities were determined in samples collected at different times, before and after induction. All experiments were performed in triplicates and mean values are presented. The standard deviations from the mean were less than 10%.

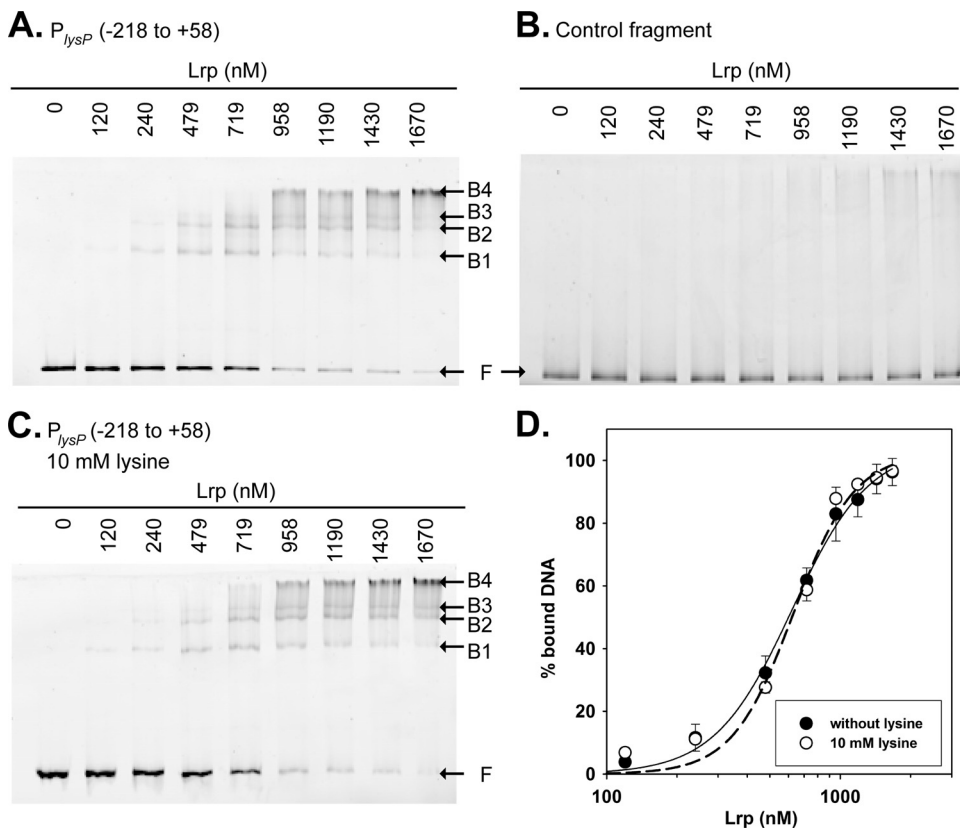


FIG. 9. Lrp binding to the *lysP* promoter region. (A and C) Electrophoretic mobility shift assays (EMSA) of the fluorescently labeled  $P_{lysP}$  fragment (positions  $-218/+58$ ) with increasing concentrations of purified His<sub>6</sub>-Lrp in the absence (A) or presence (C) of lysine. (B) EMSA performed with a DNA fragment within the *lysP* coding sequence as a control for unspecific binding. The positions of free DNA (F) and Lrp-DNA complexes (B1 to B4) are marked. (D) Binding profiles obtained after the quantification of free DNA and Lrp-bound DNA in EMSA gels were fitted using the Hill equation.

moter, EMSAs were performed with various concentrations of the purified His<sub>6</sub>-Lrp protein and a DNA fragment encompassing the  $P_{cadBA}$  control region. Accordingly, His<sub>6</sub>-Lrp is able to bind to the promoter that drives the expression of *cadBA* with an apparent dissociation constant ( $K_D$ ) of  $648 \pm 66$  nM and a Hill coefficient of 2.15 (Fig. 10C; see also Fig. S1 in the supplemental material). Together, these results indicate that Lrp upregulates expression of *cadBA*. With the identical functions of CadA and LdcC in amino acid catabolism taken into account, the opposite regulation of both genes by Lrp would be an efficient way for the cell to save energy.

**DISCUSSION**

**ArgP is responsible for the lysine-dependent control of *lysP* transcription.** While studying the mechanisms involved in the regulation of the Cad system (lysine decarboxylase system) in *E. coli*, Neely and Olson had shown that *lysP* transcription is controlled by the exogenous lysine concentration (32). Here, we identified the LysR-type transcriptional regulator ArgP as the regulator responsible for the control of *lysP* transcription. Our results indicate that under lysine-limiting growth conditions, ArgP functions as a transcriptional activator of *lysP* expression by binding to a sequence located between positions  $-91$  and  $-47$  in the *lysP* promoter/control region. Specifically,

a T-N<sub>11</sub>-A motif (nucleotides  $-65$  to  $-53$ ), characteristic for LTTR-dependent promoters (45), was identified. *In vitro* EMSAs as well as *in vivo* transcriptional studies indicated that this motif is crucial for ArgP binding. Deletion of the whole motif or substitution of the conserved nucleotides T and A prevented or reduced *lysP* induction in the absence of lysine. ArgP is a member of the LTTR protein family, which binds cofactors. Arginine and lysine were found to bind to ArgP (24). *In vitro* assays indicated that ArgP bound to the *lysP* promoter/control region irrespective of the presence of lysine and arginine, but lysine increased the affinity of ArgP by a factor of 2.

ArgP of *E. coli* is also responsible for the lysine-dependent regulation of *dapB*, which encodes one of the enzymes of the diaminopimelate and lysine biosynthesis pathway (2), and *argO*, which encodes the arginine exporter ArgO (24, 30). It is important to note that the molecular mechanisms of lysine-dependent regulation by ArgP differ between *argO* and *dapB* (2, 24). In the case of *dapB*, lysine prevents binding of ArgP to its binding site, which is located in the position  $-118/-81$  interval upstream of the transcriptional start site (2). In contrast, ArgP binds to a sequence between positions  $-85$  and  $-20$  of the *argO* operator/promoter and forms a stable binary complex in both its liganded and its unliganded forms. Arg-loaded ArgP binds to the *argO* promoter/control region and

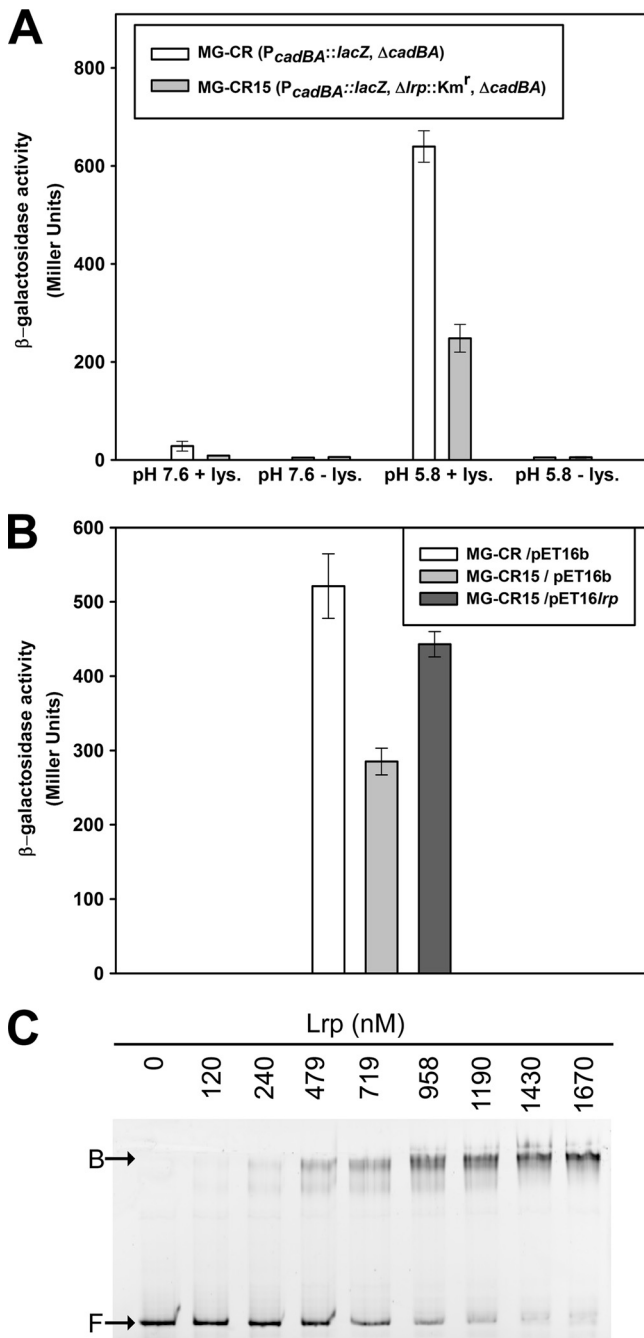


FIG. 10. Lrp stimulates *cadBA* expression. (A) Effect of *lrp* mutation on the expression of the *cadBA* operon under different growth conditions. Strains MG-CR ( $P_{cadBA}::lacZ \Delta cadBA$ ) and MG-CR15 ( $P_{cadBA}::lacZ \Delta lrp::Km^r \Delta cadBA$ ) were grown in glucose minimal medium at pH 7.6 or 5.8 with or without the addition of 5 mM lysine (lys.) under microaerobic conditions. After 7 h of incubation, samples were collected and  $\beta$ -galactosidase activities were determined. (B) Complementation of the  $\Delta lrp::Km^r$  mutant with the pET16lrp plasmid. Strains MG-CR/pET16b, MG-CR15/pET16b, and MG-CR15/pET16lrp were cultivated in glucose minimal medium (pH 5.8) with 5 mM lysine. After 7 h of incubation,  $\beta$ -galactosidase activities were determined. (C) Binding of His<sub>6</sub>-Lrp to the  $P_{cadBA}$  promoter/control region. A fragment encompassing the  $P_{cadBA}$  promoter/control region was incubated with increasing concentrations of purified His<sub>6</sub>-Lrp in the presence of salmon sperm DNA as a nonspecific competitor. The positions of free DNA (F) and the Lrp-DNA complex (B) are marked.

recruits the RNA polymerase, resulting in the induction of *argO*. Lys-loaded ArgP binds to the same sites, albeit with higher affinity, but restrains the polymerase in a molecular complex that is competent for neither productive nor abortive transcription.

Our results suggest that the mechanism for the ArgP-controlled *lysP* expression is similar to the one described for *argO*. Specifically, unloaded or Arg-loaded ArgP induces *lysP* expression, while the Lys-loaded form prevents expression. The lysine  $K_D$  value reported for ArgP is 70  $\mu$ M (24), which is in good agreement with the observed shutoff of the  $P_{lysP}::lacZ$  activity at an external lysine concentration higher than 25  $\mu$ M. In contrast to *argO*, external arginine seems not to be essential for transcription of *lysP* by ArgP. This difference might be related to the locations of the ArgP-binding sites within the promoter/control regions. The ArgP-binding site for *argO* extends up to nucleotide  $-20$  and thereby overlaps the  $-35$  promoter motif, whereas the ArgP-binding site for *lysP* extends up to position  $-47$ , which is upstream of the  $-35$  promoter site (Fig. 2). Nonetheless, an arginine effect on *lysP* expression was detectable when lysine and arginine were simultaneously added to the cultures. Under this condition, arginine overrode the inhibitory effect of lysine by a factor of 3. Laishram and Gowrishankar (24) demonstrated that arginine and lysine compete for the binding to dimeric ArgP. Therefore, the levels of *lysP* expression measured in cells that were grown in the presence of both amino acids could be attributable to the simultaneous existence of Arg-ArgP and Lys-ArgP complexes, which are effective or ineffective, respectively, in transcriptional activation.

In general, the results obtained in this work underline the importance of ArgP in the transcriptional control of genes involved in basic amino acid transport. ArgP seems to be a versatile regulator, able to control gene expression of various basic amino acid transporters by responding to low-molecular-weight effectors in order to maintain a balance in the intracellular concentration of at least lysine and arginine.

**Lrp-dependent regulation of *lysP* and *cadBA*.** The leucine-responsive protein (Lrp) was identified as another protein that specifically binds to a DNA sequence encompassing the *lysP* control region. Lrp has been designated a physiological barometer (14). Its main function is to control the expression of target genes and operons according to the nutritional status of the cell. Lrp upregulates genes during famine and downregulates genes during feast (6, 9). Most of the genes regulated by Lrp are involved in small-molecule transport and amino acid metabolism (9, 53). The amino acid L-leucine might act as a cofactor of Lrp and potentiates, overcomes, or has no effect on the function of Lrp upon its target genes (6, 33).

According to our results, Lrp potentiates the ArgP-mediated *lysP* expression when cells are cultivated in the absence of lysine, irrespective of L-leucine availability. Thus far, *lysP* has not been identified as a member of the Lrp regulon in the various genome-scale studies performed (9, 21, 53). While Lrp stimulated *lysP* expression in the absence of lysine, this global regulator did not alter the lysine-dependent repression of *lysP*. In addition, Lrp had a positive effect on the expression of the *cadBA* operon when cells were exposed to moderate acidic stress in the presence of lysine. A similar effect of Lrp has been reported for the *cadBA* operon of *Vibrio vulnificus* (42). Lrp binds cooperatively to the DNA at a degenerate consensus

sequence (11), but also with high affinity in a nonspecific manner (36). This last characteristic, together with the high abundance of Lrp in the cell and the fact that binding of Lrp to the DNA causes major conformational changes (13, 57), classifies Lrp as a nucleoid-associated protein (NAP) (14).

The results presented here demonstrate that Lrp has a stimulating effect on the expression of both *lysP* and *cadBA*. As Lrp directly interacts with the *lysP* and *cadBA* control regions in EMSAs performed with a high concentration of nonspecific competitor DNA, the possibility of an indirect effect can be discarded. We have shown that ArgP is the main regulator for *lysP*, and CadC is the major regulator for the *cadBA* operon (12, 58). These regulators activate expression of the corresponding target genes in response to lysine availability (ArgP for *lysP* and CadC via interaction with LysP for *cadBA*) (55, 58) and low pH (CadC) (58). In this scenario, Lrp would impose another level of regulation, adjusting the expression levels of *lysP* and *cadBA* in response to the physiological status of the cell.

The mechanisms employed by Lrp to regulate transcription vary and often involve interaction with other regulator proteins and NAPs. Recently, the implication of Lrp in the regulation of the gene encoding the arginine exporter (*argO*) was reported. It was shown that Lrp and ArgP behave as competitive activators able to activate *argO* expression under different conditions (35). On the other hand, expression of the *artPIQM* operon, one of the systems responsible for arginine uptake, is down-regulated by ArgR (5) and Lrp (21). Here, we found that Lrp binds to several sites at the *lysP* control region and is able to potentiate the transcriptional activation mediated by ArgP when lysine becomes limiting. Considering the *in vivo* and *in vitro* data presented in this work, the mechanism by which ArgP regulates *argO* transcription (24), and the known capacity of Lrp to alter the shape of the DNA by inducing bending and wrapping (57), a model for *lysP* regulation in which binding of Lrp to the *lysP* control region may favor and/or stabilize the ArgP-RNA polymerase-DNA complexes or introduce DNA conformational changes is conceivable.

Regarding *cadBA* transcriptional regulation, it is known that H-NS represses *cadBA* expression under noninducing conditions (46), and according to the current model, CadC binding dissolves the repressor complex formed by H-NS (22). The interplay between Lrp and other NAPs, in particular H-NS, in the regulation of several genes is well documented (10, 25, 38). According to the results described here, Lrp participates in the activation of *cadBA* under inducing conditions. It is conceivable that CadC and Lrp dissolve the repressor complex formed by H-NS in a joint action.

In conclusion, the three transporters for lysine (CadB, the LAO system, and LysP) in *E. coli* are produced under different conditions, and the corresponding genes are under the control of various regulators to meet diverse cellular needs. Expression of *cadB*, encoding the lysine/cadaverine antiporter CadB, is induced only at low pH and when external lysine is available to counteract acidic stress (27, 31). *hisJQMP*, encoding the histidine-binding protein and the ABC-type transporter of the LAO system, are repressed by Arg-loaded ArgR (4). In contrast, the arginine-ornithine-lysine binding protein which interacts with the same ABC transporter is induced under nitrogen-limiting conditions and controlled by NtrC (61), suggesting

that the LAO system serves as a scavenging system for nitrogen-rich amino acids under conditions of nitrogen starvation. As demonstrated here, *lysP* expression is induced under lysine limitation and requires ArgP and Lrp. Therefore, the main role of LysP seems to be the uptake of lysine for biosynthetic purposes. When lysine is sufficiently available, expression of *lysP* is shut off.

#### ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (JU270/5-3 and Exc114/1), the Humboldt Foundation (fellowship to J.R.), and the Elite Network of Bavaria (fellowship to I.H.). J.R. was a researcher on leave from CONICET.

We thank Tobias Kraxenberger and Britta Jungwirth for technical advice, Susanne Ude for constructing strains, and Luitpold Fried for MALDI-TOF measurements. We are grateful to Claude Gutierrez for generously providing *E. coli* strain JCP95.

#### REFERENCES

- Bachmann, B. J. 1996. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12, p. 2460–2488. In F. C. Neidhardt et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC.
- Bouvier, J., P. Stragier, V. Morales, E. Remy, and C. Gutierrez. 2008. Lysine represses transcription of the *Escherichia coli* *dapB* gene by preventing its activation by the ArgP activator. *J. Bacteriol.* **190**:5224–5229.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254.
- Caldara, M., et al. 2008. Arginine biosynthesis in *Escherichia coli*: experimental perturbation and mathematical modeling. *J. Biol. Chem.* **283**:6347–6358.
- Caldara, M., P. N. Minh, S. Bostoen, J. Massant, and D. Charlier. 2007. ArgR-dependent repression of arginine and histidine transport genes in *Escherichia coli* K-12. *J. Mol. Biol.* **373**:251–267.
- Calvo, J. M., and R. G. Matthews. 1994. The leucine-responsive regulatory protein, a global regulator of metabolism in *Escherichia coli*. *Microbiol. Rev.* **58**:466–490.
- Casadaban, M. J. 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. *J. Mol. Biol.* **104**:541–555.
- Celis, T. F., H. J. Rosenfeld, and W. K. Maas. 1973. Mutant of *Escherichia coli* K-12 defective in the transport of basic amino acids. *J. Bacteriol.* **116**:619–626.
- Cho, B. K., C. L. Barrett, E. M. Knight, Y. S. Park, and B. O. Palsson. 2008. Genome-scale reconstruction of the Lrp regulatory network in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* **105**:19462–19467.
- Corcoran, C. P., and C. J. Dorman. 2009. DNA relaxation-dependent phase biasing of the fim genetic switch in *Escherichia coli* depends on the interplay of H-NS, IHF and LRP. *Mol. Microbiol.* **74**:1071–1082.
- Cui, Y., Q. Wang, G. D. Stormo, and J. M. Calvo. 1995. A consensus sequence for binding of Lrp to DNA. *J. Bacteriol.* **177**:4872–4880.
- Dell, C. L., M. N. Neely, and E. R. Olson. 1994. Altered pH and lysine signalling mutants of *cadC*, a gene encoding a membrane-bound transcriptional activator of the *Escherichia coli* *cadBA* operon. *Mol. Microbiol.* **14**:7–16.
- de los Rios, S., and J. J. Perona. 2007. Structure of the *Escherichia coli* leucine-responsive regulatory protein Lrp reveals a novel octameric assembly. *J. Mol. Biol.* **366**:1589–1602.
- Dillon, S. C., and C. J. Dorman. 2010. Bacterial nucleoid-associated proteins, nucleoid structure and gene expression. *Nat. Rev. Microbiol.* **8**:185–195.
- Epstein, W., and B. S. Kim. 1971. Potassium transport loci in *Escherichia coli* K-12. *J. Bacteriol.* **108**:639–644.
- Fujii, T., Y. Aritoku, H. Agematu, and H. Tsunekawa. 2002. Increase in the rate of L-pipecolic acid production using lat-expressing *Escherichia coli* by *lysP* and *yetE* amplification. *Biosci. Biotechnol. Biochem.* **66**:1981–1984.
- Gabrielsen, O. S., E. Hornes, L. Korsnes, A. Ruet, and T. B. Oyen. 1989. Magnetic DNA affinity purification of yeast transcription factor  $\tau$ -a new purification principle for the ultrarapid isolation of near homogeneous factor. *Nucleic Acids Res.* **17**:6253–6267.
- Guzman, L. M., D. Belin, M. J. Carson, and J. Beckwith. 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J. Bacteriol.* **177**:4121–4130.
- Heermann, R., T. Zeppenfeld, and K. Jung. 2008. Simple generation of site-directed point mutations in the *Escherichia coli* chromosome using Red(R)/ET(R) recombination. *Microb. Cell Fact.* **7**:14.
- Henzel, W. J., et al. 1993. Identifying proteins from two-dimensional gels by



- molecular mass searching of peptide fragments in protein sequence databases. *Proc. Natl. Acad. Sci. U. S. A.* **90**:5011–5015.
21. **Hung, S. P., P. Baldi, and G. W. Hatfield.** 2002. Global gene expression profiling in *Escherichia coli* K12. The effects of leucine-responsive regulatory protein. *J. Biol. Chem.* **277**:40309–40323.
  22. **Küper, C., and K. Jung.** 2005. CadC-mediated activation of the cadBA promoter in *Escherichia coli*. *J. Mol. Microbiol. Biotechnol.* **10**:26–39.
  23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680–685.
  24. **Laishram, R. S., and J. Gowrishankar.** 2007. Environmental regulation operating at the promoter clearance step of bacterial transcription. *Genes Dev.* **21**:1258–1272.
  25. **Levinthal, M., P. Lejeune, and A. Danchin.** 1994. The H-NS protein modulates the activation of the *ilvIH* operon of *Escherichia coli* K12 by Lrp, the leucine regulatory protein. *Mol. Gen. Genet.* **242**:736–743.
  26. **Meng, S. Y., and G. N. Bennett.** 1992. Nucleotide sequence of the *Escherichia coli* *cad* operon: a system for neutralization of low extracellular pH. *J. Bacteriol.* **174**:2659–2669.
  27. **Meng, S. Y., and G. N. Bennett.** 1992. Regulation of the *Escherichia coli* *cad* operon: location of a site required for acid induction. *J. Bacteriol.* **174**:2670–2678.
  28. **Miller, J. H.** 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
  29. **Moore, J. T., A. Uppal, F. Maley, and G. F. Maley.** 1993. Overcoming inclusion body formation in a high-level expression system. *Protein Expr. Purif.* **4**:160–163.
  30. **Nandineni, M. R., and J. Gowrishankar.** 2004. Evidence for an arginine exporter encoded by *yggA* (*argO*) that is regulated by the LysR-type transcriptional regulator ArgP in *Escherichia coli*. *J. Bacteriol.* **186**:3539–3546.
  31. **Neely, M. N., C. L. Dell, and E. R. Olson.** 1994. Roles of LysP and CadC in mediating the lysine requirement for acid induction of the *Escherichia coli* *cad* operon. *J. Bacteriol.* **176**:3278–3285.
  32. **Neely, M. N., and E. R. Olson.** 1996. Kinetics of expression of the *Escherichia coli* *cad* operon as a function of pH and lysine. *J. Bacteriol.* **178**:5522–5528.
  33. **Newman, E. B., and R. Lin.** 1995. Leucine-responsive regulatory protein: a global regulator of gene expression in *E. coli*. *Annu. Rev. Microbiol.* **49**:747–775.
  34. **Patte, J. C., M. Akrim, and V. Mejean.** 1998. The leader sequence of the *Escherichia coli* *lysC* gene is involved in the regulation of LysC synthesis. *FEMS Microbiol. Lett.* **169**:165–170.
  35. **Peeters, E., P. Nguyen Le Minh, M. Foulquie-Moreno, and D. Charlier.** 2009. Competitive activation of the *Escherichia coli* *argO* gene coding for an arginine exporter by the transcriptional regulators Lrp and ArgP. *Mol. Microbiol.* **74**:1513–1526.
  36. **Peterson, S. N., F. W. Dahlquist, and N. O. Reich.** 2007. The role of high affinity non-specific DNA binding by Lrp in transcriptional regulation and DNA organization. *J. Mol. Biol.* **369**:1307–1317.
  37. **Popkin, P. S., and W. K. Maas.** 1980. *Escherichia coli* regulatory mutation affecting lysine transport and lysine decarboxylase. *J. Bacteriol.* **141**:485–492.
  38. **Pul, U., R. Wurm, and R. Wagner.** 2007. The role of LRP and H-NS in transcription regulation: involvement of synergism, allostery and macromolecular crowding. *J. Mol. Biol.* **366**:900–915.
  39. **Quay, S. C., T. E. Dick, and D. L. Oxender.** 1977. Role of transport systems in amino acid metabolism: leucine toxicity and the branched-chain amino acid transport systems. *J. Bacteriol.* **129**:1257–1265.
  40. **Reitzer, L.** 5 July 2005, posting date. Chapter 3.4.7, Catabolism of amino acids and related compounds. In R. Curtiss III (ed.), *EcoSal—Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC. doi:10.1128/ecosal.3.6.1.3.
  41. **Rey, D. A., A. Puhler, and J. Kalinowski.** 2003. The putative transcriptional repressor McbR, member of the TetR-family, is involved in the regulation of the metabolic network directing the synthesis of sulfur containing amino acids in *Corynebacterium glutamicum*. *J. Biotechnol.* **103**:51–65.
  42. **Rhee, J. E., K. S. Kim, and S. H. Choi.** 2008. Activation of the *Vibrio vulnificus* *cadBA* operon by leucine-responsive regulatory protein is mediated by CadC. *J. Microbiol. Biotechnol.* **18**:1755–1761.
  43. **Rodionov, D. A., A. G. Vitreschak, A. A. Mironov, and M. S. Gelfand.** 2003. Regulation of lysine biosynthesis and transport genes in bacteria: yet another RNA riboswitch? *Nucleic Acids Res.* **31**:6748–6757.
  44. **Sandaltzopoulos, R., and P. B. Becker.** 1994. Solid phase DNase I footprinting: quick and versatile. *Nucleic Acids Res.* **22**:1511–1512.
  45. **Schell, M. A.** 1993. Molecular biology of the LysR family of transcriptional regulators. *Annu. Rev. Microbiol.* **47**:597–626.
  46. **Shi, X., B. C. Waasdorp, and G. N. Bennett.** 1993. Modulation of acid-induced amino acid decarboxylase gene expression by *hns* in *Escherichia coli*. *J. Bacteriol.* **175**:1182–1186.
  47. **Simons, R. W., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. *Gene* **53**:85–96.
  48. **Soksawatmaekhin, W., A. Kuraishi, K. Sakata, K. Kashiwagi, and K. Igarashi.** 2004. Excretion and uptake of cadaverine by CadB and its physiological functions in *Escherichia coli*. *Mol. Microbiol.* **51**:1401–1412.
  49. **Steffes, C., J. Ellis, J. Wu, and B. P. Rosen.** 1992. The *lysP* gene encodes the lysine-specific permease. *J. Bacteriol.* **174**:3242–3249.
  50. **Stragier, P., F. Richaud, F. Borne, and J. C. Patte.** 1983. Regulation of diaminopimelate decarboxylase synthesis in *Escherichia coli*. I. Identification of a *lysR* gene encoding an activator of the *lysA* gene. *J. Mol. Biol.* **168**:307–320.
  51. **Studier, F. W., and B. A. Moffatt.** 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**:113–130.
  52. **Sudarsan, N., J. K. Wickiser, S. Nakamura, M. S. Ebert, and R. R. Breaker.** 2003. An mRNA structure in bacteria that controls gene expression by binding lysine. *Genes Dev.* **17**:2688–2697.
  53. **Tani, T. H., A. Khodursky, R. M. Blumenthal, P. O. Brown, and R. G. Matthews.** 2002. Adaptation to famine: a family of stationary-phase genes revealed by microarray analysis. *Proc. Natl. Acad. Sci. U. S. A.* **99**:13471–13476.
  54. **Tetsch, L., and K. Jung.** 2009. The regulatory interplay between membrane-integrated sensors and transport proteins in bacteria. *Mol. Microbiol.* **73**:982–991.
  55. **Tetsch, L., C. Koller, I. Haneburger, and K. Jung.** 2008. The membrane-integrated transcriptional activator CadC of *Escherichia coli* senses lysine indirectly via the interaction with the lysine permease LysP. *Mol. Microbiol.* **67**:570–583.
  56. **Thomason, L. C., N. Costantino, and D. L. Court.** 2007. *E. coli* genome manipulation by P1 transduction. *Curr. Protoc. Mol. Biol.* **79**:1.17.1–1.17.8.
  57. **Wang, Q., and J. M. Calvo.** 1993. Lrp, a major regulatory protein in *Escherichia coli*, bends DNA and can organize the assembly of a higher-order nucleoprotein structure. *EMBO J.* **12**:2495–2501.
  58. **Watson, N., D. S. Dunyak, E. L. Rosey, J. L. Slonczewski, and E. R. Olson.** 1992. Identification of elements involved in transcriptional regulation of the *Escherichia coli* *cad* operon by external pH. *J. Bacteriol.* **174**:530–540.
  59. **Weber, A., S. A. Kogl, and K. Jung.** 2006. Time-dependent proteome alterations under osmotic stress during aerobic and anaerobic growth in *Escherichia coli*. *J. Bacteriol.* **188**:7165–7175.
  60. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**:103–119.
  61. **Zimmer, D. P., et al.** 2000. Nitrogen regulatory protein C-controlled genes of *Escherichia coli*: scavenging as a defense against nitrogen limitation. *Proc. Natl. Acad. Sci. U. S. A.* **97**:14674–14679.