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Role of ArgP (IciA) in Lysine-Mediated Repression in *Escherichia coli*[▽]

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Initially identified as an inhibitor of *oriC*-initiated DNA replication *in vitro*, the ArgP or IciA protein of *Escherichia coli* has subsequently been described as a nucleoid-associated protein and also as a transcriptional regulator of genes involved in DNA replication (*dnaA* and *nrdA*) and amino acid metabolism (*argO*, *dapB*, and *gdhA* [the last in *Klebsiella pneumoniae*]). ArgP mediates lysine (Lys) repression of *argO*, *dapB*, and *gdhA in vivo*, for which two alternative mechanisms have been identified: at the *dapB* and *gdhA* regulatory regions, ArgP binding is reduced upon the addition of Lys, whereas at *argO*, RNA polymerase is trapped at the step of promoter clearance by Lys-bound ArgP. In this study, we have examined promoter-*lac* fusions in strains that were *argP*$^+$ or Δ*argP* or that were carrying dominant *argP* mutations in order to identify several new genes that are ArgP-regulated *in vivo*, including *lysP*, *lysC*, *lysA*, *dapD*, and *asd* (in addition to *argO*, *dapB*, and *gdhA*). All were repressed upon Lys supplementation, and *in vitro* studies demonstrated that ArgP binds to the corresponding regulatory regions in a Lys-sensitive manner (with the exception of *argO*, whose binding to ArgP was Lys insensitive). Neither *dnaA* nor *nrdA* was ArgP regulated *in vivo*, although their regulatory regions exhibited low-affinity binding to ArgP. Our results suggest that ArgP is a transcriptional regulator for Lys repression of genes in *E. coli* but that it is noncanonical in that it also exhibits low-affinity binding, without apparent direct regulatory effect, to a number of additional sites in the genome.

The functional role of the ArgP or IciA protein in *Escherichia coli* is an enigma in that it has been variously described as a canonical transcriptional activator, an inhibitor of chromosome replication initiation, and a nucleoid-associated protein. First identified as a protein that binds specific sequences in *oriC in vitro* to inhibit the initiation of replication (21–23, 46), ArgP was shown (46) to be a member of the family of LysR-type transcriptional regulators (LTTRs) and, subsequently, to be essential both *in vivo* and *in vitro* for transcription of the gene encoding the L-arginine (Arg) exporter ArgO (26, 32); in *Corynebacterium glutamicum*, LysG and LysE are orthologous to *E. coli* ArgP and ArgO, respectively, and LysG is required for LysE transcription (5). ArgP-regulated transcription *in vivo* has also been demonstrated for the genes *dapB* of *E. coli* (7) and *gdhA* of *Klebsiella pneumoniae* (15). (That *E. coli gdhA* may be under ArgP control had also been suggested earlier [33].) In addition, *in vitro* studies have suggested that ArgP activates the transcription of the *dnaA* and *nrdA* genes that are involved in DNA metabolism and replication (20, 27–29).

At the same time, ArgP has also been reported as a nucleoid-associated protein that shows apparently sequence-nonspecific DNA binding activity (2). The protein exhibits affinity for AT-rich and curved DNA sequences, as determined *in vitro* by DNase I footprinting or electrophoretic mobility shift assays (EMSAs) (2, 48). ArgP exists as a dimer at an estimated concentration of around 100 to 400 molecules per cell (that is, around 200 to 800 nM monomers) (3, 23).

As mentioned above, one of the well-characterized targets for transcriptional regulation by ArgP is the *argO* gene (26, 32, 37). Transcription of *argO in vivo* is activated both by Arg and its toxic analog L-canavanine, both effects being mediated by ArgP. On the other hand, *argO* transcription is drastically reduced in medium supplemented with L-lysine (Lys), to a level equivalent to that observed in Δ*argP* mutants; when both Arg and Lys are present, it is the Lys effect on *argO* which predominates. Dominant mutations in *argP* have been identified (designated *argP*^{d}) that confer an L-canavanine-resistant phenotype and act in *trans* to considerably increase *argO* transcription *in vivo* over that obtained in the *argP*$^+$ strain (32).

In vitro, the ArgP protein exhibits high-affinity binding to the *argO* regulatory region in the presence of either coeffector, Arg or Lys, as well as in their absence (7, 26, 37). Stable recruitment by ArgP of RNA polymerase to the *argO* promoter is observed in the presence of Arg or Lys, following which the two coeffectors exert dramatically opposite effects in the ternary complex; whereas productive transcription from the *argO* promoter is stimulated in the presence of ArgP and Arg, RNA polymerase is trapped at the promoter at a step after open complex formation in the presence of ArgP and Lys (26).

As with *argO*, in other ArgP-regulated promoters, such as *dapB* of *E. coli* and *gdhA* of *K. pneumoniae*, Lys supplementation is associated with a decrease in transcription *in vivo*. In these cases, however, the addition of Lys results in a decreased affinity of ArgP to bind to the corresponding regulatory regions *in vitro*, suggesting that it is the failure of RNA polymerase recruitment at the promoters which is responsible for Lys repression (7, 15).

In this study, we have used the Δ*argP* and *argP*^{d} mutations to examine ArgP's role in the regulation *in vivo* of a number of genes in *E. coli* that were chosen on the basis of either their identification in the earlier reports or their roles in Lys and Arg metabolism. EMSA experiments to determine ArgP binding with the upstream regulatory regions of these genes *in vitro*

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TABLE 1. List of *E. coli* K-12 strains

Strain[a]	Genotype[b]
MC4100	Δ(*argF-lac*)*U169 rpsL150 relA1 araD139 flbB5301 deoC1 ptsF25*
GJ4748	MC4100 *argR64 zhb-914*::Tn*10*dCm
GJ9602	MC4100 Δ*argP*
GJ9623	MC4100 Δ*lysP*
GJ9624	MC4100 Δ*argP* Δ*lysP*
GJ9647	MC4100 Δ*cadC*::Kan
GJ9648	MC4100 Δ*argP* Δ*cadC*::Kan
GJ9649	MC4100 Δ*lysP* Δ*cadC*::Kan
GJ9650	MC4100 *araD*$^+$
GJ9651	MC4100 *araD*$^+$ Δ*argP*
GJ9652	MC4100 *araD*$^+$ Δ*lysR*::Kan
GJ9653	MC4100 *araD*$^+$ Δ*argP* Δ*lysR*::Kan

[a] Strains described earlier (32) include MC4100 and GJ4748. All other strains were constructed in this study.

[b] Genotype designations are as described previously (6). All strains are F$^-$. The Δ*argP*, Δ*lysP*, Δ*lysR*, and Δ*cadC* alleles were introduced as Kan^r deletion-insertion mutations from the Keio knockout collection (4), and where necessary, the Kan^r marker was then excised by site-specific recombination with the aid of plasmid pCP20, as described previously (14). The latter mutations are shown without the Kan^r designation in the table.

were also undertaken. Our results indicate that negative regulation involving Lys as coeffector is mediated by ArgP and that the target genes include *argO*, *lysP*, *lysC*, *asd*, *dapB*, *dapD*, *lysA*, and *gdhA*. Of the genes listed above, *argO* was unique in several respects, including its responsiveness to Arg, ArgP binding even in the presence of Lys, and behavior with the *argP*^{d} mutations. Other genes, such as *dnaA* and *nrdA*, did exhibit binding to ArgP *in vitro* (that was Lys insensitive) but were not regulated by ArgP *in vivo*. These findings implicate a role for ArgP in Lys metabolism and homeostasis in *E. coli*.

MATERIALS AND METHODS

Growth media, bacterial strains, and plasmids. The routine defined and rich growth media were, respectively, glucose-minimal A (with amino acids supplemented at 40 μg/ml or otherwise as indicated below) and LB medium, as described previously (31), and the growth temperature was 37°C. The proportion of acid and basic phosphate in minimal A medium was suitably adjusted (42) to obtain a growth medium of pH 5.8. Ampicillin (Amp), kanamycin (Kan), and spectinomycin (Sp) were each used at 50 μg/ml, and trimethoprim (Tp) at 30 μg/ml. L-Arabinose (Ara) supplementation was at 0.2%.

The *E. coli* K-12 strains used in the study are listed in Table 1. Plasmids previously described include the following (salient features are in parentheses): pBAD18 (pMB9 replicon, Amp^r, for Ara-induced expression of target genes) (19), pMU575 (IncW single-copy-number replicon with promoterless *lacZ* gene, Tp^r) (1), pCP20 (pSC101-based Ts replicon encoding Flp recombinase, Amp^r) (14), and pHYD1723 (pMU575 derivative with 402-bp *argO* promoter fragment upstream of *lacZ*, Tp^r) (26). Plasmids bearing *argP*^{d} alleles encoding one of the ArgP variants A68V, S94L, P108S, V144M, P217L, or R295C and the corresponding *argP*$^+$ (pHYD915) and vector (pCL1920) controls (pSC101 replicons, Sp^r) were as described in Nandineni and Gowrishankar (32).

The promoter-*lac* fusion derivatives that were constructed using plasmid pMU575 in this study are described in Table 2. Plasmid pHYD2673 is a pBAD18 derivative carrying the *lysA*$^+$ gene from *E. coli* (genomic coordinates 2975628 to 2976968 [40]) cloned downstream of P_{ara} of the vector; the gene was PCR amplified from genomic DNA with the primer pairs 5′-ACAA*GGTACC*TTTT ATGATGTGGCGT-3′ and 5′-ACAA*TCTAGA*AGTCATCATGCAACC (KpnI and XbaI sites, respectively, are italicized in the two primers), and the KpnI-XbaI-digested product was cloned into the corresponding sites of pBAD18. The construction of plasmid pHYD2606 encoding the ArgP^d P274S variant is described below.

Construction of mutant encoding ArgP(P274S). The *argP*^{d}*(P274S)* mutant had previously been described by Celis (10) as one exhibiting dominant L-canavanine resistance but by a proposed mechanism different from that involving activation of *argO* transcription. Since all the other *argP*^{d} mutations confer L-canavanine resistance by the latter mechanism alone (32), we sought to construct and test the P274S variant of ArgP in this study.

TABLE 2. List of pMU575-derived promoter-*lac* fusion plasmids constructed in this study[a]

Plasmid	Promoter [fragment size (bp)]	Primer 1 (enzyme)	Primer 2 (enzyme)	Extent (genomic coordinates)[b]
pHYD2602	*gdhA* (435)	5′-ATTTTGATC*CTGCAG*AACGCAGCACTG-3′ (P)	5′-GTTTGATTC*GGATCC*GCTTTTGGACATG-3′ (B)	−322 to +113 (1840009–1840443)
pHYD2610	*dapD* (373)	5′-CGCCATTTTA*CTGCAG*AAACCGAAG-3′ (P)	5′-CGGGTAAC*GGATCC*TGCATTGGCT-3′ (B)	−273 to +100 (185877–186249)
pHYD2636	*lysP* (238)	5′-GCGCTTT*CTGCAG*TATTGCGATCC-3′ (P)	5′-TAGTTTC*GGATCC*CATACAAAAATGC-3′ (B)	−206 to +32 (2246550–2246787)
pHYD2647	*lysP* (158)	5′-ACAAC*TGCAG*TTCGCCAGAAAA-3′ (P)	5′-TAGTTTC*GGATCC*CATACAAAAATGC-3′ (B)	−114 to +32 (2246538–2246695)
pHYD2648	*lysP* (120)	5′-ACAAC*TGCAG*CTGGGCGATCAT-3′ (P)	5′-TAGTTTC*GGATCC*CATACAAAAATGC-3′ (B)	−76 to +32 (2246538–2246657)
pHYD2658	*artJ* (416)	5′-ACAAC*TGCAG*CAAAGCGCTGGCA-3′ (P)	5′-ACAA*GGATCC*GGATAGGTGGCTGAAA-3′ (B)	−270 to +146 (899703–900118)
pHYD2659	*artP* (288)	5′-ACAACTGCAGGAATCGCTAACGCC-3′ (P)	5′-ACAAGGATCCTATCGAACAGCGCC-3′ (B)	−192 to +96 (902896–903183)
pHYD2660	*argT* (367)	5′-ACAAC*TGCAG*ATCTCTTTGCCCGC-3′ (P)	5′-ACAA*GGATCC*GTAGCGCCGCATA-3′ (B)	−236 to +131 (2425740–2426106)
pHYD2661	*hisJ* (306)	5′-ACAAC*TGCAG*TGTCTACGGTGACTG-3′ (P)	5′-ACAA*GGATCC*TCGCAGCAAACG-3′ (B)	−187 to +119 (2424740–2425045)
pHYD2664	*lysC* (615)	5′-ACAAC*TGCAG*GTCTGCGTTGGATT-3′ (P)	5′-ACAA*TCTAGA*CGGTTCATGGCGT-3′ (X)	−244 to +371 (4231191–4231805)
pHYD2668	*asd* (343)	5′-GTATGTTTCAGT*GTCGAC*ATGAAAATAG-3′ (S)	5′-GGCGTCG*AAGCTT*CGCTCTTCAACCA-3′ (H)	−207 to +136 (3572827–3573169)
pHYD2669	*dapB* (386)	5′-CCCTGTTTTGC*TGCAG*TGGAAAC-3′ (P)	5′-AATGCCAGC*GGATCC*TGAATCAAC-3′ (B)	−285 to +101 (28057–28442)
pHYD2670	*lysA* (268)	5′-ACAAC*TGCAG*CCTCAGTCAGGCTTC-3′ (P)	5′-ACAA*GGATCC*CCCAGCCAAATTCAGC-3′ (B)	−139 to +129 (2976844–2977111)
pHYD2671	*dnaA* (668)	5′-ACAAC*TGCAG*TTTCATGGCGATT-3′ (P)	5′-ACAA*GGATCC*GCTGGTAACTCAT-3′ (B)	−460 to +208 (3881696–3882363)
pHYD2672	*nrdA* (481)	5′-ACAAC*TGCAG*CGCCATCAACAAT-3′ (P)	5′-ACAA*GGATCC*GTCGAGATTGATGC-3′ (B)	−311 to +170 (2342465–2342945)
pHYD2674	*cadB* (527)	5′-TTTAATTTACC*TGCAG*GGGGCAAAC-3′ (P)	5′-TGCAATACC*GGATCC*CCATCATATTA-3′ (B)	−387 to +140 (4357989–4358515)
pHYD2675	*lysR* (268)	5′-ACAAC*TGCAG*CAGCCAAATTCAGC-3′ (P)	5′-ACAA*GGATCC*CCCTCAGTCAGGCTTC-3′ (B)	−127 to +141 (2976844–2977111)

[a] Provided in the table for each *lac* fusion plasmid is information on primers used for PCR amplification of the indicated promoter fragment and the enzymes (B, BamHI; H, HindIII; P, PstI; S, SalI; and X, XbaI) used for cloning into plasmid vector pMU575 (1); the corresponding restriction sites in the primers are italicized.

[b] The extent of each cloned insert region, relative to the start site of transcription most proximal to the coding region of the gene, is given; genomic coordinates for the cloned regions are from Rudd (40).

Site-directed mutagenesis of the *argP*$^+$ gene on plasmid pHYD915 to *argP*(*P274S*) was done with the aid of the QuikChange kit and associated protocol (Stratagene, La Jolla, CA), using the complementary primer pairs 5′-GC ACCGCTTTGCT*T*CTGAAAGCCGCAT-3′ and 5′-ATGCGGCTTTCAG*A*AG CAAAGCGGTGC-3′ (nucleotide substitutions are italicized). The resultant plasmid, designated pHYD2606, conferred dominant L-canavanine resistance in an *argO*$^+$ strain but not in a Δ*argO* mutant (data not shown), confirming that this ArgP variant also works through ArgO in exhibiting its phenotype.

Microarray expression profiling. For microarray expression profiling experiments, RNA preparations were made from the following pair of strains after growth to mid-exponential phase in glucose-minimal A medium with 1 mM Arg (relevant genotypes are in parentheses): (i) strain MC4100 carrying the plasmid pHYD926 encoding ArgP^d(S94L) (*argP*$^+$/*argP*^{d}); and (ii) strain GJ9602 (Δ*argP*). The microarray data were generated at an outsourced facility (Genotypic Technology Pvt. Ltd., Bengaluru, India).

Other methods. The protocols for EMSA experiments with purified ArgP protein bearing a C-terminal His_6 tag (His_6-ArgP) have been described earlier (26), and the reactions were performed in EMSA binding buffer of the following composition: 10 mM Tris-Cl at pH 7.5, 1 mM EDTA, 50 mM NaCl, 5 mM dithiothreitol, and 5% glycerol. The DNA templates were obtained by PCR from *E. coli* genomic DNA with the various primer pairs listed in Table 2 and one additional control template (295-bp fragment from the *lacZ* locus, genomic coordinates 364776 to 365070 [40]) with the primer pair 5′-GTGGTGCAACG GGCGCTGGGTCGGTTAC-3′ and 5′-CAACTCGCCGCACATCTGAACTT CAG-3′; after 5′-end labeling, the PCR fragments were purified by electroelution following electrophoresis on 6% polyacrylamide gels (42).

Tolerance or sensitivity to L-canavanine was tested by the method given in Nandineni et al. (33), while resistance to thialysine was scored with 200-μg/ml supplementation in glucose-minimal A medium. Procedures for P1 transduction (31) and for PCR, *in vitro* DNA manipulations, and transformation (42) were as described previously. β-Galactosidase assays were performed by the method of Miller (31); each value reported is the average of at least three independent experiments, and the standard error was <10% of the mean in all cases.

RESULTS

Microarray profiling to identify candidate genes regulated by ArgP *in vivo*. We initially undertook a microarray-based comparison of whole-genome mRNA abundances between an isogenic pair of strains that was either Δ*argP* (GJ9602) or expressed the dominant constitutively acting S94L variant of the ArgP protein in the *argP*$^+$ strain (MC4100/pHYD926), which had been grown to exponential phase in Arg-supplemented glucose-minimal A medium. At the time that these experiments were done, the genes known to be ArgP regulated *in vivo* included the following (gene functions are indicated in parentheses): *argO* (Arg export), *dapB* (dihydrodipicolinate reductase in the diaminopimelate-Lys biosynthetic pathway), and *K. pneumoniae gdhA* (glutamate dehydrogenase for NH_4^+ assimilation and glutamate biosynthesis). All three genes are repressed upon growth in Lys-supplemented medium (7, 15, 32).

In the microarray expression experiments between the Δ*argP* and *argP*^{d}(*S94L*) strains, the mRNA abundances of all three genes listed above exceeded the $\log_2$ difference threshold of 1.0, with values of 2.3, 2.7, and 1.3, respectively. Other genes implicated in Lys metabolism (36) that crossed this threshold were (gene function and $\log_2$-fold difference are in parentheses) *lysP* (Lys permease, 2.3), *lysC* (Lys-sensitive aspartokinase, 1.9), and *asd* (aspartate semialdehyde dehydrogenase, 1.1).

Accordingly, the *E. coli* genes *argO*, *dapB*, *gdhA*, *lysP*, *lysC*, and *asd* were chosen for further analysis of *in vivo* regulation by the ArgP protein and its constitutive variants. Two additional genes, *lysA* (diaminopimelate decarboxylase) and *dapD* (tetrahydrodipicolinate synthase), were also included for the study, since both are repressed by Lys, as with the known ArgP-regulated genes (36).

Promoter-*lac* fusion studies in *argP*$^+$ and *argP* mutants. For each of the genes *dapB*, *gdhA*, *lysP*, *lysC*, *asd*, *lysA* and *dapD*, the upstream *cis* regulatory region was PCR amplified and cloned into pMU575, which is a single-copy-number IncW replicon plasmid vector for generating promoter-*lacZ* transcriptional fusions (1). The equivalent *argO-lac* fusion derivative of pMU575 (pHYD1723) has been described earlier (26).

The eight *lac* fusion constructs were then introduced into a Δ*argP* strain carrying either *argP*$^+$ or any of the constitutive *argP* mutant alleles on the pSC101-based plasmid vector pCL1920; similar derivatives of Δ*argP* with vector pCL1920 alone served as the negative controls in these experiments. β-Galactosidase assays were performed for cultures of the strains grown in medium without or with supplementation with Arg or Lys at 1 mM. The data from these experiments, presented in Table 3, permitted the following interpretations.

(i) Transcription from promoters of the genes *gdhA*, *asd*, *dapB*, *dapD*, and *lysP* in cultures of the *argP*$^+$ strains grown in medium not supplemented with Arg or Lys is higher than that in the corresponding Δ*argP* derivatives, by factors of approximately 4, 2.5, 23, 4, and 35, respectively. Arg supplementation was without effect for any of these genes.

(ii) All the genes listed above also exhibited repression upon Lys supplementation in the *argP*$^+$ but not the Δ*argP* strain, suggesting that Lys repression of their promoters is ArgP mediated. (iii) As has also been reported earlier (26, 32, 37), *argO-lac* expression was stimulated 4-fold in the *argP*$^+$ strain relative to that in Δ*argP* but only in Arg-supplemented medium. Lys supplementation was associated with repression only in the *argP*$^+$ strain.

(iv) The constitutive *argP* mutations that were used in this study had been selected to confer L-canavanine resistance (that is, for enhanced expression of ArgO, which is the exporter of Arg and L-canavanine) (10, 32); as expected, all of them displayed much higher levels of *argO-lac* expression than the *argP*$^+$ strain without or with Arg or Lys supplementation. The P274S variant in particular was the most effective for constitutive *argO* expression (with a 270-fold degree of activation).

(v) In the case of the other ArgP-regulated genes discussed above, many of the *argP* constitutive mutations behaved much like the *argP*$^+$ allele itself for both activation and Lys repression, although a few combinations exhibited differences; for example, the levels of activation obtained with the P108S variant at *gdhA* (7-fold) and *lysC* (5-fold) were higher than that with *argP*$^+$ at these promoters, but P108S was also less effective for Lys repression of *lysP*, and both V144M and P217L were ineffective for activation of *asd* and *dapD*. However, the most conspicuous discrepancy was that seen with the P274S variant; although it was the most proficient of all the ArgP^d mutants for constitutive *argO* expression, this variant was among the least effective for activation of expression from all the other ArgP-regulated promoters and, indeed, behaved like Δ*argP* at several of them (*gdhA*, *asd*, *dapD*, and *lysC*).

(vi) For *lysC*, there was a 5-fold repression by Lys in the Δ*argP* strain (that is, ArgP independent); this presumably represents the regulation imposed by the Lys-responsive riboswitch that is proposed to exist in the leader region of the *lysC* mRNA (39, 44). However, the *argP*$^+$ strain displayed 4-fold

TABLE 3. Expression of *lac* fusions in different *argP* variant derivatives[a]

ArgP variant	Expression shown as β-Gal sp act (Miller units)[b] or indicated ratio														
	argO					*argT*					*asd*				
	Nil	Arg	Lys	Arg/Lys[c]	Variant/Δ[d]	Nil	Arg	Lys	Nil/Lys	Variant/Δ	Nil	Arg	Lys	Nil/Lys	Variant/Δ
Δ	38	31	32	1.0	1.0	208	218	204	1.0	1.0	265	284	302	0.8	1.0
+	41	106	25	4.2	3.4	214	194	205	1.0	1.0	608	632	281	2.1	2.2
A68V	2,248	2,613	1,036	2.5	84	211	197	211	1.0	1.0	447	443	209	2.1	1.6
S94L	3,615	3,227	3,776	0.9	104	227	221	224	1.0	1.0	478	450	265	1.8	1.8
P108S	1,621	1,633	755	2.2	53	196	200	194	1.0	0.9	871	760	283	3.0	3.2
V144M	737	930	176	5.3	30	184	187	189	0.9	0.8	331	344	229	1.4	1.2
P217L	2,126	2,436	623	3.9	79	216	215	211	1.0	1.0	352	283	157	2.2	1.3
P274S	7,884	8,246	8,392	1.0	266	187	197	206	0.9	0.8	195	207	186	1.0	0.7
R295C	115	256	688	2.7	8.3	191	197	196	0.9	0.9	662	675	295	2.2	2.4
	dapB					*dapD*					*dnaA*				
Δ	100	108	140	0.7	1.0	161	152	162	0.9	1.0	88	89	95	0.9	1.0
+	2,344	2,380	401	5.8	23	642	572	213	3.0	3.9	85	94	90	0.8	0.8
A68V	1,074	1,199	289	3.7	11	437	322	130	3.3	2.7	87	87	82	1.0	0.9
S94L	1,561	1,468	299	5.2	16	672	567	249	2.6	4.1	72	71	85	0.8	0.8
P108S	2,891	2,882	421	6.8	28	659	757	238	2.7	4.0	78	78	91	0.8	0.8
V144M	1,692	1,592	305	5.5	17	229	278	134	1.7	1.4	98	85	85	1.1	1.1
P217L	1,143	1,024	275	4.1	11	262	216	120	2.1	1.6	92	90	95	0.9	1.0
P274S	190	173	127	1.4	1.9	160	139	144	1.1	0.9	87	85	83	1.0	0.9
R295C	3,717	3,071	572	6.4	37	301	230	152	1.9	1.8	93	87	90	1.0	1.0
	gdhA					*lysA*					*lysC*				
Δ	211	198	192	1.0	1.0	493	535	80	6.1	1.0	553	521	107	5.1	1.0
+	801	711	307	2.6	3.7	1,004	980	77	13	2.0	2,095	1,974	214	10	3.7
A68V	743	749	313	2.3	3.5	984	980	71	14	1.9	1,477	1,436	197	7.4	2.6
S94L	700	687	314	2.2	3.3	910	1,300	71	13	1.8	1,806	1,719	214	8.4	3.2
P108S	1,502	1,401	383	3.9	7.1	1,075	1,038	68	16	2.1	2,928	2,736	250	12	5.2
V144M	614	555	278	2.2	2.9	782	836	77	10	1.5	1,264	1,268	158	8.0	2.2
P217L	609	612	314	1.9	2.8	841	820	64	13	1.7	1,677	1,610	186	9.0	3.0
P274S	253	219	177	1.4	1.1	720	511	92	7.8	1.4	678	646	165	4.1	1.2
R295C	1,512	1,360	331	4.5	7.1	893	892	69	13	1.8	2,399	2,282	236	10	4.3
	lysP					*nrdA*									
Δ	51	50	48	1.0	1.0	338	348	377	0.8	1.0					
+	1,731	1,592	325	5.3	34	377	357	400	0.9	1.1					
A68V	1,558	1,455	587	2.6	31	419	399	391	1.0	1.2					
S94L	1,561	1,653	473	3.3	30	361	331	409	0.8	1.0					
P108S	1,378	1,434	922	1.4	27	386	398	426	0.9	1.1					
V144M	1,174	1,136	251	4.6	23	421	413	429	0.9	1.2					
P217L	881	858	166	5.3	17	340	418	377	0.9	1.0					
P274S	499	465	169	2.9	10	374	369	399	0.9	1.1					
R295C	1,938	1,883	987	1.9	38	389	417	387	1.0	1.1					

[a] Derivatives of the Δ*argP* strain GJ9602 each carrying two plasmids, as follows, were used in the experiments. (i) Plasmid vector pCL1920 (Δ) or its derivatives with $argP^+$ (+; pHYD915) or the different $argP^d$ variants indicated. (ii) One of the following *lac* fusion plasmids: pHYD1723 (*argO*), pHYD2660 (*argT*), pHYD2668 (*asd*), pHYD2669 (*dapB*), pHYD2610 (*dapD*), pHYD2671 (*dnaA*), pHYD2602 (*gdhA*), pHYD2670 (*lysA*), pHYD2664 (*lysC*), pHYD2636 (*lysP*), or pHYD2672 (*nrdA*).

[b] Specific activities of β-galactosidase (β-Gal) after growth in glucose-minimal A medium supplemented with 18 amino acids other than Arg or Lys (Nil), 18 amino acids and 1 mM Arg (Arg), or 18 amino acids and 1 mM Lys (Lys) are reported.

[c] Data indicate the degree of Lys repression for the *lac* fusion strain concerned, relative to Arg supplementation in the case of *argO* and to Nil supplementation for the rest.

[d] Data indicate the degree of regulation imposed by the ArgP variant concerned (or the wild-type ArgP) relative to that in the Δ*argP* strain, in medium with Arg supplementation in the case of *argO* and with Nil supplementation for the rest.

higher expression in the unsupplemented medium than the Δ*argP* strain and 10-fold repression by Lys, suggestive of an additional component of control that was ArgP dependent. The $argP^d$(P274S) mutant behaved like the Δ*argP* strain, whereas the other $argP^d$ derivatives exhibited the additional component of activated expression and Lys repression, as was obtained in the $argP^+$ strain.

(vii) Along the same lines as with *lysC*, the expression of the *lysA-lac* fusion was repressed 6- to 8-fold in the Δ*argP* and $argP^d$(*P274S*) mutants, which reflects the regulation conferred by the regulator protein LysR (see below). All other $argP^d$ derivatives, as well as the $argP^+$ strain, showed 10- to 16-fold repression upon Lys supplementation.

EMSA studies with ArgP. To determine whether the *in vivo* expression differences between $argP^+$ and *argP* mutant strains for the various promoter-*lac* fusion constructs were associated with ArgP binding to the corresponding *cis* regulatory regions *in vitro*, we performed EMSA experiments as described below. Earlier studies have shown that ArgP-mediated Lys repression can occur by two alternative mechanisms at different promot-

ers. At *dapB* or *K. pneumoniae gdhA*, ArgP binding to the regulatory region is diminished upon the addition of Lys (7, 15). At *argO*, on the other hand, Lys-liganded ArgP binds the regulatory region (7, 26, 37) but it then inhibits productive transcription at a step further downstream by trapping RNA polymerase at the promoter (26). Accordingly, in this study, EMSA experiments with His_6-ArgP were done in the absence or presence of 0.1 mM Lys, and the *cis* regulatory regions of *gdhA*, *lysC*, *dapB*, *dapD*, *asd*, *lysA*, *lysP*, and *argO* were employed. The data from these experiments are shown in Fig. 1. The apparent dissociation constants (K_ds) for binding of ArgP to the different templates in the presence or absence of Lys are given in Table 4.

Consistent with the data from earlier reports (7, 26, 37), ArgP exhibited high-affinity binding to *argO* (K_d = 15 nM) that was not diminished in the presence of Lys. Under identical experimental conditions as for *argO*, the regulatory regions of the other ArgP-regulated genes (*asd*, *dapB*, *dapD*, *gdhA*, *lysA*, *lysC*, and *lysP*) were also bound by ArgP, with apparent K_ds ranging from 55 nM to 170 nM; in all these cases (unlike the situation with *argO*), the addition of Lys was associated with an increase in the apparent K_d, indicating that ArgP binding in these instances is Lys sensitive.

Coregulation of *gdhA in vivo* by both ArgP and NaCl. In addition to its role in NH_4^+ assimilation, glutamate synthesis through the two pathways involving glutamate synthase on the one hand and glutamate dehydrogenase on the other (these are encoded by *gltBD* and *gdhA*, respectively) contribute to *E. coli* osmoregulation, that is, adaptation to growth at high osmolarity (13, 38). We found that *gdhA-lac* expression is reduced 3-fold upon growth in a medium rendered highly osmolar by NaCl supplementation; furthermore, the decrease in expression at high osmolarity was additive to that imposed by a Δ*argP* mutation, such that *gdhA-lac* transcription in the Δ*argP* mutant grown with NaCl supplementation was 10-fold lower than that in the wild-type strain grown without such supplementation (Table 5). Although the mechanism of *gdhA* transcriptional regulation by osmolarity remains to be determined, these data may provide an explanation for the earlier findings of Nandineni et al. (33) that the *gltBD argP* double mutants are growth inhibited at high osmolarity.

Analysis of *lysP* regulation by ArgP. The results described above indicated that ArgP binds the *lysP* regulatory region to mediate its nearly 35-fold transcriptional activation and that the absence of such ArgP binding either in Δ*argP* mutants or upon Lys supplementation in *argP*$^+$ strains results in very low levels of *lysP* expression. In support of this conclusion was our finding that the Δ*argP* strain is resistant to the Lys analog thialysine to the same extent (200 μg/ml) as a Δ*lysP* mutant (data not shown).

Recently, Ruiz et al. (41) had also independently identified ArgP's role both as a transcriptional activator and in mediating the repression of the *lysP* gene by Lys. However, they reported that ArgP binds the *lysP* regulatory region with similar affinities in both the absence and presence of Lys and had accordingly suggested that ArgP mediates Lys repression of *lysP* by the same mechanism of RNA polymerase trapping that it does at *argO*. Since our results were different from those of Ruiz et al. (41), we repeated the EMSA experiments with *lysP*, using a graded series of ArgP concentrations in the absence or presence of Lys (Fig. 2A); the data clearly establish that binding of ArgP to the *lysP* template is Lys sensitive. The specificity of ArgP's binding to labeled *lysP* was also established in this experiment (Fig. 2A), by demonstrating that it could be competed by unlabeled *lysP* DNA (lane 12) but not by two other nonspecific DNA fragments (lanes 13 and 14).

When nested deletion constructs of the *lysP* regulatory region were tested, a fragment extending upstream up to −114 bp (relative to the start site of transcription) was nearly indistinguishable from the larger fragment (extending up to −219 bp) in terms of both *in vivo lac* fusion regulation (Table 6) and *in vitro* Lys-sensitive ArgP binding (Fig. 2, compare panels A and B). On the other hand, a smaller fragment, extending up to −76 bp, was inactive for *lac* fusion expression *in vivo* (Table 6) and *lysP* binding *in vitro* (Fig. 2C), suggesting that the *lysP* upstream region between −114 bp and −76 bp carries critical sequence determinants for binding of and regulation by ArgP.

ArgP and *cadBA* regulation. The LysP permease has also been implicated in transcriptional regulation of the *cadBA* operon encoding Lys decarboxylase. *cadBA* expression is induced only in medium of low pH that is also supplemented with Lys and is dependent on an activator, CadC. In *lysP*-defective mutants, *cadBA* is induced at low pH even in the absence of Lys, and the model is that LysP negatively regulates *cadBA* by sequestering CadC in the absence of Lys (34, 45). Since our findings indicated that ArgP is needed for activation of *lysP* transcription, we tested whether *cadBA* expression would be rendered Lys independent even in the Δ*argP* mutant.

Accordingly, a *cadBA-lac* fusion was constructed and shown to exhibit regulation by *cadC*, *lysP*, low pH, and Lys as reported earlier (34, 45); however, the Δ*argP* mutant failed to phenocopy the Δ*lysP* strain, in that *cadBA* expression continued to be Lys dependent in the former (Table 7). These data suggest that the basal level of LysP permease present in a Δ*argP* strain is sufficient for the negative regulation of *cadBA* in the absence of Lys.

Testing for cross-regulation by LysR and ArgP genes. As mentioned above, ArgP belongs to the LTTR family, of which LysR is the prototypic member (30). Since both LysR and ArgP are involved in mediating the repression by Lys of different genes, we tested for the possibility of cross-regulation by the two transcription factors *in vivo*.

A Δ*lysR* strain fails to express *lysA* and hence is a Lys auxotroph (36). Therefore, to test for regulation in the presence or absence of Lys in the Δ*lysR* mutant, we constructed derivatives in which *lysA* was ectopically expressed from the P_{ara} promoter on a plasmid (pHYD2673). The LysR-regulated *lysA-lac* and ArgP-regulated *lysP-lac* fusions were employed for tests of *in vivo* cross-regulation (by ArgP and LysR, respectively); the data reported in Table 8 indicate that there is no cross-regulation of *lysP* by LysR and that ArgP's ability to activate *lysA* occurs only in a *lysR*$^+$ background. The expression of a *lysR-lac* fusion was also unaffected in a Δ*argP* strain, although it was increased 2-fold in a Δ*lysR* mutant (Table 8), indicative of negative autoregulation (36).

Genes for Arg import are not regulated by ArgP. Since ArgP is required for transcription of the Arg exporter ArgO, we also tested the promoters of other genes known to be involved in Arg transport (*artP*, *artJ*, *argT*, and the *hisJQMP* operon) as candidates for regulation by ArgP. The respective promoter-

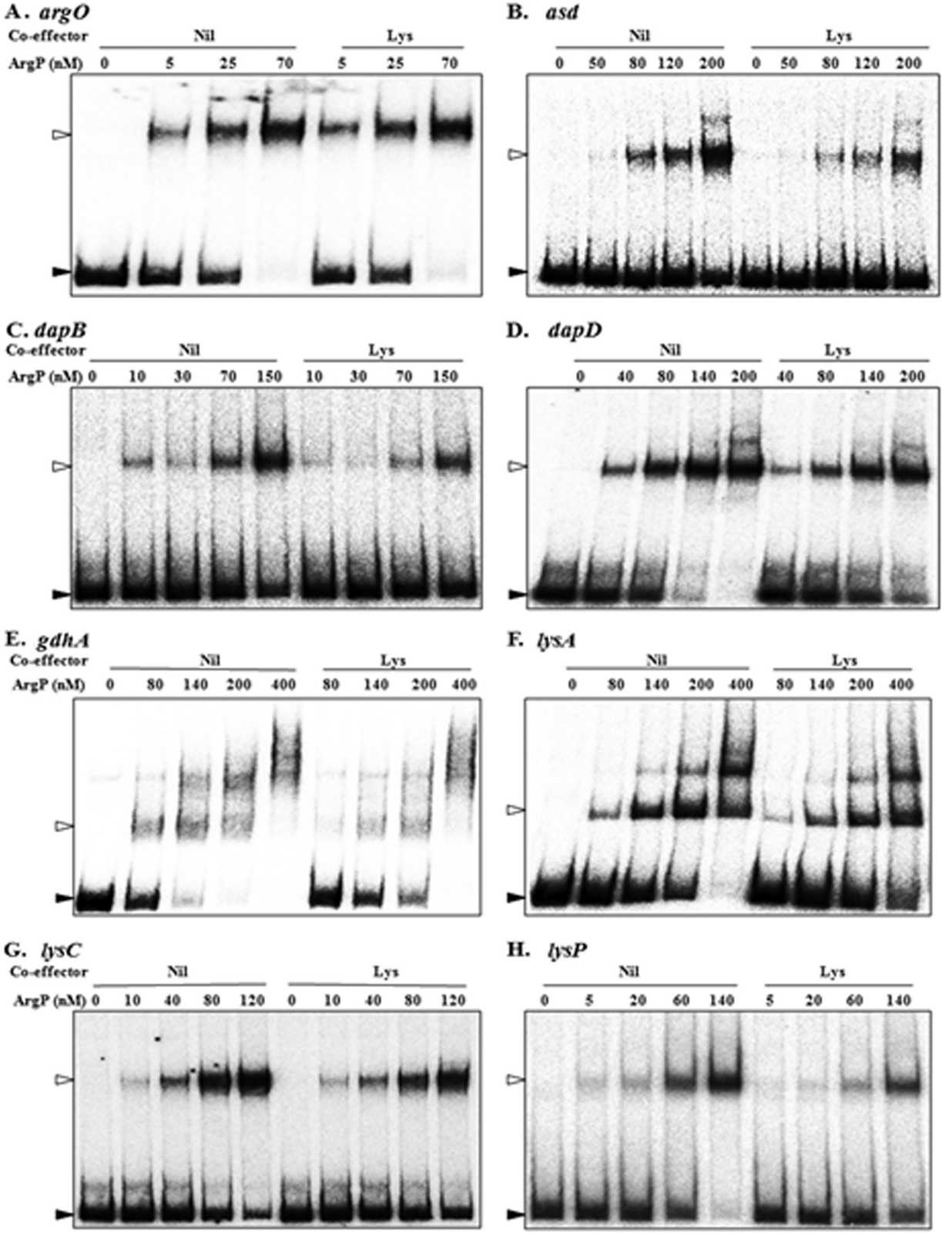

FIG. 1. EMSAs with ArgP and *cis* regulatory regions of different genes in the absence or presence of the coeffector Lys. ArgP monomer concentrations are indicated for each lane. Bands corresponding to free DNA and to DNA in binary complex with ArgP are marked by filled and open arrowheads, respectively.

TABLE 4. Apparent K_ds of ArgP binding to *cis* regulatory regions of different genes[a]

cis regulatory region	K_d (nM)	
	−Lys	+Lys
argO	15	15
argT	>400	>400
asd	170	>200
dapB	120	>150
dapD	70	120
dnaA	150	150
gdhA	80	130
lysA	150	210
lysC	70	110
lysP	55	>140
nrdA	150	150
lacZ	>400	>400

[a] From the EMSA autoradiograph for each of the DNA templates, the amounts of radioactivity in the bands corresponding to free (unbound) DNA were densitometrically determined for the different lanes, and the apparent K_d in the absence (−) or presence (+) of 0.1 mM Lys was calculated from the curve-plotting values of the unbound fraction versus the ArgP concentrations used.

lac fusions were constructed, but neither the Δ*argP* mutation nor Lys supplementation affected the expression of any of them (Table 9). Consistent with earlier reports (8, 9), the *lac* fusions with the promoters of *hisJ*, *artJ*, and *artP* exhibited repression by Arg in the wild-type strain and derepressed expression in a Δ*argR* mutant (Table 9). EMSA experiments undertaken with the regulatory region of one of the genes, *argT*, revealed a very low affinity of binding by ArgP that was Lys independent (Fig. 3 and Table 4); the expression of the *argT-lac* fusion was also not affected in any of the *argP*^{d} mutants (Table 3).

ArgP and regulation of genes of DNA replication and metabolism. In its description as IciA, ArgP has been reported to regulate *dnaA* and *nrdA* transcription (20, 27–29). We constructed and tested the promoter-*lac* fusions for both these genes, but neither was altered in expression in the Δ*argP* or *argP*^{d} mutants compared to its expression in the *argP*$^+$ strain, either without or with Lys supplementation (Table 3). Our conclusion runs contrary to that of Han et al. (20), who overproduced IciA (ArgP) and employed an RNase protection assay to show that *nrdA* transcription is ArgP regulated *in vivo*. In EMSA experiments using ArgP and the *cis* regulatory regions of both *dnaA* and *nrdA*, we observed binding at an apparent K_d of around 150 nM that was unchanged in the presence or absence of Lys (Fig. 3 and Table 4).

Finally, since our data showed that ArgP binds several DNA templates *in vitro* without a corresponding regulatory effect *in vivo*, we asked whether at high concentrations it would exhibit binding to a completely nonspecific control DNA fragment. When tested by EMSA, negligible binding of ArgP was observed even at 400 nM to an internal 295-bp DNA sequence from *lacZ* (Fig. 3 and Table 4). These findings are consistent with other reports that ArgP does not bind DNA nonspecifically (23, 41), as also with both the inability of nonspecific DNA fragments to compete with *lysP* for binding to ArgP (Fig. 2) and the very weak binding of ArgP to *argT* (Fig. 3).

TABLE 5. Regulation of *gdhA* expression by ArgP and NaCl

Strain (genotype)	β-Gal sp act (Miller units)[a]	
	−NaCl	+NaCl
MC4100 (*argP*$^+$)	1,016	331
GJ9602 (Δ*argP*)	340	102

[a] Values reported are the specific activities of β-galactosidase (β-Gal) in derivatives of the indicated strains carrying *gdhA-lac* on plasmid pHYD2602 after growth in glucose-minimal A medium without (−NaCl) or with (+NaCl) supplementation with 0.4 M NaCl and 1 mM glycine betaine.

DISCUSSION

The Arg exporter ArgO was among the first whose expression *in vivo* was identified as being under the transcriptional control of the LTTR ArgP, and the *argP*^{d} mutants were obtained as derivatives with greatly elevated *argO* expression (32). In the case of *argO*, ArgP mediates its transcriptional activation by Arg, as well as its repression by Lys (7, 26, 32, 37); the latter is achieved by a mechanism involving the active trapping at the *argO* promoter by ArgP of RNA polymerase, which is then prevented from being released to engage in productive transcription (26). Subsequently, *dapB* and *K. pneumoniae gdhA* have also been shown to be ArgP regulated and to be repressed with Lys; in both these instances, Lys repression is achieved by reduced binding of ArgP to the DNA regulatory regions in the presence of Lys (7, 15).

In the present work, we have constructed *lac* fusions to promoters of genes connected with Arg or Lys metabolism and transport and studied them in the *argP*$^+$, Δ*argP*, and *argP*^{d} strains to identify several new ArgP-regulated genes *in vivo*. These include *lysP*, *lysC*, *lysA*, *dapD*, and *asd* (in addition to *argO*, *dapB*, and *gdhA*), all of which exhibited ArgP-mediated repression upon Lys supplementation *in vivo*, as well as ArgP binding to the corresponding *cis* regulatory regions *in vitro*.

Insights from the use of *argP*^{d} mutants. For many of the newly identified targets of ArgP listed above, the magnitude of *in vivo* regulation by ArgP (and of repression by Lys) was only around 3-fold, and it was the data on *lac* fusion expression in the *argP*^{d} mutants that offered additional confidence that this regulation was indeed real.

Although the *argP*^{d} mutants had been obtained (10, 32) on the basis of their resistance to L-canavanine and increased expression in them of *argO*, the mutants exhibited different effects on the other target genes with regard both to the degree of activation relative to that in the Δ*argP* strain and to the degree of repression upon Lys supplementation (Table 3). Of these, the most significant difference was that for the P274S variant, which was almost completely defective for activation of *lysC*, *asd*, *dapD*, *gdhA*, and *dapB* and partially so for *lysP*, whereas it was indeed the most effective of all the *argP*^{d} mutants for *argO* activation. On the other hand, for the genes such as *argT*, *dnaA*, or *nrdA* that are not ArgP regulated, there was no difference in expression between the Δ*argP* derivative and any of the *argP*^{d} mutants.

The expression data with the *argP*^{d} mutants also indirectly illustrate the complexity of ArgP's binding interactions with and at the *cis* regulatory regions of its target genes, since the mutations appear to affect these interactions differently at different targets.

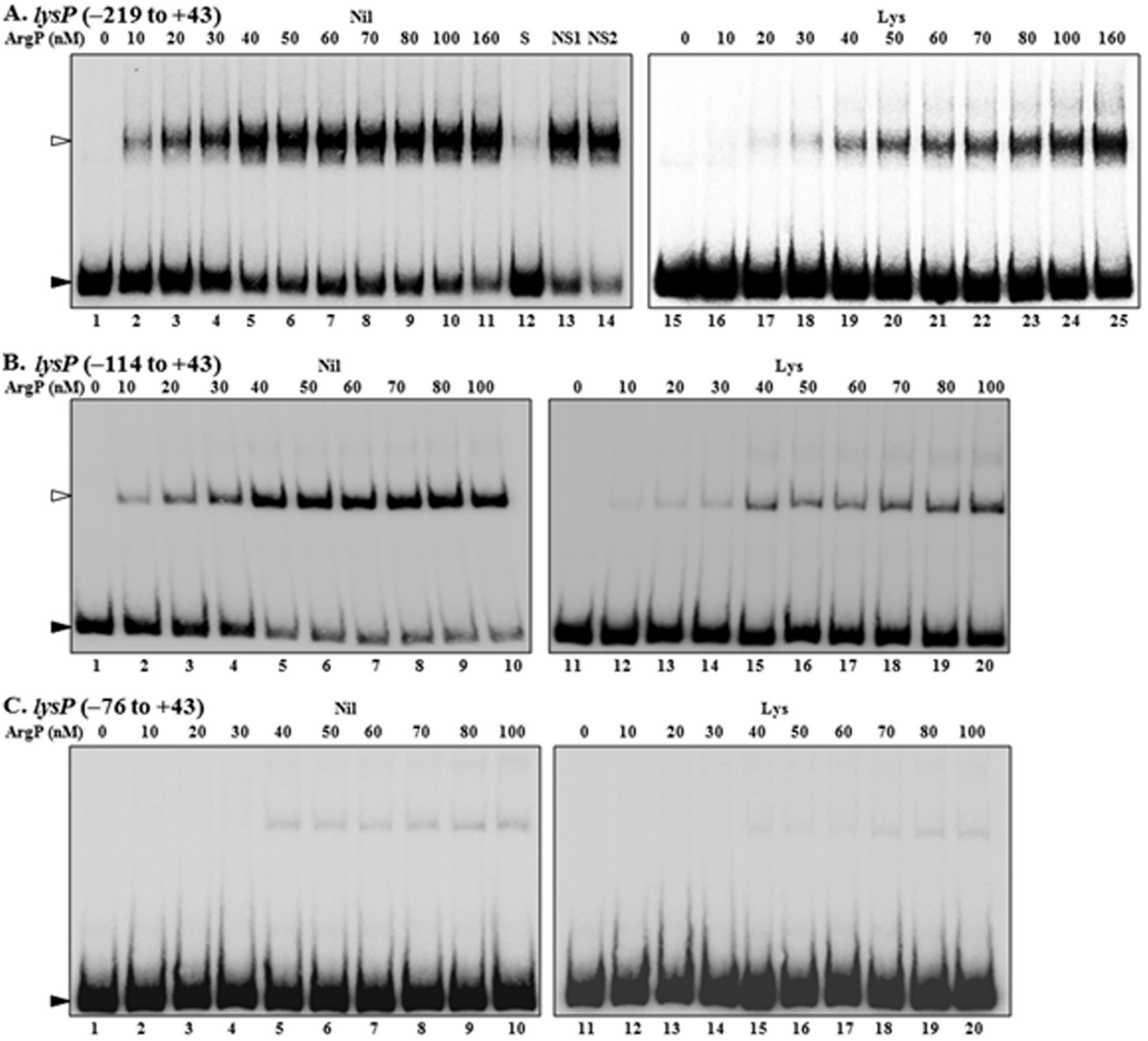

FIG. 2. EMSAs with ArgP and the full-length *cis* regulatory region of *lysP* (A) or one of its two deletion derivatives (B, C) in the absence (Nil) or presence (Lys) of the coeffector Lys. The extent in base pairs of the *lysP* regulatory region used in each experiment is given in parentheses. ArgP monomer concentrations are indicated for each lane. Bands corresponding to free DNA and to DNA in binary complex with ArgP (the latter is not observed in panel C) are marked by filled and open arrowheads, respectively. Lanes 12 to 14 of panel A depict the results of the addition of a 100-fold excess of unlabeled competitor DNA, either specific, that is the full-length *lysP* fragment (S), or one of two different nonspecific DNA fragments (NS1 and NS2), to the mixtures for EMSA reactions undertaken with 160 nM ArgP. NS1 is a 253-bp fragment from the *ilvG* locus obtained by PCR with the primers 5′-CAGCAACACTGCGCGCAGCTGCGTGATG-3′ and 5′-CTTGTGCGCCAACCGCCGCCGGTAAAC-3′ (genomic coordinates 3949570 to 3949822 [40]), while NS2 is the same 295-bp *lacZ* fragment that was used for the EMSA whose results are shown in Fig. 3.

Regulation by ArgP of Lys-biosynthetic genes. Of the ArgP-regulated and Lys-repressed genes identified earlier and in this study, five encode enzymes for biosynthesis of diaminopimelic acid and Lys (36): two in the pathway that is common for Lys, threonine, and methionine biosynthesis (*lysC* and *asd*) and three in the Lys-specific pathway (*dapB*, *dapD*, and *lysA*). Of these, *lysC* and *lysA* exhibit additional mechanisms for Lys repression, involving, respectively, a postulated riboswitch (39, 44) and the LysR regulator protein (30, 36).

Our results indicate that ArgP activates *lysA* transcription 2-fold but only in cells that are also proficient for LysR. On the other hand, ArgP-mediated regulation of *lysC* is independent of and additive to the putative riboswitch regulatory mechanism. One may speculate that Lys-dependent modulation by ArgP of multiple genes of the pathway provides a fine-tuning effect in controlling the flux of intermediates serving the biosynthesis of three different amino acids for protein synthesis, as also of diaminopimelic acid for peptidoglycan assembly.

TABLE 6. ArgP regulation of *lac* fusions to nested deletions of *lysP*

Plasmid (*lysP* extent)[a]	β-Gal sp act (Miller units)[b]			
	argP$^+$ strain		Δ*argP* strain	
	Nil	Lys	Nil	Lys
pHYD2636 (−219 to +32)	802	150	31	30
pHYD2647 (−114 to +32)	852	122	52	50
pHYD2648 (−76 to +32)	43	47	41	42

[a] Extents of *lysP* shown are in base pairs relative to the start site of transcription.

[b] Values reported are the specific activities of β-galactosidase (β-Gal) in derivatives of MC4100 (*argP*$^+$) or GJ9602 (Δ*argP*) carrying the indicated *lysP-lac* fusion plasmids after growth in glucose-minimal A without (Nil) or with (Lys) Lys supplementation at 1 mM.

Role for ArgP in Lys-mediated repression in *E. coli*. As mentioned above, all genes so far identified to be ArgP mediated are repressed by Lys, and we suggest that all Lys-liganded repression is mediated by ArgP in *E. coli*. LysR also mediates Lys repression (of *lysA*), but in this case it has been suggested that the coeffector ligand is diaminopimelic acid (which is the substrate for *lysA*) and that the latter's binding to LysR converts it into its activator conformation (30, 36). Although Lys-liganded repression is a common feature of all the ArgP-regulated genes, different mechanisms operate to achieve such repression at different genes, as discussed below.

Regulation of *lysP* by ArgP. Of the various ArgP-regulated genes, the three that exhibited >10-fold activation were *argO*, *dapB*, and *lysP*. The *lysP* gene encodes a Lys-specific permease, and our data indicate that it is around 35-fold activated by ArgP and 5-fold repressed by Lys; a Δ*argP* mutant phenocopies a Δ*lysP* mutant for resistance to the Lys analog thialysine. LysR does not participate in *lysP* transcriptional regulation. *In vitro*, ArgP binds the *lysP* regulatory region with a K_d of around 55 nM and the binding affinity is diminished upon the addition of Lys (to a K_d of >140 nM), suggesting that Lys represses *lysP in vivo* by engendering the loss of ArgP binding to the *lysP* operator region. The *in vitro* data also indicate that the *lysP* upstream sequence between −76 bp and −114 bp is required for ArgP binding.

In addition to its role in active uptake of Lys, the LysP permease also participates in Lys-dependent transcriptional regulation of the *cadBA* operon, by sequestering the CadC activator in the absence of Lys but not in its presence (34, 45). We found in this study that the Δ*argP* mutant does not phenocopy a Δ*lysP* mutant for *cadBA* expression, suggesting that the basal level of LysP which is present in a Δ*argP* strain is sufficient for mediating the sequestration of CadC in *cadBA* regulation.

While this work was being completed, Ruiz et al. (41) independently reported the identification of *lysP* as a Lys-repressed transcriptional target of ArgP. While most of our results on *lysP* are similar to those of Ruiz et al. (41), one important difference is their description that ArgP's binding to the *lysP* regulatory region *in vitro* is Lys insensitive (and therefore similar to ArgP's binding to *argO*), whereas we found that it is Lys sensitive. Perhaps this discrepancy may be related to differences in the protocols employed in the two laboratories for ArgP purification or the subsequent protein-DNA binding studies, although we emphasize that our demonstration of Lys-sensitive binding of ArgP to *lysP* was made under the identical conditions in which ArgP's binding to *argO* was Lys insensitive (Fig. 1).

Unique features of *argO* regulation by ArgP. Of the different genes under the control of ArgP *in vivo*, *argO* appears to be unique in at least two ways in its manner of regulation. First, it is the only gene that requires Arg as a coeffector for its activation. Second, it is also the only example in which ArgP's binding to the *cis* regulatory region is not Lys sensitive, so that

TABLE 7. Effects of CadC, LysP, and ArgP on *cadBA-lac* expression

Strain (genotype)	β-Gal sp act (Miller units)[a]			
	pH 7.4		pH 5.8	
	Nil	Lys	Nil	Lys
MC4100 (wild type)	25	28	24	98
GJ9623 (Δ*lysP*)	27	27	94	96
GJ9647 (Δ*cadC*)	25	26	26	24
GJ9602 (Δ*argP*)	23	22	26	96
GJ9649 (Δ*lysP* Δ*cadC*)	21	28	24	26
GJ9648 (Δ*argP* Δ*cadC*)	25	26	24	28
GJ9624 (Δ*argP* Δ*lysP*)	27	24	87	99

[a] Values reported are the specific activities of β-galactosidase (β-Gal) in the indicated strain derivatives carrying the *cadBA-lac* fusion plasmid pHYD2674 after growth in minimal A-based medium of pH 7.4 or pH 5.8 supplemented with either 19 amino acids other than Lys (Nil) or 19 amino acids and 10 mM Lys (Lys).

TABLE 8. Tests for cross-regulation by ArgP and LysR

Strain (genotype)	β-Gal sp act (Miller units)[a]						
	lysA-lac with:			*lysR-lac* with:			*lysP-lac* with Nil
	Nil	Arg	Lys	Nil	Arg	Lys	
GJ9650 (wild type)	215	300	68	30	33	33	1,004
GJ9651 (Δ*argP*)	72	75	29	29	33	34	49
GJ9652 (Δ*lysR*)	23	26	22	59	65	63	1,052
GJ9653 (Δ*argP* Δ*lysR*)	24	23	25	66	66	65	49

[a] Values reported are the specific activities of β-galactosidase in derivatives of the indicated strains carrying both the P_{ara}-*lysA* plasmid pHYD2673 and one of the following plasmids: pHYD2670 (*lysA-lac*), pHYD2675 (*lysR-lac*), or pHYD2636 (*lysP-lac*). Strains were grown in minimal A medium supplemented with 0.2% each of glycerol and l-arabinose along with one of the following: 18 amino acids other than Arg and Lys (Nil), 18 amino acids and 1 mM Arg (Arg), or 18 amino acids and 1 mM Lys (Lys).

TABLE 9. Tests for ArgP regulation of genes related to Arg transport

Plasmid (genotype)	β-Gal sp act (Miller units)[a]						
	Wild-type strain with:			Δ*argP* strain with:			*argR* with Arg
	Nil	Arg	Lys	Nil	Arg	Lys	
pHYD2658 (*artJ-lac*)	1,353	286	1,480	1,256	231	1,252	1,700
pHYD2659 (*artP-lac*)	170	105	197	184	122	210	190
pHYD2660 (*argT-lac*)	249	279	242	279	255	221	198
pHYD2661 (*hisJ-lac*)	227	115	262	244	114	296	289

[a] Values reported are the specific activities of β-galactosidase in derivatives of MC4100 (wild type), GJ9602 (Δ*argP*), and GJ4748 (*argR*) carrying the indicated *lac* fusion plasmid after growth in glucose-minimal A medium without (Nil) or with supplementation with Arg or Lys at 1 mM.

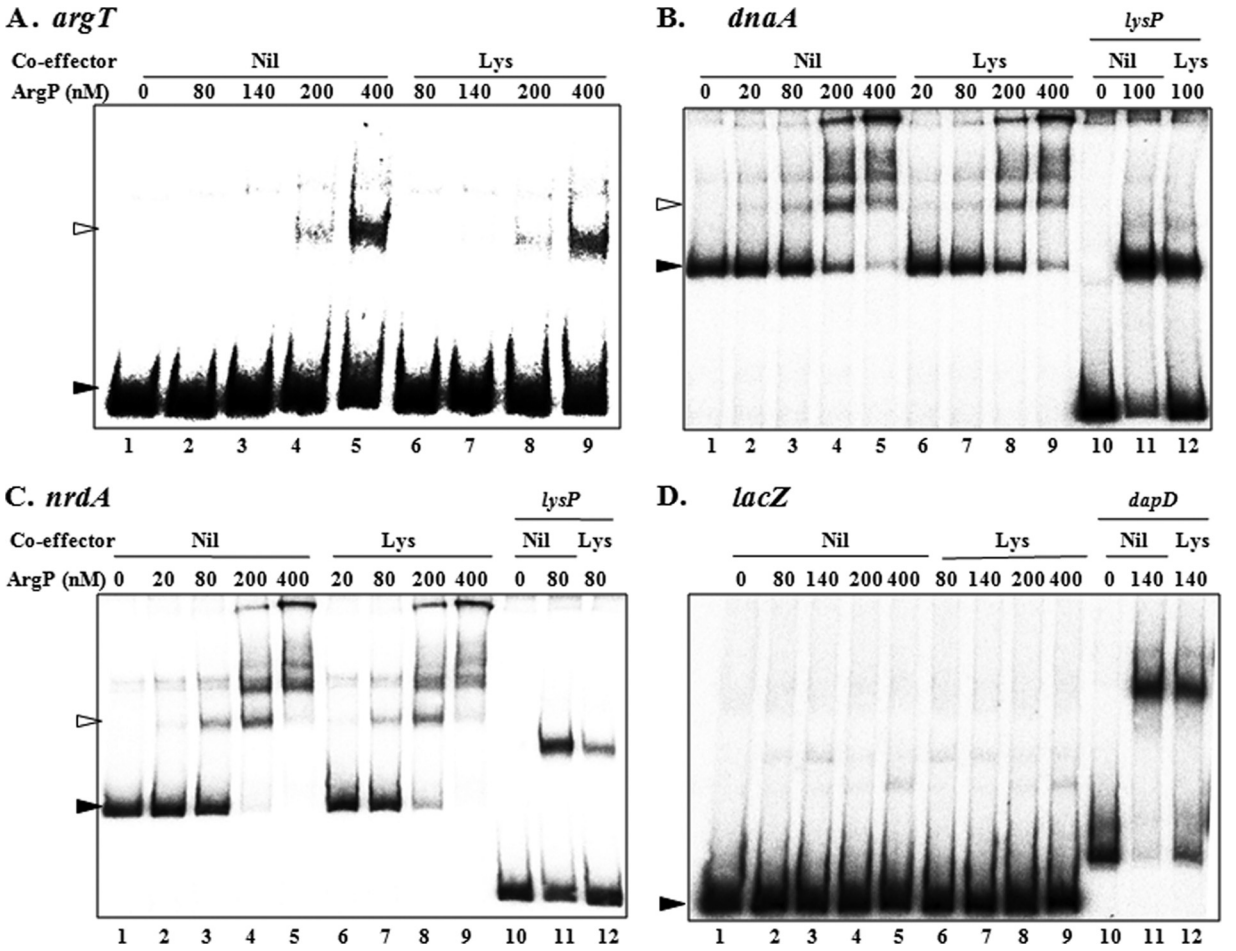

FIG. 3. EMSAs with ArgP and *cis* regulatory regions of different genes in the absence or presence of the coeffector Lys. ArgP monomer concentrations are indicated for each lane. Bands corresponding to free DNA and to DNA in binary complex with ArgP (the latter is not observed for *lacZ*) are marked by filled and open arrowheads, respectively. Lanes 10 to 12 in panels B to D represent the results of control EMSA reactions with *lysP* (full-length) or *dapD* templates as marked.

repression by Lys is achieved by an RNA polymerase-trapping mechanism at the *argO* promoter.

The differences between *argO* and the other target genes with regard to the features of their regulation by ArgP are also reflected in their differential responses to the *argP*^{d} mutations (although it must be noted that these mutations were selected on the basis of their ability to confer greatly increased *argO* expression). Perhaps the most prominent distinction is that obtained with the P274S variant of ArgP, as discussed above.

Thus, it appears that the mechanism of regulation of *argO* by ArgP is fundamentally different from that of the other genes regulated by this protein. At the same time, it is noteworthy that two of the genes that are most prominently regulated by ArgP, *argO* and *lysP*, are both also regulated by the leucine-responsive general transcriptional regulator Lrp (37, 41).

***dnaA* and *nrdA* are not regulated by ArgP *in vivo*.** In its proposed avatar as IciA, ArgP has been reported to bind *oriC* in both *E. coli* (21–23, 46) and *Mycobacterium tuberculosis* (25) and to regulate transcription from promoters of the *dnaA* and *nrdA* genes (20, 27–29). We found that ArgP does bind to the regulatory regions of *dnaA* and *nrdA* *in vitro* with moderate affinity but that it does not regulate their transcription *in vivo*. Thus, we believe that ArgP does not play a role in transactions involving DNA metabolism or replication in *E. coli*, which conclusion is supported by the facts that neither a Δ*argP* mutant nor an ArgP-overproducing strain is compromised for DNA replication or growth rate (46).

ArgP as a noncanonical transcriptional regulator. Genome-wide chromatin immunoprecipitation studies have been described recently for a number of transcriptional regulators in *E. coli*, according to which several, such as MelR (17, 18), MntR (49), NsrR (35), and PurR (12), behave canonically in that each exhibits binding at sites where it serves to control transcription of the adjacent genes or operons. Lrp also appears to be a canonical transcriptional regulator, although it binds nearly 140 sites in the genome (11).

On the other hand, DNA binding by the activator protein C-reactive protein (CRP) is apparently noncanonical, since there are about 70 sites where it binds strongly and exerts its transcriptional regulatory function and a further approximately 10^4 sites of low-affinity binding (17). CRP's behavior contrasts with that of its closely related paralog FNR, which binds canonically at around 65 locations without any noise of low-affinity binding (16). Another example of noncanonical binding

is RutR, many of whose binding sites fall within gene coding regions (43).

Our studies would suggest that the ArgP or IciA protein is also a noncanonical transcriptional regulator, in that it does exhibit specific DNA binding to mediate the transcriptional regulation of particular genes *in vivo*, but in addition, it also binds at other sites in the genome that are not associated with regulatory outcomes. The purpose of the latter category of binding by ArgP remains to be determined. It has been speculated in the case of CRP that by bending the DNA at its noncanonical binding sites, the protein may contribute to chromosome shaping and chromatin compaction (17, 47). ArgP too may similarly participate in shaping the nucleoid architecture, given its reported propensity to bind curved DNA (2). It is also possible that the putative dual functions of ArgP, in transcriptional regulation and in chromosome organization, are in some way related to its ability to dimerize in two different modes (as deduced from the crystal structure of the *M. tuberculosis* ortholog) (50).

Finally, it is noteworthy that many of the genes regulated by ArgP are controlled also by other mechanisms, such as by LysR for *lysA*, osmolarity for *gdhA*, the riboswitch for *lysC*, and Lrp for *argO* and *lysP*. These results therefore lend support to the notion that multitarget regulators and multifactor promoters represent the norm in bacterial gene regulation (24).

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REFERENCES

1. **Andrews, A. E., B. Dickson, B. Lawley, C. Cobbett, and A. J. Pittard.** 1991. Importance of the position of TYR R boxes for repression and activation of the *tyrP* and *aroF* genes in *Escherichia coli*. J. Bacteriol. **173:**5079–5085.
2. **Azam, T. A., and A. Ishihama.** 1999. Twelve species of the nucleoid-associated protein from *Escherichia coli*. Sequence recognition specificity and DNA binding affinity. J. Biol. Chem. **274:**33105–33113.
3. **Azam, T. A., A. Iwata, A. Nishimura, S. Ueda, and A. Ishihama.** 1999. Growth phase-dependent variation in protein composition of the *Escherichia coli* nucleoid. J. Bacteriol. **181:**6361–6370.
4. **Baba, T., et al.** 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. **2:**1–11.
5. **Bellmann, A., et al.** 2001. Expression control and specificity of the basic amino acid exporter LysE of *Corynebacterium glutamicum*. Microbiology **147:**1765–1774.
6. **Berlyn, M. K.** 1998. Linkage map of *Escherichia coli* K-12, edition 10: the traditional map. Microbiol. Mol. Biol. Rev. **62:**814–984.
7. **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.
8. **Caldara, M., D. Charlier, and R. Cunin.** 2006. The arginine regulon of *Escherichia coli*: whole-system transcriptome analysis discovers new genes and provides an integrated view of arginine regulation. Microbiology **152:**3343–3354.
9. **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.
10. **Celis, R. T.** 1999. Repression and activation of arginine transport genes in *Escherichia coli* K 12 by the ArgP protein. J. Mol. Biol. **294:**1087–1095.
11. **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.
12. **Cho, B. K., et al.** 2011. The PurR regulon in *Escherichia coli* K-12 MG1655. Nucleic Acids Res. **39:**6456–6464.
13. **Csonka, L. N., and W. Epstein.** 1996. Osmoregulation, p. 1210–1223. *In* F. C. Neidhardt et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. American Society for Microbiology, Washington, DC.
14. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. U. S. A. **97:**6640–6645.
15. **Goss, T. J.** 2008. The ArgP protein stimulates the *Klebsiella pneumoniae gdhA* promoter in a lysine-sensitive manner. J. Bacteriol. **190:**4351–4359.
16. **Grainger, D. C., H. Aiba, D. Hurd, D. F. Browning, and S. J. Busby.** 2007. Transcription factor distribution in *Escherichia coli*: studies with FNR protein. Nucleic Acids Res. **35:**269–278.
17. **Grainger, D. C., D. Hurd, M. Harrison, J. Holdstock, and S. J. Busby.** 2005. Studies of the distribution of *Escherichia coli* cAMP-receptor protein and RNA polymerase along the *E. coli* chromosome. Proc. Natl. Acad. Sci. U. S. A. **102:**17693–17698.
18. **Grainger, D. C., et al.** 2004. Genomic studies with *Escherichia coli* MelR protein: applications of chromatin immunoprecipitation and microarrays. J. Bacteriol. **186:**6938–6943.
19. **Guzman, L. M., D. Belin, M. J. Carson, and J. Beckwith.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. J. Bacteriol. **177:**4121–4130.
20. **Han, J. S., H. S. Kwon, J. B. Yim, and D. S. Hwang.** 1998. Effect of IciA protein on the expression of the *nrd* gene encoding ribonucleoside diphosphate reductase in *E. coli*. Mol. Gen. Genet. **259:**610–614.
21. **Hwang, D. S., and A. Kornberg.** 1990. A novel protein binds a key origin sequence to block replication of an E. coli minichromosome. Cell **63:**325–331.
22. **Hwang, D. S., and A. Kornberg.** 1992. Opposed actions of regulatory proteins, DnaA and IciA, in opening the replication origin of *Escherichia coli*. J. Biol. Chem. **267:**23087–23091.
23. **Hwang, D. S., B. Thony, and A. Kornberg.** 1992. IciA protein, a specific inhibitor of initiation of *Escherichia coli* chromosomal replication. J. Biol. Chem. **267:**2209–2213.
24. **Ishihama, A.** 2010. Prokaryotic genome regulation: multifactor promoters, multitarget regulators and hierarchic networks. FEMS Microbiol. Rev. **34:**628–645.
25. **Kumar, S., A. Farhana, and S. E. Hasnain.** 2009. In-vitro helix opening of *M. tuberculosis oriC* by DnaA occurs at precise location and is inhibited by IciA like protein. PLoS One **4:**e4139.
26. **Laishram, R. S., and J. Gowrishankar.** 2007. Environmental regulation operating at the promoter clearance step of bacterial transcription. Genes Dev. **21:**1258–1272.
27. **Lee, Y., H. Lee, J. Yim, and D. Hwang.** 1997. The binding of two dimers of IciA protein to the *dnaA* promoter 1P element enhances the binding of RNA polymerase to the *dnaA* promoter 1P. Nucleic Acids Res. **25:**3486–3489.
28. **Lee, Y. S., and D. S. Hwang.** 1997. Occlusion of RNA polymerase by oligomerization of DnaA protein over the *dnaA* promoter of *Escherichia coli*. J. Biol. Chem. **272:**83–88.
29. **Lee, Y. S., H. Kim, and D. S. Hwang.** 1996. Transcriptional activation of the *dnaA* gene encoding the initiator for *oriC* replication by IciA protein, an inhibitor of in vitro *oriC* replication in *Escherichia coli*. Mol. Microbiol. **19:**389–396.
30. **Maddocks, S. E., and P. C. Oyston.** 2008. Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. Microbiology **154:**3609–3623.
31. **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.
32. **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.
33. **Nandineni, M. R., R. S. Laishram, and J. Gowrishankar.** 2004. Osmosensitivity associated with insertions in *argP* (*iciA*) or *glnE* in glutamate synthase-deficient mutants of *Escherichia coli*. J. Bacteriol. **186:**6391–6399.
34. **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.
35. **Partridge, J. D., D. M. Bodenmiller, M. S. Humphrys, and S. Spiro.** 2009. NsrR targets in the *Escherichia coli* genome: new insights into DNA sequence requirements for binding and a role for NsrR in the regulation of motility. Mol. Microbiol. **73:**680–694.
36. **Patte, J.-C.** 1996. Biosynthesis of threonine and lysine, p. 528–541. *In* F. C. Neidhardt et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. American Society for Microbiology, Washington, DC.
37. **Peeters, E., P. N. 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.
38. **Reitzer, L.** 6 July 2004, posting date. Module 3.6.1.3, Biosynthesis of glutamate, aspartate, asparagine, L-alanine, and D-alanine. *In* R. Curtiss III et al. (ed.), EcoSal *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC. doi:10.1128/ecosal.3.6.1.3.

39. **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.
40. **Rudd, K. E.** 1998. Linkage map of *Escherichia coli* K-12, edition 10: the physical map. Microbiol. Mol. Biol. Rev. **62:**985–1019.
41. **Ruiz, J., I. Haneburger, and K. Jung.** 2011. Identification of ArgP and Lrp as transcriptional regulators of *lysP*, the gene encoding the specific lysine permease of *Escherichia coli*. J. Bacteriol. **193:**2536–2548.
42. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
43. **Shimada, T., A. Ishihama, S. J. Busby, and D. C. Grainger.** 2008. The *Escherichia coli* RutR transcription factor binds at targets within genes as well as intergenic regions. Nucleic Acids Res. **36:**3950–3955.
44. **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.
45. **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.
46. **Thony, B., D. S. Hwang, L. Fradkin, and A. Kornberg.** 1991. *iciA*, an *Escherichia coli* gene encoding a specific inhibitor of chromosomal initiation of replication in vitro. Proc. Natl. Acad. Sci. U. S. A. **88:**4066–4070.
47. **Wade, J. T., K. Struhl, S. J. Busby, and D. C. Grainger.** 2007. Genomic analysis of protein-DNA interactions in bacteria: insights into transcription and chromosome organization. Mol. Microbiol. **65:**21–26.
48. **Wei, T., and R. Bernander.** 1996. Interaction of the IciA protein with AT-rich regions in plasmid replication origins. Nucleic Acids Res. **24:**1865–1872.
49. **Yamamoto, K., A. Ishihama, S. J. Busby, and D. C. Grainger.** 2011. The *Escherichia coli* K-12 MntR miniregulon includes *dps*, which encodes the major stationary-phase DNA-binding protein. J. Bacteriol. **193:**1477–1480.
50. **Zhou, X., et al.** 2010. Crystal structure of ArgP from *Mycobacterium tuberculosis* confirms two distinct conformations of full-length LysR transcriptional regulators and reveals its function in DNA binding and transcriptional regulation. J. Mol. Biol. **396:**1012–1024.