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Lysine Represses Transcription of the *Escherichia coli dapB* Gene by Preventing Its Activation by the ArgP Activator▽†

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The *Escherichia coli dapB* gene encodes one of the enzymes of the biosynthetic pathway leading to lysine and its immediate precursor, diaminopimelate. Expression of *dapB* is repressed by lysine, but no *trans*-acting regulator has been identified so far. Our analysis of the *dapB* regulatory region shows that sequences located in the −81/−118 interval upstream of the transcription start site are essential for full expression of *dapB*, as well as for lysine repression. Screening a genomic library for a gene that could alleviate lysine repression when present in multicopy led to the recovery of *argP*, a gene encoding an activating protein of the LysR-type family, known to use lysine as an effector. An *argP* null mutation strongly decreases *dapB* transcription that becomes insensitive to lysine. Purified His_6-tagged ArgP protein binds with an apparent K_d of 35 nM to the *dapB* promoter in a gel retardation assay, provided that sequences up to −103 are present. In the presence of L-lysine and L-arginine, the binding of ArgP to *dapB* is partly relieved. These results fit with a model in which ArgP contributes to enhanced transcription of *dapB* when lysine becomes limiting.

Lysine biosynthesis in *Escherichia coli* involves nine successive enzymatic reactions, the last one being decarboxylation of diaminopimelate (DAP) by DAP decarboxylase, the *lysA* product (see Fig. S1 in the supplemental material) (reviewed in reference 19). DAP decarboxylase converts DAP, an essential molecule constituent of peptidoglycan, into L-lysine, another major cell component, and its synthesis is strictly dependent on the regulatory protein LysR, a transcriptional activator responding to the internal concentrations of DAP and lysine (24). The first eight steps are common to DAP and lysine biosynthesis. They are performed by enzymes encoded by eight genes scattered around the *E. coli* chromosome, all of which, except *dapC*, have been identified and studied in some detail. The last step is still obscure and might involve multiple nonspecific enzymes, among which is the *argD* product (15).

There is no single regulatory theme for these seven genes, and no *trans*-acting regulatory factor has been identified that could control expression of any of them in response to the lysine concentration. Two steps, performed by the *dapE* and *dapF* products, are insensitive to the lysine concentration (4, 19). Another step is controlled at the level of enzymatic activity by retroinhibition of the *dapA* product (19). The very first step, phosphorylation of aspartate, is performed by three isoenzymes, one of which, aspartokinase III, the *lysC* product, appears to be specific to the DAP-lysine pathway, whereas the other two belong to the threonine and the methionine biosynthesis pathways. Transcription of *lysC* is repressed through a riboswitch mechanism by direct binding of lysine to the 5′-terminal part of the *lysC* mRNA (25). The second step, under the control of the *asd* product, is common to the lysine, threonine, and methionine pathways, and expression of *asd* is subject to multivalent repression by these 3 amino acids. There is no attenuation-like sequence upstream of *asd* that could explain the complex regulation of its expression, which remains mysterious (13).

Finally, the steps performed by the *dapB* and *dapD* products are controlled by lysine through repression of the transcription of these two genes. Establishing the nucleotide sequences of *dapB* and *dapD* and identifying their transcription start site have ruled out the possible involvement of an attenuation mechanism (5, 21). The relatively high level of expression of *dapB* and *dapD* in the absence of lysine, as monitored from *lacZ* fusions, in contrast to their poor promoter signals, has led to the conclusion that involvement of a repressor, with lysine acting as a coeffector, was unlikely. Conversely, it was suggested that an activating molecule, antagonized by lysine, might be involved in *dapB* and *dapD* transcription (5, 21). Here, we report the identification of such an activator acting at the *dapB* promoter and target of the repressive lysine signals.

MATERIALS AND METHODS

Strains, cultures, and β-galactosidase assays. The standard wild-type strain MG1655 (F^- λ^- *rph-1*) (1) and the recipient strain for the *lacZ* fusions, pop3125 [F^- *araD139* Δ(*argF-lac*)*U169 deoC1 flbB5301 rpsL150 relA1 ptsF25 rbsR* Φ(*malP-lac*)] (7) were from the laboratory collection. Growth of bacterial cells was performed aerobically at 37°C in rich (LB) or minimal (M63) medium (16). β-Galactosidase assays were performed, and activity was quantified as previously described (16).

Recombinant DNA techniques. Isolation of plasmid DNA, PCR amplification using *Taq* DNA polymerase, digestion with restriction enzymes, ligation with T4 DNA ligase, and transformation of *E. coli* were carried out according to published protocols (22). The oligonucleotides used in this work are listed in Table 1.

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TABLE 1. Oligonucleotides used in this study

Name	Sequence (5′→3′)
B1	GCATAGCTATTCTCTTTTG
B2	GCATGCAGTCATTCATCG
B3	TCGACTCATGCCTTTCAC
B4	ATCCCTCCCTGTTTATC
B5	CATTAATTTCTAATTATCAGCG
B6	TGGCGGCGTAGCGATGCGC
B7	GGGGGAATTCAAAATGAGGGTATTTGCGCC
B8	GGGGGAATTCAACACAACCAGGGCGCG
B9	CCCGCCGACGGCTTCGGTATATGCAACCTG ACACAAAATTGTGTCATAGTGCAGGGTGT AGGCTGGAGCTGCTTC
B10	GAGGACAACGCCTGATATGTGCTATCAGGC ATTATTTGATGGATTAATCCTGACGCATA TGAATATCCTCCTTTAG
B11	GGGGGAATTCATTTACCAGGAGCAGACAA CAGC
B12	GGGGAAGCTTTTTACGTGGGTTTGTCGC CAGC
argO1	GAACTTGGTGCATCGTCG
argO2	GCATTTTGTGGACCGAGCGG

Construction of *dapB-lacZ* fusions. The 154-bp SphI-NsiI DNA fragment carrying the *dapB* promoter region in plasmid pDB17 (5) was purified and cloned in vector pSB118 (3), yielding plasmid pDB68. The resulting 212-bp EcoRI DNA fragment was cloned in the promoter-probe plasmid pOM41 (26) and inserted by homologous recombination into the chromosome of strain pop3125, upstream of the Φ(*malP-lacZ*) transcriptional fusion, as described previously (11), leading to strain JCP75. Strain JCP76 was constructed similarly, except that a 120-bp EcoRV-NsiI DNA fragment carrying the *dapB* promoter was used. The constructions in strains JCP75 and JCP76 were checked by PCR amplification from chromosomal DNA using appropriate oligonucleotides.

Construction of *argP*-carrying plasmids. Chromosomal DNA was extracted from strain MG1655 cells with the DNeasy tissue kit (Qiagen), according to the manufacturer's protocols, and partially digested with Sau3A. DNA fragments were ligated with BamHI-digested plasmid pCL57, a Bluescript derivative in which the *bla* gene has been replaced by a gene encoding spectinomycin resistance (6), and introduced by electrotransformation into strain JCP75. The transformed cells were plated on MacConkey-lactose agar in the presence of spectinomycin (100 $\mu g \cdot ml^{-1}$) and lysine (250 mM). The *argP*-carrying plasmid isolated in that experiment was then used as a template to amplify by PCR the *argP* reading frame with primers B11 and B12 (Table 1). After cleavage with EcoRI and HindIII, the *argP* fragment was cloned in pBAD18, downstream of the *araB* promoter (12).

Construction of *argP* strains. Disruption of *argP* was performed as described previously (9). A DNA fragment carrying a chloramphenicol resistance gene was amplified using plasmid pKD3 as a template and primers B9 and B10 (Table 1), which created 55-bp extensions homologous to *argP* borders. Competent JCP75 cells, containing the lambda Red recombinase expression plasmid pKD46 (9), were electrotransformed with 100 ng of amplified DNA treated with DpnI and plated on LB agar in the presence of 30 $\mu g \cdot ml^{-1}$ chloramphenicol. After the transformants were streaked on LB agar plates without antibiotics to lose the helper plasmid, Amp^s Cam^r clones were purified, leading to strain JCP95, which was checked by PCR (with primers B7 and B8) for the *argP* deletion. The *argP* mutation was then introduced into JCP76 and MG1655 by P1 transduction and selection for chloramphenicol resistance, leading to strains JCP96 and JCP97, respectively.

Electrophoretic mobility shift experiments (EMSE). ArgP protein bearing a C-terminal His_6 tag (His_6-ArgP) was produced from strain BL21(DE3) transformed with pHYD1705, a derivative of plasmid pET21b (17). Expression and purification of His_6-ArgP were done as described previously (17). Strains BL21(DE3)/pHYD1705 grown in rich medium with 0.5 mM isopropyl-β-D-thiogalactopyranoside and MG1655/pBAD18-$argP^+$ grown in rich medium with 1% arabinose overexpress His_6-ArgP and intact ArgP, respectively. Crude extracts were prepared from these strains, as described previously (2). A 392-bp DNA fragment carrying the *argO* promoter region (17) or DNA fragments carrying various segments of the *dapB* promoter region were synthesized by PCR in the presence of 20 μCi of [α-^{32}P]dATP, using appropriate pairs of oligonucleotide primers (O1 and O2 for *argO*) (Fig. 1; also see Fig. 3 for *dapB*) and chromosomal DNA or plasmid pDB17 (5) as templates. Then, 10-ng aliquots of the labeled DNA fragment were incubated for 15 min at room temperature with either various concentrations of purified His_6-ArgP or 1 μg of crude extract in 20 μl of buffer B (2) supplemented with 50 mM NaCl and 1 μg of poly(dI-dC)/poly(dI-dC) competitor DNA (Pharmacia). The binding mixture was loaded on 5% polyacrylamide gels under 6-V/cm voltage and run at a constant voltage of 12 V/cm. After electrophoresis, the gels were dried and the DNA bands were revealed by autoradiography or phosphorimager analysis (Fuji FLA-3000 and Multi Gauge v3.0 software).

RESULTS

Defining the *dapB* regulatory region. A *dapB-lacZ* translational fusion carried by a multicopy plasmid and harboring 104 bp upstream of the *dapB* transcription start site is active and repressed by lysine (5). To further delineate the region of the *dapB* promoter involved in lysine-mediated repression, we constructed two *dapB-lacZ* transcriptional fusions that were integrated into the chromosome. Both fusions share the same downstream border that overlaps the *dapB* translational start codon, whereas they extend either 81 bp or 118 bp upstream of the *dapB* transcription start site (Fig. 1). The two strains harboring these fusions were grown in minimal glucose medium, with or without lysine or arginine, and β-galactosidase was assayed (Table 2). The larger fusion (strain JCP75) behaved as the fusion carried by a multicopy plasmid and showed a fourfold reduction in β-galactosidase synthesis in the presence of lysine (5). Only chemostat-induced lysine limitation can lead to a 10-fold derepression of the *dapB* gene (20). It also exhibited a twofold reduction in the presence of arginine. The shorter fusion (strain JCP76) was expressed at a much lower level and was insensitive to the presence of lysine or arginine. Therefore, some sequences located upstream of −81 enhance *dapB* transcription and are necessary for repression by lysine or, to a lesser extent, by arginine. These data suggest that this region

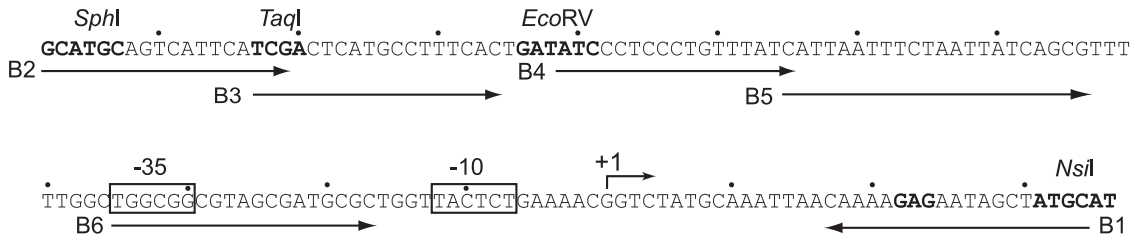

FIG. 1. Sequence of the *dapB* regulatory region. The shortest arrow indicates the *dapB* transcription start site (+1), and the corresponding −10 and −35 sequences are boxed. Relevant restriction sites are shown in boldface type, as is the ribosome binding site. The longer arrows indicate the various oligonucleotides used to amplify that region (B1 to B6).

TABLE 2. Regulation of *dapB* transcription by lysine, arginine, and ArgP

Strain	Relevant genotype[a]	β-Galactosidase activity (Miller units) in[b]:			
		Minimal glucose	Minimal glucose + lysine (10 mM)	Minimal glucose + arginine (10 mM)	Minimal glucose + arginine (10 mM) + lysine (10 mM)
JCP75	Φ(*dapBp*[−118/+35]-*lac*)	99	23	57	24
JCP95	Φ(*dapBp*[−118/+35]-*lac*) Δ*argP*::Cam^r	21	20	21	20
JCP76	Φ(*dapBp*[−81/+35]-*lac*)	10	11	10	11
JCP96	Φ(*dapBp*[−81/+35]-*lac*) Δ*argP*::Cam^r	11	11	10	11

[a] The extent of the DNA fragment carrying *dapBp* is indicated between brackets.
[b] Cells were grown aerobically at 37°C in M63 glucose. Overnight cultures were diluted 100-fold in the same medium and grown to an optical density at 600 nm of 0.8.

contains a binding site for a protein that activates transcription at the *dapB* promoter and that is the target of lysine repression.

Identification of an activator of *dapB* transcription. Repression by lysine of the larger *dapB-lacZ* fusion carried by strain JCP75 can be visualized on MacConkey lactose agar plates where colonies turn from red (Lac$^+$) to white (Lac$^-$) in the presence of a high concentration (250 mM) of lysine. We took advantage of this phenotype to screen for a gene encoding an activator of *dapB* transcription, assuming that its presence on a multicopy plasmid would reverse the negative effect of lysine. We built a genomic library of *E. coli* strain MG1655 in pCL57, a high-copy-number plasmid allowing selection for spectinomycin resistance (see Materials and Methods for details). The library was introduced by transformation in strain JCP75, and the transformants were plated on MacConkey lactose agar supplemented with spectinomycin and lysine. Among approximately 18,000 independent colonies, seven clones exhibited a reddish color. They were purified, and their plasmids were extracted and transformed back into JCP75. Only one of these plasmids was able to reconstitute the original derepressed phenotype on screening plates. It was named pDB102 and was shown to carry a 1.7-kb DNA fragment. Sequencing the borders of the insert indicated that this fragment contains only one complete reading frame, *argP*, which encodes a member of the LysR family of activating proteins (8, 23). The *argP* gene, including 183 bp of upstream regulatory sequence, was PCR amplified from the chromosome of strain MG1655, using oligonucleotides B7 and B8 (Table 1), and cloned in the unique EcoRI site of pCL57, yielding plasmid pDB105. Upon transformation into strain JCP75, this plasmid led to red colonies on MacConkey lactose agar plates supplemented with lysine, confirming that ArgP is able to enhance *dapB* transcription.

ArgP controls *dapB* transcription. To assess the importance of ArgP in *dapB* expression, we inactivated the *argP* gene using the lambda Red recombinase procedure (9), as detailed in Materials and Methods. The *argP* strains carrying the longer or the shorter fusion were named JCP95 and JCP96, respectively. They were grown in minimal glucose medium, with or without lysine, and assayed for β-galactosidase activity (Table 2). Inactivating *argP* had no effect on the shorter fusion (JCP96), which remained at the same low level as its *argP*$^+$ parent (JCP76) and was still insensitive to lysine. Conversely, the absence of ArgP strongly decreased expression of the larger fusion (compare JCP75 and JCP95) and eliminated repression by lysine.

A reciprocal experiment was conducted in which ArgP was brought back to strain JCP95. The *argP* gene was put under the control of the arabinose-inducible promoter *araBp* in plasmid pBAD18 (12). Strain JCP95 was transformed with this plasmid (as well as with the empty parent vector), and expression of the *dapB-lacZ* fusion was monitored after addition of arabinose to the growth medium. As shown in Fig. 2, induction of ArgP synthesis was soon followed by a sharp stimulation of *dapB-lacZ* transcription. Altogether, these data indicate that ArgP is able to activate transcription from the *dapB* promoter, provided that some sequences upstream of −81 are present, and is necessary for lysine to repress *dapB* expression.

ArgP binding to the *dapB* promoter. To further investigate ArgP-mediated activation of *dapB* transcription, we purified His-tagged ArgP protein (17) and used it to perform EMSE. Radioactively labeled DNA fragments encompassing the *dapB* promoter region were synthesized by PCR, using oligonucleotides that created a fixed downstream border and a variable upstream one (Fig. 1). Incubation with His_6-ArgP gave a single retarded band with DNA fragments extending up to −118 or −103 but none with the shorter fragments extending up to −81, −65, or −35 (Fig. 3A), demonstrating the existence of a binding site for ArgP located, at least in part, between positions −103 and −81.

Strain JCP97 (MG1655 Δ*argP*::Cam^r), expressing no ArgP, was transformed with the multicopy plasmid pDB105, expressing intact ArgP. The two strains were grown in rich medium until mid-exponential phase, and crude extracts were prepared and used to perform EMSE. Crude extracts enriched in ArgP

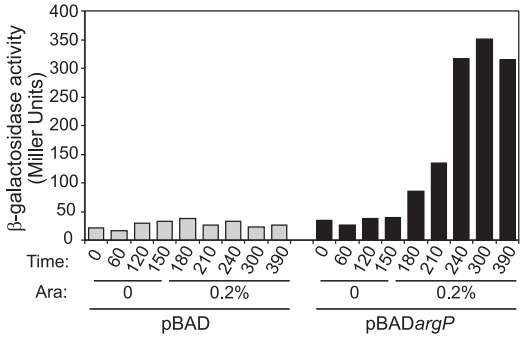

FIG. 2. ArgP activates *dapB* transcription. Strain JCP95 transformed with either pBAD or pBAD*argP* was grown in minimal glucose medium, and β-galactosidase activity was assayed at regular intervals. Arabinose (0.2% final concentration) was added 150 min after the start of the cultures.

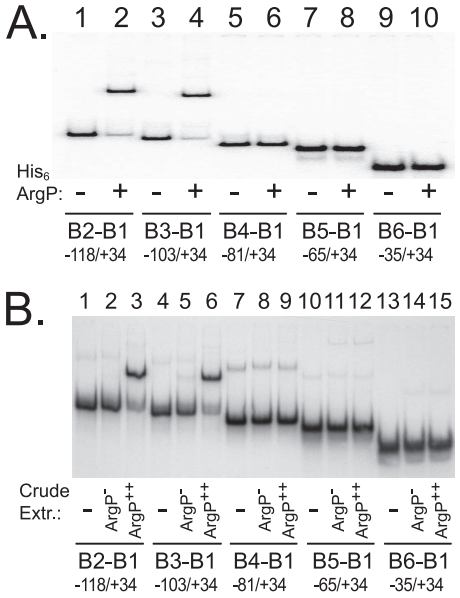

FIG. 3. ArgP binds a site upstream of the *dapB* promoter. DNA fragments, extending from +34 to the upstream locations indicated at the bottom, were incubated in plain buffer (lanes −), with 1.5 μM of purified His_6-ArgP (lanes + in panel A), or with crude extracts from the *argP* mutant JCP97 (lanes $ArgP^-$ in panel B) or from strain JCP97 harboring *argP* on a multicopy plasmid (lanes $ArgP^{++}$ in panel B). Samples were loaded on a polyacrylamide gel, run, and analyzed by autoradiography.

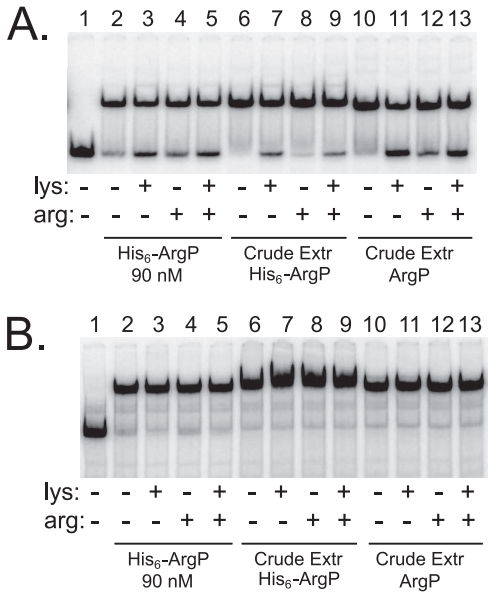

FIG. 5. Effects of lysine and arginine on binding of ArgP at *dapB* and *argO*. DNA fragments carrying the *dapB* (A) and *argO* (B) binding sites for ArgP were incubated alone (lanes 1), with 90 nM of purified His_6-ArgP (lanes 2 to 5), or with crude extracts (1 μg total protein) containing overexpressed His_6-ArgP (lanes 6 to 9) or overexpressed intact ArgP (lanes 10 to 13). Lysine or arginine (10 mM) was added as indicated. Samples were loaded on a polyacrylamide gel, run, and analyzed by autoradiography.

gave a single retarded band of mobility similar to that observed with purified His_6-ArgP protein, whereas crude extracts devoid of ArgP gave no retarded band (Fig. 3B; see also Fig. 5A), indicating that no protein other than ArgP is able to bind significantly to the *dapB* promoter region.

EMSE performed with various concentrations of His_6-ArgP demonstrated an apparent K_d of 35 nM for the binding of ArgP to the *dapB* regulatory region (Fig. 4). As a control, we also performed EMSE with a mixture of two DNA fragments, the −118/+35 *dapB* fragment, and a −293/+109 *argO* fragment shown to harbor a binding site for ArgP with an apparent K_d of 6.5 nM (17). In agreement with these data and those from Fig. 4, we observed that binding of His_6-ArgP to *argO* occurred at protein concentrations much lower than binding to *dapB* (data not shown).

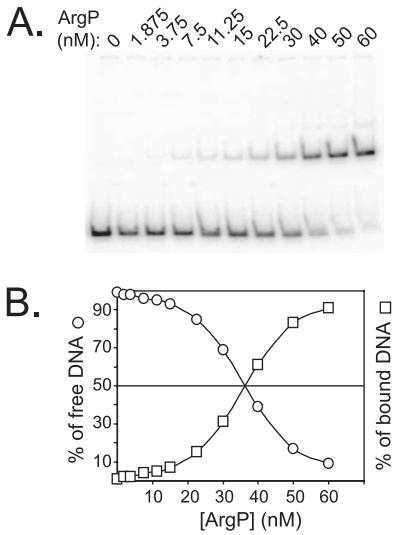

FIG. 4. Apparent K_d for ArgP binding near the *dapB* promoter. (A) The −118/+34 *dapB* DNA fragment was incubated with the indicated concentrations of purified His_6-ArgP, loaded on a polyacrylamide gel, run, and analyzed on a phosphorimager. (B) The percentages of free and retarded DNA were quantified and plotted against the concentration of His_6-ArgP.

Finally, we also investigated the effects of lysine and arginine on the binding of ArgP. As expected from earlier work (17), neither lysine nor arginine affected the binding of ArgP (or His_6-ArgP) to *argO* (Fig. 5B). In contrast, lysine and arginine could partially displace ArgP from retarded complexes with *dapB* (Fig. 5A). This effect was observed with both intact ArgP or His_6-ArgP in crude extracts and purified His_6-ArgP. Similar experiments showed that the retarded complex was insensitive to the presence of D-lysine, ornithine, or DAP (data not shown). Therefore, binding of ArgP is specifically relieved by L-lysine and L-arginine.

Altogether, these in vitro results are in good agreement with those obtained in vivo, indicating that *dapB-lacZ* fusions harboring a fragment extending up to −118 or −103 are transcribed at a higher level and are subject to lysine repression, whereas a fusion extending up to −81 is not.

DISCUSSION

A DNA region located between 81 and 118 bp upstream of the *dapB* transcription start site is required for full expression of *dapB*, as well as for its repression by lysine. Gel retardation

experiments using both purified His-tagged ArgP and crude cell extracts with or without ArgP showed that the same region is necessary for binding ArgP in a lysine-dependent manner. Altogether, these results indicate that *dapB* transcription is controlled by the activating protein ArgP, whose binding upstream of −81 is antagonized by lysine. Previous analysis of the *dapB* regulatory region had led us to propose such a regulatory mechanism, i.e., an apparent repression resulting from the loss of activation (5).

There is a strict correlation between the presence of ArgP and a high level of lysine-repressed *dapB* transcription, provided that the *dapB* regulatory region contains the necessary *cis*-acting upstream sequences. Interestingly, lysine is known to virtually abolish transcription activation of *argO*, a bona fide ArgP target. ArgP belongs to the family of LysR-type regulatory proteins, most of which use a small effector molecule as a coactivator (23), whereas lysine appears to inhibit ArgP function.

ArgP is essential for *argO* transcription (17), as LysR itself is essential for *lysA* transcription (24). However, ArgP plays only an auxiliary role in *dapB* transcription, enhancing its level about fourfold under our experimental conditions. An *argP* mutation does not lead to a DAP^- phenotype, but the associated osmosensitivity observed in some genetic backgrounds (18) might reflect a reduced level of DAP. Evidently, a full dependence on ArgP would abolish *dapB* transcription in the presence of lysine, whereas synthesis of DAP needs to be sustained. Recruiting a lysine-sensitive activating protein to boost *dapB* transcription and synthesis of a lysine precursor under growth conditions where lysine becomes limiting is an efficient and economical regulatory strategy that allows the cell to maintain a basal level of DAP synthesis independently of the lysine concentration.

The molecular mechanisms of lysine repression of ArgP-dependent transcription appear to differ betweeen *argO* and *dapB*. Lysine traps RNA polymerase at the *argO* promoter, where it is recruited by ArgP (14), whereas lysine prevents binding of ArgP to the *dapB* promoter region. These differences might reflect a different topology of interactions between ArgP and RNA polymerase at the *argO* and *dapB* promoters. A putative primary ArgP binding site, ATTAGTTTTTCT GAT, where the conserved T-N11-A motif of the LysR-type protein binding site is underlined, is centered at −63 in the *argO* promoter (14). Our results show that ArgP binding requires sequences located upstream of −81 in the *dapB* promoter. Comparison of the genomes of various *E. coli* strains indicates a 100% conservation of the *dapB* regulatory region up to −98, where it suddenly diverges (data not shown). Only one potential ArgP binding site can be found in the −81/−98 interval, ATGCCTTTCACTGAT, which is centered at −89 and is conserved in the *dapB* region of closely related enterobacteria of the genera *Shigella* and *Salmonella* (data not shown). If functional, this ArgP binding site at *dapB* would be on the opposite side of the double helix compared to *argO*, which would significantly alter ArgP interaction with RNA polymerase. While this paper was under revision, very similar results were reported for lysine-sensitive activation of the *Klebsiella pneumoniae gdhA* gene by ArgP, and similar conclusions were drawn (10).

The mechanisms of lysine repression of two other genes involved in DAP biosynthesis, *asd* and *dapD*, remain obscure. The same rationale could be at work, namely, a basal level of transcription ensuring DAP synthesis whatever the environmental conditions and its lysine-modulated enhancement by an activator. Our preliminary experiments suggest that ArgP could also play this role.

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