JOURNAL OF BACTERIOLOGY, June 2004, p. 3539–3546 Vol. 186, No. 11
0021-9193/04/$08.00+0 DOI: 10.1128/JB.186.11.3539–3546.2004

Evidence for an Arginine Exporter Encoded by *yggA* (*argO*) That Is Regulated by the LysR-Type Transcriptional Regulator ArgP in *Escherichia coli*

Madhusudan R. Nandineni[1,2] and J. Gowrishankar[2]*

Centre for Cellular and Molecular Biology, Hyderabad 500007,[1] and Laboratory of Bacterial Genetics, Centre for DNA Fingerprinting and Diagnostics, Hyderabad 500076,[2] India

Received 9 November 2003/Accepted 19 February 2004

The anonymous open reading frame *yggA* of *Escherichia coli* was identified in this study as a gene that is under the transcriptional control of *argP* (previously called *iciA*), which encodes a LysR-type transcriptional regulator protein. Strains with null mutations in either *yggA* or *argP* were supersensitive to the arginine analog canavanine, and *yggA-lac* expression in vivo exhibited *argP*$^+$-dependent induction by arginine. Lysine supplementation phenocopied the *argP* null mutation in that it virtually abolished *yggA* expression, even in the *argP*$^+$ strain. The dipeptides arginylalanine and lysylalanine behaved much like arginine and lysine, respectively, to induce and to turn off *yggA* transcription. Dominant missense mutations in *argP* (*argP*^{d}) that conferred canavanine resistance and rendered *yggA-lac* expression constitutive were obtained. The protein deduced to be encoded by *yggA* shares similarity with a basic amino acid exporter (LysE) of *Corynebacterium glutamicum*, and we obtained evidence for increased arginine efflux from *E. coli* strains with either the *argP*^{d} mutation or multicopy *yggA*$^+$. The null *yggA* mutation abolished the increased arginine efflux from the *argP*^{d} strain. Our results suggest that *yggA* encodes an ArgP-regulated arginine exporter, and we have accordingly renamed it *argO* (for "arginine outward transport"). We propose that the physiological function of *argO* may be either to prevent the accumulation to toxic levels of canavanine (which is a plant-derived antimetabolite) or arginine or to maintain an appropriate balance between the intracellular lysine and arginine concentrations.

Canavanine (CAN) is a plant-derived antimetabolite that, by virtue of its chemical similarity to arginine (Arg), inhibits the growth of bacteria after its competitive misincorporation into polypeptides in place of Arg (40). In *Escherichia coli*, mutations conferring Can^r have accordingly been obtained in the genes *argR* and *argS*, which encode the apo-repressor for enzymes of Arg biosynthesis and the arginyl-tRNA synthetase, respectively (19, 28).

Evidence exists for three different periplasmic-binding-protein-dependent Arg transporters in *E. coli* that presumably also mediate CAN uptake (reviewed in references 19 and 34). The periplasmic proteins for the three transporters are the LAO protein (which binds the basic amino acids lysine [Lys], Arg, and ornithine), the AO protein (which binds Arg and ornithine), and the ArtJ protein (which binds Arg). Can^r mutations have been identified in some genes that have been implicated in Arg uptake, including *abpS*, *argP*, and *argK*. It has been proposed that *abpS* encodes the AO protein (10; also see reference 34) and that the products of *argP* and *argK* regulate the activities of the different Arg transporters (11–14, 35, 40).

A molecular analysis of *argP* undertaken by Celis (12) surprisingly revealed that it is identical to *iciA*, whose product had previously been implicated as both an inhibitor of chromosomal replication initiation from *oriC* in vitro (23, 24, 41) and an activator of transcription from the *dnaA* 1P (29, 30) and *nrd* (21) promoters. The ArgP (IciA) protein is a member of the LysR family of transcriptional activators (39). The dominant *argP55* mutation (*argP*^{d}) conferring Can^r was deduced to cause a Pro-to-Ser substitution at residue 274 (P274S) in the 297-amino-acid protein (12). Based on data from in vitro studies, Celis suggested that wild-type ArgP both represses its own transcription and activates that of *argK*; he also sought to explain the Can^r phenotype associated with the *argP*^{d} mutation on the assumption that it is a loss-of-function (that is, dominant-negative) allele whose product is unable to efficiently activate the genes involved in Arg uptake, including *argK* (12).

In theory, perturbations in Arg or CAN efflux may also be expected to affect the CAN tolerance of a bacterial cell. However, an Arg efflux system is not known to exist in *E. coli*, and indeed only a few amino acid exporters have been characterized for any bacterium (reviewed in references 1, 9, and 17). The amino acid exporters so far identified for *E. coli* include RhtB and RhtC, for threonine and homoserine (27, 43), and YdeD and YfiK, for cysteine and *O*-acetylserine (16, 18). The exporter LysE, for both Lys and Arg, has been well characterized in *Corynebacterium glutamicum*, and the primary sequence of LysE is similar to the product of an *E. coli* open reading frame called *yggA* (3, 7, 8, 42). Interestingly, the synthesis of LysE in *C. glutamicum* is under the transcriptional control of LysG, which in turn is related in its primary sequence to *E. coli* ArgP (3, 42).

We show in this study that strains with null mutations in either *argP* or *yggA* exhibit abnormally increased sensitivities to CAN (Can^{ss}) and that ArgP is a transcriptional regulator of *yggA* that mediates the latter's induction by Arg. Intracellular Lys, on the other hand, mediates a reduction in *yggA* expression, apparently by abolishing the activating role of ArgP.

* Corresponding author. Mailing address: Laboratory of Bacterial Genetics, Centre for DNA Fingerprinting and Diagnostics, ECIL Rd., Nacharam, Hyderabad 500 076, India, Phone: 91-40-27155609. Fax: 91-40-27155610. E-mail: shankar@cdfd.org.in.

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

Strain	Genotype[b]
MC4100	Δ(*argF-lac*)*U169 rpsL150 relA1 araD139 flbB5301 deoC1 ptsF25*
SK2226	Δ(*gpt-proA*)*62 trpE3 hisG4 galK2 xylA7 lac mtl-1 glnV44* Δ*argH1 zif-290*::Tn*10*
GJ4536	MC4100 *argP202*::λp*lac*Mu55(Kan)
GJ4674	*zbh-900*::Tn*10*dKan(Ts)*1 lacZ4525*::Tn*10*dKan *lon-103*::IS*186 serA*
GJ4676 to GJ4682[c]	*serA*$^+$ *argO*::Tn*10*dTet derivatives of GJ4674
GJ4748	MC4100 *argR64 zhb-914*::*Tn10d*Cm
GJ4822	MC4100 *argO204*::Tn*10*dTet
GJ4894	GJ4748 *argO204*::Tn*10*dTet
GJ4895	GJ4748 *argP202*::λp(*lacZ*::Tn*10*dTet)Mu-Kan

[a] Strain MC4100 (20) was described earlier, and SK2226 was obtained from the *E. coli* Genetic Stock Center. The other strains were obtained in this study.
[b] Genotype designations are as described in the work of Berlyn (4). As described in the text, *argO* is the new designation proposed for the anonymous open reading frame denoted *yggA* in the work of Blattner et al. (5), and the two have been used interchangeably in this paper.
[c] Strains GJ4676 to GJ4682 are further described in Table 4.

Furthermore, Can^r *argP^d* mutations represent gain-of-function, not loss-of-function, alleles that render *yggA* expression constitutive. Derivatives with an *argP^d* mutation or with multicopy *yggA*$^+$ exhibit increased Arg excretion. Our results indicate that *yggA* encodes an ArgP-regulated Arg exporter in *E. coli*.

MATERIALS AND METHODS

Bacterial strains and growth media. The genotypes of the *E. coli* strains used for this study are listed in Table 1. The defined and nutrient media were, respectively, minimal A medium (supplemented with 0.2% glucose and the appropriate auxotrophic requirements) and Luria-Bertani (LB) medium (33), and the growth temperature was 37°C. All amino acids, all dipeptides, and CAN were L-isomers. Unless otherwise indicated, supplementation with Arg, Lys, and the dipeptides was done at a concentration of 1 mM. The concentrations of antibiotics used were described previously (38). Trimethoprim was used in nutrient and defined media at 60 and 30 μg/ml, respectively.

Phages and plasmids. The transposon vehicle phage λNK1323, used for the generation of transpositions of Tn*10*dTet, has been described elsewhere (25, 33). Phages λ 471 and λ 472, from the ordered *E. coli* genomic library of Kohara et al. (26), were obtained from K. Isono.

The plasmid vectors employed were as follows (with salient features in parentheses): (i) pMU575 (IncW based, single copy number, trimethoprim resistant, carries a promoterless *lacZ* gene for the generation of promoter-*lac* operon fusions) (2), (ii) pCL1920 (pSC101 based, low copy number, spectinomycin and streptomycin resistant) (31), (iii) pBR329 (pMB9 based; high copy number; ampicillin, tetracycline, and chloramphenicol resistant) (15), and (iv) pBluescript II KS (pMB9 based, very high copy number, ampicillin resistant) (Stratagene, La Jolla, Calif.).

The following plasmids were constructed in this study. Plasmids pHYD915, pHYD933, and pHYD949 are derivatives of pCL1920 carrying the following fragments from the λ phages described by Kohara et al. (26) subcloned into the appropriate sites of the vector: a 1.8-kb SalI fragment containing *argP*$^+$ from λ 471, a 2.2-kb BamHI fragment containing *argK*$^+$ from λ 471, and a 1.2-kb PstI-HindIII fragment containing *yggA*$^+$ from λ 472, respectively. Plasmid pHYD952 carries the 1.2-kb PstI-HindIII fragment containing *yggA*$^+$ cloned (via an intermediate step of cloning into pBluescript II KS) into the BamHI-HindIII sites of pBR329, and plasmid pHYD954 carries a 1.1-kb PstI-PvuII fragment containing *yggA′* (truncated at its 3′ end) cloned into the PstI-EcoRV sites of pBluescript II KS. The latter *yggA′* construct was also subcloned from pHYD954 on a PstI-HindIII fragment (HindIII end derived from the multiple-cloning-site region of the vector) into the corresponding sites of plasmid pMU575 to generate pHYD956 (with a *yggA-lac* operon fusion). Plasmids pHYD926 through pHYD932, with missense mutations in *argP* conferring Can^r that were obtained by nitrosoguanidine mutagenesis of pHYD915, are described below. The 1.8-kb SalI fragment containing the *argP^d* allele from pHYD926 was subcloned into the SalI site of pBR329 to generate plasmid pHYD953.

Tests for CAN tolerance. The CAN tolerance of strains was tested in glucose-minimal A medium supplemented with various concentrations of CAN, with growth being scored after 24 h at 37°C. In some cases, uracil was added at 40 μg/ml to the medium to enhance the toxicity imposed by a given CAN concentration (19). Typically, the wild-type *E. coli* strain (Can^s) was resistant to 20 μg of CAN/ml but was sensitive to 65 μg of CAN/ml in the presence of uracil. Can^{ss} strains were sensitive even to the former concentration, and Can^r strains were resistant even to the latter one.

Test for Arg cross-feeding. Approximately 10^5 cells of the Δ*argH* strain SK2226 (or its pBR329 transformant derivative), contained in a 100-μl volume of Luria-Bertani medium, were added to a petri dish with 20 ml of glucose-minimal A agar supplemented with proline, tryptophan, and histidine (that is, all of the auxotrophic requirements of SK2226 except Arg). The agar medium also contained the indicator tetrazolium chloride at 1 μg/ml (33). The test strains were spotted in quadrants on the agar surface, and Arg cross-feeding was visualized as red haloes of syntrophic growth of the auxotrophic strain around the spots.

Isolation and sequence analysis of Can^r *argP* mutants. To obtain mutations in plasmid pHYD915 (carrying *argP*$^+$) that conferred a Can^r phenotype, we mutagenized the strain MC4100/pHYD915 with nitrosoguanidine according to a previously described procedure (33). A plasmid DNA preparation made from the pool of mutagenized cells was then used to transform an *argP202* null mutant, strain GJ4536, and individual transformants were scored for Can^r. The DNA sequence of the insert region in each of the mutant plasmids conferring Can^r was determined with the aid of one pair of *lac* primers (recognizing sequences in the vector that flank the insert) as well as another pair of primers internal to *argP*, namely ARGP1 (5′-GGGCGCGAACTCGCTGAGCGA-3′) and ARGP2 (5′-GAGCAAGTTGTACGAACGCTT-3′).

Localized insertional mutagenesis near *serA* with Tn*10*dTet and molecular genetic analysis of mutants. The wild-type strain MC4100 was subjected to random insertional mutagenesis with the Tn*10*dTet transposon following infection with λ NK1323, as described elsewhere (25, 33). A phage P1 lysate prepared on a pool of Tet^r clones was used to transduce the *serA* mutant strain GJ4674 simultaneously to the phenotype Ser$^+$ Tet^r. Transductants so obtained on double selection plates were expected to have Tn*10*dTet insertion mutations in the vicinity of the *serA* locus.

To determine the sites of Tn*10*dTet insertion in the mutants, we transduced one insertion allele into MC4100 and used the resulting strain, GJ4822, for inverse PCR as follows. A template preparation of circularized fragments of Sau3A1-digested chromosomal DNA from GJ4822 was subjected to PCR with a pair of divergently oriented primers, AH1 and AH2, designed for the ends of Tn*10*dTet (22), and one of the products so obtained was sequenced with the same primers.

Upon the identification of *yggA* as the gene disrupted by Tn*10*dTet in GJ4822, the other Tn*10*dTet insertions were molecularly characterized and sequenced after PCRs with the aid of (i) two chromosomal primers flanking *yggA* and its adjacent gene, *yggB* (YGGAR, 5′-ACCTCTGGA*TC*CAAGCTTAG-3′, and YGGBF, 5′-TCCAGGAA*TT*CAACGCGATCGA-3′; mismatches to the genome sequence [5] are indicated in italics), and (ii) two more previously described primers, namely TetF (5′-TGGTCACCAACGCTTTTCCCGAG-3′) and TetR (5′-CTGTTGACAAAGGGAATCATAG-3′) (36), that read outward from either end of Tn*10*dTet.

Other techniques. The procedures for phage P1 transduction (20) and for various recombinant DNA manipulations (37) were done as previously described. β-Galactosidase specific activity measurements in *yggA′-lac* and *argP-lac* strains were made as described by Miller (33); the values are reported in Miller units and represent the averages of at least four independent determinations, with the variations between individual values being <20%.

TABLE 2. CAN tolerance or sensitivity of *argP* and *yggA* strains

Strain	Genotype[a]	CAN tolerance[b]
MC4100	WT	S
GJ4536	*argP202*	SS
GJ4536/pHYD915	*argP202*/*argP*$^+$	S
GJ4748	*argR64*	R
GJ4536/pHYD926	*argP202*/*argP*^{d}	R
MC4100/pHYD926	WT/*argP*^{d}	R
GJ4822	*yggA*	SS
GJ4822/pHYD949	*yggA*/low-copy-number *yggA*$^+$	S
GJ4822/pHYD952	*yggA*/multicopy *yggA*$^+$	R
GJ4822/pHYD954	*yggA*/multicopy *yggA′*	S
GJ4822/pHYD956	*yggA*/low-copy-number *yggA′*	SS
GJ4822/pHYD926	*yggA*/*argP*^{d}	SS
MC4100/pHYD952	WT/multicopy *yggA*$^+$	R
GJ4536/pHYD952	*argP202*/multicopy *yggA*$^+$	S

[a] WT, wild type.
[b] R, S, and SS refer, respectively, to the Can^r, Can^s, and Can^{ss} phenotypes as defined in the text.

TABLE 3. Molecular analysis of plasmid-borne mutations in *argP* conferring Can^r in *argP202* strain GJ4536

Plasmid	Amino acid change (codon alteration)[a]	Dominance of mutation[b]
pHYD926[c]	S94L (T**C**A→T**T**A)	D
pHYD927	P108S (**C**CT→**T**CT)	D
pHYD928	V144M (**G**TG→**A**TG)	D
pHYD929	P217L (C**C**C→C**T**C)	D
pHYD930	L294F (C**T**T→**T**TT)	D
pHYD931	R295C (**C**GT→**T**GT)	R
pHYD932	A68V (G**C**A→G**T**A)	D

[a] The reference *argP* (*iciA*) sequence (297 sense codons) and annotation are from the work of Blattner et al. (5) (GenBank accession number AE000375). Amino acid alterations are given in a one-letter code. Mutated nucleotides in the corresponding codons are marked in bold.
[b] The mutation was classified as dominant (D) if the corresponding plasmid conferred Can^r in MC4100 (*argP*$^+$) and as recessive (R) if it did not.
[c] Plasmid pHYD926 also carries a silent synonymous codon substitution mutation (GG**G**→GG**A**) in the penultimate codon of the divergently transcribed open reading frame *yqfE* situated upstream of *argP* (see Fig. 2).

RESULTS

***argP* null mutants are Can^{ss}.** In work to be described elsewhere (M. R. Nandineni, R. Laishram, and J. Gowrishankar, unpublished data), we identified an insertion of the transposon phage λp*lac*Mu55(Kan) at codon 35 of the *argP* structural gene (designated *argP202*) that was associated with an osmosensitive phenotype for *E. coli* strains. In light of the report by Celis (12) that a presumptively dominant-negative *argP* mutant is Can^r, we tested the null *argP202* mutant strain GJ4536 for its CAN tolerance. Unexpectedly, it was Can^{ss}, that is, more sensitive to CAN (with a CAN MIC of approximately 2 μg/ml) than the wild-type strain (Table 2). A second independent *argP* insertion mutant was also Can^{ss} (data not shown).

For complementation studies, plasmid pHYD915 carrying the cloned *argP*$^+$ gene was constructed as described above. The plasmid was able to reverse the Can^{ss} phenotype of the *argP202* mutant (Table 2), indicating that the insertion mutation is recessive to *argP*$^+$.

Identification of *argP* mutants conferring Can^r. Our findings above suggested that the loss of *argP* function is associated with a Can^{ss} phenotype, whereas the *argP* P274S mutant previously described by Celis (12) was Can^r. In order to identify additional Can^r *argP* mutants, we mutagenized the *argP*$^+$ plasmid pHYD915 with nitrosoguanidine by the procedure described above and screened transformants of the *argP202* strain GJ4536 for Can^r.

Seven of about 800 transformants tested were Can^r, and in each of them the mutation was plasmid-borne (data not shown). The plasmids were designated pHYD926 through pHYD932, and a sequence analysis indicated that each carries a GC-to-AT missense mutation at a different site in *argP*, which was deduced to result in a single amino acid residue alteration in the encoded protein (Table 3).

When the mutant plasmids were introduced into strain MC4100, which carries *argP*$^+$ chromosomally, all but one conferred a Can^r phenotype, indicating that the mutations in them were dominant (Table 3). For the work described below, the *argP* S94L mutation in plasmid pHYD926 was used as a prototypic example of a missense *argP*^{d} mutation. The *argP* R295C mutation was inferred to be recessive to *argP*$^+$, since plasmid pHYD931 did not confer Can^r to MC4100.

Identification of insertions in *yggA* conferring Can^{ss}. The *argK* (also called *ygfD*) gene is situated 2 kb away from *argP* (which in turn is situated 2 kb away from *serA*) (Fig. 1) (5); it was previously suggested both that *argK* is transcriptionally regulated by *argP* and that mutations in *argK* confer Can^r (11–13). We accordingly undertook localized transposon (Tn*10*dTet) mutagenesis of the chromosomal region in the vicinity of *serA* by the method described above and scored the mutants for altered CAN tolerance. No Can^r mutants were obtained, but seven Tet^r derivatives were identified that were Can^{ss}. In every case, the Tet^r insertion was shown (i) to be 100% cotransducible with Can^{ss} and (ii) to confer Can^{ss} in MC4100 and other strain backgrounds (data not shown). The MIC of CAN for these insertion mutants, at about 0.5 μg/ml, was even lower than that for the *argP202* null mutant.

The CAN phenotype was not complemented by either plasmid pHYD915 (carrying *argP*$^+$) or pHYD933 (carrying *argK*$^+$) in any of the mutants (data not shown). As described above, the strategy of inverse PCR (based on primers designed for the ends of Tn*10*dTet) was then adopted to determine the site of Tn*10*dTet insertion in one of the Can^{ss} mutants. DNA sequence analysis of the resultant PCR products unexpectedly revealed that the insertion was in an open reading frame called *yggA* (encoding a putative polypeptide of 211 amino acids) situated 8 kb away from *argP* (and 10 kb from *serA*) (Fig. 1). Using a pair of primers flanking *yggA* (and its adjacent gene, *yggB*) and a second pair of primers designed to read outward

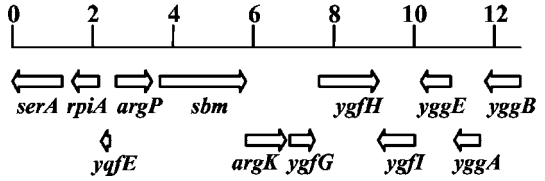

FIG. 1. Physical map of *serA-yggB* region of the *E. coli* genome at 69.5 min (clockwise going from left to right), as annotated in the work of Blattner et al. (5). Positions and transcriptional orientations of individual open reading frames are marked beneath the kilobase scale.

TABLE 4. Molecular analysis of Tn*10*dTet insertions in *yggA*

Strain	*yggA* (*argO*) allele[a]	Characteristics of Tn*10*dTet insertion		Site of YggA truncation[d]
		Coordinates of 9-bp duplicated region[b]	Orientation[c]	
GJ4676	*204*	8926–8934	I	V77
GJ4677	*205*	9046–9054	II	I37
GJ4678	*206*	8926–8934	II	V77
GJ4679	*207*	9010–9018	I	V49
GJ4680	*208*	9009–9017	II	V49
GJ4681	*209*	8923–8931	I	A78
GJ4682	*210*	9170–9178	II	—[e]

[a] Based on the results described in this paper, *yggA* has been redesignated *argO*.

[b] The coordinates of the 9-bp host sequence duplication after Tn*10*dTet insertion (25, 33), inferred from determination of the junction sequence at the counterclockwise end of the insertion, are given. The coordinates are based on the work of Blattner et al. (5) (GenBank accession number AE000375), according to which the *yggA* open reading frame extends from bp 9156 to 8521 on the complementary strand (211 sense codons).

[c] Orientation I is that in which the sequence (in the Tn*10*dTet insertion) for primer TetR is counterclockwise to that for primer TetF, and the reverse is true for orientation II.

[d] The residue number and identity (in one-letter code) of the terminal amino acid upstream of the insertion are indicated.

[e] —, the *argO210* insertion is situated 22 bp upstream of the *yggA* open reading frame.

from the two ends of Tn*10*dTet, we performed PCR experiments as described above to establish that the Tet^r insertions in all seven Can^{ss} mutants were situated in *yggA* (data not shown). The precise sites of the insertions were determined by sequencing of the PCR products, and the results summarized in Table 4 indicate that the insertions had occurred in both orientations at different sites in the proximal third of the *yggA* open reading frame (or in the region immediately upstream of its putative start codon).

Plasmids with the cloned minimal *yggA*$^+$ gene and its regulatory region in a 1.2-kb fragment (pHYD949 [low copy number, pSC101 based] and pHYD952 [high copy number, pBR329 based]) were able to complement a representative *yggA* insertion mutant, strain GJ4822, for CAN tolerance (Table 2). A smaller (1.1 kb) fragment, which was expected to encode a YggA′ polypeptide with a truncation of 14 amino acids at its C terminus, was also able to complement the *yggA* mutant, but only when it was present in a very-high-copy-number plasmid (pHYD954), not in an IncW-derived single-copy-number plasmid (pHYD956) (Table 2).

The *yggB* gene is situated immediately upstream of, and in the same transcriptional orientation as, *yggA* (Fig. 1). It encodes a mechanosensitive ion channel (32), and we determined that a Δ*yggB* mutant (kindly provided by Ian Booth) is unaffected in CAN tolerance (data not shown).

Transcriptional regulation of *yggA* by *argP* in vivo. Plasmid pHYD956 also carries the promoterless *lacZ* gene in the vector region downstream of the *yggA′* truncation, and we accordingly employed it as a *yggA-lac* operon fusion to study the transcriptional regulation of *yggA* in different strains. The results of these experiments are presented in Fig. 2, and the conclusions therefrom are summarized below.

(i) In *argP*$^+$ strains, *yggA* expression appeared to be induced by Arg, its precursor citrulline, or its analog CAN. An *argR* mutation that derepressed Arg biosynthesis also served to induce *yggA* expression, probably by increasing the intracellular Arg pools. The induction by Arg, citrulline, and in particular, CAN was most prominent in an *argR yggA* mutant strain (which, as discussed below, we believe is blocked for Arg and CAN export).

(ii) The inducing effects of Arg or its related compounds, as well as of the *argR* mutation, on *yggA* expression were completely dependent on *argP*$^+$ and were abolished by the introduction of the null *argP202* mutation. (For these experiments, the *argP-lac* fusion itself encoded by *argP202* was inactivated by a Tn*10*dTet insertion in *lacZ*.) The addition of Lys also appeared to mimic the effect of the *argP* null mutation in that it served to nearly completely repress *yggA* expression, even in the *argP*$^+$ strains.

(iii) Finally, in an *argP*^{d} S94L mutant background, *yggA-lac* expression was rendered high and constitutive by all coeffectors, including Arg, CAN, and Lys.

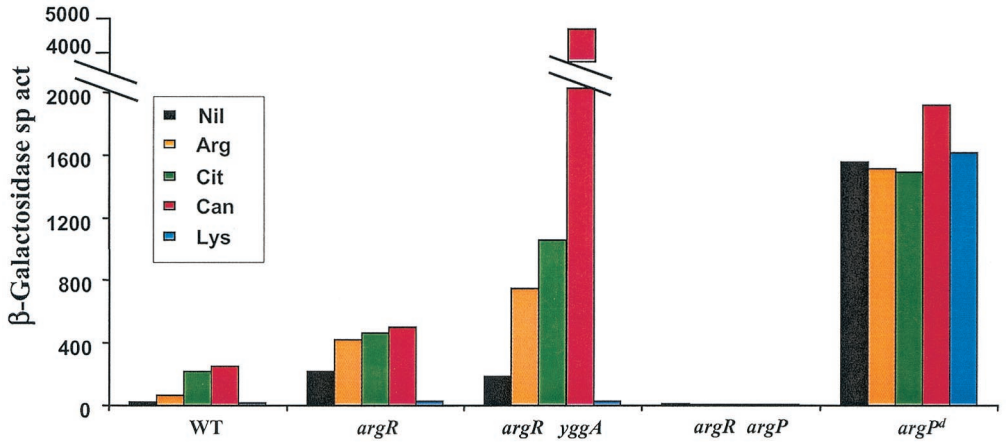

FIG. 2. Expression of *yggA-lac* in pHYD956 transformants of the wild-type (WT) strain MC4100 and the following mutant derivatives: *argR*, GJ4748; *argR yggA*, GJ4894; *argR argP*, GJ4895; and *argP*^{d}, MC4100/pHYD926. Cultures were grown to exponential phase in glucose-minimal medium supplemented with trimethoprim and additives (each at 1 mM, except for CAN, which was added to 40 μg/ml), as indicated in the inset key, for β-galactosidase assays. Enzyme specific activity values are given in Miller units (33). Cit, citrulline.

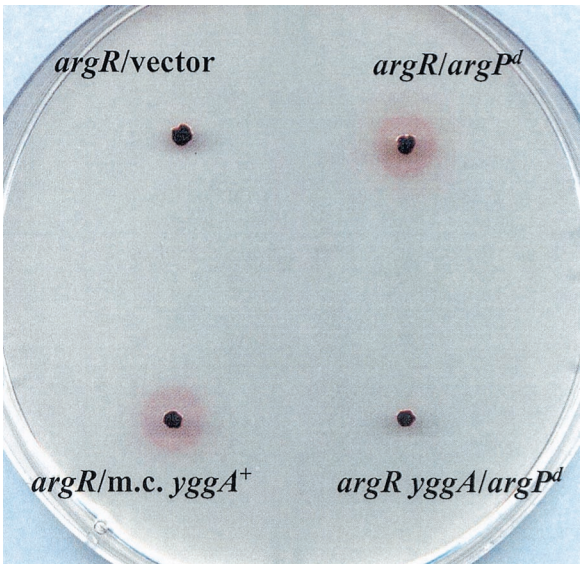

FIG. 3. Test for cross-feeding of Arg-auxotrophic strain SK2226/pBR329 by the following strains: *argR*/vector, GJ4748/pBR329; *argR*/*argP*^{d}, GJ4748/pHYD953; *argR*/m.c. (multicopy) *yggA*$^+$, GJ4748/pHYD952; and *argR yggA*/*argP*^{d}, GJ4894/pHYD953. Cross-feeding was scored after 12 h of incubation.

Evidence for increased Arg excretion in strains with *argP*^{d} mutation or multicopy *yggA*$^+$. The polypeptide deduced to be encoded by *yggA* shares significant similarity with the *C. glutamicum* protein LysE, which has been shown to function as an exporter of Lys and Arg for this bacterium (3, 42). We reasoned that the Can^{ss} and Can^r phenotypes, respectively, of *argP* null and *argP*^{d} mutants can be explained on the assumption that YggA also similarly functions in *E. coli* as an exporter of Arg and its analog CAN.

To test this hypothesis, we constructed test strains with increased levels of YggA protein by the introduction on a multicopy plasmid of either (i) *yggA*$^+$ (pHYD952) or (ii) the *argP*^{d} S94L mutation that was shown above to constitutively activate *yggA* expression (pHYD953). Both test strains also carried an *argR* mutation in order to increase the intracellular Arg pools, and their phenotypes were compared with that of a control *argR* mutant derivative that was otherwise wild type (that is, *argP*$^+$ and haploid *yggA*$^+$, transformed with the plasmid vector pBR329). Arg excretion was assayed by determining the halo of cross-feeding (that is, syntrophic growth) of the Δ*argH* strain SK2226/pBR329, which was seeded in an agar medium supplemented with ampicillin and on the surface of which each of the test and control strains had been spotted. The auxotrophic requirement of strain SK2226 (which is blocked in the last step of the Arg biosynthetic pathway) is satisfied only by Arg, not by any of its precursors, such as ornithine or citrulline (6).

By 12 h of incubation, both multicopy *yggA*$^+$ and *argP*^{d} were associated with dramatic increases in the Arg cross-feeding ability of the *argR* mutant (Fig. 3), which was suggestive of increased Arg export from the concerned test strains. In comparison with the *argR*$^+$ strain MC4100, the *argR* mutant strain GJ4748 itself exhibited increased cross-feeding of the Arg auxotroph, but only after 40 h of incubation (data not shown). We were unable to introduce the *argP*^{d} mutation into a strain with multicopy *yggA*$^+$, suggesting that the massive overexpression of YggA under these conditions is probably lethal, either directly or as a consequence of its physiological effects on intracellular Arg pools.

***yggA* is epistatic to *argP*^{d}.** If it is true that the Can^r and Arg excretion phenotypes of the *argP*^{d} mutant are a consequence of the increased and constitutive expression of YggA, then the phenotypes must be *yggA*$^+$ dependent. We found that the introduction of a null mutation in *yggA* (i) rendered an *argR*$^+$ *argP*^{d} strain Can^{ss} (Table 2) and (ii) reversed the increased Arg cross-feeding ability of an *argR argP*^{d} strain (Fig. 3). Another clue in support of the notion that *yggA* acts downstream of *argP* was the finding that multicopy *yggA*$^+$ on plasmid pHYD952 suppressed the Can^{ss} phenotype of the *argP* null mutant, even as it conferred Can^r in the *argP*$^+$ strain (Table 2); these observations also suggested that *yggA* expression from plasmid pHYD952 was driven at least in part from a constitutive vector-borne promoter.

Evidence that *yggA* expression is affected by both intracellular Arg and Lys. Although the data in Fig. 2 demonstrated that exogenously supplied Arg or CAN, on the one hand, and Lys, on the other, have opposite effects on *yggA-lac* transcription, it was unclear whether both effects are primary or only one of them is so. Lys and Arg (and also CAN) share at least one common uptake system (involving the LAO protein, whose synthesis is also repressible by Lys) (19, 34); it was therefore possible that one of the amino acids (either Arg or Lys) affected *yggA-lac* expression directly by interacting with ArgP in the cytoplasm, while the second did so indirectly by interfering with the uptake of the first. By employing *argP* and *yggA* null mutants (in which a possible confounding effect of intracellular Lys on YggA-mediated CAN export does not exist), we did obtain evidence that exogenous Lys interferes with CAN uptake, since the MIC of CAN for the two strains was increased from 1.5 and 0.8 μg/ml in the absence of Lys to 12.5 and 3 μg/ml, respectively, in its presence.

To test the direct effects, if any, of intracellular Arg and Lys on *yggA* expression and exporter function, we undertook experiments with the dipeptides arginylalanine (Arg-Ala) and lysylalanine (Lys-Ala). The dipeptides were expected to be transported into the cells through uptake systems that were different from those for the amino acids and then to be hydrolyzed within the cells to release the cognate amino acids.

β-Galactosidase expression from the *yggA-lac* fusion plasmid pHYD956 in an *argP*$^+$ *argR* strain was as substantially induced by Arg-Ala (535 Miller units) as it was by Arg (389 Miller units). Likewise, the expression of *yggA-lac* was reduced by Lys-Ala (33 Miller units) to the same extent that it was by Lys (58 Miller units). Our data are therefore consistent with the model that both Arg and Lys act directly within the cell to control *yggA* transcription in opposite ways.

Two related observations in support of a direct negative effect of intracellular Lys on *yggA* expression (and hence on Arg and CAN export) were as follows. (i) As mentioned above, the *argR* strain GJ4748 was able to cross-feed the Δ*argH* auxotrophic strain SK2226 for growth on Arg-free medium after 40 h of incubation. This cross-feeding ability was abolished upon supplementation of the medium with either Lys or Lys-Ala but not with a control dipeptide, histidylalanine (data not shown). On the other hand, neither Lys nor Lys-Ala had an

TABLE 5. *argP-lac* expression in derivatives of *argP202* strain GJ4536 with different plasmids[a]

Plasmid (genotypic description)	β-Galactosidase sp act (Miller units) with additive		
	No additive	Arg	Lys
pCL1920	125	110	115
pHYD915 (*argP*$^+$)	107	109	110
pHYD926 (*argP*^{d})	89	92	108

[a] Cultures were grown to exponential phase in glucose-minimal medium supplemented with spectinomycin. Enzyme specific activity values are given in Miller units (33).

effect on the pronounced Arg cross-feeding abilities (as depicted in Fig. 3) of the *argR* derivatives with multicopy *yggA*$^+$ or *argP*^{d}, in both of which *yggA* expression was rendered to be at a high level and constitutive. (ii) The MIC of CAN for the wild-type strain MC4100 was unaffected by histidylalanine, whereas it was sharply reduced by Lys-Ala, from >100 μg/ml in the absence of the latter to 6 μg/ml in its presence. This result also served to rule out the possibility that the effect of Lys-Ala on *yggA* expression was indirect (for example, through the release of Lys into the medium and subsequent interference with Arg uptake or reuptake), since in that case Lys-Ala would also have been expected to interfere with CAN uptake into (and hence to increase the CAN tolerance of) the wild-type strain.

***argP* is not autoregulated.** Based on data from in vitro experiments, Celis (12) had suggested that *argP* transcription may be negatively autoregulated. The *argP202* mutation represents a null insertion that also generates a *lac* operon fusion in the correct orientation with the chromosomal *argP* regulatory region (unpublished data), and we used strains with this allele to test for *argP* autoregulation in vivo. The data presented in Table 5 indicate that the *argP* promoter is of moderate strength and that its expression in the *argP202* strain derivatives is not affected by *argP*$^+$ or *argP*^{d} (carried, respectively, by plasmids pHYD915 or pHYD926), nor is it affected by potential coeffectors such as Arg or Lys. Similar results were also obtained for a second independent chromosomal *argP-lac* fusion (data not shown). We conclude that *argP* does not regulate its own transcription in vivo.

DISCUSSION

The salient findings of this study may be summarized as follows. (i) Null mutants in *argP* or *yggA* exhibit a Can^{ss} phenotype. (ii) Dominant missense gain-of-function mutations (*argP*^{d}) that confer a Can^r phenotype can occur in *argP*, but an *argP*^{d} *yggA* double mutant is Can^{ss}. (iii) The transcription of *yggA* in vivo is ArgP dependent and is induced by exogenous Arg or Arg-Ala (and also by an *argR* mutation), whereas the addition of Lys or Lys-Ala phenocopies the effect of an *argP* null mutation; furthermore, *yggA* expression is rendered constitutive in an *argP*^{d} strain. (iv) Finally, *argR*$^+$ strains with *argP*^{d} or multicopy *yggA*$^+$ are Can^r, and their *argR* derivatives (which are additionally derepressed for Arg biosynthesis) show highly increased Arg excretion levels.

YggA is similar to *C. glutamicum* LysE, which has been shown to export Arg and Lys (3, 42), whereas ArgP is a member of the LysR family of transcriptional activators (which includes LysG, the activator of LysE in *C. glutamicum*) (39). Therefore, a straightforward interpretation of our results, as further discussed below, is that (i) ArgP is a transcriptional activator of *yggA*, (ii) ArgP's activator function is enhanced by Arg and inhibited by Lys, and (iii) *yggA* encodes an Arg (and CAN) exporter in *E. coli*.

It may be emphasized here that our interpretations are based primarily on indirect lines of genetic evidence and that additional biochemical studies are necessary both to establish the role of ArgP and its coeffectors in *yggA* transcription and to examine the putative exporter function of YggA. With a native length of just 211 amino acids (from which, furthermore, the C-terminal stretch of 14 residues may be deleted without a complete loss of activity), *E. coli* YggA is even shorter than *C. glutamicum* LysE (233 residues) (3, 42); it will be interesting to determine how this class of especially small transport proteins mediates amino acid export and whether an efflux or exchange mechanism is involved in the process (7, 8).

The model proposed by Celis (11, 12) to explain the Can^r phenotype of an *argP*^{d} mutant assumed (i) that the mutation represents a loss-of-function (that is, dominant-negative) allele of *argP* and (ii) that wild-type ArgP is a transcriptional activator of *argK*, whose product in turn acts to enhance Arg uptake through two different transport systems. However, our finding in this study that *yggA* is epistatic to *argP*^{d} indicates that the Can^r phenotype associated with *argP*^{d} can be entirely accounted for by the ArgP-mediated regulation of *yggA* itself. Furthermore, even the finding of Celis (12) that *argK* is a target for transcriptional activation by ArgP may need to be reexamined, given that (i) there is a discrepancy between the expected *argK* runoff transcript size (approximately 310 bases) determined from the promoter mapping data published by Celis et al. in an earlier paper (13) and that reported by him (215 bases) for ArgP activation experiments (12) and (ii) the promoter (for *argK*) mapped by his group (13) is within the *argK* coding region per the genome sequence published by Blattner et al. (5) (GenBank accession number AE000375, wherein *argK* is annotated *ygfD*).

Coeffectors of ArgP in *yggA* regulation. The data from the *yggA-lac* expression studies suggest that Arg (or CAN) functions as a coeffector for the apo-regulator ArgP in activating *yggA* transcription. The ArgP-mediated induction of *yggA* expression is also observed upon the addition of the Arg biosynthetic precursor citrulline (Fig. 3) or ornithine (data not shown), most likely by virtue of its conversion to Arg within the cells. Similarly, *yggA-lac* induction by Arg-Ala is most simply explained as the consequence of the release of intracellular Arg after hydrolysis of the dipeptide.

The observation that *yggA* expression is elevated in an *argR* mutant raises the formal possibility that ArgR is a second regulator for *yggA*; however, the fact that the *argR* effect is also ArgP dependent (that is, it is not observed in an *argR argP* double mutant [Fig. 3]) indicates once again that it is the increased intracellular Arg concentration in the *argR* mutant which is responsible for *yggA* induction. This interpretation is supported by our observation that in a strain that was blocked in the Arg biosynthetic pathway, the introduction of an *argR* mutation was associated with only a twofold increase in *yggA-lac* expression, in contrast to a sevenfold increase in an isogenic

Arg-prototrophic strain (all cultures were grown with 1 mM Arg) (data not shown).

We also observed that the addition of Lys or the dipeptide Lys-Ala nullified the activator function of ArgP for *yggA* expression. As explained above, the dipeptide supplementation experiments served to exclude the possibility that exogenously added Lys acts merely to reduce the intracellular Arg pool by competing with the latter's uptake (or reuptake). Two alternative models, therefore, are (i) that intracellular Lys competes with and prevents Arg from binding a single coeffector binding site in ArgP and (ii) that the latter has a second independent Lys-binding site.

It may be noted that the effect of intracellular Lys on *E. coli yggA* expression (negative) is opposite that on *C. glutamicum lysE* expression (positive) (3). Furthermore, whereas the latter is reported to be induced in cultures supplemented with histidine (3) or methionine (42), we observed that the expression of the former was reduced approximately 60% in the presence of either of these amino acids (data not shown). These differences in regulation suggest that although the two exporters share substantial similarities in structure and function, their physiological roles in their respective organisms may not be identical.

Likely physiological role(s) of YggA. As mentioned above, very few amino acid export systems have so far been characterized in bacteria. It has been suggested that the physiological role of these exporters may be either to mediate the secretion of signaling molecules or to avoid the buildup of the substrate compounds to toxic levels in the cytoplasm (1, 9, 17).

In the case of *E. coli* YggA, three possibilities (not necessarily mutually exclusive) for its physiological function may be postulated. (i) The first possibility is that it acts as a safety valve to prevent the excessive accumulation of Arg following its uptake into the cells and the hydrolysis of nutrient Arg-containing peptides. Eggeling and coworkers showed that, in medium containing Lys-Ala or Arg-Ala, the *C. glutamicum lysE* mutant accumulates Lys or Arg, respectively, and is consequently growth inhibited (3, 42). We similarly found that, in comparison with an isogenic wild-type *E. coli* strain, the *argP*, and more so, the *yggA* mutants were inhibited for growth on glucose-minimal A medium supplemented with 5 mM Arg-Ala (data not shown). (ii) The second possibility is that YggA serves to maintain a correct balance between intracellular levels of the basic amino acids Arg and Lys, which may then explain why the latter acts to suppress *yggA* expression. (iii) The third possibility is that YggA has evolved to excrete CAN (which is a natural antimetabolite), which is perhaps supported by our finding that of all the substances that were tested, CAN was the most effective for inducing *yggA* transcription.

Redesignation of *yggA* as *argO*. Based on the findings described above in support of an Arg exporter function for YggA, we propose that the gene be redesignated *argO* (for "arginine outward transport").

ACKNOWLEDGMENTS

We thank Mary Berlyn, Ian Booth, K. Isono, C. G. Lerner, and Jim Pittard for strains and plasmids. The *yggA* insertion mutants were obtained by Francois Gompel, and Rakesh Laishram performed the experiments with Arg-Ala and Lys-Ala dipeptides. We acknowledge V. Vamsee Krishna and T. Giri Babu for technical assistance, Mehar Sultana for primer synthesis, N. Nagesh for DNA sequencing, and members of the J. Gowrishankar laboratory for advice and discussions.

M.R.N. was a CSIR Research Fellow. J.G. is an honorary faculty member of the Jawaharlal Nehru Centre for Advanced Scientific Research.

REFERENCES

1. **Aleshin, V. V., N. P. Zakataeva, and V. A. Livshits.** 1999. A new family of amino-acid-efflux proteins. Trends Biochem. Sci. **24:**133–135.
2. **Andrews, A. E., B. Lawley, and A. J. Pittard.** 1991. Mutational analysis of repression and activation of the *tyrP* gene in *Escherichia coli.* J. Bacteriol. **173:**5068–5078.
3. **Bellmann, A., M. Vrljiæ, M. Patek, H. Sahm, R. Krämer, and L. Eggeling.** 2001. Expression control and specificity of the basic amino acid exporter LysE of *Corynebacterium glutamicum.* Microbiology **147:**1765–1774.
4. **Berlyn, M. K. B.** 1998. Linkage map of *Escherichia coli* K-12, edition 10: the traditional map. Microbiol. Mol. Biol. Rev. **62:**814–984.
5. **Blattner, F. R., G. Plunkett III, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao.** 1997. The complete genome sequence of *Escherichia coli* K-12. Science **277:**1453–1462.
6. **Bollon, A. P., and H. J. Vogel.** 1973. Regulation of *argE-argH* expression with arginine derivatives in *Escherichia coli*: extreme nonuniformity of repression and conditional repressive action. J. Bacteriol. **114:**632–640.
7. **Bröer, S., and R. Krämer.** 1991. Lysine excretion by *Corynebacterium glutamicum.* 1. Identification of a specific secretion carrier system. Eur. J. Biochem. **202:**131–135.
8. **Bröer, S., and R. Krämer.** 1991. Lysine excretion by *Corynebacterium glutamicum.* 2. Energetics and mechanism of the transport system. Eur. J. Biochem. **202:**137–143.
9. **Burkovski, A., and R. Krämer.** 2002. Bacterial amino acid transport proteins: occurrence, functions, and significance for biotechnological applications. Appl. Microbiol. Biotechnol. **58:**265–274.
10. **Celis, R. T. F.** 1981. Chain-terminating mutants affecting a periplasmic binding protein involved in the active transport of arginine and ornithine in *Escherichia coli.* J. Biol. Chem. **256:**773–779.
11. **Celis, R. T. F.** 1990. Mutant of *Escherichia coli* K-12 with defective phosphorylation of two periplasmic transport proteins. J. Biol. Chem. **265:**1787–1793.
12. **Celis, R. T. F.** 1999. Repression and activation of arginine transport genes in *Escherichia coli* K-12 by the ArgP protein. J. Mol. Biol. **294:**1087–1095.
13. **Celis, R. T. F., P. F. Leadlay, I. Roy, and A. Hansen.** 1998. Phosphorylation of the periplasmic binding protein in two transport systems for arginine incorporation in *Escherichia coli* K-12 is unrelated to the function of the transport system. J. Bacteriol. **180:**4828–4833.
14. **Celis, R. 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.
15. **Covarrubias, L., and F. Bolivar.** 1982. Construction and characterization of new cloning vehicles. VI. Plasmid pBR329, a new derivative of pBR328 lacking the 482-base-pair inverted duplication. Gene **17:**79–89.
16. **Daßler, T., T. Maier, C. Winterhalter, and A. Böck.** 2000. Identification of a major facilitator protein from *Escherichia coli* involved in efflux of metabolites of the cysteine pathway. Mol. Microbiol. **36:**1101–1112.
17. **Eggeling, L., and H. Sahm.** 2003. New ubiquitous translocators: amino acid export by *Corynebacterium glutamicum* and *Escherichia coli.* Arch. Microbiol. **180:**155–160.
18. **Franke, I., A. Resch, T. Daβler, T. Maier, and A. Böck.** 2003. YfiK from *Escherichia coli* promotes export of *O*-acetylserine and cysteine. J. Bacteriol. **185:**1161–1166.
19. **Glansdorff, N.** 1996. Biosynthesis of arginine and polyamines, p. 408–433. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. American Society for Microbiology, Washington, D.C.
20. **Gowrishankar, J.** 1985. Identification of osmoresponsive genes in *Escherichia coli:* evidence for participation of potassium and proline transport systems in osmoregulation. J. Bacteriol. **164:**434–445.
21. **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.
22. **Higashitani, A., N. Higashitani, S. Yasuda, and K. Horiuchi.** 1994. A general and fast method for mapping mutations on the *Escherichia coli* chromosome. Nucleic Acids Res. **22:**2426–2427.
23. **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.
24. **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.
25. **Kleckner, N., J. Bender, and S. Gottesman.** 1991. Uses of transposons with emphasis on Tn*10.* Methods Enzymol. **204:**139–180.
26. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole

E. coli chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.

27. **Kruse, D., R. Krämer, L. Eggeling, M. Rieping, W. Pfefferle, J. H. Tchieu, Y. J. Chung, M. H. Saier, Jr., and A. Burkovski.** 2002. Influence of threonine exporters on threonine production in *Escherichia coli.* Appl. Microbiol. Biotechnol. **59:**205–210.
28. **LaRossa, R. A.** 1996. Mutant selections linking physiology, inhibitors, and genotypes, p. 2527–2587. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. American Society for Microbiology, Washington, D.C.
29. **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.
30. **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.
31. **Lerner, C. G., and M. Inouye.** 1990. Low copy number plasmids for regulated low-level expression of cloned genes in *Escherichia coli* with blue/white insert screening capability. Nucleic Acids Res. **18:**4631.
32. **Levina, N., S. Totemeyer, N. R. Stokes, P. Louis, M. A. Jones, and I. R. Booth.** 1999. Protection of *Escherichia coli* cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: identification of genes required for MscS activity. EMBO J. **18:**1730–1737.
33. **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, N.Y.
34. **Reitzer, L., and B. L. Schneider.** 2001. Metabolic context and possible physiological themes of σ^{54}-dependent genes in *Escherichia coli.* Microbiol. Mol. Biol. Rev. **65:**422–444.
35. **Rosen, B. P.** 1973. Basic amino acid transport in *Escherichia coli*: properties of canavanine-resistant mutants. J. Bacteriol. **116:**627–635.
36. **SaiSree, L., M. Reddy, and J. Gowrishankar.** 2000. *lon* incompatibility associated with mutations causing SOS induction: null *uvrD* alleles induce an SOS response in *Escherichia coli.* J. Bacteriol. **182:**3151–3157.
37. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Saroja, G. N., and J. Gowrishankar.** 1996. Roles of SpoT and FNR in NH_4^+ assimilation and osmoregulation in GOGAT (glutamate synthase)-deficient mutants of *Escherichia coli.* J. Bacteriol. **178:**4105–4114.
39. **Schell, M. A.** 1993. Molecular biology of the LysR family of transcriptional regulators. Annu. Rev. Microbiol. **47:**597–626.
40. **Schwartz, J. H., and W. K. Maas.** 1960. Analysis of the inhibition of growth produced by canavanine in *Escherichia coli.* J. Bacteriol. **79:**794–799.
41. **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. USA **88:**4066–4070.
42. **Vrljic, M., H. Sahm, and L. Eggeling.** 1996. A new type of transporter with a new type of cellular function: L-lysine export from *Corynebacterium glutamicum.* Mol. Microbiol. **22:**815–826.
43. **Zakataeva, N. P., V. V. Aleshin, I. L. Tokmakova, P. V. Troshin, and V. A. Livshits.** 1999. The novel transmembrane *Escherichia coli* proteins involved in the amino acid efflux. FEBS Lett. **452:**228–232.