JOURNAL OF BACTERIOLOGY, Dec. 2002, p. 6976–6986 Vol. 184, No. 24
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.24.6976–6986.2002
Copyright © 2002, American Society for Microbiology. All Rights Reserved.

The *Escherichia coli gabDTPC* Operon: Specific γ-Aminobutyrate Catabolism and Nonspecific Induction

Barbara L. Schneider, Stephen Ruback,† Alexandros K. Kiupakis, Hillary Kasbarian, Christine Pybus, and Larry Reitzer*

Department of Molecular and Cell Biology, The University of Texas at Dallas, Richardson, Texas 75083-0688

Received 5 July 2002/Accepted 19 September 2002

Nitrogen limitation induces the nitrogen-regulated (Ntr) response, which includes proteins that assimilate ammonia and scavenge nitrogen. Nitrogen limitation also induces catabolic pathways that degrade four metabolically related compounds: putrescine, arginine, ornithine, and γ-aminobutyrate (GABA). We analyzed the structure, function, and regulation of the *gab* operon, whose products degrade GABA, a proposed intermediate in putrescine catabolism. We showed that the *gabDTPC* gene cluster constitutes an operon based partially on coregulation of GabT and GabD activities and the polarity of an insertion in *gabT* on *gabC*. A Δ*gabDT* mutant grew normally on all of the nitrogen sources tested except GABA. The unexpected growth with putrescine resulted from specific induction of *gab*-independent enzymes. Nac was required for *gab* transcription in vivo and in vitro. Ntr induction did not require GABA, but various nitrogen sources did not induce enzyme activity equally. A *gabC* (formerly *ygaE*) mutant grew faster with GABA and had elevated levels of *gab* operon products, which suggests that GabC is a repressor. GabC is proposed to reduce nitrogen source-specific modulation of expression. Unlike a wild-type strain, a *gabC* mutant utilized GABA as a carbon source and such growth required σ^S. Previous studies showing σ^S-dependent *gab* expression in stationary phase involved *gabC* mutants, which suggests that such expression does not occur in wild-type strains. The seemingly narrow catabolic function of the *gab* operon is contrasted with the nonspecific (nitrogen source-independent) induction. We propose that the *gab* operon and the Ntr response itself contribute to putrescine and polyamine homeostasis.

Escherichia coli can use ammonia and a limited number of organic compounds as sole nitrogen sources (30). Ammonia supports the fastest growth rate, and a product of ammonia assimilation, glutamine, signals nitrogen sufficiency and prevents activation of the nitrogen-regulated (Ntr) response. Growth with organic nitrogen sources is slower, results in low glutamine (a signal of nitrogen limitation), and activates the Ntr response. Expression of Ntr genes often requires the alternate sigma factor σ^{54} and the response regulator nitrogen regulator I (NR_I; also called NtrC) (30, 31). Sometimes Ntr gene expression also involves a σ^{70}-dependent transcriptional activator, Nac, itself the product of an Ntr gene.

Microarray and computer analyses of *E. coli* genes have provided a catalog of Ntr genes and genes controlled by σ^{54} (29, 50). About half of the products of σ^{54}-dependent genes are involved in nitrogen metabolism (29). Ntr proteins assimilate ammonia and transport nitrogen-containing compounds, such as amino acids and ammonia. Because of the number of transport systems induced by nitrogen limitation, nitrogen scavenging has been proposed as a major function of the Ntr response (50).

The proposed assimilatory and scavenging functions of the Ntr response do not account for the induction of a few catabolic pathways. It is remarkable that the majority of catabolic pathways induced by nitrogen limitation catabolize a group of metabolically related compounds: arginine, ornithine, putrescine, and γ-aminobutyrate (GABA) (Fig. 1). Arginine and ornithine are substrates for putrescine synthesis, while GABA is an intermediate in putrescine catabolism. This pattern of gene expression suggests that Ntr genes may contribute to putrescine and polyamine homeostasis during nitrogen-limited growth.

To explore this possibility, we initially examined the *gab* operon and its relationship with GABA catabolism. GABA catabolism involves a two-step pathway that produces succinate (Fig. 1, middle line) (7, 8, 23). *gabT* codes for a GABA transaminase that generates succinic semialdehyde. *gabD* specifies an NADP-dependent succinic semialdehyde dehydrogenase, which oxidizes succinic semialdehyde to succinate (2, 7, 26). *gabP* encodes a GABA-specific permease (15, 26). Strains with missense mutations in these genes grow poorly with GABA as the nitrogen source (7, 41). In contrast to these mutations, a mutation in *gabC* stimulates GABA catabolism, which suggests that GabC is a repressor (8). The first three genes are clustered and coexpressed (50), which suggests that they form a *gabDTP* operon. Little is known about *gabC*, since previous reports on its location are not consistent (7, 23), and nothing is known about its expression.

GABA has been proposed to be an intermediate in the catabolism of arginine, agmatine, ornithine, and putrescine (Fig. 1) (41). However, recent results have shown that GABA is not an intermediate in arginine and ornithine catabolism (41). Instead, enzymes of the arginine succinyltransferase path-

* Corresponding author. Mailing address: Department of Molecular and Cell Biology, Mail Station FO 3.1, The University of Texas at Dallas, P.O. Box 830688, Richardson, TX 75083-0688. Phone: (972) 883-2502. Fax: (972) 883-2409. E-mail: reitzer@utdallas.edu.

† Present address: Cancer Immunobiology Center, University of Texas Southwestern Medical School, Dallas, TX 75235-8576.

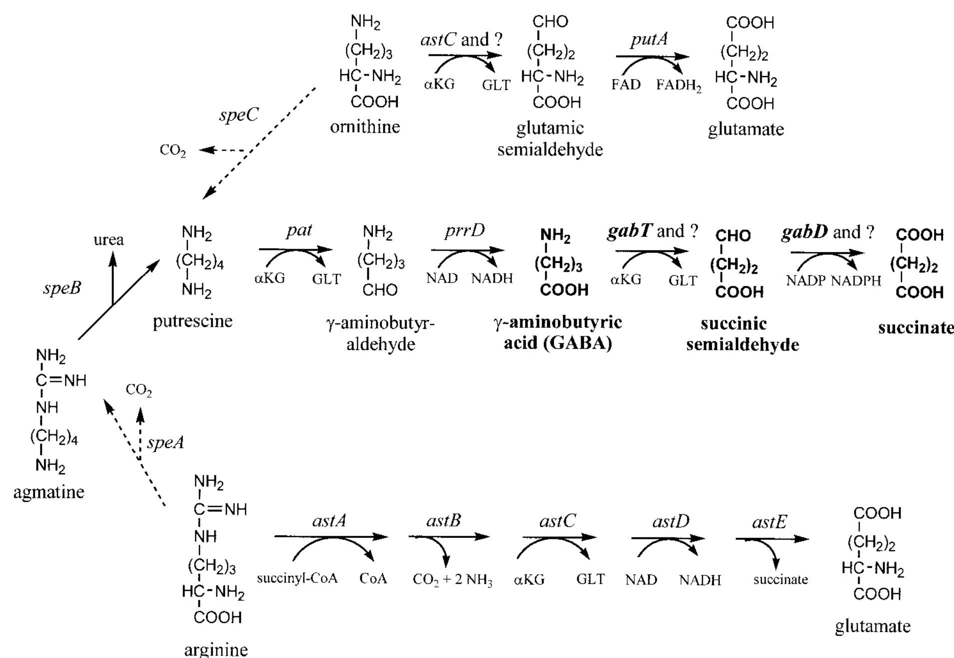

FIG. 1. GABA, putrescine, agmatine, arginine, and ornithine metabolism. The solid arrows indicate catabolic reactions, while the dashed arrows are anabolic reactions. The genes that specify the enzymes involved are shown above the reaction arrows. The question mark indicates that more than one enzyme is involved and that the gene for the second enzyme has not been identified. Details of the arginine pathway are given elsewhere (38). Abbreviations: αKG, α-ketoglutarate; GLT, glutamate; FAD, flavin adenine dinucleotide.

way (Fig. 1, bottom line), products of the *astCADBE* operon, are required for arginine catabolism (Fig. 1) (38). One product of the *ast* operon, AstC, has been implicated in ornithine catabolism, which suggests that glutamic semialdehyde, not GABA, is an intermediate in ornithine catabolism (Fig. 1) (38). Nonetheless, GABA catabolism could conceivably contribute to putrescine and agmatine metabolism.

In this paper, we describe an analysis of the structure, function, and regulation of the *gab* operon. We show that the *gab* operon has an exceptionally narrow catabolic function, considering the compounds that activate its expression and the number of pathways in which GABA might be an intermediate. We show that *gabC* is part of the *gab* operon and that its product does not participate in GABA-specific regulation. These properties are consistent with a broader physiological function than nitrogen source catabolism, such as modulation of putrescine and polyamine content during nitrogen-limited growth.

MATERIALS AND METHODS

Strains and plasmids. The strains used in this study were derivatives of *E. coli* K-12 strain W3110 (Table 1).

In strains SR1, SR2, and SR4, a gene coding for chloramphenicol resistance replaces part of a gene of interest. Their construction involved variations of the same procedure. For each, DNA was isolated from Kohara phage 443 (34), which F. Blattner (University of Wisconsin—Madison) generously supplied. The DNA of interest was cloned into the multicloning site of pWM529 (22), and part of the insert was replaced with a 1.2-kb chloramphenicol resistance cassette from pACYC184 (3). A linear fragment of the disrupted allele was electroporated into K4633 (*recD1903*::Tn*10*), and chloramphenicol resistance from the resulting strain was transduced into W3110. All constructs were confirmed by both Southern hybridization (35) and linkage to genetic markers in the vicinity (43). In the descriptions that follow, the numbers in parentheses after restriction enzymes refer to the coordinates of the sequence with GenBank accession number AE000351.

In SR1, the chloramphenicol cassette replaces 139 bp of *ygaE* (*gabC*), which deletes codons 122 to 168 of a 226-codon gene. The 3.2-kb *Bam*HI (position 5788)-*Sph*I (position 9025) fragment was isolated from Kohara phage 443 and cloned into pWM529. The resulting plasmid was digested with *Cla*I (position 7878) and *Stu*I (position 7740), and the large fragment was isolated and ligated with the chloramphenicol resistance cassette.

SR2 has a 508-bp deletion of *ygaF*. The 4-kb *Eco*47III fragment (positions 319 to 4288) from phage 443 was cloned into the *Sma*I site of pWM529. The 508-bp region between the *Sal*I (position 1746) and *Sna*BI (position 2252) sites was replaced with the 1.2-kb chloramphenicol resistance cassette. This deleted residues 38 to 206 of a putative 444-residue protein.

SR4 was constructed after ligation of the 4.8-kb *Pvu*II (position 939)-*Bam*HI (position 5788) fragment from phage 443 into pWM529. The DNA between two *Bst*EII sites (positions 3619 to 5355) was replaced with the chloramphenicol resistance gene. This deletes codons 109 to 482 of the 482-codon *gabD* gene and codons 1 to 303 of the 426-codon *gabT* gene.

SR6 and SR7 contain an operon fusion to *lacZ*, which was transferred to the chromosome as previously described (9). This method involves ligation of promoter-containing DNA into a cloning site just upstream of a promoterless *lacZ* gene on pRS551 (42). Linearization of the plasmid, transformation into strain TE2680, and isolation of kanamycin-resistant cells selected for strains with the *lacZ* fusion integrated into the chromosome. Finally, the gene for kanamycin resistance, which is adjacent to *lacZ*, was transduced from the TE2680 derivative into W3110.

SR6 contains chromosomal *lacZ* under the control of the *gabD* promoter. The promoter fragment was isolated from a plasmid containing *gab* DNA between two *Eco*47III sites (positions 319 to 4288). This plasmid was digested with *Bsa*AI (positions 2252 to 2933), and the resulting 681-bp fragment (extending from −640 to +41 with respect to the transcription start site) was ligated into the multicloning site of pWM529. This permitted removal of the promoter-containing DNA as an *Eco*RI-*Bam*HI fragment, which was ligated into the same sites of pRS551, which are just upstream of a promoterless *lacZ* gene.

SR7 contains chromosomal *lacZ* fused to DNA upstream from *gabP*. The plasmid used to construct SR1 was digested with *Bam*HI (position 5788) and *Apa*I (position 6104). This DNA, which extends from −187 to +131 relative to the 5′ end of the *gabP* structural gene, was ligated into the multicloning site of pWM529 and then ligated into pRS551 as described for SR6.

HK9 (*nac*::*cat*) was constructed by the method of Datsenko and Wanner (4).

TABLE 1. Strains, plasmids, and phage used in this study

Strain, phage, or plasmid	Relevant genotype or characteristics	Source or reference
AK3	*rpoS*::*tet* in W3110	Laboratory strain
BLS1	Δ*gabC* (*ygaE*)::*cat* Δ*nac-28*	P1 lysate from SR5 (Kan^r) into SR1
BLS2	Δ*gabC* (*ygaE*)::*cat rpoS*::*tet*	P1 lysate from SR1 (Cam^r) into AK3
CP6	Δ*gabT*	This work
EB3364	Δ*nac-28*::*kan*	25
HK1	*gabT*::(mini-Tn*5 lacZ-tet*/*1*) in W3110	P1 lysate from MT114 (Tet^r) into W3110
HK9	Δ*nac*::*cat*	This work
HK10	Δ*nac*::*cat trp*::(*kan-gabD′-′lacZ*)	P1 lysate from HK9 (Cam^r) into SR6
K4633	*recD1903*::Tn*10*	D. Friedman (University of Michigan)
MT114	*gabT*::(mini-Tn*5 lacZ-tet*/*1*)	1
SR1	Δ*gabC* (*ygaE*)::*cat* in W3110	This work
SR2	Δ*ygaF*::*cat* in W3110	This work
SR4	Δ*gabDT*::*cat* in W3110	This work
SR5	*nac-28*::Tn*5* in W3110	P1 lysate from EB3364 (Kan^r) into W3110
SR6	*trp*::(*kan-gabD′-′lacZ*) in W3110	This work
SR7	*trp*::(*kan-gabP′-′lacZ*) in W3110	This work
SR8	*trp*::(*kan-gabD′-′lacZ*) Δ*gabC* (*ygaE*)::*cat* in W3110	P1 lysate from SR1 (Cam^r) into SR6
TE2680	Δ(*lac*)*X74 recD1903*::Tn*10 trpDC700*::*putPA1303*::(*cat-′lac*)	9
W3110	*lacL8 lacI*^{q}	Laboratory strain
Kohara 443	*ygaF-gabDTP-ygaE* (*gabC*)	F. Blattner (University of Wisconsin)
pACYC184	*cat*	3
pGabp	Transcription template for *gabD* promoter	This work
pRS551	*kan-′lacZ*	42
pWM529	Cloning vector	22

The P1 primer was TTAGCTCACCAATTGCCACTGCCTTTTTTCCATCAC TGGAGAACGTGTAGGCTGGAGCTGCTTCG, and the P2 primer was CTC CTTTTATAGGGCAGGGGAACGCGACAGCTGATTAAAGGCATATGA ATATCCTCCTTAG. This deleted all of *nac* from 144 bases upstream of the structural gene to the stop codon.

CP6 (Δ*gabT*) contains a nonpolar deletion that was constructed as previously described (4). The P1 primer was GTGGGACGTTGAAGGCCGTGAGTATC TTGATTTCGCGTGTAGGCTGGAGCTGCTTCG. The P2 primer was CGG TACAAGGATGCGCAGCACGTTGTAATACGGGCCCATATGAATATCC TCCTTAGTTCC. The antibiotic resistance cassette was removed, which left an in-frame deletion of residues 47 to 391 of a 426-residue protein.

Plasmid pGabp was used as a template for transcription in vitro. It was constructed by cloning a PCR product from chromosomal DNA with primers GCTCCGAATTCACCATCTACGCTCAGGACTGG (called gabD-L) and GGTCGAAGCTTGGCGTGTAAGGCATCCACAC (called gabD-R) into the *Eco*RI/*Hin*dIII site of plasmid pTE103. We verified the DNA insert by sequencing. The insert contained the *gabD* promoter region from −280 to +64 relative to the transcription start site.

Cell growth. The minimal medium for growth was W salts (adjusted to pH 7.0) supplemented with a carbon source at 0.4% and a nitrogen source at 0.2% (33). Medium was supplemented with 100 μg of ampicillin per ml, 50 μg of kanamycin per ml, 25 μg of tetracycline per ml, or 10 μg of chloramphenicol per ml when appropriate.

Enzyme assays. Cells were harvested late in the exponential growth phase, when the A_{600} of the culture reached either 0.6 or 100 Klett units (no. 42 filter). For the succinic semialdehyde dehydrogenase and GABA transaminase assays, cell pellets were frozen, resuspended in 0.1 M KPO_4 buffer (pH 7.5)–1 mM dithiothreitol–1 mM phenylmethylsulfonyl fluoride–9% glycerol just before assay, and disrupted with three 5-s bursts of sonication. The crude extracts were ultracentrifuged for 90 min at 120,000 × *g* to remove NADH-oxidizing activity. NADP-dependent succinic semialdehyde dehydrogenase activity was assayed in 0.1 M KPO_4 buffer (pH 7.8)–0.28 mM NADP at 30°C. The reaction was started with the addition of 0.6 mM succinic semialdehyde, and the A_{340} was monitored. The assay used to measure GABA transaminase activity was identical to that described previously (49), except that 100 mM HEPES buffer at (pH 7.0), 0.15 mM NADH, and 0.4 mM succinic semialdehyde were used. β-Galactosidase was assayed as previously described (24). Specific activity is reported as nanomoles of product formed per minute per milligram of protein.

In vitro transcription. Single-round in vitro transcription reaction mixtures contained, in a final volume of 25 μl, transcription buffer (50 mM Tris · HCl [pH 7.5], 100 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM EDTA), 10 nM pGabp (template), 4 mM ATP, 0.5 mM GTP, 0.5 mM CTP, 0.1 mM UTP, 1.25 μl of [α-^{32}P]UTP (800 Ci/mmol, 10 mCi/ml; ICN), 150 μg of heparin, and 100 nM core RNA polymerase (Epicenter Technologies). When indicated, the reaction mixture also contained 300 nM σ^S, 100 nM $E\sigma^{70}$ (Epicenter Technologies), 200 nM Nac dimer, or 250 μM cyclic AMP (cAMP) and 100 nM cAMP receptor protein (CRP) dimer.

A mixture containing polymerase, cAMP, and 3 U of RNAGuard (Pharmacia) in transcription buffer was incubated for 5 min at 37°C. Nac and CRP were added together, and the mixture was incubated for another 10 min. All components were in transcription buffer, and the final volume after these additions was 20.5 μl. Transcription was initiated by addition of 4.5 μl of a solution containing GTP, CTP, labeled and unlabeled UTP, and heparin in transcription buffer and allowed to proceed for 15 min at 37°C, after which the reactions were stopped by addition of 25 μl of 50 mM EDTA–100 μg of yeast tRNA per ml and the mixtures were stored on ice. The reaction mixtures were extracted with 50 μl of acidic phenol (pH 4.2; Sigma), and 40 μl of the upper phase was added to 10 μl of sequencing loading dye. The tubes were heated to 90°C for 3 min and centrifuged briefly before being loaded onto a 5% acrylamide–8 M urea–0.5× Tris-borate-EDTA gel.

Primer extension. Primers for primer extension and sequencing reactions were GGTCGAAGCTTGGCGTGTAAGGCATCCACAC for the *gabD* region and GCCCCATCCTGAATCCTCTCGAAA for the *gabP* region. Primer extension was based on previously described methods (17, 35), with the following modifications. For determination of the transcription start site in vitro, the transcription reaction was run without labeled UTP. The nucleic acids were precipitated, resuspended in water, mixed with labeled primer, dried, and resuspended in 30 μl of an annealing buffer consisting of 40 mM PIPES (pH 6.4), 1 mM EDTA, 400 mM NaCl, and 80% formamide. The mixture was heated for 2 min at 85°C, cooled slowly to 50°C, incubated for 2.5 h, and ethanol precipitated twice. The pellet was resuspended in 15 μl of a mixture containing the buffer supplied by the manufacturer for murine leukemia virus reverse transcriptase (NEB), 100 μg of bovine serum albumin per ml, 2 mM $MgCl_2$, 1 mM each deoxynucleoside triphosphate, 7 U of RNAGuard, and 100 U of murine leukemia virus reverse transcriptase. The reaction was performed for 90 min at 40°C and stopped by

TABLE 2. Generation times for wild-type and mutant strains on various nitrogen sources

N source	Avg generation time (min) ± SE[a]						
	W3110 (wild type)	SR6 (wild type)	CP6 (Δ*gabT*)	SR4 (Δ*gabDT*)	SR1 (Δ*gabC*)	SR8 (Δ*gabC*)	SR5 (Δ*nac*)
Ammonia	ND[b]	84 ± 11	83 ± 2	ND	ND	78 ± 6	122 ± 3
Serine	ND	184 ± 8	ND	ND	ND	171 ± 8	201 ± 3
Alanine	ND	115 ± 4	ND	ND	ND	116 ± 4	180 ± 10
Proline	ND	215 ± 11	ND	ND	ND	222 ± 14	235 ± 12
Aspartate	ND	227 ± 14	ND	ND	ND	217 ± 12	191 ± 11
Glutamine	103 ± 5	98 ± 1	ND	ND	103 ± 5	92 ± 4	137 ± 1
GABA	398	374 ± 12	NG[c]	NG	155	183 ± 24	NG
GABA + aspartate	134	164 ± 13	131 ± 5	ND	139	127 ± 17	162 ± 5
Arginine	440 ± 12	405 ± 19	513 ± 31	372 ± 1	444 ± 4	381 ± 7	252 ± 4
Putrescine	172 ± 2	169 ± 8	152	168 ± 1	184 ± 7	169 ± 9	149 ± 4
Agmatine	144 ± 6	ND	ND	144 ± 6	138 ± 12	ND	168 ± 10
Ornithine	228 ± 12	ND	ND	276 ± 1	240 ± 2	ND	318 ± 10

[a] All values are for at least three separate cultures, except when no standard error is given, and these are for only one culture.
[b] ND, not determined.
[c] NG, no growth.

addition of 15 μl of sequencing loading dye. The mixture was heated for 3 min at 95°C and loaded into a sequencing gel.

Other methods. Routine recombinant DNA techniques were carried out as previously described (35). We carried out DNA sequencing by using chain-terminating dideoxy nucleotides with T7 DNA polymerase (Sequenase) on double-stranded templates (36). Sequencing reagents were from United States Biochemicals, and [γ-^{32}P]ATP (7,000 Ci/mmol) was from ICN.

RESULTS

Phenotypes of *gabT*, *gabDT*, *ygaE* (*gabC*), and *ygaF* mutants. Wild-type *E. coli* strains (W3110 or SR6) grew with a doubling time of 6 to 7 h with GABA as the sole nitrogen source (Table 2). CP6 (Δ*gabT*) and SR4 (Δ*gabDT*) could not utilize GABA as a nitrogen source. These strains grew normally with arginine, ornithine, putrescine, and agmatine, which suggests that the *gab* operon does not obviously contribute to their catabolism as nitrogen sources (Table 2). The growth with arginine and ornithine was expected (Fig. 1, top line) (38), but the growth with agmatine or putrescine was not, since GABA is a proposed intermediate in their catabolism (41).

Nitrogen limitation induces *gabD*, *gabT*, and *gabP* expression coordinately (50). This, together with their proximity, suggests that they constitute an operon. The distance and potential products of the genes adjacent to *gabD* and *gabP* (Fig. 2) suggest that a putative *gab* operon might contain more than three genes—perhaps under certain conditions that were not assessed by the microarray analysis. Only 22 bases separate *gabD* from the preceding *ygaF* gene. BLAST analysis suggested that YgaF is a putative dehydrogenase, which could conceivably oxidize either γ-aminobutyraldehyde or succinic semialdehyde (Fig. 1). However, SR2 (Δ*ygaF*) had the same doubling times (<15% difference) as W3110 (wild type) with GABA, agmatine, arginine, and ornithine as nitrogen sources (S.

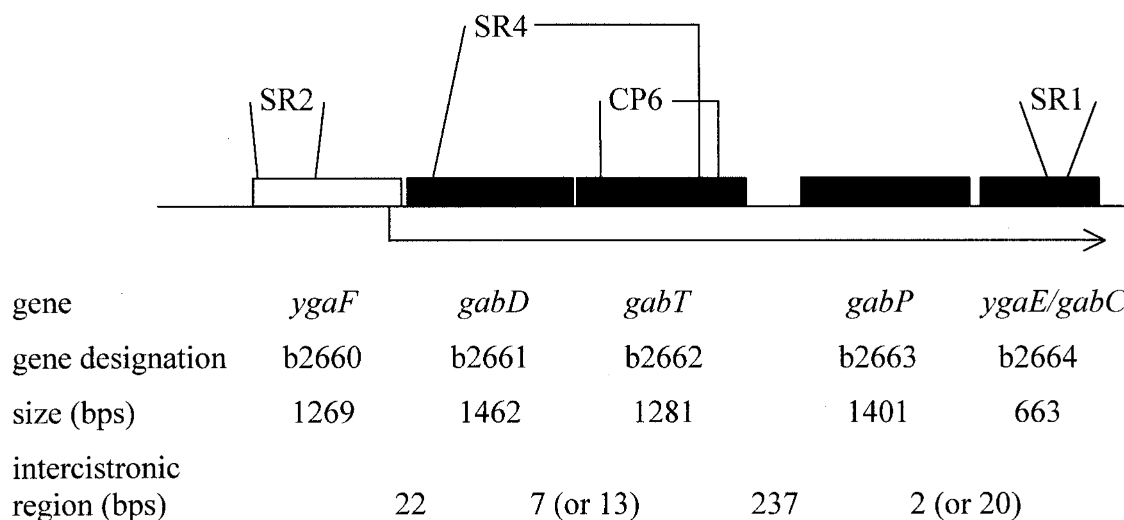

FIG. 2. The *gab* operon. The extents of the deletions and the insertion sites for SR1, SR2, SR4, and CP6 are indicated above the genes. The line below the genes indicates the proposed transcript. There is uncertainty concerning the start codons of three genes. The genes and coordinates (from the sequence with GenBank accession no. AE000351) of the first nucleotide of the start codons are as follows: *ygaF*, 1639 (GTG) or 1705 (ATG); *gabT*, 4452 or 4458 (both ATG); *gabC*/*ygaE*, 7379 or 7397 (both ATG). There are three repetitive extragenic palindromic elements between *gabD* and *gabP*.

TABLE 3. GabT (GABA-succinic semialdehyde transaminase) activity

N source[a]	Avg activity (nmol min^{-1} mg of $protein^{-1}$) ± SE (no. of determinations)			
	SR6 (wild type)	CP6 (Δ*gabT*)	HK1 (*gabT*::*lacZ*)	SR4 (Δ*gabDT*)
Ammonia	25.7 ± 1.4 (3)	1.71 ± 0.84 (3)	2.4 ± 0.6 (2)	ND[b]
Alanine	134 ± 36 (5)	ND	4.42 ± 0.24 (2)	3.7 ± 1.3 (3)
Aspartate	152 ± 17 (5)	2.9 ± 1.5 (2)	ND	ND
GABA + aspartate	150 ± 7.0 (4)	25.4 ± 0.3 (3)	3.3 ± 2.9 (3)	7.88 ± 1.0 (4)
Glutamine	265 ± 5.0 (3)	3.72 ± 2.8 (3)	ND	ND
Arginine	267 ± 11 (3)	0.77 ± 0.39 (3)	ND	ND
Putrescine	391 ± 26 (5)	111 ± 8.2 (3)	98.0 ± 2.9 (3)	102 ± 2.1 (3)

[a] All nitrogen sources were at 0.2%.
[b] ND, not determined.

Ruback and L. Reitzer, unpublished data). Therefore, YgaF is not obviously involved in GABA catabolism.

BLAST analysis indicates that the gene downstream from *gabP*, *ygaE*, codes for a putative transcriptional repressor. It is the only potential repressor gene in the immediate vicinity. SR1 and SR8 (both Δ*ygaE* mutants) grew twice as fast as W3110 and SR6 (isogenic wild-type strains) with GABA as the sole nitrogen source but otherwise grew normally with all of the other nitrogen sources tested (Table 2). The phenotype of these mutants, together with enzyme assays from these strains (results presented below), shows that *ygaE* codes for the *gab* repressor. Therefore, *ygaE* is the previously described *gabC* gene.

GabT transaminase and GabD dehydrogenase activities in nitrogen-limited wild-type and *gabT* and *gabD* mutant strains. To examine *gab* operon expression, we assayed the *gabT* product, GABA transaminase (referred to as the transaminase), and the *gabD* product, NADP-dependent succinic semialdehyde dehydrogenase (referred to as the dehydrogenase), from extracts of exponentially growing cells. Transaminase activity was low (26 U) for cells grown in ammonia-containing nitrogen-rich media and higher in all nitrogen-limited media (65 to 267 U) (Table 3; Fig. 3, lower left panel). Dehydrogenase activity paralleled transaminase activity: it was low with ammonia (28 U) and higher for all cells grown in nitrogen-limited media (52 to 142 U) (Table 4; Fig. 3, lower right panel).

CP6 (Δ*gabT*), SR4 (Δ*gabDT*), and HK1 (*gabT*::*lacZ*) had low transaminase activity (2 to 8 U) after growth with ammonia, alanine, aspartate, arginine, and glutamine as nitrogen sources (Table 3). SR4 (Δ*gabDT*) had low dehydrogenase activity (2 to 6 U) with alanine or GABA plus aspartate as the nitrogen sources (Table 4). In contrast, all of the *gabT* mutants grown with putrescine had 100 U of transaminase activity (Table 3) and the Δ*gabDT* mutant had 65 U of dehydrogenase activity (Table 4), which are about 25 and 40% of the fully induced wild-type activities, respectively. These results suggest that putrescine, but not other nitrogen sources, induces a *gab*-independent transaminase and dehydrogenase. These *gab*-independent enzymes account for the normal growth of *gab* operon mutants with putrescine and agmatine as nitrogen sources. In summary, *gabT* and *gabD* specify essentially all of the transaminase and dehydrogenase activities, respectively, except when putrescine is the nitrogen source.

GabC-dependent regulation. SR8 (Δ*gabC*) had higher levels of activities of both enzymes than those from SR6 (wild type), but only in nitrogen-limited cells (Fig. 3, top panels). The higher enzyme activities account for the faster growth with GABA as a nitrogen source and suggest that GabC is a *gab* operon-specific repressor.

Nac-dependent regulation. Most Ntr genes have either a σ^{54}-dependent promoter, which requires nitrogen regulator I (NR_I; also called NtrC), or a σ^{70}-dependent promoter, which requires Nac. *nac* is itself a σ^{54}-dependent Ntr gene. The regulatory region of the *gab* operon does not have the distinctive sequence of σ^{54}-dependent promoters, which is easily identified in all known σ^{54}-dependent promoters (29). Instead, our results suggest Nac-dependent control.

SR5 (Δ*nac*) could not grow with GABA as a nitrogen source, which suggests that *gab* operon expression requires Nac (Table 2). SR5 grew normally (<20% difference) with agmatine, aspartate, proline, or putrescine; at least 40% slower with ammonia, alanine, glutamine, or ornithine; and 43% faster with arginine (Table 2). The latter result had been previously observed (25). Transaminase and dehydrogenase activities in SR5 were dramatically lower than in the wild-type strain (Fig. 3, bottom panels). The only exception was for putrescine-grown cells, which have putrescine-inducible *gab*-independent isozymes.

Nitrogen source-specific regulation and the function of GabC. Transaminase and dehydrogenase activities in the wild-type strain varied with the nitrogen source (most easily seen in the bottom panels of Fig. 3). Serine and alanine resulted in lower activities than did the other nitrogen sources. GABA induced enzyme activity as well as did several other nitrogen sources, including those that are not metabolized to GABA (e.g., aspartate, proline, and glutamine), which is not consistent with GABA-specific induction.

The absence of GABA-specific induction suggests that GABA does not bind GabC. To account for the lack of GABA-specific induction, we considered the possibility that GabC from *E. coli* has lost its responsiveness to GABA and that GabC from other organisms has retained GABA responsiveness. Therefore, we cloned the *Klebsiella aerogenes gabC* gene into SR8. If GABA inactivated the *K. aerogenes* GabC protein, then a strain with the *K. aerogenes gabC* gene would grow faster with GABA as a nitrogen source than a similar strain with the *E. coli gabC* gene. The *K. aerogenes* and *E. coli gabC* genes on a plasmid slowed the growth of SR8 with GABA as the nitrogen source to the same extent (B. Schneider and L. Reitzer, unpublished data). We conclude that GABA or a product of GABA catabolism does not inactivate *K. aerogenes* GabC.

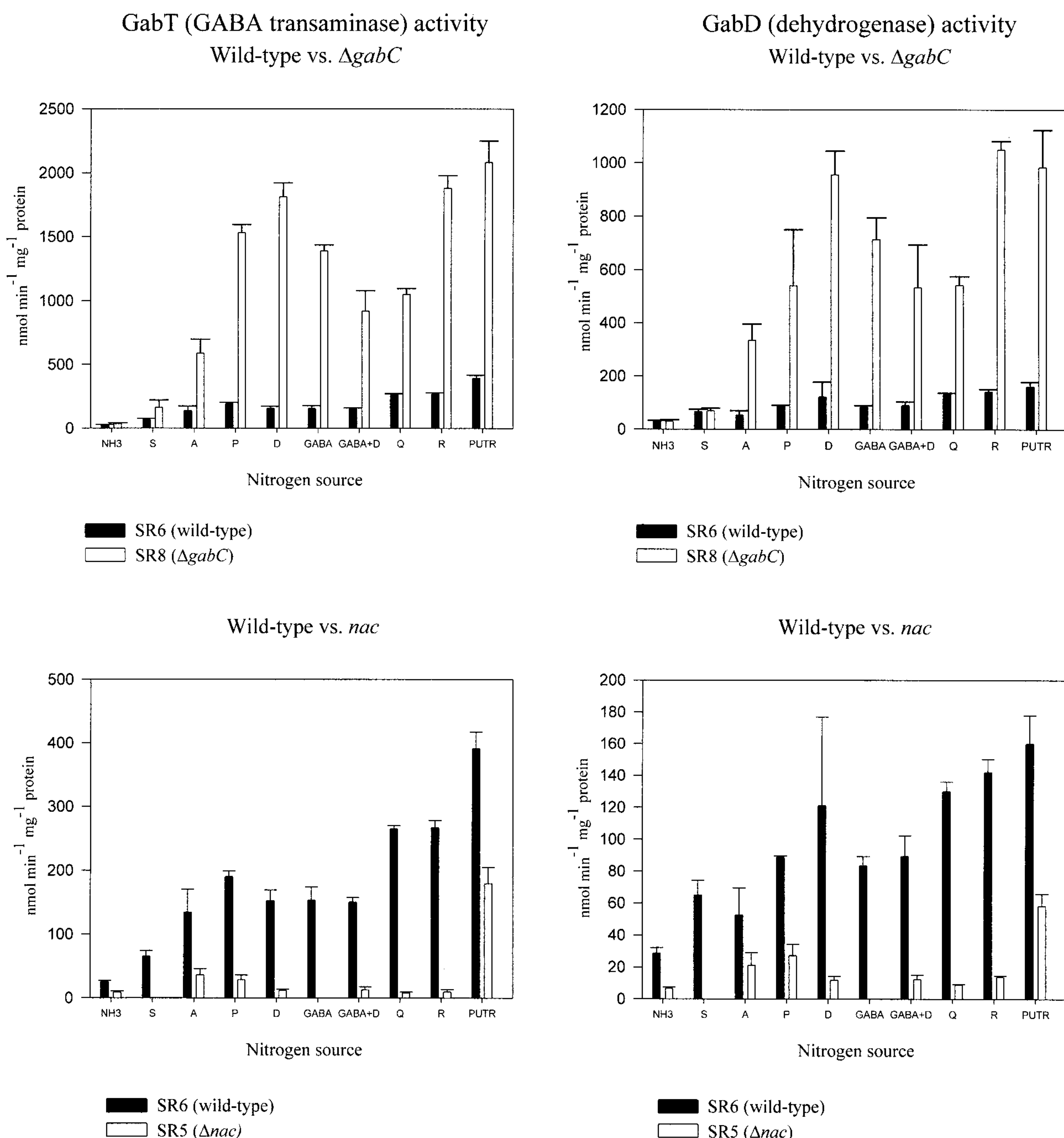

FIG. 3. GabT and GabD activities in wild-type, Δ*gabC*, and Δ*nac* strains. Cells were grown with each of the indicated nitrogen sources at 0.2%. The errors bars are the standard errors of three to five determinations. Abbreviations: NH3, ammonia; S, serine; A, alanine; P, proline; D, aspartate; GABA+D, GABA plus aspartate; Q, glutamine; R, arginine; PUTR, putrescine.

Therefore, GabC from at least two organisms fails to respond to GABA or compounds derived from GABA.

The range of the nitrogen source-specific regulation in nitrogen-limited media was much greater in SR8 (Δ*gabC*) than in SR6 (wild type) (Fig. 3). Transaminase activity from SR8 varied 12-fold (159 to 1,880 U), but that from SR6 varied only 4-fold (65 to 267 U). Dehydrogenase activity from SR8 varied 15-fold (68 to 1,050 U), but that from SR6 varied only 2.2-fold (65 to 142 U). (The values for putrescine-grown cells were excluded because of the complication of *gab*-independent isozymes.) These results suggest that the function of GabC may be to reduce nitrogen source-dependent variation.

A strain with a polar insertion in *gabT* is phenotypically GabC$^-$. GabD dehydrogenase was unexpectedly high in HK1, which contains an insertion in *gabT* (Table 4). Dehydrogenase activity in HK1 was four to eight times higher than that in SR6 (wild type) for cells grown with alanine, GABA (with aspartate), and putrescine and 70 to 100% of that from SR8 (Δ*gabC*). In other words, HK1 is phenotypically GabC$^-$. In contrast, CP6, which contains an in-frame deletion of *gabT*,

TABLE 4. GabD (succinic semialdehyde dehydrogenase) activity[a]

N source	Avg activity (nmol min^{-1} mg of protein^{-1}) ± SE (no. of determinations)			
	SR6 (wild type)	CP6 (Δ*gabT*)	HK1 (*gabT*::*lacZ*)	SR4 (Δ*gabDT*)
Ammonia	28.5 ± 3.5 (3)	13.0 ± 4.2 (3)	37.4 ± 11 (2)	ND
Alanine	52.5 ± 17 (5)	ND	396 ± 130 (2)	2.1 ± 0.6 (3)
Aspartate	121 ± 56 (5)	58.8 ± 2.5 (2)	ND	ND
GABA + aspartate	89.3 ± 13 (4)	53.3 ± 6.6 (3)	398 ± 51 (3)	6.36 ± 1.1 (4)
Glutamine	130 ± 6 (3)	109 ± 8.6 (3)	ND	ND
Arginine	142 ± 8.3 (4)	97.5 ± 5.0 (3)	ND	ND
Putrescine	160 ± 18 (5)	139 ± 11 (3)	704 ± 70 (3)	65.0 ± 3.2 (3)

[a] See the footnotes to Table 3.

invariably had less dehydrogenase activity than the wild-type strain (Table 4). The simplest explanation for the difference in dehydrogenase activity between HK1 (*gabT*::*lacZ*) and CP6 (Δ*gabT*) is the polarity of the insertion in HK1. This result suggests that *gabC* is part of the *gab* operon.

Expression from a *gabD-lacZ* fusion. We examined expression from a *gab-lacZ* fusion in order to confirm the results suggested by direct enzyme assays and to eliminate possible complications due to redundant enzymes. SR6 contains a chromosomal fusion of the *gabD* promoter (which was subsequently shown [see below] to contain DNA from −640 to +41 relative to the transcription start site) to *lacZ*. The *gabD-lacZ* fusion is inserted within the *trp* operon, which leaves the *gab* operon intact. Nitrogen limitation activated expression that varied with the nitrogen source (Table 5), which agrees with the results of direct assays (Fig. 3). β-Galactosidase activity was at least 10-fold lower in isogenic *nac* mutant cells grown in three different nitrogen-limited media (Table 5), which further confirms control by Nac.

Loss of GabC had no effect on β-galactosidase activity from cells grown with a variety of nitrogen sources and actually decreased β-galactosidase activity twofold when GABA was the nitrogen source (Table 5). This result is not consistent with the faster growth of *gabC* mutants (Table 2) or assays of transaminase and dehydrogenase activities (Fig. 3). One explanation for the discrepancy is that the promoter fragment fused to *lacZ* does not contain all of the GabC operator, even though the fusion contains DNA from −640 to +41 with respect to the *gabD* transcription start site. Attempts to clone a larger promoter fragment were unsuccessful. An alternate explanation is that the fusion's location in the *trp* operon affects the topology of the promoter and prevents GabC binding. In any case, the *gabD-lacZ* reporter recapitulated all aspects of regulation, except the control by GabC, which was lost.

GabT transaminase and GabD dehydrogenase activities in carbon-limited cells. W3110 (wild type) could not utilize GABA as a carbon source, but SR1 (*gabC*) could (Table 6). Such growth is not consistent with Nac-dependent control. Therefore, we examined transaminase and dehydrogenase activities from carbon-limited cells. Carbon limitation induced both enzymes in W3110 about twofold with aspartate versus glucose as the carbon source (Table 6). In contrast, SR1 had low activities with glucose, three- to fourfold higher activities with glycerol, and 20- to 30-fold higher activities with GABA and aspartate as carbon sources. These activities are not consistent with GABA-specific induction. Instead, slower growth correlates with higher activities. BLS1 (*gabC nac*) consistently had about twice the activities of SR1 (*gabC*) with aspartate or GABA as the carbon source, which suggests that Nac impairs expression during carbon-limited growth.

It has been reported that stationary-phase *gab* expression requires σ^S (1, 37). Our results show that *gab* expression during carbon-limited growth also requires σ^S. BLS2 (*gabC rpoS*) could not utilize GABA as a carbon source. Furthermore, BLS2 (*gabC rpoS*), compared to SR1 (*gabC*), had 8- and 30-fold less transaminase and dehydrogenase activities with glycerol and aspartate, respectively, as the carbon sources (Table 6).

***gab* operon transcripts and regulatory sites.** To confirm the results of the mutant analysis and enzyme assays, we analyzed transcription with purified core RNA polymerase (E), σ^{70}, σ^S, CRP, and Nac. We used purified Nac from *K. aerogenes*, which is stable, unlike Nac from *E. coli*, which is unstable (11, 25). The template, plasmid pGabp, produced a transcript of about

TABLE 5. β-Galactosidase activity from reporter strains with the *gabD-lacZ* operon fusion[a]

N source	Avg activity (nmol min^{-1} mg of protein^{-1}) ± SE (no. of determinations)		
	SR6 (wild type)	SR8 (Δ*gabC*)	HK10 (Δ*nac*)
Ammonia	63.6 ± 5.1	79.0 ± 15	83 ± 6
Alanine	236 ± 61	218 ± 33	ND
Serine	382 ± 86	322 ± 81	ND
GABA	1,530 ± 650	708 ± 72	ND
GABA + aspartate	1,280 ± 158	ND	120 ± 2
Arginine	2,070 ± 220	2,350 ± 420	ND
Aspartate	1,790 ± 71	1,650 ± 650	139 ± 25
Glutamine	1,660 ± 450	1,770 ± 410	ND
Putrescine	3,350 ± 140	3,220 ± 240	147 ± 11

[a] See the footnotes to Table 3. All assays were performed at least three times.

TABLE 6. Transaminase and dehydrogenase activities and rates of carbon-limited growth

Strain and genotype	Carbon source[a]	Activity (nmol min^{-1} mg of $protein^{-1}$) ± SE (no. of determinations)		Avg doubling time (min) ± SE[b]
		Transaminase	Dehydrogenase	
SR6 (wild type)	Glucose	25.7 ± 1.4 (3)	28.5 ± 3.5 (3)	84 ± 11
SR8 (*gabC*)	Glucose	34.1 ± 6.1 (3)	29.2 ± 5.1 (3)	78 ± 6.4
W3110 (wild type)	Glycerol	38.8 ± 11 (3)	32.7 ± 4.3 (3)	104 ± 2.7
SR1 (*gabC*)	Glycerol	110 ± 17 (3)	80.6 ± 9.4 (3)	100 ± 3.7
BLS1 (*gabC nac*)	Glycerol	121 ± 6.9 (3)	71.9 ± 5.2 (3)	116 ± 9.1
BLS2 (*gabC rpoS*)	Glycerol	13.9 ± 0.47 (3)	10.5 ± 0.29 (3)	130 ± 7.8
W3110 (wild type)	GABA	NG[c]	NG	NG
SR1 (*gabC*)	GABA	738 ± 50 (3)	434 ± 69 (3)	236 ± 2.7
BLS1 (*gabC nac*)	GABA	1,500 ± 51 (3)	779 ± 47 (3)	245 ± 14
BLS2 (*gabC rpoS*)	GABA	NG	NG	NG
W3110 (wild type)	Aspartate	47.2 ± 7.2 (3)	36.3 ± 4.9 (4)	256 ± 3.3
SR1 (*gabC*)	Aspartate	1,000 ± 30 (3)	534 ± 43 (2)	310 ± 14
BLS1 (*gabC nac*)	Aspartate	1,700 ± 67 (3)	961 ± 61 (3)	344 ± 3.2
BLS2 (*gabC rpoS*)	Aspartate	63.2 ± 6.7 (3)	33.1 ± 1.4 (3)	336 ± 13

[a] Cells were grown with the indicated carbon source at 0.4% and 0.2% $(NH_4)_2SO_4$.
[b] Doubling times of at least three cultures are shown.
[c] NG, no growth.

400 bases, but only with $E\sigma^{70}$ and Nac (see the two lanes next to the markers in Fig. 4). The plasmid produces a discrete transcript because of a strong transcriptional terminator downstream from the promoter region. $E\sigma^{70}$ did not direct synthesis of this transcript by itself or with cAMP and CRP. cAMP and CRP had no effect on Nac-dependent transcription. $E\sigma^{S}$ did not generate a similar transcript by itself or with either CRP or Nac. The 110- and 720-base transcripts serve as internal controls and can presumably be synthesized by either $E\sigma^{70}$ or $E\sigma^{S}$. The transcripts probably correspond to a regulatory RNA of plasmid replication and the *bla* transcript, respectively. The purified $E\sigma^{S}$ complex was active because it synthesizes these transcripts and initiates transcription from the *astCADBE* regulatory region (17).

Primer extension analysis of the in vitro transcript indicated a start site 101 bases from the structural gene (Fig. 5). (We had great difficulty detecting primer extension products from RNA extracted from cells, presumably because of extensive degradation.) Nac sites in *K. aerogenes* have a consensus of ATA(A/G)-N_3-(A/T)NT(C/G)GTAT and are centered 64 or 44 bases from the transcription start site (11, 14, 27). There is a potential Nac-binding site that is centered 44 bases from the transcription start site preceding *gabD* (Fig. 6), which is consistent with the location of a known Nac-binding site.

GabC is a member of the FadR subfamily of the GntR family of bacterial regulators, which are generally repressors (32). The extremes of the protected regions for characterized GntR-like proteins are −80 and +28 with respect to the transcription start site (6, 10, 18, 21, 28). A consensus operator site is an inverted repeat with t-GTa half-sites (capital letters indicate highly conserved bases) separated by one to three bases (32). Assuming that GabC binds a similar sequence, there are four potential half-sites with no or one mismatch from −80 to +28 (Fig. 6). A consensus half-site (no mismatches) overlaps the −10 RNA polymerase-binding region and has an adjacent half-site with two mismatches (Fig. 6).

The intercistronic *gabT-gabP* region contains 237 bases and three repetitive extragenic palindromic (REP) elements. Nonetheless, we considered the possibility that a promoter precedes the third gene of the operon, *gabP*. Primer extension indicated a transcript with a 5′ end that was 88 bases upstream of *gabP*, which was detectable only from SR1 (Ruback and Reitzer, unpublished). A binding site for σ^{70}-containing RNA polymerase was not apparent from the region upstream of this transcript. We also constructed a fusion of this region to a promoterless *lacZ* gene on the chromosome, but expression was always less than twice the background and unregulated (Ruback and Reitzer, unpublished). Considering the polar effect of an insertion in *gabT* on *gabC*, we conclude that no promoter precedes *gabP*. The primer extension results probably indicate an RNA-processing site.

DISCUSSION

The *gabDTPC* operon. Our results, combined with previously published results, suggest an operon structure for the *gab* genes and that four genes form the *gabDTPC* operon. The evidence that the first three genes are members of the *gab* operon is unambiguous. The GabT transaminase and GabD dehydrogenase activities change coordinately (Tables 3 and 4; Fig. 3 and 4). Furthermore, mutations in *gabD* have been previously shown to be polar and reduce synthesis of the GABA permease, i.e., GabP (23). Finally, microarray analysis indicates coordinate expression of *gabD*, *gabT*, and *gabP* (50).

The evidence that *gabC* is also a member of this operon is not as strong but reasonably convincing. The strongest evidence is that a strain with a polar insertion in *gabT* mimics the phenotype of a *gabC* mutant with respect to *gabD* expression. It is unusual for a repressor to be encoded within the operon that it regulates, so we considered alternative interpretations. If it is proposed that *gabC* is not part of the *gab* operon, then it becomes necessary to explain the differential effect of the

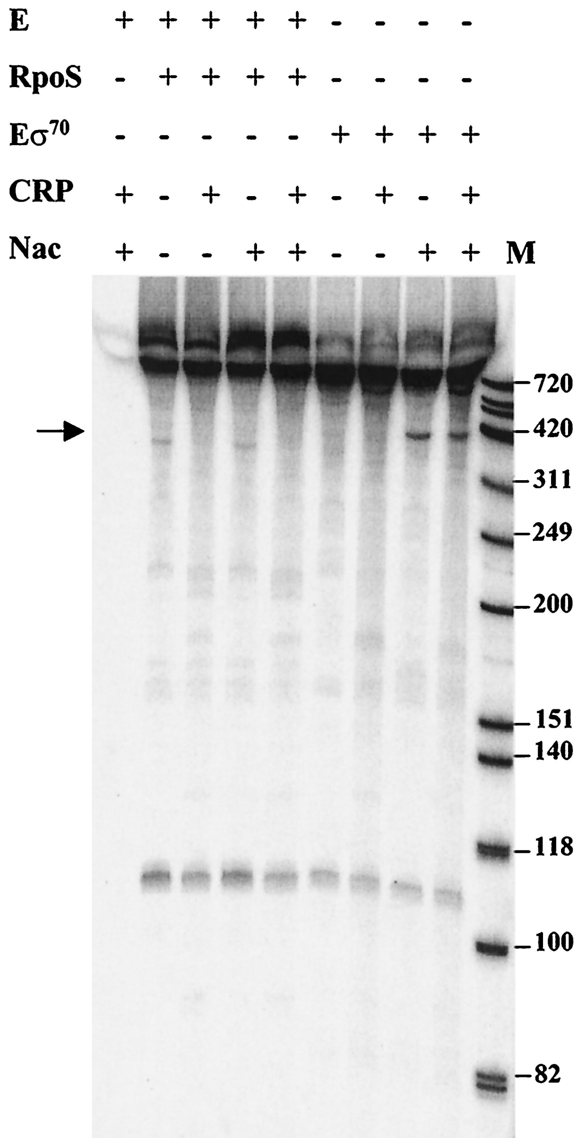

FIG. 4. Requirements for transcription in vitro from the *gabD* promoter. The arrow indicates the location of the transcript that requires $E\sigma^{70}$ and Nac. M, molecular size markers (sizes are indicated in bases on the right).

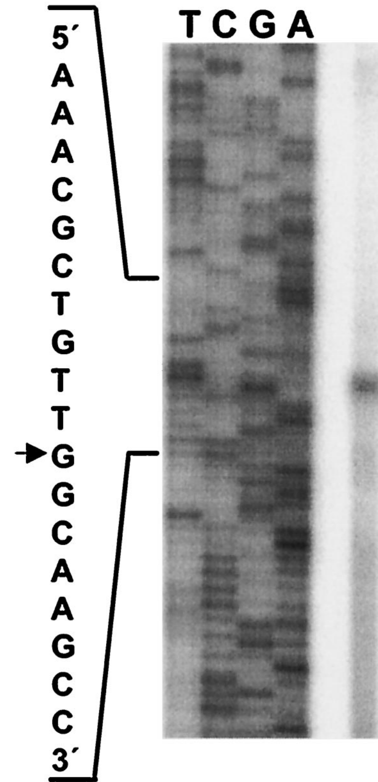

FIG. 5. Primer extension from the in vitro transcription product. The arrow indicates the initiating nucleotide. The sequence of the entire region is shown in Fig. 6.

insertion in *gabT* versus an in-frame deletion of *gabT* on GabD activity (Table 4). The only viable explanation that accounts for this difference is that polarity on *gabP* expression affects regulation. This could occur if GabP itself has a regulatory function in addition to its transport function or if impairment of transport affects accumulation of an inducer. The first possibility seems unlikely, although it is certainly not impossible. We can eliminate the second possibility, since *gab* operon expression does not require either GABA or a product of GABA catabolism. Therefore, the simplest explanation for the effect of the *gabT* lesion on GabD activity is polarity on *gabC* expression. The only uncertainty in this conclusion is the absence of evidence of a four-gene *gab* operon transcript. We tried to obtain evidence of this transcript but could not see discrete bands in a Northern blot. Even if we had observed *gab* operon transcripts, our expectation would be that the Northern blot would confirm the microarray analysis, which did not indicate *gabC* coregulation with other genes of the *gab* operon (50). This would be a reasonable expectation because transcripts of regulatory genes (e.g., *gabC*) are usually less abundant than those for structural genes (e.g., *gabD* and *gabT*). The apparent discoordinate level of *gabC* transcripts could result from less

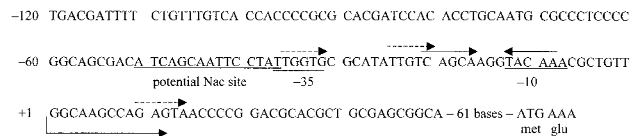

FIG. 6. Nucleotide sequence of the *gabD* promoter region. The coordinates refer to the transcription start site. The RNA polymerase −10 and −35 regions are underlined. The arrows above the sequence indicate possible GabC-binding half-sites. The dashed arrows indicate one mismatch with the t-GTa consensus for GntR-like proteins. The solid arrows show a potential palindromic GabC site that has no mismatches in the downstream half-site and two mismatches in the upstream half-site. There are also potential half-sites with one mismatch centered at +45, +58, +96, and +108 that are not shown.

frequent transcription of the distal *gabC* gene or greater susceptibility to degradation. In any case, we consider the genetic evidence to be more reliable than the biochemical evidence and therefore conclude that *gabC* is the fourth gene of the *gab* operon.

In contrast, our results argue that *ygaF*, the gene preceding *gabD*, is not part of the *gab* operon. First, a deletion of *ygaF* had no effect on catabolism of GABA or any other nitrogen source, which implies that the function of YgaF is not related to the function of *gab* operon products. Second, transcription with purified proteins indicated that a promoter with the appropriate regulation and regulatory sites precedes *gabD*. Finally, microarray analysis did not provide evidence of coregulation of *ygaF* with the *gab* operon (50), which in this case is considered meaningful because the *ygaF* transcript would be expected to be as abundant as those for other enzymes, i.e., GabD and GabT.

Control of the *gab* operon. Nac mediates the induction of the *gab* operon by nitrogen limitation. Nac also activates *codBA* (b0336-0337), a putative polyamine transport/catabolic operon (b1440-1444), *yedL* (b1932), *nupC* (b2393), and *fklB-cycA* (b4207-4208) and represses *serA* (b2913) and *gltBDF* (b3212-3214) (50). However, the phenotype of *E. coli nac* mutants tends to be subtle (25). The failure to utilize GABA as a nitrogen source is its most distinctive phenotype.

GabC does not obviously respond to a specific inducer. GabC is in the FadR subfamily of the GntR family of transcriptional regulators (32). At least one other member of the family, MatR, which regulates malonate metabolism in *Rhizobium leguminosarum*, also fails to respond to a specific inducer (21). Therefore, the absence of specific induction for these proteins has a precedent.

gab expression shows a nitrogen source modulation, and GabC reduces this modulation. This function could explain why *gabC* is a member of the *gab* operon since a modulatory function would become important only after the operon is expressed. Nitrogen source modulation of Ntr gene expression is also evident for activation of *astCADBE* (17, 38), a minimal $glnAp_2$ promoter (39), and *ygjG* (C. Pybus and L. Reitzer, unpublished data) and repression of *gltBDF* (12). The mechanism of this modulation is not known and may vary from gene to gene. For example, ArgR mediates arginine-specific stimulation of *astCADBE* expression and this control may be unique among Ntr genes (17). Control of Nac specific activity is probably not involved in nitrogen source-specific modulation, since intracellular metabolites in *K. aerogenes* do not regulate its activity (40). However, the instability of *E. coli* Nac has prevented examination of the effects of metabolic intermediates on its activity (25).

In addition to expression during nitrogen-limited growth, we also observed *gab* expression during carbon-limited growth, which required both σ^S and inactivation of *gabC*. Several groups have observed *gab* expression in environments that are not nitrogen limited (1, 37, 44, 48). When examined, such expression requires σ^S and always involves strains with insertions in the *gab* operon (1, 37, 44, 48). We propose that the common factor is loss of GabC, since we could not detect significant σ^S-dependent expression in wild-type cells during either carbon-limited growth (Table 6) or stationary phase (B. Schneider, H. Kasbarian, and L. Reitzer, unpublished data). However, loss of GabC is not sufficient for expression since we could not reconstitute σ^S-dependent *gab* expression. cAMP, growth rate, cell density, osmolarity, and starvation have been shown to affect σ^S-dependent gene expression (20). cAMP and its receptor protein did not stimulate expression (Fig. 4). Instead, our results suggest that a growth rate factor, such as guanosine tetraphosphate, may be required, which has been shown to be the case for other σ^S-dependent genes (19).

The function of the *gab* operon and polyamine metabolism. Our results show that the *gab* operon is required for GABA catabolism but does not contribute to the catabolism of any other nitrogen source. The exclusivity of nitrogen source catabolism contrasts with the nonspecificity of induction; i.e., all nitrogen sources induce. One explanation for this discrepancy is that nitrogen source catabolism is not the only function of the *gab* operon. Examination of the pattern of genes expressed during nitrogen limitation suggests a possible alternate function for the *gab* operon. Nitrogen limitation induces few catabolic enzymes, and surprisingly, most metabolize compounds that are related to putrescine. Nitrogen limitation induces b1440-1444 (putative role in both polyamine transport and catabolism), *gabDTPC* (GABA catabolism, which could remove products of putrescine catabolism), and *ygjG* (a putrescine-specific transaminase) (50; Pybus and Reitzer, unpublished). Nitrogen limitation also activates the *astCADBE* operon (17, 38), whose products metabolize both arginine and ornithine away from putrescine synthesis.

Putrescine is the major polyamine in *E. coli* (5, 45). Polyamines have been implicated in protein and nucleic acid elongation rates, translational fidelity, chromosomal structure, and other crucial functions (5). Putrescine content is higher in faster-growing cells, and loss of RpoS diminishes putrescine pools (47). The putrescine content of cells in high-ionic-strength media is also low (45). There is no agreement on the mechanisms that control the intracellular putrescine concentration, and it is likely that several mechanisms are involved. It has been argued that feedback inhibition controls putrescine synthesis (46), and the opposite has also been argued (5). It has been proposed that putrescine export controls intracellular putrescine (13, 16), but putrescine is not excreted in certain media (45). Putrescine catabolism has not been considered as a possible homeostatic control mechanism, but only because polyamine catabolism has not been extensively studied. The pattern of Ntr gene expression suggests that several catabolic Ntr enzymes act in concert to diminish putrescine levels during nitrogen-limited growth. If this is true, then enzymes of the Ntr response not only assimilate ammonia and scavenge nitrogen but also control polyamine homeostasis. Such a function would account for the seemingly nonspecific Ntr induction of the *gabDTPC* operon.

ACKNOWLEDGMENTS

National Institute of General Medical Sciences grant GM47965 and National Science Foundation grants MCB-9723003 and MCB-0077904 supported this work.

We acknowledge R. Bender and D. Friedman (University of Michigan), F. Blattner (University of Wisconsin), and P. Rather (Case Western Reserve University) for supplying strains.

REFERENCES

1. **Baca-DeLancey, R. R., M. M. South, X. Ding, and P. N. Rather.** 1999. *Escherichia coli* genes regulated by cell-to-cell signaling. Proc. Natl. Acad. Sci. USA **96:**4610–4614.
2. **Bartsch, K., A. von Johnn-Marteville, and A. Schulz.** 1990. Molecular analysis of two genes of the *Escherichia coli gab* cluster: nucleotide sequence of the glutamate:succinic semialdehyde transaminase gene (*gabT*) and characterization of the succinic semialdehyde dehydrogenase gene (*gabD*). J. Bacteriol. **172:**7035–7042.
3. **Chang, A. C., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134:**1141–1156.
4. **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. USA **97:**6640–6645.
5. **Davis, R. H., D. R. Morris, and P. Coffino.** 1992. Sequestered end products and enzyme regulation: the case of ornithine decarboxylase. Microbiol. Rev. **56:**280–290.
6. **DiRusso, C. C., T. L. Heimert, and A. K. Metzger.** 1992. Characterization of FadR, a global transcriptional regulator of fatty acid metabolism in *Escherichia coli*. Interaction with the *fadB* promoter is prevented by long chain fatty acyl coenzyme A. J. Biol. Chem. **267:**8685–8691.
7. **Dover, S., and Y. S. Halpern.** 1974. Genetic analysis of the γ-aminobutyrate utilization pathway in *Escherichia coli* K-12. J. Bacteriol. **117:**494–501.
8. **Dover, S., and Y. S. Halpern.** 1972. Utilization of γ-aminobutyric acid as the sole carbon and nitrogen source by *Escherichia coli* K-12 mutants. J. Bacteriol. **109:**835–843.
9. **Elliott, T.** 1992. A method for constructing single-copy *lac* fusions in *Salmonella typhimurium* and its application to the *hemA-prfA* operon. J. Bacteriol. **174:**245–253.
10. **Fujita, Y., and Y. Miwa.** 1989. Identification of an operator sequence for the *Bacillus subtilis gnt* operon. J. Biol. Chem. **264:**4201–4206.
11. **Goss, T. J., and R. A. Bender.** 1995. The nitrogen assimilation control protein, NAC, is a DNA binding transcription activator in *Klebsiella aerogenes*. J. Bacteriol. **177:**3546–3555.
12. **Goss, T. J., A. Perez-Matos, and R. A. Bender.** 2001. Roles of glutamate synthase, *gltBD*, and *gltF* in nitrogen metabolism of *Escherichia coli* and *Klebsiella aerogenes*. J. Bacteriol. **183:**6607–6619.
13. **Herbst, E. J., R. H. Weaver, and D. L. Keister.** 1958. The Gram reaction and cell composition: diamines and polyamines. Arch. Biochem. Biophys. **75:** 171–177.
14. **Janes, B. K., and R. A. Bender.** 1998. Alanine catabolism in *Klebsiella aerogenes*: molecular characterization of the *dadAB* operon and its regulation by the nitrogen assimilation control protein. J. Bacteriol. **180:**563–570.
15. **Kahane, S., R. Levitz, and Y. S. Halpern.** 1978. Specificity and regulation of γ-aminobutyrate transport in *Escherichia coli*. J. Bacteriol. **135:**295–299.
16. **Kashiwagi, K., and K. Igarashi.** 1988. Adjustment of polyamine contents in *Escherichia coli*. J. Bacteriol. **170:**3131–3135.
17. **Kiupakis, A. K., and L. Reitzer.** 2002. ArgR-independent induction and ArgR-dependent superinduction of the *astCADBE* operon in *Escherichia coli*. J. Bacteriol. **184:**2940–2950.
18. **Koo, J. H., I. H. Cho, and Y. S. Kim.** 2000. The malonate decarboxylase operon of *Acinetobacter calcoaceticus* KCCM 40902 is regulated by malonate and the transcriptional repressor MdcY. J. Bacteriol. **182:**6382–6390.
19. **Kvint, K., A. Farewell, and T. Nystrom.** 2000. RpoS-dependent promoters require guanosine tetraphosphate for induction even in the presence of high levels of σ^S. J. Biol. Chem. **275:**14795–14798.
20. **Lange, R., and R. Hengge-Aronis.** 1994. The cellular concentration of the σ^S subunit of RNA polymerase in *Escherichia coli* is controlled at the levels of transcription, translation, and protein stability. Genes Dev. **8:**1600–1612.
21. **Lee, H. Y., J. H. An, and Y. S. Kim.** 2000. Identification and characterization of a novel transcriptional regulator, MatR, for malonate metabolism in *Rhizobium leguminosarum* bv. trifolii. Eur. J. Biochem. **267:**7224–7230.
22. **Mandecki, W., M. A. Hayden, M. A. Shallcross, and E. Stotland.** 1990. A totally synthetic plasmid for general cloning, gene expression and mutagenesis in *Escherichia coli*. Gene **94:**103–107.
23. **Metzer, E., R. Levitz, and Y. S. Halpern.** 1979. Isolation and properties of *Escherichia coli* K-12 mutants impaired in the utilization of γ-aminobutyrate. J. Bacteriol. **137:**1111–1118.
24. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Muse, W. B., and R. A. Bender.** 1998. The *nac* (nitrogen assimilation control) gene from *Escherichia coli*. J. Bacteriol. **180:**1166–1173.
26. **Niegemann, E., A. Schulz, and K. Bartsch.** 1993. Molecular organization of the *Escherichia coli gab* cluster: nucleotide sequence of the structural genes *gabD* and *gabP* and expression of the GABA permease gene. Arch. Microbiol. **160:**454–460.
27. **Pomposiello, P. J., B. K. Janes, and R. A. Bender.** 1998. Two roles for the DNA recognition site of the *Klebsiella aerogenes* nitrogen assimilation control protein. J. Bacteriol. **180:**578–585.
28. **Quail, M. A., and J. R. Guest.** 1995. Purification, characterization and mode of action of PdhR, the transcriptional repressor of the *pdhR-aceEF-lpd* operon of *Escherichia coli*. Mol. Microbiol. **15:**519–529.
29. **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.
30. **Reitzer, L. J.** 1996. Sources of nitrogen and their utilization, p. 380–390. *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 J. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, second ed. ASM Press, Washington, D.C.
31. **Reitzer, L. J.** 1996. Ammonia assimilation and the biosynthesis of glutamine, glutamate, aspartate, asparagine, L-alanine, and D-alanine, p. 391–407. *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 J. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, second ed. ASM Press, Washington, D.C.
32. **Rigali, S., A. Derouaux, F. Giannotta, and J. Dusart.** 2002. Subdivision of the helix-turn-helix GntR family of bacterial regulators in the FadR, HutC, MocR, and YtrA subfamilies. J. Biol. Chem. **277:**12507–12515.
33. **Rothstein, D. M., G. Pahel, B. Tyler, and B. Magasanik.** 1980. Regulation of expression from the *glnA* promoter of *Escherichia coli* in the absence of glutamine synthetase. Proc. Natl. Acad. Sci. USA **77:**7372–7376.
34. **Rudd, K. E.** 1998. Linkage map of *Escherichia coli* K-12, edition 10: the physical map. Microbiol. Mol. Biol. Rev. **62:**985–1019.
35. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
36. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
37. **Schellhorn, H. E., J. P. Audia, L. I. Wei, and L. Chang.** 1998. Identification of conserved, RpoS-dependent stationary-phase genes of *Escherichia coli*. J. Bacteriol. **180:**6283–6291.
38. **Schneider, B. L., A. K. Kiupakis, and L. J. Reitzer.** 1998. Arginine catabolism and the arginine succinyltransferase pathway in *Escherichia coli*. J. Bacteriol. **180:**4278–4286.
39. **Schneider, B. L., S. P. Shiau, and L. J. Reitzer.** 1991. Role of multiple environmental stimuli in control of transcription from a nitrogen-regulated promoter in *Escherichia coli* with weak or no activator-binding sites. J. Bacteriol. **173:**6355–6363.
40. **Schwacha, A., and R. A. Bender.** 1993. The product of the *Klebsiella aerogenes nac* (nitrogen assimilation control) gene is sufficient for activation of the *hut* operons and repression of the *gdh* operon. J. Bacteriol. **175:**2116–2124.
41. **Shaibe, E., E. Metzer, and Y. S. Halpern.** 1985. Metabolic pathway for the utilization of L-arginine, L-ornithine, agmatine, and putrescine as nitrogen sources in *Escherichia coli* K-12. J. Bacteriol. **163:**933–937.
42. **Simons, R. W., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53:**85–96.
43. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:**1–24.
44. **Stancik, L. M., D. M. Stancik, B. Schmidt, D. M. Barnhart, Y. N. Yoncheva, and J. L. Slonczewski.** 2002. pH-dependent expression of periplasmic proteins and amino acid catabolism in *Escherichia coli*. J. Bacteriol. **184:**4246–4258.
45. **Tabor, C. W., and H. Tabor.** 1985. Polyamines in microorganisms. Microbiol. Rev. **49:**81–99.
46. **Tabor, H., and C. W. Tabor.** 1969. Formation of 1,4-diaminobutane and of spermidine by an ornithine auxotroph of *Escherichia coli* grown on limiting ornithine or arginine. J. Biol. Chem. **244:**2286–2292.
47. **Tweeddale, H., L. Notley-McRobb, and T. Ferenci.** 1998. Effect of slow growth on metabolism of *Escherichia coli*, as revealed by global metabolite pool ("metabolome") analysis. J. Bacteriol. **180:**5109–5116.
48. **Wang, D., X. Ding, and P. N. Rather.** 2001. Indole can act as an extracellular signal in *Escherichia coli*. J. Bacteriol. **183:**4210–4216.
49. **Zaboura, M., and Y. S. Halpern.** 1978. Regulation of γ-aminobutyric acid degradation in *Escherichia coli* by nitrogen metabolism enzymes. J. Bacteriol. **133:**447–451.
50. **Zimmer, D. P., E. Soupene, H. L. Lee, V. F. Wendisch, A. B. Khodursky, B. J. Peter, R. A. Bender, and S. Kustu.** 2000. Nitrogen regulatory protein C-controlled genes of *Escherichia coli*: scavenging as a defense against nitrogen limitation. Proc. Natl. Acad. Sci. USA **97:**14674–14679.