0021-9193/78/0133-0139$02.00/0
JOURNAL OF BACTERIOLOGY, Jan. 1978, p. 139-148
Vol. 133, No. 1
Copyright © 1978 American Society for Microbiology
Printed in U.S.A.

gltB Gene and Regulation of Nitrogen Metabolism by Glutamine Synthetase in *Escherichia coli*

GREG PAHEL, ANDREW D. ZELENETZ, AND BONNIE M. TYLER*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received for publication 1 September 1977

A mutant (*gltB*) of *Escherichia coli* lacking glutamate synthase (GOGAT) was unable to utilize a wide variety of compounds as sole nitrogen source (e.g., arginine, proline, γ-aminobutyrate, and glycine). Among revertants of these Asm⁻ strains selected on one of these compounds (e.g., arginine, proline, or γ-aminobutyrate) were those that produce glutamine synthetase (GS) constitutively (GlnC phenotype). These revertants had a pleiotropically restored ability to grow on compounds that are metabolized to glutamate. This suggested that the expression of the genes responsible for the metabolism of these nitrogen sources was regulated by GS. An examination of the regulation of proline oxidase confirmed this hypothesis. The differential sensitivities of GlnC and wild-type strains to low concentrations (0.1 mM) of the glutamine analog L-methionine-DL-sulfoximine supported the conclusion that the synthesis of a glutamine permease was also positively controlled by GS. During the course of this study we found that the reported position of the locus (*gltB*) for glutamate synthase is incorrect. We have relocated this gene to be 44% linked to the *argG* locus by P1 transduction. Further mapping has shown that the locus previously called *aspB* is in reality the *gltB* locus and that the "suppressor" of the *aspB* mutation (A. M. Reiner, J. Bacteriol. 97:1431-1436, 1969) is the locus for glutamate dehydrogenase (*gdhA*).

Previous work has established that the glutamine synthetase (EC 6.3.1.2) of *Escherichia coli* (GS_E) can regulate the expression of genes native to *Klebsiella aerogenes* (14, 30). When the structural gene for glutamine synthetase (*glnA* region) of *E. coli* is transferred into a strain of *K. aerogenes* lacking GS, the GS_E has regulatory properties similar to that of the native GS_K (30). In addition, it has been demonstrated that the GS of *E. coli* is capable of regulating the histidine dissimilation (*hut*) genes of both *K. aerogenes* and *S. typhimurium* when they are in an *E. coli* cytoplasm (7). We were therefore interested in determining whether any genes native to *E. coli* are subject to regulation by GS_E. To this end, we have taken advantage of observations on *K. aerogenes* mutants devoid of glutamate synthase (GOGAT; EC 2.6.1.15) outlined below.

In the presence of glucose, a *K. aerogenes* strain lacking GOGAT (Asm⁻) is unable to grow on a number of nitrogen sources utilized by the wild type (8). Among revertants of this strain able to use histidine as sole nitrogen source are some that produce GS constitutively (GlnC phenotype). Subsequent work has shown that in GlnC strains isolated in this manner, the synthesis of many other nitrogen-related enzymes is derepressed in ammonia excess conditions (17). In vitro studies have shown that in the case of the *hut* operon the GS of *K. aerogenes* is an activator of transcription (31). A mutant (*gltB*) of *E. coli* lacking GOGAT activity has been described by Berberich (4), who also reported its map position.

We therefore studied the genetics and physiology of this *gltB* strain and its revertants as a diagnostic tool for determining whether any genes native to *E. coli* are regulated by GS_E. This study describes our findings and also relocates the locus for *gltB*, since we were unable to confirm the reported map position. In the course of mapping this locus we have explained another long-standing observation about a locus called *aspB* (27).

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are described in Table 1. Many of the strains were provided by Barbara Bachmann of the *E. coli* Genetic Stock Center. All are *E. coli*.

Strain construction and mapping. Hfr matings were done according to the procedure of Miller (19). Generalized transductions were performed according to the procedure of Goldberg et al. (13), using coliphage P1 *cts kan*ʳ.

Media. The rich medium was Luria broth (25). The

TABLE 1. *Bacterial strains*

Strain	Genotype	Source
AB1450	F⁻ *thi-1 ilvD16 argH1 metB1 his-1 xyl-7 malP,Q1 lacPl gal-6 ara-13 mtl-13 strA9 tonA2 tsx-7 supE44 gltB (31?)*	B. Bachmann
CB100	F⁻; *thi-1 thr-1 leu-6 his-1 argH1 gltB31 gdh-1 lacY1 gal-6 malP,Q1 xyl-7 mtl-2 strA9 tonA2 tsx-68 supE44*	B. Bachmann
ET1002	F⁻ *gltB31*, sugar and phage markers as in CB100	Sequential P1 transduction of CB100
ET1003	F⁻, sugar and phage markers as in CB100	P1 transduction of ET1002
ET1004	F⁻ *gltB31 glnA299*, sugar and phage markers as in CB100	GlnC revertant of ET1002 on glucose-proline
ET1005	F⁻ *gltB31 glnA300*, sugar and phage markers as in CB100	GlnC revertant of ET1002 on glucose-arginine
ET1104	F⁻ *gltB31 gdh-1 hutC*$_K$, sugar and phage markers as in CB100	Sequential P1 transduction of CB100
ET1100	F⁻ *gltB31 hutC*$_K$[a], sugar and phage markers as in CB100	P1 transductant of ET1104
ET1101	F⁻ *hutC*$_K$, sugar and phage markers as in CB100	P1 transductant of ET1100
ET1106	F⁻ *gltB31 hutC*$_K$ *glnA302*, sugar and phage markers as in CB100	GlnC revertant of ET1100 on glucose-ornithine
ET1110	F⁻ *gltB31 hutC*$_K$ *glnA306*, sugar and phage markers as in CB100	GlnC revertant of ET1101 on glucose-arginine
PA340/T6	F⁻ *thi-1 thr-1 leu-6 his-1 argH1 aspB21 lacY1 gal-6 malP,Q1 xyl-7 mtl-2 strA9 tonA2 tsx-68 supE44*	B. Bachmann
LS519	F⁻ *rha metB* λ φ80	L. Soll
UTHG	HfrG6 *thyA his-1*	J. Suit
1025	F⁻ *argG ilv*	J. Suit
X7014-L	F⁻ *pyrC purB strA*	This laboratory
RT143	F⁻ *metB trp*(Am) *pps*	D. Fraenkel

[a] K = *K. aerogenes*.

nitrogen-free minimal medium was W salts (29). Glucose was added to 0.4%. Nitrogen sources were filter sterilized and added to a final concentration of 0.2% unless otherwise noted.

Isolation of mutants. Independent, spontaneous mutations with the Asm⁻ phenotype were isolated in separate cultures after two cycles of penicillin enrichment in glucose minimal medium containing both arginine and proline as nitrogen sources (to avoid mutations in either the specific arginine or proline pathways). Since the cultures were grown out between cycles of penicillin on medium containing glutamine, we also enriched for glutamine auxotrophs. Potential mutant strains were identified as small colonies on glucose minimal medium with 0.2% arginine and 0.005% each glutamine and aspartate as nitrogen sources.

Enzyme assays. Cells were grown in flasks with rotary shaking at 32°C to a density of 3×10^8 to 4×10^8 cells/ml, chilled on ice, and pelleted by centrifugation at 4°C. For GS assays, hexadecyltrimethylammonium bromide (CTAB) was added to a final concentration of 100 μg/ml before chilling to minimize changes in the adenylylation state of the GS during harvesting (2). GS was assayed in 10-fold-concentrated, CTAB-treated whole cells by measuring γ-glutamyl transferase activity as described by DeLeo and Magasanik (9). Enzyme activity was determined at the isoactivity point (pH 7.33) of the adenylylated and non-adenylylated enzyme (7). To rapidly determine whether a strain had high or low levels of GS activity, a colony test was developed. One hundred microliters of standard γ-glutamyl transferase reaction mix was added to each well of a disposable cell culture tray (Linbro Scientific Co., model IS-MRC-96). Cells were transferred from a plate to the well with a sterile wooden dowel. The trays were covered and incubated for 45 min at 37°C. The reaction was stopped by adding 100 μl of the standard stop mix to each well. Colonies that produce GS at a derepressed level turn dark rust color, whereas repressed colonies remain yellow.

Proline oxidase was assayed in toluene-treated cells by the method of Dendinger and Brill (10). It was found that exposure of the cells to CTAB completely abolished proline oxidase activity, so cultures to be assayed for both GS and proline oxidase were split immediately before harvesting.

Glutamate dehydrogenase (GDH) and GOGAT were assayed in sonically treated crude extracts according to the procedure of Brenchley et al. (8). It was not necessary to use the 10% correction factor for GOGAT activity suggested by these authors if freshly prepared glutamine (Calbiochem, A grade) was used. To rapidly determine whether or not a strain had GDH or GOGAT activity, a whole-cell colony test was developed. The reaction mix was a modification of that used for extracts and contained 50 mM tris(hydroxymethyl)aminomethane buffer (pH 7.6), 5 mM 2-oxoglutarate, 100 μg of CTAB per ml, and 125 μg of sodium deoxycholate per ml. Eighty microliters of this mix was added to wells of the disposable cell culture trays, followed by the addition of 10 μl of water (for nonspecific oxidase activity), 40 mM NH_4Cl (for GDH activity), or 50 mM glutamine (for GOGAT activity). Cells were transferred from freshly grown colonies on minimal plates with a wooden dowel and the tray was incubated at 37°C for 5 min before a 10-μl amount of 2.6-mg/ml reduced nicotinamide adenine dinucleotide phosphate (NADPH) was added. The trays were covered and returned to the incubator. After 10 to 30 min, 5-μl samples were removed, pipetted onto Whatman 3 MM chromatography paper, and dried under a heat lamp. When viewed under ultraviolet light, spots appearing faint purple indicated oxidation of the NADPH to $NADP^+$, whereas intense

violet spots indicated little or no oxidation. In general, all three activities were assayed for each colony in question.

Protein determinations were done according to the method of Lowry et al. (16), using bovine serum albumin as a standard.

RESULTS

Phenotype of *gltB* strains. The data in Table 2 confirm the observation that strains AB1450 and CB100 are devoid of GOGAT activity (4). Strain CB100 clearly lacks GDH activity as well and consequently is a glutamate auxotroph.

Of the three compounds found to supplement this auxotrophy, only aspartate led to a wild-type growth rate. It is transaminated with 2-oxoglutarate to yield glutamate and oxaloacetate (12). Glutamine satisfies the growth requirement somewhat less well and, in the absence of GOGAT, presumably depends on a glutaminase to produce glutamate (26). *E. coli* transports glutamate very slowly (15), and hence the glutamate auxotroph is barely supplemented at all with this compound.

To examine the phenotype of strains with only the *gltB31* mutation, we constructed the isogenic prototrophic strains ET1100 and ET1101, as described in Table 1. The former has GDH but lacks GOGAT activity, whereas the latter has both activities (Table 2).

The survey of potential nitrogen sources represented in Table 3 shows that as in *K. aerogenes* (8) and *Klebsiella pneumoniae* (21), an Asm⁻ strain cannot utilize a number of nitrogen sources that are used by the wild-type strain. Asm⁻ strains of *E. coli* can, however, assimilate ammonia at much lower concentrations (75 μM versus 1 mM) than their *K. aerogenes* or *K. pneumoniae* counterparts.

Location of the *gltB* locus. Berberich reported that the *gltB* gene is 80% linked to the *malP,Q* locus (4). In an attempt to confirm this linkage, we transduced the Mal⁻ Asm⁻ strains AB1450 and ET1100 with a P1 lysate prepared on the Mal⁺ Asm⁺ strain LS519. None of the 88 Mal⁺ transductants scored from each cross became Asm⁺, as determined by both growth on arginine as sole nitrogen source and the colony assay described in Materials and Methods.

To determine the general location of the *gltB* gene, we examined the gradient of transfer of a number of genes when strain UTHG (HfrG6; point of origin at 71 min, with clockwise transfer toward *malP,Q*) is mated with strain CB100. The frequency of recombinants shown in Table 4 indicated that the *gltB* locus maps somewhere between *malP,Q* and *thyA*. An analysis of the recombinants for unselected markers (Table 4) confirmed this result and suggested that some distance separates the *malP,Q* and *gltB* genes, since they are only 68% linked by Hfr. Several markers in the region from *thyA* to *malP,Q* were tested for linkage to *gltB* by P1 transduction, with the results shown in Table 5. The results in lines 2 and 3 imply that *gltB* is linked to

TABLE 2. *GDH and GOGAT levels in several E. coli strains*

Strain	Relevant genotype	Enzyme activity (nmol/min/mg of protein)	
		GDH	GOGAT[a]
AB1450	*gltB31*(?)[b]	598	<10
CB100	*gltB31 gdh-1*	<10	<10
ET1100	*gltB31*	518	<10
ET1101	Wild type	938	1,380

[a] These values are not corrected by 10% as suggested by Brenchley et al. (8) (see Materials and Methods). Cultures (200 ml) were grown at 37°C in glucose-ammonia-glutamine medium, and extracts were prepared as described in Materials and Methods.

[b] Bachmann has traced the geneology of this strain, and the evidence suggests that this *gltB* allele is the same as CB100.

TABLE 3. *Utilization of nitrogen sources in E. coli*

Nitrogen source[a]	Growth[b]	
	ET1101(WT)	ET1100
NH_4^+ (30 mM)	+	+
NH_4^+ (1 mM)	+	+
NH_4^+ (75 μM)	+/−	−
L-Alanine	+	−[c]
L-Arginine	+	−
L-Asparagine	+	−[c]
L-Aspartate	+	+
γ-Aminobutyrate	+	−
L-Glutamate	+/−	+/−
L-Glutamine	+	+
L-Glycine	+	−[c]
L-Methionine	+/−	−
L-Ornithine	+	−
L-Proline	+	−
L-Pyroglutamate	+	−
L-Serine	+	−[c]
D-Serine	+	+
L-Histidine	+	−[d]

[a] The wild-type (WT) ET1101 did not show significant growth with the following nitrogen sources: agmatine, citrulline, cysteine, leucine, lysine, putrescine, threonine, tryptophan, or urea.

[b] The strains were plated on 0.4% glucose solid minimal medium with 0.2% of the given nitrogen source. The growth was scored according to the following scale: +, single colonies formed within 36 h; +/−, single colonies formed after 48 h; −, no growth observed.

[c] Growth can be seen after extended incubations, about 72 h (see text).

[d] These strains carry the genes for histidine utilization from *K. aerogenes*.

argG and highly linked if not coincident with *aspB*.

Explanation of the 100% linkage of *gltB* with *aspB*. Reiner (27) mapped the *aspB* locus with respect to *pup* and *argG* and showed that it was 40% cotransducible with this second marker. However, he found that when he transduced an *argG* mutant to Arg^+ with a P1 lysate prepared on an *aspB* strain, there was no linkage of the two markers. He postulated that the recipient *argG* strain contained an unlinked suppressor of the *aspB* mutation. He mapped this "suppressor" by an interrupted mating and found that it is "about twenty minutes past *lac*." In fact, Bender et al. (3) have shown that in *K. aerogenes* the *gdh* locus is linked to *trp* by transduction. Furthermore, they have shown that an *E. coli* episome covering that region of the chromosome can complement a *gdh* mutation in *K. aerogenes*. This would imply that in *E. coli* the *gdh* locus is also near *trp*.

As discussed previously, strain CB100 has an auxotrophic requirement which can be satisfied by either glutamate or aspartate. If the *aspB* locus were really the *gltB* site, the suppressor might simply be a wild-type *gdh* allele, implying that the aspartate-requiring strain is really a double mutant, as is CB100. We tested this notion by measuring the levels of GOGAT and GDH in strain PA340/T6 (*aspB*) and found that this strain was devoid of both enzymes. Furthermore, we mapped *gdh* by P1 transduction and found that it was slightly linked to both *purB* and *trpA* (lines 4 and 5, Table 5), which is in the region "twenty minutes past *lac*." Therefore, the 100% cotransduction of the *gltB* with the *aspB* locus was a result of the wild-type *gdh* allele of the donor suppressing the aspartate

STRAIN	GENOTYPE *gdh*	*gltB*	ASPARTATE PHENOTYPE
donor	+	−	+
	X		
recipient	−	−	−
	↓		
recombinant	+	−	+

FIG. 1. *Representation of the origin of the gltB mutation in the transductant in the cross P1(ET1100) × PA340/T6. The transduction was done as described in Materials and Methods. The figure is a representation of the transduction in line 3 of Table 5. The dashed lines represent recipient DNA; the solid lines represent donor DNA. The explanation of the 100% linkage observed is that the donor could only make the recipient Asp^+ by contributing its wild-type gdh allele, since the gltB allele was mutated. Therefore, the gltB mutation in the recipient never was transduced at all.*

TABLE 4. *Mapping of gltB by Hfr cross, UTHG × CB100*[a]

Selected marker	Frequency of recombinants[b]	% Inheritance of unselected markers: *gltB*	*mal*	*mtl*	*lac*	*gal*
$gltB^+$	5.4×10^6		35 (61/176)	24 (42/176)	<1 (1/176)	<1 (1/176)
mal^+	3.8×10^6	68 (51/88)[c]		52 (16/88)	4 (4/88)	3 (3/88)
mtl^+	3.4×10^6	58 (51/88)	93 (82/88)		3 (3/88)	1 (1/88)
lac^+	1.3×10^5	48 (42/88)	68 (60/88)	45 (40/99)		ND[d]
gal^+	1.3×10^5	29 (26/88)	69 (61/88)	58 (51/88)	97 (85/88)	

[a] Strain UTGH was mated with strain CB100 as described in Materials and Methods. The GltB phenotype was selected and scored on solid medium containing glucose (0.4%), arginine (0.2%), and the amino acid growth supplements.

[b] The frequency is recombinants/donor.

[c] Numbers in parentheses give percentage with unselected phenotype.

[d] ND, Not done.

TABLE 5. *Mapping of the gdh and gltB loci by P1 transduction*[a]

Donor	Relevant genotype	Recipient	Relevant genotype	Selected markers	Unselected marker	% Cotransduction
1. ET1100	*gltB*	UTHG	*thyA*	$thyA^+$	*gltB*	0 (0/86)[b]
2. ET1100	*gltB*	1025	*argG*	$argG^+$	*gltB*	44 (38/86)
3. ET1100	*gltB*	PA340/T6	*aspB*	$aspB^+$	*gltB*	100 (86/86)
4. ET1104	*gltB, gdh*	X7014-L	*purB*	$purB^+$	*gdh*	3 (2/78)
5. ET1104	*gltB, gdh*	RT143	*trpA*	$trpA^+$	*gdh*	2 (1/52)

[a] P1 transductions were performed as described in Materials and Methods. The *gltB* mutation was scored by failure to grow on solid media with 0.4% glucose and 0.2% arginine. The GDH lesion was scored by the colony assay described in Materials and Methods.

[b] Numbers in parentheses indicate the number of transductants receiving the unselected marker over the total number scored.

requirement, and all of the recombinants still contained the mutation at *gltB* of the *aspB* recipient (Fig. 1).

Isolation of new mutants with the Asm⁻ phenotype. The geneology of strains CB0 and AB1450 (very kindly traced by B. Bachmann of the *E. coli* Genetic Stock Center) indicates that it is likely that the *gltB* alleles in these two strains and in PA340/T6 are identical. In view of the fact that GOGAT is composed of two nonidentical subunits (18, 20), we felt it would be of interest to isolate more mutants with the Asm⁻ phenotype. To this end we isolated spontaneous mutants with the Asm⁻ phenotype as described in Materials and Methods.

The mutations in these strains resulted in three easily identified phenotypic classes: tight glutamine auxotrophs, leaky glutamine auxotrophs, and Asm⁻. Mapping studies yielded the results shown in Table 6. All of the tight glutamine auxotrophs map at *glnA*; all of the others map very near *gltB*. The leaky glutamine auxotrophs are presumably similar to those reported in *Salmonella typhimurium* (11), and the locus has been designated *glnF*. Since none of the mutants with the Asm⁻ phenotype have GOGAT activity, whereas all of those with the GlnF phenotype do, further genetic and physiological analyses are being carried out on these *argG*-linked mutations.

Reversion of strains with the *gltB* mutation. It is possible to select revertants of *gltB* mutant strains at a fairly high frequency on minimal media containing those nitrogenous compounds that can be utilized by the wild-type strain but not by the mutant (see Table 3). The data in Table 7 show that the mutations that suppress the phenotype of the *gltB* mutation fall into several classes, as defined by different growth patterns.

The relatively infrequent class I revertants have a restoration of the wild-type growth pattern. This class should arise by reversion at the *gltB* locus, and all of these revertants have restored GOGAT activity as determined by the colony assay.

Class II revertants have the growth pattern expected of a GlnC mutant on the basis of the *K. aerogenes* phenotype. Indeed, the majority have derepressed γ-glutamyl transferase activity under ammonia excess conditions (glucose-ammonia-glutamine minimal medium), suggesting that GS is involved in controlling these pathways. In all of the GlnC types that have been tested, the constitutivity and *gltB* suppression are highly linked to *glnA* by P1 transduction (unpublished observations). None of the class II revertants have restored GOGAT activity. Those class II revertants that do not have derepressed γ-glutamyl transferase activity could be variations of the Gln(AC) phenotype, which has been described in *K. aerogenes* (17) and *K. pneumoniae* (28). Mutant strains with this phenotype have derepressed levels of GS protein but little or no enzymatic activity. In fact, one of the isolates in class II is a leaky glutamine auxotroph that maps at or near *glnA*.

Class III revertants are similar to those of class II except that they do not utilize proline as sole nitrogen source.

It is possible to obtain mutants (class IV-A and -B) that are apparently specifically reverted for individual pathways.

In the glutamate auxotroph CB100, reversion to glutamate independence on glucose-ammonia minimal medium occurs at a frequency of 10^{-8}/cell. The true reversion frequency of the *gltB31* allele is therefore, at most, 10^{-8}/cell. This accounts for the fact that revertants on serine as sole nitrogen source (class V) are not simply true *gltB* revertants with restored GOGAT activity as in *K. aerogenes* (8), since they occur at a 10-fold-higher frequency. These revertant strains presumably have either increased the levels of the serine-degrading enzyme(s) so that a high enough level of ammonia is produced for the GDH to assimilate or have increased the GDH level or activity so that it can utilize the low levels of NH_4^+ produced.

Revertants of the Asm⁻ strain have been selected on all of those nitrogen sources it does not utilize (Table 3). Among revertants selected on proline, pyroglutamate, ornithine, arginine, γ-aminobutyrate, and histidine (only in a hut_K derivative of *E. coli*) are some that produce GS constitutively (class II), indicating that some aspect of the degradation of each of these compounds is regulated by GS. In addition, the partially pleiotropic class III revertants have been isolated on arginine, ornithine, α-amino-

TABLE 6. *Characterization of new mutant strains*[a]

Relevant genotype	Growth phenotype[b] on:			GOGAT[c]	Linkage to *argG*	No. of independent isolates
	NH_4^+, Gln	NH_4^+	Arg			
glnA	+	−	−	+	0	4
gltB	+	+	−	−	43–55	8
glnF	+	−/+	−	+	22–35	4

[a] Mutants were isolated after penicillin enrichment as described in Materials and Methods.

[b] Growth on glucose minimal agar with nitrogen sources added to 0.2% final concentration. +, Wild-type growth; −, no single colonies after 48 h.

[c] As determined by colony assay (see Materials and Methods) from glucose-ammonia-glutamine minimal agar.

butyrate, and histidine. A GlnC revertant has never been selected on medium containing serine, alanine, glycine, asparagine, or methionine as the sole source of nitrogen, nor do GlnC revertants grow better than the Asm⁻ parental strain on any of these compounds.

Physiology of GlnC revertants and proline oxidase. In the wild-type strain ET1101 the GS level is derepressed about 10-fold when ammonia plus glutamine is replaced by glutamine alone as the nitrogen source (Table 8, lines 1 and 2). Since the proline oxidase is inducible in these strains, there is virtually no detectable activity under these conditions. When proline replaces glutamine as the sole nitrogen source, very high levels of GS are present and proline oxidase is synthesized. When ammonia is also present in this medium, the level of proline oxidase is fivefold lower in strain ET1101 (Table 8, lines 3 and 4). When succinate replaces glucose as the carbon source (in the presence of ammonia and proline), the level of proline oxidase is increased fourfold (data not shown). This indicates that as in *S. typhimurium*, the proline oxidase is regulated by catabolite repression. The "basal" induced level of proline oxidase on glucose-ammonia-proline is not reduced by the introduction of a deletion mutation of *crp* (data not shown).

Two GlnC strains, ET1106 and ET1110, make about the same levels of GS when grown with excess nitrogen as the wild type does when glutamine is used as sole nitrogen source (Table 8, lines 5 and 6). As in *K. aerogenes* (24), the GS in these GlnC strains is almost completely adenylylated. When grown in the presence of high concentrations of ammonia with proline present as inducer, these GlnC strains make two to three times more proline oxidase than the wild type (Table 8, lines 5 and 6 versus line 3).

Sensitivity of GlnC revertants to MS. Various strains were tested for their sensitivity to

TABLE 7. *Phenotypes of gltB revertants of strain ET1100*

Selected nitrogen source[a]	Frequency	Proline	Arginine/ornithine	γ-Aminobutyrate	Serine	No. isolated	% GlnC[b]	Class
Proline	10^{-6}	+[c]	+	+	+	2	0	I
		+	+	+	−	3	100	II
		+	−	−	−	97	0	IV-A
Arginine or ornithine	10^{-7}	+	+	+	+	2	0	I
		+	+	+	−	57	56	II
		−	+	+	−	45	58	III
		−	+	−	−	3	0	IV-B
Serine	10^{-6}	−	−	−	+	24	0	V

[a] Selected on glucose minimal medium with nitrogen source at 0.2%.

[b] The GlnC phenotype is defined as derepressed γ-glutamyl transferase activity (≥ wild type on glucose-glutamine) on glucose-ammonia medium as determined by the colony assay described in Materials and Methods.

[c] +, Single colonies of approximately wild-type diameter formed after 48 h; −, growth no better than that of *gltB* parent after 48 h.

TABLE 8. *GS and proline oxidase activities*

Strain	Relevant phenotype	Medium[a]	Mass doubling time (min)	GS[b]			Proline oxidase[b]
				$-Mg^{2+}$	$+Mg^{2+}$	$\bar{n}$	
ET1101	Wild type	Glutamine + ammonia	45	0.10	0.05	6	<0.1
		Glutamine	100	1.00	0.92	1	<0.1
		Proline + ammonia	50	0.13	0.05	7	1.2
		Proline	180	1.20	1.20	0	6.9
ET1106	GlnC(Asm)[c]	Proline + ammonia	50	0.83	0.04	12	2.5
ET1110	GlnC(Asm)[c]	Proline + ammonia	55	0.98	0.04	12	3.7

[a] Strains were grown at 32°C on glucose minimal medium with the indicated nitrogen sources added to 0.2% final concentration.

[b] Cells were collected and assayed as described in Materials and Methods. Enzyme activities are in micromoles of product formed per minute per milligram of protein.

[c] The GlnC strains are of the pleiotropic class II type (see text). The Asm phenotype is in parentheses since it is partially suppressed by the GlnC.

the glutamine analog L-methionine-DL-sulfoximine (MS), with the results shown in Table 9. The wild-type strain, ET1003, and the *gltB* mutant, ET1002, are resistant to the analog at much higher concentrations than GlnC revertant strains ET1004 and ET1005. Furthermore, sensitivity to the analog was not dependent on the presence of the *gltB* mutation (data not shown). There are two interpretations of this result: (i) since MS can inhibit the activity of GS it could be that the GS in the GlnC revertant was hypersensitive to MS; or (ii) the MS may have enhanced ability to enter the cell as a result of the GlnC mutation. The first alternative was directly tested by measuring the sensitivity of the GS to the MS inhibition in crude extracts of both the wild-type strain ET1003 and the GlnC strain ET1004. The results are shown in Table 10. It is clear that the activity of the GS was not differentially affected by the source of the GS or the adenylylation state. Virtually identical inhibition of the GS in crude extracts was seen for adenylylated and non-adenylylated wild-type enzymes and for GlnC enzyme.

If the second possibility is correct and high levels of GS lead to increased intracellular levels of MS, then one would predict that under conditions of nitrogen limitation the wild-type strain would become more sensitive to MS on arginine as sole nitrogen source than on ammonia. As shown in Table 9, this prediction holds true. These observations support the hypothesis that GS is involved in regulating the transport of glutamine, since at these concentrations of MS it enters the cell via a glutamine permease (5).

DISCUSSION

Various studies have shown that the GS of *E. coli* is capable of regulating the synthesis of nitrogen-utilizing enzymes when in the cytoplasm of *K. aerogenes*, and that the *hut* operons from both *S. typhimurium* and *K. aerogenes* are regulated by GS when transferred into *E. coli* (7, 14, 30). It would seem, therefore, that the GS of *E. coli* possesses the ability to regulate gene expression and that *E. coli* has all of the necessary components for the regulation of native genes. Recently, Newman and Cole (23) reported that they found no evidence implicating GS as a positive regulatory element in the synthesis of nitrite reductase and proline oxidase in *E. coli*. They conclude that *E. coli* is similar to *S. typhimurium* in its inability to regulate enzyme synthesis by GS.

The results presented here clearly contradict this conclusion of Newman and Cole and establish that native *E. coli* genes involved in the utilization of nitrogenous compounds are regulated by GS in a manner similar to *K. aerogenes*. This conclusion is based on the following results. Strains containing *gltB* mutations cannot readily assimilate ammonia at low concentrations (Asm$^-$ phenotype). However, this does not explain the inability of such strains to grow on nitrogen sources, which are degraded directly to glutamate (e.g., proline), since Asm$^-$ strains can utilize glutamate as the sole source of nitrogen. This phenotype must result from the absence of the enzymes necessary for the degradation of these glutamate-yielding compounds. Thus, the observation that in *E. coli* mutations tightly linked to *glnA* both partially suppress the growth phenotype of the *gltB* mutant and result in constitutive synthesis of GS (GlnC phenotype) suggests that the GS protein is involved in the regulation of a number of enzymes that provide the cell with glutamate. Physiological studies further support this notion. We have shown that in a wild-type strain of *E. coli* K-12, if proline is present as inducer, the levels of both proline oxidase and GS are derepressed when grown in nitrogen-limiting conditions (glucose-proline) as compared with nitrogen excess conditions (glucose-ammonia-proline). Furthermore, some GlnC revertants of Asm$^-$ strains produce higher levels of proline oxidase than the wild type when grown in nitrogen excess conditions.

When strain ET1101 is grown on succinate-

TABLE 9. *Sensitivity of various strains to MS*[a]

Strain	Relevant phenotype	Concn (mM) of MS					
		GN				Garg	
		0.05	0.1	0.5	1	0	0.1
1. ET1003	Wild type	+	+	−/+	−	+	−
2. ET1002	GltB	+	+	−/+	−	ND	ND
3. ET1004	GltB GlnC1	−	−	−	−	+	−
4. ET1005	GltB GlnC2	−	−	−	−	+	−

[a] Strains were grown on glucose-ammonia (GN) or glucose-arginine (Garg) solid media with the indicated concentrations of MS. Growth was scored from single colonies as follows: +, growth similar to the wild type under identical conditions; −/+, growth was significantly poorer than that of the wild-type strain as determined by colony size; −, no growth; ND, not determined.

TABLE 10. *Inhibition of adenylylated, non-adenylylated, and GlnC GS by MS in extracts*[a]

MS concn (mM)	E_1[b]		E_{11}[c]		E_{12}[d] (GlnC)	
	GS[e]	% I[f]	GS	% I	GS	% I
0	1,123	0	983	0	840	0
0.001	876	22	732	22	638	24
0.005	505	55	374	62	353	58
0.01	337	70	344	65	269	68

[a] The inhibition of GS activity was determined by preincubating the given amount of MS with the appropriate extract in the assay reaction mix for 10 min before adding the glutamine to start the reaction.

[b] The non-adenylylated GS was prepared by growing ET1003 at 37°C in media containing glucose (0.4%) and glycine (0.2%). Cells were harvested as described in Materials and Methods.

[c] The adenylylated GS was prepared from strain ET1003 as above, except that when the culture reached a density of 100 Klett units, $(NH_4)_2SO_4$ was added to a final concentration of 0.2%; 5 min later the cultures were harvested.

[d] The GlnC GS was prepared from ET1004 grown on glucose-ammonia minimal medium at 37°C.

[e] GS activity is in nanomoles per minute per milligram of protein.

[f] % I, Percent inhibition relative to no addition of MS.

ammonia-proline medium, the level of proline oxidase is four times higher than when it is grown on glucose-ammonia-proline. This indicates that this system is under cyclic adenosine monophosphate receptor protein (CAP)-cyclic AMP control as in *S. typhimurium* (22). However, the induced level found on glucose-ammonia-proline is not further reduced by the introduction of a deletion of the *crp* gene. One reason, therefore, that the Asm$^-$ strain reverts at such a high frequency on proline as sole nitrogen source might be that in *E. coli* the proline oxidase has a CAP-cyclic AMP and GS-independent "basal" level such that only a slight increase is needed to allow growth. This is consistent with the relatively poor derepression of proline oxidase in GlnC strains even though their inability to grow on proline is completely reversed. The reason for the large number of proline-specific revertants of Asm$^-$ strains might then be that the promoter for the proline-degradative genes may already be close to the form necessary for RNA polymerase to initiate transcription. If this is the case, then a relatively small change may allow enough initiation for growth to occur. This assumes, of course, that the enzymes necessary are in a single operon.

The other interesting feature of the proline degradation system is that some GlnC strains (class III) do not suppress the inability of an Asm$^-$ strain to grow on proline at all, while being otherwise pleiotropically reverted. Furthermore, the GlnC in strain ET1106 only allows derepression of proline oxidase to 35% of the level that the wild type attains when fully nitrogen derepressed, whereas this same mutation allows derepression of an ornithine transaminase activity to 100% of the fully nitrogen-derepressed level (B. Friedrich and G. Pahel, unpublished observations). These results indicate that the proline oxidase of *E. coli* has a requirement for GS activation in its most proficient form (i.e., that which exists under the most extreme nitrogen-limiting growth conditions). The existence of different classes (II and III) of GlnC's, which all synthesize equivalent high levels of GS but do not all activate every system regulated by GS, supports the notion that these mutations are in the structural gene for GS (*glnA*). Thus a mutation resulting in an alteration of the structure of the GS molecule would cause a change in the ability of the activation complex to recognize certain target sites but not others. Such a class of revertants has been observed in cyclic AMP-independent CAP$^+$ revertants of a *crp* mutant (1). This would imply that the conformation of the GS protein is important in its ability to stimulate transcription.

It is apparent that in both *E. coli* and *K. aerogenes* GS has the ability to activate the synthesis of enzymes involved in nitrogen utilization. However, in contrast to the situation with *K. aerogenes*, in *E. coli* GDH is not repressed when GS levels are high (30). When the *glnA* region of *E. coli* is placed in the cytoplasm of a *K. aerogenes* mutant lacking GS, the GDH levels are normally regulated. Therefore, the GS of *E. coli* is structurally competent to repress the levels of *K. aerogenes* GDH. It follows that some other factor is responsible for the failure of GDH to repress in *E. coli*. One logical place to look for this factor would be in the *gdhA* region of *E. coli*. Since a GlnC revertant of an Asm$^-$ strain in *K. aerogenes* is missing GOGAT and repressed for GDH, it is unable to make glutamate. If an *E. coli* episome carrying *gdhA* and *trp* is introduced into such a glutamate-requiring GlnC(Asm) strain of *K. aerogenes*, which is also Trp$^-$, the Trp$^+$ exconjugants are no longer glutamate requirers. This suggests that some controlling element on the episome bearing the *gdhA* gene is responsible for the failure of GDH synthesis to be repressed in *E. coli*. A likely explanation would be that the promoter-operator region of the *gdhA* gene of *E. coli* is insensitive to GS repression.

An apparent consequence of the fact that the GDH of *E. coli* is not repressed under nitrogen-limiting conditions and hence is present at higher levels than in *K. aerogenes* is that Asm$^-$

strains of *E. coli* can utilize ammonia at much lower concentrations than *K. aerogenes* (75 μM versus 1.0 mM). This correlation between GDH levels and nitrogen-scavenging ability suggests that in *E. coli* GDH plays a physiologically significant role in supplying glutamate even when there are low exogenous concentrations of ammonia. The ability of *E. coli* Asm^- strains to assimilate NH_4 at low concentrations means that upon extended incubation (72 h), strains bearing the *gltB* mutation show considerable growth on those compounds such as serine (see pathways, below) from which the nitrogen moiety does not reach glutamate either directly (e.g., proline) or by transamination (e.g., aspartate) but rather reaches it via the ammonium ion. This implies that the enzymes necessary to produce ammonia from these compounds are present. In fact, none of the strains with the GlnC phenotype grow any better than the *gltB* on any of the compounds in the pathways not regulated by GS (see below). In addition, we have never found a GlnC revertant of the Asm^- strain on any of these compounds. Thus it is unlikely that the synthesis of the degradative enzymes for serine, alanine, glycine, or asparagine is under GS control. On the other hand, if reversion of Asm^- strains on a particular compound (e.g., arginine) does yield GlnC strains, and if growth on this compound is allowed by GlnC-type mutations isolated on different compounds, then it is very likely that some aspect of the degradation of that particular compound is regulated by GS. Pathways for the degradation of proline, arginine, pyroglutamate, ornithine, and γ-aminobutyric acid (see GS-regulated pathways, below) fulfill these two criteria and are likely to be regulated by GS in a manner similar to that for degradation of proline oxidase. The regulation of nitrogen-related pathways in strain ET1101 is summarized as follows: GS regulated—arginine, γ-aminobutyrate, histidine, proline, pyroglutamate, ornithine, and glutamine transport; possibly not GS regulated—alanine, asparagine, glycine, serine, and methionine.

In none of the pathways can we rule out the possibility that the step regulated by GS is entry into the cell. The results presented here suggest that GS does have a role in regulating the transport of the glutamine and methionine analog MS. The studies of Willis et al. (32) in *E. coli* and Betteridge and Ayling (6) in *S. typhimurium* show a correlation between the levels of glutamine transport and GS and the degree of nitrogen limitation. Furthermore, Betteridge and Ayling (5) have shown in *S. typhimurium* that a high-affinity glutamine transport system is responsible for the transport of MS at low concentrations. Our results confirm and extend these observations and suggest that GS regulates a transport system for glutamine in *E. coli*.

ACKNOWLEDGMENTS

We are grateful to Barbara Bachmann for providing many of the strains used in this work. We thank Fred Bloom, Stan Streicher, and Boris Magasanik for many helpful suggestions during the course of this work and the preparation of the manuscript.

This research was supported by Public Health Service grant GM-22527 from the National Institute of General Medical Sciences. G. P. was supported by Microbiology Training grant GM-00602 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Arditti, R., T. Grodzicker, and J. Beckwith.** 1973. Cyclic adenosine monophosphate independent mutants of the lactose operon of *Escherichia coli*. J. Bacteriol. **114**:652–655.
2. **Bender, R. A., K. A. Janssen, A. D. Resnick, M. Blumenberg, F. Foor, and B. Magasanik.** 1977. Biochemical parameters of glutamine synthetase from *Klebsiella aerogenes*. J. Bacteriol. **129**:1001–1009.
3. **Bender, R. A., A. Macaluso, and B. Magasanik.** 1976. Glutamate dehydrogenase: genetic mapping and isolation of regulatory mutants of *Klebsiella aerogenes*. J. Bacteriol. **127**:141–148.
4. **Berberich, M. A.** 1972. A glutamate dependent phenotype of *E. coli* K-12: the result of two mutations. Biochem. Biophys. Res. Commun. **47**:1498–1503.
5. **Betteridge, P. R., and P. D. Ayling.** 1975. The role of methionine transport defective mutations in resistance to methionine sulphoximine in *Salmonella typhimurium*. Mol. Gen. Genet. **138**:41–52.
6. **Betteridge, P. R., and P. D. Ayling.** 1976. The regulation of glutamine transport and glutamine synthetase in *Salmonella typhimurium*. J. Gen. Microbiol. **95**:324–334.
7. **Bloom, R. R., S. L. Streicher, and B. Tyler.** 1977. Regulation of enzyme synthesis by the glutamine synthetase of *Salmonella typhimurium*: a factor in addition to glutamine synthetase is required for activation of enzyme formation. J. Bacteriol. **138**:983–990.
8. **Brenchley, J. E., M. J. Prival, and B. Magasanik.** 1973. Regulation of the synthesis of enzymes responsible for glutamate formation in *Klebsiella aerogenes*. J. Biol. Chem. **248**:6122–6128.
9. **DeLeo, A. B., and B. Magasanik.** 1975. Identification of the structural gene for glutamine synthetase in *Klebsiella aerogenes*. J. Bacteriol. **121**:313–319.
10. **Dendinger, S., and W. J. Brill.** 1970. Regulation of proline degradation in *Salmonella typhimurium*. J. Bacteriol. **103**:144–152.
11. **Garcia, E., S. Bancroft, S. G. Rhee, and S. Kustu.** 1977. The product of a newly identified gene, *glnF*, is required for synthesis of glutamine in *Salmonella*. Proc. Natl. Acad. Sci. U.S.A. **74**:1662–1666.
12. **Gelfand, D. H., and R. A. Steinberg.** 1977. *Escherichia coli* mutants deficient in the aspartate and aromatic amino acid aminotransferases. J. Bacteriol. **130**:429–440.
13. **Goldberg, R. B., R. A. Bender, and S. L. Streicher.** 1974. Direct selection for P1-sensitive mutants of enteric bacteria. J. Bacteriol. **118**:810–814.
14. **Goldberg, R. B., F. R. Bloom, and B. Magasanik.** 1976. Regulation of histidase synthesis in intergeneric hybrids of enteric bacteria. J. Bacteriol. **127**:114–119.
15. **Halpern, Y. S., and H. E. Umbarger.** 1961. Utilization of L-glutamic acid and 2-oxoglutaric acid as sole sources of carbon by *Escherichia coli*. J. Gen. Microbiol. **26**:175–183.

16. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. **Magasanik, B. M., M. J. Prival, J. E. Brenchley, B. M. Tyler, A. B. DeLeo, S. L. Streicher, R. A. Bender, and C. G. Paris.** 1974. Glutamine synthetase as a regulator of enzyme synthesis, p. 119–138. *In* B. L. Horecker and E. R. Stadtman (ed.), Current topics in cellular regulation, vol. 8. Academic Press Inc., New York.
18. **Mäntsälä, P., and H. Zalkin.** 1976. Glutamate synthase. J. Biol. Chem. **251**:3294–3299.
19. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Miller, R. E., and E. R. Stadtman.** 1972. Glutamate synthase from *Escherichia coli*. J. Biol. Chem. **247**:7407–7419.
21. **Nagatani, H., M. Shimizu, and R. C. Valentine.** 1971. The mechanism of ammonia assimilation in nitrogen fixing bacteria. Arch. Mikrobiol. **79**:164–175.
22. **Newell, S. R., and W. J. Brill.** 1972. Mutants of *Salmonella typhimurium* that are insensitive to catabolite repression of proline degradation. J. Bacteriol. **111**:375–382.
23. **Newman, B. M., and J. A. Cole.** 1977. Lack of a regulatory function for glutamine synthetase protein in the synthesis of glutamate dehydrogenase and nitrite reductase in *Escherichia coli* K-12. J. Gen. Microbiol. **98**:369–377.
24. **Prival, M. J., J. E. Brenchley, and B. Magasanik.** 1973. Glutamine synthetase and the regulation of histidase formation in *Klebsiella aerogenes*. J. Biol. Chem. **248**:4334–4344.
25. **Prival, M. J., and B. Magasanik.** 1971. Resistance to catabolite repression of histidase and proline oxidase during nitrogen limited growth of *Klebsiella aerogenes*. J. Biol. Chem. **246**:6288–6296.
26. **Prusiner, S.** 1975. Regulation of glutaminase levels in *Escherichia coli*. J. Bacteriol. **123**:992–999.
27. **Reiner, A. M.** 1969. Isolation and mapping of polynucleotide phosphorylase mutants of *Escherichia coli*. J. Bacteriol. **97**:1431–1436.
28. **Shanmugan, K. T., I. Chan, and C. Morandi.** 1975. Regulation of nitrogen fixation. Nitrogenase-derepressed mutant of *Klebsiella pneumoniae*. Biochim. Biophys. Acta **408**:101–111.
29. **Smith, G. R., Y. S. Halpern, and B. Magasanik.** 1971. Genetic and metabolic control of enzymes responsible for histidine degradation in *Salmonella typhimurium*. J. Biol. Chem. **246**:3320–3329.
30. **Streicher, S. L., A. B. DeLeo, and B. Magasanik.** 1976. Regulation of enzyme formation in *Klebsiella aerogenes* by episomal glutamine synthetase of *Escherichia coli*. J. Bacteriol. **127**:184–192.
31. **Tyler, B., A. B. DeLeo, and B. Magasanik.** 1974. Activation of transcription of *hut* DNA by glutamine synthetase. Proc. Natl. Acad. Sci. U.S.A. **71**:225–229.
32. **Willis, R. C., K. K. Iwata, and C. E. Furlong.** 1975. Regulation of glutamine transport in *Escherichia coli*. J. Bacteriol. **122**:1032–1037.