JOURNAL OF BACTERIOLOGY, Mar. 1979, p. 1111-1118
0021-9193/79/03-1111/08$02.00/0

Vol. 137, No. 3

Isolation and Properties of *Escherichia coli* K-12 Mutants Impaired in the Utilization of γ-Aminobutyrate

ESTHER METZER, RUTH LEVITZ, AND YEHESKEL S. HALPERN*

Department of Molecular Biology, Institute of Microbiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Received for publication 22 September 1978

We have isolated mutants of *Escherichia coli* K-12 CS101B that have lost the ability to utilize γ-aminobutyrate as a source of nitrogen. One class of mutants, which were not affected in the utilization of other nitrogen sources (proline, arginine, glycine), included many isolates with lesions in γ-aminobutyrate transport or in its transamination and one mutant completely devoid of succinic semialdehyde dehydrogenase activity and exhibiting low γ-aminobutyrate transport and transamination. γ-Aminobutyrate-utilizing revertants of the latter recovered full transport and transamination capacities but remained dehydrogenaseless. Another class of mutants showed pleiotropic defects in nitrogen metabolism. One such mutant was lacking glutamate synthase activity. The genes specifying the synthesis of γ-aminobutyrate permease, *gabP*, γ-aminobutyrate transaminase, *gabT*, and succinic semialdehyde dehydrogenase, *gabD*, and the control gene, *gabC*, that coordinately regulates their expression all form a cluster on the *E. coli* chromosome, linked to the *srl* and *recA* loci (at 57.5 min). The mutations with pleiotropic effects on the metabolism of nitrogenous compounds are not linked to the *gab* cluster.

A specific pathway for the utilization of γ-aminobutyrate by *Escherichia coli* K-12 has been described in earlier communications from this laboratory (9, 13). The pathway consists of a highly specific γ-aminobutyrate transport system (13) and two enzymes, glutamate-succinic semialdehyde transaminase (GSS transaminase; EC 2.6.1.19) and succinic semialdehyde dehydrogenase (SS dehydrogenase; EC 1.2.1.16), that catalyze the conversion of γ-aminobutyrate to succinic acid according to the following scheme: (i) γ-aminobutyrate + α-ketoglutarate $\xrightarrow{\text{GSS transaminase}}$ succinic semialdehyde + L-glutamate; (ii) succinic semialdehyde + NADP $\xrightarrow{\text{SS dehydrogenase}}$ succinic acid + NADPH.

The expression of γ-aminobutyrate permease and GSS transaminase and SS dehydrogenase activities is coordinately affected by mutation at a common control locus, *gabC* (8, 13). The synthesis of all three components of the γ-aminobutyrate pathway is strongly repressed by glucose, but repression can be abolished by limiting the supply of nitrogen, a condition leading to derepressed production of glutamine synthetase (EC 6.3.1.2). Evidence was presented which strongly supports the notion that glutamine synthetase acts as a positive regulator in the *E. coli* γ-aminobutyrate control system (23).

In the present paper we describe the isolation of mutants specifically impaired in γ-aminobutyrate transport and GSS transaminase and SS dehydrogenase activities, respectively. The genes which determine these activities, *gabP*, *gabT*, and *gabD*, and the pleiotropic control gene, *gabC*, are shown to be clustered on the *E. coli* chromosome in proximity to the *recA* locus.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 strains used in this study are listed in Table 1.

Growth media and cultivation of bacteria. The basal medium of Davis and Mingioli (7) lacking citrate and ammonium salts was used, supplemented with 0.5% glucose, 0.5% glycerol, or 1% sodium succinate as the carbon source and 0.1% $(NH_4)_2SO_4$ or 0.2% of another nitrogen source, as indicated. When required, L-methionine was added at a concentration of 50 μg/ml, all other L-amino acids were added at 25 μg/ml, guanine and adenine were added at 20 μg/ml, and vitamin B1 was added at 2 μg/ml. Bacteria were grown in conical flasks in a shaking water bath at 37°C, and growth was monitored in a Klett-Summerson colorimeter with a no. 54 filter.

Isolation of mutants. γ-Aminobutyrate-nonutilizing mutants were obtained by UV irradiation of utilizing strain CS101B. Mutants were isolated after two cycles of penicillin selection (11) in glucose-γ-aminobutyrate (strains D-8 and L-18) or glycerol-γ-aminobutyrate (strains M-16 and M-20) medium. Transport-negative mutants S-5 and S-9 were selected

TABLE 1. *Bacterial strains*[a]

Strain	Utilization of γ-aminobutyrate as nitrogen source	Genotype	Source/reference
CS101A	−	*metB*	Hfr Cavalli (1)
CS101B	+	*metB gabC*	UV-induced mutant of CS101A
M-16	−	*metB gabC gabD*	UV-induced mutant of CS101B
M-20	−	*metB gabC gabT*	UV-induced mutant of CS101B
S-5	−	*metB gabC gabP5*	Obtained by γ-[^{3}H]aminobutyrate suicide from UV-irradiated CS101B
S-9	−	*metB gabC gabP9*	Obtained by γ-[^{3}H]aminobutyrate suicide from UV-irradiated CS101B
D-8	−[b]	*metB gabC "nit"*	UV-induced mutant of CS101B
L-18	−	*metB gabC "nut"*	UV-induced mutant of CS101B
CS101B Met$^+$	+	*gabC*	Spontaneous mutant of CS101B
CS101A22	−		Transduction: CS101B Met$^+$ → CS101A
M-1613	−	*gabC gabD*	Transduction: CS101B Met$^+$ → M-16
M-2033	−	*gabC gabT*	Transduction: CS101B Met$^+$ → M-20
S-55	−	*gabC gabP5*	Transduction: CS101B Met$^+$ → S-5
S-93	−	*gabC gabP9*	Transduction: CS101B Met$^+$ → S-9
D-86	−[b]	*gabC "nit"*	Transduction: CS101B Met$^+$ → D-8
L-1832	−	*gabC "nut"*	Transduction: CS101B Met$^+$ → L-18
M-165	+	*metB gabC gabD gabD2*	Spontaneous γ-aminobutyrate-utilizing revertant of M-16
M-1651	+	*gabC gabD gabD2*	Transduction: CS101B Met$^+$ → M-165
KL-141	−	*pyrE argG thyA thi rpsL gltB*	(17)
CS101G2	−[b]	*metB gabC argG gltB*	Transduction: KL-141 → CS101B
CS101BD4	+	*metB gabC argG*	Transduction: KL-141 → CS101B
CS101BR	+	*metB gabC rha*	Spontaneous mutant of CS101B
D-89	−[b]	*metB gabC "nit" nalA*	Spontaneous mutant of D-8
KL-16	−	*thi-1*	Hfr, PO at 61 min, counter-clockwise transfer (1)
KL-16B	+	*thi-1 gabC*	Spontaneous mutant of KL-16
AT-2682	−	*his-1 purF guaA relA argH thi-1 fuc-4 xyl-7 malA rpsL8, L9,* or *L14* λ^r λ^- *supE44*?	B. Bachmann
KL-1699	−	*recA thi-1*	(1)
KL-1699B	+	*recA thi-1 gabC*	Spontaneous mutant of KL-1699
T100	NT[c]	*thy his srl*::Tn*10*	Derivative of Hfr G6 (16)
CS101B18	+	*gabC srl*::Tn*10*	Transduction: T100 → CS101B
CS101B25	+	*gabC recA srl*::Tn*10*	Transduction: T100 → CS101B
AT-2471	−	*tyrA thi-1 relA* λ^-	B. Bachmann
AT-2477	+	*tyrA thi-1 gabC relA* λ^-	Spontaneous mutant of AT-2471
T5	NT	*thy his trp*::Tn*10*	Derivative of Hfr G6 (16)
AT-2477/15	+	*tyrA thi-1 gabC trp*::Tn*10*	Transduction: T5 → AT-2477
L-1821	−	*metB gabC "nut" nalA*	Spontaneous mutant of L-18

[a] All the strains are derivatives of *E. coli* K-12.
[b] In the presence of glucose as the carbon source.
[c] NT, Not tested.

by the suicide method described by Lo et al. (15), using ^{3}H-labeled γ-aminobutyrate, 519 mCi/mmol (13).

Transduction. P1*kc* lysates of the desired donor bacteria were prepared by the method of Lennox (14), and transduction was carried out as described by Miller (21).

Mating experiments. The mating experiments were performed as described previously (20). When an Hfr strain was used as a recipient, mating was performed as follows. Recipient cultures were grown overnight in succinate–ammonia minimal medium at 37°C with vigorous aeration to the stationary phase (6), centrifuged, washed with basal medium, and resuspended in basal salts medium. The cells were starved for carbon and nitrogen by vigorous aeration in basal salts medium at 37°C for 5 h. The donor strain was grown in mating broth (20) without shaking at 37°C. Succinate, $(NH_4)_2SO_4$, and L-methionine were added to the recipient culture, and mating was started by mixing the parental strains to final cell densities of 8 × 10^7 donor cells and 10^9 recipient cells per ml. The mating was performed in a volume of 10 ml in a 250-ml conical flask at 37°C without shaking. Aliquots, 1

ml, were withdrawn at different times and diluted with 4 ml of basal medium containing 100 μg of nalidixic acid per ml, in order to stop immediately the transfer of Hfr DNA (24). The diluted aliquots were plated on selective media containing 20 μg of nalidixic acid per ml.

Preparation of extracts. Logarithmic cultures were harvested at a turbidity of 105 Klett units, washed with 0.85% NaCl, suspended in 0.1 M phosphate buffer (pH 7.3) and 0.01% 2-mercaptoethanol, and subjected to three 15-s treatments in an MSE ultrasonicator. The extract was centrifuged at 39,000 × *g* for 20 min to remove cell debris. Protein was determined according to Lowry et al. with bovine serum albumin as a standard (18). Enzyme activities were assayed within 2 h of preparation.

Enzyme assays. GSS transaminase, SS dehydrogenase, and glutamate synthase (EC 2.6.1.53) were assayed spectrophotometrically as described previously (23).

Transport assays. Transport of γ-aminobutyrate by intact cells was determined with suspensions of nongrowing bacteria in the presence of chloramphenicol as described previously (13).

Chemicals. ^{14}C-labeled γ-aminobutyrate, 49.4 mCi/mmol, and ^{3}H-labeled γ-aminobutyrate, 35.1 Ci/mmol, were purchased from New England Nuclear Corp., Boston, Mass. Succinic semialdehyde was prepared by synthesis and hydrolysis of dimethylformylsuccinate (12).

RESULTS

Isolation of γ-aminobutyrate-nonutilizing mutants. Penicillin treatment of glycerol-γ-aminobutyrate cultures of strain CS101B yielded two distinct classes of mutants which lost the ability to grow in a glucose-γ-aminobutyrate medium. The minority class consisted of γ-aminobutyrate-specific mutants, some of them lacking GSS transaminase activity only (e.g., strain M-20) and one mutant with no SS dehydrogenase activity and very low γ-aminobutyrate transport and transaminase activities (strain M-16). γ-Aminobutyrate-utilizing revertants of the latter regained normal transport and transamination but were still devoid of SS dehydrogenase activity (e.g., strain M-165). γ-Aminobutyrate-specific mutants, most of them with impaired transport activity (e.g., strains S-5 and S-9), were also obtained by the suicide method (see Table 2). The majority of mutants obtained by penicillin selection in the glycerol-γ-aminobutyrate medium and all of the mutants obtained by penicillin selection in a glucose-γ-aminobutyrate medium, however, were not specific and were limited in their ability to utilize other nitrogen sources as well (e.g., strains D-8 and L-

TABLE 2. *GSS transaminase (GSST) and SS dehydrogenase (SSDH) activities and V_{max} values of γ-aminobutyrate (GABA) transport in wild-type strains and in GABA-utilizing mutants and GABA-nonutilizing revertants of E. coli K-12*

Strain	Utilization of GABA as nitrogen source[a]	Sp act of GABA enzymes (nmol/mg of protein per min)[b]		GABA transport activity (V_{max})[c] (nmol/mg of protein per min)
		GSST	SSDH	
CS101A	−	60	36	0.15
CS101B	+	601	1,264	1.39
M-20	−	85	1,080	1.39
M-16	−	79	0	0.35
M-165	+	698	0	2.29
S-5	−	608	797	0[d]
S-9	−	540	1,012	0[d]
CS101G2	−	350	731	1.39
D-8	−	672	1,038	0.85
L-18	−	553	1,023	1.39

[a] In the presence of 0.5% glucose as the source of carbon.

[b] Cells were grown on succinate-NH_4^+.

[c] K_m of the transport system for GABA was the same (10 μM) in all strains tested.

[d] The radioactivity retained on the filters was not significantly different from that measured in the zero-time control mixtures.

TABLE 3. *Utilization of various nitrogen sources by γ-aminobutyrate (GABA)-utilizing and -nonutilizing mutants in the presence of glucose or succinate as the carbon source*[a]

Strain	Nitrogen source									
	$(NH_4)_2SO_4$		GABA		Proline		Aspartate		Glycine	
	Succinate[b]	Glucose	Succinate	Glucose	Succinate	Glucose	Succinate	Glucose	Succinate	Glucose
CS101A	+	+	−[c]	−[c]	+	−[c]	+	+	+	+
CS101B	+	+	+	+	+	−[c]	+	+	+	+
CS101G2	+	+	+	−	+	−	+	+	−	−
D-8	+	+	+	−	+	−	+	+	+	−
L-18	+	+	−	−	−	−	+	−	+	+

[a] Growth was tested on solid media containing either 0.5% glucose or 1% sodium succinate with 0.1% $(NH_4)_2SO_4$ or 0.2% of the other indicated nitrogen sources. The medium was supplemented with 25 μg of L-arginine per ml to enable growth of strain CS101G2.

[b] Carbon source.

[c] Slight growth after 48 h at 37°C.

18; see Table 3). To this group of nonspecific mutants unable to grow on glucose-γ-aminobutyrate also belongs strain SC101G2, a glutamate synthaseless (*gltB*) transductant of strain CS101B (see Table 3 and reference 23).

Genetic mapping of the γ-aminobutyrate utilization pathway. Earlier studies involving crosses with the γ-aminobutyrate-nonutilizing strain KL141 as the recipient placed the pleiotropic control locus *gabC* between the *argG* and *thyA* genes (10). It was noted, however, that γ-aminobutyrate-utilizing recombinants obtained by conjugation with the Hfr strain OR121 had higher levels of GSS transaminase and SS dehydrogenase and grew faster in media with γ-aminobutyrate as the nitrogen source than did γ-aminobutyrate-utilizing transductants of the same recipient (10). These findings suggested that an additional locus, transferred to the KL141 recipient before the *gabC* gene during conjugation, was involved in the utilization of γ-aminobutyrate. The location of *gabC* was, therefore, reexamined, using other γ-aminobutyrate-nonutilizing strains as recipients.

Upon interrupted mating of the guanine-requiring, γ-aminobutyrate-nonutilizing strain AT2682 as the recipient and the *gabC* mutant KL-16B as the donor (point of origin at 61 min, counterclockwise transfer), entry of *gabC* commenced a few minutes before that of *guaA*, indicating a map location between 55 and 60 min (Fig. 1; see also reference 2).

Results of mapping the *gab* genes by transduction are presented in Tables 4, 5, and 6. Table 4 demonstrates the close linkage of *gabT*, the gene which determines the synthesis of γ-aminobutyrate transaminase, to the *recA* and *srl* loci, as obtained in two-point crosses with the *gabT* recipient strain M-20 (see Table 2) and P1 lysates of *recA* and *srl* mutants. Table 5 shows three-point crosses between strains CS101A (wild-type *gabC*) and M-20 as recipients and a γ-aminobutyrate-utilizing strain as the donor. The results disclose a linkage of 49 and 46% between *recA* and *gabC* and *recA* and *gabT*, respectively, and a 31% linkage between the *gab* genes and *srl*, giving a map position for the *gab* loci of 57.5 min; the gene order, as indicated by analysis of the phenotypes of the recombinants, is: *gab-recA-srl*. The *srl-recA* cotransduction frequency obtained in these experiments (81 and 83% with strains CS101A and M-20 as recipients, respectively) is in very good agreement with recent results of McEntee (19).

***gab* gene cluster.** Methionine prototroph de-

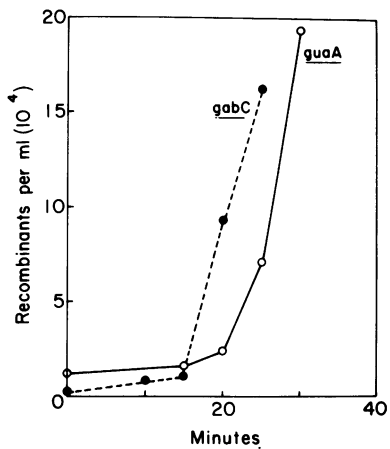

FIG. 1. *Kinetics of gabC transfer to the guanine-requiring, γ-aminobutyrate (GABA)-nonutilizing strain AT2682. The donor strain is Hfr KL-16B (gabC). Mating was performed as described in the text. Gua⁺ recombinants were selected on guanineless medium and GABA-utilizing recombinants were selected on a medium with GABA as the nitrogen source.*

TABLE 4. *Mapping of gabT by P1 transduction*

Donor strain	Recipient strain	Selected marker[a]	Unselected marker[b]	Cotransduction frequency (%)
KL-1699B (*recA gabC*)	M-20 (*gabT*)	*gabT*⁺	*recA*	55.1 (54/98)[c]
CS101B18 (*gabC srl*::Tn*10*)	M-20 (*gabT*)	*srl*	*gabT*⁺	31.1 (213/685)

[a] The selection for the *srl* genotype was carried out on MacConkey plates containing 15 μg of tetracycline per ml. Resistance to tetracycline of strain CS101B18 is due to an insertion of Tn*10* in or very close to the *srl* loci. In transduction experiments with CS101B18 as the donor, there was 100% cotransduction between the two markers.

[b] Scoring for the *recA* marker was done by assaying the UV sensitivity of the transductant colonies. Each colony was suspended in 0.2 ml of basal medium placed in one of 20 wells in a Teflon tray. The bacteria were transferred to MacConkey plates by a grid of 20 nails corresponding to the wells in the Teflon tray. The inoculated plates were UV irradiated with a germicidal lamp to a survival of 10^{-6} *recA* cells. Under these conditions the survival of the isogenic wild-type strain was 10^4 times higher. As a result, the *recA* transductants showed no visible growth after irradiation, whereas the *recA*⁺ transductants appeared in patches of confluent growth.

[c] Number of transductants/total tested is given in parentheses.

TABLE 5. *Gene order in the srl-gab region as determined by P1 transduction*[a]

Donor strain	Recipient strain	Transductant classes	No. of transductants	Cotransduction frequency (%)	
				recA	*gab*
CS101B25 (*gabC recA srl*::Tn*10*)	CS101A (wild type)	Rec⁻ Gab⁺	61		
		Rec⁻ Gab⁻	100		
		Rec⁺ Gab⁺	1	80.9	31.1
		Rec⁺ Gab⁻	37	(161/199)[b]	(62/199)
CS101B25 (*gabC recA srl*::Tn*10*)	M-20 (*gabC gabT*)	Rec⁻ Gab⁺	60		
		Rec⁻ Gab⁻	105		
		Rec⁺ Gab⁺	2	82.9	31.1
		Rec⁺ Gab⁻	32	(165/199)	(62/199)

[a] Selection of *srl* transductants was carried out as in Table 4 and the transductants were tested for their *gab* and *recA* characters (see footnote *a*, Table 4).
[b] Number of transductants/total tested is given in parentheses.

TABLE 6. *Frequency of recombination between γ-aminobutyrate-nonutilizing mutants*[a]

Recipient	Donor						
	101A22 (*gabC*⁺)	M-2033 (*gabT*)	M-1613 (*gabD*)	S-93 (*gabP9*)	S-55 (*gabP5*)	D-86 ("*nit*")	L-1832 ("*nut*")
M-20 (*gabT*)	0.0155	0	0.0039	0.0078		0.83	0.76
S-9 (*gabP9*)	0.0053	0.0096	0.0053	0	0.0029	NT[b]	0.67
S-5 (*gabP5*)	0.0088	0.0099	0.0068	0.0020	0	0.87	NT
CS101G2 (*gltB*)	0.77	0.64	0.44	0.65	0.69	0.58	0.64
D-8 ("*nit*")	1.25	0.59	0.49	1.26	0.93	0	1.00
L-18 ("*nut*")	0.50	0.98	NT	NT	0.78	0.97	0

[a] The numbers given represent the ratios of Gab⁺ transductants to *metB*⁺ transductants in the same cross.
[b] NT, Not tested.

rivatives of the different γ-aminobutyrate-nonutilizing mutants were prepared. Reciprocal transductional crosses between the different mutants were made, the methionine auxotrophs serving as recipients and the prototrophs serving as donors. γ-Aminobutyrate-utilizing transductants and methionine prototroph transductants were independently selected, and their frequencies were determined. The results are given in Table 6 and are expressed as ratios of γ-aminobutyrate-utilizing transductants to methionine-independent transductants obtained in the same cross. One can see that the nonspecific γ-aminobutyrate-nonutilizing mutants CS101G2, D-8, and L-18, when crossed with each other or with mutants with specific lesions in the γ-aminobutyrate utilization pathway, give Gab⁺/Met⁺ transductant ratios which do not significantly deviate from unity. One may conclude, therefore, that the mutations in strains CS101G2, D-8, and L-18 are linked neither to each other nor to any of the mutations in the *gab* loci. Entirely different results were obtained in crosses between γ-aminobutyrate-specific mutants. The Gab⁺/Met⁺ transductant ratios were extremely low, within the range of 2×10^{-3} to 16×10^{-3}! We conclude that the control gene *gabC* and the structural genes of the γ-aminobutyrate utilization pathway, *gabP*, *gabT*, and *gabD*, are very tightly linked. A map of the *gab* region based on the data in Table 6 is given in Fig. 2.

Isolation and properties of strain CS101G2. Strain CS101B was transduced with a P1*kc* lysate of KL141. Arginine-requiring transductants were isolated after penicillin selection and scored for their ability to grow in glucose–γ-aminobutyrate medium. One of the transductants that lost the ability to grow in that medium, strain CS101G2, was chosen for further study. It turned out that strain CS101G2 lost the ability to utilize any of a series of nitrogen sources, including γ-aminobutyrate, arginine, ornithine, putrescine, proline, and glycine, in the presence of glucose as the source of carbon. However, when succinate was the carbon source, CS101G2 utilized the above nitrogen compounds (except for glycine) and exhibited high activities of γ-aminobutyrate permease, GSS transaminase, and SS dehydrogenase but was completely devoid of glutamate synthase activity (Table 2; 23). Revertants of CS101G2 were readily selected on glucose–γ-aminobutyrate medium; the vast majority of them were still devoid of glutamate synthase activity but ex-

hibited high glutamine synthetase activity even when grown in excess ammonia, a condition highly repressive in wild-type *E. coli* (23). *E. coli* K-12 glutamate synthaseless (*gltB*) mutants with phenotypes similar to that of CS101G2 were isolated in B. Tyler's laboratory. The regulatory effects of the *gltB* mutation on the utilization of compounds which are metabolized to glutamate could be suppressed by mutations resulting in constitutive production of glutamine synthetase (GlnC phenotype). The authors found 44% linkage of *gltB* to *argG* by P1 transduction (22), contrary to an earlier report by Berberich that *gltB* was 80% linked to the *malP,Q* locus (4). Our results of P1 transduction, obtained with strain KL141 as the recipient and with four different donors (Table 7), confirm the results of Tyler and associates that *gltB* is linked to *argG*. It is noteworthy that the γ-aminobutyrate-nonutilizing donors CS101A (wild-type *gabC*) and M-20 (*gabT*) transduced KL141 to the Gab$^+$ phenotype with frequencies similar to the γ-aminobutyrate-utilizing CS101B Met$^+$ and M-1651 strains. This is in agreement with the above-reported finding that the *gab* gene cluster is located elsewhere (57.5 min) and not in the *argG* region as reported earlier (10).

γ-Aminobutyrate-nonutilizing mutants of CS101B with pleiotropic lesions in nitrogen metabolism. As already mentioned above, the majority of CS101B revertants unable to grow in γ-aminobutyrate-glucose medium, which were obtained by penicillin selection, had pleiotropic defects in the metabolism of nitrogenous compounds. Two such mutants isolated independently, D-8 and L-18, were studied in some detail. Strain D-8 behaved similarly to CS101G2 in that it could not utilize γ-aminobutyrate, proline, and glycine in the presence of glucose, but it did grow on these nitrogen sources with succinate as the source of carbon (Table 3). Interrupted mating of strain D-8 as the recipient, with strain AT2477/15 (point of origin at 96 min, clockwise transfer), places the "*nit*" locus approximately 8 to 10 min away from *trp*, i.e., at about 18 min (Fig. 3). When Hfr Cavalli (point of origin at 14 min, anticlockwise transfer) was the donor, no γ-aminobutyrate-utilizing recombinants were obtained. Mutant L-18 differed from D-8 at least in two significant respects: it was not impaired in the utilization of glycine, and it could not utilize proline and γ-aminobutyrate at all, even with succinate as the source of carbon. Mapping by interrupted mating places the "*nut*" locus very close to *trp* (less than 2 min apart; see Fig. 4). No linkage at all between the "*nit*" and "*nut*" loci was observed upon P1 transduction (Table 6). There was no P1-mediated cotransduction of "*nit*" with *argG* (0 of 130) or of "*nut*" with *argG* (0 of 188) and *rha* (0 of 230). Possible linkage to *rha* was tested because of the close linkage of the glutamine synthetase locus *glnA* to the latter (2).

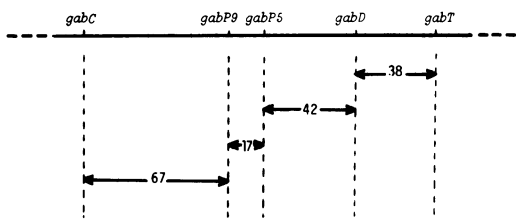

FIG. 2. *Genetic map of the gab cluster. The numbers represent average relative distances between mutant loci, based on the ratios of γ-aminobutyrate-utilizing transductants to Met$^+$ transductants (×10^4) obtained in the different crosses shown in Table 6.*

DISCUSSION

The main purpose of this work was to isolate mutants with lesions in the structural elements of the *gab* system and to use them in the genetic mapping of this system. As already mentioned in a previous paper (10), we encountered great difficulties in our attempts to isolate SS dehydrogenaseless mutants because of the lack of an effective selection method. Among other approaches, we tried to isolate mutants with specifically increased SS dehydrogenase activities

TABLE 7. *Mapping of gltB by P1 transduction*

Donor strain	Recipient strain	Selected phenotype	Unselected phenotype	Cotransduction frequency (%)
CS101B Met$^+$ (*gabC*)	KL 141 (*argG gltB*)	Arg$^+$	Gab$^+$	33 (52/157)[a]
		Gab$^+$	Arg$^+$	77 (131/170)
M-1651 (*gabC*)	KL 141 (*argG gltB*)	Arg$^+$	Gab$^+$	29 (46/158)
		Gab$^+$	Arg$^+$	76 (130/171)
CS101A (*gabC*$^+$)	KL 141 (*argG gltB*)	Arg$^+$	Gab$^+$	42 (35/84)
		Gab$^+$	Arg$^+$	73 (56/77)
M-20 (*gabC*)	KL 141 (*argG gltB*)	Arg$^+$	Gab$^+$	27 (23/85)
		Gab$^+$	Arg$^+$	76 (62/81)

[a] The numbers in parentheses represent the actual numbers of cotransductants (numerator) and the total numbers of transductants examined (denominator).

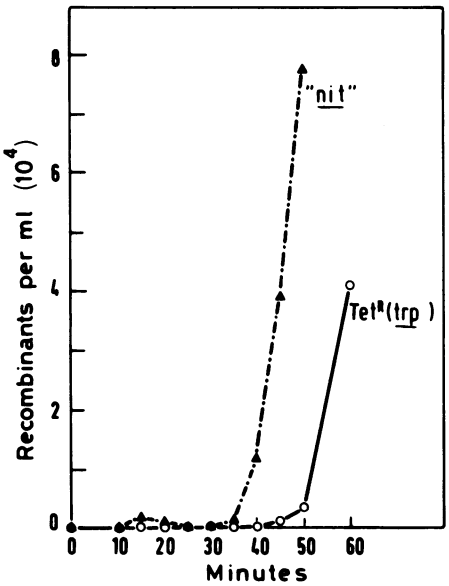

FIG. 3. *Kinetics of chromosome transfer to γ-aminobutyrate (GABA)-nonutilizing strain D-89. The donor strain is AT2477/15 (trp::Tn10). Mating was performed as described in the text. Tetracycline-resistant recombinants were selected on MacConkey plates containing 15 μg of tetracycline per ml. All of them were tryptophan auxotrophs. GABA-utilizing recombinants were selected on a medium with GABA as the nitrogen source.*

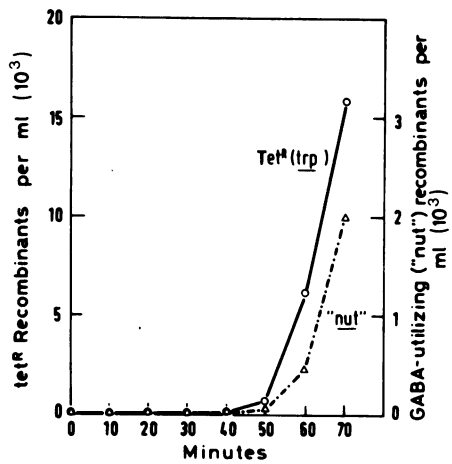

FIG. 4. *Kinetics of chromosome transfer to γ-aminobutyrate-nonutilizing strain L-1821. The donor strain is AT2477/15 (trp::Tn10). For experimental details, see text and Fig. 3.*

by selecting for resistance to the toxic effect of succinic semialdehyde, but these efforts were not successful (10). After repeated trials, we finally isolated a mutant, M-16, devoid of SS dehydrogenase activity and with strongly impaired γ-aminobutyrate transport and transaminase activities. This mutant was obtained by penicillin selection in a glycerol-γ-aminobutyrate medium. A series of revertants of M-16 able to grow in this medium was isolated. They all remained SS dehydrogenaseless but regained full γ-aminobutyrate transport and transaminase activities. Evidently SS dehydrogenase is not required for the utilization of γ-aminobutyrate as a nitrogen source and the selection of M-16 was due to the deleterious effect of the original *gabD* lesion on the expression of the other two structural genes, *gabP* and *gabT*. At first we assumed that M-16 carried a frameshift or nonsense mutation in *gabD* with polar effects on the other two *gab* genes, which presumably are in the same operon and located downstream from *gabD*. The reversions would then be due to a second frameshift mutation or deletion of the nonsense codon, abolishing the polar effect on *gabP* and *gabT*. Indeed, as shown in Table 6 and Fig. 2, the *gab* genes are all situated in one cluster, but *gabD* rather seems to be located between *gabP* and *gabT*. Furthermore, the nonsense mutation hypothesis is made unlikely by the finding that strains CS101A, CS101B, and M-16 and its γ-aminobutyrate-utilizing revertant M-165 all carry an ochre suppressor. Other explanations are, of course, possible, but it would not be useful to indulge in speculation at this point.

The pleiotropic control locus *gabC* had been previously mapped by P1 transduction, using the γ-aminobutyrate-nonutilizing strain KL141 as recipient. Its proposed location was in the *argG* region of the *E. coli* chromosome (10). As shown here, the *argG*-linked locus of *E. coli* (the mutant allele of which is inactive in strain KL141) specifies the synthesis of glutamate synthase. Glutamate synthaseless mutants cannot utilize a number of compounds, including γ-aminobutyrate, as the source of nitrogen in the presence of glucose (Table 3; 23). These results are in agreement with the recent work of Pahel et al., who demonstrated the role of glutamate synthase in the derepression of glutamine synthetase-regulated pathways in *E. coli* and the genetic linkage of *gltB* to *argG* (22). That *gabC* is not linked to *argG* or to *gltB* is seen from the fact that KL141 can be transduced to a Gab$^+$ phenotype by a lysate of the γ-aminobutyrate-nonutilizing strain CS101A at a frequency similar to that by a lysate of CS101B (Table 7). Furthermore, our results clearly show that *gabC* is closely linked to the structural genes of the γ-aminobutyrate pathway, located in the *srl-recA* region of the chromosome (Tables 4 and 5). It should be pointed out that among the γ-

aminobutyrate-nonutilizing revertants of strain CS101B examined, we did not find any true revertants to the CS101A phenotype (low basal levels of γ-aminobutyrate transport, transaminase, and SS dehydrogenase activities).

In addition to *gltB*, the utilization of γ-aminobutyrate is shown to be non-specifically controlled by two other loci, which have pleiotropic effects on the utilization of nitrogen. One of them, *nit*, is situated in the vicinity of 18 min on the *E. coli* chromosome, some 8 to 10 min preceding the *trp* loci (Fig. 3). This mutant is similar in behavior to the pleiotropic nitrogen utilization mutant of *Salmonella typhimurium* described by Broach et al. and mapped near *pyrC* and *pyrD*, some 10 min away from *trpD* (5), corresponding to the position of our *nit* mutation on the *E. coli* chromosome. The other mutation affecting the utilization of γ-aminobutyrate and of other nitrogen sources, *nut*, is located in close proximity to *trp* (Fig. 4). It is of interest that *gdhD*, the gene specifying the synthesis of glutamate dehydrogenase and another gene involved in its regulation in *Klebsiella aerogenes*, have a similar chromosomal location (3).

ACKNOWLEDGMENT

This research was supported by a grant from the United States-Israel Binational Science Foundation, Jerusalem, Israel.

LITERATURE CITED

1. **Bachmann, B. J.** 1972. Pedigree of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
2. **Bachmann, B. J., K. B. Low, and A. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
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. **Broach, J., C. Neumann, and S. Kustu.** 1976. Mutant strains (*nit*) of *Salmonella typhimurium* with a pleiotropic defect in nitrogen metabolism. J. Bacteriol. **128**: 86–98.
6. **Curtiss, R., III, L. G. Caro, D. P. Allison, and D. R. Stallions.** 1969. Early stages of conjugation in *Escherichia coli*. J. Bacteriol. **100**:1091–1104.
7. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B12. J. Bacteriol. **62**:17–28.
8. **Dover, S., and Y. S. Halpern.** 1972. Control of the pathway of γ-aminobutyrate breakdown in *Escherichia coli* K-12. J. Bacteriol. **110**:165–170.
9. **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.
10. **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.
11. **Gorini, L., and H. Kaufman.** 1960. Selecting bacterial mutants by the penicillin method. Science **131**:604–605.
12. **Jakoby, W. B.** 1962. Enzymes of γ-aminobutyrate metabolism (bacterial). Methods Enzymol. **5**:765–778.
13. **Kahane, S., R. Levitz, and Y. S. Halpern.** 1978. Specificity and regulation of γ-aminobutyrate transport in *Escherichia coli*. J. Bacteriol. **135**:295–299.
14. **Lennox, E.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
15. **Lo, T. C. Y., M. K. Rayman, and B. D. Sanwal.** 1972. Transport of succinate in *Escherichia coli*. I. Biochemical and genetic studies of transport in whole cells. J. Biol. Chem. **247**:6323–6331.
16. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
17. **Low, B.** 1973. Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K-12. J. Bacteriol. **113**:798–812.
18. **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.
19. **McEntee, K.** 1977. Genetic analysis of the *Escherichia coli* K-12 *srl* region. J. Bacteriol. **132**:904–911.
20. **Marcus, M., and Y. S. Halpern.** 1967. Genetic analysis of glutamate transport and glutamate decarboxylase in *Escherichia coli*. J. Bacteriol. **93**:1409–1415.
21. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Pahel, G., A. D. Zelenetz, and B. M. Tyler.** 1978. The *gltB* gene and the regulation of nitrogen metabolism by glutamine synthetase in *Escherichia coli* K-12. J. Bacteriol. **133**:139–148.
23. **Zaboura, M., and Y. S. Halpern.** 1978. Regulation of γ-aminobutyric acid degradation in *Escherichia coli* by nitrogen metabolism enzymes. J. Bacteriol. **133**:477–451.
24. **Zipkas, D., and M. Riley.** 1976. Simplified method for interruption of conjugation in *Escherichia coli*. J. Bacteriol. **126**:559–560.