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Characterization of the *Escherichia coli* K12 *gltS* glutamate permease gene

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Summary. The *gltS* gene is known to encode a sodium-dependent, glutamate-specific permease. We have localized the *Escherichia coli* K12 *gltS* gene with respect to the *spoT* gene, sequenced it, and recombined a null insertion-deletion allele into the chromosome without loss of viability. The *gltS* null allele gives a Glt$^-$ phenotype, i.e. it abolishes the ability of a *gltC*^{c} host to grow on glutamate as sole carbon and nitrogen source and also confers α-methylglutamate resistance. A multicopy plasmid expressing the *gltS* gene can reverse the Glt$^-$ phenotype of *gltS*$^-$ or wild-type strains while other plasmids show host-dependent complementation patterns. Induction of *gltS* gene overexpression under control of isopropyl-β-D-thiogalactoside (IPTG)-inducible promoters severely inhibits growth. The GltS protein is deduced to be a 42425 dalton hydrophobic protein with 2 sets of 5 possible integral protein domains, each flanking a central hydrophilic, flexible region.

Key words: gltS sequence – gltS deletion – Bacterial glutamate transport

Introduction

The *gltS* gene of *Escherichia coli* K12 strains encodes a glutamate permease, which is responsible for the activity of only one (GltI) of three glutamate transport systems that can be mutationally modified to facilitate transport of this amino acid (Halpern and Umbarger 1961; Marcus and Halpern 1969; Schellenberg and Furlong 1977). Transport by the *gltS* permease is glutamate-specific and sodium-dependent; the GltII and GltIII systems differ in that they transport either aspartate or glutamate and are sodium-independent (Marcus and Halpern 1969; Kahane et al. 1975; Schellenberg and Furlong 1977). Wild-type *E. coli* K12 does not grow on glutamate as a sole carbon and nitrogen source and is phenotypically Glt$^-$; selection of Glt$^+$ cells that do grow under these conditions yields mutants that overexpress either the GltI (*gltS*) or GltII systems (Marcus and Halpern 1969; Booth et al. 1989). Such mutants become sensitive to toxic compounds whose transport is system-specific, a feature that has proven useful for assigning active systems as well as for selecting secondary mutants. Activation of the GltI transport system potentiates toxicity of α-methylglutamate, D-glutamate, and homocysteic acid whereas sensitivity to the aspartate analog DL-threo-β-hydroxyaspartate is diagnostic of GltII transport system activity; functional GltII or GltIII transport systems render cells sensitive to cysteic acid (Halpern and Umbarger 1961; Schellenberg and Furlong 1977; Essenberg 1984; Booth et al. 1989).

A mutation that results in the Glt$^+$ phenotype was originally called *gltC*$^+$ (Marcus and Halpern 1967), then *gltC*^{c} (Marcus and Halpern 1969), and most recently *gltS*^{o} (Booth et al. 1989); this locus maps close to the *gltS* structural gene at 83 min (Bachmann 1990). Growth on glutamate in *gltC*^{c} mutants was thought to be regulated by a second, distal mutation *gltR*^{TL} that mapped at 92.3 min and prevented growth on glutamate at 30° but not at 42° C; *gltR*^{TL} was postulated to be a thermolabile repressor of *gltS* expression in *gltC*^{c} strains (Marcus and Halpern 1969). However, a recent analysis of mutants of wild-type *E. coli* selected for growth on glutamate yielded a mutant class that was deduced to affect the GltII system and to reside in the same locus as *gltR*^{TL} (Booth et al. 1989). Furthermore, cysteic acid-resistant suppressors of the GltII overexpression mutants could be isolated as MudI1681 insertions, suggesting that they represent disruptions of the structural gene for the membrane-bound carrier protein for glutamate or aspartate (Booth et al. 1989). The reason for the ability of the original *gltR*^{TL} mutation to make the *gltC*^{c} mutant strain Glt$^-$ at 30° C is unclear.

Recent cloning approaches have further defined some of the genes participating in glutamate transport. Genomic libraries of *E. coli* strain B prepared in multi-

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copy plasmids have been used to isolate clones enabling *E. coli* K12 strains to grow on glutamate as a sole source of carbon and nitrogen. Among these clones, the *gltS* gene region of the *E. coli* strain B was isolated, restriction mapped, and its activity as a sodium-activated membrane-associated transporter of glutamate verified (Deguchi et al. 1989). A second gene, called *gltP*, was also encountered that differs from *gltS* by: (i) its restriction enzyme map, (ii) demonstrated vesicular transport of either aspartate or glutamate, and (iii) a sodium-independent vesicular transport activity (Deguchi et al. 1989). Confirming a suggestion that *gltP* of B strains probably represents the GltII transport system of K12 strains (Booth et al. 1989), Wallace et al. (1990) have characterized and sequenced from K12 strains a gene encoding a glutamate and aspartate carrier protein with *gltP* properties which was identified among pBR322 clones conferring a Glt^+ phenotype on a $\Delta(glnH\text{-}chlE)$ strain.

The known genetic proximity of the *spo* operon and *gltS* (Bachmann 1990) has led us to search for and characterize the *gltS* gene in the course of our studies on the *spo* operon region (Sarubbi et al. 1989; Gentry and Burgess 1986, 1988; Xiao et al. 1990). This report concerns the identification of the *gltS* gene sequence and the behavior of strains in which *gltS* is either deleted or expressed from multicopy plasmid vectors.

Materials and methods

Cells, plasmids, phage and media. Table 1 shows the sources and properties of strains used in this study. Use of glutamate as sole carbon and nitrogen source was tested using M9 minimal medium (Miller 1972) lacking NH_4Cl and modified to contain 0.5% glutamate as sole carbon and nitrogen source.

Preparation of chromosomal deletions. A kanamycin-resistance element, which we abbreviate as *kan*, was substituted for the *BglII-XhoI* fragment of the *gltS* gene to yield a deletion-substitution null allele of *gltS* in plasmid pMK129 (Fig. 1). This allele was then recombined into λ_{447}, a defective specialized transducing phage carrying chromosomal DNA spanning the region between *pyrE* and the *spo* operon (An et al. 1979) by first transforming a λ_{447}, $\lambda_h cI_{857} S_7$ double lysogen (strain CF1082) with pMK129 and then inducing a phage stock. This phage stock of CF1082 was used to isolate phage recombinants as a kan^r lysogens of strain LE392. Several such isolates

Table 1. Cell strains, plasmids, and phage

Strains	Genotype	Source	{alias}
CF1082	F^-, *thi thr leu his argE pro pyrE mtl xyl gal lacY str nal supE* $\lambda_{cI857S7,d447\ pyrE\ spoT}$ [abbrev. $\lambda_{h,447}$]	J. Friesen	{JF447}
CF1636	as CF1082, but his^+, *ΔspoS3::cat*		
CF1648	F^- prototroph	B. Bachmann	{MG1655}
CS7	Hfr, *metB gltC*^{c}	Y. Halpern	
LE392	F^-, *metB1 galK2 galT22 supE44 supF58 hsdR514 hsdM trpR55*	L. Enquist	
MK411	LE392($\lambda_{h,447\Delta XhoI-BglII::kan}$)	lysogenization	
MK414	CF1648($\lambda_{h,447\Delta XhoI-BglII::kan}$)	recomb. λ_{447} × pMK129 (select kan^r lysogen)	
MK416	CF1648 Δ*XhoI-BglII::kan*	λ curing	
MK642	CS7 but Δ*XhoI-BglII::kan*	transd. × $P_{1v(MK416)}$, (select kan^r)	
Plasmids and phage:			
pBS	Bluescript	Stratagene	{pBS}
pCKR101	(pBR322 deriv. $lacI^q$ P_{tac} P_{lac})	C. Raymond and T. Stevens	
pMK14	pBS + 7.1 kb *SalI-SalI* (P_{lac}→←*gltS*)	this work	
pMK15	pBS + 7.1 kb *SalI-SalI* (P_{lac}→*gltS*→)	this work	
pMK100	pBS ΔP_{lac}(936–1148)	this work (*Hin*dIII, *Pvu*II cut)	
pMK129	pMK15 but Δ(*XhoI-BglII*)::*kan*	this work	
pMK236	pMK100 + 1.7 kb *Aha*III-*Aha*III fragment cloned in *Sma*I site	this work	
pMK242	pCKR101 + 1.7 kb *Aha*III-*Aha*III fragment cloned in *Sma*I	this work	
pMK244	pMK242 cut with *Nhe*I, filled, reclosed		
pUC4K	source of kan^r fragment	Pharmacia	
λ_{447}	defective λ with 18 kb *pyrE-spoT-gltS* fragment	(see CF1082)	
$\lambda_{447\Delta(XhoI-BglII)::kan}$		recomb. λ_{447} × pMK129	

The plasmid pCKR101 is an expression vector in whith the *tet* gene of pBR322 is replaced with a $lacI^q$ gene and P_{lac} and P_{tac} promoters (in tandem) transcribe a phage T_4 lysozyme gene in which a pUC19 multisite cloning linker is inserted. The arrows (pMK14, 15) indicate the direction of transcription of the *lac* promoter (P_{lac}) and *gltS*

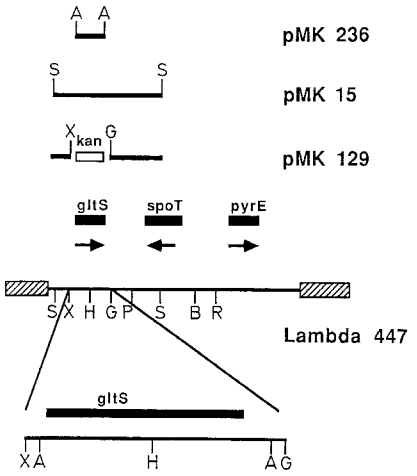

Fig. 1. Localization of the *gltS* gene. The DNA insert present in λ_{447} DNA is shown, containing the *pyrE*, *spoT*, and *gltS* genes (*filled rectangles*) together with the orientation of their transcripts. *Solid lines* correspond to chromosomal DNA inserts present in plasmids pMK236, pMK15, and pMK129. The *Xho*I-*Bgl*II deletion in pMK129 contains a substitution of a kanamycin-resistant gene (*open box*). For λ_{447} DNA, the *hatched boxes* represent λ DNA and the *solid line* represents chromosomal DNA. Restriction enzyme abbreviations: A, *Aha*III;, B, *Bam*HI; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sal*I; X, *Xho*I

were screened to obtain a purified source of $\lambda_{447\Delta BglII-XhoI::kan}$ recombinant phage that gave ampicillin-sensitive, kanamycin-resistant, temperature-sensitive doubly lysogenic strains capable of plaque formation (Sarubbi et al. 1989; Xiao et al. 1990); one such lysogen is strain MK411 (Table 1).

Lysates of strain MK411 were used to lysogenize the prototrophic strain CF1648 (alias MG1655), screening candidate isolates as just described. Heat pulse curing of such lysogens grown at 32° C was done by exposure to 42° C for 6 min, followed by 2 h outgrowth at 32° C and plating at 42° C in the presence of kanamycin. Comparison of frequencies of temperature-resistant *kan*^{r}/*kan*^{s} colonies allowed a quantitative estimate of the viability of chromosomal Δ*Bgl*II-*Xho*I::*kan* recombinants. Ratios consistent with normal recombination frequencies (0.01–0.001) indicate viability whereas lethals give ratios of $<10^{-5}$ (Sarubbi et al. 1989; Xiao et al. 1990). Strain MK416 is such an isolate bearing the deletion allele; its *kan*^{r} character was mapped by P1 phage transduction (Miller 1972), measuring linkage to *pyrE* (An et al. 1979) and to a *cat* insertion in the *rpoZ* (alias *spoS*) gene located just upstream of the *spoT* gene of the *spo* operon (Xiao et al. 1990). The effect of the chromosomal Δ*Bgl*II-*Xho*I::*kan* allele on the Glt$^+$ phenotype was measured after transduction into the *gltC*^{c} strain CS7.

DNA sequencing. The dideoxynucleotide chain-termination method of sequencing was employed, using nested deletions generated by exonuclease III digestion of the *Xho*I-*Bgl*II fragment containing the *gltS* gene inserted in both orientations into the Bluescript plasmid (Stratagene) (Sanger et al. 1977; Henikoff 1984). Both strands were sequenced.

PCR reactions. Reaction mixtures of 100 µl contained 10 ng of chromosomal DNA, 100 pmol of oligonucleotide primer(s), 10 mM TRIS-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 0.01% gelatin. One reaction cycle was 1.5 min at 94° C and 2.5 min at 72° C; 25 cycles were employed using a Coy Temperature Cycler machine (Saiki et al. 1985). Analysis of PCR reaction products was by electrophoresis of 1/20 of the reaction on 1% agarose gels.

Results

Figure 1 shows the relative chromosomal position of the *gltS* gene with the gene order *pyrE-spoT-gltS*. The orientation of the *gltS* gene (see below) is such that its transcripts are convergent an those of the *spo* operon determined previously (Gentry and Burgess 1986; Sarubbi et al. 1989). The initial localization of the *gltS* gene within the DNA insert present in λ_{447} DNA was revealed by the ability of a multicopy plasmid subclone of λ_{447} containing a 7.1 kb *Sal*I chromosomal DNA fragment (pMK15) to allow the wild-type *E. coli* K12 strain CF1648 to grow on glutamate as a sole carbon and nitrogen source (Table 2). The 7.1 kb *Sal*I fragment shown in Fig. 1 contains the 1882 bp *Xho*I-*Bgl*II fragment whose sequence is presented here. (This sequence has been deposited in the Genbank database under the accession, number X17499.) One *Sal*I site is located about 1.7 kb from the *Xho*I site and the other *Sal*I site occurs approximately in the middle of the *spoT* gene, about 3.5 kb from the *Bgl*II site (Fig. 1; Xiao et al. 1990). The Glt$^+$ phenotype mediated by the plasmid pMK15 mimics that of multicopy clones of *E. coli* strain B DNA fragments containing the *gltS* gene (Deguchi et al. 1989). Wild-type strain CF1648 is Glt$^-$ since it does not grow on glutamate under these conditions (Table 2).

Scoring the Glt$^+$ phenotype among various deletions and subclones of the 7.1 kb *Sal*I fragment enabled local-

Table 2. Abilities of plasmids to enable cell strains to utilize glutamate

Plasmid	Growth on glutamate as carbon and nitrogen source			
	CS7 *gltS*$^+$ *gltC*^{c}	MK642 Δ*gltS*	CF1648 *gltS*$^+$ *gltC*$^+$	MK416 Δ*gltS*
No plasmid	+	−	−	−
pBS vector control	+	−	−	−
pMK14	nd	+	−	−
pMK15	nd	+	+	+
pMK236	nd	+	−	−
pMK244	nd	−	−	−

As described in the text, strain MK642 is a derivative of CS7 and MK416 is a derivative of CF1648; details of the contents of plasmids listed are given in Table 1. +, Growth on glutamate as sole carbon and nitrogen source (phenotypically Glt$^+$); −, no growth on glutamate (Glt$^-$); nd, not determined

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      10                  30                  50
ACTCGAGGGTGGAAACAGACATCGAAAACAATCCCGTGGTAGCAAAGCGGCATTTTAGCT
(XhoI)

      70                  90                  110
GACCGAAAGAGAAAAGCAAACGTTTGCCACATCCTCATCTAAATATTTAAATGAGATAAA
                                          AhaIII

      130                 150                 170
TGCACTTTTTTATATAACTTTTGGTATTTTTCTGCTTAAAATCCATGCCATTTGTTCATT

      190                 210                 230  ,,,,,,,
AACAGCTGAGAATTTGACCCAGGAAAGCATAACTCGATACCCGTCATAGTTCATGAAGTT

      250 ,,,,,,,  ,,,,,,,  ,,,,,,,            290
GCCACTGCGCGATGAAGTATGACGAGTATGAAAGAGTGATGCGGATACAAAGGAGTAACT

      310                 330                 350
ATGTTTCATCTCGATACTTTAGCAACGCTTGTTGCCGCAACGCTGACGTTGCTGCTCGGG
M  F  H  L  D  T  L  A  T  L  V  A  A  T  L  T  L  L  L  G

      370                 390                 410
CGTAAGTTGGTCCATTCCGTCTCCTTTTTGAAGAAATACACCATACCGGAACCTGTTGCG
R  K  L  V  H  S  V  S  F  L  K  K  Y  T  I  P  E  P  V  A

      430                 450                 470
GGTGGTTTGTTGGTGGCGCTGGCGCTACTAGTACTGAAAAAAAGCATGGGCTGGGAAGTC
G  G  L  L  V  A  L  A  L  L  V  L  K  K  S  M  G  W  E  V

      490                 510                 530
AACTTTGATATGTCCCTGCGCGATCCGTTAATGCTGGCTTTCTTCGCCACCATTGGCCTG
N  F  D  M  S  L  R  D  P  L  M  L  A  F  F  A  T  I  G  L

      550                 570                 590
AACGCCAACATTGCCAGTTTGCGTGCCGGTGGGCGTGTGGTTGGCATCTTCTTGATTGTG
N  A  N  I  A  S  L  R  A  G  G  R  V  V  G  I  F  L  I  V

      610                 630                 650
GTTGTTGGTCTGTTGGTGATGCAAAATGCCATTGGCATTGGTATGGCTAGCTTGTTAGGG
V  V  G  L  L  V  M  Q  N  A  I  G  I  G  M  A  S  L  L  G
                                              (NheI)

      670                 690                 710
CTTGATCCGCTGATGGGGCTGTTGGCCGGTTCTATTACTCTTTCCGGCGGTCACGGTACG
L  D  P  L  M  G  L  L  A  G  S  I  T  L  S  G  G  H  G  T

      730                 750                 770
GGCGCTGCGTGGAGTAAATTGTTCATTGAACGTTATGGCTTCACCAATGCGACGGAAGTG
G  A  A  W  S  K  L  F  I  E  R  Y  G  F  T  N  A  T  E  V

      790                 810                 830
GCGATGGCCTGTGCAACGTTCGGTCTGGTGCTGGGCGGCTTGATTGGCGGTCCGGTGGCG
A  M  A  C  A  T  F  G  L  V  L  G  G  L  I  G  G  P  V  A

      850                 870                 890
CGCTATCTGGTGAAACACTCCACCACGCCGAACGGTATTCCGGATGACCAGGAAGTCCCG
R  Y  L  V  K  H  S  T  T  P  N  G  I  P  D  D  Q  E  V  P

      910                 930                 950
ACGGCGTTTGAAAAGCCGGATGTGGGACGCATGATCACCTCGTTGGTGCTGATTGAAACT
T  A  F  E  K  P  D  V  G  R  M  I  T  S  L  V  L  I  E  T
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      970                 990                 1010
ATCGCGCTGATTGCTATCTGCCTGACGGTGGGGAAAATTGTTGCGCAACTTTTGGCTGGC
I  A  L  I  A  I  C  L  T  V  G  K  I  V  A  Q  L  L  A  G

      1030                1050                1070
ACTGCTTTTGAACTGCCGACCTTCGTCTGTGTACTGTTTGTTGGCGTGATTCTGAGCAAC
T  A  F  E  L  P  T  F  V  C  V  L  F  V  G  V  I  L  S  N

      1090                1110                1130
GGTCTGTCAATAATGGGCTTTTACCGCGTCTTTGAGCGTGCGGTATCCGTGCTGGGTAAC
G  L  S  I  M  G  F  Y  R  V  F  E  R  A  V  S  V  L  G  N

      1150                1170                1190
GTAAGCTTGTCGTTGTTCCTGGCGATGGCGTTGATGGGGCTGAAACTGTGGGAGCTGGCT
V  S  L  S  L  F  L  A  M  A  L  M  G  L  K  L  W  E  L  A
  HinDIII
      1210                1230                1250
TCGCTGGCGCTGCCGATGCTGGCGATTCTGGTGGTACAGACCATCTTCATGGCGTTGTAT
S  L  A  L  P  M  L  A  I  L  V  V  Q  T  I  F  M  A  L  Y

      1270                1290                1310
GCCATCTTCGTTACCTGGCGCATGATGGGCAAAAACTACGATGCGGCAGTGCTGGCTGCG
A  I  F  V  T  W  R  M  M  G  K  N  Y  D  A  A  V  L  A  A

      1330                1350                1370
GGTCACTGTGGTTTTGGCCTCGGTGCAACGCCAACGGCAATCGCCAACATGCAGGCGATC
G  H  C  G  F  G  L  G  A  T  P  T  A  I  A  N  M  Q  A  I

      1390                1410                1430
ACTGAACGCTTTGGCCCGTCGCACATGGCGTTTTTGGTGGTGCCGATGGTCGGTGCGTTC
T  E  R  F  G  P  S  H  M  A  F  L  V  V  P  M  V  G  A  F

      1450                1470                1490
TTTATCGATATCGTCAATGCGCTGGTAATTAAGTTGTATTTGATGTTGCCGATTTTTGCC
F  I  D  I  V  N  A  L  V  I  K  L  Y  L  M  L  P  I  F  A

      1510                1530                1550
GGTTAACCGATGAAGCGGCGGTAGAAGTGCCGCCGCAACAAAGACAAATGCCTGATACGC
G  *

      1570                1590                1610
TTCGCTTATCAAGCCTGCGTATTGATTCATAATTTATTGAATTTGTAGGGTGATGAATCG

      1630                1650                1670
CATCCGGCAGGAAGGTAGGGTAACCTGAAATGGCGGTCTTCTCCATGCCGCCTTTTACGC

      1690                1710                1730
ATTCGAGTAACGTTCCGTCTCCGGCATCCAGCGTTCTATCAGGGCTTTTGCCTGTTGTGG

      1750                1770                1790
GTAACGTTCGTGAATATGGCGTGCCAGGCGCTGAACTTCCGGGATCATCGCCTGATCGCG

      1810                1830                1850
CAGTAAATCCGCCACTTTAAATTCAGCATTACCCGTCTGACGCGTGCCTAACAATTCGCC
            (AhaIII)

      1870
AGGGCCGCGAATCTCCAGATCT
             (BglII)
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Fig. 2. The sequence of the *gltS* gene. The sequence of the *Xho*I-*Bgl*II fragment is shown. The *Aha*III sites used to clone the minimal *gltS* plasmid pMK236 occur at positions 106 and 1816. The AUG codon deduced as the translational initiation site for the *gltS* gene occurs at position 301 and has a Shine-Dalgarno ribosome recognition site labeled as *bold type* characters at positions 290 to 295. Heptameric direct repeat sequences are indicated by an *apostrophe* above the designated base

ization of *gltS* activity to a 1.88 kb *Xho*I-*Bgl*II fragment; plasmid pMK129 with a *Xho*I-*Bgl*II deletion does not give a Glt$^+$ phenotype (data not shown; Fig. 1). Sequencing of this 1882 bp *Xho*I-*Bgl*II fragment indicates an open reading frame as candidate for the *gltS* structural gene with an AUG initiation codon at sequence position 301 and a UAA termination codon at position 1504 (Fig. 2). Before presenting additional features of the sequence, we will present evidence that this DNA region does in fact encode the *gltS* gene.

The viability of a Xho*I*-Bgl*II deletion in the chromosome*

The Δ(*Xho*I-*Bgl*II)::*kan* deletion-insertion allele in plasmid pMK129 was transferred to λ_{447} by recombination, then inserted into the chromosome of strain CF1648 by heat pulse curing of the lysogen and selection for kanamycin-resistant recombinants at 42° C (see Materials and methods). The titer of *kan*^{r} cured lysogens divided by the titer of total cured lysogens was found to be about 2%; apparently there is no selective pressure

against the chromosomal insertion of this allele. Strain MK416 which carries the deletion-insertion allele, was constructed in this manner.

Mapping of the Δ (XhoI-BglII) allele

Transduction of the kanamycin resistance marker in strain MK416 into strain CF1636 as the recipient was done as a three-factor cross using *pyrE*, *spoS3*::*cat*, and Δ(*BglII-XhoI*)::*kan*. The *spoS* gene, alias *rpoZ*, is immediately upstream of *spoT* within the *spo* operon (Gentry and Burgess 1988; Sarubbi et al. 1989). Selection of *kan*^{r} transductants on LB plates and screening for chloramphenicol resistance and uracil-independent recombinants gave a 43.6% linkage to *spoS* and a 21.7% linkage to *pyrE*. Conversely, selection of *pyrE*$^+$ transductants on glucose minimal medium plus casamino acids lacking uridine and scoring for inheritance of *kan*^{r} and *cat*^{r} recombinants reveals *kan*^{r} recombinants to be the minority class, confirming the placement of *kan* in CF1636 beyond *spoS* and the gene order *pyrE-spoS, T-kan*. Thus, the Δ(*XhoI-BglII*)::*kan* allele maps at the *gltS* chromosomal locus (Bachmann 1990); this mapping excludes the possibility that the *kan*^{r} property of MK416 arose by anomalous excision and reintegration of phage lambda at the chromosomal lambda attachment site.

PCR verification of the deletion-insertion allele

We have used the polymerase chain reaction (PCR) (Saiki et al. 1985) to show that the *gltS* deletion-insertion allele is a simple chromosomal substitution for the wild-type gene and to exclude the presence of a diploid. Figure 3 shows PCR amplified fragments with DNA from strain CS7 as well as otherwise isogenic DNA from the insertion-deletion strain MK642 (Table 1). Various combinations of oligodeoxynucleotide primers were used which correspond to regions flanking the *XhoI-BglII* deletion as well as a region within the *kan* insertion. The orientation of the *kan* element substitution was determined by restriction mapping (data not shown).

Single primer control lanes (Fig. 3, lanes A–F) show that a small fragment (labeled F4 in the figure) is amplified with primer O3 and also when this primer is present with or without other primers. When primers O1 and O3, which flank the nondeleted *gltS* gene region, are present an expected wild-type length 2.5 kb fragment (labeled F1, lane G) is amplified. With MK642 DNA, whenever primers O1 and O2 are present, amplification of a 637 bp fragment is expected for the deletion and is seen (F3, lanes H–J). When primers O1, O2, and O3 are all present together with strain MK642 DNA, both fragments F2 (1.88 kb) and F3, but not F1, are amplified (Fig. 3, lane I). In order to be certain that F1 could have been detected under conditions where F2 and F3 were amplified, we amplified a mixture of MK642 and CS7 chromosomal DNA with all three primers; Fig. 3, lane J shows fragments F1, F2, and F3 are indeed present.

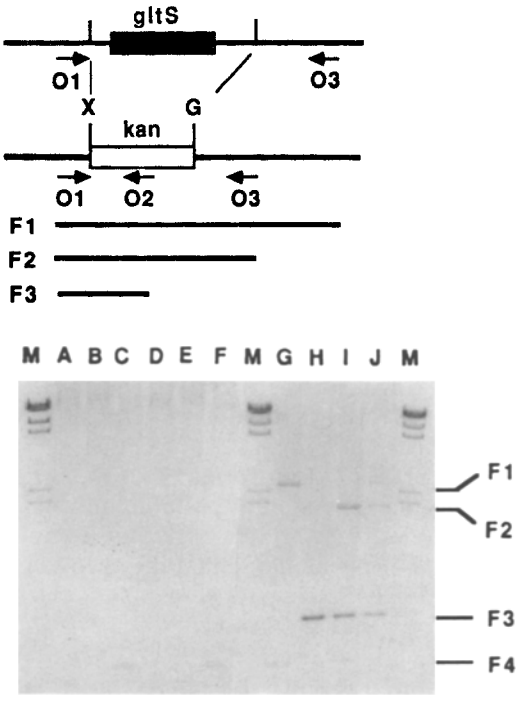

Fig. 3. Verification of a *gltS* deletion allele by PCR amplification. Three oligodeoxynucleotides used as primers are as follows: O1 hybridizes upstream to the *XhoI* site and has the sequence CGTTGCGCATTTTCTGACT; O2 hybridizes in approximately the middle of the *kan* gene insert and has the sequence CGATTCCTGTTTGTAATTGTCC; and O3 hybridizes 600 bp downstream of the *BglII* site and has the sequence AGCAGGGCTTCCATCCGC. Lanes marked M contain *Hin*dIII cut λ DNA as a marker. Lanes A–C contain strain CS7 DNA and single primers O1, O2, or O3 respectively. Lanes D–F contain strain MK642 DNA and single primers O1, O2, or O3 respectively. Lane G contains CS7 DNA and two primers (O1 and O3). Lane H contains MK642 DNA and two primers (O1 and O2). Lane I contains MK642 DNA and all three primers (O1–O3). Lane J contains the same three primers and a 1:1 admixture of CS7 and MK642 DNA

Effect of the Δ (XhoI-BglII) allele on the Glt$^+$ phenotype

If the Δ(*XhoI-BglII*)::*kan* allele deletes the glutamate permease encoded by the *gltS* gene, then it is predicted that transducing the kanamycin-resistance property from strain MK416 into the *gltC*^{c} mutant strain CS7 should be accompanied by a phenotypic change from Glt$^+$ to Glt$^-$ (Marcus and Halpern 1969). We found that all 18 *kan*^{r} recombinants indeed became Glt$^-$. Strain MK642 is an example of a Δ(*XhoI-BglII*)::*kan* derivative of strain CS7 (Table 2). From now on, for the sake of brevity, we shall refer to the Δ(*XhoI-BglII*)::*kan* allele as Δ*gltS*.

Complementation of the deletion phenotype

We have measured the ability of plasmids bearing *gltS* gene fragments to complement the Glt$^-$ phenotype of Δ*gltS* derivatives of strain CS7 and the otherwise wild-type strain MK416, as well as its parent CF1648 (Table 2). It can be seen that the Glt$^-$ phenotype is not

altered by plasmid vector controls. The ability of pMK15 (which contains the *gltS* 7.1 kb *Sal*I fragment) to elicit a Glt^+ phenotype in CF1648 mentioned above is also found with strains MK416 and MK642. In contrast, pMK14 transformants of CF1648 and MK416, but not strain MK642, remain Glt^-. The plasmid pMK15 contains a P_{lac} promoter in tandem with *gltS* and positioned about 2 kb from the deduced start of the *gltS* structural gene. In plasmid pMK14, the vector P_{lac} promoter is about 5 kb from the *gltS* start site and convergent to *gltS* sense transcripts.

The Bluescript P_{lac} sequences have been deleted in pMK100 (Table 1). Ligation of a smaller 1.7 kb *gltS* *Aha*III fragment in pMK100 yields a plasmid (pMK236) which harbors virtually a minimal *gltS* gene (Fig. 2). Table 2 shows that pMK236 transformants of the three test strains have a complementation pattern equivalent to pMK14. This implies that a source of *gltS* transcripts is present on the *Aha*III fragment and that the phenotypic defect in strain MK642 is not due to polar effects on the expression of genes downstream of the Δ*gltS* allele.

Table 2 shows that the Glt^- phenotype of strain MK642 is complemented by all plasmids containing *gltS* sequences except for a plasmid (pMK244, see below) constructed with a frame-shift mutation; in contrast, strain MK416 becomes Glt^+ only when transformed with plasmid pMK15. Implications of this strain-dependent complementation activity will be considered in the discussion. Our interpretation of the plasmid-dependence of complementation is that pMK236 has low levels of *gltS* expression, probably reflecting *gltS* promoter activity; equivalent behavior is obtained with pMK14. Presumably higher levels of *gltS* expression are required to confer a Glt^+ phenotype on strain MK416 (or CF1648) and probably result from increased transcription of *gltS* from the tandem P_{lac} promoter in pMK15, which is incompletely repressed when on high copy number plasmids (Sarubbi et al. 1989).

A growth-inhibitory effect of gltS *overexpression*

When 1 mM IPTG is added to pMK15 transformants of MK642, CF1648, or MK416, cells grow very slowly to form tiny colonies after 2 days at 35° C on LB-ampicillin plates. This property is not shared by pMK14 or pMK236. We have also been unable to recover the *gltS* *Aha*III fragment cloned in tandem with P_{lac} promoter in the Bluescript vector whereas the opposite orientation is readily obtained (data not shown). This cloning impasse is overcome when the P_{lac} region is deleted, as in pMK236. The plasmid pMK242 contains the *Aha*III fragment in a $lacI^q$-containing vector (pCKR101) which is nevertheless IPTG inducible (C. Raymond and T. Stevens, personal communication); addition of IPTG to pMK242 transformants of strains MK642, CF1648, and MK416 all show severe growth inhibition (data not shown). We take these observations to indicate that *gltS* overexpression impairs growth; there are precedents for growth inhibition accompanying overproduction of membrane proteins (Eya et al. 1989; Saier et al. 1989).

Verification of the gltS *ORF with a frameshift mutation*

We have additional evidence that the ORF assigned to the *gltS* structural gene in Fig. 2 is indeed responsible for the phenotype observed. A second *gltS* allele was constructed in which a reading frame shift is introduced in the first third of the *gltS* ORF by filling in the unique *Nhe*I site (position 646, Fig. 2) in pMK242 and religating to form plasmid pMK244. Unlike MK642/pMK242, the phenotype of MK642/pMK244 is Glt^-.

Effects of gltS *alleles on α-methylglutamate inhibition of growth*

Inhibition of cell growth by α-methylglutamate is a second phenotype known to be *gltS*-dependent, and is due to *gltS*-mediated uptake of the inhibitor (Halpern and Umbarger 1961). Generally, we have confirmed that sensitivity to 40 μg/ml α-methylglutamate on glucose minimal plates invariably accompanies the Glt^- to Glt^+ phenotypic change mediated by *gltS*-bearing plasmids (data not shown).

Sequence features of the gltS *gene region*

The complete sequence of the *Xho*I-*Blg*II fragment is presented in Fig. 2. A canonical Shine-Dalgarno sequence (AAGGAG) occurring at position 290 is located 5 bases upstream of an AUG codon, which leads us to suggest that this codon is a likely translational initiation site. The translational stop codon for the *gltS* structural gene occurs at position 1504. There are five regions of dyad symmetry downstream of this position, each with calculated free energies in excess of 20 kcal. These occur at the positions 1514–1536, 1550–1574, 1602–1634, 1649–1675, and 1765–1704. Such regions are potential transcriptional pause sites; the fourth region listed has 4 T residues on the distal stem and might serve as a transcription termination site.

At least one regulatable promoter has been deduced to be present on the *Aha*III fragment based on the complementation behavior of pMK236 (Table 2) and must therefore be located between position 108 and the initiating AUG codon at position 301 (Fig. 2). Experiments aimed at localizing promoters are in progress. This region also contains a heptameric direct repeat (ATGAAGT) that occurs with a 12 base spacing beginning at positions 233 and 252 as well as a second, but different, heptameric repeat (AGTATGA) that occurs at positions 256 and 265.

The deduced amino acid sequence of the GltS protein consists of 401 amino acids (see Fig. 2) and yields a calculated molecular mass of 42425 daltons. Comparison of the amino acid composition of the protein with average *E. coli* proteins shows an enrichment among hydrophobic amino acids: alanine (36%), phenylalanine (66%), isoleucine (55%), leucine (119%), methionine (193%), and valine (47%). Calculation of regions of integral membrane protein domains yields 10 possible inte-

Table 3. Possible integral membrane protein domains of GltS

Nucleotide positions	Amino acid residues	Amino acid sequence
310– 360	4– 20	LDTLATLVAATLTLLLG
403– 456	35– 52	IPEPVAGGLLVALALLVL
571– 621	91–107	GRVVGIFLIVVVGLLVM
637– 687	113–129	IGMASLLGLDPLMGLLA
778– 825	160–175	VAMACATFGLVLGGLI
943– 978	215–226	LVLIETIALIAI
1042–1092	248–264	FVCVLFVGVILSNGLSI
1123–1173	275–291	VSVLGNVSLSLFLAMAL
1222–1272	308–324	AILVVQTIFMALYAIFV
1405–1455	369–385	MAFLVVPMVGAFFIDIV

The regions indicated were calculated with the ALOM program found within the IDEAS set of programs of Goad and Kanehisa (1982). Nucleotide positions given refer to the numbering of Fig. 2, whereas the amino acid residue numbers given are counted with methionine encoded by the codon at nucleotides 301–303 (Fig. 2) as position 1

gral protein domains (Table 3). Protein flexibility predictions by the Protein Structure program of the University of Wisconsin Genetics Computer Group sequence analysis package using the Chou-Fasman algorithm, a hydrophobicity threshold of 1.3 and a flexibility threshold of 1.04 reveals the central portion of the protein to be enriched for both hydrophilic and flexible regions flanked on each side by five hydrophobic regions.

Discussion

One goal of these studies is to identify the DNA sequence encoding corresponding to the structural gene, *gltS*. The orientation of the *gltS* gene is known from restriction map information and is counter-clockwise on the *E. coli* chromosome (Fig. 1 and data not shown). This placement indicates that *gltS* gene transcripts are convergent with respect to known transcripts of the neighboring *spo* operon (Gentry and Burgess 1986, 1988; Sarubbi et al. 1989). The *Hin*dIII site at position 1143 in Fig. 2 is the only such site we find within the 10.6 kb region extending from the *Eco*RI site upstream of the *spoT* gene to the chromosomal-lambda DNA junction in λ_{447} (Fig. 1); this and other published mapping data (Sarubbi et al. 1989) allow placement of the *gltS* gene on the *E. coli* genomic restriction map at 3884 kb (Kohara et al. 1987).

The other goal of this effort is to construct and characterize a compelling null allele of the *gltS* gene. We have examined the phenotypic effects of a complete (Fig. 3) chromosomal deletion, and the complementing effects of plasmids expressing the *gltS* gene. Scoring the ensuing phenotypic effects either by measuring growth on glutamate as a sole source of carbon and nitrogen (Table 2) or by measuring growth inhibition by the glutamate analog, α-methylglutamate, gave mutually supportive conclusions.

A perhaps surprising observation is that the $gltC^c$ strain CS7 when transduced to Δ*gltS* (strain MK642) shows an enhanced ability to be complemented to a Glt^+ phenotype by plasmids such as pMK14 or pMK236 as compared to the otherwise equivalent strain MK416. This strain-dependent property is formally the equivalent of the activity ascribed to $gltC^c$, yet *gltS* and *gltC* are closely linked genetically (Marcus and Halpern 1969) and the retention of $gltC^c$ among the kanamycin-resistant Δ*gltS* transductants of CS7 should not be statistically favored. If strain MK642 is a rare recombinant in which $gltC^c$ is retained, then the observed phenotype can be expected to depend upon $gltC^c$ function. The $gltC^c$ locus has been proposed as an operator locus adjacent to the *gltS* gene (Marcus and Halpern 1969); in this case one would expect $gltC^c$ to be *cis*-dominant, yet apparent activation of *gltS* function is observed *in trans* in the presence of multiple plasmid copies of *gltS* (Table 2). Thus, $gltC^c$ could be an allele of a $gltC^+$ regulatory gene that is separable from *gltS* and affects *gltS* expression *in trans*. Alternatively, strain differences reflecting higher levels of glutamate transport due to nonspecific permeases could account for the enhanced ability of MK642 to be complemented by *gltS*-containing plasmids. More precise assays of *gltS* regulation will be aided by reporter gene fusions which are currently being constructed.

We have not demonstrated that the deduced *E. coli* K12 GltS protein is physically overexpressed or that it functions to transport glutamate in membrane vesicles. However, as mentioned in the Introduction, both of these features have been demonstrated by Deguchi et al. (1989) for the *E. coli* B *gltS* gene product. Their studies have revealed overproduction of a protein migrating in the 32 kDa region of acrylamide-SDS gels and demonstrated membrane vesicles prepared from cells overproducing the protein showed a marked increase in sodium-dependent glutamate-specific transport activity. It is likely that the K12 strain *gltS* gene we have sequenced is closely related to the sequence of the *gltS* gene from B strains; comparison of the restriction map of the *gltS* gene region reported by Deguchi et al. (1989) with the region we have sequenced from K12 strains reveals identities for 13 of 16 sites cleaved by 11 different restriction enzymes (data not shown).

The difference in apparent mass between the protein product we derive from the DNA sequence (42425 dalton) and that observed for the B strain GltS protein (32 kDa) by Deguchi et al. (1989) could reflect post-translational processing of the protein of anomalous electrophoretic mobility due to extensive hydrophobicity or other structural features. It is also noteworthy that the plasmid pLC26-15, listed as containing the *gltS* gene in the *E. coli* K12 gene-protein index, but could encode a product of about 37 kDa (Phillips et al. 1988). The protein whose sequence we have deduced is hydrophobic with 10 possible integral protein domains (Table 3) with 5 regions distributed on each side of a central hydrophilic, flexible region. These properties are suggestive of a membrane protein and even reminiscent of a structure for the H^+/lactose symport protein with hydrophobic

domains weaving back and forth through the membrane (see Fig. 3 in the review by Maloney 1988). While we cannot predict the structure of the GltS protein from the very limited data available, the properties deduced from sequence considerations are consistent with those expected of a membrane-bound permease. The amino acid sequence of the *gltP* encoded glutamate-aspartate carrier protein (Wallace et al. 1990) has been compared with that of *gltS* without finding extensive homology (data not shown).

We are currently attempting to localize *gltS* gene promoters so that we can characterize the regulation of *gltS* expression, which apparently occurs at low levels in wild-type cells. We are especially interested in the role of the *gltC* locus in this regard as well as other possible strain-dependent sources of regulation already discussed. The existence of repeat sequences that might influence transcription activity is also intriguing.

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Note added in proof. The nucleotide sequence of the *E. coli* strain B *gltS* region has been reported recently; (Y Deguchi, I Yamato, and Y Anraku (1990) Nucleotide sequence of *gltS*, the Na^+/glutamate symport carrier gene of *Escherichia coli* B. J Biol Chem 265:21707–21708). In the *gltS* coding region, there are six base changes between B and K-12 strains and all are translationally silent.

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