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Regulation of Glutamine Synthetase Formation in *Escherichia coli*: Characterization of Mutants lacking the Uridylyltransferase

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A λ phage (λNK55) carrying the translocatable element Tn*10*, conferring tetracycline resistance (Tet^r), has been utilized to isolate glutamine auxotrophs of *Escherichia coli* K-12. Such strains lack uridylyltransferase as a result of an insertion of the Tn*10* element in the *glnD* gene. The *glnD*::Tn*10* insertion has been mapped at min 4 on the *E. coli* chromosome and is 98% contransducible by phage P1 with *dapD*. A λ transducing phage carrying the *glnD* gene has been identified. A *glnD*::Tn*10* strain synthesizes highly adenylylated glutamine synthetase under all conditions of growth and fails to accumulate high levels of glutamine synthetase in response to nitrogen limitation. However, this strain, under nitrogen-limiting conditions, allows synthesis of 10 to 20 milliunits of biosynthetically active glutamine synthetase per mg of protein, which is sufficient to allow slow growth in the absence of glutamine. The GlnD phenotype in *E. coli* can be suppressed by the presence of a number of mutations which increase the quantity of biosynthetically active glutamine synthetase.

In *Escherichia coli* and *Klebsiella aerogenes*, the activity of glutamine synthetase (EC 6.3.1.2) (GS) is regulated by a complex system of adenylylation and deadenylylation (6, 16). A single enzyme, adenylyltransferase, is responsible for catalysis of the adenylylation-deadenylylation reactions. This reaction is governed by a regulatory protein (P_{II}). The unmodified form of P_{II} (P_{IIA}) stimulates adenylylation, and the uridylylated form (P_{IID}) is required for deadenylylation. Uridylyltransferase (UTase) catalyzes the interconversion of P_{IIA} and P_{IID}, while the uridylyl-removing enzyme (UR) catalyzes the interconversion of P_{IID} to P_{IIA}. Mutants in each of the components of this regulatory system have been obtained in *K. aerogenes*. Studies on these mutants (5, 6, 12) and on strains altered in *glnA*, the structural gene for GS (3, 23, 24), led Magasanik and co-workers to propose a model of autogenous control of GS. Recent results of Weglenski and Tyler (27) suggest that regulation of transcription of the *glnA* gene must involve activation by GS.

Although biochemical and physiological studies on GS have been carried out in *K. aerogenes*, extensive genetic studies are not yet feasible with this organism. For example, since there is no rapid way of screening for the presence of nonsense mutations, no studies exist on *K. aerogenes* strains in which alterations in GS activity are clearly due to the absence of specific gene products. The greater ease of working genetically with *E. coli* and the similarity between *E. coli* GS (GS_E) and *K. aerogenes* GS (GS_K) have stimulated our interest in *E. coli*. In this paper we report the isolation and characterization of mutants of *E. coli* defective in the gene corresponding to *glnD* in *K. aerogenes*. This mutation in *E. coli* is caused by the insertion of the Tn*10* sequence conferring tetracycline resistance (Tet^r) and results in a mutant strain devoid of the *glnD* gene product, the enzyme UTase. The loss of UTase leads to an inability to deadenylylate GS and an inability to synthesize normal levels of GS.

MATERIALS AND METHODS

Bacterial strains. The strains used are listed in Table 1. *E. coli* strains were derivatives of *E. coli* K-12.

Media. Nutrient medium (LB-gln) was Luria broth supplemented with 0.2% glutamine (Calbiochem) (4). The minimal medium was W-salts (21) supplemented with appropriate nutrients. Solid media contained 1.5% agar. Glucose was used at a final concentration of 0.4% as the source of carbon and energy; ammonium sulfate was used at 0.2% final concentration as the usual nitrogen source. Glutamine was supplied at 2 mg/ml and other amino acids at 50 μg/ml, vitamin B_1 at 2 μg/ml, tetracycline at 15 μg/ml, streptomycin and

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TABLE 1. *Strain list*[a]

Strain	Relevant genotype	Source or reference
E. coli		
AT997	*thi-1 relA1 dapC15*	B. Bachmann, CGSC457
BT1026	*thy endoI polA1 dnaE1026*	M. Gefter (8, 21)
ET1003	*tonA gal mtl*	[b]
ET1007	*tonA glnD99*::Tn*10*	P1 · ET5002 × ET1003[c]
ET1008	*tonA glnD99*::Tn*10 azi-1*	Spontaneous azide-resistant mutant of ET1007
ET1101	*rpsL*	(19)
ET1115	Prototroph *rha*^{s} *rpsL*	[d]
ET1118	*glnA202 rpsE*	ET1115[e]
ET1172	*glnD*::Tn*10 rha*^{s}	P1 · ET1007 × ET1115
ET1173	*glnD*::Tn*10 rpsE rha*^{s}	P1 · MR202 × ET1172
ET1189	*glnA*$_{K}$ *rpsE*	P1 · ET2005 × ET1118
ET1190	*glnA*$_{K}$ *rpsL*	P1 · ET1101 × ET1189
ET1191	*glnA*$_{K}$ *glnD99*::Tn*10 rpsL*	P1 · ET1008 × ET1190
ET1203	*glnD99*::Tn*10*	P1 · KT2201 × ET1003
ET2001	*rha metB glnA202*	Formerly T231 (26)
ET2029	*rha metB glnA*$_{K}$	P1 · KG2004 × ET2001 (26)
ET3001	*glnA202*	This laboratory
ET3003	*glnA*$^{+}$	P1 · ET1100 (19) × ET3001
ET3004	ColE1 *glnA*$^{+}$/*glnA202*	(Col E1 *glnA*) × ET3001[f]
ET3007	*glnA*$^{+}$ *glnD99*::Tn*10*	P1 · ET1008 × ET3003
ET3008	ColE1 *glnA*$^{+}$/*glnD99*::Tn*10 glnA202*	P1 · ET1008 × ET3004
ET5000	*thy his glnD38*::Tn*10*	λNK55 infection of UTHG
ET5001	*thy his glnD50*::Tn*10*	λNK55 infection of UTHG
ET5002	*thy his glnD99*::Tn*10*	λNK55 infection of UTHG
ET5003	*thy his glnD113*::Tn*10*	λNK55 infection of UTHG
ET7025	*dapD glnD99*::Tn*10 dnaE293*(Ts)	P1 · ET1008 × HS546
EG47	*gal lac hsr*	(26)
HS546	*polC293*(Ts) *dapD*	M. Nomura
KLF4/AB2463	F'104	B. Bachmann, CGSC 4251
MR202	Δ*(gal-bio) rpsE*	E. Signer
Q1	*thr leu supE*	E. Signer
UTHG	HfrG6 *his thy*	J. Suit
1774	*dnaE293*(Ts) *dapD* (λ*dapD1*) (λcI857*S7*)	M. Nomura, λ*dapD1* lysogen HS546 (28)
K. aerogenes		
KT2001	*glnD99*::Tn*10*	P1 · KT2202 × MK9000
KT2201	*glnA*$_{E}$ *ilv*	P1 · EG47 × MK9149
MK9000	Prototroph	B. Magasanik (23)
MK9149	*glnA ilv*	B. Magasanik
MK9159	*glnD17 leu-1 metB4*	F. Foor (5)
MK9768	*pan-1*	F. Foor (5)

[a] Symbols are those defined by Bachmann (1).

[b] Prototrophic derivative of strain CB100 (obtained from B. Bachmann).

[c] Indicates P1-mediated transduction. Phage P1 was grown on strain ET5002 and was used to transduce strain ET1003.

[d] Prototrophic derivative of CB100 except *hut*$_{K}$ and *rha*^{s} (Rha^{-} and inhibited by rhamnose).

[e] ET1115 was transduced sequentially via phage P1 first to *rpsE* and then to *glnA202*.

[f] ET3001 was transformed to a Gln^{+} phenotype with ColE1 *glnA* plasmid DNA by K. Janssen.

spectinomycin at 200 μg/ml, kanamycin sulfate at 25 μg/ml, and azide at 3 mM final concentration. Glutamine, azide, tetracycline, and kanamycin were filter sterilized.

Genetic techniques. Plate stocks of phage λNK55 were prepared by conventional methods (17) using strain Q1. Transductions were carried out with phage P1 (P1*clr*100KM) as previously described (10). Hfr crosses and F' transfer were performed with standard procedures (17).

Isolation of glutamine auxotrophs. Auxotrophs requiring glutamine for growth were isolated following insertion of a gene conferring tetracycline resistance into the host chromosome. Bacteriophage λNK55 (a gift from N. Kleckner), which carries the Tn*10* tetracycline translocatable element, was used to mutagenize *E. coli* by a modification of the procedures reported by Kleckner et al. (13) for phage P22 and *Salmonella typhimurium*. Cells from an exponentially growing culture of strain UTHG were infected with phage

λNK55 at a multiplicity of infection of 1 to 5 plaque-forming units per cell. The infected cells were first incubated for 30 min at room temperature and then at 30°C for an additional 30 min. The cells were then spread on LB-gln solid medium supplemented with tetracycline and containing 0.01 M sodium pyrophosphate to reduce background growth. Colonies appearing after a 24-h incubation at 40°C were replica plated onto solid medium containing only glucose, ammonium sulfate, vitamin B_1, and tetracycline.

Scoring of unselected markers. Transductant colonies were purified by single-colony isolation on selective plates prior to scoring for transfer of negative markers from the donor strain. Transfer of positive markers from the donor strain was tested without further purification of the transductants. Scoring for resistance to phage T5 was done by cross-streaking colonies on enriched plates with 10^9 plaque-forming units of phage T5 spread over half the area. When strain BT1026 or HS546 containing a heat-sensitive mutation in *dnaE* was transduced to tetracycline resistance, a multiplicity of infection of 0.1 was used. Only kanamycin-sensitive transductants were scored for ability to grow at 42°C. These modifications were necessary because phage P1 that confers kanamycin resistance and has a temperature-sensitive repressor was employed in this study. Kanamycin-resistant transductants were temperature-sensitive phage P1 lysogens and thus were not scoreable for the temperature-resistant phenotype of the wild-type *dnaE* allele.

Determination of the level of GS. Bacteria were grown at 30°C and harvested as described previously (24). GS biosynthetic and transferase activities were determined with intact cells as described by Bender et al. (2), except the transferase activity was assayed at pH 7.33, where the adenylylated and unadenylylated forms of GS from *E. coli* are equally active (20, 24). Protein was determined by the method of Lowry et al. (15).

Assay of UTase and UR. Cells were grown and extracts were prepared and assayed as described by Foor et al. (5). UTase activity is expressed as the difference in absorbance at 540 nm between a standard assay mixture to which extract (0.1 mg protein) had been added and a control mixture to which no extract was added. UR activity is expressed as radioactivity released from [^{3}H]UMP-P_{II} (24,600 cpm) in a standard assay mixture to which extract (0.1 mg of protein) was added. (These assays were performed in collaboration with S. G. Rhee and E. R. Stadtman).

RESULTS

Isolation and phenotype. Kleckner et al. (13) have demonstrated that gene function is abolished when a *tet*^{r} translocatable element (Tn*10*) inserts into a gene. Consequently, this method of mutagenesis was employed in this study. A derivative of phage lambda, λNK55 (*b221cI857Dam29*), carrying the *tet*^{r} translocatable element, Tn*10*, was used to infect cells of strain UTHG(HfrG6 *his thy*). In this strain background, λNK55 is incapable of virulent or lysogenic growth. Therefore Tet^r transductants could only result from the transposition of the Tn*10* element from the phage genome into the *E. coli* chromosome.

Approximately 11,000 Tet^r transductants were obtained from 200 independent adsorption mixtures. Replica plating these transductants from nutrient agar (LB-gln) to solid minimal agar identified at least 146 independent auxotrophs. Four of these were glutamine auxotrophs. When these mutants (strains ET5000, ET5001, ET5002, and ET5003) were incubated at 30°C on plates without glutamine no growth occurred for 3 days, after which time papillae became visible. These papillae were due to reversion to glutamine independence, since the strains reverted at a frequency of approximately 10^{-5} to the Gln$^+$ phenotype. This high frequency is due to suppressor mutations (see below).

Genetic analysis of Gln$^-$ mutants. Evidence that the acquisition of glutamine auxotrophy and of resistance to tetracycline were the consequence of the same event was obtained by growing phage P1 on the Gln$^-$ mutants (strains ET5000 to ET5003). The P1 lysates were used to transduce tetracycline-sensitive prototrophic strains (ET1003 and LS519) to tetracycline resistance. In each case at least 80 transductants were examined for acquisition of the Gln$^-$ phenotype, which was generally 100% co-inherited. Occasionally a lower linkage (98%) was observed; this may be due to the high frequency of reversion of the Gln$^-$ phenotype. Nevertheless the high linkage clearly demonstrates that the tetracycline-resistant mutants did not harbor the Tn*10* element at multiple sites on the cell genome and that the glutamine auxotrophy resulted from insertion of the Tn*10* element.

The close linkage between the gene conferring resistance to tetracycline and the locus responsible for the Gln$^-$ phenotype provided a powerful tool for genetic analysis. Strains ET5000 to ET5003, which contain the various *gln*::Tn*10* mutations and which are derivatives of UTHG(HfrG6), were mated with female strains to determine the location of the mutations on the *E. coli* genome. These experiments demonstrated that the *gln*::Tn*10* mutations were all located between *mtl* and *gal* on the segment of the genome that includes the *tonA* locus (80 to 10 min).

Since in *K. aerogenes* the *glnD* gene, which codes for UTase, lies within this region (5), it seemed reasonable to determine whether our mutation could be complemented by the same episome that covers the *K. aerogenes glnD* locus. Therefore, F$^-$ strains carrying the *gln*::Tn*10* mutations were obtained by infecting strain ET1003 with phage P1 grown on the Hfr strains ET5000 to ET5003 and selecting for tetracycline

resistance. In *K. aerogenes*, the *glnD* locus is covered by the *E. coli* episome F'104 (5). When this episome was introduced into strain ET1007 (F^-, *gln*::Tn*10*), the Gln^+ phenotype was restored. Similar results were obtained with the three other *gln*::Tn*10* derivatives of ET1003.

The chromosomal position of one of the *gln*::Tn*10* mutations was determined more accurately by transduction with phage P1 grown on strain ET1008 (*tonA azi-1 gln*::Tn*10*). Cross 1 (Table 2) shows that *gln*::Tn*10* is linked approximately 65% with *tonA* and 2% with *azi*. Another cross (Table 2, cross 2) was then carried out to order *gln*::Tn*10* in relation to *tonA*. We selected transductants that had acquired both azide resistance and tetracycline resistance from the donor strain and found that 95% of the transductants had also obtained the donor *tonA* gene. Thus this cross suggests that *tonA* lies between *azi* and *gln*::Tn*10*, as indicated in Fig. 1. A third cross was performed to order *gln*::Tn*10* with respect to *dnaE*. Strain BT1026, carrying a heat-sensitive mutation in the *dnaE* gene, was used (Table 2, cross 3). The results of this cross showed that the *gln*::Tn*10* locus is linked approximately 45% with *dnaE* (Table 2). Furthermore, the data reveal that *dnaE* is not between *tonA* and *gln*::Tn*10* because only 44% (10 of 23) of the transductants that had inherited both the donor *tonA* and *gln*::Tn*10* markers were able to grow at 42°C, demonstrating that they had simultaneously acquired the donor $dnaE^+$ locus (compare Table 2, lines 5 and 7). Similar reasoning shows that *tonA* is not between *dnaE* and *gln*::Tn*10* because only 62% (10 of 16) of the transductants acquiring both the *gln*::Tn*10* mutation and the wild-type $dnaE^+$ allele from the donor strain (ET1008) had also inherited the *tonA* mutation from this strain (compare Table 2, lines 6 and 7). Finally, the linkage of the *gln*::Tn*10* locus to *dapD* was found (Table 3, cross 4) to be 97%. Thus the final gene order is *azi..tonA..(dapD,glnD*::Tn*10)..dnaE* (Fig. 1).

The close linkage of *dapD* and *glnD* suggested that any specialized transducing phage that is able to transduce the *dapD* gene should be able to transduce the *glnD* gene. Strain 1775 (obtained from M. Nomura), which carries the specialized transducing phage λ*dapD1* (28), was subjected to a heat shock to obtain a lysate of this transducing phage. The phage lysate was used to transduce strain ET7025 (*dapD glnD*) to dap^+ at a multiplicity of infection of approximately 0.1. All (26 of 26) dap^+ transductants were also gln^+, indicating that λ*dapD1* transducing phage is also able to transduce the *glnD* gene.

Assay of UTase and UR activities. Since *glnD* mutants of *K. aerogenes* lack both UTase and UR activities (5, 6), extracts of the *E. coli* mutant were tested for these enzymes. The results presented in Table 3 show that insertion of the Tn*10* element at this site eliminated both activities. Other experiments have shown that

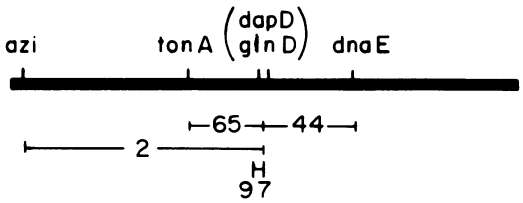

FIG. 1. *Map of the glnD region of E. coli derived from the data of Table 3. Numbers below the map represent the average cotransduction frequency of the markers.*

TABLE 2. *Cotransduction of glnD::Tn10 with tonA, dnaE, and dapD*[a]

Cross no.	Recipient		Recombinants				
	Strain	Relevant genotype	Selected phenotype	No. examined	Unselected genotype	No. of colonies with indicated genotype	Frequency of indicated genotype (%)
1	AT997	$tonA^+$ azi^+	Tet^r	194	*tonA*	126	65
					azi-1	4	2
2	AT997	$tonA^+$ azi^+	Tet^r Azi^r	40	*tonA*	38	95
3	BT1026	$tonA^+$ *dnaE*		36	$tonA^+$ *dnaE*	7	19
					tonA dnaE	13	36
					$tonA^+$ $dnaE^+$	6	17
					tonA $dnaE^+$	10	28
4	HS546	*dapD*	Tet^r	73	$dapD^+$	71	97

[a] Phage P1 grown on strain ET1008 (*tonA glnD99*::Tn*10 azi-1*) was used to transduce recipient strains for the indicated phenotype. The recombinants were checked for unselected traits as described in the text.

these strains contain normal adenylyltransferase and P_{II} (data not shown).

Physiology of GlnD mutants. Strain ET1007 was grown on a medium containing excess or growth-rate-limiting levels of nitrogen to determine the effect of the *glnD* mutation on GS levels. At the end of each experiment, the cultures were plated on glucose-ammonia and glucose-ammonia-glutamine to determine the proportion of Gln^+ revertants. The number of Gln^+ revertants was not large enough (approximately 0.01%) to account for any changes in the GS levels in the assayed cultures. The results of the transferase and biosynthetic assays, which measure, respectively, the total amount of GS present and the amount of nonadenylylated GS, are summarized in Table 4. When cultures were grown under conditions of nitrogen excess, the level of total GS protein in cells of the *glnD* strain, ET1007, was approximately sixfold lower than that observed in the wild-type strain, ET1003, grown under comparable conditions (Table 4). The relative amount of biosynthetically active GS in ET1007 was undetectable, whereas the wild-type strain grown under the same conditions had significant activity. Under derepressing (nitrogen-limiting) conditions, total GS protein in the GlnD mutants was roughly 13% of that observed in the wild-type strain, and the biosynthetic activity was about 5% (Table 4). Although GS levels were significantly reduced in the GlnD mutant, ET1007, regulation of enzyme synthesis was not seriously affected because the derepression ratio (glucose-glutamine level/glucose-ammonia-glutamine level) of 5 was similar to the ratio of 8 found with the wild-type strain.

TABLE 3. *Assay of UTase and UR*

Extract from strain	Genotype of strain	Enzyme activity[a]	
		UTase (A_{540})	UR (cpm)
ET1003	Wild type	1.46	1,790
ET1007	*glnD99*::Tn*10*	0.01	70

[a] Values for UR activity have been corrected by a control value of 1,300 cpm. A_{540}, Absorbance at 540 nm.

The GlnD mutant grown under nitrogen-limiting conditions contained significant GS biosynthetic activity, 20 milliunits/mg of protein. This observation suggested that strain ET1007 might be able to grow under derepressing conditions in the absence of glutamine if a suitable initial period of growth in glucose-glutamine minimal medium was permitted to achieve elevated GS levels. This prediction was tested by growing the wild-type strain ET1003 and strain ET1007 on a nitrogen-limiting medium (glucose-glutamine) for several generations before shifting to growth on glucose-aspartate medium (a nitrogen-limiting growth medium). Figure 2 shows that cells of strain ET1007 pregrown on glucose-glutamine medium could grow on glucose-aspartate medium at the same rate as wild-type cells. However, cultures of strain ET1007 pregrown on glucose-ammonia-glutamine medium did not grow at all when shifted to glucose-aspartate medium. After growth of these cultures for 10 h on glucose-aspartate minimal medium, the cells were harvested as described by Bender et al. (2) and assayed for GS activity. No change in the level of transferase and biosynthetic activity was observed in either culture of the *glnD* mutant, whereas the levels in both cultures of the wild type were those seen in wild-type cells grown on glucose-glutamine. Although the level of biosynthetic activity in the mutant pregrown on glucose-glutamine was far less than that seen in the wild type, this was apparently sufficient to allow growth in the absence of glutamine. Examination of each of the cultures of ET1007 for revertants to glutamine independence demonstrated that reversion was not responsible for these observations since the number of Gln^+

TABLE 4. *GS levels in a mutant lacking UTase activity*[a]

Strain	Growth medium	GS activity[b]			
		Transferase	% Wild-type[c]	Biosynthetic	%Wild-type
ET1003	Glucose-glutamine	0.82	100	0.42	100
	Glucose-ammonia-glutamine	0.10	12	0.05	12
ET1007	Glucose-glutamine	0.11	13	0.02	5
	Glucose-ammonia-glutamine	0.02	2	<0.01	

[a] Strains were grown at 30°C in a minimal medium containing glucose as the sole carbon source and either glutamine or glutamine and ammonia as the nitrogen source. At 100 Klett units (approximately 4×10^8 cells/ml) the cells were CTAB harvested (2) and assayed for total GS activity (transferase assay) and for nonadenylylated GS activity (biosynthetic assay).

[b] GS activity in micromoles per minute per milligram of protein as described in the text.

[c] Percentage of GS activity, with 100% taken as the GS activity of strain ET1003 grown on glucose-glutamine minimal medium.

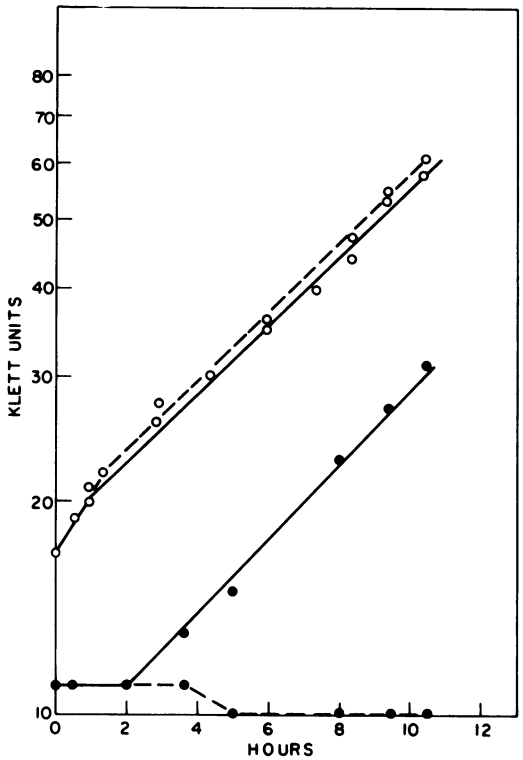

FIG. 2. *Growth of strains ET1003 and ET1007 on glucose-aspartate minimal medium. Cells of ET1003 (GlnD$^+$) and ET1007 (GlnD$^-$) were each pregrown at 30°C on glucose-ammonia-glutamine and glucose-glutamine medium for several generations. The cells were then centrifuged, washed once in 0.85% NaCl, and resuspended in glucose-aspartate medium. Growth was continued at 30°C.* (——) *Cells pregrown in glucose-glutamine;* (- - -) *cells pregrown in glucose-ammonia-glutamine;* (○) *strain ET1003;* (●) *strain ET1007.*

revertants was only 2×10^3/ml.

Suppression of the GlnD phenotype. Approximately 10 to 50% of the Gln$^+$ revertants of strain ET1007 had high levels of GS (GlnC phenotype) regardless of the nitrogen source in the medium. The presence or absence of tetracycline in the growth medium did not alter the frequency (10^{-5}) of revertants. This implies that one or more loci independent of *glnD*, rather than exact excision of the Tn*10* element (which occurs at a frequency of 10^{-7}), is responsible for suppression of the glutamine requirement.

The GlnD phenotype of *E. coli* is also suppressed by the presence of a ColE1 *glnA*$^+$ plasmid which results in a higher level of GS than found in the haploid strain. Derivatives of strain ET3001 (*glnA202*) containing either the ColE1 *glnA*$^+$ plasmid (strain ET3004) or the wild-type *glnA* allele on the chromosome (strain ET3003) were transduced to *glnD99*::Tn*10*. All *glnD* transductants with *glnA* on the chromosome (Table 5, strain ET3007) are glutamine auxotrophs. In contrast, the *glnD* transductants containing the plasmid are Gln$^+$ (Table 5, strain ET3008). Thus the ColE1 *glnA*$^+$ plasmid suppresses the glutamine auxotrophy resulting from the *glnD*:Tn*10* insertion.

We introduced the *glnD*:Tn*10* mutation via P1 transduction into isogenic strains of *E. coli* (ET1115 and ET1190) differing only in the origin of the *glnA* region. Strain ET1172, containing the native *E. coli glnA* gene (*glnA*$_E$) and the *glnD99*::Tn*10* insertion, was, as expected, a glutamine auxotroph, while strain ET1191, containing the *glnA* region of *K. aerogenes* and the *glnD*:Tn*10* mutation, grew slowly in the absence of glutamine (Table 5). We conclude that the presence of the *glnA*$_K$ gene in *E. coli* partially suppresses the GlnD phenotype.

The *glnD99*::Tn*10* allele was transferred via P1 transduction into a strain of *K. aerogenes* (KT2201) that contained the *glnA*$_E$ gene. In this way we sought to examine the effect of the cytoplasm of *K. aerogenes* and *E. coli* on the combination of the *glnA*$_E$ and the *glnD99*::Tn*10* insertion. Of 24 tetracycline-resistant transductants of strain KT2201, 23 grew as well as wild-type strains in the absence of glutamine. It is likely that the Tn*10* insertion translocated to another point on the genome in these Tet^r Gln$^+$ transductants. One transductant (strain KT2202) was a partial glutamine auxotroph (Table 5). Extracts of this strain have been shown to lack UTase (S. G. Rhee and F. Foor, unpublished data). The *glnD*::Tn*10* insertion in strain KT2202 had integrated at or near the *glnD* site, since the insertion was linked to *pan* by P1 transduction, as is the case for *glnD* point mutants in *K. aerogenes*. The *glnD*::Tn*10* insertion in strain KT2202 was transduced via P1 into other *K. aerogenes* strains. Of 16 Tet^r transductants of *K. aerogenes* strain MK9000, 15 were partial glutamine auxotrophs (Table 5) on glucose-ammonia medium and were unable to utilize nitrate as a source of nitrogen. These results show that the *glnD*::Tn*10* allele in *K. aerogenes* results in only a partial Gln$^-$ phenotype regardless of the source of the *glnA* region. Finally, the *glnD*::Tn*10* insertion was transduced from the *K. aerogenes* strain, KT2202, to the *E. coli* strain, ET1003. Eight Tet^r transductants (for example, strain ET1203, Table 5) were shown to be complete glutamine auxotrophs, indicating that the insertion had not been altered by its passage through the *K. aerogenes* cytoplasm.

The GlnD phenotype can also be partially suppressed by the introduction of a mutation (*rpsE*) conferring spectinomycin resistance from

TABLE 5. *Suppression of the glnD mutation*

Strain[a]	Relevant genotype[b]			Growth on minimal medium containing[c]:		
	glnD allele	*glnA* allele	Other	G-N-g	G-N	G-NO_3
ET3003	+	E		++	++	
ET3004	+	*202*	ColE1 *glnA*	++	++	
ET3007	*99*	E		++	−	
ET3008	*99*	*202*	ColE1 *glnA*	++	++	
ET1115	+	E		++	++	
ET1172	*99*	E		++	−	
ET1190	+	K		++	++	
ET1191	*99*	K		++	+	
KT2201	+	E		+++	++	++
KT2202	*99*	E		+++	+	−
MK9000	+	K		+++	+++	++
MK9792	*99*	K		++	+	−
ET1203	*99*	E		++	−	
ET1173	*99*	E	*rpsE*	++	+	

[a] The prefix ET designates *E. coli* strains; MK and KT, *K. aerogenes* strains.

[b] E indicates the presence of the *glnA* region of *E. coli* on the chromosome; K, the *glnA* region of *K. aerogenes* on the chromosome; ColE1, the ColE1 *glnA* plasmid with the *glnA202* allele on the chromosome.

[c] Bacteria were streaked for single colonies on the indicated media. Abbreviations are: G, 0.4% glucose; N, 0.2% $(NH_4)_2SO_4$; g, 0.2% glutamine; NO_3, 0.2% $NaNO_3$. Growth of *E. coli* strains was determined after 72 h of incubation, *K. aerogenes* after 48 h, at 30°C. −, No visible growth; +++, a colony diameter of about 4 mm; + and ++, intermediate sizes.

strain MR202 into strain ET1172 (*glnD*::Tn*10*). Strain ET1172 is a glutamine auxotroph (Table 5); the spectinomycin-resistant transductants (10 of 10) are partial glutamine auxotrophs (strain ET1173, Table 5). The levels of GS in these *rpsE* strains are consistently 20% higher than the GS levels of *rpsE*$^+$ strains under a wide variety of growth conditions (A. Zelenetz, G. Pahel, and B. Tyler, unpublished data). Sixteen spontaneous spectinomycin-resistant mutants of ET1172 were also selected. Of these, four were able to suppress the Gln$^-$ phenotype in the *glnD*::Tn*10* strain. At present the exact nature of the suppression of the GlnD phenotype by the *rpsE* mutations is not clear.

DISCUSSION

We have isolated and characterized independent strains of *E. coli* in which the Tn*10* element (which confers resistance to tetracycline) is inserted into or near the *glnD* gene. This gene specifies UTase, an enzyme which is involved in the adenylylation system regulating the activity of GS (9). These *glnD*::Tn*10* strains have an absolute growth requirement for glutamine; the auxotrophy in general cotransduces 100% with the Tn*10* insertion. The map position of the *glnD* gene in *E. coli* has been determined by utilizing the cotransduction of *glnD* with the Tn*10* insertion. The *glnD* gene maps at min 4 on the revised *E. coli* map (1) and is 98% linked to *dapD* by P1 transduction. Lysates of a λ*dapD1* specialized transducing phage were also able to transduce the *glnD* gene. Biochemical evidence indicates that the Tn*10* insertion in *glnD* results in the loss of both UTase and UR (Table 3). This result is consistent with the idea that these activities are due to the product of a single gene (5) or to products of neighboring genes.

The experiments of Foor et al. (6) indicate that the synthesis of GS occurs at a lower rate in *K. aerogenes* if the enzyme is in the adenylylated conformation. These results are consistent with the autogenous regulation of GS synthesis. The levels of GS have been measured in the *glnD*::Tn*10* strain ET1007. We find that total GS activity is always lower in the mutant strain than in the wild-type strain (ET1003) regardless of the growth conditions. In response to nitrogen limitation, the *glnD* mutants increase the rate of GS synthesis fivefold but still contain a smaller percentage of biosynthetically active GS than do wild-type cells. The differences in the levels of GS can be explained in several ways. According to the model of Foor et al. (6), the *glnD*::Tn*10* insertion would result in the synthesis of largely adenylylated GS, which would in turn increase the repression of *glnA* transcription. The results are also consistent with the data of Weglenski and Tyler (27), which suggest that the *glnD* mutation in these strains would lead to decreased activation of *glnA* transcription. Alternatively, the decreased rate of GS synthesis in these GlnD mutants compared to that in the wild-type strain could be due to an alteration in the ratio of *glnA* repressor to

glnA activator.

The *glnD*::Tn*10* *E. coli* strain ET1007 is able to grow in the absence of glutamine provided the strain is pregrown under conditions of nitrogen limitation (Fig. 2). Our data indicate that approximately 20 milliunits of biosynthetically active GS is sufficient to allow slow growth of *E. coli* in the absence of glutamine. Therefore a small increase in the amount of biosynthetically active GS may be sufficient to partially suppress the glutamine auxotrophy due to the *glnD*::Tn*10* insertion.

A number of different suppressors of the *glnD* phenotype have been described. The GlnD$^-$ phenotype in strain ET1172 is suppressed by the presence of an *rpsE* mutation conferring spectinomycin resistance due to the alteration of a ribosomal protein. Strains containing this *rpsE* mutation contain approximately 20% more GS activity than *rpsE*$^+$ strains (A. Zelenetz, G. Pahel, and B. Tyler, unpublished data). Ribosomal suppression (11) of some gene product may be involved in this effect. It is of interest that *gltE* and *gltM* mutations which affect the glutamyl-tRNA synthetase were isolated as streptomycin-suppressible lethal mutations (18). Consequently, it is tempting to speculate that some *rpsE* mutations may be affecting either synthesis or the activity of the glutamyl-tRNA synthetase, which in turn may affect the rate of GS synthesis, possibly through an alteration in the level of charged tRNA (14). The *glnD*::Tn*10* insertion is also suppressed by second-site mutations which allow the synthesis of high levels of GS even in the presence of excess ammonia. Finally, the *glnD* phenotype is suppressed by the presence of a ColE1 *glnA*$^+$ plasmid which increases the level of GS (E. Bartnik and B. Tyler, unpublished data).

GlnD mutants of *K. aerogenes* (5) and *K. pneumoniae* (S. L. Streicher, F. R. Bloom, F. Foor, M. Levin, and B. Tyler, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, K88, p. 200) which lack UTase have also been isolated. In contrast to the GlnD mutants in *E. coli*, the GlnD mutants in both *Klebsiella* strains exhibit only a partial glutamine auxotrophy. We sought to investigate the reason for this difference by constructing various hybrid strains of *E. coli* and *K. aerogenes* containing the *glnD*::Tn*10* insertion. The *glnA* region had previously been transduced via phage P1 between *K. aerogenes* and *E. coli* (26). Now we have succeeded in transducing the *glnD*::Tn*10* insertion via phage P1 from *E. coli* to *K. aerogenes*. However, in this case only one of 24 tetracycline-resistant colonies contained the insertion at the *glnD* site. At least two factors contribute to this low frequency: restriction of the incoming DNA and nonhomology between the *glnD* regions of *E. coli* and *K. aerogenes*. In keeping with this idea we found that, once Tn*10* integrated in the *glnD* gene of *K. aerogenes*, the frequency of obtaining partial glutamine auxotrophs in transduction to other *K. aerogenes* strains improved dramatically; apparently these transductants had received the *glnD*::Tn*10* mutation.

These genetic experiments revealed that an *E. coli* strain containing the $glnA_K$ gene and the *glnD*:Tn*10* insertion grows slowly without glutamine, whereas a strain containing the native $glnA_E$ gene and the *glnD*:Tn*10* insertion does not grow in the absence of glutamine. In addition, the *K. aerogenes* strains containing the *glnD*::Tn*10* insertion and either the $glnA_E$ (strain KT2202) or the $glnA_K$ gene (strain KT2001) grow slowly in the absence of glutamine.

One explanation for the results involving hybrid strains containing the *glnD*::Tn*10* insertion might be the inability of the adenylylation system of *E. coli* (or *K. aerogenes*) to interact with heterologous GS. According to this hypothesis, the GS synthesized from the $glnA_E$ gene in the *K. aerogenes* cytoplasm would not be adenylylated as readily as the native *K. aerogenes* GS. Consequently, more nonadenylylated GS would accumulate in the $glnA_E$ *glnD*::Tn*10* mutants of *K. aerogenes*, resulting in the suppression of the *glnD* phenotype. However, *K. aerogenes* strain KT2001 contains the native $glnA_K$ gene together with the *K. aerogenes* adenylylation system enzymes except for the *glnD*::Tn*10* insertion. This strain is also a partial glutamine auxotroph; consequently, additional explanation is needed. It is possible that the adenylylation system may also be somewhat less efficient in *K. aerogenes* than in *E. coli*. In other words, in a *K. aerogenes* *glnD* mutant the rate of GS synthesis is greater than the rate of adenylylation. In the *E. coli* *glnD* strains, the opposite situation occurs.

On the other hand, other explanations exist for the results we obtained with the hybrid strains. One intriguing possibility is that transcription may occur more frequently from the $glnA_K$ promoter than from the $glnA_E$ promoter. Thus in the *E. coli* cytoplasm the stronger $glnA_K$ promoter would result in a rate of synthesis of GS protein which is faster than the rate of adenylylation. Consequently, such strains would contain more nonadenylylated GS than GlnD mutants of *E. coli* with the $glnA_E$ gene, and suppression of the GlnD phenotype would occur. However, this hypothesis does not explain the fact that the *E. coli* strains containing the $glnA_E$ gene and the *glnD*::Tn*10* mutation are complete glutamine auxotrophs while derivatives of *K. aerogenes* with these same *E. coli* loci ($glnA_E$,

glnD::Tn*10*) are only partial glutamine requirers. These observations can be explained by suggesting that another factor in the cytoplasm of *K. aerogenes* may increase transcription of the *glnA* gene relative to the rate of transcription in *E. coli*. One candidate for this cytoplasmic factor is the *glnF*-gene product which is required for the synthesis of GS (7).

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