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Genetics

The product of a newly identified gene, *glnF*, is required for synthesis of glutamine synthetase in *Salmonella*

(regulation of enzyme synthesis/covalent modification/adenylylation)

EMILIO GARCIA*, SHARON BANCROFT*, SUE GOO RHEE†, AND SYDNEY KUSTU*

* Department of Bacteriology, University of California, Davis, California 95616; and † Laboratory of Biochemistry, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014

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ABSTRACT **The product of a newly identified gene, *glnF*, which is distinct from the glutamine synthetase structural gene (*glnA*), is required for synthesis of glutamine synthetase [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] in *Salmonella typhimurium* and probably in *Escherichia coli*. *Salmonella* strains with ICR (2-chloro-6-methoxy-9-[3-(2-chloroethyl)aminopropylamino]acridine dihyodrochloride)-induced (frameshift) mutations in *glnF* are glutamine auxotrophs; they have less than 10% of wild-type glutamine synthetase activity or antigen and are unable to derepress the synthesis of the enzyme. The mutant allele is recessive to the wild-type allele, indicating that the *glnF* gene encodes a diffusible product. Mutant *glnF* strains have normal activities of all proteins involved in covalent modification of glutamine synthetase: adenylyltransferase (EC 2.7.7.42), P_{II}, uridylyltransferase, and uridylyl removing enzyme. In addition, they have glutamate synthase (EC 1.4.1.13) and glutamate dehydrogenase (EC 1.4.1.4) activities. Thus, *glnF* does not encode the structure of any of these proteins. The above evidence suggests that the product of the *glnF* gene is (or produces) a positive regulatory factor that is required for synthesis of glutamine synthetase; it indicates that autoregulation cannot account for control of the synthesis of glutamine synthetase in *Salmonella*.**

Glutamine synthetase [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] (GS) is covalently modified by adenylylation in a variety of Gram-negative bacteria (1, 2). As demonstrated by the elegant studies of Stadtman (3), Holzer (4), and their colleagues, adenylylation converts the enzyme to a catalytically inactive or less active form. Adenylylation is reversible and is catalyzed by the enzyme glutamine-synthetase adenylyltransferase (EC 2.7.7.42) working in conjunction with a second protein P_{II} (reviewed in ref. 5); P_{II} itself is covalently modified by uridylylation (6), which is catalyzed by uridylyltransferase (Fig. 1). Recently, Magasanik and his colleagues have proposed that glutamine synthetase controls transcription of its own structural gene (*glnA*) in *Klebsiella aerogenes* (7–9) and that covalent modification of the enzyme alters its function as a regulator of transcription (10). These conclusions are based in part on the findings that mutant strains [*glnB* (P_{II}) and *glnD* (uridylytransferase)] that produce highly adenylylated glutamine synthetase (10) also contain small amounts of the enzyme (11, 12), whereas other strains [*glnE* (adenylyltransferase)] that are unable to adenylylate glutamine synthetase (10) contain elevated amounts (13).

We have previously defined the structural gene for glutamine synthetase (*glnA*) in *Salmonella typhimurium* (14). Glutamine synthetase in enteric bacteria is composed of 12 identical subunits (reviewed in refs. 15 and 16) and thus there should be a single structural gene. We now report that the product of a newly identified gene (*glnF*) is required for synthesis of glutamine synthetase in *Salmonella* and probably in *E. coli*. This gene is well separated from the *glnA* and *glnD* (uridylyltransferase) genes on the *Salmonella* chromosome, and is not in a region of the chromosome corresponding with that of the *glnB* (P_{II}) and *glnE* (adenylyltransferase) genes in *Klebsiella* (8, 10) (Fig. 2). In addition, biochemical studies indicate that all proteins involved in covalent modification of glutamine synthetase are present in *glnF* extracts in normal amounts.

Abbreviations: GS, glutamine synthetase; $GS_{\bar{n}}$, the subscript $\bar{n}$ (0–12) indicates the average number of adenylylated subunits per dodecamer; adenylyltransferase, glutamine-synthetase adenylyltransferase (EC 2.7.7.42); P_{IIA}, unmodified protein component that stimulates adenylylation of GS; P_{IID}, uridylylated P_{IIA}, which stimulates deadenylylation of GS; uridylyltransferase, UTP: P_{IIA} uridylyltransferase; UR, uridylyl removing enzyme, which catalyzes the conversion of P_{IID} to P_{IIA}; ICR191E, 2-chloro-6-methoxy-9-[3-(2-chloroethyl)aminopropylamino]acridine dihydrochloride.

MATERIALS AND METHODS

Chemicals and Growth Media. The frameshift mutagen ICR191E (17) was kindly donated by Dr. H. J. Creech. Medium E of Vogel and Bonner (18) containing 0.4% glucose was used as the minimal medium for most experiments. For some experiments a second mineral salts-glucose mixture was used (19); NH_4Cl (final concentration 20 mM) was added to this mixture as the nitrogen source for growth curves.

Bacterial Strains: Genetic Analysis by Recombination. Strains constructed for this work were derived from *S. typhimurium* strain LT2 and are listed in Table 1. Glutamine auxotrophs were isolated by mutagenesis and penicillin counterselection. Phage was P22 (int-4) (20) or P22 (HT *int*$^-$) (21). Recombination analysis of Gln$^-$ strains by transduction and approximate conjugation mapping of the *glnF* and *glnD* genes were carried out as described (14). Transductional linkage of *glnF* to *argE* was determined reciprocally using strains SK99, SK100, and *argE116*, *argE72*, and JL2413. (A total of 500 transductants was scored.) Strain SK176 (containing *glnA60* and *glnF75*) was identified by its failure to revert to glutamine prototrophy; the presence of the *glnF75* mutation was confirmed by showing that it could be recovered genetically on the basis of transductional linkage to *argE*.

Construction of Merodiploids and Complementation Analysis. Phage grown on strains with insertions of a tetracycline-resistance element (Tn 10) in *argE* or *argF* were used to transduce strains SK99 and SK100 to tetracycline resistance and simultaneously to arginine auxotrophy (22). Episomes were transferred from *E. coli* strains to *Salmonella* recipients by selecting for arginine-independence. The resulting *Salmonella* merodiploids were tested for glutamine independence and were shown to remain tetracycline-resistant and auxotrophic for histidine. They were shown to segregate the episomes after growth on nonselective medium and to transfer them to appropriate recipient strains.

Enzyme Assays. For assays of glutamine synthetase under repressing conditions and for assays of glutamate synthase (EC

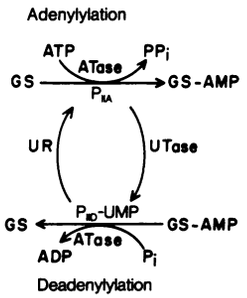

FIG. 1. Proteins involved in covalent modification of glutamine synthetase. Adapted from Ginsburg and Stadtman (5). ATase, adenylyltransferase; UTase, uridylyltransferase; UR, uridylyl removing enzyme; P_{IIA}, protein component that stimulates adenylylation of GS; P_{IID}, uridylylated P_{IIA}, which stimulates deadenylylation of GS.

1.4.1.13) and glutamate dehydrogenase (EC 1.4.1.4), cells were grown in medium E plus glucose, appropriate supplements, and 3 mM glutamine. For assays of glutamine synthetase under derepressing conditions, cells were grown in minimal-glucose medium with glutamine (10 mM) as sole nitrogen source. [It has been postulated that derepression occurs because the cells are "nitrogen-limited" as a result of poor glutamine transport (23).] Cell extracts with a protein concentration of about 50 mg/ml were prepared as described (14, 19). Total glutamine synthetase activity independent of the degree of adenylylation was measured using the γ-glutamyl transfer assay of Stadtman *et al.* (24) with the modifications described previously (14). Activities were corrected for "blank activity" in the absence of ADP and arsenate (25) and are expressed in units of μmol of γ-glutamyl hydroxamate formed per min. Glutamate synthase and glutamate dehydrogenase activities were measured as described (19).

For assays of the proteins required for covalent modification of glutamine synthetase, cells were grown in medium E plus glucose, histidine, and 10 mM glutamine. Adenylyltransferase and uridylyltransferase activities were assayed in crude extracts or supernatants obtained after treatment of extracts with streptomycin sulfate. Uridylyl removing enzyme (UR) activity was assayed in streptomycin supernatants after dialysis. Since P_{IIA} and P_{IID} activities could not be clearly demonstrated up to this point, these proteins were partially purified as follows. The dialyzed streptomycin supernatants were treated with mercaptoethanol (final concentration 26%, vol/vol) according to an unpublished procedure of E. R. Stadtman, E. Engleman, P. B. Chock, and S. G. Rhee. Dialyzed supernatants were concentrated by ion-exchange chromatography on diethylaminoethyl-cellulose DE52.

Activities of adenylyltransferase, P_{IIA}, P_{IID}, uridylyltransferase, and UR were assayed spectrophotometrically by unpublished procedures of E. R. Stadtman, B. Park, S. G. Rhee, and P. B. Chock. Assays are based on the ability to measure selectively the activity of the adenylylated or the unadenylylated form of glutamine synthetase. For adenylyltransferase assays, *Salmonella* extracts were first incubated with glutamine and saturating amounts of unadenylylated glutamine synthetase from *E. coli* ($GS\overline{1.5}$); the increase in activity of adenylylated glutamine synthetase was then determined. (In the presence of glutamine, adenylyltransferase activity is independent of P_{II}.) For P_{IIA} and P_{IID} assays, *Salmonella* fractions were incubated with saturating amounts of *E. coli* adenylyltransferase and *E. coli* $GS\overline{1.5}$ or $GS\overline{12.0}$, respectively, and the increase in activity of adenylylated or unadenylylated GS, respectively, was then determined. For uridylyltransferase and UR assays, extracts were incubated with partially purified *E. coli* P_{IIA} or P_{IID}, respectively, and the increase or decrease in the activity of P_{IID}, respectively, was then determined as described above.

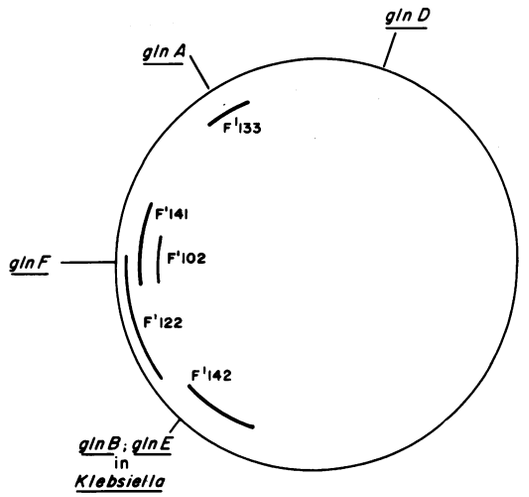

FIG. 2. Locations of *gln* genes on the *Salmonella* chromosome; *E. coli* F′ factors used for complementation analysis. The *glnA*, *glnD*, and *glnF* genes in *Salmonella* lie at 125, 7, and 102 min on the chromosome, respectively; the *glnA* (8) and *glnD* (29) genes in *Klebsiella* lie at homologous chromosomal positions. The *glnB* gene in *Klebsiella* is covered by F′142 (8) and the *glnE* gene is linked to *glnB* by P1-mediated transduction (10).

Immunology. Ouchterlony immunodiffusion experiments were performed by placing 8 μl of rabbit antiserum to purified *Salmonella* glutamine synthetase in the center well and 0.15–0.6 mg of crude bacterial protein in the outer wells. The minimum amount of wild-type glutamine synthetase that gave a precipitin band under these conditions was 0.035 unit, the amount contained in 0.05 mg or 0.25 mg of crude protein under derepressing or repressing growth conditions, respectively.

The amount of inactive antigen in mutant extracts was determined by their ability to inhibit precipitation of wild-type glutamine synthetase by antibody [method of Hamers and Hamers-Casterman (26) as described by Kaminskas *et al.* (27)]. (Cells were grown under repressing conditions.) In each case two experiments were run in parallel. In the first experiment, fixed aliquots of antiserum (5 μl) were titrated with increasing amounts of wild-type crude extract; after overnight incubation, precipitate was removed by centrifugation and glutamine synthetase activity of the supernatant was measured. The second experiment was identical except that antiserum was incubated overnight with a fixed amount of mutant extract before being exposed to wild-type extract. For each experiment, activity in the supernatant was plotted as a function of the amount of wild-type extract added. The y-intercept (negative) is a measure of the amount of wild-type glutamine synthetase precipitated by the antibody and the x-intercept is a measure of the amount of crude protein in which it is contained; the difference between the x-intercepts for the two experiments can be used to calculate the percentage of inactive antigen in the mutant extract.

RESULTS

Phenotype and Genetic Analysis of *glnF* and *glnD* Strains. Crosses by recombination using P22-mediated transduction indicated that mutations in 23 independent glutamine auxotrophs were distributed among three well-separated loci [*glnA* (12 strains), *glnD* (6 strains), and *glnF* (5 strains)]. Preliminary conjugation mapping of the *glnF* gene indicated a peak of coinheritance (60%) of *glnF75* with the *argE* gene at 102 min

Table 1. Bacterial strains

Strain	Genotype	Parents recipient, donor	Source
TA831	*hisF645*		G. F.-L. Ames
TA2192	*glnA55 hisF645 dhuA1 hisJ5601*		(14)
SK35	*glnA60 hisF645*	TA831	ICR mutagenesis
SK99	*glnF75 hisF645*	TA831	ICR mutagenesis
SK100	*glnF76 hisF645*	TA831	ICR mutagenesis
SK106	*glnF92 hisF645*	TA831	DES mutagenesis
SK108	*glnF93 hisF645*	TA831	DES mutagenesis
SK101	*glnD77 hisF645*	TA831	ICR mutagenesis
SK103	*glnD79 hisF645*	TA831	ICR mutagenesis
argE1828::Tn10			G. F.-L. Ames
argF1823::Tn10			G. F.-L. Ames
SK162	*glnF75 hisF645 argE1828*::Tn10	SK99, *argE1828*	Transduction
SK163	*glnF76 hisF645 argE1828*::Tn10	SK100, *argE1828*	Transduction
SK162/F'102 coli		SK162, CGSC4247	Episome transfer
SK162/F'122 coli		SK162, CGSC4257	Episome transfer
SK162/F'141 coli		SK162, CGSC4248	Episome transfer
SK163/F'102 coli		SK163, CGSC4247	Episome transfer
SK163/F'122 coli		SK163, GCSC4257	Episome transfer
SK163/F'141 coli		SK163, CGSC4248	Episome transfer
SK170	*glnF75 hisF645 argF1823*::Tn10	SK99, *argF1823*	Transduction
SK173	*glnA60 hisF645 argF1823*::Tn10	SK35, *argF1823*	Transduction
SK175	*glnA60* HfrK5 *argE1828*::Tn10	SK174, *argE1828*	Transduction
SK176	*glnA60 glnF75 hisF645*	SK170, SK175	Conjugation
SK177	*glnA60 glnF75 hisF645 argF1823*::Tn10	SK176, *argF1823*	Transduction
SK173/F'133		SK173, CGSC4265	Episome transfer
SK177/F'133		SK177, CGSC4265	Episome transfer
argE116			B. N. Ames
JL2413	*argE15 ara-9*		J. L. Ingraham
argE72			J. L. Ingraham
CGSC4247	*argG6 metB1 his-1 leu-6*		B. Bachmann, *E. coli* Genetic Stock Center
KLF2/JC1553	*recA1 mtl-2 xyl-7 malA1* / *gal-6 lacY1 strA104 tonA2* / *tsx-1* λRλ⁻ *supE44*	F'102 *argR*⁺ argG⁺*	
CGSC4248	*argG6 metB1 his-1 leu-6*		B. Bachmann, *E. coli* Genetic Stock Center
KLF41/JC1553	*recA1 mtl-2 xyl-7 malA1* / *gal-6 lacY1 strA104 tonA2* / *tsx-1* λRλ⁻ *supE44*	F'141 *asd*⁺ *argG*⁺*	
CGSC4265	*argG6 metB1 his-1 leu-6*		B. Bachmann, *E. coli* Genetic Stock Center
KLF33/JC1553	*recA1 mtl-2 xyl-7 malA1* / *gal-6 lacY1 strA104 tonA2* / *tsx-1* λRλ⁻ *supE44*	F'133 *ilv*⁺ *argH*⁺†	
CGSC4257	*argG6 metB1 his-1 leu-6*		B. Bachmann, *E. coli* Genetic Stock Center
KLF22/KL110	*recA1 mtl-2 xyl-7 malA1* / *gal-6 lacY1 strA104 tonA2* / *tsx-1* λRλ⁻ *supE44 thyA23*	F'122 *argG*⁺ *metC*⁺ *cysC*⁺	

* The homologous locus in *Salmonella* is *argE*.
† The homologous locus in *Salmonella* is *argF*.

(28); *glnF* is about 15% linked to *argE* by P22 (int-4)-mediated transduction (Fig. 2). The *glnA* and *glnD* genes lie at 125 min (14) and 7 min (S. Bancroft and S. Kustu, unpublished results) on the *Salmonella* chromosome, respectively (Fig. 2).

Strains with *glnF* mutations (SK99 and SK100) failed to grow in minimal medium and had a doubling time of 68 min in medium supplemented with 5 mM glutamine; the doubling times of a strain with an ICR-induced mutation in *glnA* (SK35) and the parent strain (TA831) in glutamine-supplemented medium were 79 and 45 min, respectively. The long doubling times of glutamine auxotrophs in glutamine-supplemented medium suggested that their growth was limited by the rate of glutamine transport. Consistent with this, the doubling times of both *glnF* and *glnA* strains were decreased by about 12 min if the medium was further supplemented with arginine and uracil, major end products that contain a nitrogen atom derived from glutamine. Strains with ICR-induced *glnD* mutations are glutamine bradytrophs; they had doubling times of 72 and 54 min in minimal medium without and with added glutamine, respectively.

Glutamine Synthetase Activity and Antigen. Glutamine synthetase activity in extracts of four *glnF* strains (<0.01 unit/mg) was less than 10% of wild-type glutamine synthetase activity under repressing growth conditions and less than 2% under derepressing conditions (Table 2). Activity of glutamine synthetase in *glnF* strains did not increase under derepressing conditions whereas that in the wild-type strain increased 3- to 5-fold. Appropriate mixing experiments indicated that *glnF*

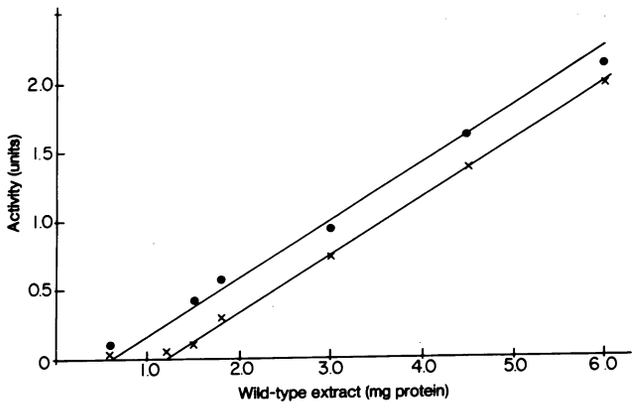

FIG. 3. Competition of a *glnF* extract for binding to antibody against glutamine synthetase. Antiserum against glutamine synthetase was titrated with extract from a wild-type strain (TA831) with (●) or without (×) prior exposure to extract from a *glnF* strain (SK99). Exposure of antiserum to 6.6 mg of crude mutant protein shifted the x-intercept by 0.6 mg, indicating that the mutant extract contained 10% as much antigen as the wild-type extract. (One milliliter of serum precipitated 100 units of glutamine synthetase; the specific activity of the wild-type extract was 0.4 unit/mg.)

extracts did not inhibit the activity of glutamine synthetase in the wild-type extract. A spontaneous glutamine-independent revertant of strain SK99 regained wild-type glutamine synthetase activity. The *glnF75* mutation present in strain SK99 could not be recovered genetically from the revertant. By contrast to *glnF* strains, extracts of five *glnD* strains had 30–60% of wild-type glutamine synthetase activity under both repressing and derepressing growth conditions; activity of glutamine synthetase in *glnD* strains increased about 4-fold under derepressing conditions like that in the wild-type strain (Table 2).

Extracts of four *glnF* strains (grown under repressing or derepressing conditions) failed to produce a precipitin band with antibody against wild-type glutamine synthetase on immunodiffusion plates. (The strains included two with diethylsulfate-induced mutations.) In addition, an extract of strain SK99 inhibited precipitation of wild-type glutamine synthetase by antibody only slightly (Fig. 3); the inhibition indicated that the *glnF* extract contained at most 10% of the antigen present in a wild-type extract. An extract of a control strain with an ICR-induced mutation in *glnA* (SK35) gave the same slight degree of inhibition as the *glnF* extract. By contrast, an extract of a *glnA* strain that produces inactive antigen (strain TA2192) inhibited precipitation of glutamine synthetase by antibody significantly (data not shown); the degree of inhibition indicated that this *glnA* extract contained 92% as much antigen as a wild-type extract.

Activity of Other Enzymes of Ammonia Assimilation. Extracts of *glnF* strains (SK99 and SK100) had about 85% (0.14 μmol/min–mg) of wild-type glutamate synthase activity and about 65% (0.17 μmol/min–mg) of wild-type glutamate dehydrogenase activity. A spontaneous revertant of strain SK99 regained 100% of wild-type glutamate dehydrogenase activity. Like *glnF* strains, a strain with a *glnA* mutation (SK35) also had lower glutamate synthase and glutamate dehydrogenase activities than the wild type.

Complementation of *glnF*. ICR-induced mutations in *glnF* were complemented by the *E. coli* episomes F′102, F′122, and F′141 (Fig. 2) (30), indicating that the *glnF* gene produces a diffusible product. Merodiploid strains were glutamine prototrophs; the four that were tested had 90–142% of wild-type glutamine synthetase activity (0.22 unit/mg), whereas a segregant had less than 10% of wild-type activity.

Table 2. Glutamine synthetase activities* of *glnF* and *glnD* strains

Strain	Growth conditions†	
	Repressing	Derepressing
TA831 (wild type)	0.27	1.12
SK99 (*glnF*)	<0.01	<0.01
SK100 (*glnF*)	<0.01	<0.01
SK101 (*glnD*)	0.12	0.50
SK103 (*glnD*)	0.09	0.36

* The assay is described in *Materials and Methods*. Activities are in units/mg of protein.
† Media are described in *Materials and Methods*.

Analysis of strains that were diploid for the *glnA* region (but had a functional *glnA* gene only from *E. coli*) indicated that the product of the *glnF* gene was required for function of the *E. coli glnA* gene, at least when this gene was present in the *Salmonella* cytoplasm. Thus, the *glnF* strain SK177/F′133$_{coli}$ had low levels of glutamine synthetase activity under both repressing and derepressing growth conditions, whereas the *glnF*$^+$ strain SK173/F′133$_{coli}$ had high levels of glutamine synthetase activity (Table 3).

Covalent Modification of Glutamine Synthetase. Preliminary assays indicated that extracts of *glnF* strains (SK99 and SK100) could catalyze deadenylylation of purified glutamine synthetase *in vitro* (data not shown). This activity requires the function of three of the four proteins known to be involved in covalent modification of glutamine synthetase: adenylyltransferase, P_{II}, and uridylyltransferase (E. Stadtman, personal communication). The presence of adenylyltransferase, P_{II}, uridylyltransferase, and UR in an extract of strain SK99 was confirmed by assaying the activity of each separately. The data in Table 4 indicate that each of these proteins is present in the *glnF* extract in normal amounts. By contrast, there is no detectable uridylyltransferase activity in an extract of a *glnD* strain, SK103, and, as expected, this extract contains only one form of the P_{II} protein P_{IIA} (Table 4).

DISCUSSION

The product of a newly identified gene, *glnF*, is required for synthesis of glutamine synthetase in *Salmonella*. The *glnF* gene lies at 102 min on the *Salmonella* chromosome, well separated from the structural gene for the glutamine synthetase subunit,

Table 3. Effect of a *glnF* mutation on the function of the *E. coli glnA* gene

Strain	Growth conditions*	Glutamine synthetase activity (units/mg of protein)
SK173/F′133$_{coli}$ (*glnA60 hisF645*/F′ *glnA*$^+$)	Repressing	0.110
	Derepressing	0.516
SK177/F′133$_{coli}$ (*glnF75 glnA60 hisF645*/F′ *glnA*$^+$)	Repressing	0.017
	Derepressing	0.014

* Media are given in *Materials and Methods*.

Table 4. Activities of proteins involved in covalent modification of glutamine synthetase

	Wild-type activity (ΔOD_{540}/10 mg of protein)	% of wild-type activity	
		SK99 (*glnF*)	SK103 (*glnD*)
Adenylyltransferase	0.87	76	91
P_{IIA}	0.84	87	123
P_{IID}	3.04	74	Not detectable
Uridylyltransferase	0.45	169	Not detectable
UR	0.26	115	103

glnA (14). The following observations suggest that the *glnF* product is required for synthesis of glutamine synthetase in *E. coli* also: (*i*) merodiploid analysis indicates that the *glnF* gene is present in *E. coli* at a chromosomal position homologous to that in *Salmonella* and (*ii*) the *E. coli glnA* gene (carried on F'133) cannot function in the *Salmonella* cytoplasm in *glnF* mutant strains. The product of the *glnF* gene has not been identified; however, *glnF* does not encode glutamine synthetase, glutamate synthase, glutamate dehydrogenase, or any of the proteins involved in covalent modification of glutamine synthetase.

In enteric bacteria, synthesis of glutamine synthetase is derepressed in response to nitrogen limitation (4, 31–33). Magasanik and his colleagues have proposed that glutamine synthetase itself mediates this derepression in *Klebsiella aerogenes* (7–9); they propose that the function of the enzyme in controlling transcription of its own structural gene depends on its degree of adenylylation (10). Glutamine synthetase alone does not mediate derepression of its own synthesis in *Salmonella* because the product of the *glnF* gene is required for synthesis of glutamine synthetase. In addition, the degree of adenylylation of glutamine synthetase apparently does not affect derepression of its synthesis. Thus, *glnD* strains, which lack uridylyltransferase activity (Table 4) and have highly adenylylated glutamine synthetase (S. Bancroft and S. Kustu, unpublished results), are able to derepress synthesis of the enzyme normally (Table 2).

The function of the *glnF* gene product is not known. A simple hypothesis is that the *glnF* product functions directly as an activator of transcription for the glutamine synthetase structural gene, *glnA*. However, among other possibilities, the *glnF* product may be required for synthesis of a small molecule coregulator of transcription or it may function in a posttranscriptional process. Analysis of other alleles in *glnF* and of mutations that suppress the loss of the *glnF* product should help to clarify the function of this product.

Note Added in Proof. Eighty spontaneous suppressors of deletion mutations in *glnF* have been mapped; all were closely linked to the *glnA* gene. The four strains containing suppressor mutations that have been tested had low glutamine synthetase activity and could not derepress synthesis of the enzyme. These results suggest that suppressor mutations may lie in a regulatory region adjacent to *glnA* and are consistent with the hypothesis that the *glnF* product functions as an activator of transcription for *glnA*.

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