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Complex *glnA-glnL-glnG* Operon of *Escherichia coli*

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The *glnG* gene product is both a positive regulator and a negative regulator of the expression of *glnA*, the structural gene for glutamine synthetase, as well as a positive regulator of the expression of a number of genes whose products are involved in the uptake and degradation of nitrogen-containing compounds. The regulation of β-galactosidase in various strains containing a Mu *d*1 (*lac bla*) insertion within *glnG* leads to the following conclusions regarding the expression of this gene: (i) like the synthesis of glutamine synthetase, the synthesis of the *glnG* product is regulated in response to the nitrogen source; (ii) high-level expression of *glnG* under nitrogen-limiting growth conditions depends on transcription initiated at the *glnA* promoter; and (iii) there is a second, *glnA*-distal promoter for *glnG*, whose activity is negatively controlled by the *glnG* product. Thus, the *glnG* product regulates the synthesis of the *glnG* product at two distinct promoters (positively and negatively at the *glnA* promoter and negatively at the *glnA*-distal promoter). In addition, a high level of *glnG* product, corresponding to the level produced by initiation of transcription at the *glnA* promoter under nitrogen-limiting conditions, is necessary for activation of histidase synthesis. The lower level of *glnG* product originating from transcription initiated at the *glnA*-distal promoter is not sufficient to activate histidase synthesis, but is sufficient to activate fully and to repress *glnA* expression.

The related species *Klebsiella aerogenes* (20), *Escherichia coli* (19), *Salmonella typhimurium* (12), and *Klebsiella pneumoniae* (24) all have the ability to regulate the expression of certain genes in response to the quantity and quality of the nitrogen source available for growth. When cells are inoculated into medium containing glucose as the carbon source and any of a number of growth rate-limiting nitrogen sources (25), the levels of the enzyme glutamine synthetase (L-glutamate:ammonia ligase [ADP forming]; EC 6.3.1.2) are 5-to 10-fold higher than when the preferred nitrogen source (ammonia) is included in the same medium. In addition, the expression of a large and diverse group of genes coding for enzymes responsible for the metabolism of nitrogen compounds to yield ammonia or glutamate can be activated under nitrogen-limiting conditions but not under nitrogen-excess conditions. The presence of the well-characterized *hut* operons from *K. aerogenes* in the *E. coli* strains used in this work allowed us to use the levels of histidase (L-histidine:ammonia lyase; EC 4.3.1.3) to monitor the state of this activation.

In this work we studied the regulation of the expression of the *glnA*-linked regulatory gene *glnG*. The product of the *glnG* gene is necessary for both positive and negative control of expression of the *glnA* gene (the structural gene for glutamine synthetase) and for the activation of expression of genes such as *hutH*, the structural gene for histidase (9, 11, 18). The loss of the *glnG* product by insertion leads to unregulated production of glutamine synthetase at a low level (the GlnR phenotype) and to the failure to activate the expression of *hutH* and similar genes under nitrogen-limiting growth conditions (the Reg^- phenotype) (18). The low constitutive level of glutamine synthetase found in *glnG* mutant strains is not altered due to the introduction of mutated alleles in the unlinked *glnA* regulatory genes *glnB* (9), *glnD* (18), and *glnF* (9, 11, 18). Strains which have lost the *glnF* function are glutamine auxotrophs (10). The suppression of the Gln^- phenotype of *glnF* mutant strains by the loss of *glnG* function was the basis for the selection of Mu *d*1 (*lac bla*) insertions in *glnG* described in this paper. The expression of the fused *lacZ* gene was used to monitor the expression of *glnG* in various genetic backgrounds under both nitrogen-limiting and nitrogen-excess growth conditions.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains used are listed in Table 1. A virulent derivative of phage P1 was used for all transductions (18). Mu *d*1 (*lac* Ap^r) phage were prepared as a heat-induced lysate of strain Mal103 (4). Episome transfers were done as

TABLE 1. Bacterial strains

Strain[a]	Genotype and/or phenotype	Source or derivation	Reference
ET6059	*glnF208*::Tn*10*		18
GP8000	Wild type	Gln^+ Tc^s transductant of ET6059	
GP8001	*gln-2002*::Mu *d*1 *glnF208*::Tn*10*	Mu *d*1 infection of ET6059	
GP8002	*glnG2003*::Mu *d*1 *glnF208*::Tn*10*	Mu *d*1 infection of ET6059	
GP8003	*gln-2004*::Mu *d*1 *glnF208*::Tn*10*	Mu *d*1 infection of ET6059	
GP8004	*gln-2005*::Mu *d*1 *glnF208*::Tn*10*	Mu d1 infection of ET6059	
GP8005	*gln-2006*::Mu *d*1 *glnF208*::Tn*10*	Mu *d*1 infection of ET6059	
GP8006	*gln-2007*::Mu *d*1 *glnF208*::Tn*10*	Mu *d*1 infection of ET6059	
GP8026	*gln-2002*::Mu *d*1	Ap^r Reg^- transductant of GP8000 (see text)	
GP8027	*glnG2003*::Mu *d*1	Ap^r Reg^- transductant of GP8000 (see text)	
GP8028	*gln-2004*::Mu *d*1	Ap^r Reg^- transductant of GP8000 (see text)	
GP8029	*gln-2005*::Mu *d*1	Ap^r Reg^- transductant of GP8000 (see text)	
GP8030	*gln-2006*::Mu *d*1	Ap^r Reg^- transductant of GP8000 (see text)	
GP8031	*gln-2007*::Mu *d*1	Ap^r Reg^- transductant of GP8000 (see text)	
GP8047	*glnG10*::Tn*5*		22
GP8070	Δ(*glnA-G*)*51 srl-1300*::Tn*10 recA51 thyA*		17
GP8500	*metBJKL*?::Tn*5* (Mu c^+)		18
GP8501	*metBJKL*?::Δ(Tn*5*) (Mu c^+)		This paper
GP8512	*gln-2002*::Mu *d*1	Ap^r Reg^- transductant of GP8501 (see text)	
GP8513	*glnG2003*::Mu *d*1	Ap^r Reg^- transductant of GP8501 (see text)	
GP8514	*gln-2004*::Mu *d*1	Ap^r Reg^- transductant of GP8501 (see text)	
GP8515	*gln-2005*::Mu *d*1	Ap^r Reg^- transductant of GP8501 (see text)	
GP8516	*gln-2006*::Mu *d*1	Ap^r Reg^- transductant of GP8501 (see text)	
GP8517	*gln-2007*::Mu *d*1	Ap^r Reg^- transductant of GP8501 (see text)	
GP8523	*glnG2003*::Mu *d*1 *srl-1300*::Tn*10 recA51*	Tc^r UV^s transductant of GP8513	
GP8531	*glnA21*::Tn*5* *glnG2003*::Mu *d*1	Km^r Gln^- transductant of GP8513	
GP8533	*glnA21*::Tn*5* *glnG2003*::Mu *d*1 *srl-1300*::Tn*10 recA51*	Tc^r UV^s transductant of GP8531	
DR6301	*glnA21*::Mu *d*1 Δ*lacU169*		22
Mal103	Mu *d*1 (*lac*, *bla*), Mu c^{ts}	M. Casadaban	
DB6895	*argE metB*/F'114 ts lac^+ *zzz-536*::Tn*10*	D. Botstein	
SG117	*ilv met* Nal^r	S. Guterman	
MX727	*glnA21*::Tn*5*	F. Bastarachea	
YMC10	Δ*lacU169 hutC thi endA hsr*		2
YMC15	Δ*lacU169 hutC thi endA hsr glnL302*		6
CGSC 4265	*leuBC hisG1 recA1 argG6 metB1 rpsL104*/F'133 *ilv-argH* = KLF33/JC1553	B. Bachmann	

[a] All strains in the GP8000 series were derived from strain ET6059 and contain the following markers in addition to those listed: *rhaD100* Δ*lacU169 thi* $hutC_{Klebs}$. All strains numbered from GP8502 upward were derived from strain GP8501 and contain (in addition to the GP8000 markers and listed markers) the *metBJKL*?::Δ[Tn*5*] marker and a Muc^+ prophage.

previously described (18). The episomes bearing the *glnA21*::Tn*5* and *glnG10*::Tn*5* alleles were constructed by transferring F'133 from strain CGSC 4265 into strains MX727 and GP8047, respectively. After homogenotization, the Tn*5*-bearing episomes were isolated by mating into strain GP8070, selecting for kanamycin resistance (Km^r), and counter selecting for tetracycline resistance (Tc^r). To construct merodiploid

strains for complementation analysis, the episomes were transferred into various *metB* mutant strains, and Met^+ Thy^+ exconjugants were selected.

Media and growth conditions. The minimal and broth media used have been described previously (18, 19). All growth for enzyme assays was at 32°C in minimal medium containing 0.4% D-glucose as the carbon source and either 0.2% freshly prepared filter-sterilized Calbiochem grade A L-glutamine (nitrogen-limiting medium) or 0.2% L-glutamine and 0.2% $(NH_4)_2SO_4$ (nitrogen-excess medium). The Reg^- phenotype was scored on glucose minimal medium containing 0.2% L-arginine hydrochloride as the sole nitrogen source.

Isolation of suppressors of *glnF208*::Tn*10*. A culture of strain ET6059 (*glnF208*::Tn*10*) was grown to a density of 5×10^8 cells per ml in LB broth medium at 32°C and centrifuged, and the cells were suspended in 0.1 volume of SM^+ (86 mM NaCl, 1 mM $MgSO_4$, 5 mM $CaCl_2$, 20 mM Tris, pH 7.5). To separate samples of these cells equal volumes of a fresh heat-induced lysate of strain Mal103 were added undiluted and in several 10-fold dilutions. After a 20-min incubation at 30°C for adsorption, the cells were plated onto glucose ammonia minimal medium plates containing 25 μg of ampicillin per ml and 40 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) per ml. Colonies appeared on these plates at frequencies between 10^{-4} and 10^{-5} per surviving cell. No colonies appeared on such selective plates if ET6059 was plated without Mu *d*1 infection. Colonies were scored and picked for retesting from plates prepared from the most dilute infection by the Mal103 lysate which yielded Gln^+ ampicillin-resistant (Ap^r) colonies. This represented the lowest effective multiplicity of infection and thus the highest probability of a single infection per cell.

Cell harvest and enzyme assays. Cells were harvested at a density of approximately 4×10^8 cells per ml by adding hexadecyltrimethyl-ammonium bromide (CTAB) to a final concentration of 100 μg/ml and $MnCl_2$ to a final concentration of 1.0 mM, followed by continued shaking for 2 min at 32°C before the cultures were placed on ice. The cultures were centrifuged, and the cells were suspended in cold 1% KCl, centrifuged, suspended in 0.1 to 0.05 volume of a solution containing 20 mM imidazole (pH 7.5), 0.3 mM $MnCl_2$, and 100 μg of CTAB per ml, and held on ice. These CTAB-treated "whole" cells were used for all enzyme assays. All enzyme activities are expressed as nanomoles of product formed per minute per milligram of protein. Protein determinations were done by the method of Lowry et al. (14). Histidase activity was determined at 37°C with a Zeiss recording spectrophotometer at a wavelength of 277 nm. Each reaction mixture contained 5 to 25 μl of whole cell suspension, 20 μg of CTAB per ml, 100 mM diethanolamine, 5 mM glutathione, and 10 mM L-histidine in a total volume of 1.0 ml; the pH was 9.4. The γ-glutamyl transferase activity of glutamine synthetase was determined at 37°C by using 2 to 30 μl of whole cell suspension in a 0.5-ml (total volume) reaction mixture containing 150 mM imidazole, 0.3 mM $MnCl_2$, 20 mM potassium arsenate, 20 mM NH_2OH, 0.4 mM ADP, 20 mM L-glutamine, and 100 μg of CTAB per ml; the pH was 7.27. The reaction was stopped by adding 1 ml of 0.2 M $FeCl_2$–0.12 M trichloroacetic acid–0.2 N HCl. Cells and cell debris were removed by centrifugation, and the optical density was determined at 540 nm.

β-Galactosidase activity was determined at 28°C by using 5 to 50 μl of a "whole" cell suspension in a 1-ml (total volume) reaction mixture containing 50 mM sodium phosphate, 50 mM 2-mercaptoethanol, 10 mM KCl, 1 mM $MgSO_4$, 100 μg of sodium deoxycholate, 200 μg of CTAB, and 1.11 mM *o*-nitrophenyl-β-galactoside (pH 7.0). The reaction was stopped by adding 0.5 ml of 1 M Na_2CO_3, the cells and debris were removed by centrifugation, and the optical density was determined at 420 nm.

DNA biochemistry. Plasmid DNA preparation, restriction endonuclease digestion, gel electrophoresis, and transformation of cells were all performed by using the buffers and reaction conditions described by Davis et al. (8), and chromosomal DNA was prepared by the procedure of Clarke and Carbon (7).

Chromosome mobilization. F'114 ts *lac*$^+$ *zzz-536*::Tn*10* was transferred by conjugation from strain DB6895 into each of the Rec^+ Mu *d*I lysogenic strains to be tested, as well as into control strain GP8000. Selection for the episome was via resistance to 25 μg of tetracycline per ml, with counterselection against the methionine and arginine requirements of the donor.

Mobilization of the chromosome after reciprocal recombination between homologous *lac* regions on the chromosomal Mu *d*I prophage and the episome was detected by mating into strain SG117 (Ilv^- Met^- nalidixic acid resistant [Nal^r]). Each mating was performed by mixing 1 volume of an LB broth overnight culture of strain SG117 grown to saturation with aeration at 32°C (2×10^9 to 4×10^9 cells per ml) with 10 volumes of a standing 32°C LB broth overnight culture of the donor (4×10^8 to 8×10^8 cells per ml). The cells were shaken gently at 32°C for 40 min, blended vigorously with a Vortex mixer, diluted into cold 0.85% saline, and plated immediately. The donor strain was counterselected by adding 50 μg of nalidixic acid per ml to selection plates. Spontaneous Nal^r mutants arose in less than 1 cell per 10^7 cells. Met^+ exconjugants were selected on glucose ammonia nalidixic acid plates containing 50 μg of L-isoleucine per ml and 50 μg of L-valine per ml. Spontaneous Met^+ revertants occurred at a frequency of less than 10^{-7}. Ilv^+ exconjugants were selected on glucose ammonia nalidixic acid minimal medium plates containing 50 μg of L-methionine per ml. Spontaneous Ilv^+ revertants arose at a frequency of 5×10^{-7}.

RESULTS

Isolation of suppressors of *glnF208*::Tn*10* via Mu *d*1(*lac bla*) insertion. Cells of strain ET6059 (*glnF208*::Tn*10*) were infected with a lysate from strain Mal103, and Gln^+ Ap^r lysogens were selected as described above. After 36 h of incubation on plates containing the β-galactosidase indicator dye X-gal, about 40% of the colonies were of widely variable but distinct shades of blue, 50% were white, and the remaining 10% were very light blue. After an additional 24 h of incubation, all of the white colonies were very faintly blue colored. A total of 50 independent colonies were purified on selective plates (glucose ammonia minimal medium containing

ampicillin) and nonselective plates (LB broth containing glutamine) and then tested for β-galactosidase production on glucose ammonia plates containing X-gal. The range of color in the blue colonies and the eventual appearance of the light blue color in the white colonies were the same as in the original selection. Four distinctly blue colonies (designated strains GP8001 through GP8004) and two white colonies (strains GP8005 and GP8006) were chosen for further study.

Linkage of Mu *d*1 insertions and the Reg⁻ phenotype to *glnA21*::Tn*5*. A P1 phage lysate was grown on each of the six insertion strains (GP8001 through GP8006). Phage from each lysate were used to transduce two different recipient strains to Ap^r. The first of these strains was the Met⁻ Mu *c*$^+$ lysogenic strain GP8501. The presence of the wild-type Mu repressor prevents induction of the Mu *d*1 prophage in incoming DNA. In such cases more than 95% of the Ap^r transductants from each of the Mu *d*1 donors failed to grow on glucose minimal medium containing arginine as the sole nitrogen source (the Reg⁻ phenotype). This is the phenotype previously reported for *glnA*-linked suppressors of *glnF* (18). One Ap^r Reg⁻ colony from each of the above-described transductions was chosen, and the strains were designated GP8512 (Mu *d*1 from GP8001) through GP8517 (Mu *d*1 from GP8006).

The second strain transduced to Ap^r with the P1 lysates grown on the Mu *d*1 insertion strains was wild-type strain GP8000. In this case only 5 to 15% of the Ap^r transductants had the Reg⁻ phenotype, presumably due to induction and transposition of the incoming Mu *d*1 prophage. One Ap^r Reg⁻ colony from each of these transductions was chosen, and the strains were designated GP8026 (Mu *d*1 from GP8001) through GP8031 (Mu *d*1 from GP8006).

The parental strain (GP8000) and each of the Reg⁻ Mu *d*1 strains were transduced to Tc^r with a P1 lysate grown on strain ET6059 (*glnF208*::Tn*10*). All of the Tc^r transductants of strain GP8000 required glutamine for growth on glucose ammonia minimal medium. None of the Tc^r transductants of Reg⁻ Mu *d*1 strains GP8026 through GP8031 was a glutamine auxotroph. Therefore, both the Reg⁻ phenotype and the *glnF* suppression phenotype are associated with the Mu *d*1 insertions.

The insertion strains GP8026 through GP8031 were transduced to Km^r with a P1 lysate grown on strain MX727 (*glnA21*::Tn*5*) (Table 2). For each of these strains, more than 89% of the Gln⁻ Km^r transductants had lost Ap^r, color on X-gal plates, and the temperature-sensitive growth phenotypes caused by the Mu *d*1 prophage. Therefore, each of these six strains bears a single Mu *d*1 prophage which is closely linked to *glnA*.

Direction of transcription of *lacZ* in the Mu *d*1 insertion strains. Both the *glnA* gene (22) and the *glnG* gene (2) are transcribed in a counterclockwise direction on the *E. coli* chromosome. Therefore, the orientations of the various Mu *d*1 prophages were determined with respect to the chromosome. This was accomplished by mobilization of the chromosome via the *lac* homology on F'114 (see above). Depending on the orientation of the Mu *d*1 prophage and, hence, the *lac* genes in the chromosome, either Met$^+$ (clockwise orientation of *lac* genes) or Ilv$^+$ (counterclockwise orientation) would be transferred as an early marker to the appropriate recipient strain

TABLE 2. Linkage of Mu *d*1 insertions to *glnA* and direction of transcription

Strain[a]	Relevant genotype	Transductional analysis[b]			Chromosome mobilization[c]		
		No. Ap^s	Total no.	% Linkage	No. Ilv$^+$	No. Met$^+$	Ilv$^+$/Met$^+$ ratio
GP8000	Wild type	NT[d]	NT		<0.5	<0.2	
DR6301	*glnA21*::Mu *d*1	NT	NT		56	~0.5	112
GP8026	*gln-2002*::Mu *d*1	227	233	97	14	~0.5	28
GP8027	*glnG2003*::Mu *d*1	82	92	89	50	1.5	33
GP8028	*gln-2004*::Mu *d*1	191	200	96	72	3	24
GP8029	*gln-2005*::Mu *d*1	186	195	95	64	1	64
GP8030	*gln-2006*::Mu *d*1	226	231	98	2	18	0.11
GP8031	*gln-2007*::Mu *d*1	190	191	99	~0.6	16	0.04

[a] The strains were the recipients in P1 transductions and the donors in chromosome mobilizations.

[b] The donor in all crosses was strain MX727 (*glnA21*::Tn*5*). Selection was for Km^r. Approximately 5% of the Km^r transductants were Gln$^+$ and were not counted. Ap^s indicates the loss of the Mu *d*1 phage. In addition, the Ap^s derivative had lost β-galactosidases activity and the temperature-sensitive growth phenotype due to the Mu *d*1 prophage.

[c] The F'114 *lac* episome from strain DB6895 was introduced into each of the strains, which in turn were used as donors in matings with SG117. Exconjugants were selected as either Ilv$^+$ Nal^r or Met$^+$ Nal^r colonies (see text). Numbers of Ilv$^+$ and Met$^+$ per 10^6 cells of the donor are given.

[d] NT, Not tested.

(Fig. 1). In all cases the recipient was SG117 (Ilv$^-$ Met$^-$ Nal^r). Strain DR6301 containing the *glnA21*::Mu *d*1 insertion (22) with Mu *d*1 in a known orientation served as control donor. In such an experiment the mobilization of either Ilv$^+$ or Met$^+$ was negligible in the absence of a Mu *d*1 prophage (Table 2, strain GP8000). Strain DR6301 generated a 100-fold excess of Ilv$^+$ exconjugants over Met$^+$ exconjugants. The insertion strains which were blue (strains GP8026 through GP 8029) all generated at least a 24-fold excess of Ilv$^+$ exconjugants over Met$^+$ exconjugants (Table 2). On the other hand, the ratios of Ilv$^+$ exconjugants to Met$^+$ exconjugants in the two white isolates (strains GP8030 and GP8031) were 0.11 and 0.04, respectively. Therefore, the *lacZ* gene in each of the four blue insertion strains (GP8026 through GP8029) is transcribed in the same direction as *glnA* and *glnG*, whereas the *lacZ* gene in each of the two white insertion strains (GP8030 and GP8031) is transcribed in the opposite direction.

Exact location of the site of insertion in strain GP8523. Since recent evidence (6, 16; T. MacNeil, D. MacNeil, and B. Tyler, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, K83, p. 151) indicates that there is a second regulatory gene in the *glnA* region, it was essential to locate any insertion precisely before undertaking an extension physiological characterization of it. To this end, DNA was prepared from strain GP8523, hydrolyzed with restriction endonuclease *Bam*HI, and ligated into the *Bam*HI site in plasmid pBR322. The rationale for this approach is explained in the legend to Fig. 2. The ligated mixture was used to transform strain YMC10 to Ap^r on LB plates containing ampicillin and X-gal. There were 2 blue colonies among 2,300 Ap^r transformants. Miniscreens of the plasmids from the two blue transformants indicated that each contained the 4.4-kilobase pair pBR322 *Bam*HI fragment and an insertion approximately 11 kilobase pairs long. One of these two plasmids was chosen and designated pGP301. Blue pigment formation on X-gal plates and Ap^r were 100% cotransformed. From the size of the insertion

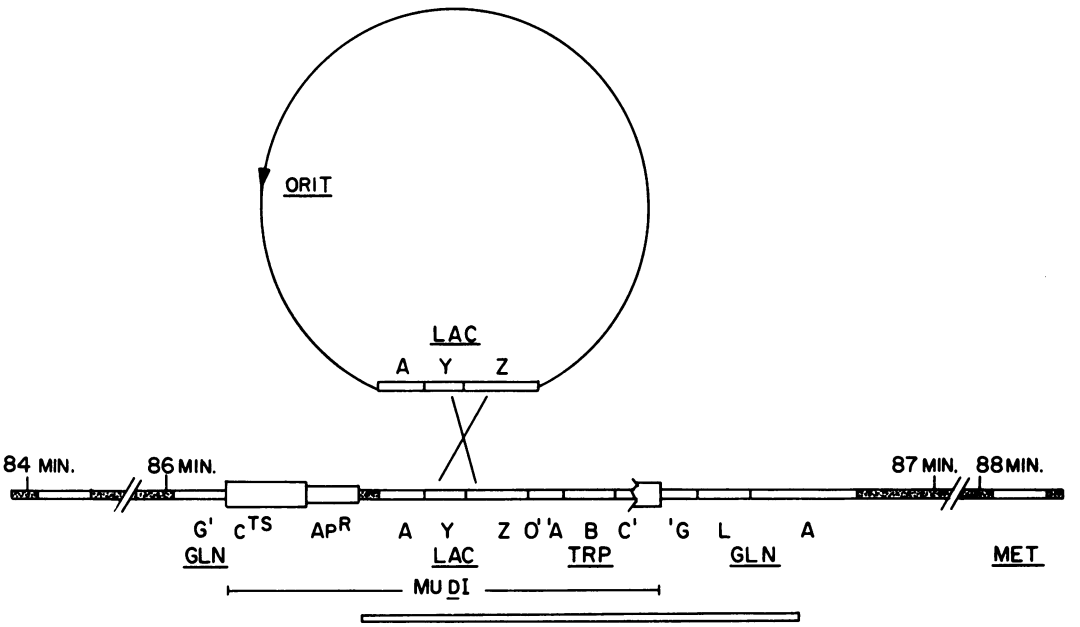

FIG. 1. Determination of the direction of *lacZ* transcription in Mu *d*1 insertions via chromosome mobilization. The bottom line represents the *E. coli* chromosome from the *ilv* gene cluster (~84 min) to the *met* gene cluster (~88 min)(1). A Mu *d*1 (*lac bla*) prophage inserted within the *glnG* gene is shown. A prime next to a genetic symbol indicates that the gene is interrupted on the side on which the prime is written. Diagonal slashes intersecting the map indicate discontinuities in the diagram. The genetic regions are not drawn to scale. The *glnA* and *glnG* genes are transcribed from right to left in this diagram, as are the prophage *lac* genes in the orientation depicted. The circle represents the F'114 ts *lac*$^+$ *zzz*::Tn*10* episome. The location of the Tn*10* insertion is not known. The origin of transfer (*oriT*) relative to the *lac* genes is from reference 13. A reciprocal recombination between the homologous *lac* regions (shown by crossed lines on the figure) followed by conjugal transfer initiated at *oriT* and continuing in the direction of the arrow would result in Ilv$^+$ being transferred as an early marker to the appropriate recipient (see text). Reversing the orientation of the Mu *d*1 prophage would result in Met$^+$ being transferred as an early marker. (The open bar at the bottom of the figure represents the region cloned on pGP301 [see Fig. 2].)

and the known contribution of Mu *d*1 DNA in any *lacZ*-containing *Bam*HI insertion (9.3 kilobase pairs), we estimated that the Mu *d*1 prophage is inserted near the amino-terminal portion of the *glnG* gene. This was confirmed by demonstrating the presence of the predicted *Cla*I, *Sal*I, *Pvu*II, and double digestion restriction fragments from the *glnALG* region on plasmid PGP301 (data not shown). The results of this mapping are shown in Fig. 2, along with partial restriction maps of the *glnA* region (2) and the Mu *d*1 (*lac* Ap^r) phage (5). Because of the uncertainty inherent in determining the sizes of DNA fragments and polypeptides by gel electrophoresis, the previous results might conceivably place the *gln-2003*::Mu *d*1 insertion as far to the right as the carboxy terminus of *glnL* (Fig. 2). This is unlikely for the following reason. Plasmid pGP301 was transformed into GlnC (high-level constitutive) strain YMC15 (*glnL302*) (6), and the cells were grown in LB broth medium containing glutamine. The transformants of YMC15 produced levels of glutamine synthetase comparable to those in Gln$^+$ strain YMC10, whereas untransformed recipient strain YMC15 produced high constitutive levels of this enzyme. The low level of glutamine synthetase produced by pGP301-containing derivatives of YMC15 was due to complementation of the recessive *glnL302* mutation rather than recombination since all segregants of such strains which had lost the plasmid (detected by the loss of β-

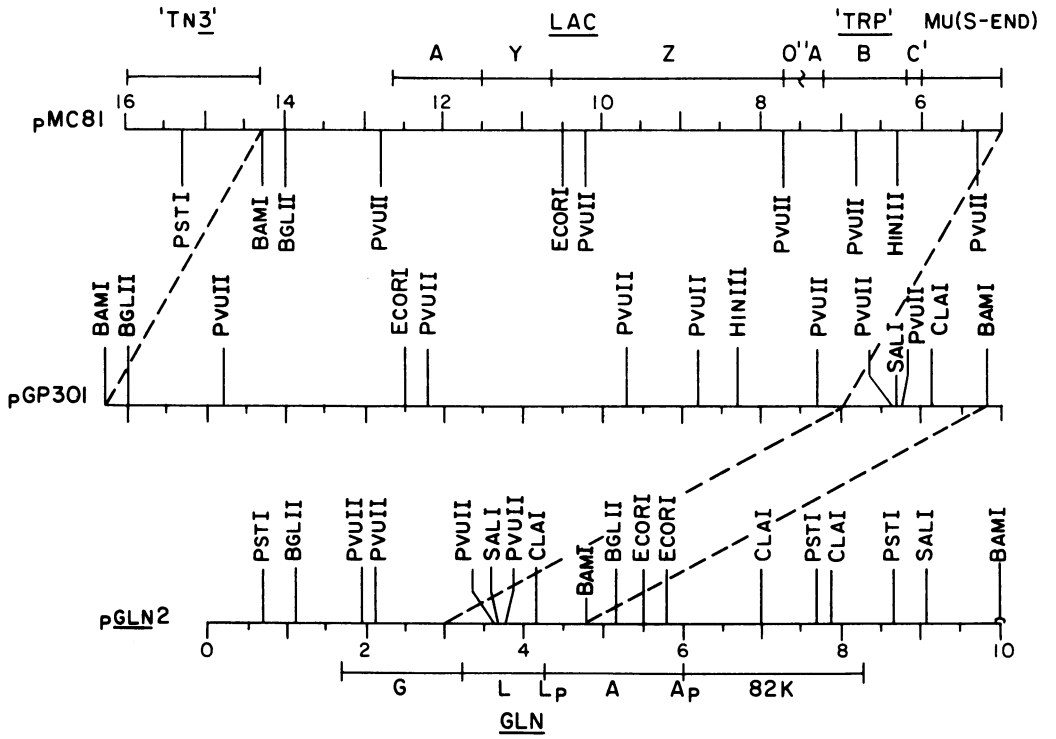

FIG. 2. Physical location of the Mu *d*1 insertion in GP8523. The top line shows a partial genetic and physical map of the S end of the Mu *d*1 (*lac bla*) prophage (on pMC81) (5). The bottom line shows a partial genetic and physical map of the *glnALG* region (on p*gln2*) (2). The *glnA*-distal promoter (L_P) is tentatively placed between *glnA* and *glnL* due to arguments made by Chen et al. (6). The kilobase pair scales for each of these maps are from the original references. Note that in the Mu *d*1 prophage there is no *Bam*I site from the S end through the *lac* genes. Thus, it is possible to clone *lac* from the *Bam*I site at 14.3 kilobase pairs rightward to the first *Bam*I site outside the prophage DNA. The bottom diagram (p*gln2*) shows that the only *Bam*I site within the *glnALG* genes is at 4.8 kilobase pairs within *glnA*. Therefore, for any Mu *d*1 insertion in the *glnL* or *glnG* genes in the orientation shown, it is possible to clone the *lac* genes and whatever bacterial material remains between the site of insertion and the *Bam*I site in *glnA* (see Fig. 1). In our experiments such clones were identified easily by their blue color on X-gal-containing plates due to the presence of the *glnA*-distal promoter (L_P) on the cloned fragment. Also shown (middle line) is a partial restriction map of pGP301, which was prepared as described above and in the text. There are two unmapped *Cla*I sites within the Mu *d*1 material on pGP301 (and pMC81). Note that *Bgl*II and *Pst*I could also be utilized in a similar procedure to clone Lac$^+$ inserts from Mu *d*1 insertions.

galactosidase and, hence, blue color formation on X-gal-containing plates) regained the GlnC phenotype. Since the genes carried on plasmid pGP301 complement *glnL302*, it is unlikely that the *gln-2003*::Mu *d*1 insertion is in the *glnL* gene.

Evidence that *gln-2003*::Mu *d*1 is likely to be in *glnG* was provided by the following experiments. Precise excision of Mu *d*1, which restores gene function, occurs at a frequency of less than 1 per 10^{10} cells, whereas imprecise excision occurs frequently. In addition, Chen et al. have demonstrated that deletion of *glnL* results in a strain with the Reg^+ phenotype (6). Therefore, the *glnF*-suppressing *gln*::Mu *d*1 insertions should be divisible into two classes. One class should consist of insertions that are not located in *glnG*, but are polar on it; these mutants should therefore be able to revert to growth on arginine as the sole nitrogen source by any deletion allowing expression of *glnG*. The other class should consist of insertions that are in *glnG*; these mutants should therefore be able to revert to Reg^+ only by precise excision, a very rare event. The *glnA*-linked *gln*::Mu *d*1 insertions divided into these two classes approximately equally. Strains containing the *gln-2003*::Mu *d*1 insertion reverted to growth on arginine as the sole nitrogen source at a frequency of less than 1 per 10^{10} cells, even if the cultures were first heated to 43°C to induce the Mu *d*1 prophage and encourage deletion formation. Therefore, the *gln-2003*::Mu *d*1 insertion seems to be in a region which is absolutely required for the Reg^+ phenotype and which is likely to be *glnG*. Therefore, the insertion in strain GP8523 was designated *glnG2003*::Mu *d*1.

Physiology of the Mu *d*1 insertion strains. Glutamine synthetase, histidase, and β-galactosidase levels were determined in *glnF208*::Tn*10*-bearing strains ET6059 and GP8001 through GP8006, as well as in $glnF^+$ strains GP8501 and GP8512 through GP8517 grown on glucose glutamine and glucose ammonia glutamine minimal media. With the exception of Gln^+ strain GP8501, the levels of each enzyme were very similar on the two media. For simplicity therefore, only the results from the glucose glutamine growth medium are presented in Table 3. Strain ET6059 produced very low levels of glutamine synthetase (10 U/mg of protein), as previously reported (18). The levels of glutamine synthetase in all six of the *glnF*::Tn*10* *gln*::Mu *d*1 insertion strains (50 to 100 U/mg of protein [Table 3, strains GP8001 through GP8006]) were in the range previously reported for *glnA*-linked *glnF* suppressors (18); these levels were sufficient to reverse the glutamine auxotrophy caused by the loss of the *glnF* product. The wild-type control strain GP8501 produced 900 U of glutamine synthetase per mg of protein when it was grown on glucose glutamine minimal medium. The level dropped to 150 U/mg of protein on glucose ammonia glutamine medium. The GlnF-positive *gln*::Mu *d*1 insertion strains (GP8512 through GP 8517) produced from 50 to 80 U of glutamine synthetase activity per mg of protein, once again similar to previously reported values (18). The *gln*::Mu *d*1 insertion strains all have the GlnR phenotype (i.e., low constitutive levels of glutamine synthetase in both *glnF*-positive and *glnF*-negative backgrounds).

Histidase synthesis was activated in response to nitrogen-limited growth (glucose glutamine medium) only in wild-type strain GP8501 (320 U/mg of protein) (Table 3). The absence of the *hut*-specific repressor due to the *hutC7* mutation made it unnecessary to add inducer in order to measure histidase levels. When grown on repressing medium (glucose ammonia glutamine medium), strain GP8501 produced 70 U of histidase per mg of protein, similar to the nonactivated levels observed in all of the other strains in Table 3. The fact that the *glnF*-positive *gln*::Mu *d*1 insertion strains (GP8512 through GP8517) failed to activate histidase synthesis confirmed the Reg^- phenotype inferred from the failure of these strains to utilize arginine as the sole nitrogen source.

The β-galactosidase levels in the *glnF208*::Tn*10* *gln*-Mu *d*1 insertion strains with *lacZ* oriented in the correct direction for *glnG* transcription (blue strains GP8001 through GP8004) varied from 440 U/mg of protein (GP8001) to 105 U/mg of protein (GP8004), as predicted from the range of blue pigment formation on plates containing X-gal. The *glnF*-positive *gln*::Mu *d*1 strains (GP8512 through GP8515) uniformly produced slightly higher (23 to 61%) levels of the enzyme than the corresponding *glnF*::Tn*10* strains.

Strains GP8005 and GP8006, which have Mu *d*1 insertions in which *lacZ* transcription is in the opposite direction from *glnA* and *glnG*, produced 2.0 and 3.0 U of β-galactosidase per mg of protein, respectively. These very low levels of activity did not change in *glnF*-positive strains GP8516 and GP8517 (Table 3). We do not know whether this transcription originates within the Mu *d*1(*lac bla*) prophage or outside it.

Regulation of β-galactosidase in *glnG2003*::Mu *d*1 insertion strains with and without the wild-type *glnA-glnG* region on an episome. The levels of glutamine synthetase, histidase, and β-galactosidase were determined in *recA56 met*::Δ[Tn*5*] *glnG2003*::Mu *d*1 strain GP8523 grown on glucose glutamine and glucose ammonia glutamine minimal media containing 25 μg of L-methionine per ml. The previously described pattern of enzyme synthesis was observed (Table 4, experiments 1a and b). Glutamine synthetase and

TABLE 3. Enzyme levels in haploid Mu *d*1 insertion strains

Strain	Relevant genotype	Enzyme levels[a]		
		Glutamine synthetase	Histidase	β-Galactosidase
ET6059	*glnF208*::Tn*10*	~10	60	0
GP8501[b]	Wild type	900	320	0
GP8001	*gln-2002*::Mu *d*1 *glnF208*::Tn*10*	80	ND[c]	440
GP8512[b]	*gln-2002*::Mu *d*1	110	55	550
GP8002	*glnG2003*::Mu *d*1 *glnF208*::Tn*10*	70	ND	265
GP8513[b]	*glnG2003*::Mu *d*1	60	60	380
GP8003	*gln-2004*::Mu *d*1 *glnF208*::Tn*10*	70	ND	115
GP8514[b]	*gln-2004*::Mu *d*1	90	65	185
GP8004	*gln-2005*::Mu *d*1 *glnF208*::Tn*10*	60	ND	105
GP8515[b]	*gln-2005*::Mu *d*1	180	65	135
GP8005	*gln-2006*::Mu *d*1 *glnF208*::Tn*10*	70	ND	2.0
GP8516[b]	*gln-2006*::Mu *d*1	60	55	2.0
GP8006	*gln-2007*::Mu *d*1 *glnF208*::Tn*10*	60	ND	3.0
GP8517[b]	*gln-2007*::Mu *d*1	180	70	3.0

[a] Enzyme levels are expressed as nanomoles of product formed per minute per milligram of protein. Growth was on 0.4% glucose–0.2% glutamine minimal medium at 32°C, which are nitrogen-limiting conditions.
[b] L-Methionine at a concentration of 25 μg/ml was added to these cultures.
[c] ND, Not determined.

histidase levels were low and not regulated by the supply of nitrogen (GlnR and Reg⁻ phenotypes), and approximately 300 to 400 U of β-galactosidase per mg of protein was produced. When the wild-type *glnA-glnG* region was introduced into strain GP8523 on F'133, the regulation of glutamine synthetase and histidase was restored to the wild-type pattern (Table 4, experiments 2a and b). Interestingly, the levels of β-galactosidase in this merodiploid strain were also regulated by the supply of nitrogen. When the merodiploid strain was grown under nitro-

TABLE 4. Enzyme levels in *glnG2003*::Mu *d*1 merodiploid strains

Expt	Relevant genotype[a]		Growth conditions[b]	Enzyme levels[c]		
	Chromosome	Episome		Glutamine synthetase	Histidase	β-Galactosidase
1a	*glnG2003*::Mu *d*1	None	Excess	70	90	320
1b			Limiting	60	80	400
2a		*glnA*⁺ *glnG*⁺	Excess	160	52	70
2b			Limiting	2,000	320	570
3a		*glnA21*::Tn*5* *glnG*⁺	Excess	100	60	105
3b			Limiting	1,550	65	1,230
4a		*glnA*⁺ *glnG10*::Tn*5*	Excess	140	60	250
4b			Limiting	140	60	215
5a	*glnA21*::Tn*5* *glnG*2003::Mu *d*1	None	Excess	<10	85	360
5b			Limiting[d]	<10	50	265
6a		*glnA*⁺ *glnG*⁺	Excess	130	65	45
6b			Limiting	1,000	270	9
7a		*glnA21*::Tn*5* *glnG*⁺	Excess	<10	60	45
7b			Limiting	<10	75	50
8a		*glnA*⁺ *glnG10*::Tn*5*	Excess	90	65	215
8b			Limiting	110	60	200

[a] The parental strain in experiments 1 through 4 was GP8523 [*glnG2003*::Mu *d*1 *metBJKL*?::Δ(Tn*5*) *recA*]. The parental strain in experiments 5 through 8 was GP8533 [*glnA21*::Tn*5* *glnG2003*::Mu *d*1 *metBJKL*?::Δ(Tn*5*) *recA*].
[b] All growth was at 32°C on glucose minimal medium containing 0.2% glutamine (nitrogen-limiting conditions) or 0.2% glutamine plus 0.2% $(NH_4)_2SO_4$ (nitrogen-excess conditions). L-methionine (25 μg/ml) was added to the cultures of the two haploid strains. The mass doubling times of all strains were between 1 and 1.5 h under nitrogen-excess conditions and between 3 and 3.5 h under nitrogen-limiting conditions, except as noted below.
[c] Enzyme levels are expressed as nanomoles of product formed per minute per milligram of protein.
[d] This strain grew very poorly on glucose glutamine minimal medium at 32°C (mass doubling time, ~16 h) and entered growth stasis just before reaching harvest density. Therefore, the β-galactosidase level is subject to question.

gen-excess conditions, the level of this enzyme was only 70 U/mg of protein, compared with 320 U/mg of protein in the haploid strain grown under the same conditions. Therefore, under nitrogen-excess conditions a product provided by the episome acted in *trans* to repress synthesis from the promoter responsible for *lacZ* transcription.

Regulation of β-galactosidase in haploid and merodiploid strains containing *glnA21*::Tn*5* in *cis* to *glnG2003*::Mu *d*1. The *glnA* and *glnG* genes are transcribed in the same direction, with *glnG* distal to *glnA* (2, 22). Furthermore, the results described above indicated that in the presence of the Gln^+ episome, the synthesis of the *glnG* product is regulated in parallel to the synthesis of glutamine synthetase. Therefore, the transcription of *lacZ* in the *glnG2003*::Mu *d*1 insertion strain could have been initiated from the *glnA* promoter.

To examine this question, we constructed strain GP8533 with the *glnA21*::Tn*5* insertion in *cis* to the *glnG2003*::Mu *d*1 prophage. In such a strain transcription of *lac* from the *glnA* promoter should be blocked by the presumed polar insertion, so that β-galactosidase production would depend on a promoter distal to *glnA*::Tn*5*. As shown in Table 4, experiments 5a and b, the levels of β-galactosidase in haploid strain GP8533 were similar to those found in *glnA*-positive strain GP8523. This result indicates that in the absence of *glnG* the synthesis of β-galactosidase can proceed from a promoter independent of the *glnA* promoter.

When the wild-type *glnA-glnG* region was introduced into strain GP8533, the expected wild-type pattern of regulation of glutamine synthetase and histidase was observed (Table 4, experiments 6a and b). The level of β-galactosidase in this merodiploid strain grown under nitrogen-excess conditions was 45 U/mg of protein. Since the haploid strain grown under the same conditions produced 360 U of the enzyme per mg of protein, the activity of the *glnA*-distal promoter was repressed in *trans* by a product from the episome. When this merodiploid strain was grown under nitrogen-limiting conditions, only 9 U of β-galactosidase was found, indicating even stronger repression of the *glnA*-distal promoter. This low level of β-galactosidase may have reflected the expression of the fully repressed *glnA*-distal promoter, or it could have been due to spurious low-level transcription initiated within the Tn*5* element or elsewhere between it and *glnG*. The level of β-galactosidase in the $glnA^+$ *glnG2003*::Mu *d*1 merodiploid strain grown under the same conditions was 570 U/mg of protein (Table 4, experiment 2b). Thus, it appears that the Tn*5* insertion in *cis* to *glnG2003*::Mu *d*1 did have a drastic polar effect on the expression of *lacZ* from transcription initiated at the *glnA* promoter. Therefore, in a wild-type cell the expression of *glnG* under nitrogen-limiting growth conditions presumably depends almost entirely on the *glnA* promoter. The expression of *glnG* from the second, *glnA*-distal promoter apparently is stronger when cells are grown under nitrogen-excess conditions than when they are grown under nitrogen-limiting conditions.

Complementation of *glnG2003*::Mu *d*1 with an episome bearing the *glnA21*::Tn*5* insertion. The merodiploid strain containing wild-type *glnA* and *glnG* genes in the *trans* position displayed normal regulation of glutamine synthetase, producing 1,550 U/mg of protein under nitrogen-limiting conditions and 100 U/mg of protein under nitrogen-excess conditions (Table 4, experiments 3a and b). As previously reported for similar merodiploids (18), histidase synthesis was not activated in this strain when it was grown under nitrogen-limiting conditions. The level of β-galactosidase in this merodiploid strain was 1,230 U/mg of protein under nitrogen-limiting conditions and 105 U/mg of protein under nitrogen-excess conditions (Table 4, experiments 3a and b). Thus, the level of β-galactosidase varied in parallel with the level of glutamine synthetase, indicating initiation of transcription at the *glnA* promoter, as previously suggested. Despite the high level of β-galactosidase, we might expect the level of functional *glnG* product to be low in this strain since its transcription should be initiated only at the *glnA*-distal promoter. This assumption was tested by examining a merodiploid strain with Tn*5* inserted into both the chromosomal and episomal *glnA* genes. In this case expression of both the wild-type *glnG* gene and the *glnG-lacZ* fusion should have depended solely on the *glnA*-distal promoter. This strain produced 50 U of β-galactosidase per mg of protein under nitrogen-limiting conditions (Table 4, experiment 7b) and presumably a corresponding amount of wild-type *glnG* product from the episome. Therefore, in the $glnA^+$ *glnG2003*::Mu *d*1 merodiploid strains containing the *glnA21*::Tn*5* episome, a similar level of functional *glnG* product was apparently sufficient to activate fully expression of *glnA* but not sufficient to activate expression of *hut*.

Complementation of *glnG2003*::Mu *d*1 with an episome bearing the *glnG10*::Tn*5* insertion. The restoration of nitrogen regulation to *glnG2003*::Mu *d*1 strains may be due to the introduction of wild-type *glnG* product. This assumption was confirmed by introducing an episome bearing the *glnG10*::Tn*5* insertion into strains GP8523 and GP8533. As shown in Table 4, experiments 4a and b and 8a and b, glutamine

synthetase, histidase, and β-galactosidase syntheses were all unregulated, with levels similar to those found in haploid strain GP8523 (Table 4, experiments 1a and b). Plasmid and deletion mapping experiments have indicated there is no essential regulator distal to *glnG* with respect to *glnA* (2; MacNeil et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1981). Therefore, activation at the *hutUH* promoter, activation and repression at the *glnA* promoter, and repression at the secondary *glnG* promoter all depend on the presence of the wild-type *glnG* product.

DISCUSSION

The experimental results described above led us to conclude that *glnA*, the structural gene for glutamine synthetase, and *glnG*, whose product regulates the expression of *glnA*, as well as the expression of other unlinked genes, such as *hut*, are members of a single complex operon that is transcribed in the direction from *glnA* to *glnG*.

This relationship of *glnA* to *glnG* was illustrated best by the results of experiments 2 and 6 (Table 4). In these experiments the cells carried an episome with functional *glnA* and *glnG* genes and had the structural gene for β-galactosidase fused to the chromosomal *glnG* gene, destroying the function of this *glnG* gene and enabling us to monitor its expression by measuring β-galactosidase. We found that the levels of both β-galactosidase and glutamine synthetase were much higher in cells grown with nitrogen limitation than in cells grown with nitrogen excess (Table 4, experiment 2). The ability of the cells to increase the expression of the chromosomal *glnG* gene in response to nitrogen limitation was completely abolished by the polarity resulting from the insertion of Tn*5* into the neighboring *glnA* gene (Table 4, experiment 6). Furthermore, the results of other experiments showed that the sensitivity of both *glnA* expression and *glnG* expression to the nitrogen source of the medium required a functional *glnG* product (Table 4, experiments 4 and 8). In cells carrying functional *glnA* but not *glnG* on the episome, neither the level of glutamine synthetase nor the level of β-galactosidase was altered significantly in response to the nitrogen source in the medium. We also confirmed that *glnA* and *glnG* are transcribed in the same direction, from *glnA* to *glnG* (Fig. 2). Therefore, it appears that both of these genes can be controlled jointly at the *glnA* promoter by the *glnG* product. In view of the fact that *glnG* is a member of an operon starting with *glnA*, we prefer this designation to the designation *ntrC* proposed by McFarland et al. (16).

Our measurements of the levels of β-galactosidase, glutamine synthetase, and histidase in cells having different constitutions under conditions of nitrogen limitation and nitrogen excess permitted us to analyze the physiological consequences of the autogenous regulation of *glnG* expression in some detail. We found that in a haploid strain carrying the *glnG-lacZ* fusion and a Tn*5* insertion in the adjacent *glnA* gene, the level of β-galactosidase is approximately 300 U/mg of protein (Table 4, experiment 5). We observed that the presence of a functional *glnG* product arising from transcription of the *glnA*-distal promoter on an episome decreases the expression of the identical promoter on the chromosome to approximately 50 U of β-galactosidase per mg of protein (Table 4, experiment 7b). If we assume that each cell contains, on the average, 1.5 episomes per chromosome (22), then we can estimate that each cell contains a level of *glnG* product equivalent to approximately 75 U of β-galactosidase per mg of protein. Apparently this level of *glnG* product is sufficient for a sixfold decrease in transcription initiated at the *glnA*-distal promoter.

We assume that a similar amount of *glnG* product is present in cells carrying the *glnG*$^+$ *glnA21*::Tn*5* episome with a functional *glnA* gene and the *glnG-lacZ* fusion on the chromosome (Table 4, experiment 3). Apparently, this amount of *glnG* product is sufficient for full activation of *glnA* transcription in the nitrogen-limiting medium and for its repression in the nitrogen-excess medium; however, it is insufficient for activation of *hut* transcription since histidase production was not activated by nitrogen limitation.

Examination of this strain also revealed that activation by the episomal *glnG* product of the chromosomal *glnA* promoter results in a β-galactosidase level of 1,230 U/mg of protein (Table 4, experiment 3b), reflecting the amount of *glnG* product that can be produced by transcription initiated at the activated *glnA* promoter. We would expect to find functional *glnG* product at a similar level in cells carrying a *glnA*$^+$ *glnG*$^+$ episome and *glnA*$^+$ and the *glnG-lacZ* fusion on the chromosome. The fact that in this strain the level of β-galactosidase was lower than the level found in the strain carrying the *glnA21*::Tn*5* *glnG*$^+$ episome reflects the previously observed homeostatic ability of merodiploids to maintain a constant level of glutamine synthetase despite an increase in the copy number of the *glnA* gene (23). A level of functional *glnG* product equivalent to approximately 1,200 U of β-galactosidase per mg of protein should also be present in cells of the wild-type strain grown on glucose glutamine minimal medium. It appears that this amount of *glnG* product, which is approximately 15 times greater than the amount produced from transcription originating at the negatively controlled *glnA*-distal promot-

er, does not result in any further activation of *glnA* expression, but allows the activation of expression of other enzymes, such as histidase (Table 4, experiments 3b and 7b). We also found that at this high level of functional *glnG* product, expression of *glnG* initiated at the *glnA*-distal promoter is repressed almost completely (Table 4, experiment 6b). The requirement of juxtaposition of the *glnA* and *glnG* genes for the activation of histidase synthesis has been observed previously in *E. coli* (18, 22) and has also been observed in *K. aerogenes* (21).

It may be advantageous or even necessary for a cell to be able to initiate transcription of the *glnG* product from two promoters. Repression by the *glnG* product of transcription starting at the *glnA* promoter should still permit sufficient transcription of the *glnG* product from the second promoter to maintain the repression at the *glnA* promoter and to allow immediate activation of transcription from the *glnA* promoter, if the nitrogen source of the medium becomes growth limiting. However, the level of the *glnG* product in cells growing with an excess of nitrogen would not be sufficient to allow activation of transcription at promoters of other genes of enzymes capable of providing the cell with ammonia, such as the *hut* genes. Consequently, deprivation of ammonia leads to rapid formation of glutamine synthetase, the enzyme required for the assimilation of ammonia, followed more slowly by the synthesis of enzymes capable of forming ammonia from other nitrogen sources, presumably as sufficient *glnG* product accumulates to activate this expression (F. Foor and B. Magasanik, unpublished data).

At present we can not assess whether the system permits an even more subtle regulation of *glnG* expression in response to changes in the environment. We have shown previously that in cells carrying a *glnA-lacZ* fusion, approximately 2.5 U of β-galactosidase per mg of protein corresponds to 1 U of glutamine synthetase per mg of protein (22). We found that in strain GP8523 during growth on glucose glutamine medium only 0.8 U of β-galactosidase is produced for each 1 U of glutamine synthetase (Table 4, experiment 3b). In both instances, the production of β-galactosidase presumably depends on transcription initiated at the promoter of *glnA*. If we assume that in general the levels of β-galactosidase accurately reflect the relative levels of transcription of different genes, then there is a reduction in transcription between the *glnA* and *glnG* genes of roughly 60 to 70%. Therefore, it is possible that the continuation of transcription distal to *glnA* is regulated. We found no evidence for such control in our experiments with strains containing different amounts of wild-type *glnG* product grown under different conditions. However, we must consider another regulator of *glnA* expression, the product of the *glnL* gene (also called *ntrB*), which is located between *glnA* and *glnG* (6, 16; MacNeil et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1981) and may play a role in transcription termination or in regulation of the *glnA*-distal promoter.

Finally, our results are compatible with the view that the products of the *glnF* and *glnG* genes may alone be responsible for activation of the synthesis of enzymes capable of supplying the cells with ammonia in response to nitrogen starvation. The view that this activation is due to glutamine synthetase (15) was in part based on the observation that many glutamine auxotrophs whose lesions are in *glnA* lose the ability to increase the levels of these enzymes in response to ammonia limitation (Reg^- phenotype) (3, 18, 22). It is likely that these are polar mutants, which are restricted to a low level of *glnG* product because they are not able to initiate transcription of this gene from the *glnA* promoter (S. Guterman, G. Singer, G. Pahel, and B. M. Tyler, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, K153, p. 152). Thus, not the product but transcription of the *glnA* gene may be required for the cellular response to ammonia deprivation. A definitive solution to these problems must await the isolation of the *glnG* product and investigation of this product at the molecular level.

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