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Genetics

Regulation of expression from the *glnA* promoter of *Escherichia coli* in the absence of glutamine synthetase

(nitrogen metabolism/operon fusion/*glnF*/*glnG*/*glnD*)

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ABSTRACT **One of the suspected regulators of glutamine synthetase [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] in enteric bacteria is glutamine synthetase itself. We isolated *Escherichia coli* strains carrying fusions of the β-galactosidase structural gene to the promoter of the glutamine synthetase gene, with the aid of the Casadaban Mud1 (Ap^R, *lac*, cts62) phage. Some aspects of regulation were retained in haploid fusion strains despite the absence of glutamine synthetase, whereas other aspects required glutamine synthetase catalytic or regulatory activity or both. The direction of transcription of the glutamine synthetase gene was also determined.**

The *glnA* gene in enteric bacteria codes for glutamine synthetase [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] (1, 2). Expression of *glnA* is controlled by the products of several genes (3–5). The complexity of *glnA* control may be related to the central role that glutamine synthetase plays in nitrogen metabolism and its regulation. When ammonia, the preferred nitrogen (N) source of enteric bacteria, is in excess in the culture medium its assimilation is carried out by glutamate dehydrogenase (6, 7) and the synthesis of glutamine synthetase is repressed (8). When ammonia is present in low concentrations, glutamine synthetase levels are high (8), and glutamine synthetase with glutamate synthase in a coupled reaction is responsible for all ammonia assimilation (6, 7). In the absence of ammonia, enteric bacteria can grow on alternative (growth-limiting) N sources. Various studies have led to the hypothesis that glutamine synthetase, independently of its enzymatic activity, is a positive control element for the synthesis of enzymes involved in the utilization of alternative N sources, such as histidase in *Klebsiella aerogenes* (9, 10). [Histidase expression is also activated in *Escherichia coli* after transfer of the *hut* genes from *K. aerogenes* (11).] In the absence of ammonia, glutamine synthetase synthesis is high, even when glutamine (Gln), the end product of the glutamine synthetase reaction, is the sole N source (3).

Among the proteins suspected of regulating *glnA* expression is glutamine synthetase. Because mutations resulting in constitutive synthesis of glutamine synthetase in *K. aerogenes* are located in the *glnA* region, between mutations resulting in glutamine auxotrophy (Gln$^-$), glutamine synthetase was assumed to regulate its own synthesis (12). The recent discovery of a gene closely linked to *glnA* (3, 4), called *glnG* in *E. coli*, caused us to reexamine the hypothesis of autoregulation. The *glnG* product is necessary for both positive and negative control of *glnA*, and could be responsible for control functions attributed to glutamine synthetase. The product of *glnF*, a gene unlinked to *glnA*, is necessary for activation of glutamine synthetase synthesis, because in its absence glutamine synthetase is expressed at very low levels, resulting in a Gln$^-$ phenotype (3, 4). The P_{II} protein, a component of the glutamine synthetase adenylylation system, also plays a part in regulating glutamine synthetase biosynthesis (5).

We have isolated strains of *E. coli* with the *lac* genes fused to the promoter of the *glnA* gene. Thus we can measure expression from the *glnA* promoter by assaying for β-galactosidase, both in haploid fusion strains that lack glutamine synthetase and in merodiploid fusion strains that contain a wild-type *glnA* gene as well as the *glnA–lac* fusion.

Our results indicate that glutamine synthetase is not essential for positive or negative control of *glnA* expression. However, glutamine synthetase may play a modulating role in the regulation of *glnA* expression.

MATERIALS AND METHODS

Growth conditions were as described (13). Indicator plates contained 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) at 40 μg/ml (14).

All bacterial strains are Δ(*lac*)U169 *thi strA* and contain the *rhaD* allele and the *hut* operon from *K. aerogenes*, which contains *hutC* (3). Merodiploid strains contained F′133, or a derivative F′, containing the *glnA* region (from CGSC 4265), and contained *metBJK?136*::Tn5 on the chromosome (3). Strain DR6317 also contained *met136*::Tn5. All merodiploid strains, and strain DR6317, were made *recA56* by cotransduction with *sr11300*::Tn*10*. The *glnF* marker (3) and the *glnD* marker (15) result from Tn*10* insertion. The selection for Tn5 insertion within *glnG* is described below.

Strains bearing fusions of *lacZ* to the *glnA* promoter were isolated with the aid of Casadaban's Mud (Ap^R, *lac*, cts62) phage (16), referred to as Mud1. *Method 1.* After infection, a culture of strain ET6002 was inoculated on LB plates (14) containing ampicillin (Ap) at 25 μg/ml but no added glutamine. Such plates contain limiting glutamine. Small colonies were tested for glutamine auxotrophy. *Method 2.* Strain ET6002 was grown in glucose (Glc)/ammonia/Gln medium and infected with Mud1. Cells were immediatedly diluted into five cultures. Each culture was grown out, and then the cells were washed and resuspended in Glc/ammonia medium (lacking glutamine) for two generations. Cycloserine was added to 100 μg/ml for 2 hr. After washing, cells were treated as in method 1.

Transductions were carried out with phage P1vir (17). Plate matings were done as described (14).

Isolation of strain DR6362, containing *glnG*::Tn5, was accomplished by mating ET6059 (3) with strain DB4291 and

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Abbreviations: Ap, ampicillin; Km, kanamycin; R and S, resistance (resistant) and sensitivity (sensitive); Gln$^+$ and Gln$^-$, glutamine-requiring and glutamine-independent; Lac$^+$ and Lac$^-$, lactose-utilizing and lactose-nonutilizing; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

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selecting for glutamine prototrophy (Gln^+) and kanamycin resistance (Km^R) at 39°C (5). Isolation of DR6359, a strain carrying *glnA-lac* on an episome, was by mating F'133 (from CGSC4265) into the recombination-proficient fusion strain DR6301 and incubating for several days on a Glc/ammonia plate containing Ap to allow recombination. A mixture of cells from the plate was used as donor in a subsequent mating with a tetracycline-resistant recipient, resulting in strain DR6359.

The enzyme assays in whole cells for histidase and for glutamine synthetase by its transferase activity have been described (13), except that transferase assays were at pH 7.27, the isoactivity point for *E. coli* K-12. β-Galactosidase was assayed as described by Miller (14), except that samples of whole cells or cells treated with hexadecyltrimethylammonium bromide (CTAB) were added directly to Z buffer that contained sodium deoxycholate at 0.1 mg/ml and CTAB at 0.2 mg/ml. A derivative of strain DR6360 containing an intact *lac* operon produced 21,000 units of β-galactosidase per mg of protein when fully induced by growth on succinate/ammonia/Gln with isopropyl thiogalactoside (1 mM) (compare with values in Tables 3 and 4).

RESULTS

Constructing *glnA-lac* Fusions. We infected strain ET6002 with the Casadaban Mud (Ap^R, *lac*, cts62) phage (16), which contains the *lac* structural genes, but no promoter, within the Mu phage DNA ends. The defective phage, subsequently referred to as Mud1, is capable of random integration characteristic of Mu and can be used to insert the *lac* genes within the *glnA* gene in a single step.

One potential fusion strain of Mud1 to the *glnA* promoter (DR6301) was isolated by method 1. Forty Gln^- derivatives from five separate cultures were isolated by method 2. Twenty-one of the 40 produced dark blue color on minimal plates containing X-Gal, indicating high β-galactosidase activity (Lac^+), and 19 produced white colonies (Lac^-). This is consistent with the notion that the Mud1 phage had integrated randomly with regard to its orientation. One blue clone was chosen from each culture for further study (strains DR6302–DR6306).

To confirm that the Mud1 phage was inserted in the *glnA* region of the chromosome, we transduced the mutants with a stock of phage P1 grown on strain ET1214 (3), which contains a Tn*10* insertion closely linked to *glnA*. The majority of tetracycline-resistant transductants of each fusion strain were Gln^+, indicating that the Mud1 insertion in the *glnA* region was responsible for the Gln^- phenotype. All tetracycline-resistant Gln^+ transductants were Ap^S and were Lac^- on X-Gal indicator plates, indicating that each fusion strain contained a single Mud1 insertion integrated in the *glnA* gene.

Strains containing the six independent *glnA-lac* fusions were grown on glucose (Glc) minimal medium containing glutamine as sole N source or on Glc/Gln with added ammonia. When samples from each culture were assayed, there was no significant difference in β-galactosidase activity among the fusion strains when grown on comparable media (Table 1).

Table 2 shows that none of the six strains containing the *glnA-lac* allele was capable of stimulating the synthesis of histidase during N-limited growth. In a fusion strain carrying an episome containing the wild-type *glnA* region, however, histidase formation was stimulated about 5-fold in response to N limitation. Thus haploid fusion strains lacked *hut* regulatory capacity as well as glutamine synthetase catalytic activity.

If the *lac* genes are in fact fused to the *glnA* promoter, then synthesis of β-galactosidase and glutamine synthetase should be regulated in parallel in fusion strains merodiploid for the *glnA* region. We isolated two merodiploid strains containing *glnA21*::Mud1; in strain DR6327 the fusion was located on the chromosome and the wild-type glutamine synthetase structural gene on the episome, whereas in strain DR6359 the fusion was on the episome and the $glnA^+$ gene on the chromosome. When glutamine was the sole N source, both β-galactosidase and glutamine synthetase activities were high. Growth in the presence of ammonia had a strong repressing effect on expression of both glutamine synthetase and β-galactosidase. Whether cells were grown on Glc/Gln, Glc/ammonia, or Glc/ammonia/Gln medium, the ratio of activities of β-galactosidase to glutamine synthetase remained relatively constant, indicating good coordination of β-galactosidase and glutamine synthetase expression (Table 3). Finally, in strain DR6374, which contains a *glnF* allele, synthesis of both glutamine synthetase and β-galactosidase was reduced drastically, indicating that β-galactosidase expression was regulated by the *glnF* product (Table 3). We concluded that β-galactosidase expression was regulated by the control region of the *glnA* gene.

Table 1. β-Galactosidase activity in cultures of strains containing *glnA*::Mud1 fusions

Strain	*glnA*::Mud1 allele	β-Galactosidase activity, nmol/min per Klett unit* Glc/Gln†	Glc/ammonia/Gln‡
DR6301	21	15 5	7.0
DR6302	22	16.9	7.6
DR6303	23	17.1	5.7
DR6304	24	16.9	4.8
DR6305	25	16.9	6.6
DR6306	26	17.7	5.9

* Samples from cultures were added directly to the assay mix and standardized per Klett unit (no. 44 filter).
† Glc/Gln medium: 0.4% glucose/0.2% glutamine.
‡ Glc/ammonia/Gln medium: 0.4% glucose/0.2% ammonia/0.2% glutamine.

Because the control regions of both $glnA^+$ and *glnA-lac* in the merodiploid strains were identical, we could evaluate the expression of β-galactosidase in terms of glutamine synthetase activity. We calculated that 2.2–2.8 units of β-galactosidase correspond to 1 unit of glutamine synthetase (see Table 3).

β-Galactosidase Expression in Haploid Fusion Strains. We could evaluate expression from the *glnA* promoter in haploid fusion strains, because we knew that 2–3 units of β-galactosidase activity was equivalent to the expression of 1 glutamine synthetase unit. It was immediately apparent that the haploid strain DR6317 could produce a high level of β-galactosidase in the absence of glutamine synthetase (Table 4, experiment 1). It produced the equivalent of 5000 units of glutamine synthetase per mg of protein when grown on Glc/Gln, and 3700 units when grown on Glc/ammonia/Gln, indicating stronger expression of *glnA-lac*, even in the presence of ammonia, than *glnA* expression in the wild-type grown in N-limiting medium.

Table 2. Histidase activity of strains grown on Glc/Gln medium

Strain	*gln* alleles	Histidase activity, nmol/min per mg protein
DR6301	*glnA21*::Mud1	66
DR6302	*glnA22*::Mud1	68
DR6303	*glnA23*::Mud1	77
DR6304	*glnA24*::Mud1	78
DR6305	*glnA25*::Mud1	61
DR6306	*glnA26*::Mud1	68
DR6327	F'*glnA*$^+$/*glnA21*::Mud1	270

Table 3. Glutamine synthetase and β-galactosidase activities of merodiploid strains that contain the *glnA21*::Mud1 fusion as well as *glnA*+

Strain	*gln* alleles: Episome	*gln* alleles: Chromosome	Growth medium*	Activity, nmol/min per mg protein: Glutamine synthetase	Activity, nmol/min per mg protein: β-Galactosidase	β-Galactosidase/glutamine synthetase activity†
DR6327	*glnA*+	*glnA21*::Mud1	Glc/Gln	1680	3440	2.0
			Glc/ammonia	230	500	2.2
			Glc/ammonia/Gln	160	250	1.5
DR6359	*glnA21*::Mud1	*glnA*+	Glc/Gln	1480	5610	3.8
			Glc/ammonia	190	660	3.5
			Glc/ammonia/Gln	140	440	3.3
DR6374	*glnA*+	*glnA21*::Mud1 *glnF*::Tn*10*	Glc/ammonia/Gln	14	20	1.4

* Growth conditions are as indicated in Table 1.

† One can estimate how many units of β-galactosidase activity correspond to 1 unit of glutamine synthetase activity (glutamine synthetase, β-galactosidase equivalence) by calculating the ratio of the two activities in merodiploid strains. Genes on an episome, however, are likely to be present in more copies than those on the chromosome. To compensate for this possibility, we divide the activity coded by the episomal gene by the copy number (C) of the plasmid relative to the chromosome. Thus for strain DR6359, with *glnA–lac* on the episome and *glnA*+ on the chromosome, the equivalence (E) = (β-galactosidase activity/glutamine synthetase activity) ÷ C_1. For strain DR6327, however, which contains *glnA*+ on the episome and *glnA–lac* on the chromosome, E = (β-galactosidase activity/glutamine synthetase activity) × C_2. Assuming that the relative copy numbers in the strains are the same, $C_1 = C_2$. When the two merodiploids are grown on Glc/Gln, then $E = 2.0 \times C$ and $E = 3.8 \div C$; $E^2 = 2.0 \times 3.8$; $E = 2.8$. We calculate that $E = 2.8$ for cells grown on Glc/ammonia, and 2.2 for cells grown on Glc/ammonia/Gln. It is not necessary to assume that the *glnA* promoter is equally active on the chromosome and episome, because C can be regarded as a composite parameter of copy number times the efficiency of transcription.

Because strain DR6317 contained such high levels of β-galactosidase when grown in the presence or absence of ammonia, it was conceivable that in cells lacking glutamine synthetase expression of *glnA–lac* would not be repressed even if they harbored a mutation in *glnF*. We found, however, that strain DR6331, a haploid fusion strain containing a Tn*10* insertion in *glnF*, synthesized β-galactosidase at a level equivalent to only 14 units of glutamine synthetase activity (Table 4, experiment 2). This was comparable to *glnA* expression in strain DR6374, the merodiploid carrying a mutated *glnF* allele.

If the *glnG* product were responsible for negative control in haploid fusion strains, then a *glnF* strain that also contained an insertion in *glnG* (strain DR6372), should produce noticeably more β-galactosidase compared to the isogenic *glnG*+ strain DR6331. Strain DR6372 produced 5 times as much β-galactosidase as strain DR6331: β-galactosidase production in strain DR6372 was equivalent to 65 units of glutamine synthetase (Table 4, experiment 3), comparable to glutamine synthetase expression in *glnA*+ strains lacking *glnG* and *glnF* products (3). Thus, the wild-type *glnG* product of the *glnF* strain DR6331 was necessary, independent of glutamine synthetase, for severe repression of expression from the *glnA* promoter.

The *glnG* product in Gln+ strains has a positive as well as negative regulatory function, because *glnG* strains that contain a wild-type *glnF* gene fail to respond to N limitation and produce only about 60 glutamine synthetase units when grown on Glc/Gln medium (3). In haploid fusion strains, too, loss of *glnG* product resulted in the inability to produce high levels of β-galactosidase. Strain DR6363, a *glnG* fusion strain, produced a level of β-galactosidase equal to that of strain DR6372 (*glnG*, *glnF*), despite the presence of the *glnF*+ allele (Table 4, experiment 4), far below the rate of synthesis of β-galactosidase of the haploid fusion strain carrying wild-type *glnG* and *glnF* alleles (Table 4, experiment 1).

Table 4. β-Galactosidase activity of haploid fusion strains grown on Glc/ammonia/Gln

Exp.	Strain	*gln* genotype: *A*	*F*	*G*	*D*	β-Galactosidase activity, nmol/min per mg protein
1	DR6317	*A21*::Mud1	+	+	+	8170*
2	DR6331	*A21*::Mud1	−	+	+	30
3	DR6372	*A21*::Mud1	−	−	+	140
4	DR6363	*A21*::Mud1	+	−	+	150
5	DR6336	*A21*::Mud1	+	+	−	660

* Strain DR6317 produced 13,900 units of β-galactosidase per mg of protein when grown on Glc/Gln and 1050 units when grown on histidine/ammonia/Gln.

Recent work in *K. aerogenes* indicates that at least one component of the glutamine synthetase adenylylation system, the P_{II} protein, appears to be involved in the regulation of glutamine synthetase synthesis as well (5). Strains of *E. coli* containing an insertion in *glnD*, resulting in nonfunctional P_{II} modifying enzyme, synthesize glutamine synthetase at repressed levels, even in N-limiting medium (15). In such strains P_{II} is found predominantly in the P_{IIA} (adenylylating) form, which may account for *glnA* repression. We found that haploid fusion strains containing a Tn*10* insertion in *glnD* synthesized β-galactosidase at a much reduced rate compared to strain DR6317 (compare experiments 5 and 1 in Table 4,). The *glnD* strain produced the equivalent of 300 units of glutamine synthetase from *glnA-lac*, compared to 3700 units for the *glnD*+ strain DR6317.

Direction of Transcription. We determined the direction of transcription of *glnA* by deletion analysis starting with fusion strain DR6363, which contains a Tn5 insertion (coding for Km^R) in the *glnG* gene, closely linked to *glnA21*::Mud1. Most temperature-resistant survivors of Mud1 induction still produced β-galactosidase. Ten of 101 such survivors, however, contained deletions from *kil* in Mud1 into *glnG*, because they were simultaneously temperature resistant, Mu sensitive, and Km^S (Fig. 1). Because the *lacZ* gene is still present and operational in these deletions, *glnA* must be transcribed toward *glnG*. This method has been used previously to determine the direction of transcription of other genes (B. Wanner, personal communication). The existence of deletions extending from *rha* through *glnA*, some of which retain the *glnG*::Tn5 Km^R de-

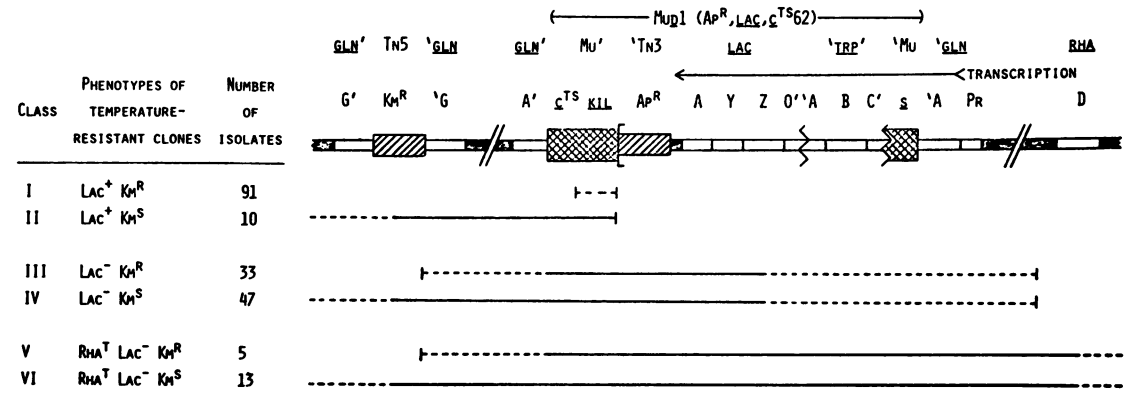

FIG. 1. Deletion analysis using strain DR6363. A detailed map of the *glnA* region in strain DR6363 shows the positions of Mud1 within the *glnA* structrual gene and of the Tn5 element within *glnG*. Pr indicates the *glnA* promoter. A prime next to a genetic symbol indicates that the gene has a deletion or is interrupted on the side the prime is written. Diagonal slashes intersecting the map indicate a discontinuity in the diagram. These genetic regions are not necessarily drawn to scale. Clones of DR6363 were incubated at 44°C on LB/X-Gal plates to select for loss of *kil* gene function (18). Most survivors were Lac+, and one Lac+ survivor per clone was scored for Ap^R, Mu immunity, and Km^R. Of 101 survivors (class I) 91 were not large deletions, because they were Ap^R and Km^R, and 10 of 10 tested remained Mu-immune. The remaining 10 Lac+ survivors, however, all had lost Mu immunity, and had become Km^S. The loss of function due to deletion formation is indicated by the solid lines: deletions could extend through regions indicated by broken lines. Lac- survivors were assumed to have deletions extending at least from *lacZ* through *kil* (class III), or from *lacZ* through the Tn5 marker within *glnG* (class IV). Class V and class VI deletion strains were selected by incubating DR6363 clones on succinate/ammonia/Gln minimal plates containing 2% rhamnose at 44°C. Only *kil*-defective, rhamnose-tolerant (Rha^T) derivatives survived, and each was Lac-.

terminant, confirms the order *glnG-glnA-rha* (3). Thus *glnA* is transcribed counterclockwise on the *E. coli* chromosome away from *rha* and toward *glnG*. The direction of transcription is the same as that suggested for *glnA* of *K. aerogenes* (19).

DISCUSSION

It has been proposed that glutamine synthetase is an activator of the formation of many enzymes capable of providing the cell with ammonia or glutamate, as well as a regulator of the expression of its own structural gene, *glnA* (12). We tested the validity of this view by examining the expression of β-galactosidase from the *glnA* promoter and of histidase in strains lacking glutamine synthetase. We fused the *lac* genes to the promoter of *glnA*, using the Mud1 phage (16). In merodiploid cells carrying the fusion as well as a normal *glnA* region, β-galactosidase and glutamine synthetase responded in parallel to regulation by the *glnF* product and by the nitrogen source of the medium, with 2–3 units of β-galactosidase equivalent to 1 unit of glutamine synthetase. These results clearly establish that *lacZ* has been fused to the promoter of *glnA*.

The haploid cells carrying only the fusion were devoid of glutamine synthetase catalytic function and unable to activate the formation of histidase in response to ammonia deprivation. On the other hand, they produced β-galactosidase in the presence or absence of ammonia at a level considerably higher than that of merodiploid cells grown under corresponding conditions. Glutamine synthetase, or a hypothetical downstream gene product in the same operon, appears to be essential for activation of *hut*, but not of *glnA* expression. A similar conclusion regarding *hut* has been drawn previously from observations on strains with phage Mu inserted in *glnA* (3).

Even in the absence of glutamine synthetase, full expression of *glnA-lac* depends on the products of *glnF* and *glnG*. Moreover, because complete repression of *glnA-lac* in a *glnF* strain depends on an intact *glnG* gene, the *glnG* product is both a repressor and an activator of *glnA-lac*. Of particular interest is the observation that a Tn*10* insertion in *glnD* lowered the expression of *glnA-lac* in the haploid fusion strain. The loss of the *glnD* product, uridylyltransferase, prevents the conversion of P_{II}, an activator of the adenylylation of glutamine synthetase, to its uridylylated form, an activator of deadenylylation. It has previously been shown that P_{II}, but not its uridylylated derivative, prevents derepression of glutamine synthetase. Our results now show that this effect of P_{II} is exerted to some extent in the absence of glutamine synthetase.

Although intact glutamine synthetase is required neither for repression nor for activation of *glnA-lac* expression by the product of the *glnG* gene, we cannot exclude the possibility that the fusion strains contain a fragment of glutamine synthetase devoid of enzymatic activity but retaining regulatory activity. However, this is unlikely, because we find that in six independently isolated fusion strains β-galactosidase is synthesized at the same high level (Table 1) and that three fusion strains into which a defective *glnF* gene has been introduced produce β-galactosidase at the same very low level (data not shown). In view of the fact that phage Mu integrates at random sites (20), it is unlikely that each fusion strain should contain a fragment with the same regulatory ability.

Considering now the expression of the *glnA* gene in strains that contain *glnG* and *glnF* products, we find that functional glutamine synthetase plays an important role. The level of β-galactosidase in the haploid fusion strain is much higher than that of the merodiploid strain containing a functional *glnA* gene in addition to the fusion: 4 times higher when both strains are grown on Glc/Gln, and more than 30 times higher when ammonia has been added to the medium. The loss of glutamine synthetase catalytic function may result in a deficiency in the intracellular level of glutamine. It is likely that glutamine is required for the repression of *glnA* expression (21) and that, even in cells grown with exogenous glutamine, the enzymatic activity of glutamine synthetase may be essential for raising the internal concentration of glutamine to the level required for full repression of glutamine synthetase. However, the assumption that the enzymatic activity of glutamine synthetase is alone responsible for this repression is not in complete accord with our observations. In wild-type cells grown on histidine/ammonia/Gln, *glnA* expression is low (80 units), and most of the glutamine synthetase produced is enzymatically inactive, due to adenylylation (unpublished results). The contribution of glutamine from glutamine synthetase enzyme activity in

such cells is probably minimal. Similarly, in mutants lacking the *glnD* product, uridylyltransferase, glutamine synthetase is almost completely adenylylated and therefore inactive (15). In both instances, removal of glutamine synthetase by the introduction of the *glnA-lac* fusion increases gene expression from the *glnA* promoter about 5-fold (Table 4). This 5-fold difference in expression may therefore be due to a regulatory function of glutamine synthetase that is missing in haploid fusion strains.

In conclusion, our results show that *glnA* can be activated and repressed in the absence of functional glutamine synthetase. Nevertheless, glutamine synthetase appears to play a role, which may only be in part due to its enzymatic activity, in modulating the expression of *glnA* in response to the availability of nitrogen in the growth medium.

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