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Glutamine Synthetase Mutations Which Affect Expression of Nitrogen Fixation Genes in *Klebsiella pneumoniae*

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Previous studies have implicated glutamine synthetase (L-glutamate:ammonia ligase [adenosine diphosphate forming], EC 6.3.1.2) as a major controlling element of the nitrogen fixation (*nif*) genes in *Klebsiella pneumoniae*. We report here the isolation of a new class of *K. pneumoniae* mutants which exhibit altered patterns of *nif* and *hut* (histidine utilization) regulation. The expression of *nif* in these mutants, which were isolated as Gln^+ (glutamine nonrequiring) revertants of a particular *glnA* mutation, is extremely sensitive to ammonia repression. These mutants have a Nif^- Hut^- phenotype at external ammonia concentrations at which wild-type strains are Nif^+ Hut^+. On the other hand, these mutants can be fully derepressed for *nif* at very low ammonia concentrations. We adopted the nomenclature "$GlnR^-$ (Nif^- Hut^-)" to facilitate discussion of the phenotype of these mutant strains. The mutations in these strains which confer the $GlnR^-$ phenotype map at or near *glnA*, the structural gene for glutamine synthetase.

In at least four species of enteric bacteria, *Klebsiella aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*, derepression of many enzymes involved in nitrogen assimilation occurs only when the bacteria are starved for ammonia (26). These enzymes include glutamine synthetase (GS; L-glutamate: ammonia ligase [ADP forming], EC 6.3.1.2) in all the species (5, 6, 16, 18, 24), the enzymes responsible for histidine utilization (*hut*) in *K. pneumoniae* (24) and *K. aerogenes* (6, 18), and the enzymes for nitrogen fixation (*nif*) in *K. pneumoniae* (24, 25). The enzyme glutamine synthetase plays a particularly important role in nitrogen assimilation. Accumulating evidence indicates that GS is intimately involved in a centralized cellular control system which mediates derepression of many nitrogen assimilation enzymes (15). GS is a dodecamer of 12 identical subunits, each of which can be reversibly modified by adenylylation of specific tyrosine residues (21). It is possible that both the intracellular levels and adenylylation state of GS are important in determining its regulatory activity (10, 27).

An active role for GS in regulating nitrogenase (Nif) expression in *K. pneumoniae* was inferred from the Nif phenotypes conferred by two classes of mutations mapping at or near the site of the GS structural gene (24). One class of mutations resulted in glutamine auxotrophy and in the absence of GS enzymatic activity ($GlnA^-$ phenotype). The other class of mutations resulted in the constitutive synthesis of GS in the presence of ammonia ($GlnC^-$ phenotype). Streicher et al. (24) found that $GlnA^-$ strains were unable to derepress nitrogenase during ammonia-limited anaerobic growth and that some $GlnC^-$ strains were partly constitutive for nitrogenase in the presence of ammonia. These experiments were analogous to experiments which had previously demonstrated that GS is involved in regulating *hut* derepression in *K. aerogenes* (6, 18).

To investigate the regulatory role of GS in *nif* derepression, we produced additional classes of *glnA*-linked mutations which affect the *nif* and *hut* regulatory functions of GS. A general feature of strains carrying these mutations is that they retain some GS biosynthetic activity (are $GlnA^+$), but have abnormal patterns of *nif* and *hut* derepression when grown in the presence of low levels of ammonia. We have adopted the nomenclature "$GlnR^-$ (Nif^- Hut^-)" to facilitate discussion of the phenotypes of strains carrying

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gln mutations which result in abnormal patterns of *nif* and *hut* derepression. In all cases, the mutations responsible for the GlnA$^-$ and GlnR$^-$ phenotypes are very closely linked. (Previously, we have used the nomenclature "GlnA$^+$R$^-$" to describe the same mutants [1].)

In this paper, we characterize the physiological and genetic properties of strains with GlnR$^-$ phenotypes. We show that nitrogenase is much more sensitive to ammonia repression in one class of GlnR$^-$ strains. From these data we infer that GS may not be absolutely required for *nif* derepression.

(A preliminary report of some of the experiments described here was presented at the Second International Symposium on Dinitrogen Fixation, Salamanca, Spain, 1976 [1].)

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. All strains are derivatives of *K. pneumoniae* M5a1 (24). In a previous study involving the effect of *gln* mutations on *nif* expression (24), it was assumed that mutations conferring the GlnA$^-$ phenotype were located in the GS structural gene (*glnA*) (9). Moreover, genetic evidence has been presented which indicates that *glnA*-linked mutations which confer a GlnC$^-$ phenotype map within *glnA* (3, 4, 22). Accordingly, in Table 1, strain KP5069 (GlnC$^-$ phenotype) is listed as carrying the *glnA29* mutation. In fact, however, no direct biochemical evidence exists which demonstrated that *glnA29*, used by Streicher et al. (24), actually maps within the GS structural gene as opposed to mapping within an adjacent regulatory gene (11, 13; G. Pahel and B. Tyler, Proc. Natl. Acad. Sci., in press). Similarly, no direct biochemical evidence exists which demonstrates that other *glnA* mutations used in this study are actually located in the GS structural gene. These mutations were assigned to the *glnA* locus on the basis of their linkage to *rha* and *metB* (22) and the fact that strains carrying these mutations do not have GS enzymatic activity.

Media and culture of bacteria. The rich medium used was LB (1% Tryptone [Difco Laboratories, Detroit, Mich.], 0.5% yeast extract, 0.5% NaCl, pH 7.2). The minimal basal salts (BS) medium used for the isolation of mutants and selection of transductants consisted of 60 mM K_2HPO_4, 33 mM KH_2PO_4, 0.5 mM $MgSO_4 \cdot 7H_2O$, 0.17 mM NaCl, 0.01 mM $CaCl_2 \cdot 2H_2O$, and 0.4% glucose. Bacteria for enzyme assays were grown in liquid BS minimal medium, supplemented when appropriate with 15 mM $(NH_4)_2SO_4$ (high ammonia level) or with 0.75 mM $(NH_4)_2SO_4$ (low ammonia level). Histidine and glutamine were provided as amino acid sources at 20 μg/ml. Glutamine (Calbiochem A grade) was prepared freshly for each experiment and sterilized by filtration. Bacteria for nitrogenase assays were grown anaerobically in 10 ml of nitrogen-deficient media (NFDM) (7) in a 25-ml tube and were sparged with dinitrogen at room temperature. LB and BS media were solidified with 1.5% agar (Difco Laboratories, Detroit, Mich.). NFDM medium was solidified with Serva ultrapure agar (no. 11396) obtained from Accurate Chemical and Scientific Corp., Hicksville, N.Y.

Bacteria for nitrogenase assays were first grown

TABLE 1. *List of K. pneumoniae strains and their characteristics*[a]

Strain	Genotype	Relevant phenotype	Source
KP18	Δ(*gal-hut-bio*)	Hut$^-$	S. Streicher
KP5022	*hisD2 hspR1*	GlnA$^+$ GlnR$^+$	24
KP5060	*hisD2 hspR1 glnA100*	GlnA$^-$ GlnR$^-$ (Nif$^-$ Hut$^-$)	24
KP5061	*hisD2 hspR1 glnA101*	GlnA$^-$ GlnR$^-$ (Nif$^-$ Hut$^-$)	24
KP5062	*hisD2 hspR1 glnA102*	GlnA$^-$ GlnR$^-$ (Nif$^-$ Hut$^-$)	24
KP5064	*hisD2 hspR1 glnA104*	GlnA$^-$ GlnR$^-$ (Nif$^-$ Hut$^-$)	24
KP5065	*hisD2 hspR1 glnA105*	GlnA$^-$ GlnR$^-$ (Nif$^-$ Hut$^-$)	24
KP5066	*hisD2 hspR1 glnA106*	GlnA$^-$ GlnR$^-$ (Nif$^-$ Hut$^-$)	24
KP5067	*hisD2 hspR1 glnA107*	GlnA$^-$ GlnR$^-$ (Nif$^-$ Hut$^-$)	24
KP5069	*hisD2 hspR1 glnA29*	GlnC$^-$	24
KP5016-3	*hisD2 hspR1 glnA100 metB100 rha-103*	GlnA$^-$ Met$^-$ Rha$^-$	EMS[b] of KP5060
KP5120	*hisD2 hspR1 metB100 rha-103*	Met$^-$ Rha$^-$ GlnA$^+$ GlnR$^+$	P1(KP5022) × KP5016-3
KP5218-2	*hisD2 hspR1 gln-2182*	GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$)	Spontaneous from KP5060
KP502-3	*hisD2 hspR1 gln-5023*	GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$)	EMS of KP5060
KP503-2	*hisD2 hspR1 gln-5032*	GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$)	EMS of KP5060
KP507-2	*hisD2 hspR1 gln-5072*	GlnA$^+$ GlnR$^-$ (Nif$^+$ Hut$^-$)	NTG[b] of KP502-3
KP507-4	*hisD2 hspR1 gln-5074*	GlnA$^+$ GlnR$^-$ (Nif$^+$ Hut$^-$)	NTG of KP502-3
KP5011	*hisD2 hspR1*	GlnA$^+$ GlnR$^+$	P1(KP5022) × KP5060
KP5412	*hisD2 hspR1 gln-5023*	GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$)	P1(KP502-3) × KP5060
KP5415	*hisD2 hspR1 gln-2182*	GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$)	P1(KP5218-2) × KP5060
KP5413	*hisD2 hspR1 gln-5072*	GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$)	P1(KP507-2) × KP5060
KP5058	*hisD2 hspR29 nifB213*	Nif$^-$	8

[a] All strains are derivatives of *K. pneumoniae* strain M5a1 (24).
[b] EMS, EMS mutagenesis; NTG, NTG mutagenesis.

aerobically in LB at 32°C to saturation, and then 0.1 ml of this culture was inoculated into 10 ml of NFDM supplemented with 500 μg of glutamine per ml in a 25-ml-capacity test tube sealed with a serum stopper and sparged with dinitrogen at 30°C. The nitrogen-deficient medium used in chemostat cultures was the same as NFDM except that $MgCl_2$ replaced $MgSO_4$ and Na_2SO_4 was added to 0.05 mM to limit growth. Chemostat medium was supplemented with NH_4Cl at various concentrations and when appropriate 20 μg of histidine per ml.

Preparation of P1 lysogens, P1 lysates, and P1-mediated transductions. Lysogens of *K. pneumoniae* carrying the P1*clr*100Km prophage were isolated on LB solid medium supplemented with 5 mM $CaCl_2$ and 20 μg of kanamycin per ml, and P1 lysates were prepared by thermal induction at 42°C in LB liquid medium supplemented with 5 mM $CaCl_2$ (12). P1-mediated transductions were performed as previously described (23, 24).

Enzyme assays. Nitrogenase activity was measured in whole cells with the acetylene reduction procedure described earlier (8, 23). All other enzyme assays were performed with both whole-cell and crude-extract preparations. For whole-cell assays, exponentially growing cultures (100 turbidity units measured on a Klett-Summerson colorimeter equipped with a green filter) were harvested according to the method of Bender et al. (2). For preparation of crude enzyme extracts, exponentially growing cells were harvested by centrifugation at 15,000 × *g* for 10 min and then resuspended in 10 mM imidazole–2 mM $MgCl_2$–3 mM β-mercaptoethanol, pH 7.5. The cells were sonically disrupted in an ice bath and then centrifuged at 27,000 × *g* for 45 min to remove cell debris; the supernatant fluid was used for enzyme assays.

The γ-glutamyltransferase activity of GS was assayed as described for *K. aerogenes* (2). Glutamine synthetase consists of 12 identical subunits, each of which can be reversibly adenylylated (21). The adenylylation state can vary from fully unadenylylated ($\bar{n}$ = 0) to fully adenylylated ($\bar{n}$ = 12). Both the adenylylated and unadenylylated forms of GS are equally active in catalyzing the transferase reaction under the proper assay conditions. These assay conditions include a particular pH at which both forms of GS have equal activity (isoactivity point). We have determined the isoactivity point of GS from *K. pneumoniae* strains KP5022, KP502-3, and KP507-2 to be 7.6 (R. Skvirsky and K. Durbin, unpublished data). In the presence of 60 mM $MgCl_2$, the γ-glutamyltransferase activity of the adenylylated GS subunits is completely inhibited, but the unadenylylated subunits retain activity. Thus, the ratio of GS transferase activity in the presence and absence of 60 mM $MgCl_2$ is a measure of the adenylylation state of GS (2, 21).

Procedures for assaying histidase (18, 20), glutamate dehydrogenase (17), glutamate synthase (6), and GS cross-reacting material (9) have been described elsewhere. In general, we used the methods described for *K. aerogenes* for each of these assays.

Protein determinations were performed according to the method of Lowry et al. using bovine serum albumin as a standard (14).

Mutagenesis of bacteria. Mutagenesis by ethyl methane sulfonate (EMS) has been described by Prival et al. (17). Mutagenized bacteria were grown overnight in LB medium at 32°C, diluted, and plated on the appropriate screening or selection medium. Mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was performed by spreading a 0.1-ml amount of a rich broth (LB) culture at a density of 5×10^7 cells per milliliter on the selection medium (NFDM agar) and placing a small crystal of NTG on the agar in the center of the plate. After anaerobic incubation of a $GlnR^-$ (Nif^- Hut^-) strain at 30°C for 5 days, a ring of Nif^+ colonies appeared, surrounding the dissolved crystal of NTG. Control plates containing no NTG had no colonies.

Growth of bacteria in chemostat. Medium supply (1.5 liters) and four culture tubes (27 ml each, with side arms) were maintained at 30°C and were kept anaerobic by bubbling with dinitrogen. A dilution rate of 0.2 h^{-1} (5 ml/h) was maintained with a Büchler polystatic pump fitted with Tygon tubing, internal diameter 0.8 mm. Sterility was maintained by assembling the chemostat in a Baker-Edgegard laminar-flow hood. Each chemostat culture tube was inoculated with 3 ml of bacteria grown anaerobically in NFDM medium. The pump was turned on, and the chemostat cultures were allowed to equilibrate for at least 18 h. Growth was monitored anaerobically by collecting the efflux from the chemostat culture tubes in dinitrogen-sparged serum-stoppered Klett tubes. The absorbance at 540 nm was determined in a Klett colorimeter. Once equilibrated, each culture maintained a constant density. For each ammonia concentration tested, four 5-ml efflux samples of each strain were collected anaerobically at intervals of 1 h in Klett tubes and assayed as described above. At selected time points, the effluent bacteria were diluted and plated on solid LB media. Isolated colonies were tested for His^+ and $GlnR^+$ phenotypes. No His^+ or $GlnR^+$ revertants were found.

RESULTS

Isolation of mutants with altered GS regulatory activity. This section describes the isolation of strains carrying mutations in *glnA* (the structural gene for GS) which affected the *nif* and *hut* regulatory functions of GS but which had little or no effect on the synthesis of glutamine (biosynthetic function of GS). Strains carrying such mutations would have a $GlnA^+$ $GlnR^-$ (Nif^- Hut^-), $GlnA^+$ $GlnR^-$ (Nif^+ Hut^-), or $GlnA^+$ $GlnR^+$ (Nif^- Hut^+) phenotype. Our strategy for obtaining such strains was to isolate $GlnA^+$ revertants of *glnA* strains and then to screen these $GlnA^+$ revertants for those having a Nif^- or Hut^- phenotype or both. The *glnA* strains we used for these reversion studies were glutamine auxotrophs and were unable to derepress nitrogenase or histidase. We predicted that second-site reversions in *glnA* might result in a GS protein with biosynthetic activity but which lacked regulatory activity.

We isolated GlnA$^+$ revertants of seven independent *glnA* strains which had been obtained previously by EMS mutagenesis of strain KP5022 (24). The seven *glnA* strains were divided into two classes on the basis of their reversion frequency to GlnA$^+$. Class 1 strains, KP5061, KP5064, KP5065, KP5066, and KP5067, had spontaneous reversion rates to GlnA$^+$ of approximately 10^{-6} to 10^{-7}. Class 2 strains, KP5060 and KP5062, had a spontaneous reversion frequency to GlnA$^+$ of approximately 10^{-10}. We chose one strain from class 2, KP5060, for more extensive study. To obtain a large number of GlnA$^+$ revertants of KP5060, the strain was mutagenized with EMS as described above. After mutagenesis, GlnA$^+$ revertants of KP5060 were obtained at a frequency of 10^{-6}.

We screened a total of 1,200 GlnA$^+$ revertants isolated from all seven *glnA* strains for aerobic growth on solid BS medium containing 0.4% glucose and 0.2% histidine as sole nitrogen source (Hut$^+$ phenotype), and for anaerobic growth on NFDM solid medium supplemented with 10 μg of histidine per ml (Nif$^+$ phenotype). In class 1 *glnA* strains (high frequency GlnA$^+$ reversion rate), 1,000 GlnA$^+$ revertants (200 from each strain) were all Nif$^+$ Hut$^+$. In class 2, we screened six spontaneous GlnA$^+$ revertants of KP5060 and 100 EMS-induced GlnA$^+$ revertants each of KP5060 and KP5062. We found that 4 of the 6 spontaneous revertants and 52% of the EMS-induced revertants were Nif$^+$ Hut$^+$ but that two of the spontaneous revertants and 48% of the EMS-induced revertants retained a GlnR$^-$ (Nif$^-$ Hut$^-$) phenotype. One spontaneous GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$) revertant of KP5060 (KP5218-2) and two EMS-induced GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$) revertants of KP5060 (KP502-3 and KP503-2) were selected for further study.

All GlnA$^+$ revertants of KP5060 which exhibited a GlnR$^-$ phenotype were both Nif$^-$ and Hut$^-$. To obtain strains with a GlnA$^+$ GlnR$^-$ (Nif$^+$ Hut$^-$) phenotype, we selected directly for growth of strain KP502-3 on solid NFDM medium. No spontaneous Nif$^+$ revertants were obtained from among 10^{11} cells plated on NFDM agar. Approximately 50 Nif$^+$ revertants per plate were readily obtained, however, after mutagenesis of strain KP502-3 with NTG as described above. A total of 48 independent Nif$^+$ revertants obtained in this way were purified and screened for the Hut phenotype by testing their ability to grow on solid BS medium containing 0.2% glucose and 0.2% histidine. Five of these Nif$^+$ revertants were Hut$^-$ and two of these (KP507-2 and KP507-4) were studied further.

We also attempted to isolate revertants of GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$) strains to Hut$^+$ but found that the GlnR$^-$ (Hut$^-$) phenotype was too leaky to allow direct selection of Hut$^+$ revertants. All GlnA$^-$ GlnR$^-$ (Nif$^-$ Hut$^-$) and all GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$) strains tested formed microcolonies on 0.4% glucose-0.2% histidine medium.

The growth characteristics of GlnA$^+$ GlnR$^-$ mutants on solid BS and NFDM minimal media are summarized in Table 2. The GlnR$^-$ (Hut$^-$) phenotype was leaky and was comparable to the Hut$^-$ phenotype of *glnA* strains when they were grown in media (supplemented with 0.05% glutamine) containing glucose as the carbon source and histidine as the major nitrogen source (data not shown). On the other hand, when histidine was the sole carbon and nitrogen source, GlnR$^-$ strains grew as well as GlnR$^+$ strains. This was expected since under the latter carbon starvation conditions, *hut* is derepressed by catabolite activator protein. The slight amount of growth observed for strain KP5058 on NFDM medium was probably due to trace amounts of ammonia

TABLE 2. *Growth characteristics of mutant strains with a GlnR$^-$ phenotype on various media*[a]

Strain	Relevant phenotype	Growth on medium:[b]						
		GNgln	GN	GH	GP	H	P	NFDM
KP5022	GlnA$^+$ GlnR$^+$	+	+	+	+	+	+	+
KP5060	GlnA$^-$ GlnR$^-$	+	−	−	−	−	−	−
KP18	Hut$^-$	+	+	−	+	−	+	+
KP5058	Nif$^-$	+	+	+	+	+	+	+/−
KP502-3	GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$)	+	+	+/−	+/−	+	+	+/−
KP503-2	GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$)	+	+	+/−	+/−	+	+	+/−
KP507-2	GlnA$^+$ GlnR$^-$ (Nif$^+$ Hut$^-$)	+	+	+/−	+/−	+	+	+
KP507-4	GlnA$^+$ GlnR$^-$ (Nif$^-$ Hut$^-$)	+	+	+/−	+/−	+	+	+

[a] All strains were streaked on solid BS or NFDM medium and incubated at 30°C.

[b] Medium abbreviations: GNgln, GN, GH, and GP are BS (plus 20 μg of histidine per ml when necessary) plus 0.2% glucose, G; 0.05% glutamine (gln) and 15 mM $(NH_4)_2SO_4$, N; 0.2% histidine, H; or 0.2% proline, P; H and P are BS (plus 20 μg of histidine per ml when necessary) plus 0.4% histidine, H; or 0.4% proline, P. +, Normal colonies after 24 h (BS medium) or 5 days (NFDM); −, no growth after 48 h (BS medium) or 5 days (NFDM); and +/−, microcolonies after 48 h (BS medium) or 5 days (NFDM).

in the agar. This may also explain the slight amount of growth observed for strains KP502-3 and KP503-2 on NFDM medium.

Mapping the mutations which confer a GlnR⁻ phenotype. In *K. aerogenes*, *glnA* is linked by P1 cotransduction to *rha* and *metB* (22). We have shown in *K. pneumoniae* that the order of the markers is *glnA rha metB*, and that *rha* is about 10% linked to *glnA* and that *metB* is about 4% linked to *glnA* (1). To map mutations conferring a GlnR⁻ phenotype with respect to *glnA*, we determined the linkage of mutations conferring a GlnR⁻ phenotype to *metB* and *rha* in crosses in which GlnA⁺ was not selected. Then we determined the linkage of mutations conferring a GlnR⁻ phenotype to *glnA* in crosses in which GlnA⁺ was selected directly. In crosses 3 to 5 (Table 3) in which the donor strain carried a mutation which conferred a GlnR⁻ (Nif⁻ Hut⁻) phenotype, the GlnR⁻ phenotype was 100% cotransducible with the GlnA⁺ phenotype. Similarly, in crosses 6 to 8, in which the donor carried a mutation which conferred a GlnR⁻ (Nif⁺ Hut⁻) phenotype, the GlnR⁻ was 100% cotransducible with the GlnA⁺ phenotype.

Since strains KP502-3 and KP503-2 (GlnR⁻ [Nif⁻ Hut⁻]) were isolated as GlnA⁺ revertants of strains KP5060 (*glnA100*), it was possible that the reversion was due to a second-site mutation which suppressed the *glnA100* mutation and that *glnA100* was still present in the revertants. If this was true, it should be possible to recover the *glnA100* mutation by crossing strain KP502-3 to a *gln*⁺ *metB* recipient. However, among 1,000 Met⁺ transductants (cross 9, Table 3), none had a GlnA⁻ phenotype. We conclude that if strain KP502-3 contains two *gln* mutations, the mutation conferring the GlnR⁻ (Nif⁻ Hut⁻) phenotype is closely linked to *glnA100*.

The conclusion that the mutation conferring the GlnR⁻ (Nif⁻ Hut⁻) phenotype is closely linked to *glnA100* is supported by comparing the degree of *metB* and *glnA100* linkage observed in cross 2 with the degree of *metB* linkage to the mutation(s) conferring the *GlnA*⁺ phenotype in cross 5. (In cross 5, recipients become GlnA⁺ only by incorporating the mutation conferring the GlnR⁻ phenotype.) If this latter mutation is relatively far from *glnA100* and lies between *glnA100* and *metB100*, one would expect the percentage of Met⁺ GlnA⁺ transductants in cross 5 to be higher than the percentage in cross 2. In contrast, if the mutation lies beyond *glnA* relative to *metB*, then the percentage of Met⁺ GlnA⁺ transductants obtained in cross 5 would be lower than in cross 2. In fact, the Met⁺ GlnA⁺ transductants obtained in cross 5 occurred at the same frequency as those obtained in cross 2, indicating that the mutation conferring the GlnR⁻ (Nif⁻ Hut⁻) phenotype is tightly linked to *glnA100*.

Regulation of enzyme synthesis (activity) in GlnR⁻ strains. To examine the regulatory properties of GlnR⁻ strains, we measured the activities of several enzymes involved in ammonia assimilation in cells grown either in the presence or absence of ammonia (Table 4). To assist comparison among the strains, we transduced all primary mutations into an isogenic background (see Table 1). The general pattern observed for wild type (KP5022) showed concomitant derepression of GS, histidase, and nitrogenase, and repression of glutamate dehydrogenase during growth in limiting ammonia (0.75 mM $[NH_4]_2SO_4$ as nitrogen source). Conversely, when ammonia was plentiful, GS, histidase, and nitrogenase activities were repressed while glutamate dehydrogenase was derepressed. As controls, we measured enzyme activities in two *glnA* mutants, KP5060 (GlnA⁻) and KP5069 (GlnC⁻), whose properties had been previously described (24). Strains KP5412, KP5413, and KP5415 (all

TABLE 3. *Mapping the mutations which confer a GlnR⁻ phenotype by P1 transductions*[a]

Cross	Strain	P1 donor (relevant phenotype)	Recipient[b]	Selected[c] phenotype	% with unselected phenotype: GlnA⁺	GlnR⁻ (Nif⁻ Hut⁻)	GlnR⁻ (Nif⁺ Hut⁻)	Rha⁺	Met⁺
1	KP5022	GlnA⁺ GlnR⁺	GlnA⁻ Rha⁻ Met⁻	Gln⁺	(100)	0	0	9	5
2	KP5022	GlnA⁺ GlnR⁺	GlnA⁻ Rha⁻ Met⁻	Met⁺	4	0	0	46	(100)
3	KP502-3	GlnA⁺ GlnR⁻ (Nif⁻ Hut⁻)	GlnA⁻ Rha⁻ Met⁻	Gln⁺	(100)	100	0	7	5
4	KP503-2	GlnA⁺ GlnR⁻ (Nif⁻ Hut⁻)	GlnA⁻ Rha⁻ Met⁻	Gln⁺	(100)	100	0	9	4
5	KP502-3	GlnA⁺ GlnR⁻ (Nif⁻ Hut⁻)	GlnA⁻ Rha⁻ Met⁻	Met⁺	4	4	0	48	(100)
6	KP507-2	GlnA⁺ GlnR⁻ (Nif⁺ Hut⁻)	Gln⁻ Rha⁻ Met⁻	Gln⁺	(100)	0	100	8	n.t.[d]
7	KP507-4	GlnA⁺ GlnR⁻ (Nif⁺ Hut⁻)	Gln⁻ Rha⁻ Met⁻	Gln⁺	(100)	0	100	8	n.t.
8	KP507-2	GlnA⁺ GlnR⁻ (Nif⁺ Hut⁻)	Gln⁻ Rha⁻ Met⁻	Met⁺	5	0	5	44	(100)
9	KP502-3	GlnA⁺ GlnR⁻ (Nif⁻ Hut⁻)	Rha⁻ Met⁻	Met⁺	100	5	0	47	(100)

[a] P1 transductions were performed as described in the text.
[b] The recipient strains were KP5016-3 and KP5120.
[c] One hundred transductants were screened in each cross except in cross 9 in which 1,000 were screened.
[d] n.t., Not tested.

TABLE 4. *Regulation of ammonia assimilation enzymes in wild-type and mutant GlnR⁻ strains*[a]

Strain	[NH₄][a]	GS activity	GS $\bar{n}$[b]	GS CRM[b]	Histidase activity	Glutamate dehydrogenase activity	Glutamate synthase activity	Nitrogenase activity
KP5022 (*gln*⁺) (GlnA⁺)	Low	100 (860)[c]	4	100	100 (480)[c]	100 (123)[c]	100 (84)[c]	100 (5.7)[c]
	High	24	9	n.t.[d]	11	450	71	<0.1
KP5060 (*glnA100*) [GlnA⁻ GlnR⁻ (Nif⁻ Hut⁻)]	Low	<1	n.t.	n.t.	23	400	95	<0.1
	High	<1	n.t.	n.t.	21	400	80	<0.1
KP5069 (*glnA29*) (GlnC⁻)	Low	135	10	n.t.	95	24	50	100
	High	126	10	n.t.	105	<1	35	30
KP5412 (*gln-5023*) [GlnA⁺ GlnR⁻ (Nif⁻ Hut⁻)]	Low	40	3	45	22	1300	98	2
	High	13	10	n.t.	47	430	n.t.	<0.1
KP5415 (*gln-2182* [GlnA⁺ GlnR⁻ (Nif⁻ Hut⁻)]	Low	35	4	40	49	1450	107	8
	High	10	9	n.t.	44	1130	100	<0.1
KP5413 (*gln-5072*) [GlnA⁺ GlnR⁻ (Nif⁺ Hut⁻)]	Low	55	5	55	23	1250	79	52
	High	8	9	n.t.	16	790	80	<0.1

[a] Cultures for nitrogenase assays were grown anaerobically in NFDM supplemented with 0.05% glutamine and 15 mM (high) or 0.75 mM (low) ammonium sulfate and harvested at a density of 100 Klett units as described in the text. Cultures for all other assays were grown aerobically to 100 Klett units in BS supplemented with 0.2% glucose, 0.05% glutamine, and 15 mM (high) or 0.75 mM (low) ammonium sulfate. All of the latter assays were performed on the same batch of cells. Assays were performed on whole-cell preparations as well as on crude extracts (except for nitrogenase assays), and no significant differences were obtained between intact-cell and crude-extract assays. Cultures growing in BS were harvested in the presence of 100 μg of hexadecyltrimethylammonium bromide per ml (2). The doubling time of all strains (except KP5069) growing in BS medium varied between 95 and 105 min (low ammonia) and between 50 and 55 min (high ammonia). The doubling time of strain KP5069 in BS was 90 min (low ammonia) and 45 min (high ammonia). The doubling time of all strains growing in NFDM medium (supplemented with 0.05% glutamine) varied between 110 and 120 min both in the presence and absence of ammonia.

[b] $\bar{n}$, Adenylylation state of GS; CRM, cross-reacting material (see the text for details).

[c] All activities are presented as percentages of wild-type activity (KP5022). Specific activities for KP5022 are shown in parentheses and are expressed as nanomoles produced per minute per milligram of protein except for nitrogenase which is expressed as micromoles of ethylene produced per hour per milligram of protein.

[d] n.t., Not tested.

GlnR⁻) showed normal regulation trends for GS, although the repression levels were about half that of the wild type. To determine whether GS activity accurately reflected the amount of enzyme present in these strains, we measured the amount of GS cross-reacting material in these strains (Table 4). In all cases, cross-reacting material specific for GS antibody correlated closely with the amount of enzymatic activity observed.

The regulation patterns of histidase and glutamate dehydrogenase were similar for all three GlnR⁻ strains; regardless of the ammonia concentration, histidase levels remained low while glutamate dehydrogenase levels were high. The glutamate synthase activities changed very little in any of the strains, a result which was in accord with previous observations (6). Nitrogenase activities in strains KP5412 and KP5415 (GlnR⁻ [Nif⁻ Hut⁻]) were 12- to 15-fold repressed compared to the wild type (KP5022) in the absence of ammonia and presence of 0.05% glutamine, whereas nitrogenase activity was repressed more than 200-fold in strain KP5060 (GlnA⁻). Nitrogenase activity in strain KP5413 (GlnR⁻ [Nif⁺ Hut⁻]) rose to half that of the wild type (KP5022) in the absence of ammonia and was completely repressed in the presence of ammonia. The GlnC⁻ strain (KP5069) grown in the presence of ammonia produced nitrogenase at only partially (10 to 40%) derepressed levels.

Regulation of GS and nitrogenase activity during chemostat-limited growth. The nitrogenase assays described in Table 4 were performed on GlnR⁻ strains grown in the presence of 0.05% glutamine. This level of glutamine does not repress nitrogenase in wild-type strains and was included in the growth media because strain KP5060 requires this level of glutamine supplementation for growth. Subsequently, we found that strains KP5412 and KP5415 would derepress nitrogenase more fully in NFDM medium lacking glutamine if care was taken not to introduce any ammonia or glutamine in the in-

oculation added to the NFDM medium. As a result of these observations, we postulated that GlnR⁻ strains could be derepressed for nitrogenase but that they were considerably more sensitive to ammonia repression than wild-type strains. To test this hypothesis, we determined the amount of nitrogenase in cells grown in a continuous-flow chemostat apparatus in which growth was limited by the availability of sulfur in the medium. Under these conditions, a constant ammonia concentration could be maintained in the growing cultures.

The results in Table 5 contrast markedly with those presented in Table 4. In the previous experiments, nitrogenase was markedly repressed in GlnR⁻ strains KP5412 and KP5415 when these strains were grown in NFDM minimal medium containing 0.05% glutamine. Similarly, strains KP502-3 and KP503-2 had a Nif⁻ phenotype when grown on solid NFDM medium containing 0.05% glutamine (Table 2). As shown in Table 5, when KP502-3 was grown in the chemostat in the complete absence of ammonia or glutamine, nitrogenase was fully derepressed to wild-type levels. In contrast to the wild type, however, nitrogenase activity in KP502-3 dropped to 20% of the fully induced level at 1 mM ammonia and was undetectable at 2 mM ammonia. At 2 mM ammonia, nitrogenase activity in the wild type was 42% of the fully induced level.

The pattern of GS activities and adenylylation states in chemostat cultures (Table 5) were comparable to those observed in aerobically grown cultures (Table 4). The general trends of GS repression and adenylylation as a function of ammonia concentration were similar in all strains, although the absolute levels of GS activity was higher in the wild type. To facilitate comparison of GS adenylylation states in the

TABLE 5. *Nitrogenase and GS activities of GlnR⁻ strains in a sulfur-limited chemostat*

Strain	Relevant phenotype	NH_4^+ (mM)	GS sp act[a]	$\bar{n}$[b]	Unadenylylated[c] GS (% of KP4511 level at 0 NH_4^+)	Nitrogenase[d] sp act
KP5022 (*gln*⁺)	GlnA⁺ GlnR⁺	0.0	n.t.[e]			5.6
		1.25	n.t.			3.0
		2.0	n.t.			2.3
		3.0	n.t.			1.3
		3.5	n.t.			1.1
		4.0	n.t.			0.0
KP502-3 (*gln-5023*)	GlnA⁺ GlnR⁻ (Nif⁻ Hut⁻)	0.0	n.t.			5.8
		1.25	n.t.			1.2
		2.0	n.t.			0.0
KP5411 (*gln*⁺)	GlnA⁺ GlnR⁺	0.0	1640	4	(100)	6.0
		1.0	1580	5	86	3.4
		2.0	1340	6	63	2.7
		2.5	915	8	29	2.0
KP5412 (*gln-5023*)	GlnA⁺ GlnR⁻ (Nif⁻ Hut⁻)	0.0	815	3	57	5.8
		1.0	680	5	37	1.0
		2.0	665	6	31	0.0
KP5413 (*gln-5072*)	GlnA⁺ GlnR⁻ (Nif⁺ Hut⁻)	0.0	485	4	30	6.0
		1.0	416	5	23	2.7
		2.0	328	6	15	2.5
		2.5	322	7	13	1.7

[a] GS activity is expressed as nanomoles of product formed per minute per milligram of protein.

[b] Adenylylation state of GS (see the text).

[c] The relative total amount of unadenylylated GS present in each culture is expressed as the percentage of unadenylylated GS present in the wild-type extract (KP5411) grown in the absence of ammonia. For example, for strain KP5411 in the presence of 0 NH_4^+, (7 + 12) (1,620) or 1,085 units of GS transferase activity is due to unadenylylated enzyme. For strain KP5412 at 1.0 mM NH_4^+, (7 + 12) (680) or 397 units of GS transferase activity is due to unadenylylated enzyme. Thus, the amount of unadenylylated GS in KP5412 at 1 mM NH_4^+ relative to the amount of unadenylylated GS at 0 NH_4^+ is equal to 397 + 1,085, or 37%.

[d] Nitrogenase activity is expressed as micromoles of ethylene produced per hour per milligram of protein.

[e] n.t., Not tested.

different strains, and at different ammonia levels, we have normalized the absolute level of unadenylylated GS in each culture to the level found in wild-type cells at 0 M NH_3 (Table 5). The levels of unadenylylated GS in the various strains at different ammonia concentrations did not correlate well with the levels of *nif* derepression.

DISCUSSION

Previous results, which showed that mutations mapping within or adjacent to the structural gene for GS (*glnA*) affect *nif* expression, implicated GS as a major regulatory element of *nif* expression (24). We have, in part, verified this conclusion by demonstrating the specific alteration of *nif* expression in mutants whose lesions are tightly linked to *glnA*. Unlike the mutants reported earlier, however, the strains described in this paper appear to genetically distinguish various regulatory functions from the biosynthetic activity of GS; strains KP5412 and KP5415 are phenotypically GlnR$^-$ (Nif$^-$ Hut$^-$), whereas strain KP5413 is phenotypically GlnR$^-$ (Nif$^+$ Hut$^-$). In all three strains, the lesion responsible for the altered Nif or Hut phenotype is tightly linked to *glnA* (Table 3).

Our data do not allow us to determine whether the mutations responsible for the GlnR$^-$ phenotype map within *glnA* or within a closely linked gene. Three other groups have recently reported regulatory mutants in closely related bacterial species whose lesions are tightly linked to *glnA* (11, 13; Pahel and Tyler, in press). Gaillardin and Magasanik described GlnR$^-$ mutants of *K. aerogenes* which are unable to fully induce GS, to repress glutamate dehydrogenase, or to turn on histidase (11). Pahel and Tyler isolated mutant strains of *E. coli* carrying phage Mu insertions in the *glnA* region which have a GlnR$^-$ phenotype (Pahel and Tyler, in press). At least one of these insertions has been shown by complementation analysis to map in *glnG*, a gene tightly linked to *glnA*. Similarly, Kustu et al. reported the isolation of GlnR$^-$ mutants in *S. typhimurium* which appear to map in a gene distinct from *glnA* (13). In light of these results, it is possible that mutations which we have isolated which confer a GlnR$^-$ phenotype are located in a *gln* regulatory gene which maps adjacent to *glnA*. On the other hand, because our GlnR$^-$ strains were isolated as GlnA$^+$ revertants of *glnA100*, it is most probable that the mutations which confer the GlnR$^-$ phenotype are located within the same gene as *glnA100*. One further possibility is that *glnA100* itself is not located within *glnA* but rather is located in a regulatory gene required for *glnA*, *nif*, and *hut* expression. If this were the case, revertants of *glnA100* selected to be glutamine nonrequirers could conceivably still be defective in *nif* or *hut* regulation or both.

The results presented in Table 5 show that nitrogenase in GlnR$^-$ (Nif$^-$ Hut$^-$) strains, although extremely sensitive to ammonia repression, can become fully induced in the absence of ammonia. The atypical degree of ammonia sensitivity cannot be explained simply in terms of decreased levels of unadenylylated GS in GlnR$^-$ (Nif$^-$ Hut$^-$) strains compared to the wild type. At 2 mM added ammonia, strain KP5412 (GlnR$^-$ [Nif$^-$ Hut$^-$]) was fully repressed for nitrogenase when the unadenylylated GS level was 31% of the fully derepressed levels of GS in the wild-type strain. In contrast, when the unadenylylated GS level in the wild-type strain fell to 29% of the maximum level (at 2.5 mM added ammonia), nitrogenase was only 67% repressed. Similarly, nitrogenase was fully derepressed to wild-type levels in GlnR$^-$ strains KP5412 and KP5413 when the unadenylylated GS levels were 57 and 30% of the GS levels associated with full nitrogenase derepression in the wild type.

Thus, the adenylylation state of GS does not correlate with the extent of nitrogenase derepression. If nitrogenase derepression in GlnR$^-$ strains is dependent on the intracellular levels of unadenylylated GS, we must conclude that the level of unadenylylated GS measured in vitro is not an accurate measure of the level of GS in a form active for regulating nitrogenase expression. For example, GlnR$^-$ strains may contain mutations which alter the regulatory activity of GS without affecting its capacity to become adenylylated.

The change in *nif* regulation in GlnR$^-$ (Nif$^-$ Hut$^-$) strains could result from a decreased affinity of GS or a *gln* regulatory protein for *nif* promoter binding sites. For example, if the concentration of GS or a *gln* regulatory protein in a *nif* activating form were inversely proportional to the external ammonia concentration, then, at particular ammonia concentrations, a GlnR$^-$ strain could have less regulatory protein bound to *nif* activation sites. This would result in an apparent hypersensitivity to ammonia repression. In this type of model, one can readily postulate mutant forms of GS or a *gln* regulatory protein which regulate *glnA* or *hut* normally, but are defective in *nif* regulation.

An alternative explanation which accounts for the hypersensitivity of GlnR$^-$ strains to ammonia repression is based on a model in which GS or a *gln* regulatory protein is postulated to be

one of two independent systems which are both responsible for *nif* regulation. The model postulates (i) that *nif* expression is regulated primarily by a specific repression system which blocks *nif* transcription above 1 mM ammonia; (ii) GS or a *gln* regulatory protein acts as an activator which competes with the repression system at ammonia concentrations between 1 and 3.5 mM; and (iii) GS or a *gln* regulatory protein is not absolutely required for *nif* derepression. In terms of this model, $GlnR^-$ (Nif^- Hut^-) strains may contain mutations which result in complete loss of the activator, leaving *nif* expression under the sole control of the repression system. This results in hypersensitivity to ammonia repression. Thus, one interpretation of the data presented in Tables 4 and 5 is that the lack of nitrogenase derepression in $GlnR^-$ strains at specific ammonia (or glutamine) concentrations is due to repression by ammonia (or glutamine) in the medium rather than failure of GS or a *gln* regulatory protein to activate *nif* transcription.

A prediction of the model that GS or a *gln* regulatory protein is not essential for *nif* derepression is that *nif* should become derepressed in *glnA* mutants at extremely low ammonia concentrations. It is difficult to demonstrate the validity of this prediction, however, because the level of glutamine required to supplement the auxotrophy of a *glnA* strain is sufficient to repress nitrogenase in $GlnR^-$ strains although not in wild-type strains. Nevertheless, in support of this prediction, Shanmugam et al. reported preliminary results which indicate that derepression of nitrogenase in *glnA* strain KP5060 can occur at very low glutamine concentrations (19).

In conclusion, our results are consistent with a model in which ammonia affects *nif* regulation independently of its effects on GS levels and GS adenylylation states.

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LITERATURE CITED

1. **Ausubel, F. M., R. F. Margolskee, and N. M. Maizels.** 1977. Mutants of *Klebsiella pneumoniae* in which expression of nitrogenase is independent of glutamine synthetase control, p. 347-356. *In* W. Newton, J. R. Postgate, and C. Rodriquez-Barrueco (ed.), Recent developments in nitrogen fixation. Academic Press Inc., New York.
2. **Bender, R. A., K. A. Janssen, A. D. Resnick, M. Blumenberg, F. Foor, and B. Magasanik.** 1977. Biochemical parameters of glutamine synthetase from *Klebsiella aerogenes*. J. Bacteriol. **129**:1001-1009.
3. **Bender, R. A., and B. Magasanik.** 1977. Autogenous regulation of the synthesis of glutamine synthetase in *Klebsiella aerogenes*. J. Bacteriol. **132**:6-112.
4. **Bender, R. A., and B. Magasanik.** 1977. Regulatory mutations in the *Klebsiella aerogenes* structural gene for glutamine synthetase. J. Bacteriol. **132**:100-105.
5. **Brenchley, J. E., C. A. Baker, and L. G. Patil.** 1975. Regulation of the ammonia assimilatory enzymes in *Salmonella typhimurium*. J. Bacteriol. **124**:182-189.
6. **Brenchley, J. E., M. J. Prival, and B. Magasanik.** 1973. Regulation of the synthesis of enzymes responsible for glutamate formation in *Klebsiella aerogenes*. J. Biol. Chem. **248**:6122-6128.
7. **Cannon, F. C., R. A. Dixon, J. R. Postgate, and S. B. Primrose.** 1974. Plasmids formed in nitrogen fixing *Escherichia coli-Klebsiella pneumoniae* hybrids. J. Gen. Microbiol. **80**:241-251.
8. **Cannon, F. C., G. R. Riedel, and F. M. Ausubel.** 1977. Recombinant plasmid that carries part of the nitrogen fixing (*nif*) gene cluster of *Klebsiella pneumoniae*. Proc. Natl. Acad. Sci. U.S.A. **74**:2963-2967.
9. **DeLeo, A. B., and B. Magasanik.** 1975. Identification of the structural gene for glutamine synthetase in *Klebsiella aerogenes*. J. Bacteriol. **121**:313-319.
10. **Friedrich, B., and B. Magasanik.** 1977. Urease of *Klebsiella aerogenes*: control of its synthesis by glutamine synthetase. J. Bacteriol. **131**:446-452.
11. **Gaillardin, C. M., and B. Magasanik.** 1978. Involvement of the product of the *glnF* gene in tbe autogenous regulation of glutamine synthetase formation in *Klebsiella aerogenes*. J. Bacteriol. **133**:1329-1338.
12. **Goldberg, R. B., R. A. Bender, and S. L. Streicher.** 1974. Direct selection for P1-sensitive mutants of enteric bacteria. J. Bacteriol. **118**:810-814.
13. **Kustu, S. G., N. G. McFarland, S. P. Hui, B. Esmon, and G. Ferro-Luzzi Ames.** 1979. Nitrogen control in *Salmonella typhimurium*: co-regulation of synthesis of glutamine synthetase and amino acid transport systems. J. Bacteriol. **138**:218-234.
14. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
15. **Magasanik, B. M., M. J. Prival, J. E. Brenchley, B. M. Tyler, A. B. DeLeo, S. L. Streicher, R. A. Bender, and C. G. Paris.** 1974. Glutamine synthetase as a regulator of enzyme synthesis. Curr. Top. Cell. Regul. **8**:119-138.
16. **Pahel, G., A. D. Zelenetz, and B. M. Tyler.** 1978. *gltB* gene and regulation of nitrogen metabolism by glutamine synthetase in *Escherichia coli*. J. Bacteriol. **133**: 139-148.
17. **Prival, M. J., J. E. Brenchley, and B. Magasanik.** 1973. Glutamine synthetase and the regulation of histidase formation in *Klebsiella aerogenes*. J. Biol. Chem. **248**:4334-4344.
18. **Prival, M. J., and B. Magasanik.** 1971. Resistance to catabolite repression of histidase and proline oxidase during nitrogen limited growth of *Klebsiella aerogenes*. J. Biol. Chem. **246**:6288-6296.
19. **Shanmugam, K. T., F. O'Gara, K. Anderson, and R. C. Valentine.** 1978. Biological nitrogen fixation. Annu. Rev. Plant Physiol. **29**:263-276.
20. **Smith, G. R., S. Halpern, and B. Magasanik.** 1971. Genetic and metabolic control of enzymes responsible for histidine degradation in *Salmonella typhimurium*. J. Biol. Chem. **246**:3320-3329.
21. **Stadtman, E. R., A. Ginsberg, J. E. Ciardi, J. Yeh, S. B. Henning, and B. M. Shapiro.** 1970. Multiple molecular forms of glutamine synthetase produced by en-

zyme catalyzed adenylylation and deadenylylation reactions. Adv. Enzyme Regul. **8**:99–118.

22. **Streicher, S. L., R. A. Bender, and B. Magasanik.** 1975. Genetic control of glutamine synthetase in *Klebsiella aerogenes*. J. Bacteriol. **121**:320–331.
23. **Streicher, S. L., E. Gurney, and R. C. Valentine.** 1971. Transduction of the nitrogen fixation genes in *Klebsiella pneumoniae*. Proc. Natl. Acad. Sci. U.S.A. **68**:1174–1177.
24. **Streicher, S. L., K. T. Shanmugam, F. Ausubel, C. Morandi, and R. B. Goldberg.** 1974. Regulation of nitrogen fixation in *Klebsiella pneumoniae*: evidence for a role of glutamine synthetase as a regulator of nitrogenase synthesis. J. Bacteriol. **120**:815–821.
25. **Tubb, R. S.** 1974. Glutamine synthetase and ammonia regulation of synthesis in *Klebsiella*. Nature (London) **251**:481–485.
26. **Tyler, B.** 1978. Regulation of the assimilation of nitrogen compounds. Annu. Rev. Biochem. **47**:1127–1162.
27. **Tyler, B., A. B. DeLeo, and B. Magasanik.** 1974. Activation of transcription of *hut* DNA by glutamine synthetase. Proc. Natl. Acad. Sci. U.S.A. **71**:225–229.