Proc. Natl. Acad. Sci. USA
Vol. 78, No. 4, pp. 2135–2139, April 1981
Biochemistry

Nitrogen regulatory locus "*glnR*" of enteric bacteria is composed of cistrons *ntrB* and *ntrC*: Identification of their protein products

(regulation of gene expression/positive and negative control/nitrogen utilization/glutamine synthetase/*in vitro* protein synthesis)

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Communicated by P. K. Stumpf, December 23, 1980

ABSTRACT The nitrogen regulatory locus "*glnR*" of *Escherichia coli* and *Salmonella typhimurium* is composed of two cistrons, which we propose to call *ntrB* and *ntrC* (*nitrogen regulation* B and C). Frameshift mutations in *ntrB* and *ntrC* were isolated on a λ phage that carries the *E. coli ntrB* and *ntrC* genes and the closely linked *glnA* gene, the structural gene encoding glutamine synthetase [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2]; mutations were selected as suppressors of *glnF* (which we propose to rename *ntrA*), a selection used previously to isolate *glnR* mutations. Phage DNA from one mutant (*ntrB*) failed to direct synthesis of a 36-kilodalton (kDal) protein whose synthesis was directed by DNA from the parent phage ($ntrB^+$) in a coupled *in vitro* transcription/translation system. DNA from three other mutants (*ntrC*) failed to direct synthesis of a 54-kDal protein; DNA from two of these mutants instead directed synthesis of smaller proteins, 53 and 50 kDal, respectively. In all four cases, DNA from frameshift revertants directed synthesis of both the 36-kDal and 54-kDal proteins. These results suggested that *ntrB* and *ntrC* were separate genes which encoded 36-kDal and 54-kDal protein products, respectively. Frameshift mutations in *ntrB* and *ntrC* complemented each other with regard to regulation of *glnA* expression *in vivo* and growth on arginine as nitrogen source, another nitrogen-controlled phenotype; this confirmed that *ntrB* and *ntrC* are separate cistrons that encode diffusible products. The *ntrB* and *ntrC* genes were also defined in *S. typhimurium*. Studies of mutant strains provided information on the roles of the *ntrB* and *ntrC* products in activation and repression of *glnA* expression and raised the possibility that these products function as a protein complex in regulating expression of nitrogen-controlled genes.

In enteric bacteria, synthesis of several proteins including glutamine synthetase [Gln synthetase; L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] is controlled by availability of nitrogen in the growth medium; synthesis of these proteins is increased under nitrogen-limiting conditions (1, 2) (reviewed in ref. 3). The products of two positive regulatory genes, *glnF* and *glnR* [called *glnG* in *Escherichia coli* (4)] are required for nitrogen control (5, 6). Mutations to loss of function of *glnF* and several previously characterized mutations to loss of function of *glnR* result in loss of ability to express nitrogen-controlled genes at high levels (5). We have proposed the following working model for the functions of the *glnF* and *glnR* products (5) (Fig. 1). The *glnF* product converts the *glnR* product to a form with positive regulatory character. By analogy with other bacterial regulatory mechanisms, the *glnF* product may catalyze synthesis of a low molecular weight signal of nitrogen limitation which binds to the *glnR* product, or the *glnF* product may interact directly with the *glnR* product.

In addition to having positive regulatory character, the *glnR* product has negative regulatory character (5). This was inferred because mutations to loss of function of *glnR* suppress the glutamine requirement caused by mutations to loss of function of *glnF*. That is, $glnR^-$ $glnF^-$ strains make more Gln synthetase than do $glnF^-$ strains and can grow in the absence of glutamine. Again by analogy with other bacterial regulatory mechanisms, the *glnR* product may be a macromolecular regulator of transcription which exists in two forms—one that represses transcription of *glnA*, the structural gene for Gln synthetase, and one that activates transcription of *glnA* and other genes subject to nitrogen control [in *Salmonella typhimurium* these include genes for amino acid transport components (7)]. The *glnF* product would lead to formation of the activator form. In a $glnF^-$ background the *glnR* product would have exclusively negative regulatory character.

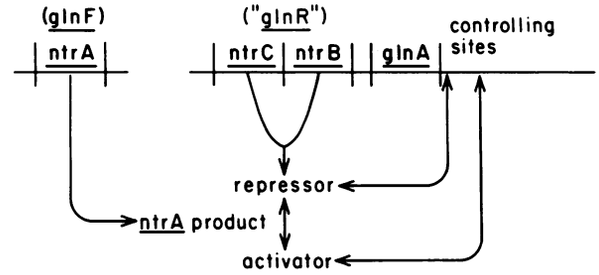

FIG. 1. Working model for control of *glnA* expression by the *ntrA* (previously called *glnF*) and *ntrB* and *ntrC* (previously called "*glnR*") products. The *ntrB* and *ntrC* products can repress transcription of *glnA* or activate transcription of *glnA* (and other genes under nitrogen control). The *ntrA* product leads to formation of activator. The model accommodates the possibility that the *ntrB* and *ntrC* products may work as a protein complex.

We have now found that mutations at the "*glnR*" locus which suppress *glnF* lie in two cistrons rather than one. We have identified the protein products of these cistrons and have studied their individual functions in nitrogen regulation *in vivo*. Because the "*glnR* gene" consists of two cistrons, nomenclature for this gene must be revised. We propose to rename *glnF* as *ntrA* and "*glnR*" as *ntrB* and *ntrC* because these genes have a pleiotropic role in nitrogen regulation which is not restricted to control of the *glnA* gene (*ntr* for *nitrogen regulation*).

MATERIALS AND METHODS

Specialized λ Transducing Phages. Seven λ*glnA* transducing phages carrying the *E. coli glnA* region ($\lambda glnA_1$–$\lambda glnA_7$) isolated by the technique of Shrenk and Weisberg (8) were obtained from James Friesen. Physical characterization of the

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Abbreviations: Aut, ability to utilize arginine as nitrogen source; Gln synthetase, glutamine synthetase; kDal, kilodalton; ICR, 2-chloro-6-methoxy-9-[3-(2-chloroethyl)aminopropylamino]acridine dihydrochloride.

phages indicated that each carried a different amount of bacterial DNA adjacent to *glnA* (unpublished data). To determine by complementation analysis whether λ*glnA* phages carried "*glnR*" (*ntrB* and *ntrC*) and to isolate "*glnR*" mutations on them, they were transferred to well-characterized *S. typhimurium* mutant strains (5, 6). For transfer they were first lysogenized on an *E. coli* F'*gal* episome (F'100-12) (9) by infecting *E. coli* strain NCM79 (*glnA3 recA56 galT23 argA22 srl300*::Tn*10*/F'100-12) with a heat-induced lysate of strain NCM6 [*glnA3 trpA9825* (λ cI857 S7; λ cI857 S7 $glnA_6$)] or a similar double lysogen and selecting Gln^+ transductants. Episomes carrying λ*glnA* phages were then transferred to *S. typhimurium* strain SK72 [Δ(*glnA-ntrB*)*60** *hisF645 galE1794*] by selecting Gal^+ transconjugants and screening for those that were also Gln^+. Subsequent transfers were made intragenerically. In all cases, segregation of episomes carrying transducing phages was demonstrated.

Isolation of Mutant Strains. *Salmonella ntrB* and *ntrC* mutations were isolated spontaneously by suppression of the glutamine requirement of strain SK100 carrying *ntrA76* (5). *E. coli ntrB* and *ntrC* mutations were isolated on λ$glnA_6$ by suppression of *ntrA* after mutagenesis (with 2-chloro-6-methoxy-9-[3-(2-chloroethyl)aminopropylamino]acridine dihydrochloride [ICR]) of *S. typhimurium* strain SK720 [*ntrA75* Δ(*glnA-ntrB*)*60 galE1823 metB869*::Tn*10*/F'100-12 (λ cI857 S7; λ cI857 S7, $glnA_6$)]. ICR-induced revertants were also isolated. Because λ does not grow in *S. typhimurium* (10), episomes carrying phages were transferred to an *E. coli* λ lysogen (NCM107) in order to isolate DNA template for use *in vitro*.

Complementation in *E. coli*. To perform intrageneric complementation analysis of *E. coli ntrB* and *ntrC* mutations, such mutations were initially recombined into the *E. coli* chromosome. To do this, lysates were prepared from *E. coli* λ$glnA_6$ lysogens carrying *ntrB* or *ntrC* mutations on the phage. The *ntrB* and *ntrC* mutations were recombined into the chromosome of *E. coli* strain NCM155 (*glnA3 galT23*) by selecting Gln^+ transductants at 42°C and screening for those which were Aut^- (unable to grow on arginine as nitrogen source) (5) and λ sensitive. The Aut^- phenotype (*ntrB* or *ntrC* lesion) was linked by P1-mediated transduction to a Tn*10* element inserted near *glnA* (obtained from S. Kushner). For complementation analysis, these strains were made *recA* (11), and λ$glnA_6$ carrying an *ntrB* or *ntrC* mutation was introduced (from an *E. coli* strain) on F'100-12. Gal^+ transconjugants were scored for Aut phenotype and resistance to λ. Complementing strains were Aut^+ and λ resistant. Noncomplementing strains were λ resistant but Aut^-; these strains were shown to contain λ$glnA_6$ by their ability to produce $glnA^+$ transducing phages after heat induction.

Gln Synthetase Assays. Cells were grown to stationary phase at 30°C and cell extracts were prepared as described (12) in 10 mM imidazole, pH 7.3/1 mM $MnCl_2$. Total activity of Gln synthetase was assayed as described (13, 14). *E. coli* Gln synthetase was also assayed by using the Mn^{2+} triethanolamine-dimethylglutaric acid assay mixture (pH 7.57) of Stadtman *et al.* (15) with similar results. Levels of Gln synthetase antigen were determined as described (7).

***In Vitro* Protein Synthesis.** Template DNA was extracted from λ*glnA* phages purified after heat induction of *E. coli* double lysogens (16). DNA was used at a concentration of 100–200 μg/ml. Standard conditions for coupled transcription/translation assays have been described (17). Assays were done at optimal concentrations of amino acids, tRNA, Mg^{2+}, K^+, and S-30 (to be described elsewhere). S-30 cell extracts were prepared from *S. typhimurium* strain SK417 (*ntrA76 relA1 hisT1504 his*Δ*2253*) or SK416 [Δ(*glnA-ntrB*)*60 zig-205*::Tn*10 relA1 hisT1504 his*Δ*2253*]. Proteins synthesized *in vitro* were labeled with [^{35}S]methionine (5 μCi per reaction; 1 Ci = 3.7×10^{10} becquerels) and were separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (18). Gels were fixed, stained (19), dried, and placed under film (Kodak X-Omat XR-2).

Chemicals. The frameshift mutagen ICR 191E was kindly donated by H. J. Creech. L-[^{35}S]Methionine (1017.8 Ci/mmol) and ^{14}C-labeled molecular weight standards were obtained from New England Nuclear.

RESULTS

Characterization of λ*glnA* Phages for the Presence of the "*glnR*" Locus by Complementation Analysis. Seven different λ transducing phages that carry the *E. coli glnA* region were characterized by complementation analysis for presence of the "*glnR* gene." Phages were transferred to *S. typhimurium* recipients containing a chromosomal deletion of part of the *glnA*–"*glnR*" region (see footnote *). All merodiploids were glutamine independent, confirming that all seven phages carried an intact *glnA* gene. We will refer to these phages as λ$glnA_1$–λ$glnA_7$. A merodiploid carrying one of these phages, λ$glnA_2$, failed to utilize arginine as nitrogen source, indicating that λ$glnA_2$ lacked a functional "*glnR* gene" (5).

Table 1 summarizes Gln synthetase activities for several of the *S. typhimurium* strains harboring λ*glnA* phages. Strain SK595 carrying λ$glnA_6$ ("$glnR^+$") was able to increase synthesis of Gln synthetase approximately 30-fold in response to nitrogen limitation, similar to a control strain (SK726) carrying the *E. coli* episome F'133, which covers the *glnA* region. The presence of a chromosomal *ntrA* mutation in such strains resulted in synthesis of low, unregulated levels of Gln synthetase (strains SK727 and SK588). In contrast to the above "$glnR^+$" merodiploids, strain SK574 carrying λ$glnA_2$ ("$glnR^-$") was unable to synthesize high levels of Gln synthetase under nitrogen-limiting conditions. The low level of synthesis in this strain was independent of *ntrA* (see strain SK584) as expected based on previous studies of *S. typhimurium* "*glnR*" mutant strains (5). Activation of synthesis of Gln synthetase from λ$glnA_2$ was restored in a merodiploid with a functional "*glnR*" locus on the chromosome (strain SK801) and was dependent on a functional *ntrA* gene (strain SK834). These results confirm that the "*glnR* product" works in *trans* and therefore is diffusible (5). They also indicate that *cis*-acting regulatory sites adjacent to *glnA* on λ$glnA_2$ are intact.

Identification of Two Protein Products of the "*glnR*" Locus (*ntrB* and *ntrC*). When DNA from λ$glnA_6$ was used as template in a coupled *in vitro* transcription/translation system, it directed synthesis of six major bacterial proteins [75, 68.5, 56, 54, 47.5, and 36 kilodaltons (kDal), respectively] (Fig. 2, lane 2) (Gln synthetase, the product of the *glnA* gene, was identified by precipitation with specific antiserum as the 56-kDal protein). DNA from λ$glnA_2$, which was "$glnR^-$" as judged by complementation analysis, failed to direct synthesis of the 54- and 36-kDal proteins and of a 47.5-kDal protein (Fig. 2, lane 1). Synthesis of the 54- and 36-kDal proteins was directed by six independent λ$glnA^+$ "$glnR^+$" phages, including λ$glnA_6$. The 47.5-kDal protein was not correlated with the presence or absence of a functional "*glnR*" locus because several λ$glnA^+$ "$glnR^+$" phages as well as λ$glnA_2$ failed to direct its synthesis.

* Previously called Δ(*glnA-glnR*)*60* (5). This deletion apparently extends into *ntrB* because it fails to recombine with *ntrB* point mutations; it does recombine with all *ntrC* point mutations that we have tested and therefore may or may not extend into *ntrC*. The deletion was phenotypically $NtrC^-$ with respect to isolation of mutations on λ$glnA_6$.

Table 1. Analysis of λ*glnA* phages for the presence of "*glnR*"

Strain*	Relevant genotype	Gln synthetase,† μmol/min per mg	
		N excess	N limiting
SK726	Δ(*glnA*-"*glnR*")‡/F′133 *glnA*$^+$ "*glnR*$^+$"§	0.05	1.0
SK727	Δ(*glnA*-"*glnR*")*ntrA75*/F′133 *glnA*$^+$ "*glnR*$^+$"	0.02	0.02
SK595	Δ(*glnA*-"*glnR*")/λ*glnA*$_6$ ("*glnR*$^+$")	0.04	1.04
SK588	Δ(*glnA*-"*glnR*")*ntrA75*/λ*glnA*$_6$ ("*glnR*$^+$")	0.02	0.02
SK574	Δ(*glnA*-"*glnR*")/λ*glnA*$_2$("*glnR*$^-$")	0.06	0.17
SK584¶	Δ(*glnA*-"*glnR*")*ntrA75*/λ*glnA*$_2$ ("*glnR*$^-$")	0.05	0.15
SK801	*glnA192* "*glnR*$^+$"/λ*glnA*$_2$ ("*glnR*$^-$")	0.15	1.86
SK834	*glnA192* "*glnR*$^+$" *ntrA202*::Tn*10*/λ*glnA*$_2$ ("*glnR*$^-$")	0.07	0.08

* For *ntrA*$^+$ strains there were <4% λ segregants; for *ntrA*$^-$ strains there were <17% λ segregants. In both *ntrA*$^+$ and *ntrA*$^-$ strains, segregation of F′133 occurred at <4%.

† N excess: minimal glucose medium (20) with 20 mM NH_4^+ and 3 mM glutamine as nitrogen sources. N limiting: 3 mM glutamine as sole nitrogen source. Both media were supplemented with 0.2 mM arginine, uracil, and hypoxanthine (and 2 μM thiamin), major end products that contain a nitrogen atom derived from glutamine, to stimulate the growth rate of mutant strains.

‡ Strain SK35 [Δ(*glnA-ntrB*)*60*] had no detectable Gln synthetase activity. For explanation of genotype see text footnote *.

§ Strains carrying F′133 also contain the *metE863*::Tn*10* mutation, which is covered by this episome.

¶ This strain was demonstrated to contain an *ntrA* mutation by genetic analysis (6).

To determine whether either the 54-kDal or 36-kDal protein was the "*glnR*" product, we isolated "*glnR*" frameshift mutations on λ*glnA*$_6$. DNA from one such mutant (*ntrB1*) failed to direct synthesis of the 36-kDal protein *in vitro* (Fig. 2, lane 3). DNA from three other mutants (*ntrC3*, *ntrC4*, and *ntrC5*) failed to direct synthesis of the 54-kDal protein (Fig. 2, lanes 5, 7, and 9); DNA from two of these mutants (*ntrC4* and *ntrC5*) instead directed synthesis of smaller proteins, 53 and 50 kDal, respectively (presumably, premature termination products). In all cases, DNA from frameshift revertants directed synthesis of both the 36-kDal and 54-kDal proteins (Fig. 2, lanes 4, 6, 8, and 10). DNA from both frameshift mutants and revertants directed synthesis of all other proteins, including Gln synthetase, whose synthesis was directed by DNA from λ*glnA*$_6$. These results indicated that the "*glnR* gene" (5) might, in fact, be two genes (*ntrB* and *ntrC*) that encoded 36-kDal and 54-kDal protein products, respectively.

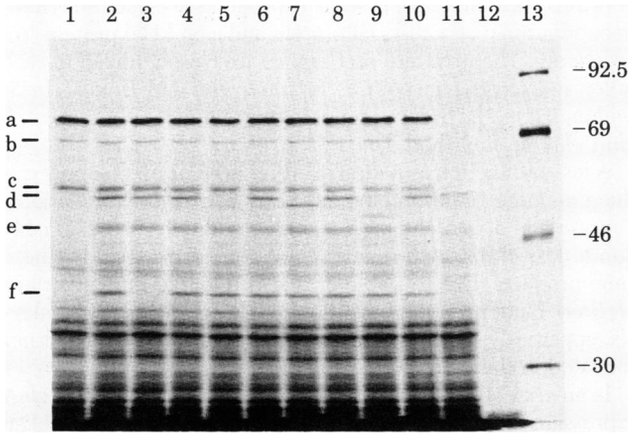

FIG. 2. Autoradiogram of [^{35}×S]methionine-labeled proteins synthesized *in vitro* after separation by sodium dodecyl sulfate/9% polyacrylamide gel electrophoresis. Templates: λ*glnA*$_2$ (*ntrB*$^-$ *ntrC*$^-$) (lane 1); λ*glnA*$_6$ (*ntrB*$^+$ *ntrC*$^+$) (lane 2); λ*glnA*$_6$ *ntrB1* (lane 3); revertant of *ntrB1* (lane 4); λ*glnA*$_6$ *ntrC3* (lane 5); revertant of *ntrC3* (lane 6); λ*glnA*$_6$ *ntrC4* (lane 7); revertant of *ntrC4* (lane 8); λ*glnA*$_6$ *ntrC5* (lane 9); revertant of *ntrC5* (lane 10); λcI857S7 (lane 11); no DNA (lane 12). Letters a–f at the left indicate bacterial proteins: a, 75 kDal; b, 68.5 kDal; c, 56 kDal; d, 54 kDal, e, 47.5 kDal; and f, 36 kDal. Molecular weights of standard proteins (New England Nuclear) are indicated at the right; in order of decreasing molecular weight the standards (lane 13) were phosphorylase b, bovine serum albumin, ovalbumin, and carbonic anhydrase. Because there were no protein bands of molecular weight higher than phosphorylase b, the top portion of the gel is not included in the figure.

Complementation Analysis of *E. coli ntrB* and *ntrC* Mutations. ICR-induced mutations *ntrB1*, *ntrB2*, *ntrC3*, and *ntrC4* isolated on λ*glnA*$_6$ were recombined into the *E. coli* chromosome and analyzed for their effects on synthesis of Gln synthetase (Table 2.) Strain NCM67 (*ntrB*$^+$ *ntrC*$^+$) increased synthesis of Gln synthetase 10-fold under N-limiting conditions. The two *ntrC* strains (NCM 131 and -133) produced low levels of Gln synthetase and failed to increase its synthesis under N-limiting conditions. The two *ntrB* strains (NCM 130 and -134) produced high levels of Gln synthetase even when grown with a high concentration of ammonia.

Merodiploid strains with an *ntrB* mutation on the chromosome and an *ntrC* mutation on λ*glnA*$_6$ (or vice versa) regained the ability to regulate synthesis of Gln synthetase over about a 10-fold range in response to availability of ammonia (Table 2, strains NCM 147, -145, -150, and -152) and to utilize arginine as nitrogen source. Control strains carrying *ntrB* or *ntrC* mutations on both chromosome and λ did not regain the ability to regulate synthesis of Gln synthetase (strains NCM 149, -151, -146, and -148) or grow on arginine. Thus, mutations in *ntrB* and *ntrC* complement each other, indicating that *ntrB* and *ntrC* are separate cistrons that encode diffusible products and that both products are required for what had previously been defined as "*glnR*" function.

λ*glnA*$_2$, which was "*glnR*$^-$" and failed to direct synthesis of both the 54-kDal and 36-kDal proteins, was demonstrated to lack both *ntrB* and *ntrC* function genetically because it failed to complement either the *ntrB1* or *ntrC4* mutation on the *E. coli* chromosome for growth on arginine as nitrogen source (data not shown).

Analysis of *S. typhimurium ntrB* and *ntrC* Mutations. Having isolated *E. coli ntrB* and *ntrC* mutations, we wanted to determine whether similar mutations occurred in *S. typhimurium*. We therefore studied a number of spontaneous *ntrA* suppressors in *S. typhimurium*. These were crossed into an *ntrA*$^+$ back-

Table 2. Complementation of *E. coli ntrB* and *ntrC* mutations for regulation of synthesis of Gln synthetase and growth on arginine as nitrogen source

Strain	Relevant genotype*	Gln synthetase,† μmol/min-mg N excess	N limiting	Fold increase‡	Aut§
NCM67	*ntrB*$^+$ *ntrC*$^+$	0.08	0.81	10.1	+
NCM131	*ntrC3*	0.05	0.05	1.0	−
NCM133	*ntrC4*	0.06	0.04	0.7	−
NCM130	*ntrB1*	0.86	2.1	2.4	−
NCM134	*ntrB2*	0.75	2.3	3.1	−
NCM147	*ntrB1*/λ*glnA*$_6$ *ntrC3*	0.27	3.3	12.2	+
NCM145	*ntrB1*/λ*glnA*$_6$ *ntrC4*	0.14	1.6	11.4	+
NCM150	*ntrC4*/λ*glnA*$_6$ *ntrB1*	0.19	1.91	10.1	+
NCM152	*ntrC4*/λ*glnA*$_6$ *ntrB2*	0.17	3.7	21.8	+
NCM149	*ntrC4*/λ*glnA*$_6$ *ntrC4*	0.16	0.13	0.8	−
NCM151	*ntrC4*/λ*glnA*$_6$ *ntrC3*	0.20	0.18	0.9	−
NCM146	*ntrB1*/λ*glnA*$_6$ *ntrB1*	1.26	1.8	1.4	−
NCM148	*ntrB1*/λ*glnA*$_6$ *ntrB2*	2.45	3.8	1.6	−

* The subscript in λ*glnA*$_6$ designates the particular transducing phage and is not a mutant allele number. Gal$^-$ segregants of strains NCM147 and NCM152 were also Aut$^-$ and λ sensitive. Segregation occurred at <15%. Gal$^-$ segregants of strains NCM145 and NCM150 remained Aut$^+$ and λ resistant; because the strains were *recA56*, this suggests that λ had integrated into the chromosome.

† For all haploid strains and for merodiploid strains NCM146 and NCM147, levels of Gln synthetase activity and antigen were correlated. Media were as in Table 1.

‡ Ratio of Gln synthetase activity under N limiting conditions to that under N excess conditions.

§ Ability to use arginine as sole nitrogen source. Glucose was the carbon source.

ground and had properties similar to those of *E. coli ntrB* or *ntrC* mutations. Strains with mutations that recombined with Δ(*glnA-ntrB*)*60* and were therefore distal to *glnA* failed to increase synthesis of Gln synthetase under N-limiting conditions (Table 3, strains SK639 and -652) and thus resembled *E. coli ntrC* strains. Several "*glnR*" strains characterized previously had the same properties and failed to increase synthesis of nitrogen-controlled proteins (amino acid transport components) in addition to Gln synthetase (5). Strains with mutations that did not recombine with Δ(*glnA-ntrB*)*60* and were therefore proximal to *glnA* synthesized high levels of Gln synthetase even when grown with high ammonia (strains SK611 and -622) and thus resembled *E. coli ntrB* strains. Such strains have not been characterized previously. In an *ntrA*$^-$ background, both *ntrB* and *ntrC* mutations resulted in synthesis of low, unregulated levels of Gln synthetase (strains SK517, -530, -488, and -500). These levels were higher than those in an *ntrA* strain (strain SK100), presumably accounting for suppression of its glutamine requirement. The properties of *ntrB* and *ntrC* mutations were essentially the same in *S. typhimurium* and *E. coli* (Tables 2 and 3 and unpublished data). Based on the above results the *ntrB* and *ntrC* cistrons apparently occur in *S. typhimurium* as well as *E. coli*, and the functions of both are required for normal nitrogen regulation.

Table 3. Gln synthetase levels in *Salmonella ntrB* and *ntrC* strains

Strain	Relevant genotype	Gln synthetase,* μmol/min-mg N excess	N limiting	Aut†
TA831	*ntrB*$^+$ *ntrC*$^+$	0.13	1.8	+
SK639	*ntrC302*	0.03	0.05	−
SK652	*ntrC315*	0.02	0.06	−
SK611	*ntrB243*	1.2	2.7	−
SK622	*ntrB285*	1.2	2.6	−
SK100	*ntrA76*	<0.01	<0.01	−
SK517	*ntrA76 ntrC302*	0.02	0.05	−
SK530	*ntrA76 ntrC315*	0.02	0.06	−
SK488	*ntrA76 ntrB243*	0.03	0.06	−
SK500	*ntrA76 ntrB285*	0.03	0.06	−

* Levels of Gln synthetase activity and antigen were correlated. Media were as in Table 1.

† Ability to use arginine as sole nitrogen source.

DISCUSSION

The previously defined nitrogen regulatory locus ("*glnR*") of *E. coli* and *S. typhimurium* is not a single gene but is composed of two cistrons, *ntrB* and *ntrC*. The products of these genes have been identified as a 36-kDal protein and a 54-kDal protein, respectively. The *ntrB* and *ntrC* genes are closely linked to each other and to *glnA* in both *S. typhimurium* and *E. coli*. In *S. typhimurium*, the order of genes in this region is *polA ntrC ntrB glnA rha* (unpublished data).

A loss-of-function mutation in either *ntrB* or *ntrC* suppresses the glutamine requirement caused by *ntrA* (*glnF*) mutations. Suppression of *ntrA* by *ntrB* or *ntrC* mutations is presumably due to loss of negative regulation of *glnA* expression by these gene products (Fig. 1; Table 3; ref. 5). Double-mutant strains *ntrB ntrA* and *ntrC ntrA* synthesize Gln synthetase at low levels; apparently, *glnA* expression can be neither repressed nor activated in these strains.

In an *ntrA*$^+$ background, *ntrB* and *ntrC* mutations affect *glnA* expression differently. An *ntrC* strain, which lacks the 54-kDal protein, appears to have no residual nitrogen regulatory function (5). It neither activates nor represses *glnA* expression—Gln synthetase is synthesized at low levels. An *ntrB* strain, which lacks the 36-kDal protein, retains ability to activate *glnA* expression fully and, in fact, synthesizes Gln synthetase at high levels in a defined minimal medium containing a high concentration of ammonia (sufficient to decrease synthesis in a wild-type strain) (Tables 2 and 3). From these results we infer that the *ntrC* product (54-kDal protein) without the *ntrB* product (36-kDal protein) is sufficient to mediate *ntrA*-dependent activation of *glnA* expression. Both the *ntrB* and *ntrC* products appear to be required for negative regulation of *glnA* expression.

A working model that can account for participation of the *ntrA*, *ntrB*, and *ntrC* products in regulating *glnA* expression

and that of other nitrogen-controlled genes (5) is outlined in Fig. 1. It is clear that *ntrB* and *ntrC* mutations alter expression of nitrogen-controlled genes. An attractive hypothesis is that the *ntrB* and *ntrC* products function as a protein complex and act directly at the level of transcription.

We are grateful to Dr. James Friesen for sending us the λ*glnA* phages, M. K. McKinley for expert assistance in performing some of the complementation studies, and G. F.-L. Ames, B. Bochner, L. N. Csonka, and J. L. Ingraham for thoughtful criticisms of the manuscript. This work was supported by U.S. Public Health Service Grants GM21307 and GM27307 to S.K. and S.A., respectively.

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