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Coordinated Regulation of Amino Sugar-Synthesizing and -Degrading Enzymes in *Escherichia coli* K-12

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The intracellular concentration of the enzyme glucosamine-6-phosphate synthase, encoded by the gene *glmS* in *Escherichia coli*, is repressed about threefold by growth on the amino sugars glucosamine and *N*-acetylglucosamine. This regulation occurs at the level of *glmS* transcription. It is not due just to the presence of intracellular amino sugar phosphates, because mutations which derepress the genes of the *nag* regulon (coding for proteins involved in the uptake and metabolism of *N*-acetylglucosamine) also repress the expression of *glmS* in the absence of exogenous amino sugars.

The amino sugars D-glucosamine (GlcN) and *N*-acetyl-D-glucosamine (GlcNAc) are essential components of the peptidoglycan of bacterial cell walls and of the lipopolysaccharide of the outer membrane in gram-negative bacteria. When present in the environment both compounds are taken up and used for cell wall and lipid A synthesis (9). In the absence of amino sugars in the environment the bacteria must synthesize glucosamine-6-phosphate from fructose-6-phosphate and glutamine via the enzyme glucosamine-6-phosphate synthase (L-glutamine:D-fructose-6-phosphate amidotransferase) (EC 2.6.1.16). This enzyme is encoded by the gene *glmS*, which maps to 84 min on the *Escherichia coli* chromosome (39). It is a dimer of identical, 68-kDa subunits showing classic properties of amidotransferases (4, 5, 12, 18). It is subject to weak product inhibition at millimolar concentrations of GlcN-6-P but is not subject to allosteric regulation (36, 40), unlike the equivalent eukaryotic enzymes, which are allosterically inhibited by UDP-GlcNAc (for examples, see references 10, 17, 19, and 41).

The amino sugars are also sources of carbon and nitrogen to the bacteria; in particular, GlcNAc produces growth rates comparable to those produced by glucose. Growth on amino sugars induces the enzymes necessary for the uptake and absorption of the sugars encoded by the divergent *nagE* and *nagBACD* operons located at 15 min on the *E. coli* map. *nagE* encodes the GlcNAc-specific enzyme II of the phosphoenolpyruvate-dependent phosphotransferase system, and *nagA* and *nagB* encode the two enzymes, *N*-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25) and glucosamine-6-phosphate deaminase (glucosamine-6-phosphate:fructose-6-P isomerase [deaminating]) (EC 5.3.1.10), necessary for the degradation of GlcNAc-6-P to GlcN-6-P and hence to fructose-6-P (Fig. 1) (26, 29, 33, 37, 40). GlcN is taken up primarily by the generic hexose phosphotransferase system transporter, encoded by the genes *manXYZ* (7, 15), to give GlcN-6-P and is then converted to fructose-6-P via the *nagB*-encoded deaminase.

The deaminase is a well-characterized allosteric enzyme (6). It is a hexamer of identical subunits with a molecular mass of 29.7 kDa which exist in a trimer-of-dimers structure with one threefold axis of symmetry and three local twofold axes (2, 13). It is a K-type allosteric enzyme activated by GlcNAc-6-P (6). The deacetylase is a tetramer of identical 41-kDa subunits which has only recently been purified to homogeneity (34).

The early work of White (40) showed that growth on GlcNAc induced both the deaminase and deacetylase activities about 10-fold. Peri et al. (26) found similar values, although Vogler and Lengeler (37) found much higher induction ratios. The use of protein fusions between *nagE*, *nagB*, or *nagA* and *lacZ* showed that all three *nag* genes are induced, in parallel, about 20-fold (28, 30). mRNA analysis (29) has shown that the induction occurs at the transcriptional level, as is usual for operons encoding inducible catabolic functions. The expression of the genes for deaminase and deacetylase is controlled by a repressor encoded by the *nagC* gene. The signal for the induction of the regulon is the presence of GlcNAc-6-P, i.e., the intracellular product of the transport of GlcNAc. This phosphorylated form of GlcNAc prevents the repressor from binding to its operators (31).

The enzyme activity measurements of White (40) and Vogler et al. (38) suggested that the activity of GlcN synthase decreased during growth on the amino sugars about fourfold. However, these measurements are complicated by the fact that the high level of deaminase induced by growth on GlcNAc masks the GlcN-6-P-synthesizing activity of the synthase in crude extracts. In this work we have reinvestigated this question but used protein levels measured by Western blotting (immunoblotting) to confirm that externally supplied amino sugars not only induce the synthesis of the degradative enzymes but also repress the synthesis of GlcN-6-P synthase. We have also examined various mutant strains and show that the regulation responds to the activated status of the *nag* regulon and not just to the presence of GlcNAc-6-P.

MATERIALS AND METHODS

Bacteria. The bacterial strains used are given in Table 1. Bacteria were grown at 37°C in the synthetic minimal A medium (23) or morpholinepropanesulfonic acid (MOPS)

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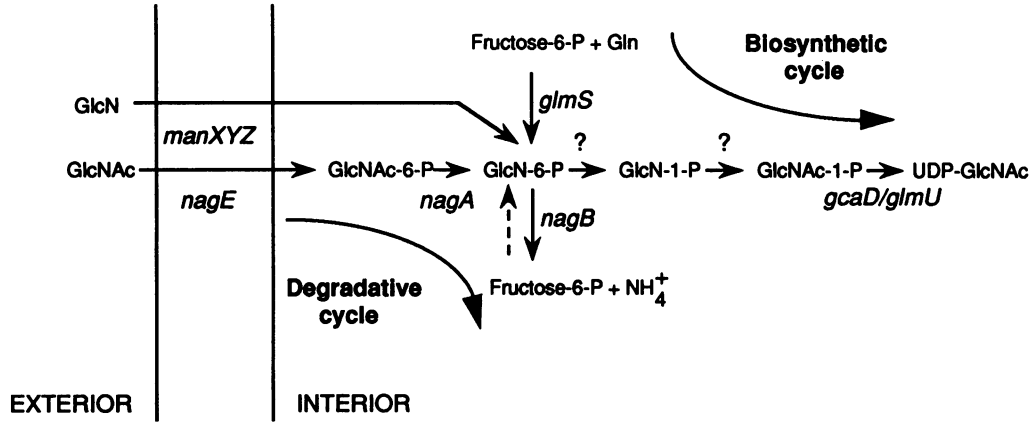

FIG. 1. Metabolism of glucosamine and *N*-acetylglucosamine. The intermediates and genes encoding the enzymes involved in the biosynthetic and degradative pathways are indicated. The genes and/or enzymes that have not been identified are indicated with a question mark. *gcaD* encodes GlcNAc-1-P uridyltransferase; this gene has been identified in *E. coli* as the gene (previously called *urf*) immediately upstream of *glmS* on the chromosome and will be named *glmU* (14, 20).

medium (25) supplemented with 0.2% glucose, GlcNAc, or GlcN or 0.4% glycerol and with the amino acids arginine and histidine at 50 μg/ml or 0.5% Casamino Acids.

Immunoblotting. Bacteria were grown to an optical density at 650 nm (A_{650}) of about 0.8, harvested by centrifugation, and frozen at −80°C. The cells were lysed by sonication, debris was removed by centrifugation, and the protein extracts were adjusted to 10 mg/ml. Aliquots (20 to 50 μg) were analyzed on small (9 by 6 cm^2) sodium dodecyl sulfate (SDS)-polyacrylamide (12.5%) gels. The proteins were electrophoretically transferred to nitrocellulose (Hybond-C; Amersham). The blotting time was kept short (2.5 to 4 h at 300 mA) since we discovered that deaminase was poorly retained on the membrane and a large fraction passed through the primary membrane and was detectable on subsequent layers of nitrocellulose. The proteins transferred to the membrane were revealed by Ponceau S red. The membranes were blocked with 5% nonfat milk or 3% gelatin and treated with polyclonal antibodies raised against GlmS, NagB, or NagA in phosphate-buffered saline–1% milk–0.2% Tween. Initial experiments showed no cross-reaction with other proteins under the conditions used, and subsequently the membranes were incubated with the three antibodies together, diluted 1/10,000 or 1/5,000. The bound antibodies were detected by reaction with ^{125}I-labelled protein A (Amersham) or colorimetrically with a peroxidase-linked secondary antibody. The ^{125}I-protein A blots were quantitated either with a PhosphorImager (Molecular Dynamics) or by determining the radioactivity in individually excised bands in a gamma counter. Gels run with different amounts of the culture extracts were quantitated and gave comparable results for the level of expression of the different proteins, except that the basal levels of NagB and NagA were not measurable with these antibodies.

TABLE 1. Bacterial strains used

Strain	Genotype	Reference
IBPC5321	*thi-1 argG6 argE3 his-4 mtl-1 xyl-5 rpsL tsx-29*? Δ*lacX74*	29
IBPC546	IBPC5321 *nagB*::Km	30
IBPC524	IBPC5321 *nagA*::Cm	30
IBPC529C	IBPC5321 *nagC*::Cm	30
IBPC590	IBPC5321 Δ*nagEBACD*::Tc	32
IBPC574	IBPC5321 *nagA1 nagC11 zbf-507*::Tn*10*	32

Measurements of gene-specific mRNA levels. Total RNA was extracted from exponentially growing cultures of IBPC5321 by the hot-phenol method. Abortive reverse transcription experiments (1) were carried out with 5′-end-labelled oligonucleotide primers for *nagB* (GATCGGCAGT CGGTTTGAACGC) and for *glmS* (CAGAGTCATATCCG CGGTATTC). They hybridized to regions from nucleotides 75 to 97 and 71 to 93 after the start codon of the two genes, respectively. Primer extension with avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) was carried out as described elsewhere (35) in the presence of ddCTP instead of dCTP (*nagB*) or ddTTP instead of dTTP (*glmS*) to produce extension products of 30 and 32 nucleotides, respectively. Transcripts were analyzed on 15% denaturing acrylamide gels and quantitated by cutting out the radioactive band from the gel and by Cerenkov radiation counting.

Enzymes. The purification to homogeneity of deacetylase and its assay will be reported elsewhere (34). Deaminase (3, 6) and synthase (4) were purified as described previously. Antibodies were produced by injecting New Zealand White rabbits according to a standard immunological regimen.

RESULTS

Growth on glucosamine or *N*-acetylglucosamine represses GlcN-6-P synthase levels. Extracts of exponentially growing cultures of a wild-type *E. coli* strain in minimal medium with glucose, glucosamine, or *N*-acetylglucosamine as the carbon source were analyzed on polyacrylamide gels, electroblotted to nitrocellulose membranes, and treated with antibodies raised against the three purified proteins (GlcN-6-P deaminase, NagB; GlcNAc-6-P deacetylase, NagA; and GlcN-6-P synthase, GlmS). The presence of amino sugars in the growth medium produces high levels of both deacetylase and deaminase. Unfortunately, our antibodies are not sensitive enough to detect the uninduced levels of these two proteins (Fig. 2A, lanes 5 and 6). This is partly due to the fact that the response of these antibodies is not linear with increasing

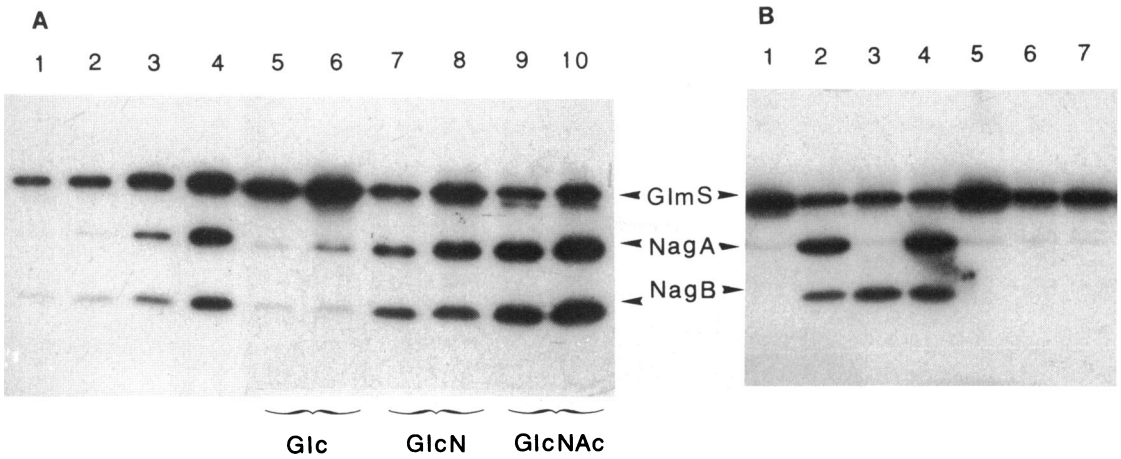

FIG. 2. Immunoblotting analysis of GlcN-6-P deaminase (NagB), GlcNAc-6-P deacetylase (NagA), and GlcN-6-P synthase (GlmS) in bacteria grown in the presence of amino sugars (A) or carrying mutations in the *nag* genes (B). (A) Bacteria (IBPC5321) were grown in minimal medium with 0.2% glucose (lanes 5 and 6), 0.2% GlcN (lanes 7 and 8), or 0.2% GlcNAc (lanes 9 and 10), and extracts were made as described in Materials and Methods. Aliquots containing 50 μg (lanes 5, 7, and 9) or 100 μg (lanes 6, 8, and 10) were analyzed on an SDS-acrylamide gel. Lanes 1 to 4 contained a mixture of the three purified proteins, NagB, NagA, and GlmS, so that the lanes contain 5, 10, 20, or 50 ng of each protein, respectively. The separated proteins were electrophoretically transferred (3 h at 300 mA) to nitrocellulose (Hybond-C; Amersham). The figure shows an autoradiograph of the membrane treated with antibodies against GlmS, NagA, and NagB as revealed with reaction with ^{125}I-labelled protein A. (B) Exponential-phase cultures of the various mutant strains grown in minimal medium with 0.2% glucose were harvested and treated as described for panel A. Lane 1, IBPC5321 (wild type grown in glucose); lane 2, IBPC5321 (grown in GlcNAc); lane 3, IBPC524 (*nagA*::Cm); lane 4, IBPC529C (*nagC*::Cm); lane 5, IBPC574 (*nagA1 nagC11*); lane 6, IBPC546 (*nagB*::Km); lane 7, IBPC590 (Δ*nagEBACD*).

amounts of the pure proteins in the range from 5 to 50 ng (Fig. 2A, lanes 1 to 4). On the other hand, the antibody raised against the synthase does respond linearly in the same concentration range (Fig. 2A, lanes 1 to 4). Quantitation of the amount of ^{125}I-labelled protein A bound to the nitrocellulose shows that growth on GlcN or GlcNAc represses the synthase level about two- to threefold, respectively (Fig. 2B, lanes 5 to 10, and Table 2). It is not possible to quantitate the induction of deaminase and deacetylase by this technique because of the insensitivity of their respective antibodies. However, previous experiments using enzyme assays (26, 37, 40) or gene fusions with *lacZ* (30) have shown that *nagB* induction is about 5-fold with GlcN and 20-fold with GlcNAc. The experiments of White (40) suggested that GlcN did not induce the deacetylase. However, the immunoblotting experiments detected a significant increase in the NagA protein in the GlcN-grown extracts. Enzymatic tests for deacetylase activity either coupled to the deaminase to follow the synthesis of fructose-6-P or by spectrophotometric measurement of the loss of GlcNAc-6-P (34) verified that the induced deacetylase protein in our strain grown on GlcN is active. We have no explanation for this discrepancy with the data of White, unless it is due to a difference in genetic background.

TABLE 2. Relative levels of glucosamine-6-phosphate synthase in different strains

Strain	Genotype	Carbon source[a]	Relative GlmS[b]
IBPC5321	Wild type	Glucose	1
		GlcN	0.49
		GlcNAc	0.32
IBPC546	*nagB*	Glucose	0.65
IBPC524	*nagA*	Glucose	0.51
IBPC529C	*nagC*	Glucose	0.60
IBPC590	Δ*nagEBACD*	Glucose	0.67
IBPC574	*nagA1 nagC11*	Glucose	1.5

[a] Bacteria were grown in minimal medium with 50 μg of arginine and histidine per ml and the carbon source indicated at 37°C.

[b] Estimated from immunoblots treated with ^{125}I-protein A. Values are the mean of results calculated from four gels (IBPC5321 results) or two gels (other strains) and are relative to the value for the wild-type strain grown in glucose.

Growth on the amino sugars decreases *glmS* mRNA levels. The immunoblotting experiments show that the decrease in synthase activity detected by White (40) is due to a decrease in the amount of synthase protein (rather than an effect on the specific activity of the protein). The abortive primer extension technique (1) was used to compare the levels of mRNA in these different strains. This technique was employed because it yields more quantitative data concerning mRNA levels at a particular position within the transcript. Reverse transcriptase is very sensitive to secondary structures within the mRNA, which reduces the intensity of signals from longer transcripts. Oligonucleotides complementary to near the 5′ end of the *nagB* and *glmS* mRNAs, within their structural genes, were used to prime reverse transcriptase reactions in the presence of three deoxynucleotides and one dideoxynucleotide. The short transcript corresponding to the stop at the position of the dideoxynucleotide is a measure of the amount of mRNA corresponding to that part of the gene. Growth on either glucosamine or *N*-acetylglucosamine produces a considerable increase in *nagB* mRNA (Fig. 3, lanes 1 to 8) and a significant decrease in the *glmS* mRNA (Fig. 3, lanes 9 to 16) compared with mRNA extracted from a strain grown on glucose or glycerol. Quantitation of these bands on different gels gives an increase in *nagB* of about 5- and 20-fold for growth on glucosamine and *N*-acetylglucosamine. A similar induction in *nagA* mRNA was measured during growth on either amino sugar (28), while either sugar decreased *glmS* mRNA about two- to threefold. These results imply that the regula-

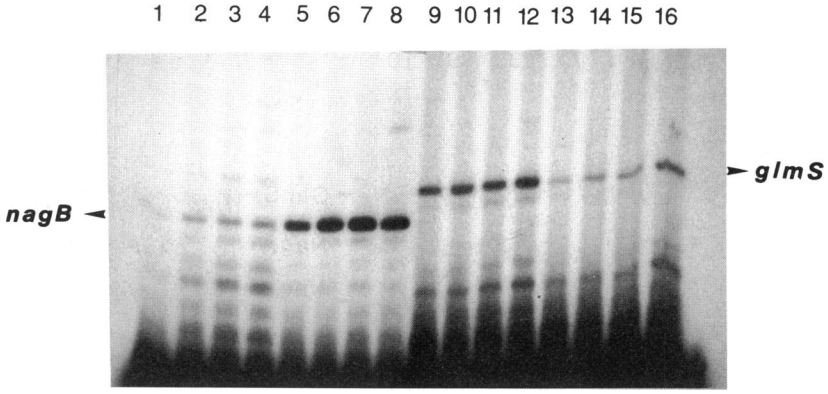

FIG. 3. Abortive primer extension analysis of mRNA levels of GlcN-6-P deaminase and GlcN-6-P synthase during growth on amino sugars. Total RNA from bacteria grown on minimal medium supplemented with 0.4% glycerol (lanes 1, 2, 9, and 10), 0.2% glucose (lanes 3, 4, 11, and 12), 0.2% GlcN (lanes 5, 6, 13, and 14), and 0.2% GlcNAc (lanes 7, 8, 15, and 16) were used as templates for reverse transcriptase with oligonucleotide primers specific for *nagB* (lanes 1 to 8) or *glmS* (lanes 9 to 16) as described in Materials and Methods. Lanes 1, 3, 5, 7, 9, 11, 13, and 15, 15 μg of RNA; other lanes, 30 μg of RNA. The short transcripts observed in the presence of ddCTP (*nagB*) or ddTTP (*glmS*) are indicated.

tion of *glmS* expression occurs at the transcriptional level. Glucose and GlcNAc give similar growth rates (doubling times of 50 to 55 min), and both exert catabolite repression (8), while glycerol and GlcN give doubling times of 90 to 100 min at 37°C and exert no catabolite repression. Since there is a clear decrease in the level of *glmS* mRNA in bacteria grown on GlcNAc or GlcN-containing medium compared with that in bacteria grown on a medium with a similar growth rate and catabolic repression level, i.e., glucose and glycerol, respectively, the regulation of *glmS* expression is specifically due to the presence of the amino sugar and is not the consequence of one of the global control mechanisms like catabolite repression or growth rate regulation.

Mutations which derepress the degradative enzymes repress the biosynthetic enzyme. In an attempt to further understand the regulation of *glmS* expression, we tested the levels of deaminase, deacetylase, and synthase in strains carrying mutations in various genes. A mutation in the gene for the repressor provokes the derepression of the *nag* regulon (31). This produces a level of deaminase and deacetylase during growth on glucose equivalent to that during growth on GlcNAc (Fig. 2B, lanes 2 and 4); moreover, there is a decrease in the level of synthase to about 60% of that of the wild-type strain, i.e., similar to that observed during growth on GlcNAc (Fig. 2B, lanes 1, 2, and 4, and Table 2). This result is in some ways surprising since the overproduced deaminase, in the absence of exogenous amino sugars, could be expected to degrade the GlcN-6-P synthesized by the synthase. This demonstrates that the separation of the biosynthetic and degradative enzymes and their products within the cell is sufficiently strict that a futile cycle (as discussed previously [38, 40, 42]) involving the synthesis and degradation of GlcN-6-P with net conversion of glutamine to ammonia is avoided, even under conditions of excess deaminase. It should be noted that the turnover numbers of deaminase and synthase differ by a factor of about 100 (4, 6), with deaminase being the more active enzyme.

A mutation in the gene for the deacetylase also provokes the derepression of the *nag* regulon by allowing the accumulation of GlcNAc-6-P. A strain carrying the *nagA*::Cm mutation (IBPC524) produces no deacetylase but synthesizes high levels of deaminase and an amount of synthase similar to that in the *nagC* strain (Fig. 2B, lane 3, and Table 2). An insertion mutation in *nagB* (IBPC546), the gene for deaminase, provokes a partial derepression of the regulon, and this was proposed to be because it is polar on the expression of the downstream *nagA* gene (31). The immunoblotting experiment confirmed this interpretation: neither deaminase nor deacetylase is detected in the *nagB*::Km strain (Fig. 2B, lane 6), and the level of synthase is also diminished (Table 2). In a strain where the whole *nag* regulon is deleted (IBPC590), there is no deaminase and deacetylase and the level of synthase is also reduced compared with that in the wild-type strain grown on glucose (Fig. 2B, lane 7, and Table 2). These results concerning the effect on *glmS* expression were also verified by direct GlcN-6-P synthase activity measurements on extracts of the strains in which deaminase was not overproduced. In strains IBPC546 and IBPC590 there was about 30% of the glucosamine synthase activity of the wild-type strain (IBPC5321) grown on glucose. Qualitatively similar effects were observed in strains grown on glycerol-Casamino Acids medium (data not shown).

We also examined the strain (IBPC574) which carries two point mutations in the *nag* regulon: a mutation (*nagA1*) which renders the deacetylase inactive and a second mutation in the repressor gene (*nagC11*) which confers a superrepressor phenotype. The bacteria accumulate GlcNAc-6-P because of the deacetylase defect, but the *nag* regulon is not induced because the superrepressor is not displaced from its operators by the presence of GlcNAc-6-P, the normal inducer of the regulon (32). This strain contains elevated levels of synthase (Fig. 2B, lane 5, and Table 2). This shows that the presence of high intracellular concentrations of GlcNAc-6-P per se is not the signal to repress the expression of the synthase.

DISCUSSION

The data presented show that the *glmS* gene is subject to a control mechanism which causes its expression to be reduced when the level of the *nag* regulon genes is derepressed. As the factor of regulation is quite small (no more than fourfold), it was possible that the reduction was just due to changes in enzyme activity or translational efficiency. However, the mRNA levels parallel the protein levels,

showing that the control mechanism is likely to be true transcriptional regulation.

The repression of *glmS* expression occurs not only when the cells are growing on GlcNAc or GlcN, when there is no need for the cell to synthesize amino sugars, but also when the *nag* regulon is derepressed either because of a mutation in the Nag repressor or via endogenous induction. The latter phenomenon occurs because of the accumulation of the inducer, GlcNAc-6-P, in a *nagA* strain (31). We wondered whether the molecule(s) transmitting the signal to reduce *glmS* expression was the same one that turned up *nag* expression. The facts that both IBPC524 (*nagA*::Cm) and IBPC574 (*nagA1 nagC11*) contain high (millimolar) concentrations of GlcNAc-6-P and that only in IBPC524 is the *glmS* level repressed suggest that the molecular signal is not GlcNAc-6-P. It is more likely to be a dedicated component of the biosynthetic pathway, e.g., GlcN-1-P or UDP-GlcNAc. It is interesting to note that the glucosamine synthase of eukaryotes is inhibited by UDP-GlcNAc (10, 17, 19, 41) while that of *E. coli* is not (17, 36). It would be interesting if the same compound was acting in two completely different ways, as a transcriptional effector in prokaryotes and as an enzymatic inhibitor in eukaryotes.

The Nag repressor is clearly not acting as a repressor for the synthase, since *nagC* strains show the repressed level of *glmS*. The data do not eliminate the possibility that NagC is an activator, enhancing the expression of the synthase above a basal level observed during growth on amino sugars. For this scenario to be true, the superrepressor encoded by the *nagC11* mutation in IBPC574 would still need to be an activator of *glmS* expression and maybe a superactivator, since the synthase level in IBPC574 is higher than that in IBPC5321 (wild type) (Table 2). It would also mean that at the same time as NagC is bound to *nagEB* operators and repressing their expression, it is also bound to the *glmS* operator to activate its expression. The entry of amino sugars into the cell would simultaneously displace NagC from all its binding sites. There are many examples of a regulatory protein acting as either a repressor or an activator, depending upon the location of the operator site or the presence of low-molecular-weight ligands, e.g., cyclic AMP receptor protein (16) or TyrR (27). A particularly relevant example is the repressor of the fructose phosphotransferase system which seems to activate the expression of the *pps* gene encoding phosphoenolpyruvate synthase (11).

Under the conditions of a *nagA* or *nagC* mutation, the level of the deaminase is very high and it might be expected that GlcN-6-P synthesized by GlmS is degraded by the overproduced deaminase so that the cells are starving for amino sugars. This seems not to be the case, since the *nagA* and *nagC* strains grow perfectly normally on glucose, as observed previously (38). One possible explanation for this is that the overproduced deaminase is now acting anabolically and actually synthesizing GlcN-6-P. The enzyme was long known to be reversible in vitro (6), and recently it has been shown that overproduced deaminase with either excess ammonium ions or a mutation in an unidentified gene, *glmX*, allowed a *glmS* strain to grow in the absence of external amino sugars (38). It is possible in the *nagA* and *nagC* strains that NagB is contributing to the GlcN-6-P pool rather than degrading it. However, this explanation is not possible in the strains IBPC546 (*nagB*) and IBPC590 (Δ*nagEBACD*), and in both of these strains the amount of GlmS protein is reduced about twofold compared with that in the wild-type IBPC5321.

We can calculate that even under the repressed conditions the amount of GlcN-6-P synthase is sufficient to ensure the synthesis of enough GlcN-6-P for cell wall and lipopolysaccharide synthesis. From the blot of Fig. 2 we can estimate that for 50 μg of protein in the sonicated extracts there is 10 ng of GlmS; i.e., it represents 0.02% of the soluble protein. This value is comparable with that obtained by purification of the protein (0.1 g/3.3 kg [wet weight] of cells, i.e., about 0.03% of the soluble protein [36]). Assuming that the weight of one cell is 9.5×10^{-13} g and the protein content is 15% of the total wet weight (24), the amount of GlmS can be estimated as 3×10^{-14} mg per cell. From the specific activity of the enzyme (10 μmol of GlcN-6-P formed per min per mg of protein [4]) and a cell water content of about 10^{-15} liter per cell (21, 24), we calculate that GlmS can maintain an intracellular concentration of GlcN-6-P of 3×10^{-4} M, which is the same order of magnitude as the pool sizes of the glucosamine-containing peptidoglycan precursors in the cell, evaluated as 3×10^{-4} to 4×10^{-4} M from the data of Mengin-Lecreulx et al. (21, 22). This approximate calculation is consistent with the observation that the strains in which the expression of GlmS is repressed (*nagA* and *nagC* strains) grow efficiently in the absence of exogenous amino sugars but suggests that the pools of peptidoglycan precursors are likely to be reduced under these conditions.

There must be a strong directionality of GlcN-6-P towards the synthesis of UDP-GlcNAc, the primary cytoplasmic intermediate in the synthesis of lipid A and peptidoglycan. The steps from GlcN-6-P to UDP-GlcNAc are poorly characterized but presumably are via GlcN-1-P. Dobrogosz (9) showed that GlcNAc had to be deacetylated before it could be incorporated into cell walls, suggesting that any isomerase converting GlcNAc-6-P to GlcNAc-1-P has insignificant activity and that the major flux goes via GlcN-1-P. If the isomerase had a very high affinity for GlcN-6-P with equilibrium completely on the GlcN-1-P side, this could account for forcing the GlcN-6-P into the biosynthetic pathway. These are, however, not normal properties for an isomerase, and it is more likely that an energy-consuming reaction, phosphorolysis of UTP or acetylation, is the irreversible step. This could indicate that GlcN-6-P synthase, the isomerase, the acetylase, and the UDP-joining enzyme (GlcNAc-1-P uridyltransferase) are all part of a multienzyme complex. Interestingly, the gene for the UDP-joining enzyme, *gcaD*, has recently been identified in *Bacillus subtilis* (14) and is homologous to an open reading frame, *urf*, immediately upstream of *glmS* on the *E. coli* chromosome (39). Subcloning of this region and enzyme activity tests have confirmed that the protein encoded by *urf* is GlcNAc-1-P uridyltransferase, which will be named GlmU (20). The short intergenic distance between *urf/glmU* and *glmS* and the absence of an obvious promoter consensus on the DNA sequence upstream of *glmS* suggest that these genes are cotranscribed. This could mean that the regulation of *glmS* expression described in this work is also applicable to *urf/glmU*. An analysis of the transcription in this region is essential to identify the regulatory sites implicit in the results described here.

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