A dominant mutation in the gene for the Nag repressor of *Escherichia coli* that renders the *nag* regulon uninducible

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The gene *nagC* encodes the repressor for the *nag* regulon. A point mutation within the gene, which confers a super-repressor phenotype and makes the repressor insensitive to the inducer, *N*-acetylglucosamine 6-phosphate, has been characterized. The mutation is semi-dominant since heterozygous diploids have reduced growth rates on glucosamine and *N*-acetylglucosamine compared to the wild-type strain.

Introduction

The *nag* regulon of *Escherichia coli*, located at 15.5 min on the genetic map (Bachmann, 1990) encodes genes necessary for the uptake and degradation of the amino sugars, D-glucosamine (GlcN) and *N*-acetyl-D-glucosamine (GlcNAc). It consists of divergent operons *nagE* and *nagBACD* (Fig. 1). *nagE* encodes the GlcNAc-specific transporter (EII^{Nag}) of the phosphoenolpyruvate-dependent phosphotransferase system (PTS) (Jones-Mortimer & Kornberg, 1980; Lengeler, 1980; Peri & Waygood, 1988; Rogers *et al.*, 1988) and produces intracellular GlcNAc 6-phosphate. GlcN is primarily transported by the generic hexose transporter of the PTS, encoded by the genes *manXYZ* located at 40 min, which also transports GlcNAc (Curtis & Epstein, 1975; Erni *et al.*, 1987; Jones-Mortimer & Kornberg, 1980; Saris & Palva, 1987). Expressed in the opposite direction to *nagE* (anticlockwise) are *nagB* and *nagA* encoding the two enzymes which degrade GlcNAc 6-phosphate to fructose 6-phosphate: GlcNAc-6-phosphate deacetylase (*nagA*) and GlcN-6-phosphate deaminase (*nagB*) (Holmes & Russell, 1972; Plumbridge, 1989; Vogler & Lengeler, 1989; White, 1968). The third gene, *nagC*, encodes a repressor for the regulon (Plumbridge, 1991; Vogler & Lengeler, 1989). Two binding sites for the repressor have been located in the intergenic *nagE*–*nagB* region overlapping the *nagE* and *nagB* promoters (Plumbridge & Kolb, 1991). A gene for a fourth ORF, provisionally called

nagD, is located downstream of the *nagC* gene. No function has been attributed to this gene.

Mutations preventing growth on GlcNAc were first isolated by White (1968). He characterized two alleles in detail, *nagB2* and *nagA1*. The *nagB2* mutation prevented growth on both GlcNAc and GlcN while the *nagA1* mutation prevented growth on GlcNAc but not GlcN. The *nagA1* mutation was pleiotropic; the strains were Nag^S, had increased levels of GlcN-6-phosphate deaminase, enhanced GlcNAc transport activity and accumulated high concentrations of GlcNAc 6-phosphate, the substrate of the deacetylase. [Nag^S is the phenomenon where the presence of GlcNAc inhibits growth on other carbon sources. This is presumably due to the accumulation of toxic levels of GlcNAc 6-phosphate which cannot be further metabolized in the *nagA* strain (White, 1968; Bernheim & Dobrogosz, 1970).] The same set of phenotypes are apparent with other *nagA* mutations, both those constructed *in vitro* by the insertion of antibiotic resistance cassettes, (Plumbridge, 1991) or by λ placMu mutagenesis *in vivo* (Vogler & Lengeler, 1989). The enhanced transport and deaminase activities of these strains are the result of endogenous induction from the accumulation of GlcNAc 6-phosphate which is the intracellular inducer of the regulon. In the presence of this phosphorylated form of GlcNAc, the repressor is prevented from binding to the operator sites of the *nagE*–*nagB* intergenic region so that expression of the two operons is induced (Plumbridge, 1991).

White (1968) noted that the strain carrying the *nagA1* mutation was unstable and segregated bacteria which had lost the Nag^S phenotype, i.e. they became Nag^R. They were still Nag[–] but now grew slowly or not at all on GlcN, presumably due to the acquisition of secondary

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Abbreviations: GlcN, D-glucosamine; GlcNAc, *N*-acetyl-D-glucosamine; PTS, phosphoenolpyruvate-dependent phosphotransferase system.

Fig. 1. Organisation of the chromosome in the vicinity of the *nag* genes and the metabolism of GlcNAc. (a) Location of the *nag* genes and their direction of transcription, together with that of the surrounding genes; *glnS*, encoding glutamyl-tRNA synthetase (GlnRS) and *asnB*, encoding asparagine synthetase. The transposon *zbf507::Tn10* is located on the clockwise-side of the genes (right on the figure). Underneath is shown the structure of the *nagD-lacZ* fusion carried by pEBg1 and pEBgN4. B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI. (b) Scheme showing the metabolism of GlcNAc and GlcN and the functions of the *nag* genes. *glmS* encodes glucosamine synthetase but the normally catabolic *nagB* gene product can also function anabolically under certain conditions (Vogler *et al.*, 1989).

mutations. The strain carrying the *nagA1* mutation, JP5053, available from the CGSC (*E. coli* Genetic Stock Center, Yale University, New Haven, CT, USA) is of this type, it does not grow on GlcNAc, grows very slowly on GlcN and is insensitive to GlcNAc in the medium. This paper describes the analysis of the strain carrying the *nagA1* mutation and identifies the secondary mutation as a mutation in the *nagC* gene giving a super-repressor phenotype.

Methods

Bacteriological methods. The *E. coli* strains used in this work are listed in Table 1. The *nagB-lacZ* fusion carried by a λ 857 transducing phage, λ NagB-lacZ, and its use in measuring expression of the *nag* regulon have been described previously (Plumbridge, 1990). The same fusion transferred to a *cI⁺ imm²¹* bacteriophage (λ RS45) by the method of Simons *et al.* (1987) is called λ RS/NagB-lacZ. β -Galactosidase activities, expressed in the units described by Miller (1972) are the average of four samples per bacterial culture taken at two different optical densities. Generally, several independent cultures were measured and the results differed by less than 10%. Bacteria were grown at 30 °C in minimal MOPS medium (Neidhardt *et al.*, 1974) supplemented with 50 μ g arginine and histidine ml⁻¹ and 0.2% carbon source, except glycerol which was 0.4%. Cultures of bacteria carrying plasmids were grown in MOPS medium with 0.5% Casamino acids and 500 μ g ampicillin ml⁻¹. Nag^s was conveniently monitored on MacConkey-GlcNAc plates. Nag⁺ colonies are red, Nag⁻ colonies are white, while Nag^s bacteria do not grow. Loss of the transposon Tn10 was selected as described by Maloy & Nunn (1981).

Recombinant DNA techniques. The 4.2 kb *Eco*RI-*Bgl*II fragment of pB30-1 (Plumbridge, 1989) carrying the 5' end of *nagE*, *nagBAC* and the beginning of *nagD* (Fig. 1) was cloned into the *lacZ* fusion vector pMC1403 (Casadaban *et al.*, 1980), digested with *Eco*RI and *Bam*HI,

and the ligated mixture transformed into IBPC574 (*nagA1*, *nagC11*). Since the *lacZ* gene at the *Bam*HI site is in the same phase as the *nagD* gene at the *Bgl*II site, the resulting colonies were expected to be blue on X-gal-containing plates and since the plasmid insert carried the *nagA* gene, the plasmids were expected to complement the *nagA1* mutation and produce Nag⁺ bacteria. Some of the blue clones were however Nag⁻, e.g. IBPC574(pEBg1), while others were Nag⁺. Restriction enzyme analysis of the plasmid DNA showed that the plasmids conferring either a Nag⁺ or Nag⁻ phenotype contained the same DNA insert. When the transformation was repeated in the Nag⁺ strain IBPC5321 all the resulting Lac⁺ colonies were Nag⁺, e.g. IBPC574(pEBgN4)). The plasmids conferring Nag⁺ and Nag⁻ phenotypes were cycled through a *recA1* strain, wild-type for *nag*, and retested for their ability to complement the *nagA1* mutation in IBPC574CR. The plasmid inserts were transferred to λ RS45 to give λ EBg1 and λ EBgN4. The *nagA* and *nagC* genes from pEBg1 were subcloned to pUC19 and pBR322 as described for the wild-type proteins (Plumbridge, 1989). In pBR322 only low levels of the cloned genes are expressed in the absence of the pUC19 *lac* promoter. The same fragments were cloned into M13mp18 and M13mp19 and sequenced using Sequenase (US Biochemicals) and [α^{35} S]thio-dATP.

A chromosomal replacement of the whole *nag* region between *Hpa*I sites in *nagE* and *asnB* with a tetracycline resistance (tc) cassette was constructed using the *recBC sbcBC* strain JC7623 (Jasin & Schimmel, 1984) to give strain IBPC590 as described previously for the *nagE*, B, A, C and D insertion mutations (Plumbridge, 1991).

Results and Discussion

Localization of the mutation conferring Nag^R in the *nagA1* strain

The *nagA1* mutation was transduced out of JP5053 using the Tn10 marker *zbf507::Tn10*, which is about 50% co-transducible with the *nag* region, into the Δ *lac* strain

Table 1. *Bacterial strains*

Strain*	Relevant genotype	Reference or source
JP5053	<i>argH1 metB1 nagA1 nagC11 rpsL155 rpoB352 λ^R λ⁺</i>	B. Bachmann, CGSC
JP5061	<i>thi-1 leuB6 purE42 nagB2 galE45 mtl-1 xyl-5 ara-14 rpsL109 azi-6? rpoB301 tonA23 tsx-67 supE44</i>	B. Bachmann, CGSC
IBPC5321	<i>thi-1 argG6 argE3 his-4 mtl-1 xyl-5 rpsL tsx-29? ΔlacX74</i>	Plumbridge (1989)
IBPC571	IBPC5321 <i>nagB2 zbf507::Tn10</i>	
IBPC574	IBPC5321 <i>nagA1 nagC11 zbf507::Tn10</i>	Plumbridge (1989)
IBPC574C	IBPC5321 <i>nagA1 nagC11</i>	
IBPC524	IBPC5321 <i>nagA::cm1</i>	Plumbridge (1991)
IBPC531	IBPC5321 <i>nagA::cm2</i>	Plumbridge (1991)
IBPC529C	IBPC5321 <i>nagC::cm</i>	Plumbridge (1991)
IBPC590	IBPC5321 <i>ΔnagEBACD::tc</i>	This work
IBPC424	IBPC5321 <i>glnS1 supE44</i>	Plumbridge & Söll (1989)
IBPC527	Similar to IBPC524	
IBPC527-1S	Nag ^R derivative of IBPC527	
IBPC583	IBPC5321 <i>nagE::km nagA::tc</i>	This work
IBPC711	IBPC5321 <i>nagA1 λRS/NagB-lacZ</i>	This work
IBPC712	IBPC5321 <i>glnS1 nagC11 zbf507::Tn10 λRS/NagB-lacZ</i>	This work
JM101Δ <i>nag</i>	JM101Δ <i>nagEBACD::tc</i>	This work

* The letter R after a strain number (e.g. IBPC574CR) indicates the *recA1* derivative of the strain, normally constructed by P1-mediated co-transduction with the adjacent *srl::Tn10* marker.

Table 2. *The effect of the nagA1 nagC11 mutations on activation of the nag regulon*

The bacterial strains indicated, carrying the λ NagB-lacZ fusion to monitor the activity of the *nag* regulon, were grown continuously in minimal MOPS medium with the carbon sources indicated and β -galactosidase activity, expressed in units described by Miller (1972), was measured as described in Methods. The values given are the mean of at least two independent cultures. Three transductants of the type IBPC712, but only one isolate of the type IBPC711, were found (Table 5) and tested. Strain IBPC712 carries in addition the *glnS1* (ts) mutation.

		β -Galactosidase activity of the λ NagB-lacZ fusion				
Strain	Genotype	Glucose	Glucose + GlcNAc	Glycerol	Glycerol + GlcNAc	GlcN
IBPC5321	Wild-type	55	630	81	1090	295
IBPC574	<i>nagA1 nagC11</i>	57	55	120	87	130
IBPC711	<i>nagA1 nagC⁺</i>	1550	ND	ND	2370	ND
IBPC712	<i>nagA⁺ nagC11</i>	35	ND	ND	38	ND

ND, Not determined.

IBPC5321. The resulting strains (e.g. IBPC574) all had the same characteristics as JP5053; they were Nag⁻, Nag^R and grew very slowly on GlcN (doubling time 5–6 h compared to 100 min for IBPC5321 at 37 °C). It was apparent, therefore, that the mutations causing the Nag⁻ and Nag^R phenotypes had co-transduced into IBPC574. The growth defects were complemented by plasmids carrying the entire *nagBACD* operon, e.g. pB30-1, but not by a plasmid expressing just *nagA* [pBR(NagA)], which complements an *in vitro*-constructed null mutation in *nagA*, *nagA::cm* (Plumbridge, 1991). This suggested

that a mutation within the *nag* operon but outside *nagA* is responsible for the Nag^R phenotype. Expression of the λ NagB-lacZ fusion in IBPC574 was very low and addition of GlcNAc to a culture growing on glucose or glycerol failed to increase the expression (Table 2) showing that the secondary mutation was acting *in trans* on expression of the NagB-lacZ fusion. These properties of the secondary mutation are consistent with a super-repressor mutation in the *nagC* gene. The experiments described below, confirmed this interpretation and the mutation has been named *nagC11*.

Table 3. *Effect of the nagA1 nagC11 mutations on growth on GlcN and GlcNAc in diploids*

The table gives the doubling times at 30 °C in minimal MOPS medium containing 50 µg arginine and histidine ml⁻¹ and 0.2% of the carbon sources indicated. The strains are all diploid for the *nag* region and the alleles carried by the chromosome and the λ lysogen are indicated. Strains were precultured in the same medium overnight and diluted to give a starting OD₆₅₀ of about 0.02, and growth was followed spectroscopically until OD₆₅₀ = 0.5. The values given are the average of two independent experiments for growth on GlcNAc or GlcN. They differed by less than 12% (for the slow growth rates) and most by less than 5%.

Strain	Genotype		Doubling times for growth on carbon source (min)		
	Chromosome	Lysogen	Glucose	GlcN	GlcNAc
IBPC574CR(λ EBg1)	<i>nagA1 nagC11</i>	<i>nagA1 nagC11</i>	86	580	NG
IBPC574CR(λ EBgN4)	<i>nagA1 nagC11</i>	<i>nagA⁺C⁺</i>	88	255	340
IBPC524R(λ EBg1)	<i>nagA::cm nagC⁺</i>	<i>nagA1 nagC11</i>	82	190	NG
IBPC524R(λ EBgN4)	<i>nagA::cm nagC⁺</i>	<i>nagA⁺C⁺</i>	77	120	83
IBPC5321R(λ EBg1)	<i>nagA⁺C⁺</i>	<i>nagA1 nagC11</i>	82	265	270
IBPC5321R(λ EBgN4)	<i>nagA⁺C⁺</i>	<i>nagA⁺C⁺</i>	85	145	89

NG, No growth.

Effect of the nagA1 nagC11 mutations in cells diploid for the nag region

The *nagA1 nagC11* mutations were cloned by homologous recombination to give plasmid pEBg1 and bacteriophage λ EBg1 as described in Methods. pEBgN4 and λ EBgN4 carry the equivalent wild-type DNA. Three strains were lysogenized with λ EBg1 and λ EBgN4: IBPC5321R (wild-type for *nag*), IBPC574CR (*nagA1 nagC11*) and IBPC524R carrying a null mutation in the *nagA* gene. Analysis of the growth rates (Table 3) of the six diploid strains showed that the mutations conferring both the Nag⁻ and the Nag^R character were present on λ EBg1. IBPC574CR(λ EBg1), the homozygous *nagA1 nagC11*/*nagA1 nagC11* diploid grew very slowly on GlcN (doubling time about 580 min) and not at all on GlcNAc. For both the heterozygous *nagA1 nagC11/nagA⁺C⁺* diploids IBPC5321(λ EBg1) and IBPC574CR(λ EBgN4), growth rates on both GlcN and GlcNAc were slower, two- and three-fold, respectively, than with the wild-type *nagA⁺C⁺/nagA⁺C⁺* diploid showing the dominant nature of the *nagA1 nagC11* mutations which act *in trans* on the expression of the wild-type operon. The presence of the *nagA::cm* allele had no effect on the activity of the wild-type *nag* genes, IBPC524R(λ EBgN4) grew normally on GlcNAc and GlcN. However, the *nagA::cm nagC⁺/nagA1 nagC11* diploid, IBPC524R(λ EBg1), did not grow on GlcNAc and grew slowly on GlcN. All the strains grew identically on glucose (Table 3).

Effect of individually cloned nagA1 and nagC11 alleles

The *nagA* and *nagC* genes were cloned from pEBg1 into pUC19 and pBR322. The cloned *nagA1* allele complemented neither the *nagA1* nor the *nagA::cm* mutations. The effect of the individual *nagA1* and *nagC11* alleles on expression of the *nag* regulon was tested by measuring their effect on the λ NagB-lacZ fusion and their ability to complement null mutations in the *nagA* and *nagC* genes (Table 4). Disruption of either *nagA* (IBPC531R) or *nagC* (IBPC529CR) by insertion of a Cm^R cassette produces a derepression of the regulon (Table 4, row 1) which is complemented by the cloned wild-type *nagA* or *nagC* genes, respectively (Table 4, rows 2 and 4; Plumbbridge, 1991). The cloned *nagA1* allele has no effect on the derepression provoked by an insertion in *nagA* (or *nagC*) (Table 4, row 5). This is consistent with the mutation producing a non-functional protein. On the other hand the cloned *nagC11* allele not only complements the derepression provoked by the *nagC::cm* mutation but also complements the derepression produced by the *nagA::cm* mutation or a deletion of the *nagEBACD* genes, which the wild-type *nagC* gene cannot do (Table 4, rows 2 and 3). Derepression of the *nagA::cm* strain is due to the accumulation of the inducer GlcNAc 6-phosphate which cannot be degraded in the absence of an active GlcNAc-6-phosphate deacetylase encoded by *nagA* (Plumbbridge, 1991). Strains carrying the *nagA1 nagC11* mutation accumulate high levels of GlcNAc

Table 4. Effect of the cloned *nagA1* and *nagC11* alleles on expression of the *nag* regulon

The bacterial strains indicated, lysogenized with λ NagB-lacZ to monitor activity of the *nag* regulon, were transformed with the five pBR322-derived plasmids. pBR(NagA) and pBR(NagC) are plasmids expressing low levels of the wild-type proteins. pBR(NagA1) and pBR(NagC11) are the equivalent plasmids carrying the cloned mutant alleles from pEBg1. The transformants were grown continuously in minimal MOPS medium with the carbon sources indicated, supplemented with 0.5% Casamino acids and 500 μ g ampicillin ml⁻¹. The table gives the β -galactosidase activities expressed in units described by Miller (1972) from a representative experiment.

Strain (IBPC): Genotype: Grown on:	β -Galactosidase activities of the λ NagB-lacZ fusion				
	5321R Wild-type Glucose	5321R Wild-type GlcNAc	529CR <i>nagC::cm</i> Glucose	531R <i>nagA::cm</i> Glucose	590 Δ <i>nagEABCD</i> Glucose
Plasmid					
pBR322	61	840	1120	1145	1170
pBR(NagC)	34	695	35	1260	1012
pBR(NagC11)	23	167	25	51	30
pBR(NagA)	42	435	1050	60	1171
pBR(NagA1)	66	770	1005	1200	1032

6-phosphate as observed for the *nagA::cm* strain (Plumbridge, 1991) and the original *nagA1* isolate (White, 1968). The *nagC1* protein is apparently insensitive to this inducer and can bind to the *nag* operators and repress in the presence of high levels of GlcNAc 6-phosphate present in the *nagA* strains.

The insensitivity of the mutant repressor to GlcNAc 6-phosphate was confirmed by an *in vitro* test (Fig. 2). Both the wild-type (lanes 2–5) and NagC11 (lanes 6–9) repressors form complexes with *nag* DNA under similar conditions as visualized by gel mobility shift assay, but only the wild-type repressor is dissociated by the presence of GlcNAc 6-phosphate (lanes 10–12) whilst the NagC11 complex is unaffected (lanes 13–15).

The identification of the *NagC11* mutation as one producing an inducer-insensitive super-repressor, permitted a genetic selection for the separated mutations *nagA1* and *nagC11* based on their effect on the λ NagB-lacZ fusion (Table 5). The NagB-lacZ fusion in strain IBPC711 (*nagA1 nagC+*) was derepressed while that in IBPC712 (*nagA+ nagC11*) was uninducible (Table 2).

Sequencing the cloned inserts of pBR(NagA1) and pBR(NagC11) located two point mutations. The *nagA1* mutation resulted in a change from Asp to Asn (GAT to AAT) at position 248 (total 382) and the *nagC11* mutation resulted in a change from Leu to Pro (CTG to CCG) at position 125 (total 406). It is interesting to note that the *nagA1* mutation, a +1 charge change, results in a protein with an apparent molecular mass on SDS-polyacrylamide gels more in agreement with the molecular mass predicted by the DNA sequence (41.9 kDa) than the wild-type protein which migrates with an apparent molecular mass of 44 kDa (Plumbridge, 1989).

Fig. 2. Insensitivity of the NagC11 protein to inducer, GlcNAc 6-phosphate. Complexes between *nag* DNA and the wild-type or NagC11 repressor were detected by gel retardation as described previously (Plumbridge & Kolb, 1991). JM101 Δ *nag* carrying either pUC(NagC) or pUC(NagC11) was grown in LB plus 500 μ g ampicillin ml⁻¹ and induced for 3 h with 1 mM-IPTG to overproduce the repressor. Extracts were sonicated, clarified by centrifugation, adjusted to 10 mg protein ml⁻¹ and tested for DNA binding on a 200 bp fragment covering the intergenic *nagE-B* region uniformly labelled with [α ³²P]dCTP. Dilutions of the extracts were mixed with DNA in 25 mM-HEPES, 100 mM-sodium glutamate buffer (pH 8.0) containing 500 μ g BSA ml⁻¹, 200 μ M-cAMP and 5 mM-GlcNAc 6-phosphate where indicated. After 15 min at room temperature the complexes were analysed on a 5% (w/v) acrylamide gel. The figure shows an autoradiograph of the dried gel. Lanes: 1, free DNA; 2–5 and 10–12, extract from pUC(NagC); 6–9 and 13–15, extract from pUC(NagC11). Protein concentrations due to the bacterial extracts are 100 μ g ml⁻¹ (lanes 2, 6, 10 and 13), 50 μ g ml⁻¹ (lanes 3, 7, 11 and 14), 25 μ g ml⁻¹ (lanes 4, 8, 12 and 15) and 12.5 μ g ml⁻¹ (lanes 5 and 9). Arrowheads indicate the migration positions of free-*nag* DNA (D) and the complex *nag* DNA-cAMP/CAP-NagC (C).

Table 5. Cotransduction frequencies between *glnS*, *nagA*, *nagC* and *zbf507::Tn10*

Strain		Marker		Co-transduction (%)
Donor	Recipient	Selected	Unselected	
IBPC574 (<i>nagA1 nagC11 zbf507::Tn10</i>)	IBPC424 λ RS/NagB-lacZ (<i>glnS1 supE44</i>)	<i>zbf507::Tn10</i>	<i>glnS</i> ⁺	35 (35/100)
			<i>nagA1 nagC11</i> *	33 (33/100)
			<i>nagA</i> ⁺ <i>nagC11</i> †	1 (3/300)
		<i>glnS</i> ⁺	<i>zbf507::Tn10</i>	51 (53/103)
			<i>nagA1 nagC11</i> *	85 (87/103)
			<i>nagA1 nagC</i> ⁺ ‡	0.4 (1/260)

* Phenotype; Nag⁻, uninducible.

† Phenotype; very slow growth on GlcNAc, NagB-lacZ fusion repressed, resulting strain called IBPC712.

‡ Phenotype; Nag⁻, NagB-lacZ fusion derepressed, resulting strain called IBPC711.

Other mutations resulting in a Nag^R phenotype

Nag^R derivatives of strains carrying the *nagA::cm* mutation were selected. Several bacteria were obtained which exhibited properties different from the *nagA1 nagC11* strains, e.g. IBPC527-1S: in particular the NagB-lacZ fusion was still derepressed suggesting that GlcNAc 6-phosphate was accumulating and inducing the regulon. The Nag^R character was lost in the presence of plasmids expressing *nagE* implying a defect in the *nagE* (transport) function. To verify that a *nagE* mutation could confer a Nag^R phenotype on a *nagA* strain, a double *nagE::km nagA::tc* mutant strain was constructed (IBPC583). This strain was indeed Nag⁻ Nag^R and the NagB-lacZ fusion was still derepressed. Analysis of Nag^R derivatives of IBPC711 (*nagA1*) identified a third class where the deacetylase activity has been partially or fully restored which prevents the intracellular accumulation of the inducer, GlcNAc 6-phosphate.

It should be noted that the *nagC11* super-repressor mutation is not a new class of mutation. Mis-sense mutations in the *lacI* gene coding for the Lac repressor, which produce super-repressor phenotypes, have been known for a long time (reviewed in Miller, 1980). Recently Kleina & Miller (1990) have detected additional amino acid substitutions in the Lac repressor using an elegant system of complementation of amber mutations with suppressors inserting many different amino acids. Other examples of super-repressor phenotypes have been described: e.g. *gal* (Saedler *et al.*, 1968), *trp* (Kelley & Yanofsky, 1985), λ (Hecht & Sauer, 1985), *Tn10tet* (Smith & Bertrand, 1988), *fadR* (Hughes *et al.*, 1988) and *nadI* (Zhu & Roth, 1991).

The complete insensitivity of the *nagC11* protein to inducer and its strongly inhibitory effect when present in a diploid could be indicative that the mutation is directly

affecting the GlcNAc 6-phosphate binding site. However, it is to be admitted that the change from Leu to Pro at position 125 in *nagC1* could produce a major structural change in the protein, for example preventing the allosteric transition to the form inactive in DNA binding. As yet nothing is known about the structural domains in the Nag repressor. A helix-turn-helix DNA binding motif was tentatively identified in the C-terminal part of the protein (Plumbridge, 1989). The mutation at amino acid 125, about one third of the way from the N-terminal, could locate another functional domain.

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References

- BACHMANN, B. J. (1990). Linkage map of *Escherichia coli* K12, edition 8. *Microbiological Reviews* **54**, 130–197.
- BERNHEIM, N. J. & DOBROGOSZ, W. J. (1970). Amino sugar sensitivity in *Escherichia coli* mutants unable to grow on *N*-acetylglucosamine. *Journal of Bacteriology* **101**, 384–391.
- CASADABAN, M. J., CHOU, J. & COHEN, S. N. (1980). *In vitro* gene fusions which join an enzymatically active β -galactosidase segment to amino-terminal fragments of exogenous proteins: *Escherichia coli* plasmids vectors for the detection and cloning of translational initiation signals. *Journal of Bacteriology* **143**, 971–980.
- CURTIS, S. J. & EPSTEIN, W. (1975). Phosphorylation of D-glucose in *E. coli* mutants defective in glucosephosphotransferase, mannosephosphotransferase and glucokinase. *Journal of Bacteriology* **122**, 1189–1199.
- ERNI, B., ZANOLARI, B. & KOCHER, H. P. (1987). The mannose permease of *E. coli* consists of three different proteins. *Journal of Biological Chemistry* **262**, 5238–5247.
- HECHT, M. H. & SAUER, R. T. (1985). Phage lambda repressor revertants: amino acid substitutions that restore activity to mutant proteins. *Journal of Molecular Biology* **186**, 53–63.

- HOLMES, R. P. & RUSSELL, R. R. B. (1972). Mutations affecting amino sugar metabolism in *Escherichia coli* K12. *Journal of Bacteriology* **111**, 290–291.
- HUGHES, K. T., SIMONS, R. W. & NUNN, W. D. (1988). Regulation of fatty acid degradation in *Escherichia coli*: *fadR* superrepressor mutants are unable to utilise fatty acids as sole carbon source. *Journal of Bacteriology* **170**, 1666–1671.
- JASIN, M. & SCHIMMEL, P. (1984). Deletion of an essential gene in *Escherichia coli* by site specific recombination with linear DNA fragments. *Journal of Bacteriology* **159**, 783–786.
- JONES-MORTIMER, M. C. & KORNBERG, H. L. (1980). Amino-sugar transport systems in *Escherichia coli* K12. *Journal of General Microbiology* **117**, 369–376.
- KELLEY, R. L. & YANOFSKY, C. (1985). Mutational studies with the *trp* repressor of *Escherichia coli* support the helix-turn-helix model of repressor recognition of operator DNA. *Proceedings of the National Academy of Sciences of the United States of America* **82**, 483–487.
- KLEINA, L. G. & MILLER, J. H. (1990). Genetic studies of the lac repressor. XIII. Extensive amino acid replacements generated by the use of natural and synthetic nonsense suppressors. *Journal of Molecular Biology* **212**, 295–318.
- LENGELER, J. (1980). Characterisation of mutants of *Escherichia coli* K12, selected by resistance to streptomycin. *Molecular and General Genetics* **179**, 49–54.
- MALLOY, S. R. & NUNN, W. D. (1981). Selection for loss of tetracycline resistance by *Escherichia coli*. *Journal of Bacteriology* **145**, 1110–1112.
- MILLER, J. H. (1972). In *Experiments in Molecular Genetics*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- MILLER, J. H. (1980). In *The Operon*, pp. 31–88. Edited by J. H. Miller & N. S. Reznikoff. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- NEIDHARDT, F. C., BLOCH, P. L. & SMITH, D. F. (1974). Culture medium for enterobacteria. *Journal of Bacteriology* **119**, 736–747.
- PERI, K. G. & WAYGOOD, E. B. (1988). Sequence of cloned *EnzymeII^{Nag}* of the phosphoenolpyruvate:N-acetylglucosamine phosphotransferase system of *Escherichia coli*. *Biochemistry* **27**, 6054–6061.
- PLUMBRIDGE, J. A. (1989). Sequence of the *nagBACD* operon in *Escherichia coli* K12 and pattern of transcription within the *nag* regulon. *Molecular Microbiology* **3**, 506–515.
- PLUMBRIDGE, J. A. (1990). Induction of the *nag* regulon of *Escherichia coli* by N-acetylglucosamine and glucosamine: Role of the cAMP-catabolite activator protein complex in expression of the regulon. *Journal of Bacteriology* **172**, 2728–2735.
- PLUMBRIDGE, J. A. (1991). Repression and induction of the *nag* regulon of *Escherichia coli* K12: the roles of *nagC* and *nagA* in maintenance of the uninduced state. *Molecular Microbiology* **5**, 2053–2062.
- PLUMBRIDGE, J. A. & KOLB, A. (1991). CAP and Nag repressor binding to the regulatory regions of the *nagE-B* and *manX* genes of *E. coli*. *Journal of Molecular Biology* **217**, 661–679.
- PLUMBRIDGE, J. A. & SÖLL, D. (1989). Characterisation of cis-acting mutations which increase the expression of a *glnS-lacZ* fusion in *Escherichia coli*. *Molecular and General Genetics* **216**, 113–119.
- ROGERS, M. J., OHGI, T., PLUMBRIDGE, J. A. & SÖLL, D. (1988). Nucleotide sequences of the *Escherichia coli* *nagE* and *nagB* genes: the structural genes for the N-acetylglucosamine transport protein of the bacterial phosphoenolpyruvate:sugar phosphotransferase system and for glucosamine-6-phosphate deaminase. *Gene* **62**, 197–207.
- SAEDLER, H., GUILLON, A., FIETHEN, L. & STARLINGER, P. (1968). Negative control of the galactose operon of *E. coli*. *Molecular and General Genetics* **102**, 79–88.
- SARIS, P. E. J. & PALVA, E. T. (1987). The *ptsL*, *pel/ptsM* (*manXYZ*) locus consists of three genes involved in mannose uptake in *E. coli*. *FEMS Microbiology Letters* **44**, 371–376.
- SIMONS, R. W., HOUMAN, F. & KLECKNER, N. (1987). Improved single and multicopy *lac* based cloning vectors for protein and operon fusions. *Gene* **53**, 85–96.
- SMITH, L. D. & BERTRAND, K. P. (1988). Mutations in the Tn10 *tet* repressor that interfere with induction: location of the tetracycline-binding domain. *Journal of Molecular Biology* **203**, 949–959.
- VOGLER, A. P. & LENGELER, J. W. (1989). Analysis of the *nag* regulon from *Escherichia coli* K12 and *Klebsiella pneumoniae* and of its regulation. *Molecular and General Genetics* **219**, 97–105.
- VOGLER, A. P., TRENTMAN, S. & LENGELER, J. W. (1989). Alternative route for biosynthesis of amino sugars in *E. coli* K12 mutants by means of a catabolic isomerase. *Journal of Bacteriology* **171**, 6582–6592.
- WHITE, R. J. (1968). Control of aminosugar metabolism in *Escherichia coli* and isolation of mutants unable to degrade amino sugars. *Biochemical Journal* **106**, 847–858.
- ZHU, N. & ROTH, J. R. (1991). The *nadI* of *Salmonella typhimurium* encodes a bifunctional regulatory protein. *Journal of Bacteriology* **173**, 1302–1310.