A mutation which affects both the specificity of PtsG sugar transport and the regulation of *ptsG* expression by Mlc in *Escherichia coli*

Jacqueline Plumbridge

Tel: +33 1 58 41 50 00. Fax: +33 1 58 41 50 20. e-mail: plumbridge@ibpc.fr

Institut de Biologie Physico-chimique (UPR9073), 13 rue Pierre et Marie Curie, 75005 Paris, France

Normally glucosamine (GlcN) is not a substrate for EIICB^{Glc} of the glucose phosphotransferase system (PTS), encoded by *ptsG*, but it is transported by the mannose (Man) PTS, encoded by *manXYZ*. A mutation, *umgC*, has been described in *Escherichia coli* which allows a strain mutated in the Man PTS to grow on GlcN. The *umgC* mutation was mapped to the *ptsG* region and was proposed to make *ptsG* expression constitutive. Transcription of *ptsG* is regulated by the repressor Mlc so that mutations in *mlc* enhance the expression of *ptsG*. An *mlc* mutation, however, is not sufficient to allow good growth on GlcN, unlike the *umgC* mutation. The *umgC* mutation is shown to enhance expression of *ptsG* even in the absence of any PTS sugar transport, but the increase is greater in the presence of GlcN or Man. The *umgC* mutation also increases expression of the *ptsHI* and *manXYZ* operons, which are both regulated by Mlc. The *umgC* mutation was sequenced and two mutations were found: one, G176D, within the IIC membrane domain and the second, E472K, within the soluble IIB domain of PtsG. The cloned *UmgC* allele shows the enhanced transport and regulatory characteristics of the chromosomal mutation. Analysis of the two mutations present individually on plasmids shows that the IIC mutation is responsible for both the effect on sugar specificity and regulation.

Keywords: Mlc, glucosamine, glucose induction, phosphotransferase system, catabolite repression

INTRODUCTION

The phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) is the major sugar transport system in bacteria. It consists of a series of proteins which transfer a phosphate from PEP to a sugar as it enters the cell. There are two cytoplasmic components, Enzyme I (EI) and HPr (encoded by the *ptsI* and *ptsH* genes) which are common to the transport of most sugars and which pass the phosphate to the sugar-specific membrane components called Enzyme IIs (EIIs). The EIIs are responsible for the phosphorylation of the sugar during its transport across the inner membrane. The EIIs are multidomain proteins where the different

domains (named IIA, IIB, IIC) are situated on one or several polypeptides (reviewed by Postma *et al.*, 1996). The IIC domain is an intrinsic membrane-spanning protein, whilst the IIA and IIB domains are soluble proteins which in some cases are anchored to the cytoplasmic side of the inner membrane by the IIC domain.

The EIIs can be specific for one sugar or capable of transporting several different sugars. For example, the only known sugar substrate of EIIN^{ag} from *Escherichia coli*, encoded by *nagE*, is *N*-acetylglucosamine (GlcNAc), although EIIN^{ag} does transport the antibiotic streptozotocin (Lengeler, 1980). GlcNAc is also transported by the mannose (Man) PTS, EIABCD^{Man}, encoded by the *manXYZ* operon. This transporter has a wide substrate specificity, transporting a number of hexoses, including Man, glucose (Glc), fructose and the amino sugars, glucosamine (GlcN) and GlcNAc. On the other hand, the Glc PTS (EIICB^{Glc}), encoded by *ptsG*,

Abbreviations: DT, doubling time; Glc, glucose; GlcN, glucosamine; GlcNAc, *N*-acetylglucosamine; Man, mannose; PTS, phosphoenolpyruvate (PEP)-dependent phosphotransferase system; Cm, chloramphenicol; Km, kanamycin; Tc, tetracycline.

Fig. 1. A model for the regulation of Mlc-controlled promoters by the activity of PtsG. (a) In the absence of Glc, IICB^{Glc} (PtsG) is predominately in its phosphorylated form and Mlc-controlled genes are repressed. (b) Glc is transported across the cytoplasmic membrane via IIC^{Glc}, it is concomitantly phosphorylated by IIB^{Glc} and Mlc-controlled genes are derepressed. One hypothesis to explain the correlation between unphosphorylated IICB^{Glc} and derepression of Mlc-controlled genes invokes a physical interaction between Mlc and membrane-associated IICB^{Glc}. It is proposed that the UmgC mutant described in this work partially mimics the conformation normally adopted by IICB^{Glc} during transport of Glc and hence leads to derepression of Mlc-controlled genes.

has a more limited range of substrates; it is the major transporter for Glc but it is also capable of transporting Man and the non-metabolizable Glc analogue α -methylglucoside (Curtis & Epstein, 1975; Kornberg & Jones-Mortimer, 1975). PtsG is, however, normally incapable of transporting the amino sugars so that in a wild-type *E. coli* strain GlcN is only transported by the Man PTS.

A spontaneous mutation was selected which allowed an *E. coli manXYZ* (*ptsM*) mutant strain to grow on GlcN (Jones-Mortimer & Kornberg, 1980). This mutation, called *umgC*, mapped to the *ptsG* loci (*ptsG* was previously called *umg*, uptake of α -methylglucoside). It was believed that this mutation rendered the expression of *ptsG* constitutive and thus capable of transporting GlcN which was not itself capable of inducing *ptsG* expression, unlike Glc (Jones-Mortimer & Kornberg, 1980).

It has been shown that expression of *ptsG* is controlled by the *mlc*-encoded transcription factor. Growth on Glc induces *ptsG* expression (Erni & Zanolari, 1986; Korn-

Table 1. *E. coli* strains

Strain	Genotype*	Source/reference
JM2053	<i>thyA ilvD mglP ptsF ptsG umgC nag kga ptsM pel(am) uhp^c galP::Tn10</i>	Jones-Mortimer & Kornberg (1980); CGSC
IBPC5321	<i>thi-1 argG6 argE3 his-4 mtl-1 xyl-5 rpsL ΔlacX74 mlc-1</i>	Plumbridge & Vimr (1999)
IBPC522	<i>IBPC5321 ptsM8 nagE467 ptsG22 zcf229::Tn10</i>	Plumbridge (1990)
IBPC703	<i>IBPC5321 ptsM8 nagE467 umgC zcf229::Tn10</i>	IBPC522 $\times$ P1 (JM2053) = GlcN ⁺
IBPC704	<i>IBPC5321 nagE467 ptsG22 zcf229::Tn10</i>	IBPC522 $\times$ P1 (JM2053) = GlcN ⁺
IBPC707	<i>IBPC5321 manXYZ::Tn9</i>	Plumbridge & Vimr (1999)
JM101	<i>F' traD36 lacI^a Δ(lacZ)M15 proA⁺B⁺/supE Δ(lac-proAB)</i>	
IBPC1012	<i>JM101 mlc::Tc</i>	
KD622	<i>MC4100 ϕ(malK-lacZ)1113 (λplacMu50) mlc::Tn10Km</i>	Decker <i>et al.</i> (1998)
CAG12122	<i>MG1655 zea3125::Tn10Km rph-1</i>	Singer <i>et al.</i> (1989)
JM-G1	<i>JM101 λRS/ptsG-lacZ</i>	Plumbridge (1998b)
JM-G2	<i>JM-G1 mlc::Tc</i>	Plumbridge (1998b)
JM-G11	<i>JM-G1 manXYZ::Tn9</i>	
JM-G19	<i>JM-G1 manXYZ::Tn9 mlc::Tc</i>	
JM-G35	<i>JM-G1 manXYZ::Tn9 umgC zcf229::Tn10</i>	
JM-G55	<i>JM-G1 manXYZ::Tn9 umgC zcf229::Tn10 mlc::Tn10Km</i>	
JM-G88	<i>JM-G1 umgC zcf229::Tn10 zea3125::Tn10Km</i>	
JM-G62	<i>JM-G1 ΔptsG::Cm</i>	
JM-G68	<i>JM-G1 ΔptsG::Cm ΔptsHlcr::Km</i>	
JM-G77	<i>JM-G1 ΔptsG::Cm ΔptsHlcr::Km ΔnagEBACD::Tc</i>	
JM-G96	<i>JM-G1 ΔptsG::Cm ptsM8 zdj225::Tn10</i>	
JM-H1	<i>JM101 λRS/ptsH-lacZ</i>	Plumbridge (1999)
JM-H2	<i>JM-H1 mlc::Tc</i>	Plumbridge (1999)
JM-H6	<i>JM-H1 manXYZ::Tn9</i>	
JM-H30	<i>JM-H1 manXYZ::Tn9 umgC zcf229::Tn10</i>	
JM-M1	<i>JM101 λRS/manX-lacZ</i>	Plumbridge (1998a)
JM-M2	<i>JM-M1 mlc::Tc</i>	Plumbridge (1998a)
JM-M10	<i>JM-M1 manXYZ::Tn9</i>	
JM-M13	<i>JM-M1 manXYZ::Tn9 umgC zcf229::Tn10</i>	

* Cm, chloramphenicol; Km, kanamycin; Tc, tetracycline.

Fig. 2. The *ptsG* gene and location of the *umgC* mutation. The relative locations of the IIC and IIB domains are indicated. The two changes found in the *umgC* strain (G176D and E472K) are shown and the site of phosphorylation, Cys421, within IIB is indicated. *ptsG* is expressed from two promoters, p1 and p2, and controlled by Mlc binding to two operators located upstream as shown. The locations of the oligonucleotides Glc2, Glc5 and Glc9 are shown with an asterisk at their 5' end. Only relevant restriction sites are indicated. The *EcoRI* site shown with a dotted line is derived from pBR322 during the cloning.

berg & Reeves, 1972), presumably by relieving Mlc repression (Kimata *et al.*, 1998; Plumbridge, 1998b, 1999). Mlc has also been shown to control the expression of the *manXYZ* transporter (Plumbridge, 1998a) and the central genes of the PTS, *ptsHI* (Kim *et al.*, 1999; Plumbridge, 1999; Tanaka *et al.*, 1999). These experiments suggested there was a correlation between the activity of PtsG, in particular the state of phosphorylation of IIB^{Glc}, and the derepression of Mlc-controlled genes (Fig. 1). In the light of this information, a possible explanation for the effect of the *umgC* mutation was that it rendered PtsG expression constitutive by affecting Mlc regulation. In this work the *umgC* mutation has been characterized and its effect on Mlc regulation of *ptsG* investigated.

METHODS

Bacteriological methods and verification of the *umgC* phenotype. The bacterial strains used are listed in Table 1. The *ptsG-lacZ*, *ptsH-lacZ* and *manX-lacZ* fusions carried on λ bacteriophage have been described previously (Plumbridge, 1998a, b, 1999). The effect of the different mutations on expression of these fusions was tested by introducing the mutations into lysogenic strains by P1 transductions. The *umgC* mutation was isolated from strain JM2053 (Table 1). A P1 lysate grown on JM2053 was used to transduce the *ptsG22 ptsM8* (GlcN⁻) strain, IBPC522, selecting for GlcN⁺. There were two classes of GlcN⁺ bacteria as judged by their colour on MacConkey/GlcN plates and both were Glc⁺ Man⁺. The two classes corresponded to those which had become *manXYZ*⁺ or *umgC* (*ptsG*⁺). Those which had received the *umgC* mutation (pink-red on MacConkey/GlcN, e.g. IBPC703) remained GlcN⁺ when the *manXYZ::Tn9* mutation was introduced, whilst those which had acquired a *manXYZ*⁺ allele (red on MacConkey/GlcN, e.g. IBPC704) lost the ability to grow on GlcN when the *manXYZ::Tn9* mutation was introduced. The *umgC* (GlcN⁺) mutation was shown to be located near to *ptsG* since it was co-transducible (5–10%) with *zcf229::Tn10*. The work described here shows that the *umgC* mutation is in fact an allele of *ptsG*. However, for simplicity and clarity it will be referred to as *umgC* to distinguish it from other *ptsG* mutations. The *zcf229::Tn10* transposon was used to introduce the *umgC* mutation into *manXYZ::Tn9* strains, screening for GlcN⁺ bacteria. The *manXYZ::Tn9* mutation was removed by selecting for *zea3125::Tn10*Km and screening for Cm^r. The *ptsM8*

(*manXYZ*) mutation was introduced using *zdf225::Tn10* (Plumbridge, 1990). Bacteria were grown in minimal MOPS medium at 30 °C and β -galactosidase activities were measured as described previously (Miller, 1972; Plumbridge, 1998a).

S1 mapping. Total RNA was prepared from strains JM-G1, JM-G2, JM-G35 and JM-G55 growing on minimal MOPS medium with glycerol or GlcN as carbon source. The probe used was the PCR fragment, Glc5-Glc2 (Fig. 2), labelled at Glc2 to monitor expression from the chromosomal copy of *ptsG*. A second probe covering the *ptsG-lacZ* junction (Glc1-Lac22) was used to monitor mRNA from the *ptsG-lacZ* fusion. Hybridization and S1 analysis were carried out as described previously (Plumbridge, 1998b).

DNA sequencing and cloning of the *umgC* mutation. PCR fragments covering the *ptsG* gene (Glc5 to Glc9, Fig. 2) were amplified from chromosomal DNA of the wild-type and the *umgC* mutated allele (IBPC703), and sequenced using the Thermosequencase Radiolabelled Terminator Cycle Sequencing Kit (Amersham Pharmacia) and a series of internal primers. Plasmid pTZ(*ptsG*) has been described previously (Plumbridge, 1999). pTZ(*umgC*) was made in an analogous way starting from IBPC703. Both plasmids contain DNA corresponding to the *MluI* to Glc9 oligonucleotide (see Fig. 2) so that the *ptsG* promoter and Mlc operator region is absent and *ptsG* is expressed from the *lac* promoter. To separate the G176D mutation from the E472K mutation on pTZ(*umgC*), the N-terminal and C-terminal halves of the gene were recloned using the *SacII* restriction site located near aa 285. The presence of the mutations on the plasmids was verified by sequencing.

RESULTS

The relative effects of *umgC* and *mlc* mutations on growth on GlcN and Man

The *umgC* mutation of JM2053, conferring growth on GlcN in a *manXYZ* background, was initially transduced into a strain derived from IBPC5321 (IBPC703). IBPC5321 was subsequently shown to carry a spontaneous *mlc* (*mlc-1*) mutation (Plumbridge & Vimr, 1999). To verify that the *mlc-1* mutation in IBPC703 was not necessary for the GlcN⁺ phenotype, the *umgC* mutation was transduced out of IBPC703 using *zcf229::Tn10* into JM-G11, a *manXYZ::Tn9 mlc*⁺ strain derived from JM101, to give JM-G35. Both JM-

Table 2. Effect of the *umgC* mutation on growth rates on GlcN and Man, and on expression of *ptsG-lacZ*

JM101 was lysogenized with λ RS/*ptsG-lacZ* and the mutations indicated were introduced by P1 transduction. All strains are *ptsG*⁺ except those which carry the *umgC* mutation. Bacteria were grown in minimal MOPS medium with 0.4% glycerol or 0.2% GlcN, Man or Glc at 30 °C as indicated. β -Galactosidase activities (Miller units, MU) were measured at four points during exponential growth. DTs were calculated by linear regression during the period when the cultures were sampled for β -galactosidase activity measurements. The data are the means of at least two and mostly four to six independent cultures. NG, No growth.

Strain	Genotype	Glycerol		GlcN		Man		Glc	
		<i>ptsG-lacZ</i> (MU)	DT (min)	<i>ptsG-lacZ</i> (MU)	DT (min)	<i>ptsG-lacZ</i> (MU)	DT (min)	<i>ptsG-lacZ</i> (MU)	DT (min)
JM-G1	Wild-type	8	110	25	150	35	140	59	66
JM-G2	<i>mlc</i>	140	115	100	100	98	95	59	74
JM-G11	<i>manXYZ</i>	9	115	—	NG	23	300	58	68
JM-G19	<i>manXYZ mlc</i>	130	120	230	600	190	270	63	65
JM-G35	<i>manXYZ umgC</i>	40	125	160	150	190	160	85	71
JM-G55	<i>manXYZ umgC mlc</i>	150	125	160	155	175	170	82	72
JM-G88	<i>umgC</i>	55	130	125	110	100	95	73	75

G11 and JM-G35 carry a *ptsG-lacZ* fusion on a λ phage as reporter for *ptsG* expression. About 5% of the Tc^R bacteria became GlcN⁺ Man⁺ while retaining the *manXYZ::Tn9* mutation. This shows that the *umgC* mutation is sufficient to confer growth on GlcN to a *manXYZ* strain in the absence of an *mlc* mutation. As will be shown, the *umgC* mutation is an allele of *ptsG* but for simplicity it will be called *umgC* to distinguish it from *ptsG*⁺ and *ptsG*[−] alleles.

The capacities of the *manXYZ*- or *umgC*-encoded transporters to allow growth on Man and GlcN were investigated by measuring the growth rates of strains using either of these systems in the presence or absence of the *mlc* mutation (Table 2). Mlc controls the expression of both *manXYZ* and *ptsG*. An *mlc* mutation increases their expression 4- and 20-fold respectively, in low catabolite repression media like glycerol (Plumbridge, 1998a, b, 1999). The effect of the *mlc* mutation on *manXYZ* expression is detected by an increase in the growth rate on both GlcN and Man. The introduction of the *mlc* mutation into a wild-type strain decreases the doubling time (DT) on GlcN from about 150 to 100 min (30 °C) (Table 2, line 2). A similar decrease in DT is seen with Man. This decrease is almost exclusively due to the effect of the *mlc* mutation on *manXYZ* expression since the PtsG-encoded transporter is effectively incapable of allowing growth on GlcN and allows only slow growth on Man. This is shown by the fact that introduction of a *manXYZ* mutation into a wild-type strain (*ptsG*⁺*mlc*⁺) eliminates measurable growth on GlcN and increases the DT on Man from about 140 to 300 min (Table 2, line 3). Subsequent introduction of an *mlc* mutation into the *manXYZ* strain has only a very small effect on growth on Man or GlcN. The growth rate on Man is slightly improved and growth on GlcN can now be measured, but with a DT of about 600 min (Table 2, line 4). This shows that GlcN can be transported, but very in-

efficiently, by the PtsG transporter when its expression is increased.

The effect of the introduction of the *mlc* mutation into a *manXYZ* strain can be contrasted with the introduction of the *umgC* mutation. The latter allows growth rates comparable to the wild-type *manXYZ*-encoded transporter. The DT of the *manXYZ umgC* strain on GlcN is about 150 min compared to 600 min for the *manXYZ mlc* strain, using the wild-type PtsG transporter (Table 2, line 5). Further introduction of an *mlc* mutation into these *manXYZ umgC* strains had no appreciable effect on the growth rates. The *manXYZ umgC* and the *manXYZ umgC mlc* strains exhibit similar growth rates on all sugars tested. The effect of the *umgC* and *mlc* mutations was also tested during growth on glycerol and Glc. The *umgC* mutation produced a slight decrease in the growth rate on glycerol and possibly on Glc, but the changes were relatively small (Table 2).

These experiments strongly suggest that constitutive expression of wild-type *ptsG*, as produced by the *mlc* mutation, is not sufficient to allow PtsG to transport GlcN efficiently and so imply that the *umgC* mutation is acting in a different, specific way to permit GlcN and Man uptake and that the *umgC* mutation is not just enhancing *ptsG* expression as expected for a constitutive mutation.

The *umgC* mutation enhances *ptsG* expression

The effect of the *umgC* mutation on growth rates compared to that of the transcription factor *mlc* strongly suggests that the *umgC* mutation is altering PtsG sugar specificity and thus allowing it to transport GlcN. However, the *umgC* mutation does also increase *ptsG* expression as shown by an effect *in trans* of the *umgC* mutation on the *ptsG-lacZ* fusion. Growth of a wild-

type strain on either GlcN or Man increases *ptsG* expression three- to fourfold but not so much as growth on Glc (eightfold; Table 2, line 1). These and other PTS sugars are capable of inducing *ptsG* expression presumably by partially relieving Mlc repression, although the molecular mechanism of the derepression has not been fully elucidated (Plumbridge, 1999). The presence of the *mlc* mutation derepresses expression in all four media relative to *mlc*⁺, but the level of *ptsG-lacZ* expression observed is different in each medium (Table 2, line 2). The P1 promoter of the *ptsG* gene is dependent upon cAMP/CAP and the level of expression in the different sugars is related to the level of catabolite repression generated by growth on the different sugars (Epstein *et al.*, 1975; Hogema *et al.*, 1998; Plumbridge, 1999).

The effect of the *umgC* mutation on the level of expression of the *ptsG-lacZ* fusion was compared to that of the *mlc* mutation (Table 2, lines 2 and 5). Two effects are discernible. Even in a non-PTS medium like glycerol, the *umgC* mutation causes about a fourfold increase in *ptsG-lacZ* expression. Introduction of an *mlc* mutation to the *umgC* strain increased expression in glycerol to the same level as in the *mlc* strain. In GlcN or Man media the *umgC* mutation also increases *ptsG-lacZ* expression about five- to sixfold (i.e. at least 20-fold compared to the basal level of the wild-type strain in glycerol), producing levels of expression even higher than that produced by the *mlc* mutation in the same medium. Subsequent introduction of the *mlc* mutation into the *umgC* strain had no further effect on expression during growth of the PTS sugars (Table 2, line 6). This can be correlated with the similar growth rates of strains carrying the *umgC* and *umgC mlc* mutations. Although the presence of the *umgC* mutation affects *ptsG* expression in the absence of any PTS substrate, expression is considerably increased in the presence of the UmgC substrates, Man or GlcN. It can also be noted that even in Glc medium the *umgC* mutation appears to increase *ptsG* expression somewhat, producing about a 30% increase in *ptsG-lacZ* expression.

The strong effect of the *umgC* mutation during growth on GlcN and Man in the *manXYZ* background on expression of the *ptsG-lacZ* reporter is reduced by the presence of a *manXYZ*⁺ allele. The presence of a second transporter for Man and GlcN reduces the level of expression of the *ptsG-lacZ* fusion, presumably by reducing the flux through the UmgC transporter. On the other hand the level of expression of *ptsG-lacZ* during growth on glycerol is not affected by the presence of the *manXYZ*⁺ allele.

The *umgC* mutation enhances *ptsG* mRNA levels

The increase in *ptsG-lacZ* expression is paralleled by an increase in chromosomal *ptsG* mRNA levels as measured by an S1 protection experiment using the Glc5-Glc2 probe (Fig. 2). The *umgC* mutation produces a small increase during growth on glycerol (Fig. 3, lane

Fig. 3. S1 analysis of the effect of *umgC* mutation on chromosomal *ptsG* mRNA. The probe used was the Glc5-Glc2 fragment labelled at Glc2. It was hybridized overnight in 40 mM PIPES (pH 6.9), 400 mM NaCl, 80% formamide (52 °C) with 25 µg total RNA extracted from JM-G1 (lanes 1 and 5), JM-G35 (*umgC*; lanes 2 and 6), JM-G2 (*mlc*; lanes 3 and 7) and JM-G55 (*umgC mlc*; lanes 4 and 8) grown in minimal glycerol medium (lanes 1-4) or minimal GlcN medium (lanes 5-8). C indicates the strain carried the *umgC* mutation. Lane 9 shows the control with 25 µg tRNA. After digestion with S1 nuclease (250 units ml⁻¹ in 30 mM sodium acetate, pH 4.5, 250 mM NaCl, 1 mM ZnCl₂, 5% glycerol) for 40 min at 37 °C, S1-resistant hybrids were analysed on a 6% denaturing acrylamide gel. The major and minor *ptsG* transcripts, P1 and P2, are indicated.

2) but a considerable increase during growth on GlcN (Fig. 3, lane 6). The *mlc* mutation in glycerol produces a comparable enhancement in *ptsG* mRNA (Fig. 3, lanes 3 and 4,) but the *mlc* mutation in GlcN (Fig. 3, lane 7) seems to produce a somewhat lower level of *ptsG* mRNA than the *umgC* mutation, with or without the *mlc* mutation (Fig. 3, lanes 6 and 8). This correlates with a lower value for the *ptsG-lacZ* fusion in the *mlc* strain grown on GlcN than for the *umgC* strain in the same medium (Table 2). The position of the two transcription start sites for *ptsG*, the major P1 transcript and the minor P2 transcript, are not changed by the *umgC* mutation and both mRNAs are similarly affected, which is the expected observation if *umgC* is altering Mlc regulation, since Mlc controls both promoters (Plum-

Table 3. Effect of the *umgC* mutation on *ptsH-lacZ* and *manX-lacZ* expression

JM101 was lysogenized with λ RS/*ptsH-lacZ* or λ RS/*manX-lacZ* and the mutations indicated were introduced by P1 transduction. Bacteria were grown in minimal MOPS medium with 0.4% glycerol (Gly) or 0.2% GlcN, Man or Glc at 30 °C as indicated. β -Galactosidase activities were measured at four points during exponential growth. The data are the means of at least two and mostly three to six independent cultures

Genotype	β -Galactosidase activity (Miller units)							
	<i>ptsH-lacZ</i>				<i>manX-lacZ</i>			
	Gly	GlcN	Man	Glc	Gly	GlcN	Man	Glc
Wild-type	420	790	840	1680	180	350	310	130
<i>mlc</i>	1450	1730	1850	1620	700	540	590	150
<i>manXYZ umgC</i>	670	2020	1670	1590	240	930	740	200

Table 4. Effect of the *umgC* mutations on plasmids on growth rates and expression of the *ptsG-lacZ* fusion

JM-G96 (λ RS/*ptsG-lacZ*, Δ *ptsG ptsM8*) was transformed with plasmids carrying pTZ(*PtsG*) wild-type and the mutant alleles indicated. The *UmgC* plasmid carries both G176D and E472K. Bacteria were pregrown in minimal MOPS medium with 0.4% glycerol and 0.5 mg ampicillin ml⁻¹, then diluted into medium with 0.2% carbon source indicated, 0.5 mg ampicillin ml⁻¹ and 10 μ M IPTG. Exponentially growing bacteria were then rediluted into the same medium and growth rates (DT) and β -galactosidase activities (Miller units, MU) were measured. Data are the means of at least two independent cultures. There was more variability in both growth rates on Man and GlcN and β -galactosidase activities in these experiments than when the *ptsG* mutations were present on the chromosome, presumably due to variations in copy number and IPTG induction. Higher IPTG concentrations did not improve growth rates.

Plasmid <i>ptsG</i> allele	Glycerol		GlcN		Man		Glc	
	<i>ptsG-lacZ</i> (MU)	DT (min)	<i>ptsG-lacZ</i> (MU)	DT (min)	<i>ptsG-lacZ</i> (MU)	DT (min)	<i>ptsG-lacZ</i> (MU)	DT (min)
<i>PtsG</i> ⁺	8	120	70	300	60	230	60	80
<i>UmgC</i>	30	125	80	110	80	100	62	80
G176D	22	120	80	80	70	100	50	75
E472K	8	125	–	> 500	–	> 500	50	80

bridge, 1998b). The S1 analysis was repeated using a probe specific for the *ptsG-lacZ* fusion. The pattern of expression was the same as for the chromosomal copy of *ptsG* (data not shown).

The *umgC* mutation enhances *ptsH* and *manX* expression

Both the *ptsHlcr* and *manXYZ* operons are controlled by Mlc but the factors of induction, three- to fourfold, are lower than that for *ptsG* (Plumbridge, 1998a, b, 1999; Table 3). The *umgC* mutation produces a small increase in expression of both *ptsH* and *manX* during growth on glycerol and larger increases during growth on GlcN and Man, comparable to the presence of the *mlc* mutation (Table 3). The observation that *umgC* has an equivalent effect on *ptsG*, *ptsH* and *manX* is in agreement with the idea that the *umgC* mutation is affecting their expression via Mlc regulation. Expression of the *nagE*-encoded GlcNAc transporter is not con-

trolled by Mlc and is not significantly affected by the *umgC* mutation (data not shown).

The *umgC* allele has two mutations, one in the IIC domain and one in the IIB domain of EIICB^{Glc}

Sequencing the *ptsG* chromosomal locus defined by oligonucleotides Glc5 to Glc9, which include 200 bp upstream of the start codon, of the *umgC* strain detected two point mutations in the *ptsG* ORF: G176D in what is predicted to be a periplasmic loop of the IIC domain (Buhr & Erni, 1993), and E472K near the C-terminal end of the protein (total 477 aa) within the soluble IIB domain (Fig. 2). The *ptsG* locus (from *MluI* site to oligo Glc9) carrying the *umgC* mutation was cloned into a plasmid vector under control of the *lac* promoter. Plasmids carrying each mutation individually were also constructed. The ability of the plasmids carrying the single and double mutations to allow growth on GlcN and Glc was measured. Both plasmids carrying the

original double *umgC* mutation and the G176D single mutation in the IIC domain allowed growth on GlcN but not the plasmid carrying the E472K IIB mutation (Table 4). In fact, the plasmid carrying the E472K mutation appears to have a negative effect on growth on GlcN and Man. The G176D and double *UmgC* plasmids also produced an increased expression of *ptsG-lacZ* during growth on glycerol, showing that it is the IIC mutation which is responsible for both the change in sugar specificity and the increase in *ptsG* expression.

DISCUSSION

The *umgC* mutation affects PtsG sugar transport specificity

The PtsG protein consists of two domains: an N-terminal membrane-bound EIIC domain predicted to include eight membrane-spanning helices, which is responsible for sugar transport across the cytoplasmic membrane, and a soluble EIIB domain located on the cytoplasmic side of the membrane (Buhr & Erni, 1993). The EIIB domain contains the Cys421 residue which is phosphorylated by EIIA^{Glc} and then transfers the phosphate to the transported sugar concomitantly with its passage through the cytoplasmic membrane. Several mutations have been described which affect the sugar specificity of EIIGlc in *E. coli*. G320V, located in the predicted transmembrane helix VIII (Buhr & Erni, 1993), allows PtsG to take up mannitol (Begley *et al.*, 1996), five mutations (F37Y, G176D, G281D, I283T and L289Q) have been characterized which allow PtsG to transport ribose (Oh *et al.*, 1999) and one mutation, V12F, has been described which allows the transport of fructose (Kornberg *et al.*, 2000). Ribose is not a PTS sugar and both ribose and fructose were taken up as free sugars by facilitated diffusion rather than by phosphorylation-accompanied transport. Interestingly, the V12F mutation, located in what is predicted to be an N-terminal α -helix on the cytoplasmic side of the membrane, was previously isolated in a selection for strains which grew better in a Glc- and oxygen-limited environment (Manché *et al.*, 1999). It shows enhanced Glc transport characteristics as well as the ability to transport fructose (Kornberg *et al.*, 2000; Manché *et al.*, 1999). Two of the ribose-positive mutations were found in regions predicted to be in periplasmic loops of the membrane-spanning region (F37Y and G176D), whilst the three others were clustered in a sequence predicted to form transmembrane helix VII (G281D, I283T and L289Q). In addition, mutations which impair transport but do not affect phosphorylation (Buhr *et al.*, 1992) and mutations which partially uncouple transport from phosphorylation (Ruijter *et al.*, 1992) are found throughout the IIC domain. The wide distribution of these mutations affecting sugar specificity or phosphorylation implies a flexible structure where changes at many locations, both within the membrane and in the connecting loops, affect transport characteristics.

Interestingly, the IIC mutation found in the *umgC* strain studied here is the same as one of the ribose-positive

mutations, G176D (Oh *et al.*, 1999). Moreover, recent experiments, which repeated the selection for a GlcN⁺ phenotype, yielded several different mutations, including changes in both V12 and G176 (Notley-McRobb & Ferenci, 2000; Zeppenfeld *et al.*, 2000). As in the case of V12F, the *umgC* mutation also allows uptake of fructose (data not shown), implying that V12F and G176D allow a general reduction in sugar specificity and transport-phosphorylation coupling. It should be noted that the *umgC* mutation does not eliminate all sugar specificity. It does not, for example, allow the uptake of GlcNAc; introduction of the *umgC* mutation to a *manXYZ nagE* (GlcNAc⁻) strain does not enable the strain to grow on GlcNAc.

The *umgC* mutation affects Mlc regulation

Two distinct, but interrelated, phenotypes can be assigned to the *umgC* mutation: an enhanced transport capacity for GlcN or Man and an increase in the expression of *ptsG* and other genes regulated by Mlc. Several pieces of evidence support the hypothesis that the *umgC* mutation affects regulation by Mlc: the *umgC* mutation produces parallel effects *in cis* on *ptsG* mRNA levels and *in trans* on *ptsG-lacZ* expression; three different Mlc-controlled operons, *ptsG*, *manXYZ* and *ptsHI*, are similarly affected; and the *mlc* mutation is epistatic to *umgC* mutations. During growth on GlcN and Man, when the *umgC* mutation has its maximum effect, there is no additional increase in expression when an *mlc* mutation is introduced, consistent with the idea that the *mlc* and *umgC* mutations are affecting the same regulatory pathway. However, it is clear that the increase in *ptsG* expression produced by the *umgC* mutation is not the only reason that growth on GlcN and Man improves since an *mlc* mutation, which produces increased *ptsG* expression, does not allow good growth on GlcN or Man. Notice that there is relatively little effect of the *umgC* mutation during growth on Glc. This is presumably because the level of catabolite repression generated by Glc dominates the expression level of *ptsG* and other Mlc-controlled genes, all of which are strongly cAMP/CAP-dependent.

The G176D mutation both causes the change in sugar specificity and the increase in *ptsG* expression

The analysis of the two mutations found in the *umgC* strain, G176D and E472K, individually on plasmids show that it is the G176D mutation which is responsible for both phenotypes, i.e. growth on GlcN and increased *ptsG* expression. This same mutation has already been shown to permit the uptake of ribose. Since several other mutations affecting PtsG sugar specificity map to the IIC domain, it is not surprising that the G176D mutation is responsible for the change in sugar specificity. It is perhaps less expected that the regulatory phenotype, increased expression of Mlc-controlled genes, is also associated with this same mutation. Previous results (Plumbridge, 1999) implicated the soluble IIB domain and the level of PtsG phosphorylation in the regulation of Mlc-controlled genes. Only in the presence of a *ptsG*⁺

allele were Mlc-regulated genes derepressed by growth on PTS sugars or by mutations which interrupted the flow of phosphates from PEP to the sugar.

Growth on Glc reasonably leads to induction by relieving Mlc repression but unlike other classical repressors no low-molecular-mass metabolite has been identified which is capable of displacing Mlc from its DNA targets. One hypothesis to explain the role of PtsG in Mlc induction is that transport of Glc permits an interaction between Mlc and membrane-bound PtsG, thus sequestering Mlc away from its DNA targets (Fig. 1; Plumbridge, 1999). Recent experiments have indeed shown that such an interaction between membrane-bound PtsG and Mlc exists (S.-J. Lee, W. Boos and J. Plumbridge, unpublished). The predicted location of G176D in a periplasmic loop strongly suggests that there is no direct interaction between G176D and Mlc which is presumably confined to the cytoplasm or the cytoplasmic side of the inner membrane. Instead it would imply that the G176D mutation has a global effect on IICB^{Glc} structure which is monitored by Mlc in the soluble part of the cell. Since the G176D mutation affects regulation in the absence of PTS sugar transport, the mutation might be mimicking an active transport conformation which is able to signal to the IIB domain. There is evidence for such a conformational coupling between the IIC and IIB domains of the mannitol-specific PTS permease (Meijberg *et al.*, 1998). The V12F mutation in the N-terminal cytoplasmic helix of the IIC domain, which shows similar characteristics to G176D (increased *ptsG* expression and the ability to transport fructose and GlcN), could, however, be in direct contact with Mlc.

The E472K mutation near the C terminus of the IIB domain appears to have no direct effect on *ptsG* expression levels. As the G176D mutation in the *umgC* strain is sufficient for growth on GlcN, one can wonder what is the origin of the E472K mutation. The presence of a plasmid carrying E472K appears to have a detrimental effect during growth on GlcN or Man compared to the wild-type *ptsG* allele (Table 4), suggesting that E472K might decrease PtsG activity. If the G176D mutation produces a very strong increase in PtsG transport activity, as has been shown for V12F (Kornberg *et al.*, 2000; Manché *et al.*, 1999), a second mutation reducing this hyperactivity might have arisen. This hypothesis is supported by the observation that when another example of strain JM2053, which had been stored independently for many years, was analysed, it was also found to carry two mutations: G176D and a UAG stop codon at position E472 (L. Notley-McRobb & T. Ferenci, personal communication) rather than the AAG lysine found in JM2053 obtained from the CGSC and analysed in this study.

NOTE ADDED IN PROOF

Biochemical evidence for the model in Fig. 1 comes from the demonstration that Mlc binds to PtsG-containing membranes (Lee *et al.*, 2000; Tanaka *et al.*, 2000).

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