

The Complete Phosphotransferase System in *Escherichia coli*

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Abstract

We here tabulate and describe all currently recognized proteins of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) and their homologues encoded within the genomes of sequenced *E. coli* strains. There are five recognized Enzyme I homologues and six recognized HPr homologues. A nitrogen-metabolic PTS phosphoryl transfer chain encoded within the *rpoN* and *ptsP* operons and a tri-domain regulatory PTS protein encoded within the *dha* (dihydroxyacetone catabolic) operon, probably serve regulatory roles exclusively. In addition to several additional putative regulatory proteins, there are 21 (and possibly 22) recognized Enzyme II complexes. Of the 21 Enzyme II complexes, 7 belong to the fructose (Fru) family, 7 belong to the glucose (Glc) family, and 7 belong to the other PTS permease families. All of these proteins are briefly described, and phylogenetic data for the major families are presented.

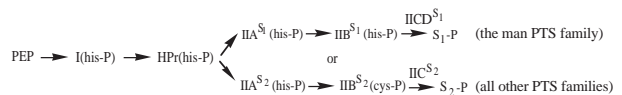
Introduction

Availability of the completely sequenced genome of an organism such as *E. coli* (Blattner *et al.*, 1997) or *Bacillus subtilis* (Kunst *et al.*, 1997) allows an evaluation of the complete complement of proteins synthesized within that cell. Previous publications from this laboratory, representing in large measure the efforts of Jonathan Reizer and his wife Aiala, have described individual operons encoding PTS proteins revealed by the *E. coli* and *B. subtilis* genome sequencing efforts. In this paper we present an integrated overview of the PTS in the enteric Gram-negative bacterium *E. coli* and make brief comparisons between the PTS in this bacterium and that in the Gram-positive bacterium, *B. subtilis*.

The last four years have witnessed an explosion in the amount of sequence data available for analysis.

Genome sequencers sometimes deposit new sequences into the databases without rigorous scrutiny. Frequently, sequencing errors conceal important Orfs, and distant phylogenetic relationships are not noticed. Our laboratory has analyzed sequence data relevant to the PTS, particularly in *E. coli*. We have carried out definitive sequence comparisons of PTS proteins, have carried out collaborative 3-dimensional analyses of several of these proteins and are currently integrating all available 1°, 2° and 3° structural information to obtain a detailed picture of the structure-function-phylogenetic relationships of PTS proteins (Reizer and Saier, 1997).

The first established phosphoryl transfer chains of the PTS are as follows:



[S₁ and S₂ are two distinct PTS sugars.]

Enzyme I (I) and HPr are general energy coupling proteins of the system while IIA, IIB and IIC (and sometimes, IID) are the protein or domain constituents of the individual sugar permeases. Primary structural analyses have shown that the C-terminal domains of Enzyme I are homologous to those of PEP synthases and pyruvate:phosphate dikinases, but that the N-terminal domains of Enzyme I and all of the other PTS proteins are not demonstrably homologous to other enzymes of known catalytic function. On the other hand, PTS-like proteins and protein domains are encoded within many *E. coli* operons and are found covalently attached to several enzymes, non-PTS transport proteins and transcription factors in a variety of bacteria where they may play regulatory roles (Poolman *et al.*, 1992, 1995; Saier and Reizer, 1994). Such PTS-like proteins or protein domains almost always bear well-conserved sequences surrounding the phosphorylation sites, exhibit the recognized catalytic residues, and in virtually all tested cases, can be phosphorylated via the PTS phosphoryl transfer chain.

Primary, secondary and tertiary structural analyses have established that the PTS arose as a chimeric system from many different sources. Thus, not all IIA proteins/domains or all IIB proteins/domains share a common evolutionary origin (see Reizer and Saier, 1997 for an overview). For example, the primary, secondary and tertiary structures of the glucose, mannose, and mannitol IIA proteins of *E. coli* are entirely different even though the IIC domains of two of these permeases (glucose and mannitol) share sufficient sequence similarity to clearly suggest homology. Further, the N,N'-diacetylchitobiose (formerly known as that for cellobiose), mannose and glucose IIB proteins are non-homologous based on similar 3-dimensional criteria, and the first of these three IIB proteins

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shows structural and mechanistic similarity with tyrosyl protein phosphate phosphatases which like IIB^{Chb} have cysteyle-P intermediates (Ab *et al.*, 1997; von Montfort *et al.*, 1997). The PTS is thus a mosaic system, having arisen from many different ancestral proteins (Reizer and Saier, 1997).

Our computational analyses of the completely sequenced *E. coli* genome have revealed the following novel *pts* genes/operons: (1) a gene cluster possibly concerning anaerobic carbon metabolism including a fused HPr, Enzyme I and IIA (H-I-IIA; FrwA) and a fructose-like Enzyme II complex (FrwBCD) (Reizer *et al.*, 1995), (2) a structurally similar system, FryA (domain order H-I-IIA)-FryBC (Reizer and Saier, 1997; this report), (3) a "nitrogen-regulatory Enzyme I" with a NifA-like domain (I^{Ntr}) (Crasnier *et al.*, 1994; Reizer *et al.*, 1995), as well as previously identified NPr (nitrogen-related HPr) and IIA^{Ntr} encoded within the *rpoN* (σ^{54}) operon, (4) a novel fused PTS-related gene product encoded within the dihydroxyacetone utilization operon including an N-terminal regulatory domain (R), an HPr domain and the HPr-recognition/histidyl phosphorylation domain of an Enzyme I homologue (Paulsen *et al.*, 2000; Reizer and Saier, 1997), (5) an operon (*frv*) encoding a fructose-like PTS (Reizer *et al.*, 1994a), (6) an operon (*glv*) encoding a glucoside-like PTS (Reizer *et al.*, 1994a), (7) a gene cluster encoding two PTS Enzymes II plus metabolic enzymes specific for galactosamine and N-acetylgalactosamine (Brinkkötter *et al.*, 2000; Reizer *et al.*, 1996a), (8) two operons (*sga* and *sgc*) concerned with pentitol/pentose metabolism and encoding an array of PTS proteins (Reizer *et al.*, 1996b), (9) the *fruBMH* gene of *E. coli* (Reizer *et al.*, 1994a), and (10) a fructose-like Enzyme II, II^{FrX} (unpublished). Altogether, five complete fructose-like Enzyme II complexes are known, Fru, Frv, Frx, Frw and Fry. The function of only one of these (Fru) is known (see Table 1).

We have also analyzed the complete PTS genetic complement in other Gram-negative bacterial pathogens such as *H. influenzae* (Reizer *et al.*, 1996c), *Treponema pallidum* (Reizer *et al.*, 1998a), *Neisseria meningitidis* (Reizer *et al.*, 1998a), and *Pseudomonas aeruginosa* (Reizer *et al.*, 1999a). Each of these organisms exhibits a unique complement of PTS proteins with distinctive structures and domain orders. For example, in *P. aeruginosa*, each of 3 PTS systems has its own EI and HPr (Reizer *et al.*, 1999a). Additionally, a hybrid response regulator with a PTS protein domain was detected in Clostridia (Reizer *et al.*, 1999b), and we have recently identified novel PTS proteins probably involved in transcriptional and metabolic regulation in a variety of bacteria (J. Tshieu and M.H. Saier, Jr., unpublished). The topology of the unusual glucitol permease has been examined (Reizer *et al.*, 1996d), and we have recently identified previously unrecognized distant evolutionary relationships between the IIA proteins specific for fructose and galactitol (J. Tshieu and M.H. Saier, Jr., unpublished). PTS proteins have been systematically classified into the transporter classification (TC) system (Saier, 2000).

We have analyzed HPr phosphoryl transfer interactions using the technique of site-specific mutagenesis, revealing probably sites of protein-protein interaction (Koch *et al.*, 1996). Phosphoryl transfer via the *E. coli* nitrogen-related

PTS has been studied biochemically (Rabus *et al.*, 1999). Altogether, five Enzyme I and six HPr paralogues are encoded within the *E. coli* genome. Most are poorly characterized in terms of their physiological functions, their targets of phosphorylation, their specificities, their regulatory mechanisms, and their involvement in regulation of physiological processes. In this review, all of these proteins will be briefly described.

The *ptsP* and *rpoN* Operons

A connection between nitrogen and carbon utilization was recognized over 25 years ago (Contesse *et al.*, 1969), yet the molecular mechanisms linking these two assimilatory processes remain poorly defined. Our computational studies led to the proposal that proteins of the PTS may provide such a link. A gene coding for a PTS protein, now designated IIA^{Ntr} (nitrogen-related Enzyme IIA), was identified within the *rpoN* operon (Jones *et al.*, 1994; Powell *et al.*, 1995; Reizer *et al.*, 1992). The *rpoN* gene encodes the sigma factor σ^N or σ^{54} of RNA polymerase (Magasanik, 1993; Merrick, 1993). In addition to the *ptsN* gene that encodes IIA^{Ntr}, the *npr* gene, also present in the *rpoN* operon, encodes an HPr-like protein designated NPr. The *ptsP* gene, encoding EI^{Ntr}, maps elsewhere (Reizer *et al.*, 1996e).

Further efforts revealed a connection, mediated by I^{Ntr}, NPr and IIA^{Ntr}, between carbon and nitrogen metabolism in *E. coli* (Powell *et al.*, 1995; Rabus *et al.*, 1999; Reizer *et al.*, 1996e). Recent work in several laboratories has shown that this complex regulatory system is apparently operative in many other Gram-negative bacteria where it similarly coordinates nitrogen metabolism with carbon utilization. In some cases it has been shown to be essential for virulence, and it plays additional regulatory roles as well (Cases and de Lorenzo, 2000; Jin *et al.*, 1994; Michiels *et al.*, 1998; O'Toole *et al.*, 1997; Segura and Espin, 1998; P.H. Edelstein, personal communication; see below). The apparent interaction of IIA^{Ntr} with the essential, ubiquitous, RAS-like GTP binding protein, Era (Powell *et al.*, 1995), suggests a role in the coordination of cell division (Lu and Inouye, 1998; Meier *et al.*, 1999, 2000; Powell *et al.*, 1999; Sze and Shingler, 1999).

A *ptsN* null mutant (*ersB1::kan*; Powell *et al.*, 1995) of *E. coli* exhibited poor utilization of various amino acids. Moreover, several sugars and dicarboxylates inhibited growth of the *ptsN* disruption mutant on media containing an amino acid (e.g., alanine, aspartate) as the sole source of nitrogen. This growth inhibition was relieved by supplying (a) the *ptsN* gene in trans or (b) an ammonium salt to the growth medium, but not by the addition of exogenous cAMP (Powell *et al.*, 1995). These results supported our earlier proposal (Reizer *et al.*, 1992) of a novel mechanism linking carbon and nitrogen assimilation.

We have studied expression of a σ^{54} -dependent transcriptional *xyIS-lacZ* fusion from the *Pseudomonas putida* TOL plasmid in *E. coli* (Du *et al.*, 1996) and of several σ^{54} -controlled genes in *K. pneumoniae* (Merrick *et al.*, 1995). *xyIS* operon expression is subject to carbon source-dependent cAMP-independent regulation in *E. coli* (Holtel *et al.*, 1994). We showed that 3-methyl benzyl alcohol-induced β -galactosidase levels were enhanced 1.5-3.3-

fold, depending on the carbon source present, by the *ptsN* null mutation (the *ersB1* mutation; Powell *et al.*, 1995). This enhancement was reversed by introduction of the cloned *ptsN* gene into the *ersB1* strain (Du *et al.*, 1996). Comparable effects were observed in studies with the *glnA* operon of *K. pneumoniae* (Merrick *et al.*, 1995). Opposite effects were observed with an *npr* null mutant (Merrick *et al.*, 1995). The results showed that IIA^{Ntr} and NPr influence σ^{54} -dependent transcription. Since *P. aeruginosa* and many other Gram-negative bacteria possess EI^{Ntr}, NPr and IIA^{Ntr} orthologues (Reizer *et al.*, 1999a and refs. cited above), it seems clear that this mechanism is widespread in evolutionarily divergent bacteria.

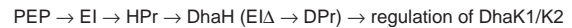
EI^{Ntr} as well as NPr and IIA^{Ntr} were purified to homogeneity and characterized biochemically in parallel with EI, HPr and IIA^{Glc} (Rabus *et al.*, 1999). The former 3 proteins comprise a phosphoryl transfer chain that functions independently of and in parallel with the latter 3 proteins (Rabus *et al.*, 1999). This was the first demonstration of specificity at the levels of EI and HPr of the PTS in a single organism. Two recent papers (Michiels *et al.*, 1998; Segura and Espin, 1998) have identified EI^{Ntr} homologues in *Azotobacter vinlandii* and *Rhizobium etli*, respectively. While inactivation of the former homologue in *A. vinlandii* altered β -hydroxyl butyrate accumulation, inactivation of the homologue in *R. etli* affected melanin production. In the latter case, the EI^{Ntr} homologue, NPr and IIA^{Ntr} may comprise part of a single regulatory cascade (Michiels *et al.*, 1998). In *P. putida*, IIA^{Ntr} controls sensitivity of the toluene-responsive, σ^{54} -dependent promoter Pu to glucose repression (Cases and de Lorenzo, 2000). In *L. pneumophila*, EI^{Ntr} controls virulence (P.H. Edelstein, personal communication). Thus, both biochemical data (Rabus *et al.*, 1999) and physiological data suggest the presence of PTS "general energy-coupling proteins", EI^{Ntr} and NPr, that function exclusively in regulation rather than in sugar transport. This suggestion is substantiated by genome analyses showing that some bacteria have regulatory PTS proteins but not sugar transporting PTS permeases (Saier and Paulsen, 2000). This fact suggests (but does not prove) that the nitrogen regulatory PTS phosphoryl transfer chain is independent of PTS sugar transport.

Since IIA^{Ntr} appears to be the terminal phosphoryl acceptor in the pathway, it seems likely that this protein is the central regulatory protein that interacts with various targets. In view of this possibility, we have collaboratively determined its 3-dimensional structure (Bordo *et al.*, 1998) in preparation for protein-protein interaction and protein phosphorylation studies. However, the mechanism of its action remains essentially unknown.

The *dha* Operon

DhaK1/K2 (YcgTS) together comprise the full-length putative dihydroxyacetone kinase which is the proposed target of DhaH regulation (Jin and Lin, 1984; Paulsen *et al.*, 2000). *Deinococcus radiodurans* has a *dha* operon encoding an R^{Dha} protein (corresponding to the N-terminal R-domain of DhaH but lacking the PTS domains of DhaH; 133 aas; 40% identity) as well as DhaK1 and DhaK2. This organism lacks DhaR, clearly suggesting that DhaH

regulates DhaK1/K2 and not DhaR. The proposed phosphoryl transfer pathway in *E. coli* is:



It should be noted that the involvement of DhaH in DhaK regulation is still hypothetical (Paulsen *et al.*, 2000).

Operons Encoding Enzyme I and HPr Homologues in Addition to Those Described Above

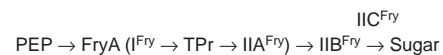
As described in Table 1, the *pts* operon encodes the classical Enzyme I (PtsI) and HPr (PtsH). Homologues are found (1) in the *ptsP* operon (EI^{Ntr}), the *rpoN* operon (NPr) and the *dha* operon DhaH (R-DPr- Δ I). An HPr homologue is also encoded within the *fruB* gene product (IIA^{Fru}-M-FPr). Finally, in both the *frw* and *fry* operons triphosphoryl transfer proteins of the same domain order and including both Enzyme I and HPr domains are to be found (see Table 1).

The proposed phosphoryl transfer pathway involving proteins encoded in the *frw* operon (Charbit *et al.*, 1996; Reizer *et al.*, 1995; see bmb.med.miami.edu/ecogene for the correct gene sequence for FrwA; K. Rudd, personal communication) is:



Because enzymes of anaerobic metabolism (Pfl; GldA) are encoded within the *frw* gene cluster, we suggest that this pathway may normally function under anaerobic conditions and may regulate anaerobic metabolism (Reizer *et al.*, 1995).

The proposed phosphoryl transfer pathway involving proteins encoded in the *fry* operon is:



The inclusion of homologues of endoglucosidases and peptidases in the *fry* gene cluster suggests that the proposed substrate sugar(s) may be oligosaccharides such as cell wall degradation products (J. Tchieu and M.H. Saier, unpublished observations).

The Sugar Transporting Energy-Coupling PTS Enzymes, Enzyme I and HPr

Enzyme I and HPr are the two sugar-non-specific protein constituents of the PTS that function in sugar transport. They initiate phosphoryl transfer to sugar in a reaction that also requires a sugar-specific Enzyme II complex (Postma *et al.*, 1993). Enzyme I uses phosphoenolpyruvate (PEP) as the phosphoryl donor, autophosphorylates on the N3 position of a histidyl residue, and transfers the phosphate to the small heat stable PTS protein, HPr.

Enzyme I includes C-terminal PEP recognition and autophosphorylation catalytic domains that are homologous to the corresponding domains in PEP synthases and pyruvate:phosphate dikinases (Saier, 1987). These three homologous enzymes all catalyze PEP-dependent autophosphorylation reactions employing the same mechanism. They are all homodimeric enzymes with a requirement for a divalent metal ion. This requirement is

Table 1. Proteins of the PTS in *E. coli*

Protein Names	Family	Alternative designation	#a.a.	Domain order	SwissProt (P) acc. or gi no.	(Proposed) Function
<i>aga (gam) operon</i>						
AgaB	Man		158	IIB	P42909	Galactosamine IIB
AgaC	Man		267	IIC	P42910	Galactosamine IIC
AgaD	Man		263	IID	P42911	Galactosamine IID
AgaF	Man		144	IIA	gi8895750	Galactosamine IIA
<i>aga operon</i>						
AgaV	Man		157	IIB	P42904	N-Acetylgalactosamine IIB
AgaW	Man		259	IIC	gi8895748	N-Acetylgalactosamine IIC
AgaE	Man		292	IID	gi8895749	N-Acetylgalactosamine IID
AgaF	Man		144	IIA	gi8895750	N-Acetylgalactosamine IIA
<i>asc operon</i>						
AscF	Glc		485	IIBIICIIA	P24241	Arbutin/Cellobiose/Salicin IIBABC
<i>bgl operon</i>						
BglF	Glc		625	IIBIICIIA	P08722	Beta-Glucoside IIBABC
<i>chb operon</i>						
ChbA	Lac		116	IIA	P17335	Di-N-acetylchitobiose/Cellobiose IIA
ChbB	Lac		106	IIB	P17409	Di-N-acetylchitobiose/Cellobiose IIB
ChbC	Lac		452	IIC	P17334	Di-N-acetylchitobiose/Cellobiose IIC
<i>cmt operon</i>						
CmtA	Fru		462	IIBC	P32059	Mannitol (cryptic) IIBC
CmtB	Fru		147	IIA	P32058	Mannitol (cryptic) IIA
<i>dha operon</i>						
DhaH	HPr, E I	ADI;YcgC	473	RH(DPr)(I)	P37349	Dihydroxyacetone kinase regulator (putative)
DhaK1			366		P76015	Dihydroxyacetone kinase subunit I
DhaK2			210		P76014	Dihydroxyacetone kinase subunit II
DhaR			639		P76016	Dihydroxyacetone operon regulator (putative)
<i>fru operon</i>						
FruA	Fru		563	IIB'BC	P20966	Fructose IIB'BC
FruB	Fru, HPr	DTP; FPr	376	IIMH(FPr)	P24217	Fructose IIA/FPr
<i>frv operon</i>						
FrvA	Fru		148	IIA	P32155	Fructose-like IIA
FrvB	Fru		485	IIBC	P32154	Fructose-like IIBC
<i>frw operon</i>						
FrwA	E I, HPr, Fru	TTP; YijH	833	H-I-IIA	P32670	Enzyme I, HPr, Fructose-like IIA
FrwB	Fru	YijK	106	IIB	P32673	Fructose-like IIB
FrwC	Fru	YijJ	359	IIC	P32672	Fructose-like IIC
FrwD	Fru	YijM	113	IIB'	P32676	Fructose-like IIB'
<i>frx operon</i>						
FrxF	Fru	HsrA	658	IIBABC	P54745	Fructose-like IIBABC
<i>fry operon</i>						
FryA	E I, HPr, Fru	TTP; YpdD	831	H-I-IIA	P77439	Enzyme I, HPr, Fructose-like IIA
FryB	Fru	YpdH	108	IIB	P77579	Fructose-like IIB
FryC	Fru	YpdG	415	IIC	P76525	Fructose-like IIC
<i>gat operon</i>						
GatA	Gat		150	IIA	P37187	Galactitol IIA
GatB	Gat		94	IIB	P37188	Galactitol IIB
GatC	Gat		451	IIC	P37189	Galactitol IIC
<i>glv operon</i>						
GlvB	Glc		161	IIB	P31451	Arbutin IIB
GlvC	Glc		368	IIC	P31452	Arbutin IIC (Probably uses IIA ^{Glc})
<i>gut operon</i>						
GutA	Gut	SrlA	187	IIC1	P56580 (gi1789055)	Glucitol IIC1
GutB	Gut	SrlB	123	IIA	P05706	Glucitol IIA
GutE	Gut	SrlE	319	IIB-IIC2	P56580 (gi3915813)	Glucitol IIB-IIC2

<i>mal</i> operon						
MalX	Glc		530	IICB	P19642	Maltose/Glucose IIBC
<i>man</i> operon						
ManX	Man		323	IIAB	P08186	Mannose/Glucose/Glucosamine/Fructose IIAB
ManY	Man		266	IIC	P08187	Mannose/Glucose/Glucosamine/Fructose IIC
ManZ	Man		286	IID	P08188	Mannose/Glucose/Glucosamine/Fructose IID
<i>mtl</i> operon						
MtlA	Fru		637	IIC-IIB-IIA	P00550	Mannitol IIABC
<i>nag</i> operon						
NagE	Glc		648	IIC-IIB-IIA	P09323	Acetylglucosamine IIABC
<i>ptsG</i> operon						
PtsG	Glc		477	IIC-IIB	P05053	Glucose IIBC
<i>pts</i> operon						
PtsH	HPr	HPr	85	H(HPr)	P07006	Sugar transport/phosphorylation
PtsI	E I	E I	575	I	P08839	Sugar transport/phosphorylation
Crr	Glc	IIA ^{Glc}	169	IIA	P08837	Glucose IIA
<i>rpoN</i> operon						
PtsN	Fru	II A ^{Ntr}	163	IIA	P31222	Nitrogen regulation
NPr	HPr		90	H(NPr)	P33996	Nitrogen regulation
<i>ptsP</i> operon						
PtsP	E I	I ^{Ntr}	748	NifA-I	P37177	Nitrogen regulation
<i>sga</i> operon						
SgaA	Fru		159	IIA	P39303	Putative Pentitol/Pentose IIA
SgaB	?		101	IIB	P39302	Putative Pentitol/Pentose IIB
(SgaT)	SgaT		484		P39301	Putative Pentitol/Pentose12 TMS Transporter
<i>sgc</i> operon						
SgcA	Fru		143	IIA	P39363	Putative Pentitol/Pentose IIA
SgcB	Gat		92	IIB	gi1361235	Putative Pentitol/Pentose IIB
SgcC	Gat		437	IIC	P39365	Putative Pentitol/Pentose IIC
<i>tre</i> operon						
TreB	Glc		473	IIBC	P36672	Trehalose IIBC (Probably uses IIA ^{Glc})

provided by Mg²⁺ under normal physiological conditions. Enzymes I of the PTS differ from PEP synthases and pyruvate:phosphate dikinases in that the Enzymes I have an N-terminal HPr-recognition domain and lack the ATP-recognition domain of the latter enzymes. They are each therefore mosaic enzymes with domains derived from at least two different sources. Because the PTS is thus far found only in bacteria (not in archaea or eukaryotes), the PTS is thought to have arisen after the three domains of life diverged, and little gene transfer between these domains of living organisms has been documented. PEP synthase can be considered to be the precursor of Enzyme I. Truncated PEP synthase domains are found as regulatory domains in pyruvate kinases of Gram-positive bacteria (Nguyen and Saier, 1995) while truncated Enzyme I domains are found in the DhaH protein of *E. coli* that is thought to regulate the heterooligomeric dihydroxyacetone kinase of this organism (Paulsen *et al.*, 2000).

HPr (the small, heat stable, histidyl phosphorylatable protein of the PTS) is the second of the two sugar non-specific protein constituents of the PTS (Postma *et al.*, 1993). It accepts a phosphoryl group from Enzyme I-P, becomes phosphorylated on a histidyl residue (histidine 15, phosphorylated on position N1), and transfers the

phosphate to a histidyl residue (N3) in any one of the many sugar-specific Enzyme IIA proteins/domains (Saier, 1987). HPr is a small (85 residue), monomeric, single domain protein that is relatively heat stable. It serves as an intermediary phosphoryl transfer protein between Enzyme I and the various sugar-specific Enzyme II complexes of the PTS. Although five Enzyme I-like proteins and six HPr-like proteins or protein domains are encoded within the *E. coli* genomes, no non-PTS homologues of HPr, or of any of the Enzyme II domains have been identified to date. Of the six HPr paralogues in *E. coli*, only two appear to function in sugar transport in normal, wild-type cells. These two proteins, HPr and FPr (encoded within the *fru* operon as a domain in FruB (Table 1)) are both phosphorylated by the "classical" Enzyme I, and they transfer their phosphoryl groups to the sugar-specific IIA proteins with differing relative rates (Sutrina *et al.*, 1998).

The phylogenetic tree for the 5 *E. coli* Enzyme I paralogues together with the single *B. subtilis* EI orthologue are shown in Figure 1A while that for the six *E. coli* HPr paralogues together with the sole *B. subtilis* HPr orthologue are shown in Figure 1B. In Figure 1A, the *B. subtilis* protein clusters loosely with the genuine *E. coli* Enzyme I. These two proteins serve the same functions and can substitute

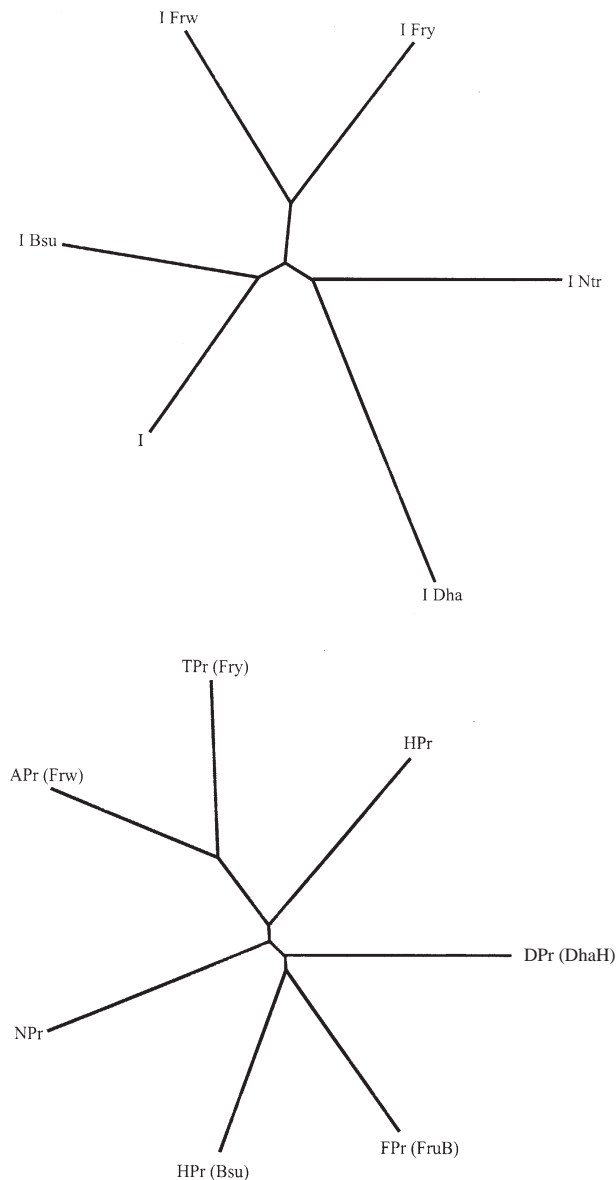


Figure 1. Phylogenetic trees for A, the *E. coli* Enzyme I (I) paralogues as well as the *B. subtilis* Enzyme I, and B, the *E. coli* HPr paralogues as well as the *B. subtilis* HPr. The Clustal X program was used to generate the trees shown here and in Figures 2 and 3 (Thompson *et al.*, 1997).

for each other enzymatically (Sutrina *et al.*, 1990). In Figure 1B, the *B. subtilis* orthologue clusters loosely with the *E. coli* FPr domain. These two proteins as well as the *E. coli* HPr are known to exhibit enzymatic cross-reactivity. Except for the consistent clustering of the Frw and Fry proteins in both trees, there is no appreciable clustering of *E. coli* paralogues.

Phylogenetic Analyses of *E. coli* Paralogues Within the Fru Family (TC #4.A.2)

The seven Fru family members encoded within the *E. coli* genome are (1) CmtAB, (2) FruAB, (3) FrvAB, (4) FrwABC(D), (5) Frx (HsrA), (6) FryABC, and (7) MtlA.

Except for the IIA components, the two-mannitol systems (Cmt and Mtl) are very divergent in sequence from the five fructose-like systems. Phylogenetic trees for the five *E. coli* paralogues of the Fru family, together with the *B. subtilis* Fru orthologues, are shown in Figures 2A (IIA), B (IIB) and C (IIC). The tree depiction suggests that the probable gene duplication events that gave rise to all of these *E. coli* paralogues occurred at about the same time that Gram-positive bacteria diverged from Gram-negative bacteria. The two Mtl and Cmt systems probably diverged from the Fru systems at an earlier time in evolutionary history (data not shown).

Phylogenetic Analyses of *E. coli* Paralogues Within the Glc Family (TC #4.A.1)

The seven Glc family members encoded within the *E. coli* genome are (1) AscF-Crr, (2) BglF, (3) GlvBC, (4) MalX-Crr, (5) NagE, (6) PtsG-Crr and (7) TreB-Crr. Note that four of these seven PTS permeases use IIA^{Glc} encoded by the *crr* gene. The phylogenetic trees showing the probable evolutionary relationships of these paralogues to each other are presented in Figures 2D (IIA), E (IIB) and F (IIC). Only four IIA proteins are presented since the Glc, Asc, Mal and Tre system all use the same IIA protein (Crr or IIA^{Glc}). Both the IIB and IIC trees reveal two primary sequence divergent clusters: the Glc/Nag/Mal clusters, to which the *B. subtilis* Glc protein domains belong, and the Asc, Bgl, Tre, Scr clusters. In both of these latter trees, the *B. subtilis* Glc proteins cluster with the *E. coli* orthologues. Thus, it is predicted that all gene duplication events giving rise to the *E. coli* paralogues of the Glc family occurred prior to the divergence of Gram-positive bacteria from Gram-negative bacteria.

Phylogenetic Analyses of *E. coli* Paralogues Within the Man Family (TC #4.A.6)

Three Enzyme II complexes comprise the Man family, (1) Aga(Gam)BCDF, (2) AgaVWEF and (3) ManXYZ. These three systems are specific for galactosamine, N-acetyl galactosamine and hexoses, respectively (Brinkkötter *et al.*, 2000; Reizer *et al.*, 1996a). These systems comprise the so-called "splinter group" of PTS permeases that are non-homologous to all other PTS systems and require the presence of an auxiliary IID constituent (see Introduction). Because the Gam and Aga systems use the same IIA protein (AgaF), only two IIA^{Man} paralogues are found encoded within sequenced *E. coli* chromosomes.

The four phylogenetic trees of the Man family Enzyme II complex constituents of *E. coli* are presented in Figure 3. In the IIB, IIC and IID trees, the pairs of Aga proteins cluster together, distant from the homologous Man proteins. Thus, parallel evolution of all constituents of these three systems can be proposed. The two Aga systems diverged from each other following extragenic duplication of the primordial gene cluster encoding the precursor of these two systems, and this event followed the one that gave rise to the Man and Aga systems. It is interesting to note that the Man system can be used as an entry pathway for phage lambda DNA (see article by Esquinas-Rychen and Erni in this symposium). The possible involvement of one

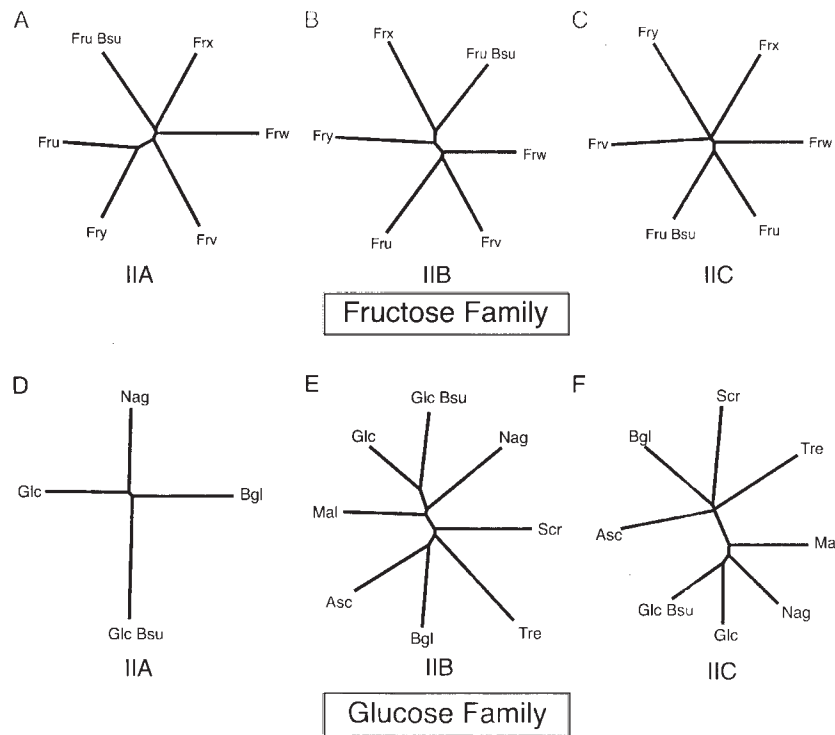


Figure 2. Top: Phylogenetic trees for the fructose-like PTS protein paralogues of *E. coli* as well as the *B. subtilis* orthologues: A (IIA), B (IIB) and C (IIC). Bottom: Comparable phylogenetic trees for the *E. coli* protein paralogues of the Glc family as well as the *B. subtilis* orthologues: D (IIA), E (IIB) and F (IIC). The Clustal X program was used for the construction of all trees (Thompson *et al.*, 1997).

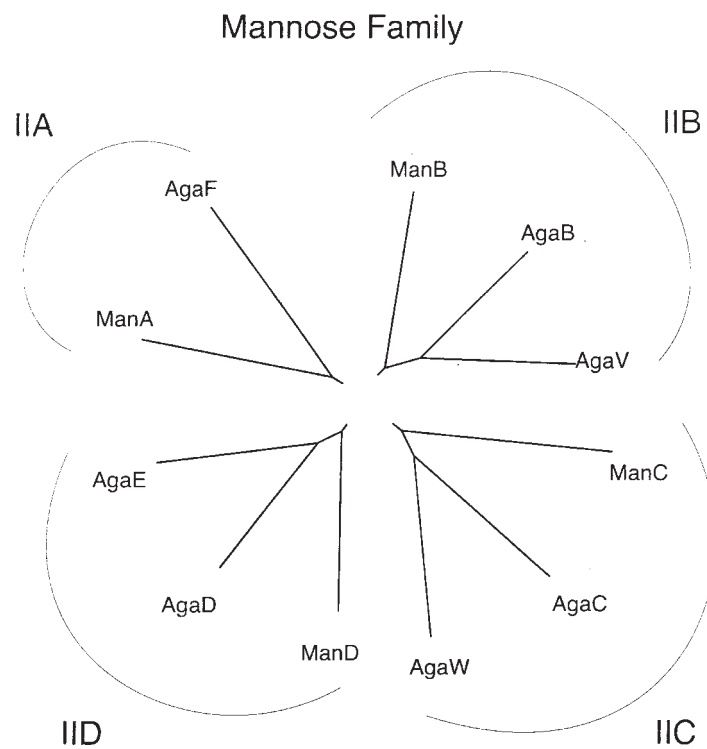


Figure 3. Phylogenetic trees for the proteins of the Man family. The trees for the IIA paralogues (upper left), the IIB paralogues (upper right), the IIC paralogues (lower right) and the IID paralogues (lower left) are presented as derived with the Clustal X program (Thompson *et al.*, 1997).

Table 2. Proteins encoded within the *fru* gene cluster

Protein	No. of Residues	Acc. No.	NCBI gi No.	Homolog(s)	Proposed Function
GldA Eco	367	P32665	7427674	GldA Ppu	Glycerol Dehydrogenase
TalC Eco	220	P32669	418514	MipB Eco	Transaldolase
FrwA Eco	833	P32670	418515	YpdD (FryA) Eco	H-I-IIA
FrwC Eco	359	P32672	418517	IIC Fru	IIC
FrwB Eco	106	P32673	418518	IIB Fru	IIB
PflD Eco	765	P32674	418519	PflE Eco	Pyruvate/Formate Lyase (PFL)
PflC Eco	292	P32675	585665		PFL-activating Enzyme
FrwD Eco	113	P32676	418521	IIB' Fru	IIB'
YjiO Eco	283	P32677	418522	PocR Sty XylR Lia	Transcriptional Regulator

or more of the Aga proteins in phage lambda infection has not been tested.

The Fructose (Fru) Family

The Fructose (Fru) Permease

FruAB, the fructose PTS permease, takes up exogenous fructose, releasing the 1-phosphate ester into the cell cytoplasm in preparation for metabolism, primarily via glycolysis (Postma *et al.*, 1993). This Enzyme II^{Fru} complex possesses three domains in the FruA protein with the domain order IIB'-IIB-IIC (Prior and Kornberg, 1988) and three domains in its FruB protein, also named diphosphoryl transfer protein (DTP), with the domain order IIA-M-H where IIA is the first phosphorylation site domain, M is a central domain of unknown function, and H is an HPr-like domain called FPr (fructose-inducible HPr) (Geerse *et al.*, 1989). FruAB is homologous to MtlA (the mannitol-specific PTS Enzyme II) which has been reported to possess 6 transmembrane α -helical segments in its IIC domain. The IIA, IIB and IIB' domains are localized to the cytoplasmic side of the membrane. IIB' is required for high affinity binding of FruB to FruA but does not participate in phosphoryl transfer (Charbit *et al.*, 1996).

The *fru* operon is inducible in wild-type *E. coli* K12 due to the presence of the fructose repressor, FruR, also known as the catabolite repressor/activator (Cra) protein. Cra is a member of the LacI-GalR family (Feldheim *et al.*,

1990; Ramseier *et al.*, 1993). This operon is also subject to positive control by the cyclic AMP-cyclic AMP receptor protein (CRP) complex. The *fru* operon contains the *fruB* gene, the *fruK* gene (encoding fructose-1-P kinase) and the *fruA* gene in that order. The *fruR* gene does not map near the *fru* operon. FruK is a distant homologue of phosphofructokinase.

The Putative Frv Permease

FrvAB, a PTS permease of unknown specificity, presumably takes up an exogenous PTS sugar, releasing the phosphate ester into the cell cytoplasm in preparation for metabolism (Postma *et al.*, 1993; Reizer *et al.*, 1994b). This Enzyme II^{Frv} complex possesses two domains in a single polypeptide chain (FrvB) with the domain order IIB-IIC and one domain in FrvA that corresponds to a IIA protein. It is homologous to the fructose-specific PTS Enzyme II. The *frv* operon (*frvABXR*) encodes, in addition to FrvAB, a probable hydrolase (FrvX) and a transcriptional regulatory protein (FrvR). It is presumably cryptic, but nothing is known regarding its expression.

The Putative Frw Permease

FrwACBD, a putative PTS permease presumably takes up unknown exogenous sugars, releasing the phosphate esters into the cell cytoplasm in preparation for metabolism (Postma *et al.*, 1993). FrwA (an HPr-Enzyme I-Enzyme IIA^{Fru} hybrid protein), FrwB (Enzyme IIB^{Fru}), FrwC (Enzyme

Table 3. Proteins encoded within the *fry* operon and by potential downstream regulatory genes

Protein	No. of Residues	Acc. No.	NCBI gi No.	Homolog(s)	Proposed Function
YpdA	565	P76523	7429361	LytS Bsu	Sensor Kinase
YpdB	244	P77742	7449506	LytT Bsu AlgR Pae	Response Regulator
YpdC	285	P77396	3915529	MrkE Kpn YjiO Eco RhaR Eco AraC Eco	Transcriptional Activator (Sugar Metabolism)
FryA (YpdD)	831	P77439	6226488	PtsA	Tpr-I-IIA
YpdE	345	P77585	7435575	YsdC Bsu	Endo-Beta-Glucanase or Peptidase
YpdF	361	P76524	7430113	Orf1 Pfu	Proline Peptidase
FryC (YpdG)	415	P77579	7466348	FruB	Enzyme IIC
FryB (YpdH)	108	P76525	7449331	FruA	Enzyme IIB
Glk	321	P46880	3183526	Glk Nme	Glucokinase

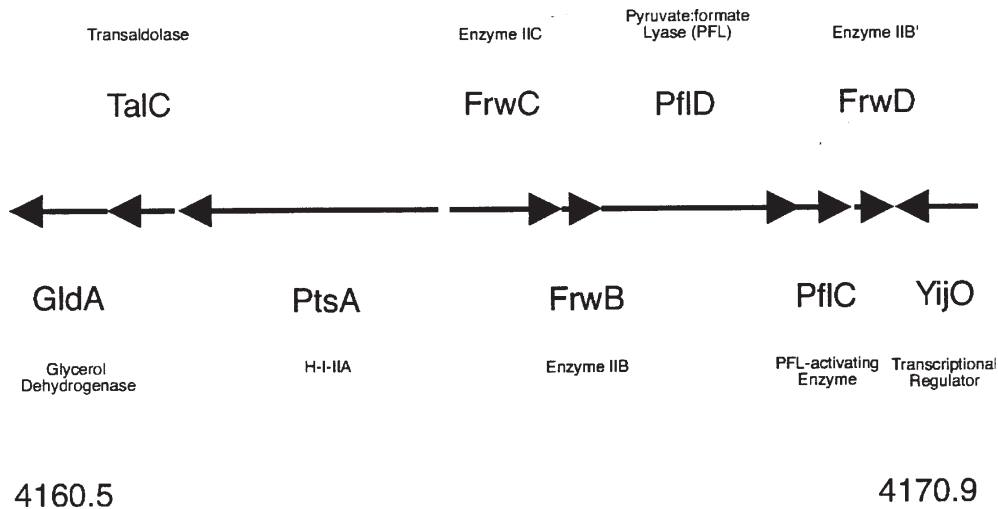


Figure 4. Operon structure of the *frw* gene cluster. Putative gene products are indicated above or below the arrows that depict the individual genes. All gene products are tabulated in Table 2. Nucleotide positions are indicated at the bottom of the Figure.

IIC^{Frw}), and FrwD (Enzyme IIB^{Frw}) are all encoded within the *frw* gene cluster. The Frw proteins and protein domains are homologous to constituents of the fructose Enzyme II complex. The *frw* gene cluster also encodes several enzymes concerned with anaerobic carbon metabolism. These enzymes include glycerol dehydrogenase, a putative transaldolase, a pyruvate-formate lyase and its activating enzyme (Table 2). At least two divergently transcribed operons are present in the *frw* gene cluster (Figure 4), but the operon structures are not clearly defined.

The Putative Frx Permease

Fr_x (HrsA), a putative PTS permease, presumably takes up an exogenous sugar, releasing the phosphate ester into the cell cytoplasm in preparation for metabolism. Fr_x is a

fructose-like PTS permease with the domain order IIA-IIB-IIC (Reizer and Reizer, 1996). Nothing is known about its sugar specificity, its function or the regulation of its synthesis. However, it has been given the alternative gene designation HrsA (heat responsive gene A) because when expressed on a plasmid, the *hrsA* gene, encoding Fr_x, suppressed the impaired thermoinduction of an outer membrane porin, OmpC in an *E. coli envZ micF* double mutant (R. Utsumi, personal communication). The basis for this observation is unknown. Fr_x appears to be the first gene in a bicistronic operon in which the second gene, YbgG, encodes a homologue of α -mannosidase. This observation suggests that Fr_x may transport a glycoside, possibly a mannoside.

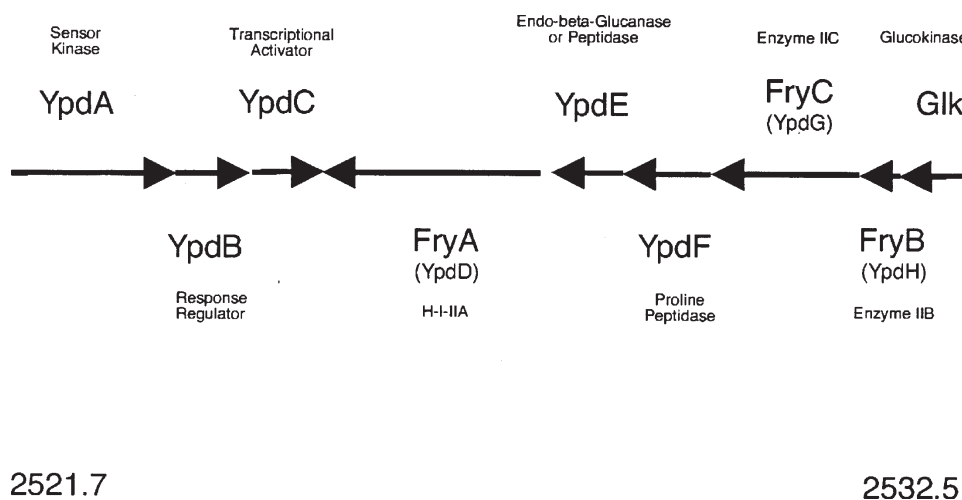


Figure 5. Operon structure of the *fry* gene cluster. Putative gene products (see Table 3) are indicated above or below the arrows that depict the individual genes. The format of presentation is as for Figure 4.

The Putative Fry Permease

FryABC (YpdDGH) comprises a complete PTS phosphoryl transfer chain with an Enzyme I, an HPr and a IIA^{Fru}-like domain incorporated in a single triphosphoryl transfer protein. FryA is homologous to FrwA throughout its length. FryB and FryC are the IIB and IIC components, respectively, of a putative PTS permease (Tables 1 and 3).

The operon structure of the *fry* operon and upstream regulatory genes are shown in Figure 5. At the left hand side of Figure 5 are depicted a putative sensor kinase (YpdA) with 6 putative transmembrane α -helical segments, a response regulator (YpdB), and a putative transcriptional activator (YpdC) homologous to several involved in sugar metabolism (the AraC/RhaR family). Between the *fryA* and *fryB* genes are found *ypdE*, encoding a putative endo- β -glucanase or peptidase, and *ypdF*, encoding a putative proline peptidase. On the basis of these observations, it can be proposed that the *fry* operon gene products are concerned with glycoprotein or peptidoglycan metabolism. The presence of the *glk* gene encoding glucokinase suggests that glucose or glucosamine may be a product of this metabolism.

The Mannitol (Mtl) Permease

MtlA, the mannitol PTS permease, takes up exogenous mannitol with low micromolar affinity, releasing the phosphate ester, mannitol-1-P, into the cell cytoplasm in preparation for oxidation to fructose-6-P by the NAD-dependent mannitol-P dehydrogenase (MtlD). Subsequent metabolism is primarily via glycolysis.

MtlA, the Enzyme II^{Mtl} complex, possesses three domains in a single polypeptide chain with the domain order IIC-IIB-IIA (Lee and Saier, 1983). It is homologous to FruAB, the fructose-specific PTS Enzyme II although only the IIA domains show extensive sequence similarity. The secondary structure of IIA^{Mtl} has been solved by NMR (Kroon *et al.*, 1993). MtlA has been reported to possess 6 transmembrane α -helical segments in its IIC domain (Sugiyama *et al.*, 1991).

The *mtl* operon (*mtlADR*) is inducible (~20x) by growth of wild type *E. coli* K12 in the presence of mannitol. The MtlR protein is a negative transcriptional regulator of the operon (Figge *et al.*, 1994). The operon is also positively controlled by the cyclic AMP-cyclic AMP receptor protein (CRP) complex and negatively by the catabolite repressor/activator (Cra) protein.

The Cryptic Mannitol (Cmt) Permease

CmtAB, the "cryptic mannitol" PTS permease, presumably functions in the uptake of an exogenous sugar. Thus, CmtA (Enzyme IIBC^{Cmt}) and CmtB (Enzyme IIA^{Cmt}) encode a complete mannitol-like PTS permease. The two genes complemented a mannitol-negative *E. coli* mutant when expressed using a heterologous promoter (Sprenger, 1993). The *cmt* genes are therefore believed to be cryptic in wild-type *E. coli*. The natural substrate(s) of the "cryptic mannitol" permease are not known.

Downstream of *cmtB* and *cmtA* are found two genes encoding proteins homologous to carbohydrate metabolic enzymes: *yggP* encodes a sorbitol or alcohol dehydrogenase homologue, and *yggF* encodes a fructose-

1,6-bisphosphatase homologue. The next gene, *yggD*, encodes the putative *cmt* operon transcriptional regulator, homologous to MtlR of *E. coli*.

The Glucose (Glc) Family

The Arbutin/Salicin/Cellobiose (ASC) Permease

AscF, the Arbutin/Salicin/Cellobiose PTS permease, takes up exogenous β -glucosides, releasing the phosphate esters into the cell cytoplasm in preparation for hydrolysis and metabolism, primarily via glycolysis. AscF, the Enzyme II^{Asc} complex, possesses three domains in a single polypeptide chain with the domain order IIB-IIC-IIA. It is homologous to PtsG (the glucose-specific PTS Enzyme II) which has been reported to possess 8 transmembrane α -helical segments in its IIC domain (Buhr and Erni, 1993).

The *ascFB* operon is cryptic in wild type *E. coli* K12. It has been reported to be activated by the insertion of IS186 into the divergently transcribed *ascG* (repressor) gene in some *E. coli* isolates (Hall and Xu, 1992). Crypticity of this and other *E. coli* β -glucoside metabolic operons presumably serves as a protective device against toxic β -glucosides found in nature. The *asc* operon contains the *ascF* gene encoding the Enzyme IIBCA and the *ascB* gene encoding a phospho- β -glucosidase that hydrolyzes the aglycone from the glycoside phosphate ester. The *ascFB* operon and the monocistronic *ascG* gene, encoding the repressor of the *ascFB* operon are transcribed from divergent promoters. AscF and AscB are paralogues of BglF and BglB, respectively (see below). AscG is paralogous to GalR. The *bgl* and *asc* operons have been estimated to have arisen by operon duplication about 3×10^8 years ago.

The β -Glucoside (Bgl) Permease

BglF, the aromatic β -glucoside (Arbutin/Salicin) PTS permease, takes up exogenous β -glucosides, releasing the phosphate esters into the cell cytoplasm in preparation for hydrolysis and metabolism. BglF, the Enzyme II^{Bgl} complex, possesses three domains in a single polypeptide chain with the domain order IIB-IIC-IIA (Chen and Amster-Choder, 1998a,b). It is homologous to AscF and to PtsG (the glucose-specific PTS Enzyme II). The latter protein has been reported to possess 8 transmembrane α -helical segments in its IIC domain. It serves not only to transport sugars but also as the sugar sensor to control expression of the *bgl* operon via BglG (Chen *et al.*, 1997; Görke and Rak, 1999).

The *bgl* operon is cryptic in wild type *E. coli* K12 (Free *et al.*, 1998; Mukerji and Mahadevan, 1997; Schnetz, 1995). It has been reported to be activated by the insertion of an IS element into the region upstream of the operon in some *E. coli* isolates (Schnetz and Rak, 1992). However, it has been reported to be expressed *in vivo* in mouse liver (Khan and Isaacson, 1998). Crypticity of this and other *E. coli* β -glucoside metabolic operons may serve as a protective device against toxic β -glucosides found in nature.

The *bgl* operon contains the *bglG* gene encoding a *bgl* operon-specific antiterminator, the *bglF* gene encoding the Enzyme II^{Bgl}, the *bglB* gene encoding a phospho- β -glucosidase that hydrolyzes the aglycone from the glycoside phosphate ester, and YeiC (538 aas; spP26218)

encoding a putative outer membrane protein homologous to LamB of *E. coli*. Other nearby genes encode a variety of carbohydrases that may function in the hydrolysis of exogenous macromolecular carbohydrates (Schnetz *et al.*, 1987). Insertion of an IS element upstream of the operon activates a promoter, and the operon is then subject to β -glucoside induction by a mechanism in which BglF-mediated phosphorylation of BglG controls its transcriptional antitermination activity (Amster-Choder *et al.*, 1989). The operon is subject to positive control by cyclic AMP and the cyclic AMP receptor protein (CRP).

The Putative Glucoside (Glv) Permease

GlvCB, a PTS permease of unknown specificity (Reizer *et al.*, 1994b) may be the biochemically characterized arbutin-specific PTS permease (Hall *et al.*, 1983). The latter system functions in the uptake of exogenous sugar(s) in conjunction with a IIA protein such as IIA^{Glc}, releasing the phosphate esters into the cell cytoplasm in preparation for metabolism. GlvC, the Enzyme IIC^{Glv} and GlvB, the Enzyme IIB^{Glv} are homologous to the IIC and IIB domains in PtsG (the glucose-specific PTS Enzyme II) which has been reported to possess 8 transmembrane α -helical segments in its IIC domain. The IIB domain is presumably localized to the cytoplasmic side of the membrane and may function with the glucose IIA protein (Crr).

The *glv* operon (*glvCBG*) may be cryptic in wild-type *E. coli* K12. GlvG probably encodes an α - or β -phosphoglucosidase as it is homologous to such enzymes. Nothing is known about the expression or regulation of the operon (Reizer *et al.*, 1994b). However, upstream and divergently transcribed is the *yidP* gene, encoding a putative transcriptional regulator of the operon, homologous to GntR and TreR of *B. subtilis* and to FavR and PhnF of *E. coli*. The *yidL* gene encodes another putative transcriptional regulator. *YidL* is downstream of and divergently transcribed from *glvCBG*.

The Maltose/Glucose (MalX) Permease

MalX, the maltose-glucose PTS permease, presumably takes up exogenous sugar, releasing the phosphate ester into the cell cytoplasm in preparation for metabolism. MalX (Enzyme IICB^{Mal}) can use glucose and maltose as substrates. It may catalyze facilitated diffusion of free sugar as well as group translocation (Reidl and Boos, 1991). The protein presumably functions with the glucose Enzyme IIA and is homologous to the glucose- and N-acetylglucosamine-specific Enzyme IICBs. The physiological function of MalX is not known (Reidl and Boos, 1991).

The N-Acetylglucosamine (NagE) Permease

NagE, the N-acetylglucosamine PTS permease, takes up exogenous N-acetyl-glucosamine, releasing the phosphate ester into the cell cytoplasm. The Enzyme II^{Nag} complex possesses three domains in a single polypeptide chain with the domain order IIC-IIB-IIA (Peri and Waygood, 1988). It is homologous to PtsG/Crr (the glucose-specific PTS Enzyme II). NagE transports N-acetylglucosamine with low micromolar affinity. It can also transport antibiotics such as streptozotocin and nojiramicin (Lengeler, 1980).

The monocistronic *nagE* operon and the adjacent

nagBACD operon comprise part of the *nag* regulon. They are transcribed from divergent promoters. The *nagBACD* operon encodes (a) glucosamine-6-P deaminase (NagB), (b) N-acetylglucosamine-6-P deacetylase (NagA), (c) the *nag* regulon transcriptional regulator (NagC) and (d) a gene of unknown function which is, however, homologous to functionally characterized phosphatases (NagD) (Plumbridge, 1989). The NagC repressor together with the cyclic AMP-cyclic AMP receptor protein (CRP) complex controls expression of the *nag* regulon (Plumbridge, 1991; Plumbridge, and Kolb, 1995). Complexities of the regulation are described in the symposium article by J. Plumbridge.

The Glucose (PtsG/Crr) Permease

PtsG/Crr, the glucose-specific PTS permease, takes up exogenous glucose with low micromolar affinity, releasing the phosphate ester into the cell cytoplasm. It can also transport the nonmetabolizable glucoside, methyl α -glucoside with 10-fold lower affinity.

The Enzyme II^{Glc} complex possesses two domains in a single polypeptide chain with the domain order IIC-IIB (PtsG), and it functions with an additional polypeptide chain, the Crr or IIA^{Glc} protein. The IIC domain of PtsG has been reported to possess 8 transmembrane α -helical segments (Buhr and Erni, 1993).

The *ptsG* operon in wild-type *E. coli* K12 is 5-10x inducible by growth in the presence of glucose, but some *E. coli* strains synthesize PtsG constitutively. IIA^{Glc} is synthesized constitutively from its own promoter, but it is also slightly inducible as a result of read through from the weaker *ptsH* promoter of the *pts* operon. *ptsG* but not *crr* is subject to positive control by the cyclic AMP-cyclic AMP receptor protein (CRP) complex. Regulation of *ptsG* expression involves a complex mechanism whereby PtsG directly binds its transcriptional regulator, Mlc (Kimata *et al.*, 1999; Plumbridge, 1998a, 1999, 2000; Tanaka *et al.*, 2000; Zeppenfeld *et al.*, 2000). The *ptsG* operon contains only the *ptsG* gene encoding the Enzyme IIC^{Glc}. Both the IIA^{Glc} protein and the IIB^{Glc} domain of the PtsG protein have been solved in 3-dimensions by x-ray crystallography and NMR spectroscopy. The 3-dimensional structure of the complex of IIA^{Glc} with HPr has also been solved (Chen *et al.*, 1993; Eberstadt *et al.*, 1996; Liao *et al.*, 1991).

The Trehalose (Tre) Permease

TreB, the trehalose PTS permease, together with IIA^{Glc} takes up exogenous trehalose with micromolar affinity. Hydrolysis occurs via phosphotrehalase (TreA) releasing glucose and glucose-6-P. Subsequent metabolism occurs primarily via glycolysis. TreB, which together with IIA^{Glc} (Crr) comprises the Enzyme II^{Tre} complex, possesses two domains in a single polypeptide chain with the domain order IIB-IIC (Klein *et al.*, 1995). The IIB and IIC domains are both homologous to the IIB and IIC domains of PtsG, the glucose-specific PTS Enzyme II.

The *treRBC* operon is inducible in wild-type *E. coli* K12 by the presence of low concentrations of trehalose. The *treBC* operon contains the *treB* gene encoding the Enzyme II^{Tre} and the *treC* gene encoding the phospho-trehalase that hydrolyzes the α,α -glycosidic bond in trehalose-6-phosphate (Rimmele and Boos, 1994). The monocistronic

Table 4. Comparison of types of PTS and PTS-associated proteins in *E. coli* with those in *B. subtilis*

Organism	Energy Coupling Proteins		No. Family Members in PTS Permease Family								Auxiliary PTS Proteins		
	Enzyme I	HPr	Fru	Glc	Man	Lac	Gut	Gat	SgaT	Total	Transcriptional Activators	Transcriptional Antiterminators	Enzymes
<i>E. coli</i>	5	6	7	7	3	1	1	2	(1)	21 or 22	0	1	0
<i>B. subtilis</i>	1	2	3	9	1	2	0	0	0	15	4	4	4

treR operon, encoding the repressor of the *treBC* operon is upstream of the *treBC* operon and is transcribed in the same direction. *tre* operon expression is under the control of the cyclic AMP-cyclic AMP receptor protein (CRP) complex as well as that of TreR. Metabolism of trehalose can occur either by a PTS-dependent (low trehalose concentrations) or a PTS-independent (high trehalose concentrations) mechanism (Boos *et al.*, 1990; Maréchal, 1984). The latter process involves periplasmic hydrolysis of trehalose to glucose.

The Mannose (Man) Family

The Mannose (Man) Permease

ManXYZ, the Enzyme I^{Man} complex (the mannose PTS permease) takes up exogenous hexoses (mannose, glucose, glucosamine, fructose, 2-deoxyglucose, mannosamine, N-acetylglucosamine, etc.), releasing the phosphate esters into the cell cytoplasm. ManXYZ possesses four domains in three polypeptide chains, ManX=IIA^{Man}, ManY=IIC^{Man} and ManZ=IID^{Man}. They are

members of the mannose PTS permease family, the "splinter group", which is not homologous to most other PTS permeases. The IIB and IIA domains (ManX) form a homodimer that is localized to the cytoplasmic side of the membrane (Stolz *et al.*, 1993). IIC and IID are integral membrane proteins with six and one transmembrane α -helical spanner(s), respectively (Huber and Erni, 1993). The 3-dimensional structure of IIA^{Man} and the secondary structure of IIB^{Man} have been determined (Nunn *et al.*, 1996; Seip *et al.*, 1997).

The *manXYZ* operon is either constitutively expressed or inducibly expressed in response to extracellular sugar substrates, depending on the *E. coli* strain examined. The Mlc protein plays a primary role in transcriptional regulation of this operon in inducible strains (Plumbridge, 1998b; Plumbridge and Kolb, 1991). The *manXYZ* operon is also subject to positive control by the cyclic AMP-cyclic AMP receptor protein (CRP) complex.

Table 5. Suggested homology based on PSI-BLAST searches.

IIC Retrieved:	Glc	Fru	Mtl	Lac	Cel	Gat	Sga/Sgc	LicR/CelR/BglG
Query:								
Glc (Scr)	+	+	+	+	+			
Fru	+	+	+	+	+			
Mtl	+	+	+	+	+			
Lac				+	+			
Cel				+	+			
Gat						+	+	
Sgc						+	+	
IIA	Glc	Fru	Mtl	Lac	Cel	Gat	Sga/Sgc	LicR/CelR/BglG
Glc (Scr)	+							
Fru		+	+			+	+	+
Mtl (Ntr)		+	+			+	+	+
Lac				+	+			
Cel				+	+			
Gat		+	+			+	+	+
Sga/Sgc		+	+	+	+	+	+	+
LicR/CelR/BglG						+		+
IIB	Glc	Fru	Mtl	Lac	Cel	Gat	Sga/Sgc	LicR/CelR/BglG
Glc (Scr)	+							
Fru		+						
Mtl			+					
Lac				+	+			
Cel				+	+			
Gat			+		+	+	+	+
Sga/Sgc			+	+	+	+	+	+
LicR/CelR/BglG						+		+

The Galactosamine (Gam) Permease

AgaBCDF, the galactosamine (Gam) PTS permease, takes up exogenous galactosamine, releasing the phosphate ester into the cell cytoplasm in preparation for metabolism (Brinkkötter *et al.*, 2000; Postma *et al.*, 1993). AgaB is an Enzyme IIB; AgaC is an Enzyme IIC, AgaD is an Enzyme IID, and AgaF is an Enzyme IIA. AgaF is shared by the N-acetylgalactosamine permease, AgaVWEF (Brinkkötter *et al.*, 2000). All of the constituents of these two systems are homologous to the proteins of the mannose Enzyme II complex (Reizer *et al.*, 1996a).

The first of two *aga* operons (*kbaYagaBCDI*) (see below) encodes KbaY which together with KbaZ comprises a two polypeptide tagatose-bis-phosphate aldolase, three of the four constituents of the Gam Enzyme II complex, and Agal, a putative isomerase. The *agaR* gene, encoding a transcriptional regulator, precedes and is divergently transcribed from the *kbaZagaVWEFA* operon that is adjacent to the *kbaYagaBCDI* operon. The *kbaZagaVWEFA* operon encodes the four N-acetylgalactosamine Enzyme II constituents (AgaVWEF) as well as the second polypeptide chain of tagatose-bis-phosphate aldolase, kbaZ, and AgaA, an N-acetylgalactosamine 6-P deacetylase. The repressor, AgaR, responds positively to exogenous N-acetylgalactosamine and galactosamine.

E. coli strains B, C and EC3132 can utilize both sugars, but *E. coli* K-12 can use neither due to a deletion covering genes *agaW'EF'A*. *E. coli* K-12 therefore lacks the complete set of metabolic enzymes for both N-acetylgalactosamine and galactosamine metabolism, yielding a negative phenotype for the utilization of these two sugars. Remnants of the putative recombination site flank the deleted DNA in *E. coli* K-12. *E. coli* K-12 "suppressor" mutants can be isolated that regain the ability to utilize N-acetylgalactosamine but not galactosamine. They retain the original *agaW'EF'A* deletion and carry suppressor mutations in the *gat* (galactitol) operon or the *nag* (N-acetylglucosamine) operon (Brinkkötter *et al.*, 2000).

The N-Acetylgalactosamine (Aga) Permease

AgaVWEF, the N-acetylgalactosamine (Aga) PTS permease, takes up exogenous N-acetylgalactosamine, releasing the phosphate ester into the cell cytoplasm in preparation for metabolism (Brinkkötter *et al.*, 2000). AgaV is an Enzyme IIB; AgaW is an Enzyme IIC, AgaE is an Enzyme IID, and AgaF is an Enzyme IIA. AgaF is shared by the galactosamine permease, AgaBCDF (Brinkkötter *et al.*, 2000). All of the constituents of these two systems are homologous to the proteins of the mannose Enzyme II complex (Reizer *et al.*, 1996a).

The second of two *aga* operons (*kbaZagaVWEFA*) (see above) encodes KbaZ, which together with KbaY comprises a two polypeptide tagatose-bis-phosphate aldolase, all four of the constituents of the Aga Enzyme II complex, and AgaA, an N-acetylgalactosamine-6-P deacetylase. The *agaR* gene, encoding a transcriptional regulator, precedes and is divergently transcribed from the *kbaZagaVWEFA* operon. This operon is adjacent to the *kbaYagaBCDI* operon. The *kbaYagaBCDI* operon encodes three of the four galactosamine Enzyme II constituents

(AgaBCD) as well as the second polypeptide chain of tagatose-bis-phosphate aldolase, kbaY, and Agal, a putative isomerase. The repressor, AgaR, responds positively to exogenous N-acetylgalactosamine and galactosamine.

E. coli strains B, C and EC3132 can utilize both sugars, but *E. coli* K-12 can use neither due to a deletion covering genes *agaW'EF'A* (see above). They lack the complete set of metabolic enzymes for both N-acetylgalactosamine and galactosamine, yielding a negative phenotype for the utilization of these sugars. *E. coli* K-12 "suppressor" mutants can be isolated that regain the ability to utilize N-acetylgalactosamine but not galactosamine. They retain the original *agaW'EF'A* deletion and carry suppressor mutations in the *gat* (galactitol) operon or the *nag* (N-acetylglucosamine) operon (Brinkkötter *et al.*, 2000).

Other PTS Permease Families

The N,N'-Diacetylchitobiose (Chb) Permease

ChbA-ChbB-ChbC, the N,N'-diacetylchitobiose PTS permease (previously designated CelABC, the Cellobiose/Arbutin/Salicin PTS permease), takes up exogenous N,N'-diacetylchitobiose, releasing the phosphate ester into the cell cytoplasm in preparation for hydrolysis and metabolism. Only this disaccharide is an inducer of the system, but other β -glucosides such as cellobiose are substrates (Keyhani and Roseman, 1997). The Enzyme II^{Chb} complex possesses three polypeptide chains, ChbA, ChbB and ChbC (Parker and Hall, 1990a; Reizer *et al.*, 1990). It is homologous to the well-characterized lactose-specific PTS Enzymes II of Gram-positive bacteria. IIC (ChbC) is an integral membrane transport protein while IIA (ChbA) and IIB (ChbB) are localized to the cytoplasmic side of the membrane.

As noted above, the *chb* operon is inducible in wild-type *E. coli* K12, but it has been reported to be activated for β -glucoside uptake by the insertion of an IS1 insertion sequence elements upstream of the coding region of the *chb* operon in some *E. coli* isolates (Parker and Hall, 1990b). The *chb* operon contains in addition to *chbA*, *chbB* and *chbC* which encode the Enzyme II^{Chb} complex, the *chbR* gene encoding a negative regulatory protein, and the *chbF* gene encoding an enzyme that can function as either a phospho- β -N,N'-diacetylchitobiase or a phospho- β -glucosidase. The operon also includes the *chbG* gene of unknown function. The operon gene order is *chbBCARFG*. ChbR and ChbF are paralogues of MelR, the repressor of the *E. coli* melibiose operon, and *melA*, the α -galactosidase of the melibiose operon, respectively (Parker and Hall, 1990a). The 3-dimensional structure of IIB^{Chb} has been determined (von Montfort *et al.*, 1997). The *chb* operon is subject to positive control by the cyclic AMP-cyclic AMP receptor protein complex.

The Galactitol (Gat) Permease

GatABC, the galactitol PTS permease, takes up exogenous galactitol, releasing the phosphate ester into the cell cytoplasm in preparation for oxidation and further metabolism, primarily via a modified glycolytic pathway, the tagatose-6-P glycolytic pathway (Nobelmann and Lengeler, 1995, 1996; Postma *et al.*, 1993). GatABC, the

Enzyme II^{Gat} complex, possesses three polypeptide chains, GatA (IIA^{Gat}), GatB (IIB^{Gat}) and GatC (IIC^{Gat}). GatA is probably homologous for the IIA constituents of the Fru, Mtl and Sga/Sgc systems (see Table 5 below). GatB is homologous to IIB^{Sga} and IIB^{Sgc} (see below) and shows limited sequence similarity to the IIB proteins of the mannitol and cryptic mannitol permeases (IIB^{Mtl} and IIB^{Cmt}, respectively) (see Table 5 below and Reizer *et al.*, 1996b, 1997). GatC is homologous to the SgcC (IIC^{Sgc}) protein and shows very limited sequence similarity to IIC^{Fru}. The latter domain has been reported to possess 6 transmembrane α -helical segments. GatD, the galactitol 1-P-5-dehydrogenase, is homologous to several dehydrogenases including the glucitol (sorbitol) dehydrogenases of *Bacillus halodurans* and many other organisms.

The *gat* operon (*gatYZABCD*) contains the *gatY* gene encoding tagatose 1,6-bis-P aldolase and the *gatZ* gene encoding tagatose 6-P kinase as well as *gatD*, the NAD-dependent galactitol 1-P-5-dehydrogenase (Nobelmann and Lengeler, 1995, 1996). *gatR* encodes the repressor of the *gat* operon. The *gat* operon is either constitutively expressed or galactitol inducible in various wild-type *E. coli* strains. In *E. coli* strains that express the *gat* operon constitutively, the *gatR* gene is truncated. The *gat* operon is subject to positive control by the cyclic AMP-cyclic AMP receptor protein (CRP) complex.

The Glucitol (Gut) Permease

GutABE, the glucitol PTS permease or the glucitol Enzyme II^{Gut} complex, takes up exogenous glucitol, releasing the phosphate ester into the cell cytoplasm in preparation for oxidation to fructose-6-P and subsequent metabolism, primarily via glycolysis (Postma *et al.*, 1993; Reizer *et al.*, 1996d, 1998b; Yamada and Saier, 1987).

The enzyme possesses a split IIC domain unlike all other characterized Enzyme II complexes of the PTS (Table 1) (Reizer *et al.*, 1998b; Tangney *et al.*, 1998). GutA is a (putative) 4 TMS integral membrane protein of 187 amino acid residues. GutE is a larger protein of 319 residues that includes the hydrophilic IIB domain fused to a hydrophobic (putative) 4 TMS domain (Tangney *et al.*, 1998; Yamada and Saier, 1988). GutB is the hydrophilic IIA domain. Thus, the integral membrane IIC constituent of the glucitol permease is split in half and encoded by two distinct genes, *gutA* and *gutE*.

The *gut* operon is inducible in wild-type *E. coli* K12 by the presence of exogenous glucitol. The *gut* operon (*gutAEBDMRQ*) not only encodes the Enzyme II^{Gut} complex and glucitol-6-P dehydrogenase, it also encodes GutM and GutR, positive and negative transcriptional regulators of *gut* operon expression, respectively (Yamada and Saier, 1988), and GutQ, a functionally uncharacterized protein (Yamada *et al.*, 1990). As expected, *gut* operon expression is under the control of the cyclic AMP-cyclic AMP receptor protein (CRP) complex.

The Potential SgaTBA Pentitol/Pentose Permease

SgaTBA, a possible PTS permease, may take up one or more exogenous sugars. The *sga* operon encodes SgaT, an integral membrane putative transporter protein with 12 putative transmembrane α -helical spanners that might

function as a PTS Enzyme IIC or as a non-PTS permease. It is not homologous to a functionally characterized protein, but as of January, 2001, homologues are found in *Vibrio cholerae*, *Mycoplasma pneumoniae*, *Bacillus halodurans*, *Streptomyces coelicolor*, and *Corynebacterium diphtheriae* (unpublished observations and F. Titgemeyer, personal communication; see symposium contribution by the Titgemeyer group). SgaA is the putative Enzyme IIA^{Sga}, and SgaB is the putative Enzyme IIB^{Sga} (Reizer *et al.*, 1996d). They have close homologues in the same five bacteria. Thus, an intact SgaTBA system is present in these six organisms. SgaA and SgaB may be distantly related to the IIA and IIB domains of several other PTS permease families (see below). SgaA is clearly homologous to the IIA domains of IIA^{Cmt}, showing 39% identity. Little is known regarding the function of these enzymes or expression of the *sga* operon in which the encoding genes are found. However, the *sga* genes may allow metabolism and possibly interconversion of pentose and hexose phosphate esters (Reizer *et al.*, 1997). A putative hexulose-6-P synthase (SgaH), hexulose-6-P isomerase (SgaU) and sugar isomerase (SgaE) are encoded within the *sga* operon.

The Potential SgcABC Pentitol/Pentose Permease

SgcABC, a putative PTS permease, presumably has the capacity to take up an exogenous sugar, releasing the phosphate ester into the cell cytoplasm. SgcA (Enzyme IIA^{Sgc}) is homologous to the IIA domains of the fructose- and mannitol-specific PTS permeases. SgcB (Enzyme IIB^{Sgc}) is most closely related to the IIB domain of the galactitol-specific PTS permease, and SgcC (Enzyme IIC^{Sgc}) is homologous to the IIC domain of the galactitol PTS permease (Reizer *et al.*, 1996d). The function of this Enzyme II complex is not known, but it has been suggested to function in the transport and phosphorylation of 5-carbon sugars (Reizer *et al.*, 1997). Expression of the *sgc* operon in which the encoding genes are found has not been studied. The operon also encodes a putative transcriptional regulator (SgcR; homologous to AgaR), a putative ribulose-5-P-3-epimerase (SgcE), a putative SGS region protein (SgcQ) and a putative endoglucanase (SgcX, homologous to FrvX).

Comparison of the PTS in *E. coli* and *B. subtilis*

E. coli and *B. subtilis* represent the best characterized member of the Gram-negative and Gram-positive bacterial kingdoms, respectively, and these two organisms have genomes that are of about the same size (Blattner *et al.*, 1997; Kunst *et al.*, 1997; Reizer and Reizer, 1996). In Table 4 we summarize the occurrence of PTS proteins and their targets of phosphorylation. While *E. coli* encodes within its genome either 21 or 22 putative PTS permeases (depending on whether SgaT proves to be a PTS Enzyme IIC), *B. subtilis* has 15. Although the genes encoding these proteins are distributed throughout the *E. coli* chromosome, all PTS permease-encoding genes are found on just one half of the *B. subtilis* chromosome. *E. coli* encodes seven PTS permeases in the Fru family, seven in the Glc family and 7 in all other families. Of these remaining families, 3 are in the Man family, 2 in the Gat family and one each in

the Lac and Gut families. While four of the Glc family Enzyme II complexes share the same IIA protein (IIA^{Glc}) two of the Man family Enzymes II share the same IIA protein (IIA^{Aga}).

In *B. subtilis*, the 15 PTS permeases include nine Glc family members, three Fru family members, two Lac family members and one Man family member (Table 4; Reizer *et al.*, 1999c). The Gut, Gat and SgaT families are not represented. Of the nine Glc family members, only three have an associated IIA domain. However, two IIA^{Glc}-like domains (one apparently truncated) are encoded in the *B. subtilis* genome. Thus, while *E. coli* has three IIA^{Glc}-like proteins/domains for seven permeases, *B. subtilis* has either four or five IIA^{Glc}-like proteins/domains for nine permeases (Reizer *et al.*, 1999c).

While *E. coli* has five Enzyme I-like proteins/domains and six HPr-like proteins/domains, *B. subtilis* has only one and two such proteins, respectively. The second HPr in *B. subtilis*, the Crh protein, lacks the active site histidine and presumably functions exclusively in regulation. The proliferation of Enzymes I and HPrs in *E. coli* presumably reflects regulatory functions, but functional aspects of these proteins are still poorly defined. Only the EI^{Ntr} → NPr → IIA^{Ntr} has been biochemically studied and shown to function independently of the EI → HPr → IIA^{Sugar} phosphoryl transfer pathway (Rabus *et al.*, 1999). The DhaH tripartite protein of *E. coli* that contains a truncated Enzyme I domain as well as an HPr domain probably functions as an operon-specific regulator (Paulsen *et al.*, 2000). Finally, *E. coli* contains a single PTS phosphorylated antiterminator, BglG, the operon-specific transcriptional regulator of the *bgl* (β-glucoside) operon (Amster-Choder *et al.*, 1989).

The deficiency of Enzyme I and HPr paralogues in *B. subtilis* correlates with the presence of numerous PTS phosphorylatable transcriptional activators and antiterminators in this organism. Eight such proteins are found encoded within the *B. subtilis* genome. Further, two enzymes, glycerol kinase, GlpK, and pyruvate kinase, PykA, contain PTS phosphorylation domains, and another PTS auxiliary enzyme, the HPr(ser)kinase/phosphatase, PtsK may function in PTS-mediated regulation. Except for the single PTS-antiterminator, BglG, and DhaH, *E. coli* lacks all such PTS auxiliary proteins. It therefore seems clear that while both *E. coli* and *B. subtilis* use the PTS for regulatory purposes, they have chosen very different mechanisms to effect this regulation.

Distant Sequence/Motif Similarities in PTS Proteins Detected Using the PSI-BLAST Search Tool

PTS permeases have been grouped into six families in the TC system (Saier, 2000). These families are (1) the glucose/sucrose (Glc) family, (2) the fructose/mannitol (Fru) family, (3) the lactose/diacetylchitobiose/cellobiose (Lac) family, (4) the glucitol (Gut) family, (5) the galactitol (Gat) family and (6) the mannose/sorbose/fructose (Man) family. Each of the protein domains (the IIA, IIB, IIC, and in the case of the Man family, the IID domain) in each of these six families was PSI-BLASTed with iterations to convergence. The PTS protein constituents of the Gut and Man families brought up none of the constituents of the other families, and blasting constituents of the other families

did not bring up constituents of these two families. All constituents of these two families are therefore phylogenetically distinct from other PTS proteins on the basis of this criterion.

When constituents of the other four families were blasted, members of other families were often retrieved. The results are summarized in Table 5. When the IIC constituents of the Glc (Scr) or Fru (Mtl) families were PSI-BLASTed, IIC constituents of these two families as well as those of the Lac (Chb/Cel) family were retrieved. By contrast when IIC constituents of the Lac (Chb/Cel) family were blasted, only members of their own family were retrieved. We therefore suggest that the IIC constituents of the Glc (Scr), Fru (Mtl) and Lac (Chb/Cel) families are all related, though distantly, and that the IIC proteins of the Lac family are most distant. The IIC^{Gat}, IIC^{Sga} and IIC^{Sgc} proteins retrieved each other but no other PTS protein.

Similar analyses of the IIA constituents revealed that those of the Glc (Scr) family never retrieved members of other families, and members of other families never retrieved them. By this criterion, the IIA^{Glc} family is therefore distinct. By contrast, when IIA constituents of the Fru (Mtl), Gat (Sga/Sgc) families were PSI-BLASTed, all of the IIA constituents of these families, and occasionally those of the Lac (Chb/Cel) family, were retrieved. Members of the IIA^{Lac} family never retrieved other family members. Thus, for the IIA constituents, the Fru (Mtl) and Gat (Sga/Sgc) families are most closely related, and the Lac (Chb/Cel) family is more distant. Retrieval of transcriptional regulators and antiterminators of the BglG/LicR/CelR family revealed the presence of IIA-like domains in these proteins (Table 5).

Comparable analyses of the small IIB domains revealed independence of both the Glc (Scr) and the Fru (Mtl) families, but IIB^{Mtl} proteins are apparently distantly related to IIB^{Gat}, IIB^{Sga} and IIB^{Sgc}. IIB domains were also found in the LicR/CelR/BglG family of regulators. The domain order in these regulators is IIB-IIA, and these two domains occur at the extreme C-termini of these proteins. These results are consistent with available 3-dimensional structural data showing that IIA^{Glc}, IIA^{Ntr} and IIA^{Man} as well as IIB^{Glc}, IIB^{Cel} and IIB^{Man} all have different 3-D structures. They support the contention that the multidomain PTS Enzyme II complexes represent mosaic systems derived, through evolution, from a variety of protein sources (Reizer and Saier, 1997; Saier and Reizer, 1994).

Genes Encoding Phosphotransferase System Proteins in Other Bacteria

The contrast between the complete PTS in *E. coli* and that in *B. subtilis* may be representative of a wider range of diversity. For example, among the Gram-positive bacteria, none has yet been found to possess more than one Enzyme I, and only *Bacillus* species have so far been found to encode a Crh (catabolite repression HPr). *Streptococcus pneumoniae*, with a small genome, less than half the size of the *E. coli* genome, encodes as many PTS Enzyme II complexes as does *E. coli*. All of the low G+C Gram-positive bacteria use the HPr(ser)kinase/phosphatase (PtsK) for regulatory purposes. Surprisingly, *Treponema pallidum* and several species of *Neisseria* encode PtsKs, suggesting that

the kinase-mediated regulatory system studied in Gram-positive bacteria is present in these Gram-negative pathogens. *Treponema pallidum* also has one Enzyme I-like protein and one HPr homologue, but no Enzyme II complexes. These proteins probably function exclusively in regulation, possibly interconnecting regulatory circuits for carbon and nitrogen metabolism (Fraser *et al.*, 1998). Finally, *Pseudomonas aeruginosa*, with a 6 Mpb genome encodes three complete PTS phosphoryl transfer chains, one regulatory chain (Enzyme I^{Ntr}, NPr and IIA^{Ntr}) that may coordinate nitrogen and carbon metabolism (Rabus *et al.*, 1999) and two that are likely to allow uptake and phosphorylation of fructose and N-acetylglucosamine, respectively (Reizer *et al.*, 1999a). These last two systems each have their own sugar-specific Enzyme I and HPr protein domains incorporated into triphosphoryl transfer proteins analogous to FrwA and FryA of *E. coli*. From these comparative analyses, it is clear that the PTS has diverged both structurally and functionally depending on the organism analyzed. Understanding the functional consequences of these divergent systems will provide molecular biologists with exciting challenges for years to come.

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