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Minireview

Parallel PTS systems ☆

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More than forty years ago, Kundig, Ghosh, and Roseman discovered the system for sugar transport that is now generally referred to as the phosphoenolpyruvate:sugar phosphotransferase system (PTS)[1] [1]. This multi-protein system (see Fig. 1, carbohydrate PTS), found in numerous microorganisms, effects a PEP-dependent transport/phosphorylation of numerous sugars. There is a sequential phosphoryl transfer to two cytosolic proteins, enzyme I (EI) and HPr, then to membrane-associated enzymes II, which may consist of 3–4 domains (A, B, C, and D). Over the years, this system has become established as a central component in bacterial physiology as a result of the identification of multiple regulatory functions, superimposed on the transport functions, for essentially all the components of the glucose arm of the PTS. The varied aspects of PTS structure, function, and regulation have been previously reviewed [2–5].

Jonathan Reizer, who spent three years (1985–1988) in the laboratory of A.P., was a brilliant scientist who devoted essentially his entire career uncovering interesting aspects of the PTS. He was blessed with a devoted wife, Aiala, who was an expert in the emerging field of bioinformatics. Their hobby, which stretched late into the evening, was to search through databases for genes homologous to PTS sequences. The results of some of their efforts were the discovery of the pathway paralagous to the carbohydrate PTS that we now refer to as the nitrogen-metabolic PTS (see Fig. 1) [6]. They identified proteins homologous to EI (EI^{Ntr}), HPr (NPr), and $EIIA^{Glc}$ ($EIIA^{Ntr}$). Based on the location of the genes encoding NPr and IIA^{Ntr} on the operon encoding *rpoN* as well as the growth properties of a disruption mutant, Reizer and his colleagues suggested that the nitrogen-metabolic PTS functioned as a regulator of nitrogen metabolism [7]. Tragically, Jonathan Reizer's career was cut short; he died on the last day of 1999. He would undoubtedly have been a major scientific influence on the further development of understanding of the nitrogen-metabolic PTS.

While the biology of the carbohydrate PTS has previously been reviewed, there have been sparse summaries of the status of understanding of the nitrogen-metabolic PTS. It is therefore our intention to make a comparative analysis in this review of these two parallel phosphotransferase systems.

Gene organization

EI and HPr are cytosolic components of the PTS that can funnel phosphoryl groups to many sugar acceptors via specific enzymes II (see Fig. 1). In the case of the glucose arm of the PTS, the IIA domain is a cytosolic protein. It is of interest that the genes encoding HPr (*pts*H), EI (*pts*I), and IIA^{Glc} (*crr*) are encoded in a single operon (see Fig. 2). In contrast, the membrane-associated components ($IICB^{Glc}$) of the glucose PTS are encoded in a different region of the *Escherichia coli* genome (*pts*G).

The organization of the genes of the nitrogen-metabolic PTS is depicted in Fig. 3. In contrast to the arrangement of the carbohydrate PTS, none of the genes encoding EI^{Ntr} (*pts*P), NPr (*pts*O) or IIA^{Ntr} (*pts*N) are contiguous on the *E. coli* chromosome. *pts*N and *pts*O are found on the same operon as

☆ This article is dedicated to Ann Ginsburg who has, for many years, been both a close friend and important collaborator.

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[1] *Abbreviations used:* PTS, phosphotransferase system; EI, enzyme I; EIN, N-terminal domain; PEP, phosphoenolpyruvate.

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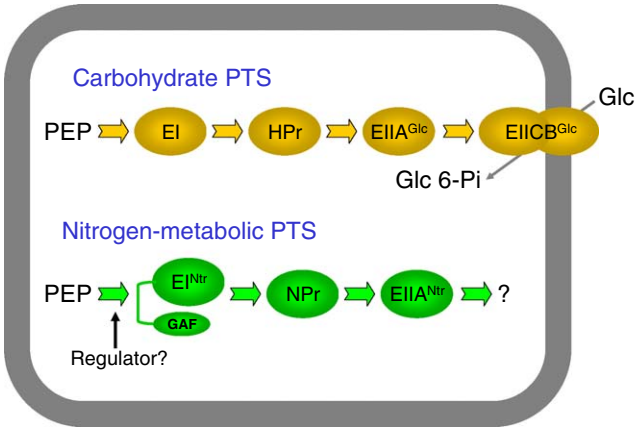

Fig. 1. Two distinct phosphotransferase systems in *E. coli*. (For color illustration, the reader is referred to the web version of this paper.)

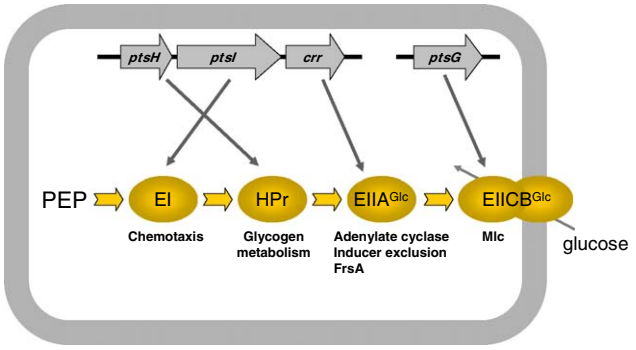

Fig. 2. Organization of the genes of the carbohydrate PTS. (For color illustration, the reader is referred to the web version of this paper.)

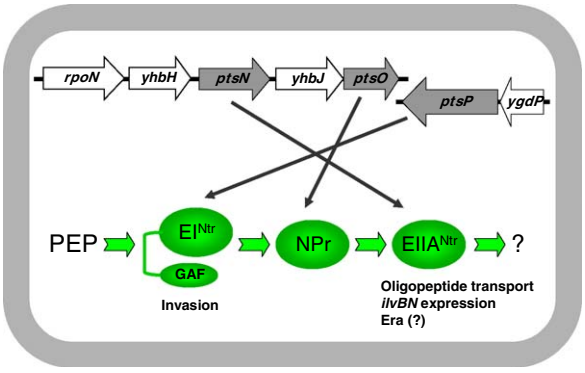

Fig. 3. Organization of the genes of the nitrogen-metabolic PTS. (For color illustration, the reader is referred to the web version of this paper.)

*rpo*N (encoding sigma N); this has formed part of the basis for the idea that this pathway is, in some way, involved with regulation of nitrogen metabolism. *pts*P, encoding EI^{Ntr}, is found on a different region of the chromosome and is transcribed in the opposite orientation to *pts*N and *pts*O.

Structures

The carbon PTS

The three-dimensional structures of all the cytosolic proteins and domains of the glucose arm, as well as those for some other sugar-specific paths, of the carbon PTS have been elucidated using both NMR and X-ray crystallographic methods [5]. As depicted in Fig. 4A, the first step of the PTS involves the autophosphorylation of EI by PEP. The binding site for PEP is located in the C-terminal domain while the active site His189 is in the N-terminal domain (EIN) where the binding site for HPr also resides. The active site His15 of HPr is the recipient of the phosphoryl group from EI. The ~40 kDa EIN-HPr complex (Fig. 4B) was solved by NMR [8]. EIN comprises two domains: there is an α subdomain, corresponding to residues 33–143, containing four helices and an α/β subdomain which is a β-sandwich and three short helices. HPr consists of three helices and a four-stranded antiparallel β-sheet. The structures of the two proteins are essentially the same in the free and complexed state. The majority of the contacts between EI and HPr are hydrophobic supplemented by some electrostatic interactions (see Fig. 4B).

Phosphorylated HPr can donate its phosphoryl group to numerous sugar-specific enzymes II. For the glucose arm of the PTS, IIA^{Glc} is the phosphoacceptor from HPr. Since three-dimensional structures of HPr and IIA^{Glc} had previously been solved by both X-ray crystallography and NMR [9], the solution structure of the HPr-IIA^{Glc} complex (Fig. 4C) was solved by NMR, including the use of residual dipolar coupling data as well as direct use of the coordinates of the crystal structures of the free proteins [10]. This was possible because no significant structural alteration of either protein is observed as a result of complex formation. The HPr (open-faced α/β sandwich) and the IIA^{Glc} (sandwich of β-strands with six antiparallel strands on each side) interaction involves a complementary fit of a convex region on HPr with a concave region on IIA^{Glc}. The central portion of the interface is mainly hydrophobic; the border of the surface is supplemented with electrostatic interactions, positive for HPr and negative for IIA^{Glc}.

The last phosphotransfer protein–protein complex in the glucose arm of the PTS involves $IIA^{Glc}$and the membrane-bound glucose transporter $IICB^{Glc}$ (Fig. 4D). $IICB^{Glc}$ consists of an N-terminal transmembrane domain (IIC) with eight putative transmembrane helices connected to a C-terminal cytoplasmic domain (IIB) by a linker [11–13]. The NMR structure of IIB^{Glc} is an α/β protein with a trigonal pyramid-like appearance [13]. There is a base of a β-sheet topped by three α-helices. NMR was also used to solve the structure between the IIB domain and IIA^{Glc}, including a structure refinement of IIB using residual dipolar coupling data [14]. The same concave depression on IIA^{Glc} was shown to interact with a convex surface on the IIB domain of $IICB^{Glc}$. The active site residues, His90 of IIA^{Glc} and Cys35 of IIB^{Glc}, are buried at the center of the interface. As with the other complexes, hydrophobic intermolecular contacts are supplemented with electrostatic interactions involving charged residues on the surface of both proteins (Fig. 4D). Based on these data, a structural model for the entire complex was proposed (Fig. 4D). Note that there is a disorder-to-order structural conversion at the N-terminus

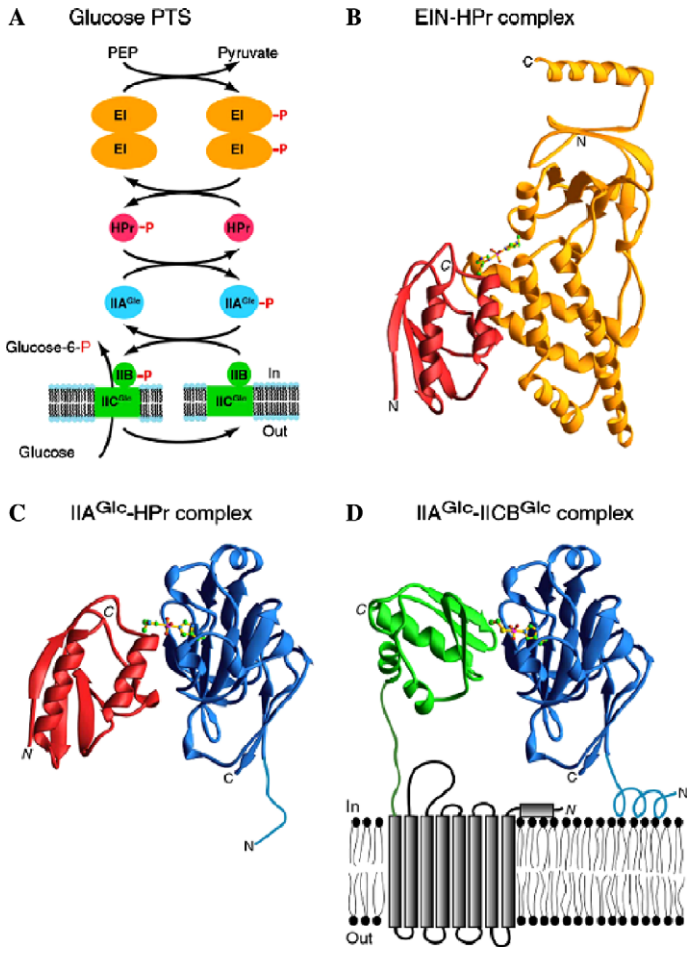

Fig. 4. Three-dimensional structures of the protein–protein complexes of the glucose arm of the carbohydrate PTS. (For color illustration, the reader is referred to the web version of this paper.)

of IIA^{Glc} depending on its location (cf., Figs. 4C and D). The solution structure of the essential membrane anchor of the amphitropic IIA^{Glc} bound to short chain phosphatidyl glycerols has also been determined by NMR [15,16].

Some important deductions that have come from the structural studies on the proteins of the carbon PTS are that any given interaction of the complex makes only a small contribution to the overall stability; thus, mutations in the proteins may be tolerated without total loss of function. It is also important to note that HPr, IIA^{Glc}, and IIB^{Glc} utilize similar surfaces for interaction with multiple partners although the actual structural folds of the individual partner proteins are quite different. Consequently, it would be impossible to predict from homologies the repertoire of protein pairs that actually form.

In contrast to EI, containing 575 amino acid residues, EI^{Ntr} has 748 amino acid residues (Fig. 5A). EI^{Ntr} contains an N-terminal domain of 127 amino acid residues that exhibits homology to the N-terminal sensory domain of NifA as well as other GAF domains. The C-terminal domain of EI^{Ntr} corresponds to 578 amino acids and is homologous to other enzymes I. The two domains are connected by flexible linkers. It was suggested that the N-terminal domain of EI^{Ntr} functions in sensory transduction of some signal that eventually regulates the phosphorylation of downstream proteins. The region around the active site histidine (residue 356) is well conserved between EI and EI^{Ntr} [17] (Fig. 5). Throughout the sequences, there is a high degree of sequence identity of EI and EI^{Ntr} (see residues highlighted in red), suggesting evolution from a common precursor. Further in silico analyses [7] led to the identification of the HPr homologue, NPr. In contrast to HPr (85 residues), NPr has a length of 90 residues (Fig. 5B). The two proteins have well-conserved sequences in the vicinity of the phosphorylation sites (His15 for HPr and His16 for NPr) as well as that of the regulatory serine residues (Ser46 in HPr and Ser48 in NPr).

The same operon (that for *rpoN*) also encodes IIA^{Ntr} (163 residues), a homologue of IIAs specific for fructose and mannitol (148 residues). As expected, the region in the vicinity of the phosphorylation site (His73) is strongly conserved among these proteins (Fig. 5C). The area around a second His residue (His120 of IIA^{Ntr}) also exhibits some degree of conservation. The sequence identity of IIA^{Mtl} with IIA^{Ntr} is about 20%.

Full-length EI^{Ntr} as well as a variety of fragments have been cloned and expressed (unpublished studies of C.-R. Lee, Y.-J. Seok, and A. Peterkofsky). Structural

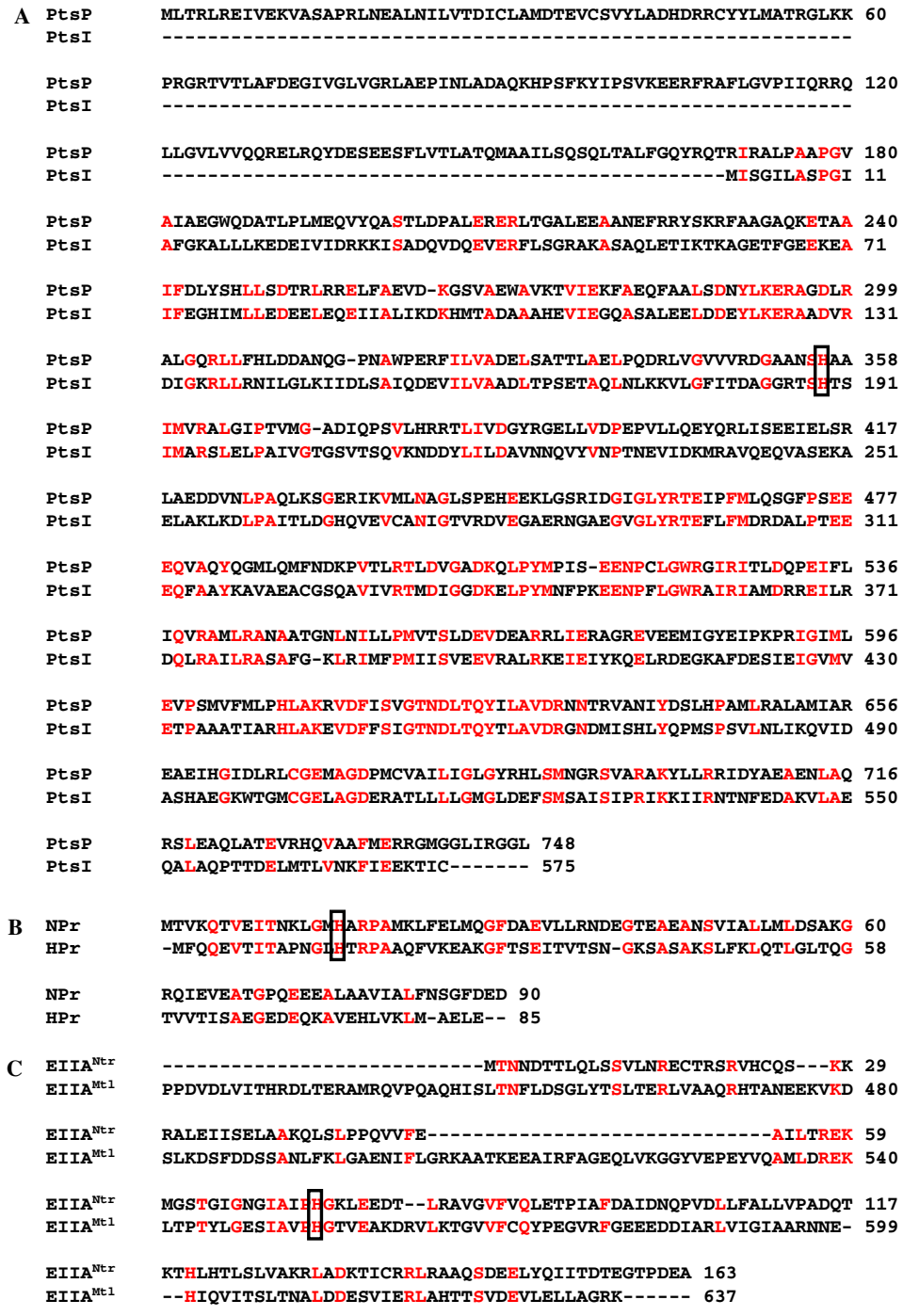

Fig. 5. Sequence alignments of the proteins of the nitrogen-metabolic PTS with its paralogs. (A) enzyme I^{Ntr} (*pts*P) aligned with EI (*pts*I) of the carbohydrate PTS; (B) NPr aligned with HPr; (C) IIA^{Ntr} ($EIIA^{Ntr}$) aligned with the IIA domain ($EIIA^{Mtl}$) of the mannitol transporter. Residues in red are identical in the alignments. The boxed residues correspond to the active-site phosphorylatable histidines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

studies, by NMR, on some of these fragments are under way (G. Wang).

The three-dimensional structure of IIA^{Ntr} has been solved both by X-ray crystallography [18] and by NMR [19]. The enzyme is characterized by a novel fold involving a central mixed parallel/antiparallel β-sheet surrounded by six α-helices (Fig. 6). The overall shape of the molecule is generally spherical. The active site His73 is located in the center of a β-sheet. As is the case with IIA^{Glc}, this active site residue is situated in a shallow depression, designed for a specific interaction with its partner, NPr. The folds of IIA^{Ntr} and IIA^{Mtl} are similar [20]. It was noted that, in IIA^{Ntr}, His120 is found on the surface, while the homologous residue of IIA^{Mtl}, His111, forms part of the active site. In the crystal structure, the protein is found as a dimer (labeled molecule A and B in Fig. 6). It was

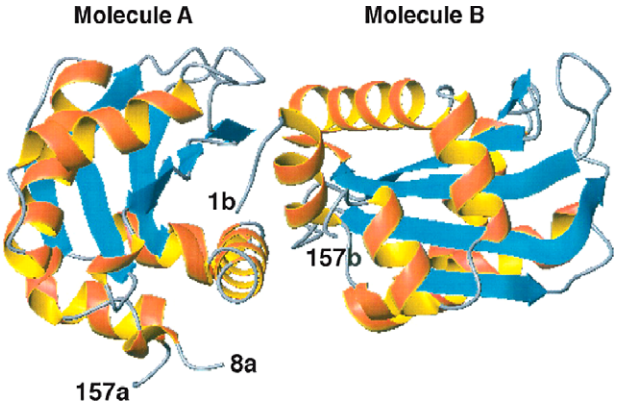

Fig. 6. The three-dimensional structure of IIA^{Ntr} (For color illustration, the reader is referred to the web version of this paper).

suggested that this might be a consequence of crystal packing [20].

Both IIA^{Ntr} and IIA^{Mtl} have essential Arg residues (Arg57 in IIA^{Ntr} and Arg49 in IIA^{Mtl}). It is presumed that this residue stabilizes the phosphorylated His residue at the active site. An additional residue that is conserved in both proteins is His111 of IIA^{Mtl}, corresponding to His120 of IIA^{Ntr}. In the case of IIA^{Mtl}, it was proposed that this residue is essential for phosphoryl transfer from IIA to IIB. However, His120 of IIA^{Ntr} is not near the site of phosphorylation and it is not yet clear that there is a downstream phosphoryl acceptor from P-IIA^{Ntr}.

Diffusion studies on IIA^{Ntr} established that it is, in fact, monomeric in solution [19], establishing that the dimeric structure of the crystal is an artifact. The NMR data strongly suggest that the physiological structure corresponds to molecule A of Fig. 6. Titration of unlabeled NPr into a solution of ^{15}N-labeled IIA^{Ntr} resulted in selective chemical shift changes, which identified the surface on IIA^{Ntr} for interaction with NPr; the small NMR shifts imply that there is little change in the structure of IIA^{Ntr} when forming a complex with NPr. Deletion analysis indicated that the N-terminal tail of IIA^{Ntr}, corresponding to residues 1–8, is unnecessary for interaction with NPr.

As alluded to above, both full-length and a truncated version of NPr have been overexpressed [19]. While it is anticipated that the three-dimensional structure of this protein will resemble that of HPr, as yet that structure has not been reported.

Function and regulation

The carbohydrate PTS

As outlined in Fig. 1, the carbohydrate PTS functions to concomitantly transport and phosphorylate a wide variety of sugars (classified as PTS sugars). Energetically, it would appear that the sugar specific, membrane-embedded permease should be sufficient to catalyze the coupled transport/phosphorylation reaction. The rationale for the existence of the otherwise extraneous proteins EI, HPr, and cytoplasmic IIA proteins or domains has become apparent; the carbohydrate PTS functions, not only as a transport system, but as a global regulatory network (see Fig. 7).

Evidence has been presented that EI can reversibly phosphorylate acetate kinase and it was suggested that acetate kinase may be a regulator of the PTS and provide a link between the PTS and the Krebs cycle [21]. Another report has linked the state of phosphorylation of EI to the

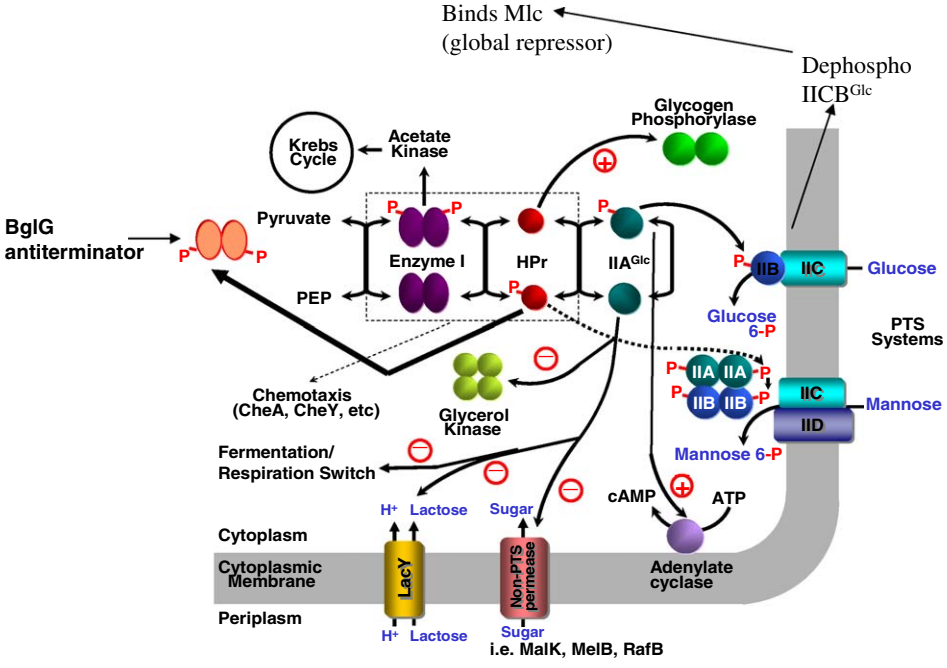

Fig. 7. The multiplicity of interactions of the proteins of the carbohydrate PTS.

chemotactic response. Based on the finding that unphosphorylated EI inhibits the autophosphorylation of CheA, a model for PTS-dependent chemotactic signalling was proposed [22]. It was suggested that the dephosphorylation of EI associated with the uptake of a PTS sugar inhibits CheA phosphorylation which might affect the flagellar motor.

A phosphorylation-state regulatory function has also been ascribed to HPr. BglG is a transcriptional regulator, governing the expression of the *bgl* operon. Proteins encoded by *bgl* deal with the transport and metabolism of β-glucosides. When sugar substrates are absent, EII^{Bgl} phosphorylates and thereby inactivates BglG; the opposite process takes place in the presence of sugars. Additional studies [23] showed that activation of BglG requires EI and HPr; in the absence of EII^{Bgl}, BglG becomes phosphorylated. The model was proposed that activation of BglG requires both dephosphorylation by EII^{Bgl} as well as phosphorylation by HPr at a distinct site. This is consistent with a form of catabolite control in which β-glucosides are utilized only in the absence of PTS sugars, when the state of phosphorylation of HPr is high.

A high-affinity interaction (association constant $\sim 10^8\,M^{-1}$) was demonstrated for HPr with a protein that, after purification, was identified to be *E. coli* glycogen phosphorylase [24] (see Fig. 7). The interaction appears to be physiological, since unphosphorylated HPr and, to a lesser degree, phosphorylated HPr stimulate the glycogen phosphorylase activity. NMR analysis revealed that HPr uses a common surface to interact with glycogen phosphorylase and other proteins [25]. Interestingly, the paralog of HPr, NPr, has no effect on glycogen phosphorylase activity. A regulation model was proposed suggesting that, when PTS sugars are being transported and HPr is primarily in the dephospho-form (as is the case at growth onset), glycogen phosphorylase is activated and glycogen degradation is enhanced. This degraded glycogen can serve as an extra source of energy during early stages of growth. When PTS sugars are not being transported (as is the case in stationary phase) and HPr is primarily in the phospho-form, glycogen phosphorylase is in a less active form and glycogen accumulates.

By far the greatest number of regulatory interactions associated with the PTS has involved IIA^{Glc}. The phenomenon of inducer exclusion, whereby transport of a PTS sugar blocks the simultaneous uptake of non-PTS sugars, is explained by the interaction of the dephospho-form of IIA^{Glc} with a variety of membrane-associated permeases. The interaction that has been most studied in this regard is that involving lactose permease [26–28]. The binding of IIA^{Glc} to the permease is stimulated by the addition of substrates of the permease. A study of the topology of the interaction suggested that interaction of lactose permease with its substrate promotes a conformational change that brings several cytoplasmic loops (IV/V and VI/VII) into an arrangement optimal for interaction with IIA^{Glc}. Mutagenesis of lysine residues of IIA^{Glc} indicated that the region around Lys69 is important for the interaction with lactose permease. It was proposed that the contact patch involves Lys69, Phe71, His75, Ala76, Ser78, Glu86, His90, Asp94, and Glu97.

Another important interaction attributed to IIA^{Glc} involves the regulation of adenylyl cyclase activity (see Fig. 7). It was proposed that the explanation for the phenomenon of catabolite repression is associated with that interaction. Transport of PTS sugars markedly depresses cellular levels of cAMP [2]. It has been proposed that this is due to the stimulation of adenylyl cyclase activity by interaction with phosphorylated IIA^{Glc}. Over the years, attempts have been made to directly demonstrate this regulation in vitro; such attempts have been only partially successful [29–32]. There is reason to believe that there is at least one other factor involved in the interaction with and regulation of adenylyl cyclase activity.

A ligand-fishing approach uncovered still another protein with a high-affinity interaction with IIA^{Glc} [33]. The 47 kDa protein, named FrsA (for fermentation/respiration switch) forms a 1:1 complex with the unphosphorylated form of IIA^{Glc} (see Fig. 7). Disruption of the gene encoding FrsA resulted in increased cellular respiration; overexpression of FrsA resulted in increased fermentation. Consequently, it was proposed that IIA^{Glc} regulates the flux between respiration and fermentation by sensing the availability of sugar metabolites by a phosphorylation-dependent interaction with FrsA.

In contrast to the significant level of understanding of regulation mechanisms associated with the carbohydrate PTS, there is substantially less known about potential regulatory mechanisms connected with the nitrogen-metabolic PTS. This is especially significant since phosphoryl transfer to a specific substrate has not yet been demonstrated for the nitrogen PTS and it has therefore been suggested that its role is in regulation [34]. A possible regulatory function for EI^{Ntr} has been proposed in *Bradyrhizobium japonicum* [35] where a role for this protein in oligopeptide transport was suggested. Purified aspartokinase interacts with EI^{Ntr}. A variety of phosphorylation studies culminated in the proposal that aspartokinase regulates EI^{Ntr} function via a regulation of the phosphorylation state of EI^{Ntr}. As yet, no such mechanism has been deduced for EI^{Ntr} in *E. coli*.

Azotobacter vinelandii does not encode a carbohydrate PTS, but its genome does encode EI^{Ntr} [36]. A mutant deficient in this protein is deficient in the accumulation of poly-β-hydroxybutyrate and in nitrogen fixation. The precise mechanism connecting poly-β-hydroxybutyrate accumulation and nitrogenase activity has yet to be elucidated.

A *ptsP* gene was identified in *Legionella pneumophila* [37]. A mutant defective in that gene grew well in culture media, but was virulence defective. The mutant was able to invade macrophages normally, but showed poor intracellular growth. The precise mechanism of the growth defect associated with EI^{Ntr} remains to be worked out.

While no direct regulatory role for NPr has emerged, it has been proposed that the major function of this protein is to control the state of phosphorylation of IIA^{Ntr}; there is evidence for participation of IIA^{Ntr} in some regulation mechanisms. A mutant in which the *ptsN* gene, encoding IIA^{Ntr}, was disrupted exhibited suppression of a conditionally lethal era^{ts} mutation [7]. Some sugars or Krebs cycle intermediates inhibited growth of the *ptsN* mutant grown in a defined medium and the growth inhibition was relieved when the *ptsN* gene was expressed. This was taken as evidence for some connection of IIA^{Ntr} to nitrogen regulation.

Phenotype microarray analysis led to a further understanding of a regulatory role for IIA^{Ntr} [38]; a deletion mutant for IIA^{Ntr} became extremely sensitive to peptides containing leucine while mutants encoding EI^{Ntr} or NPr were resistant to those peptides. This indicated that the dephospho-form of IIA^{Ntr} was, in some way, associated with the phenomenon of leucine toxicity. Molecular biological studies led to the findings that the dephospho-form of IIA^{Ntr} is required for the derepression of the operon which encodes the first enzyme in the pathway for the biosynthesis of branched chain amino acids. It was proposed that dephospho-IIA^{Ntr} binds to some undefined transcription inhibitor for that operon.

Concluding remarks

As is evident from the discussion above, there has accumulated a wealth of information on both the biochemistry of the transport processes driven by the carbohydrate PTS as well as the fascinating regulatory interactions in which a number of these proteins participate. Comparable studies on the nitrogen-metabolic PTS are in their relative infancy. It is not yet clear whether there is a downstream phosphoryl acceptor from IIA^{Ntr}. Since regulatory interactions involving IIA^{Ntr} are certainly dependent on the state of phosphorylation of that protein, it will be of great interest to define the mechanisms controlling that process. In the next few years, it is predictable that significant new information concerning the structural and molecular biology and the biochemistry of the nitrogen-metabolic PTS will be deduced.

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