Group Translocation – PEP:PTS

Secondary article

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The phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) is a bacterial transport system that phosphorylates its carbohydrate substrates during their transport, leading to intracellular accumulation of the respective phosphoryl esters. In mixtures of carbon sources, preferential growth on PTS substrates often occurs, as the phosphorylation state of PTS proteins coordinates the activity and synthesis of enzymes of intermediary metabolism.

Overview

In 1964 Roseman and coworkers discovered the PTS during experiments designed to explore the presence of adenosine triphosphate (ATP)-dependent kinases in *Escherichia coli*. Shortly thereafter, PTS action was linked to glucose transport in this cell, and in the intervening years we have come to appreciate that this system plays a major role in the transport and metabolism of many carbohydrates by eubacteria, most prominently in Gram-negative and Gram-positive anaerobes and facultative anaerobes, less so in strict aerobes (Meadow *et al*., 1990). The PTS is not present in the archaebacteria, nor is it found in eukaryotes. The substrate specificity of the PTS, along with its restriction to the eubacteria, suggests it arose comparatively late in evolution, probably in response to the presence of significant quantities of environmental carbohydrate. In fact, simple polymers of glucose (starch, cellulose) or glucosamine (chitin) represent a sizeable nutritive reserve. It should not be surprising that selective pressure rewarded development of an efficient system for handling such material.

The PTS functions to transport substrates according to the following overall reaction:

$$\text{carbohydrate}_{out} + \text{PEP}_{in} \text{—(…PTS proteins…)} \rightarrow \text{carbohydrate-P}_{in} + \text{pyruvate}_{in}$$

PTS substrates are usually mono- or disaccharides or straight chain carbohydrates, whose phosphorylation typically yields the 6-phosphate ester, fructose 1-phosphate being the main exception.

The PTS proteins are of two kinds. Two of them, known as Enzyme I (EI) and HPr (heat-stable or histidine-containing protein), are common to nearly all PTS-mediated reactions. (Fructose transport can sometimes use a separate, HPr-like protein, known as FPr.) These common players act to transfer the phosphoryl group of PEP to a second cohort of proteins, the various Enzymes II (EIIs), which sets substrate specificity and directs transport and phosphorylation.

Because transport and phosphorylation are linked, the term 'group translocation' has been used to describe the PTS, indicating that at least one participant (e.g. the phosphoryl group) can be viewed as moving along a reaction coordinate partly oriented across the membrane.

Components of the PTS and Their Functions

The PTS proteins

Enzyme I

Most Enzymes I are of similar size (63 kDa in *E. coli* or *Salmonella typhimurium*) and functional organization. Present studies suggest the EI dimer, in the presence of PEP and Mg^{2+}, undergoes autophosphorylation at a histidine in the N-terminal portion of the molecule. On dissociation, the phosphorylated monomer transfers its phosphoryl group to a histidine on HPr. The EI-HPr complex has been modelled on the basis of the known structures of HPr and the EI N-terminal domain. That model suggests formation of the complex does not require major conformational changes in either partner, consistent with the fact that phosphoryl transfer is readily reversible. Indeed, of all PTS-mediated phosphorylations, only the terminal step, substrate phosphorylation, is accompanied by a significant loss of free energy.

HPr

HPr, about 9 kDa, has been purified from both Gram-negative and Gram-positive bacteria. In a few cases the structure of HPr and its phosphorylated product (phospho-His-HPr) have been determined, either by X-ray crystallography, nuclear magnetic resonance or both. A

comparison of these structures shows that changes attributable to phosphorylation are limited to a region immediately surrounding the active site residue, His15.

Substrate-specific Enzymes II

Specificity for carbohydrate is conferred by the Enzyme II complexes, each of which uses a modular design incorporating three functionally distinct domains (Lengeler *et al*,. 1994). There is (1) a hydrophilic A domain having the first phosphorylation site; (2) a hydrophilic B domain with the second phosphorylation site; and (3) a hydrophobic C domain, usually with 6–8 transmembrane segments and a large internal hydrophilic loop that may form the transport pathway. These three domains may be linked together in a single polypeptide, or they may exist as separate proteins. Thus, in *E. coli* one finds a single Enzyme II for mannitol transport ($EIICBA^{Mtl}$), but separate proteins representing the Enzyme II domains for glucose ($EIIA^{Glc}$ and $EIICB^{Glc}$) or mannose ($EIIAB^{Man}$, $EIIC^{Man}$ and $EIID^{Man}$) transport (**Figure 1**).

Sequence comparisons suggest four distantly related EII families, each named for its most prominent substrate: $EII^{Glucose}$, $EII^{Mannitol}$, $EII^{Lactose}$ and $EII^{Mannose}$. (In the $EII^{Mannose}$ family, which also includes the fructose PTS of *Bacillus subtilis*, the hydrophobic C domain is comprised of two proteins, EIIC and EIID.) It also appears that contemporary Enzymes II reflect varying degrees of gene duplication and fusion along with domain splicing and shuffling. Thus, even within the $EII^{Glucose}$ family, one finds examples in which domain order is different ($EIICBA^{Nag}$ and $EIIBCA^{Bgl}$, specific for *N*-acetylglucosamine and β-glucosides, respectively) or in which polypeptide number differs ($EIICBA^{Nag}$ and $EIIA^{Glc}$ plus $EIICB^{Glc}$).

Studies in *E. coli*, *B. subtilis* or *Lactococcus lactis* give tertiary structures for EIIA and EIIB domains representative of each of these four families. As yet, however, there is no clear understanding of the C domain, since it has been possible to study carefully only a few examples. Nevertheless, it has been shown that $EIICBA^{Mtl}$ and $EIICB^{Glc}$ of *E. coli* function as dimers, and that dimerization is likely to be nucleated by the hydrophobic C domain. This implies that in other cases at least the hydrophobic C domain is a dimer.

Table 1 illustrates PTS diversity by showing representative examples grouped according to Enzyme II specificity. In viewing this list, however, one must keep in mind several things. Note that despite a diversity of structure, Enzymes II display a common reaction sequence in which the

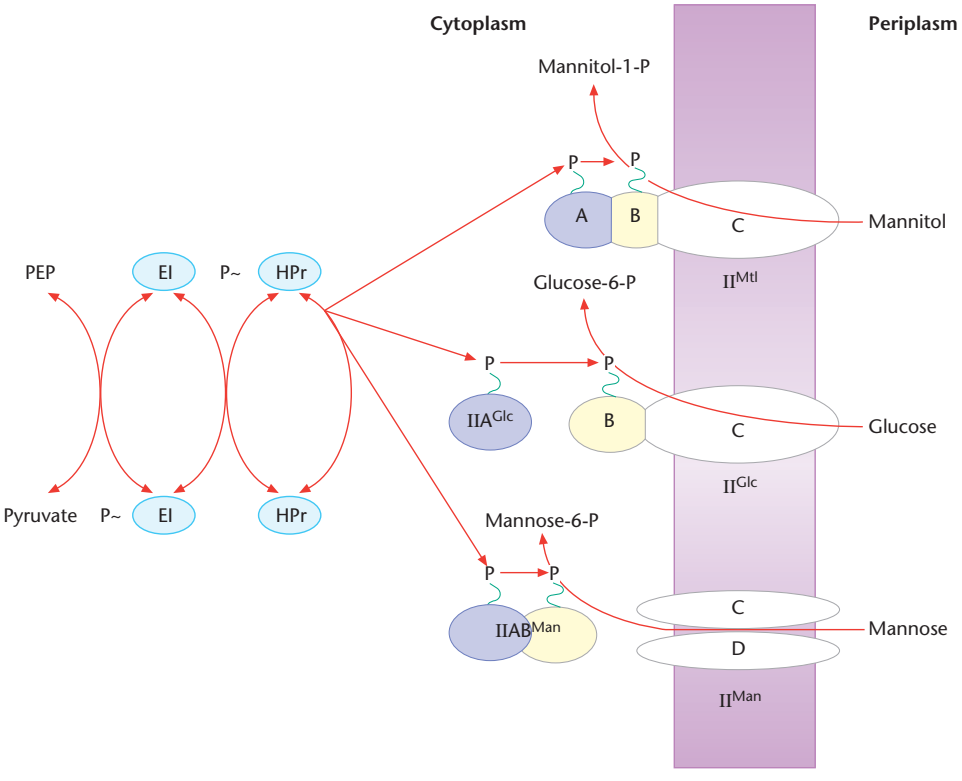

Figure 1 The phosphotransferase system (PTS). The general PTS proteins are Enzyme I (EI) and HPr. Only three of many carbohydrate-specific EII complexes are shown: $EIICBA^{Mtl}$ is specific for mannitol; $EIICB^{Glc}/EIIA^{Glc}$ for glucose; $EIIAB^{Man}/EIIC^{Man}/EIID^{Man}$ for mannose. The phosphorylated forms of EI and HPr are indicated, respectively, as P ~ EI and P ~ HPr. The phosphorylated forms of the EIIA and EIIB domains are also shown. PEP, phosphoenolpyruvate.

Table 1 Representative Enzymes II of the PTS

EII family	Substrate	Organism	EII domains
Glucose	Glucose	*Escherichia coli*	IIA, IICB
	Glucose	*Bacillus subtilis*	IICBA
	N-acetylglucosamine	*Escherichia coli*	IICBA
	β-Glucosides	*Escherichia coli*	IIBCA
	Sucrose	*Klebsiella pneumoniae*	IIA, IIBC
Mannitol	Mannitol	*Escherichia coli*	IICBA
	Mannitol	*Staphylococcus carnosus*	IIA, IICB
	Fructose	*Escherichia coli*	FPr, IIBC
Mannose	Mannose	*Escherichia coli*	IIAB, IIC, IID
	Sorbose	*Klebsiella pneumoniae*	IIA, IIB, IIC, IID
	Fructose	*Bacillus subtilis*	IIA, IIB, IIC, IID
Lactose	Lactose	*Lactococcus lactis*	IIA, IICB
	Cellobiose	*Escherichia coli*	IIA, IIB, IIC

phosphoryl group moves in an orderly way from EIIA, to EIIB, and then to substrate within an EIIC domain. This is true even for fructose transport in *E. coli*, as the N-terminal part of FPr functions as $EIIA^{Fru}$, accepting a phosphoryl group from the HPr-like C-terminal fraction. Note also that most Enzymes II probably have a broader range of substrate than cited here. For example, in *E. coli* $EIICB^{Glc}$ is named for its primary passenger, glucose, without indication that it also accepts glucosamine, sorbose, methyl-α-glucoside and mannose. Finally, remember that bacteria have other transport mechanisms. Within any single cell, both PTS and non-PTS pathways may be available to a substrate. And a substrate which moves by the PTS in one cell type, may move by an alternate route elsewhere.

The PTS genes

Genes for Enzyme I and HPr

In most bacteria, the genes encoding HPr and EI are closely linked; in the enteric bacteria, they constitute a *ptsHI* operon. Growth on a PTS substrate generally increases the levels of HPr and EI by 3–5-fold. In *E. coli* and *S. typhimurium*, transcription of *ptsHI* relies in part on the CRP (cAMP receptor protein)/cAMP (cyclic adenosine monophosphate) complex, and since cAMP levels can vary during growth on PTS substrates (below), it is not always easy to predict the precise levels of HPr and EI. It is not clear whether Gram-positive cells, some of which have no cAMP, use an equivalent transcriptional regulation of *ptsH* or *ptsI* genes.

Genes for Enzymes II

Genes for the EII complexes are often found (with a notable exception in the enteric bacteria) within an operon, or regulon, that includes the enzyme used in initial metabolism of the PTS carbohydrate. An important exception is the carbohydrate repression resistant (*crr*) gene of Gram-negative cells. This gene, which encodes $EIIA^{Glc}$, is linked to *ptsHI*, far from the gene (*ptsG*) encoding $EIICB^{Glc}$. Most *crr* transcription originates from promoter elements at the end of the *ptsI* gene, despite the fact that *ptsHI* and *crr* genes form an operon (in *E. coli*).

Phosphoryl transfer

Phosphorylation of Enzyme I, HPr and the Enzyme II domains

The complete chain of phosphoryl transfer has been traced in a few model systems. In most cases, phosphorylation occurs at histidine. Thus, the phosphoryl group of PEP is bound first to the N-3 of a histidine in each monomer of dimeric EI. On dissociation, the phosphoryl group on EI is transferred to the N-1 of His15 in HPr. The phosphoryl group then moves to the first of two phosphorylation sites in an EII complex. The first of these (in an EIIA domain) is always a histidine, but the second (in an EIIB domain) may be a cysteine ($EII^{Glucose}$, $EII^{Mannitol}$ and $EII^{Lactose}$ families) or a second histidine (the $EII^{Mannose}$ family). Sequence similarity and the results of directed mutagenesis support the idea that all EII complexes are phosphorylated twice. This means that, including substrate phosphorylation, each PTS-mediated event is the product of five distinct phosphorylations, consistent with earlier chemical arguments predicting an odd number of phosphotransfers. Since some Enzymes II are domain fusions, one might question in these cases whether phosphoryl transfer is an intra- or intersubunit event. Findings with the $EIICBA^{Mtl}$ dimer show that intersubunit transfer can occur, but that optimal rates are achieved only for the phosphotransfer within the single subunit.

Transport and the phosphorylation of carbohydrate

Substrate phosphorylation concomitant with its transport distinguishes the PTS from other transport mechanisms. This might be viewed as a simple facilitated transport step followed by a rapid internal phosphorlation, but it appears that PTS-mediated entry of free substrate, without phosphorylation, is very slow indeed. For example, measurements in *S. typhimurium* mutants lacking EI and HPr suggest that, for substrates of the EII^{Man} or EII^{Gle} systems, this 'uncoupled' rate is 0.1% of that found for the intact system. This means that phosphorylation at an EII complex somehow lowers the energy barrier for substrate release by domain C, so that nearly all transported substrate appears inside as the phosphate ester. Nevertheless, in *S. typhimurium* carrying a *ptsHI* deletion, glucose-positive mutants can arise by mutation in $EIICB^{Glc}$. For this reason, some suggest the C domain is a closed 'pore' until phosphorylation occurs, and that such mutants have $EIICB^{Glc}$ fixed in its 'open' conformation. It follows that substrate accumulated by an alternative mechanism might be phosphorylated by an EIIB domain, and this has been observed in a few instances. This general view may also explain why PTS substrates can sometimes be extruded in their nonphosphorylated forms (below).

Regulatory Activities Associated with the PTS

Diauxie and catabolite repression

In early studies with *E. coli* and *B. subtilis*, Monod noted what he called 'diauxic growth' or 'diauxie', during which cells displayed a hierarchy of preference when grown in mixtures of carbohydrates, such that some (now known to be PTS substrates) were completely consumed before an alternative carbon source (a non-PTS substrate) was used. For example, when grown in a mixture of glucose and lactose, *E. coli* completely consumes the glucose before induction of lactose permease (LacY) and β-galactosidase (LacZ), two proteins required for lactose transport and metabolism. This response is broadly described as 'catabolite repression' and illustrates the complex manner in which bacteria adapt to their environment and optimize their metabolic capacities. As we shall see, entirely different mechanisms are at work in Gram-negative and Gram-positive cells.

Regulation of transport and metabolism in Gram-negative bacteria

Pleiotropy of *ptsH* and *ptsI* mutants

In the enteric bacteria, clues as to the origins of catabolite repression came first from studies of *E. coli* and *S. typhimurium* mutants lacking HPr or EI (*ptsH,I* strains). As expected, these cells fail to grow on PTS carbohydrates. Unexpectedly, they are negative for many additional substrates, including lactose, maltose, melibiose and glycerol (known as class I compounds), and xylose, rhamnose and even Krebs-cycle intermediates (class II compounds). Growth on the non-PTS substrates is restored in either of two ways: (1) growth on class I compounds is enabled by mutation of the *crr,* encoding $EIIA^{Glc}$; (2) growth on *both* class I and class II substrates is rescued by addition of cAMP to the medium.

Inducer exclusion

Why does elimination of $EIIA^{Glc}$ allow growth on class I substrates? Much work has shown that in wild-type cells, but not *crr* mutants, transport of class I substrates is inhibited by glucose or its nonmetabolizable analogue, α-methylglucoside. This effect, known as 'inducer exclusion', suggests *ptsH,I* mutants are negative for class I substrates because accumulation or processing of an external inducer is prevented, and that *ptsH,I crr* double mutants are positive for these same substrates because this block has been eliminated. Indeed, the phosphorylation state of $EIIA^{Glc}$ itself is key to PTS-mediated regulations in enteric bacteria (**Figure 2**).

$EIIA^{Glc}$ phosphorylation is determined by a balance between its appearance (as driven by PEP via EI and HPr) and its disappearance (as driven by external glucose via $EIICB^{Glc}$). Since $EIIA^{Glc}$ is dephosphorylated in *ptsH,I* mutants or in pts^+ strains growing on glucose, it would appear that dephosphorylated $EIIA^{Glc}$ promotes inhibition of transport and/or metabolism of class I substrates, or that these elements require activation by phospho-$EIIA^{Glc}$. In fact, for a few test cases, inhibition of a target transporter is known to be a direct result of $EIIA^{Glc}$ binding (**Figure 2**). Moreover, binding and inhibition occur only if the target substrate is present; this restricts $EIIA^{Glc}$ binding to a conformation adopted in the presence of a competing carbohydrate and avoids unnecessary depletion of the $EIIA^{Glc}$ pool (about 10 μmol L^{-1} in *E. coli*). The same tactic is used to exert control over processing of glycerol, whose permeation is rapid even without its transporter – $EIIA^{Glc}$ binds to and inhibits glycerol kinase (GlpK), preventing the initial step in glycerol metabolism. Recall also that phosphoryl transfer among PTS proteins is reversible; this means dephosphorylated $EIIA^{Glc}$ is favoured by any PTS substrate that places a sufficient drain on the pool of phospho-His-HPr.

Adenylate cyclase

The phosphorylation state of $EIIA^{Glc}$ exerts still other regulatory phenomena. In particular, phospho-$EIIA^{Glc}$ activates adenylate cyclase (in a way not yet understood). Thus, in *crr* mutants lacking $EIIA^{Glc}$ adenylate cyclase activity is 10–20% that of crr^+ cells. Since cAMP, when

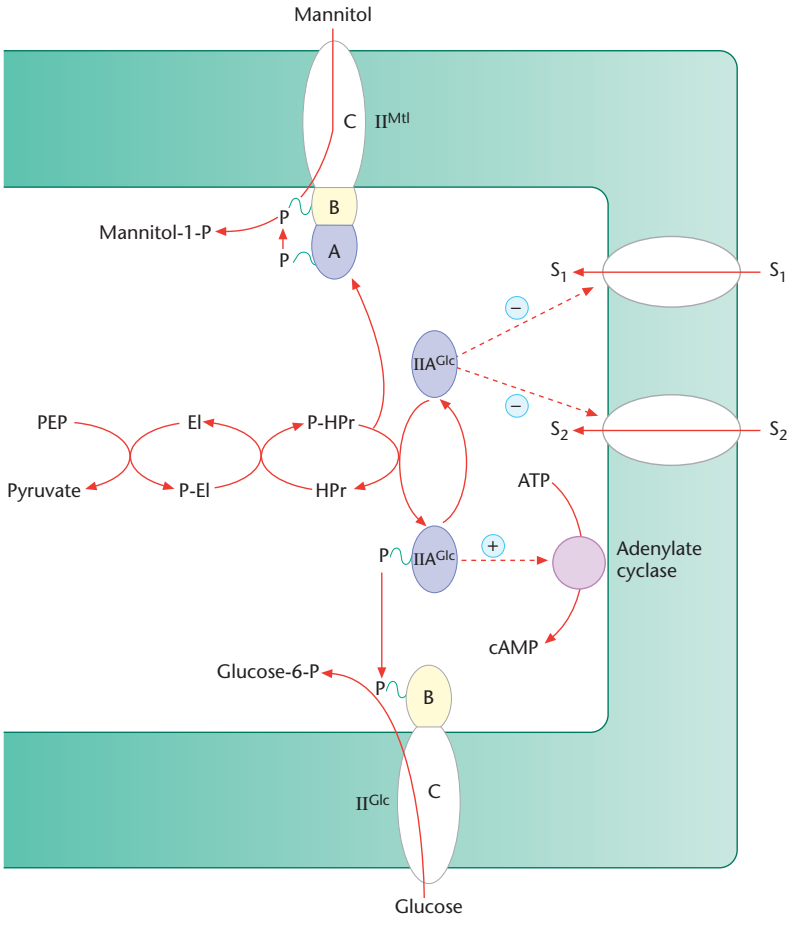

Figure 2 Regulation by the phosphotransferase system (PTS) in enteric bacteria. $EIIA^{Glc}$ and $P \sim EIIA^{Glc}$ represent the dephosphorylated and phosphorylated forms of $EIIA^{Glc}$. $P \sim EIIA^{Glc}$ activates (+) adenylate cyclase, while $EIIA^{Glc}$ inhibits (−) non-PTS class I substrate transporters, denoted by S_1 and S_2. Other symbols as in **Figure 1**.

bound to its receptor protein, CRP, allows transcription of a large number of catabolic operons, a low rate of cAMP synthesis will reduce or even block gene expression, depending on the properties of the individual promoters.

A likely mechanism of diauxie

This section was introduced by discussion of glucose/lactose diauxie, a phenomenon often viewed as a paradigm for cAMP-dependent regulation of gene expression. Indeed, glucose does regulate cAMP synthesis, and cAMP is important for *lac* operon expression. But recent experiments suggest the major determinant of glucose/lactose diauxie is related to inducer exclusion rather than lowered cAMP. Arguing from this result, inducer exclusion may be the crucial element for PTS regulation of the processing of other class I substrates.

Pathways into and out of catabolite repression

The transport and metabolism of class I substrates is regulated in two ways. On the one hand, $EIIA^{Glc}$ binds directly to its various targets, initiating inducer exclusion. In addition, there is a reduced expression of catabolic operons, owing to the lowered level of cAMP. By contrast, regulation directed to class II compounds occurs only at the level of gene expression and is determined solely by levels of the cAMP/CRP transcriptional activator. Such regulation is most often initiated by an external PTS substrate, but this need not always be the case (below).

Alternative pathways to catabolite repression

Collateral pathways may also effect inducer exclusion. For example, growth on glucose 6-phosphate (a non-PTS substrate) is associated with a low PEP:pyruvate ratio. In such cases, the PTS effectively reverses, generating PEP by draining the pool of phospho-EIIs (including $EIIA^{Glc}$) to favour their dephosphorylated forms. This leads seconda-

rily to increased inducer exclusion and lowered cAMP. In yet another instance, strains lacking $EIIA^{Glc}$ may still show inducer exclusion (and glucose transport) if closely related EIIA domains in the $EII^{Glucose}$ family are sufficiently elevated.

Escape from catabolite repression

In either its phosphorylated or dephosphorylated form, $EIIA^{Glc}$, the main player in PTS-mediated regulation in Gram-negative bacteria, acts by forming stoichiometric complexes with at least three distinct sets of proteins: (1) its PTS partners, HPr and $EIICB^{Glc}$; (2) the uncharacterized element(s) responsible for activation of adenylate cyclase (possibly the enzyme itself); and (3) in the presence of their substrates, the multiple targets responsible for transport or metabolism of class I compounds. Clearly, then, cells might escape catabolite repression if the number of targets is larger than the available pool of $EIIA^{Glc}$. In fact, one may verify this prediction by varying the amounts of $EIIA^{Glc}$ or a selected target, or by presenting several class I substrates at the same time. As expected, transport of class I compounds becomes insensitive to inhibition by PTS carbohydrates when the level of $EIIA^{Glc}$ falls below the level of its targets. The same is found for glycerol metabolism, which remains blocked only so long as cells maintain an $EIIA^{Glc}$:GlpK ratio greater than about 1, consistent with *in vitro* study showing a 1:1 binding between $EIIA^{Glc}$ and the GlpK monomer.

Regulation of transport and metabolism in Gram-positive bacteria

In Gram-negative cells, the phosphorylation state of $EIIA^{Glc}$ coordinates metabolic priorities, but in Gram-positive organisms, the indexing signal is a new phosphorylation state of HPr. In *B. subtilis*, for example, HPr is phosphorylated at His15 (by EI) and dephosphorylated (by an EII) as usual. But HPr can also be phosphorylated at Ser46 by a separate ATP-dependent protein kinase, the product of the *ptsK* (or *hprK*) gene. Recent findings show this phosphoryl group is removed by the same protein, which therefore functions as a bifunctional kinase/phosphatase.

Phospho-Ser-HPr is less readily phosphorylated by phospho-EI than is HPr itself, and phospho-His-HPr is a poor substrate for the ATP-dependent kinase. Moreover, the HPr kinase is activated by fructose 1,6-diphosphate (and possibly other glycolytic intermediates). This means the presence of a PTS carbohydrate will generate the substrate of the kinase (dephosphorylated HPr) as well as an activator of the kinase (fructose 1,6-diphosphate). Accordingly, one anticipates that growth on PTS substrates will lead to appearance of phospho-Ser-HPr, together with all the regulatory programmes ascribed to it (below).

Inducer expulsion

Formation of phospho-Ser-HPr correlates with 'inducer expulsion', a process by which addition of a PTS precursor (e.g. external glucose) leads to hydrolysis of a second PTS product (e.g. internal lactose phosphate). The carbohydrate so liberated is then expelled by way of its own EII, while the released phosphate replenishes the internal phosphate pool and is available for phosphorylation of the preferred substrate (here, glucose). This clearly establishes a priority among various metabolizable PTS carbohydrates, and although direct experimental evidence is still to come, one presumes that phospho-Ser-HPr is directly involved in such events.

Regulation of gene expression

In Gram-positive cells, regulation of gene expression during catabolite repression relies importantly, although not exclusively, on the phosphorylation state of HPr (Hueck and Hillen, 1995). A number of genes and operons contain a conserved sequence, the catabolite-responsive element (CRE), which provides a binding site for the catabolite control protein, CcpA, a deoxyribonucleic acid (DNA)-binding protein first discovered in *B. subtilis* (Henkin, 1996). Transcription of CRE-containing genes is prevented on binding of CcpA. *In vitro* study suggests that CcpA binds tightly only in the presence of phospho-Ser-HPr, the concentration of which could thereby tune the expression of CcpA-regulated genes. *B. subtilis* also has an HPr-like protein, Crh. Crh does not itself participate in core PTS activities, because it has Glu15 rather than His15. On the other hand, Crh has Ser46, and phospho-Ser-Crh does seem to take part in CcpA regulation, since in these cells only HPr/Crh double mutants are fully relieved of catabolite repression.

Regulation of the genes encoding Enzymes II

Knowing how the PTS regulates non-PTS systems also helps us understand how expression of the various Enzymes II is controlled. The nature of this control is of varying complexity and at times involves feedback signals which reflect the phosphorylation state of one or more PTS components.

Some Enzymes II are present at significant levels in the absence of their substrates; this is true for the glucose and mannose PTS in enteric bacteria (some further increase does occur with substrate). But in most instances, substrate-specific components are induced by growth on the specified carbohydrate. In the simplest cases, the intracellular product (e.g. a sugar phosphate) binds to a specific repressor, promoting its release from DNA. This occurs in *E. coli* with fructose 1-phosphate, which binds FruR, the fructose PTS repressor, and with *N*-acetylglucosamine 6-phosphate, which binds NagC, a repressor of the *N*-acetylglucosamine PTS.

A more complex example, involving regulation by antitermination at the RNA level(Rutberg, 1997), occurs for the *E. coli bgl* operon, which encodes the β-glucoside Enzyme II (EII^{Bgl}), the antiterminator (BglG), and the phospho-β-glucosidase. With no β-glucoside in the medium, *bgl* expression is limited by premature termination, and the low basal level of EII^{Bgl} is in its phosphorylated state. As it happens, phospho-$EIIB^{Bgl}$ can directly transfer its phosphoryl group to a histidine on BglG, the antiterminator, generating inactive and monomeric phospho-BglG. When β-glucosides are present, however, phospho-EII^{Bgl} is depleted as the phosphoryl group is transferred to substrate, and phospho-BglG is diminished as it contributes its own phosphoryl group to EII^{Bgl}. This encourages increased dimerization of dephosphorylated BglG, and since the dimer binds messenger ribonucleic acid (mRNA) to prevent formation of the terminator structure, the *bgl* operon becomes fully transcribed. In this way, the *bglF* gene product, EII^{Bgl}, acts as both a kinase and a phosphatase during regulation of its own synthesis.

The *sac* operon (encoding the sucrose PTS) of *B. subtilis* self-regulates in similar fashion. Still other *B. subtilis* operons are regulated by phosphorylation of a DNA- or RNA-binding protein, but more than a single regulatory phosphorylation may take place, with the phosphoryl group(s) originating from the associated phospho-EIIs or from phospho-His-HPr. As may be expected, EII-mediated phosphorylation typically generates an inactive regulator, while HPr-mediated phosphorylation gives an activation. And in cases where both occur, dual inhibition and activation is presumed to allow for a more precise tuning of gene expression and enzyme activity (Stülke *et al.*, 1998).

Summary of principles underlying PTS-mediated regulation

In Gram-negative bacteria, phosphorylation of carbohydrate drains the pool of phospho-$EIIA^{Glc}$, leading to increased concentrations of $EIIA^{Glc}$. This, in turn, sets in motion two sorts of control. $EIIA^{Glc}$ is itself an inhibitor of a variety of proteins, including those involved in transport and metabolism (of class I compounds). At the same time, lack of phospho-EII^{Glc} reduces adenylate cyclase activity, and reduced levels of a transcriptional activator, the cAMP/CRP complex, lowers expression of proteins used in alternative catabolic pathways (of class I and II compounds).

Entirely different mechanisms achieve much the same results in Gram-positive cells. Here, metabolism of PTS carbohydrates generates phospho-Ser-HPr. This, too, is accompanied by regulation at the level of transport (mediated at least in part by inducer expulsion) and gene expression (mediated by formation of a transcriptional inhibitor, the CcpA/phospho-Ser-HPr complex).

These different mechanisms broadly distinguish PTS-based global regulatory networks in Gram-negative and Gram-positive cells, but it is unlikely that this is the whole story; other differences will assuredly emerge in individual circumstances. Thus, we already know that in Gram-negative cells, glycerol kinase is inhibited by $EIIA^{Glc}$, but in Gram-positive cells it is activated by phosphotransfer from phospho-His-HPr. Again, the same end is achieved by mechanistically distinct pathways, in each case exploiting an appropriate PTS element as the guiding signal.

Integration with a Wider Metabolic Network

Previous sections described the PTS and showed how it coordinates a preferential utilization of its substrates. Are other interactions directed to the remainder of cellular metabolism?

A link to gluconeogenesis

Possible links are suggested by the behaviour of FruR in enteric bacteria. FruR was first identified as the repressor of *fruFKA*, the operon encoding fructose PTS proteins (FPr, fructose 1-phosphate kinase and EII^{Fru}). But FruR has a much more extended role, and for this reason it has also been called Cra, the catabolite repressor/activator. FruR-negative mutants do not grow on pyruvate or lactate, due to reduced expression of PEP synthase and other enzymes required in gluconeogenesis. FruR is now known to be a transcriptional activator of, among others, the gene encoding PEP synthase, by virtue of an appropriate binding site upstream of the promoter. Other work shows more genes with a FruR-binding site downstream of the promoter, and in these latter cases, FruR acts as a transcriptional repressor. Further, *in vitro* study shows that fructose 1-phosphate displaces FruR from its binding sites (see above), so that carbon sources yielding high levels of fructose 1-phosphate (e.g. fructose itself) may inhibit expression of FruR-activated genes while stimulating expression of FruR-inhibited genes.

PTS-related genes

Genome projects allow one to identify PTS-related genes by sequence homology, and this should prove extraordinarily productive (Reizer and Reizer, 1996). Thus, we know that *E. coli* contains some 50 genes encoding known and putative PTS proteins and that *B. subtilis* has more than 30 such genes. Each new bacterial genome provides fresh ground for such work. Considering what has already been learned from studying how PTS proteins coordinate metabolic priorities, even if a cell were to lack a full PTS, one suspects any remnants might be involved in a

regulatory capacity, independent of their 'usual' role in transport.

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