Title

The involvement of transport proteins in transcriptional and metabolic regulation

Authors

Västermark, Åke
Saier, Milton H

Publication Date

2014-04-01

DOI

10.1016/j.mib.2014.01.002

Peer reviewed

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

The involvement of transport proteins in transcriptional and metabolic regulation

Åke Västermark and Milton H Saier Jr

Transport proteins have sometimes gained secondary regulatory functions that influence gene expression and metabolism. These functions allow communication with the external world via mechanistically distinctive signal transduction pathways. In this brief review we focus on three transport systems in *Escherichia coli* that control and coordinate carbon, exogenous hexose-phosphate and phosphorous metabolism. The transport proteins that play central roles in these processes are: first, the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS), second, the glucose-6-phosphate receptor, UhpC, and third, the phosphate-specific transporter, PstSABC, respectively. While the PTS participates in multiple complex regulatory processes, three of which are discussed here, UhpC and the Pst transporters exemplify differing strategies.

Addresses
Department of Molecular Biology, University of California at San Diego, La Jolla, CA 92093-0116, United States

Corresponding author: Saier, Milton H (msaier@ucsd.edu)

Current Opinion in Microbiology 2014, **18**:8–15

This review comes from a themed issue on **Cell regulation**

Edited by **Cecília Maria Arraiano** and **Gregory M Cook**

For a complete overview see the Issue and the Editorial

Available online 8th February 2014

1369-5274/$ – see front matter,

http://dx.doi.org/10.1016/j.mib.2014.01.002

'No man ever steps in the same river twice' — Heraclitus

Introduction

Transport proteins evolved early as their functions are essential to all life, but regulation was late-evolving and consequently is often phylum-specific. Therefore, although transport mechanisms are universal, regulation is not. For example, while the constituents of the prokaryotic sugar-transporting phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) are essentially the same in all bacterial and archaeal phyla that possess the system, its involvement in regulation differs drastically depending on the organismal type, often even within a single phylum. Similarly, glucose is taken up by the same transport mechanism in most eukaryotes, from yeast to humans, but its regulation is completely different depending on the organismal type. In this brief review, we examine the roles played by transport systems in the regulation of carbon, glucose-6-phosphate (G6P) and phosphorous (Pho) metabolism in a single organism, *Escherichia coli*. We anticipate that while the principles revealed by the studies described here will prove to be universal, the specific mechanisms will be organism-specific.

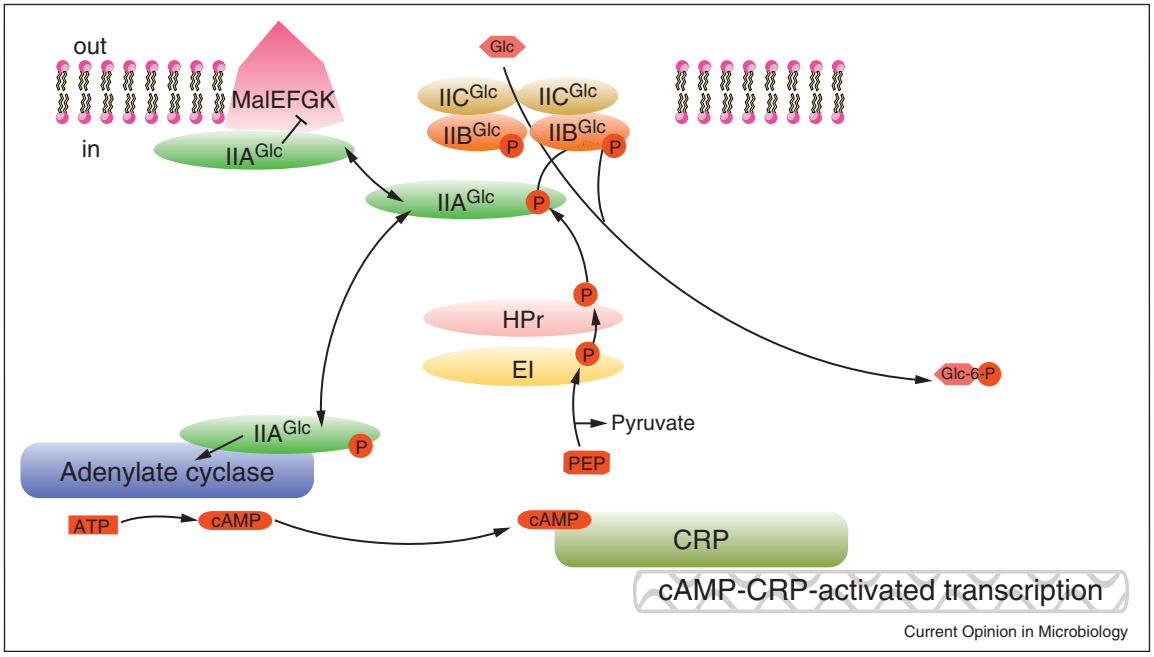
The *E. coli* PTS

The PTS, discovered just 50 years ago [1••,2••], consists of several proteins and/or protein domains: first, a PEP-dependent protein phosphorylating kinase, termed Enzyme I; second, a heat-stable phosphoryl carrier protein, HPr, and third, sugar-specific Enzyme II complexes which consist of IIA, IIB, IIC and sometimes IID proteins and/or protein domains [3–6,7•]. Enzyme I and HPr are the general energy-coupling proteins, common to all Enzyme II complexes of the PTS, catalyzing phosphoryl transfer from PEP to HPr. Phosphorylated HPr then transfers the phosphoryl group to IIA, which phosphorylates IIB. The IIC permease then transfers the phosphoryl group from IIB ~ P to a sugar in a process that couples sugar phosphorylation to the concomitant IIC-mediated transport of the extracellular sugar into the cytoplasm. The five-step phosphoryl transfer process can be schematically described as: PEP → EI ~ P → HPr ~ P → IIA ~ P → IIB ~ P — (IIC) → sugar-P. In all PTS phosphoryl transfer proteins, the phosphoryl moiety is linked to a histidyl residue, except for some of the IIB proteins in which a cysteyl residue is phosphorylated. In each case, a high energy phosphoryl protein forms reversibly with energies of hydrolysis close to that of PEP [8], and hence, only the last step, sugar phosphorylation, is physiologically irreversible. If the relevant sugar of a PTS transporter is present in the extracellular medium, the sugar drains the phosphoryl groups off of all the PTS phosphoryl carrier proteins and induces synthesis of the EII complex, a prerequisite for rapid sugar uptake (Figure 1).

Normally, a distinction is made between the so-called PTS sugars (mostly hexoses and hexitols, e.g. glucose, mannose, fructose and mannitol) and non-PTS sugars (e.g. maltose, lactose, glycerol and melibiose) [8]. For the hexoses, the main enzymes II are II^{Glc}, II^{Man} and II^{Fru}. The phosphorylated forms of these substrates are converted to fructose-1.6-bisphosphate in preparation for glycolytic metabolism. There is considerable organismal variation concerning the sugars transported via the PTS. For example, in *Rhodospirillum rubrum*, only fructose is utilized by the PTS while in *Spirochaeta aurantia*, only mannitol is utilized via this system [8,9].

Phosphorylated sugars, released from their Enzyme II complexes into the cytoplasm and metabolized via glycolysis,

Figure 1



PTS-mediated catabolite repression and inducer exclusion. Principal signal transduction pathways for carbon regulation in *E. coli*. A sugar such as glucose (Glc) is transported into the cell cytoplasm and concomitantly phosphorylated by the PTS, resulting in dephosphorylation of IIA^{Glc}. Nonphosphorylated IIA^{Glc} inhibits non-PTS sugar catabolic enzymes and transporters causing inducer exclusion. Phosphorylated IIA^{Glc}, on the other hand, promotes cAMP formation by binding to adenylate cyclase and allosterically activating it. cAMP-driven transcription results when cAMP binds to CRP, a transcriptional activator. The rate-limiting step in the PTS phosphoryl transfer chain appears to be Enzyme I-catalyzed phosphorylation of HPr. ⊥, inhibition; →, activation.

generate both PEP and ATP. Hence, the glycolytic pathway, including the PTS, constitutes a cycle starting with PEP and generating PEP. However, in some bacteria, PTS EII-mediated transport may not always be coupled to sugar phosphorylation. Exceptions to the usual mechanism of group translocation in which transport is tightly coupled to substrate modification can be found in references [10,11,12•,13].

There can be many different Enzyme II complexes in any given bacterial cell, and each one exhibits specificity for just one or a few closely related sugars. In fact, the IIC constituents have evolved independently at least four different times, giving rise to proteins of differing topologies (0, 6, 8 and 11 α-helical transmembrane segments (TMSs), respectively) [12•]. The Enzyme II transporter genes are in general localized to their respective operons which also contain the genes required for conversion of the sugar substrate into a common glycolytic intermediate. Two exceptions to this generalization are the operons encoding the glucose and mannose Enzyme II complexes. These operons encode only the PTS Enzyme II constituents.

PTS-mediated catabolite repression and inducer exclusion

The name carbon catabolite repression derives from the notion that the catabolites of sugar metabolism are responsible for repression. In fact, the metabolism of any sugar can cause repression, and in general, the more rapidly an exogenous sugar is transported, the stronger the catabolite repression. However, in *E. coli*, the phosphorylation state of IIA^{Glc} provides the dominant mechanism. This mechanism is operative in enteric Gram-negative bacteria closely related to *E. coli*, but different versions are present in Gram-positive firmicutes and actinobacteria, in spirochaetes and even in other proteobacteria. For example, in the firmicute, *Bacillus subtilis* [14•], the PTS plays a role in catabolite repression, but the mechanism involves an ATP-dependent HPr kinase, not the phosphorylation state of IIA^{Glc}. In all bacteria, including *E. coli*, there are probably multiple mechanisms of catabolite repression [15•,16,17•].

In its phosphorylated forms, IIA^{Glc} binds to and activates adenylate cyclase, which converts ATP to cyclic adenosine 3′,5′-monophosphate (cAMP), a cytoplasmic second messenger indicating the extracellular availability of a PTS carbon and energy source [18•]. It is noteworthy that although most Enzyme II complexes contain the full complement of IIA, IIB, IIC and sometimes IID proteins or protein domains, IIA^{Glc} can be promiscuous within the glucose family, replacing the sugar-specific IIA for some systems. Thus, for example, the *E. coli* sucrose Enzyme II does not have its own IIA and uses IIA^{Glc}. This facilitates

the direct regulation of the IIA^{Glc} phosphorylation state by several related sugars. Furthermore, if phosphoryl groups are drained off of HPr, all of the systems that use HPr are affected. To the extent that signals are conveyed through the same second messenger (cAMP), signals from different complexes cannot be distinguished [19,20].

A decrease in intracellular cAMP leads to the dissociation of the cAMP–cAMP receptor protein (CRP) complex from its operator sites in the control regions of operons subject to CRP control. This thereby deactivates cAMP–CRP-directed transcription of catabolite repressible genes responsible for the catabolism of exogenous carbon sources [21,22]. Non-PTS carbohydrate enzymes and transporters, including glycerol kinase (GlpK), the maltose permease (MalEFGK) and the lactose permease (LacY), of *E. coli* are regulated by direct binding of IIA^{Glc} [23], inhibiting their activities if a PTS sugar is abundant, a phenomenon called 'inducer exclusion' (Figure 1).

Regulation of glycogen breakdown by HPr of the PTS

In addition to its role in the PTS phosphorylation cycle, HPr allosterically regulates the enzyme, glycogen phosphorylase, which catalyzes the rate-limiting step of glycogenolysis [24], the release of glucose-1-phosphate from cellular glycogen, a storage form of energy consisting of glucose polymers linked α-1,4. When phosphate is drained off of HPr because a PTS sugar is available in the medium, HPr binds to glycogen phosphorylase, inhibiting its activity so that utilization of glycogen is blocked [24] (Figure 2). Thus, the PTS proteins, HPr, IIA^{Glc}, and $IIBC^{Glc}$, all have direct but distinct signaling activities, secondary functions that undoubtedly evolved late, after the evolution of the PTS as a concerted sugar transport/kinase system.

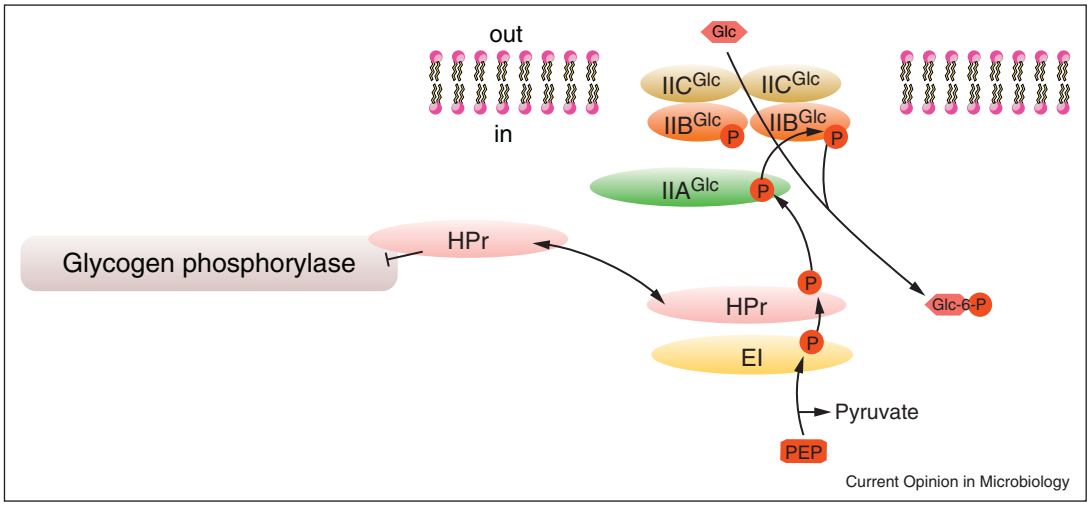
Direct binding of a transcriptional repressor, Mlc, to the PTS Glucose Enzyme II

In addition to the signal transduction pathways described above for the regulation of non-PTS sugar metabolism, another mechanism, influencing primarily the expression of *pts* genes, involves the transcription factor, Mlc, which binds to and is regulated by $IIBC^{Glc}$. Mlc primarily regulates the *pts* operon, encoding Enzyme I and HPr, as well as genes encoding the glucose (PtsG) and mannose (Man) EII complexes [25]. If glucose is transported by $IIBC^{Glc}$, this transporter sequesters Mlc. Then, Mlc cannot diffuse to the DNA to repress transcription. This results in glucose activation (or more accurately, derepression) of the *ptsG*, *man* and *pts* operons, all of which are concerned with glucose metabolism. The PTS thus exhibits autogenous induction [26••,27], where synthesis of Enzyme I, HPr, II^{Glc} and II^{Man} is enhanced by transport of glucose through IIC^{Glc}. The sequestration of Mlc by $IIBC^{Glc}$ when exogenous glucose is available thus mediates the autogenous induction of the entire glucose PTS [28] (Figure 3).

G6P regulation

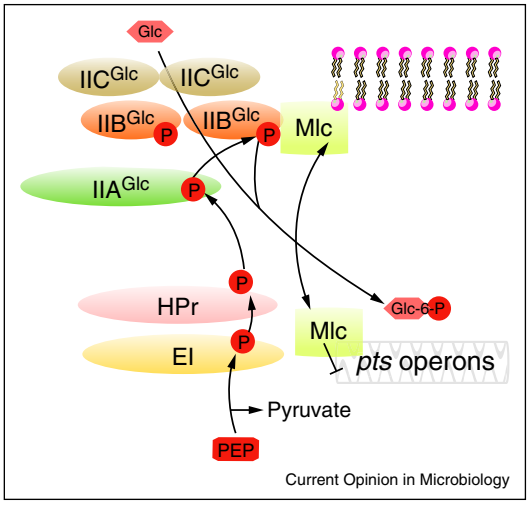
The Uptake hexose phosphate Transport (UhpT) system of *E. coli* is specifically induced by G6P, although the transporter itself has broad specificity for a wide range of sugar phosphates [29–31] (Figure 4). Although the transporter consists of a single protein, UhpT, the regulatory system controlling its expression is composed of three proteins, UhpA, B and C [32]. UhpT (TC# 2.A.1.4.1) and UhpC (TC# 2.A.1.4.4) are homologous sugar

Figure 2



Regulation of glycogen breakdown by HPr of the PTS. HPr, the phosphate donor to PTS IIA proteins, becomes dephosphorylated when exogenous PTS sugars such as glucose are available. Dephospho-HPr then binds to glycogen phosphorylase, preventing glucose-1-phosphate release from glycogen. ⊥, inhibition.

Figure 3

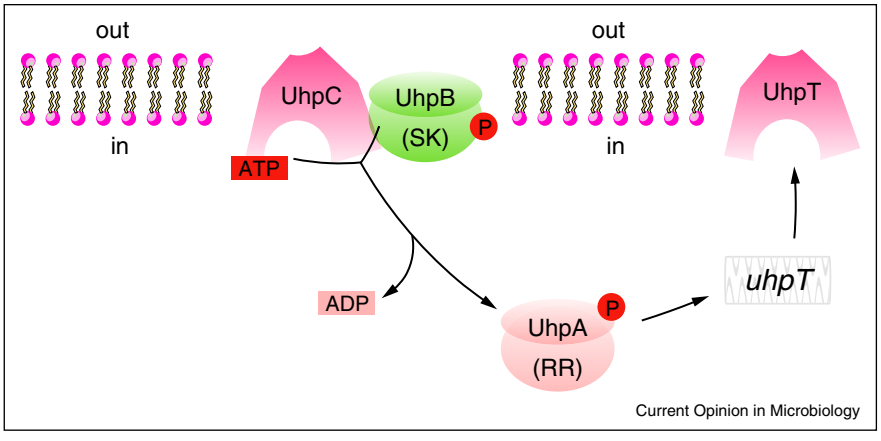


Direct binding of a transcriptional repressor, Mlc, to the PTS Glucose Enzyme II. $IIBC^{Glc}$ in the unphosphorylated state (i.e. when glucose is present) sequesters Mlc, preventing repression of *pts* genes. However, when glucose is absent, $IIBC^{Glc}$ is phosphorylated, causing release of Mlc so it represses expression of these genes. ⊥, repression.

phosphate:inorganic phosphate (P_i) exchangers functioning as transporter and receptor/transporter, respectively. They are close homologues within the Organophosphate:P_i Antiporter (OPA) family of the Major Facilitator Superfamily of secondary carriers (TC# 2.A.1.4). The differences between UhpT and UhpC are that while the low specificity UhpT is an efficient transporter expressed at high levels following induction by exogenous G6P, the G6P-specific UhpC works as a receptor expressed at extremely low levels [33]. UhpC has high affinity for G6P despite its low rate of transport relative to UhpT, conveying a signal to UhpB and UhpA that upregulates the expression of the *uhpT* gene when external G6P is available. UhpC was first characterized in 1980 as a receptor (initially called UhpR) that could induce UhpT expression [34], and a high degree of sequence similarity between UhpT and UhpC was demonstrated in 1992 [35].

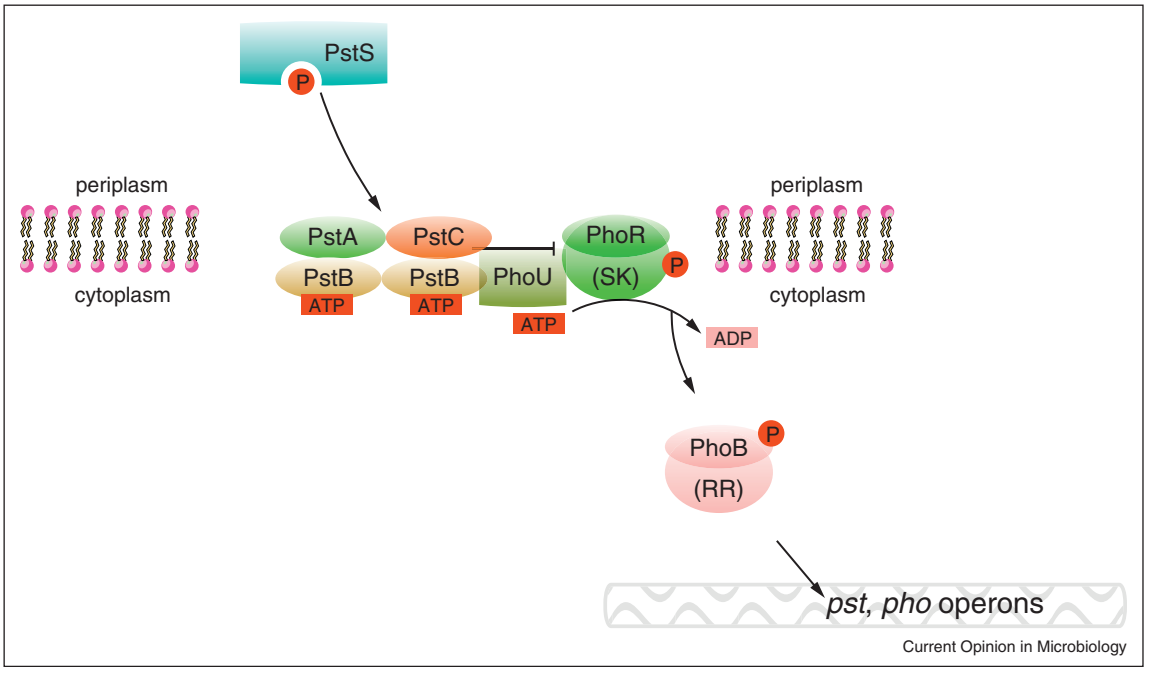
UhpB is a sensor kinase (SK) while UhpA is a DNA-binding response regulator (RR) of a two-component (SK/RR) regulatory system [36]. UhpB, which interacts directly with UhpC in its N-terminal 2 TMS membrane domain, has a kinase activity in its C-terminal domain [37] that phosphorylates a histidyl residue in its central domain, H313, using ATP as the phosphoryl donor. UhpB is activated only when G6P is bound to the receptor. Phosphorylated UhpB then transfers the phosphoryl group to an aspartyl residue in UhpA, which promotes gene expression of *uhpT* using σ^{70} RNA polymerase [38], by binding to multiple sites in positions between −80 and −32 upstream of the *uhpT* gene in the transcriptional regulatory promoter region. UhpA ~ P functions as an enhancer, binding upstream of the promoter. The mechanism of genetic regulation of the synthesis of UhpT [39] is analogous to the mechanism by which hormones, such as insulin, regulate gene expression in mammals, and is an example of a transporter that directly controls gene expression. In humans, deficiencies in a close homologue, TC# 2.A.1.4.5, are responsible for glycogen storage diseases (Gierke's disease) [40].

Figure 4



The uptake hexose phosphate (Uhp) transport system. The Uhp system consists of four proteins: UhpA, B, C, and T. UhpC and UhpT are homologous members of the major facilitator superfamily, serving the function of receptor/transporter and transporter, respectively. UhpB and A are the sensor kinase and response regulator of the regulatory two-component system, serving to convey the signal of glucose-6-phosphate (G6P) availability to upregulate the expression of the *uhpT* gene. The 2 TMS membrane domains of the UhpB protein sense when the substrate, G6P, for which UhpC is highly specific, is bound to its external surface. Then with G6P bound to UhpC, and with UhpC in direct contact with UhpB, UhpB utilizes ATP to autophosphorylate its central domain. Once UhpB is phosphorylated, UhpB can donate its phosphoryl group to UhpA, which binds to multiple sites between −80 and −32 upstream of the start site of the *uhpT* gene, functioning as an enhancer in the promoter region. →, transcriptional activation.

Figure 5



The phosphate-specific transporter, PstSABC, a transporter functioning in signal transduction. The phosphate (*pho*) regulon uses PstS, a periplasmic inorganic phosphate (P_i) binding receptor that serves as a constituent of the ABC transport complex, PstSABC. It also serves as a sensor of external P_i concentration. PstS senses the phosphate concentration in the periplasmic space and transmits a signal via PstABC and PhoU to the sensor kinase, PhoR, independently of the transport activity of PstSABC, thereby influencing expression of *pho* regulon genes. Therefore, the transporter is a sensor that senses extracellular phosphate to control gene expression. ⊥, inhibition; →, activation.

It has been shown that higher levels of G6P are needed to induce UhpT transcription under phosphate limitation. Products of the *pho* regulon can hydrolyze G6P in the periplasm (inducer degradation), to glucose and free phosphate [41]. In an example of Uhp cross-talk, adenylate cyclase activity is inhibited by hexose phosphate transport, although the mechanism has not been defined [42]. The more UhpT that is expressed in the presence of exogenous G6P, the lower the cytoplasmic cAMP concentration. If glucose is transported, IIA^{Glc} in the free form is generated, and hence less cAMP is produced. Since the *uhpT*, *ptsHI*, *ptsG* and *man* genes are all under cAMP–CRP control, this mechanism allows glucose to inhibit sugar phosphate uptake and sugar phosphate to inhibit sugar uptake [43,44]. Thus, rates of cAMP–CRP-activated transcription are reduced by transport of either glucose or G6P.

Phosphorous (*pho*) regulation

In the presence of excess external P_i, this anion can pass the outer membrane of *E. coli* through the PhoE porin [45] and then enter the cytoplasm via the low affinity PiT transporter. Under these conditions, the high affinity phosphate-specific transporter, Pst is repressed. In the case of phosphate limitation (sub-μM external levels), a periplasmic phosphate sensory binding protein, PstS, a constituent of the high affinity ABC phosphate transporter, PstSABC, binds inorganic phosphate and facilitates transport across the cytoplasmic membrane. PstA and PstC are the integral membrane proteins of the system while PstB hydrolyzes ATP to energize uptake. This transport system transmits a signal, probably via the accessory protein, PhoU, to the sensor histidine kinase, PhoR, which positively controls transcription of the many phosphate-yielding (*pho*) operons via the phosphorylated RR, PhoB (Figure 5). PhoU may also regulate both P_i uptake via the Pst system and Pst-mediated signal transduction to PhoR [46]. Interestingly, full activation of the *pho* regulon requires the nonphosphorylated form of a cytoplasmic PTS regulatory protein, $EIIA^{Ntr}$ [47], suggesting that the transcriptional responses to phosphate limitation are dependent on nitrogen and carbon sufficiency, mediated by PTS^{Ntr} [48].

PhoR self-phosphorylates on a histidyl residue before transferring the phosphoryl group to an aspartyl residue in PhoB [49•]. PhoB ~ P then binds to so-called PHO boxes upstream of *pho* operons to activate transcription of the many *pho* genes, including those in the *pst* operon [50]. Most of the enzymes encoded by *pho* genes degrade organic phosphates, converting them to P_i, which is the preferred source of phosphorous.

Under conditions of excess exogenous phosphate, with P_i bound to PstS, the products of the *pst* operon, together with PhoU, transmit an inhibitory signal to the PhoR kinase and thereby inhibit PhoB phosphorylation and hence *pho* regulon expression. Deleting any one gene in the *pst* operon, including *phoU*, results in constitutive expression of *pho* operons [51]. This shows that the signal transmitted by the Pst-PhoU system to PhoR is a negative one, dependent on all constituents of the Pst transporter, causing inhibition of the otherwise constitutively high level of PhoR activity [46]. Surprisingly, the effect of signal transduction via the Pst transporter on gene regulation is not a consequence of P_i transport. Although the same proteins are involved in both transport and signal transduction, these two functions are independent of each other as shown by experiments demonstrating that specific point mutations in the *pst* genes can eliminate either of these functions without affecting the other [46]. Thus, while the two functions use the same proteins, they do not use the same mechanism [52].

Conclusions and perspectives: integration of, and parallels between carbon, Glc6P, and phosphorous regulation in *E. coli*

In the sugar-transporting PTS, effects on transcription are carried out: first, by the binding of Mlc to $IIBC^{Glc}$, second, by controlling cytoplasmic inducer levels by direct interaction of IIA^{Glc} with target non-PTS catabolic enzymes and transporters, and third, by regulating cAMP levels by direct interaction of $IIA^{Glc} \sim P$ with adenylate cyclase. Additionally, fourth, the PTS limits the utilization of other carbon sources such as exogenous non-PTS sugars and endogenous glycogen when glucose or another PTS sugar is available and transported. These mechanisms allow *E. coli* to create a hierarchy of preferred carbon sources, enabling rapid growth while preventing waste.

Regulation of G6P utilization is controlled by the Uhp signal transduction system. UhpC, which is a major facilitator superfamily transporter, highly specific for G6P, works as a receptor, conveying a signal through a two-component system, the UhpB SK and the UhpA RR, to increase rates of synthesis of UhpT, the low specificity sugar-P transporter. This mechanism allows the bacteria to respond to exogenous G6P, preventing induction by intracellular G6P and leakage of sugar phosphates from the cell.

Fewer details are established for phosphate (*pho*) gene regulation via the ABC transporter complex, PstSABC. In the presence of sufficient external phosphate, the PstS receptor binds periplasmic inorganic phosphate, which binds to the PstABC complex, transmitting via PhoU an inhibitory signal to PhoR, the SK. Inhibition of the phosphorylation state of PhoR causes PhoB to be dephosphorylated, preventing expression of the entire *pho* regulon by a negative feedback mechanism that once again prevents energy wastage.

There are reciprocal effects of PTS-mediated transport on *uhp* transcription, and vice versa. UhpT is under catabolite repression [53], as are all PTS enzymes. When UhpT is transporting sugar phosphates, catabolite repression results from inhibition of adenylate cyclase activity [42], just as glucose transport by the PTS induces catabolite repression. Thus, the systems repress themselves and each other, maintaining a controlled rate of carbon uptake that allows optimal growth with minimal wastage.

The transcriptional responses to phosphate limitation are dependent on carbon and nitrogen sufficiency, mediated by PTS proteins [48], again revealing interconnectivity of the regulatory systems. These interactions prevent wasteful metabolism of nutrients under conditions that are not beneficial to the cell. Moreover, *pho* regulon periplasmic phosphatases break down G6P, inhibiting *uhpT* induction when inorganic phosphates are present in excess [41].

These examples emphasize the interconnectivity of the systems considered in this review article. Future work is likely to reveal additional aspects of the integrative control systems that allow coordination of these different metabolic processes. Additional details concerning the involvement of key transporters in metabolic and transcriptional regulation are also likely to come to light.

Acknowledgements

The work reported was supported by NIH Grants GM077402-05A1 and GM 094610-01. We thank Professors Alex Ninfa and Mike Merrick for critically reading the manuscript before submission.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Kundig W, Ghosh S, Roseman S: **Phosphate bound to histidine in a protein as an intermediate in a novel phospho-transferase system**. *Proc Natl Acad Sci U S A* 1964, **52**:1067-1074.
Represents the discovery of the PTS in 1964.

2. •• Saier MH Jr, Reizer J: **The bacterial phosphotransferase system: new frontiers 30 years later**. *Mol Microbiol* 1994, **13**:755-764.
Summarizes the developments until 1994.

3. Herzberg O, Reddy P, Sutrina S, Saier MH Jr, Reizer J, Kapadia G: **Structure of the histidine-containing phosphocarrier protein HPr from *Bacillus subtilis* at 2.0-A resolution**. *Proc Natl Acad Sci U S A* 1992, **89**:2499-2503.

4. Lee CA, Saier MH Jr: **Mannitol-specific enzyme II of the bacterial phosphotransferase system. III. The nucleotide sequence of the permease gene**. *J Biol Chem* 1983, **258**:10761-10767.

5. Fairbrother WJ, Gippert GP, Reizer J, Saier MH Jr, Wright PE: **Low resolution solution structure of the *Bacillus subtilis* glucose permease IIA domain derived from heteronuclear three-dimensional NMR spectroscopy**. *FEBS Lett* 1992, **296**:148-152.

6. Ab E, Schuurman-Wolters GK, Nijlant D, Dijkstra K, Saier MH, Robillard GT, Scheek RM: **NMR structure of cysteinyl-phosphorylated enzyme IIB of the N,N′-diacetylchitobiose-specific phosphoenolpyruvate-dependent phosphotransferase system of *Escherichia coli***. *J Mol Biol* 2001, **308**:993-1009.

7. • Barabote RD, Saier MH Jr: **Comparative genomic analyses of the bacterial phosphotransferase system**. *Microbiol Mol Biol Rev* 2005, **69**:608-634.
Provides a comprehensive survey of PTS proteins encoded within dozens of bacterial genomes, revealing the diversity of such proteins and impying the existence of many yet-to-be discovered regulatory mechanisms.

8. Saier MH Jr: **Bacterial phosphoenolpyruvate: sugar phosphotransferase systems: structural, functional, and evolutionary interrelationships**. *Bacteriol Rev* 1977, **41**:856-871.

9. Saier MH Jr, Newman MJ, Rephaeli AW: **Properties of a phosphoenolpyruvate: mannitol phosphotransferase system in *Spirochaeta aurantia***. *J Biol Chem* 1977, **252**:8890-8898.

10. Rodriguez-Diaz J, Rubio-del-Campo A, Yebra MJ: **Lactobacillus casei ferments the N-acetylglucosamine moiety of fucosyl-alpha-1,3-N-acetylglucosamine and excretes L-fucose**. *Appl Environ Microbiol* 2012, **78**:4613-4619.

11. Hvorup R, Chang AB, Saier MH Jr: **Bioinformatic analyses of the bacterial L-ascorbate phosphotransferase system permease family**. *J Mol Microbiol Biotechnol* 2003, **6**:191-205.

12. • Saier MH, Hvorup RN, Barabote RD: **Evolution of the bacterial phosphotransferase system: from carriers and enzymes to group translocators**. *Biochem Soc Trans* 2005, **33**:220-224.
Provides evidence that PTS Enzyme II complexes evolved independently several times.

13. Saier MH Jr, Simoni RD, Roseman S: **The physiological behavior of enzyme I and heat-stable protein mutants of a bacterial phosphotransferase system**. *J Biol Chem* 1970, **245**:5870-5873.

14. • Saier MH Jr, Chauvaux S, Deutscher J, Reizer J, Ye JJ: **Protein phosphorylation and regulation of carbon metabolism in Gram-negative versus Gram-positive bacteria**. *Trends Biochem Sci* 1995, **20**:267-271.
Compares two PTS-mediated carbon regulatory mechanisms in two phylogenetically divergent bacteria.

15. • Lengeler JW, Jahreis K: **Bacterial PEP-dependent carbohydrate: phosphotransferase systems couple sensing and global control mechanisms**. *Contrib Microbiol* 2009, **16**:65-87.
Summarizes PTS signal transduction in *E. coli*.

16. Deutscher J: **The mechanisms of carbon catabolite repression in bacteria**. *Curr Opin Microbiol* 2008, **11**:87-93.

17. • Saier MH Jr: **Cyclic AMP-independent catabolite repression in bacteria**. *FEMS Microbiol Lett* 1996, **138**:97-103.
Discusses catabolite repression other than those dependent on cAMP.

18. • Aboulwafa M, Saier MH Jr: **Biophysical studies of the membrane-embedded and cytoplasmic forms of the glucose-specific Enzyme II of the *E. coli* phosphotransferase system (PTS)**. *PLoS ONE* 2011, **6**:e24088.
Reveals that PTS Enzyme II complexes can exist in multiple principal states inside the *E. coli* cell.

19. Bywater RP: **Membrane-spanning peptides and the origin of life**. *J Theor Biol* 2009, **261**:407-413.

20. Bywater RP, Sorensen A, Rogen P, Hjorth PG: **Construction of the simplest model to explain complex receptor activation kinetics**. *J Theor Biol* 2002, **218**:139-147.

21. Escalante A, Salinas Cervantes A, Gosset G, Bolivar F: **Current knowledge of the *Escherichia coli* phosphoenolpyruvate–carbohydrate phosphotransferase system: peculiarities of regulation and impact on growth and product formation**. *Appl Microbiol Biotechnol* 2012, **94**:1483-1494.

22. Peterkofsky A, Wang G, Garrett DS, Lee BR, Seok YJ, Clore GM: **Three-dimensional structures of protein-protein complexes in the *E. coli* PTS**. *J Mol Microbiol Biotechnol* 2001, **3**:347-354.

23. Chen S, Oldham ML, Davidson AL, Chen J: **Carbon catabolite repression of the maltose transporter revealed by X-ray crystallography**. *Nature* 2013, **499(7458)**:364-368.

24. Seok YJ, Koo BM, Sondej M, Peterkofsky A: **Regulation of *E. coli* glycogen phosphorylase activity by HPr**. *J Mol Microbiol Biotechnol* 2001, **3**:385-393.

25. Joyet P, Bouraoui H, Ake FM, Derkaoui M, Zebre AC, Cao TN, Ventroux M, Nessler S, Noirot-Gros MF, Deutscher J *et al.*: **Transcription regulators controlled by interaction with enzyme IIB components of the phosphoenolpyruvate: sugar phosphotransferase system**. *Biochim Biophys Acta* 2013, **1834**:1415-1424.

26. •• Plumbridge J: **Regulation of gene expression in the PTS in *Escherichia coli*: the role and interactions of Mlc**. *Curr Opin Microbiol* 2002, **5**:187-193.
Summarizes details of the transcriptional regulation of *pts* genes by Mlc.

27. Plumbridge J: **Regulation of PTS gene expression by the homologous transcriptional regulators, Mlc and NagC, in *Escherichia coli* (or how two similar repressors can behave differently)**. *J Mol Microbiol Biotechnol* 2001, **3**:371-380.

28. Schlegel A, Bohm A, Lee SJ, Peist R, Decker K, Boos W: **Network regulation of the *Escherichia coli* maltose system**. *J Mol Microbiol Biotechnol* 2002, **4**:301-307.

29. Shattuck-Eidens DM, Kadner RJ: **Exogenous induction of the *Escherichia coli* hexose phosphate transport system defined by uhp-lac operon fusions**. *J Bacteriol* 1981, **148**:203-209.

30. Shattuck-Eidens DM, Kadner RJ: **Molecular cloning of the uhp region and evidence for a positive activator for expression of the hexose phosphate transport system of *Escherichia coli***. *J Bacteriol* 1983, **155**:1062-1070.

31. Weston LA, Kadner RJ: **Role of uhp genes in expression of the *Escherichia coli* sugar-phosphate transport system**. *J Bacteriol* 1988, **170**:3375-3383.

32. Weston LA, Kadner RJ: **Identification of uhp polypeptides and evidence for their role in exogenous induction of the sugar phosphate transport system of *Escherichia coli* K-12**. *J Bacteriol* 1987, **169**:3546-3555.

33. Schwoppe C, Winkler HH, Neuhaus HE: **Properties of the glucose-6-phosphate transporter from Chlamydia pneumoniae (HPTcp) and the glucose-6-phosphate sensor from *Escherichia coli* (UhpC)**. *J Bacteriol* 2002, **184**:2108-2115.

34. Goldenbaum PE, Farmer KS: **uhp-directed, glucose 6-phosphate membrane receptor in *Escherichia coli***. *J Bacteriol* 1980, **142**:347-349.

35. Island MD, Wei BY, Kadner RJ: **Structure and function of the uhp genes for the sugar phosphate transport system in *Escherichia coli* and *Salmonella typhimurium***. *J Bacteriol* 1992, **174**:2754-2762.

36. Wright JS 3rd, Kadner RJ: **The phosphoryl transfer domain of UhpB interacts with the response regulator UhpA**. *J Bacteriol* 2001, **183**:3149-3159.

37. Island MD, Kadner RJ: **Interplay between the membrane-associated UhpB and UhpC regulatory proteins**. *J Bacteriol* 1993, **175**:5028-5034.

38. Olekhnovich IN, Kadner RJ: **RNA polymerase alpha and sigma(70) subunits participate in transcription of the *Escherichia coli* uhpT promoter**. *J Bacteriol* 1999, **181**:7266-7273.

39. Kadner RJ, Webber CA, Island MD: **The family of organo-phosphate transport proteins includes a transmembrane regulatory protein**. *J Bioenerg Biomembr* 1993, **25**:637-645.

40. Pan CJ, Chen SY, Jun HS, Lin SR, Mansfield BC, Chou JY: **SLC37A1 and SLC37A2 are phosphate-linked, glucose-6-phosphate antiporters**. *PLoS ONE* 2011, **6**:e23157.

41. Hoffer SM, van Uden N, Tommassen J: **Expression of the pho regulon interferes with induction of the uhpT gene in *Escherichia coli* K-12**. *Arch Microbiol* 2001, **176**:370-376.

42. Dumay V, Danchin A, Crasnier M: **Regulation of *Escherichia coli* adenylate cyclase activity during hexose phosphate transport**. *Microbiology* 1996, **142(Pt 3)**:575-583.

43. Olekhnovich IN, Kadner RJ: **Mutational scanning and affinity cleavage analysis of UhpA-binding sites in the *Escherichia coli* uhpT promoter**. *J Bacteriol* 2002, **184**:2682-2691.

44. Olekhnovich IN, Dahl JL, Kadner RJ: **Separate contributions of UhpA and CAP to activation of transcription of the uhpT promoter of *Escherichia coli***. *J Mol Biol* 1999, **292**:973-986.

45. Jansen C, Heutink M, Tommassen J, de Cock H: **The assembly pathway of outer membrane protein PhoE of *Escherichia coli***. *Eur J Biochem* 2000, **267**:3792-3800.

46. Muda M, Rao NN, Torriani A: **Role of PhoU in phosphate transport and alkaline phosphatase regulation**. *J Bacteriol* 1992, **174**:8057-8064.

47. Luttmann D, Gopel Y, Gorke B: **The phosphotransferase protein EIIA(Ntr) modulates the phosphate starvation response through interaction with histidine kinase PhoR in *Escherichia coli***. *Mol Microbiol* 2012, **86**:96-110.

48. Pfluger-Grau K, Gorke B: **Regulatory roles of the bacterial nitrogen-related phosphotransferase system**. *Trends Microbiol* 2010, **18**:205-214.

49. • Hsieh YJ, Wanner BL: **Global regulation by the seven-component Pi signaling system**. *Curr Opin Microbiol* 2010, **13**:198-203.

Summarizes the transcriptional regulatory system for the *pho* regulon.

50. Aguena M, Spira B: **Transcriptional processing of the pst operon of *Escherichia coli***. *Curr Microbiol* 2009, **58**:264-267.

51. Torriani A: **From cell membrane to nucleotides: the phosphate regulon in *Escherichia coli***. *Bioessays* 1990, **12**:371-376.

52. Rao NN, Torriani A: **Molecular aspects of phosphate transport in *Escherichia coli***. *Mol Microbiol* 1990, **4**:1083-1090.

53. Kadner RJ, Murphy GP, Stephens CM: **Two mechanisms for growth inhibition by elevated transport of sugar phosphates in *Escherichia coli***. *J Gen Microbiol* 1992, **138**:2007-2014.