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The Bacterial Phosphoenolpyruvate:Carbohydrate Phosphotransferase System: Regulation by Protein Phosphorylation and Phosphorylation-Dependent Protein-Protein Interactions

Josef Deutscher,[a,b,c] Francine Moussan Désirée Aké,[b,c] Meriem Derkaoui,[b,c] Arthur Constant Zébré,[b,c] Thanh Nguyen Cao,[b,c] Houda Bouraoui,[b,c] Takfarinas Kentache,[b,c] Abdelhamid Mokhtari,[b,c] Eliane Milohanic,[b,c] Philippe Joyet[b,c]

Centre National de la Recherche Scientifique, FRE3630 Expression Génétique Microbienne, Institut de Biologie Physico-Chimique, Paris, France[a]; INRA, Microbiologie de l'Alimentation au Service de la Santé Humaine, UMR1319, Jouy en Josas, France[b]; AgroParisTech, Microbiologie de l'Alimentation au Service de la Santé Humaine, Jouy en Josas, France[c]

SUMMARY 231
INTRODUCTION 232
PTS-Mediated Sugar Transport and Phosphorylation 232
Regulatory Functions of the PTS 233
PTS-MEDIATED REGULATION BY PHOSPHORYLATION 234
Proteins Containing Their Own PTS-Recognized Phosphorylation Sites 234
Proteins Containing a PTS Component Fused to the N or C Terminus 235
Transcriptional activators 235
The lactose-specific transporter LacS 237
HPr domain-containing proteins 237
Proteins fused to other PTS domains 239
Proteins Containing a Specific PTS-Recognized Phosphorylation Domain, the PRD 239
Structures of PRD and PRD-containing proteins 239
PRD-containing antiterminators 240
PRD-containing transcription activators 240
The Mga regulator of *S. pyogenes* 240
REGULATION BY PROTEIN-PROTEIN INTERACTION 241
Interaction with Unphosphorylated PTS Proteins 241
Inducer exclusion in *Enterobacteriaceae* 241
Other EIIA^{Glc} interaction partners 242
The EIIA^{Glc} interface in protein-protein interactions 243
PTS^{Ntr} 243
Regulation of K^+ transport 244
Other EIIA^{Ntr} interaction partners 244
Interaction with EIIB components 245
Interaction with HPr and its paralogues Crh and NPr 246
Interaction with EI and EI^{Ntr} 247
Interaction with Phosphorylated PTS Proteins 247
Interaction with phosphorylated EIIA components 247
Interaction with phosphorylated EIIB components 247
Interaction with histidyl-phosphorylated HPr 248
Interactions with P-Ser-HPr and P-Ser-Crh 248
CONCLUSIONS AND PERSPECTIVES 249
ACKNOWLEDGMENTS 250
REFERENCES 250

SUMMARY

The bacterial phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) carries out both catalytic and regulatory functions. It catalyzes the transport and phosphorylation of a variety of sugars and sugar derivatives but also carries out numerous regulatory functions related to carbon, nitrogen, and phosphate metabolism, to chemotaxis, to potassium transport, and to the virulence of certain pathogens. For these different regulatory processes, the signal is provided by the phosphorylation state of the PTS components, which varies according to the availability of PTS substrates and the metabolic state of the cell. PEP acts as phosphoryl donor for enzyme I (EI), which, together with HPr and one of several EIIA and EIIB pairs, forms a phosphorylation cascade which allows phosphorylation of the cognate carbohydrate bound to the membrane-spanning EIIC. HPr of firmicutes and numerous proteobacteria is also phosphorylated in an ATP-dependent reaction catalyzed by the bifunctional HPr kinase/phosphorylase. PTS-mediated regulatory mechanisms are based either on direct phosphorylation of the target protein or on phosphorylation-dependent interactions. For regulation by PTS-

Address correspondence to Josef Deutscher, Josef.Deutscher@grignon.inra.fr.

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mediated phosphorylation, the target proteins either acquired a PTS domain by fusing it to their N or C termini or integrated a specific, conserved PTS regulation domain (PRD) or, alternatively, developed their own specific sites for PTS-mediated phosphorylation. Protein-protein interactions can occur with either phosphorylated or unphosphorylated PTS components and can either stimulate or inhibit the function of the target proteins. This large variety of signal transduction mechanisms allows the PTS to regulate numerous proteins and to form a vast regulatory network responding to the phosphorylation state of various PTS components.

INTRODUCTION

PTS-Mediated Sugar Transport and Phosphorylation

Many bacteria (1, 2) as well as some archaea (3) take up sugars and sugar derivatives, such as sugar alcohols, amino sugars, glycuronic acids, disaccharides, and numerous other carbon sources, via the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS). The PTS is usually composed of one membrane-spanning protein and four soluble proteins. Enzyme I (EI) and HPr are the general cytoplasmic PTS components, which in most organisms are involved in the uptake of all PTS carbohydrates; their genes are usually organized in the *ptsHI* operon, with *ptsH* coding for HPr and *ptsI* for EI. In contrast, the EIIA, EIIB, and EIIC (and in mannose-type PTSs also EIID) proteins are usually specific for one substrate or, in a few cases, for a small group of closely related carbohydrates (1, 2, 4). To indicate their substrate specificity, a three letter code is used, which is added as superscript to the corresponding protein name (5). For example, $EIIA^{Glc}$ stands for the glucose-specific EIIA component, $EIIB^{Fru}$ for the fructose-specific EIIB, $EIID^{Man}$ for the mannose-specific EIID, etc. The genes encoding the PTS components specific for a certain sugar are normally organized in an operon, which frequently also contains the genes required for the catabolism of the transported substrate.

With the exception of several actinobacteria, such as *Agromyces italicus*, *Cellulomonas flavigena*, or *Actinomyces turicensis*, which possess a regular HPr in addition to a DhaM protein composed of an HPr-like domain and an $EIIA^{Man}$-like domain, Gram-positive bacteria usually contain only one EI and one HPr. In contrast, *Enterobacteriaceae* usually produce several EI and HPr homologues or paralogues, such as nitrogen-related EI^{Ntr} and NPr and the fructose-specific FPr. Both types of bacteria normally contain several operons encoding the sugar-specific PTS components. According to the sequence and composition of the membrane components, seven families of PTS can be distinguished: the glucose, fructose, lactose, glucitol, galactitol, mannose, and ascorbate families (6). Interestingly, numerous alpha-, beta-, gamma-, and deltaproteobacteria also contain an incomplete PTS lacking any known EIIB and EIIC component. These PTSs are therefore probably not involved in carbohydrate transport but carry out only regulatory functions. Finally, in certain bacteria the phosphorylation of intracellular dihydroxyacetone requires EI, HPr, and an EIIA of the mannose family (7).

PTS proteins are often fused to each other, thus forming multifunctional polypeptides made up of two or more domains. Frequently, one or two of the soluble PTS components are fused to the N or C terminus of the membrane-spanning transport protein and are therefore located at the cytosolic side of the membrane. For example, the oligo-β-glucoside-specific PTS of *Bacillus subtilis* is composed of three distinct proteins, whereas in the mannitol-specific PTS permease of the same organism, the EIIB component is fused to the C terminus of the membrane-spanning EIIC and only $EIIA^{Mtl}$ is a distinct protein (Fig. 1). The mannose-type PTSs are an exception because they possess two transmembrane proteins, as is the case for most mannose/glucose-specific PTSs of firmicutes as well as the low-efficiency fructose-specific levan PTS of *B. subtilis* (Fig. 1).

The PTS uses PEP as an energy source for the uptake of its substrates and as a phosphoryl donor for their phosphorylation. In order to phosphorylate the carbohydrates during their transport, the soluble PTS components form a phosphorylation cascade beginning with EI, which autophosphorylates at the N-ε3 position of a conserved histidyl residue at the expense of PEP (Fig. 1) (8). Phosphorylated EI (P∼His-EI) transfers the phosphoryl group to the N-δ1 position of His-15 in HPr (9), and histidyl-phosphorylated HPr (P∼His-HPr) passes the phosphoryl group on to one of several sugar-specific EIIAs usually present in a bacterium. (The symbol "∼" indicates an energy-rich phosphoryl bond, in contrast to, for example, seryl-phosphorylated proteins, which are written as P-Ser throughout this article.) EIIAs are also phosphorylated at the N-ε3 position of a histidyl residue (10, 11). P∼EIIAs, however, phosphorylate their cognate EIIB at a cysteyl residue (12), except the EIIBs of the mannose PTS family, which are phosphorylated at the N-δ1 position of a conserved histidine (13). In the last step, P∼EIIB transfers its phosphoryl group to a carbohydrate molecule bound to the cognate EIIC. There is so far only one carbohydrate, fucosyl-α-1,3-*N*-acetylglucosamine, which is transported by a PTS (that of *Lactobacillus casei*) without being phosphorylated (14). All other carbohydrates are phosphorylated during their transport and subsequently converted into phosphorylated intermediates of either the Embden-Meyerhof-Parnas, pentose phosphate, or Entner-Doudoroff pathway. Only maltose taken up via a PTS by *Enterococcus faecalis* cells was reported to be first dephosphorylated to maltose by the maltose-6′-phosphate (maltose-6′-P)-specific phosphatase MapP (15). Intracellular maltose is subsequently cleaved by the enzyme maltose phosphorylase into glucose-1-P and glucose, which are converted into glycolytic intermediates.

The crystal structure of the *N*,*N*′-diacetylchitobiose-specific EIIC component of *Bacillus cereus* with the disaccharide bound to the active site has been determined and indeed confirmed that the phosphorylatable hydroxyl group at the C-6′ position of the disaccharide is located close to the cytoplasm and can therefore be accessed by the P∼EIIB component (16). Phosphorylation of the carbohydrate is thought to lower its affinity for EIIC, and the phosphorylated carbohydrate is therefore released into the cytosol.

Among the about 230 archaea for which the genome sequence has been determined, more than 50 contain the general PTS components EI and HPr; most of these belong to the genus *Haloferax*. It seems that these organisms use the PTS mainly to transport and phosphorylate fructose (3, 17) or to phosphorylate dihydroxyacetone (18). A previous deduction that archaea are devoid of any PTS is therefore no longer valid (6). *Methanopyrus kandleri* AV19 possesses a protein (MK1512) corresponding to the short form of HprK/P present in alphaproteobacteria (19). As will be explained below, HprK/P phosphorylates HPr at Ser-46 (Fig. 1). The N-terminal part of MK1512 exhibits 55% sequence identity to a ca. 85-amino-acid-long region of HprK/P from firmicutes compris-

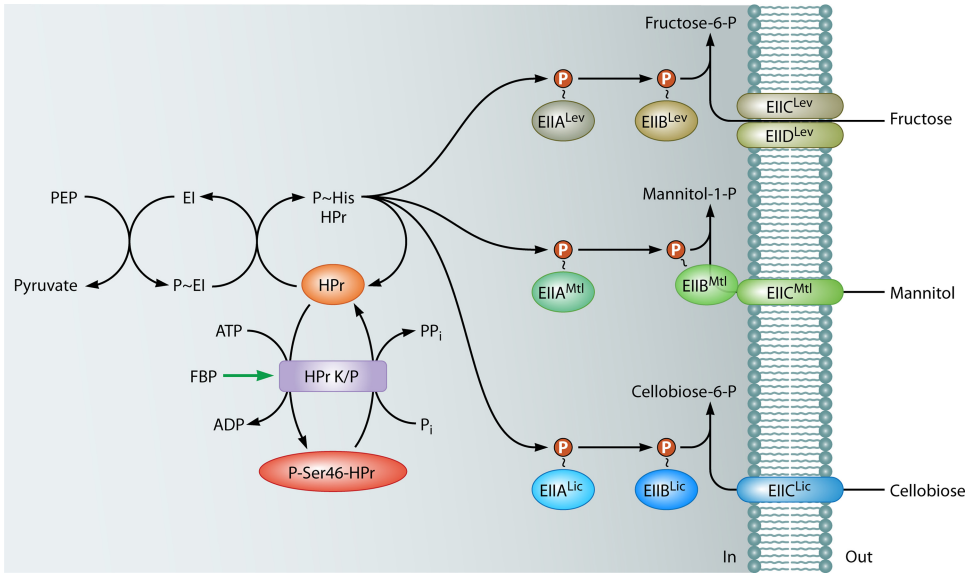

FIG 1 Schematic presentation of the phosphorylation cascade formed by the *B. subtilis* PTS components necessary for the uptake of fructose (PTS^{Lev}), mannitol (PTS^{Mtl}), and cellobiose (PTS^{Lic}). The two general PTS components EI and HPr phosphorylate the EIIAs, which are specific for a certain carbohydrate. *B. subtilis* contains nine entire PTSs, six PTSs lacking an EIIA component, and one PTS lacking EIIA and EIIB components (49). For the seven incomplete PTSs, EIIA and EIIB components of other PTS, most likely from the same family, probably complement the transport and phosphorylation functions. Nevertheless, the PTSs usually exhibit different sugar specificities. The P~EIIAs transfer the phosphoryl group to their cognate EIIB, which finally phosphorylates the carbohydrate bound to the corresponding membrane-integral EIIC or, for the fructose-specific levan PTS, to EIIC and EIID. The phosphorylated carbohydrate is subsequently released into the cytoplasm. While the PTS phosphorylation cascades for cellobiose and fructose are formed by EI, HPr, and two distinct EIIA and EIIB proteins, the EIIB component of PTS^{Mtl} is fused to the C terminus of the EIIC domain and is therefore attached to the cytoplasmic side of the membrane. Shown are also the fructose-1,6-bisphosphate (FBP)-stimulated and ATP-requiring phosphorylation of HPr at Ser-46 as well as the dephosphorylation of P-Ser-HPr, which follows a phosphorolysis reaction with P-Ser-HPr and P_i being the substrates and HPr and pyrophosphate (PP_i) the products (22). The ATP-dependent phosphorylation of HPr occurs in firmicutes but also in many proteobacteria containing HprK/P and the HPr paralogue NPr.

ing the active site with the P loop. This organism also contains an HPr-like domain present in a protein of unknown function (MK0716). Interestingly, in the HPr-like domain the phosphorylatable His is replaced with a Glu, whereas the ATP-dependent phosphorylation site around Ser-46 is conserved. It is therefore likely that in some archaea PTS proteins also carry out regulatory functions, which are associated with phosphorylation of this serine residue.

Regulatory Functions of the PTS

Soon after the discovery of the PTS in *Escherichia coli* (20), it was realized that it not only transports and phosphorylates carbohydrates but also carries out regulatory functions. In *Enterobacteriaceae*, $EIIA^{Glc}$ (also called Crr, for catabolite repression resistance) was found to be the central regulator of carbon metabolism. In firmicutes, this function is carried out by HPr, which in these organisms becomes phosphorylated not only at His-15 by PEP and EI but also at Ser-46 by ATP and the metabolite-controlled bifunctional kinase/phosphorylase HprK/P (Fig. 1) (21, 22). The latter HPr modification plays no role in sugar uptake and phosphorylation but serves exclusively regulatory functions. In addition to $EIIA^{Glc}$ and HPr, many other PTS proteins also play regulatory roles. Intriguingly, numerous proteobacteria possess EI, HPr, and one or two EIIAs but lack any known EIIB and EIIC proteins. Although *E. coli* and other *Enterobacteriaceae* are devoid of HprK/P and the region around Ser-46, the ATP-dependent phosphorylation site in HPr, is not conserved, HPr proteins of proteobacteria with an incomplete PTS usually possess an HprK/P, and the region around Ser-46 strongly resembles that in firmicutes. These observations suggest that in these proteobacteria, the incomplete PTS is not involved in carbohydrate transport but carries out only regulatory functions by interacting with or by phosphorylating non-PTS target proteins.

Indeed, in most cases phosphorylation of non-PTS proteins by PTS components serves regulatory functions. There are nevertheless at least two reported cases where PTS proteins phosphorylate a non-PTS protein or its bound cofactor for enzymatic purposes. First, the phosphoryl group of P~EI from *Salmonella enterica* serovar Typhimurium can be transferred to the intermediately phosphorylated active-site residue of acetate kinase (23). Second, P~EIIA of the dihydroxyacetone phosphorylation system of numerous bacteria and several archaea (24) transfers its phosphoryl group to an ADP molecule tightly bound to the active site of the dihydroxyacetone kinase subunit DhaL, which subsequently phosphorylates dihydroxyacetone bound to DhaK (18). Some enterobacteria possess a more complex dihydroxyacetone-specific multidomain protein composed of an EIIA-like domain and an HPr-like domain followed by a truncated EI-like domain containing the conserved phosphorylatable histidine. This protein is phosphorylated by PEP, EI, and HPr at the truncated EI-like domain, and after primarily intramolecular transfer of the phosphoryl group to the HPr- and EIIA-like domains, it phosphorylates the ADP bound to DhaL (7).

In most cases, however, phosphorylation of non-PTS proteins by PTS components serves regulatory purposes. To allow the PTS to carry out its regulatory functions, several different mechanisms evolved. They include direct phosphorylation of the target pro-

teins by PTS components. Phosphorylation can occur at PTS domains fused to the target proteins or at specific phosphorylation sites or domains that evolved in or were fused to the target protein. Alternatively, PTS components, in either phosphorylated or unphosphorylated form, interact with their target proteins. PTS-catalyzed phosphorylation of a target protein can stimulate or inhibit its activity, and this also holds for the interaction of phosphorylated and unphosphorylated PTS components with their target proteins. We describe these different scenarios in detail below.

PTS-MEDIATED REGULATION BY PHOSPHORYLATION

Since all cytoplasmic PTS proteins or membrane-associated hydrophilic PTS domains undergo transient phosphorylation, it is not surprising that numerous regulatory functions of the PTS are mediated by phosphoryl group transfer to the target proteins. Identical to the case for PTS proteins, all presently known PTS-regulated target proteins also become phosphorylated at histidine (phosphoamidate) or cysteine (thiophosphate) residues. This renders all phosphorylation steps reversible, because the standard free energies of the various high-energy phosphate bonds (P∼Nδ-1 or P∼Nε-3 in P∼histidines and P∼S in cysteines) are very similar (25–27). Consequently, if the PTS proteins are present primarily in phosphorylated form, the PTS-regulated target proteins are also mainly phosphorylated.

Two major factors were so far reported to affect the phosphorylation state of the PTS proteins in *Enterobacteriaceae* and probably most other bacteria. First, the uptake of an efficiently metabolized PTS sugar, such as glucose, and its concomitant phosphorylation lead to dephosphorylation of the PTS components. The phosphoryl group transfer from most P∼EIIBs to their cognate substrates seems to be more rapid than rephosphorylation via the PTS cascade. Second, changes in the ratio of PEP to pyruvate, the two compounds which are the substrate and product of the autophosphorylation of EI (Fig. 1), also affect the phosphorylation state of the PTS proteins (28, 29). The PEP-to-pyruvate ratio changes in response to the metabolic state of the cell. Starving cells have a high PEP-to-pyruvate ratio, and under resting conditions the PTS proteins are therefore mainly phosphorylated (28, 30) and the cells are primed to take up any PTS substrate they might encounter. In metabolically active cells, the PEP-to-pyruvate ratio is low and the PTS proteins are barely phosphorylated at His and Cys residues (30). Very low phosphorylation of the PTS proteins is therefore usually observed in cells efficiently metabolizing PTS sugars, such as glucose (28, 31). However, growth on non-PTS sugars can also lower the PEP-to-pyruvate ratio and therefore the phosphorylation state of the PTS proteins (28). Finally, α-ketoglutarate, which accumulates in *E. coli* cells exposed to nitrogen-limiting conditions, inhibits the autophosphorylation activity of EI. Nitrogen limitation therefore inhibits glucose uptake. In contrast, sudden nitrogen availability almost immediately increases glucose uptake and consumption without significantly altering the concentration of glycolytic intermediates (32).

In firmicutes, the metabolite-controlled HprK/P-mediated phosphorylation of HPr at Ser-46 (33) during the rapid metabolism of a carbohydrate slows the phosphoryl group transfer within the PTS phosphorylation cascade (34). The kinase function of HprK/P is enhanced during growth on efficiently utilized carbon sources (30, 35), and the resulting seryl-phosphorylated HPr (P-Ser-HPr) is a poor substrate for the PEP-dependent phosphorylation by P∼EI (36, 37). This mechanism further lowers the poor PEP-requiring phosphorylation of HPr and the EIIA and EIIB components in firmicutes growing on an efficiently metabolizable carbon source. The alterations of the phosphorylation state of the general and sugar-specific PTS components in response to the metabolic state of the cell and/or to the presence of a distinct carbohydrate are used to control numerous cellular functions.

Proteins Containing Their Own PTS-Recognized Phosphorylation Sites

One might expect that the development of a phosphorylation site recognized by PTS components would be the most straightforward and simplest way to render a protein "PTS controlled." However, there is so far only one well-established example in which a protein developed a specific regulatory site (as opposed to the enzymatic sites described above) for phosphorylation by a PTS protein. This is glycerol kinase (GlpK) from firmicutes (38). GlpKs from firmicutes possess a surface-exposed flexible loop close to the dimer interface, which contains a histidyl residue surrounded by three aromatic amino acids, Aro-His-Aro-Aro (with Aro being Tyr or Phe in arbitrary order, depending on the organism) (39). This sequence does not resemble any known phosphorylation site in PTS proteins. The histidine residue in the above tetrapeptide (His-230 in GlpK from *B. subtilis*) (40) becomes phosphorylated at the N-ε3 position by PEP, EI, and HPr (38, 41). Despite a usually 60% amino acid sequence identity between GlpKs from firmicutes and other bacterial phyla, the phosphorylatable His is present only in GlpKs of firmicutes. Accordingly, GlpKs of no other phylum were so far found to be phosphorylated by PTS proteins, suggesting that this regulatory mechanism developed relatively late in evolution. In fact, GlpKs from *Enterobacteriaceae* possess at the same position a similar but histidine-less flexible loop for the binding of fructose-1,6,-bisphosphate (FBP), which leads to the formation of inactive tetramers (42). Proteins strongly resembling GlpKs from firmicutes but phosphorylating other carbohydrates, such as the presumed fuculose kinase Lmo1034 of *Listeria monocytogenes* (43), also lack the phosphorylation loop with the conserved histidine. The phosphorylation of GlpKs from firmicutes is reversible, and in contrast to the inhibitory effect of FBP binding on GlpKs of *Enterobacteriaceae*, it stimulates the GlpK-catalyzed ATP-dependent phosphorylation of glycerol 10- to 15-fold (44). Addition of an excess of HPr to purified phosphorylated GlpK leads to rapid dephosphorylation of the kinase and consequently lowers its activity (38). The uptake of efficiently metabolized PTS sugars, which leads to the dephosphorylation of PTS components and therefore also of GlpK, inhibits the activity of the kinase. Indeed, firmicutes in which EI or HPr have been inactivated not only are unable to utilize PTS carbon sources but also are unable to grow on glycerol as the sole carbon source (40, 45). The absence of GlpK phosphorylation leads to inducer exclusion, one of the regulatory mechanisms contributing to carbon catabolite repression, which will be discussed in detail in the section "Inducer exclusion in *Enterobacteriaceae*." The regulatory His of GlpK is located about 20 Å from the active site. PTS-mediated GlpK stimulation therefore follows a long-range activation mechanism. Studies with an *E. faecalis* GlpK mutant protein in which the regulatory His-232 had been replaced with an arginine, which leads to a fully active enzyme without phosphorylation (46), suggested that phosphorylation induces structural rearrangements along the dimer interface that allow an

TABLE 1 Proteins fused to PTS domains with proven or presumed regulatory functions

Organism(s)	Protein function	Fused PTS domain	Location of PTS domain	Conserved phosphorylation site	Effect of phosphorylation
Clostridium acetobutylicum	NtrC-type response regulator HprR CA_C3088	HPr	N terminus	His-15	?
Eubacterium limosum	BkdR-like transcription regulator	HPr	N terminus	His-16	?
Tepidanaerobacter acetatoxydans Re1	BkdR-like transcription regulator	HPr	N terminus	His-15	?
Streptococcus thermophilus	Lactose/galactose antiporter LacS	$EIIA^{Glc}$	C terminus	His-552	+
Streptococcus salivarius	Lactose/galactose antiporter LacS	$EIIA^{Glc}$	C terminus	His-552	?
Several *Borrelia* species	Na^+/H^+ symporter	$EIIA^{Ntr}$	C terminus	His-622	?
Pasteurella multocida	Triosephosphate isomerase	$EIIB^{Glc}$	C terminus	Cys-304	?
Actinobacillus succinogenes	Triosephosphate isomerase	$EIIB^{Glc}$	C terminus	Cys-297	?
Several *Vibrio* species	Cyclic diguanylate phosphodiesterase-like	$EIIC^{Lac}$	C terminus	171–432[a]	?
Firmicutes, actinobacteria, and a few proteobacteria	LevR-type transcription activators	$EIIA^{Man}$	Fourth domain	His-585[b]	+
		$EIIB^{Gat}$	Penultimate domain	Cys-718	−
Numerous *Clostridiales*, *Clostridium beijerinckii* NCIMB 8052	LevR-type transcription activator, YP_001309609.1, YP_001307365.1, YP_001307684.1	$EIIA^{Man}$	Fourth domain	His-629[c]	?
		$EIIB^{Gat}$	Penultimate domain	Cys-760	?
		$EIIA^{Mtl}$	C terminus	His-919	?
Numerous *Clostridiales*, *Thermoanaerobacter ethanolicus* JW200	LevR-type transcription activator, EGD53072.1	$EIIA^{Man}$	Fourth domain	His-606	?
		$EIIB^{Gat}$	Penultimate domain	Cys-740	?
		$EIIA^{Mtl}$	C terminus	His-896	?
Several *Selenomonadales* and other firmicutes, *Pelosinus fermentans* B3	LevR-type transcription activator, EIW31722.1	$EIIA^{Man}$	Fourth domain	His-604	?
		$EIIB^{Gat}$	Penultimate domain	Cys-744	?
		$EIIA^{Mtl}$	C terminus	His-898	?
Firmicutes, actinobacteria, and a few proteobacteria	MtlR/LicR-type transcription activators	$EIIB^{Gat}$	Penultimate domain	Cys-419[d]	−
		$EIIA^{Mtl}$	C terminus	His-599	−

[a] The number refers to the region corresponding to the $EIIC^{Lac}$ domain.
[b] The numbers refer to the *B. subtilis* LevR sequence.
[c] The numbers refer to the *C. beijerinckii* LevR-like protein with the ID YP_001309609.1.
[d] The numbers refer to the *B. subtilis* MtlR sequence.

optimal positioning of a crucial arginine residue (Arg-18) in the active site (47).

Proteins Containing a PTS Component Fused to the N or C Terminus

Another mechanism of PTS-mediated regulation that developed during bacterial evolution was the fusion of a PTS component to a target protein. In most cases the phosphorylatable His or Cys is conserved in the regulatory PTS domain and can therefore become phosphorylated by the proteins of the PTS phosphorylation cascade. For some proteins it was not an entire PTS domain that was fused to the target polypeptide but only a relatively small fragment containing the phosphorylatable histidine or cysteine. Phosphorylation or dephosphorylation of the PTS domain induces structural changes in the target protein, which lower or stimulate its activity. Proteins possessing a regulatory PTS domain include non-PTS transporters (48), various types of transcription activators (49–51), and two-component system response regulators (52). Several proteins and protein families containing a clearly identifiable PTS domain are listed in Table 1. There are also numerous proteins of unknown function that contain a PTS domain (6). The PTS-mediated regulation of the lactose transporters from *Streptococcus thermophilus* (53) and *Streptococcus salivarius* (54) and of several transcription activators from firmicutes (1) and some *Enterobacteriaceae*, such as FrzR of *E. coli* (55), has been studied in detail.

Transcriptional activators. The PTS domain-containing transcription activators are mainly found in firmicutes and in actinobacteria but are less frequent in proteobacteria. Some of these bacteria possess transcription activators that can contain up to three EIIA and EIIB domains (Table 1). In fact, two types of EII domain-containing transcription activators can be distinguished. The LevR-type activators possess an N-terminal DNA binding helix-turn-helix motif followed by a homologue of the central ATP binding cassette-containing domain of NtrC-type regulators and four regulatory domains; two or, in a few cases, three of the regulatory domains are EIIA and EIIB domains of the mannose/glucose, galactitol, and mannitol/fructose PTS classes (1). These transcription activators contain other regulatory domains (Fig. 2), which are discussed below (see "Proteins Containing a Specific PTS-Recognized Phosphorylation Domain, the PRD"). The NtrC-type regulators containing three EII domains belong primarily to the *Clostridiales*, including *Clostridia*, *Thermoanaerobacter*, *Carboxydibrachia*, and a few other firmicutes (Table 1). The members of the second type of EII domain-containing transcription activators are composed of a DeoR-like helix-turn-helix motif followed by a winged helix-turn-helix domain resembling that in the *Streptococcus pyogenes* virulence gene regulator Mga and

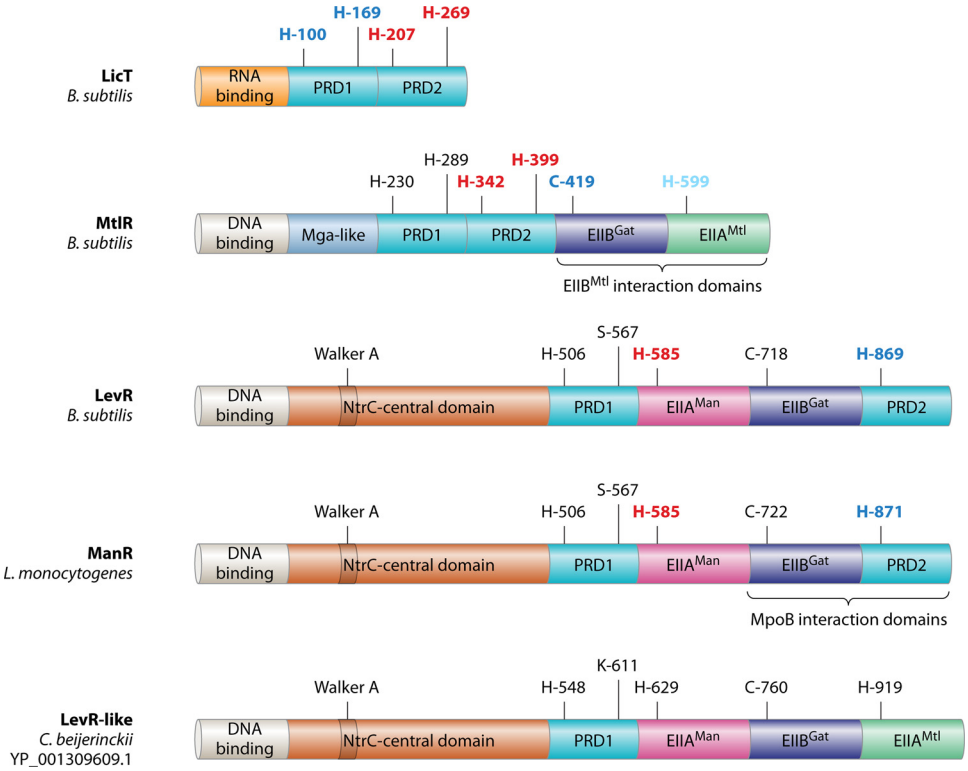

FIG 2 Schematic presentation of the different regulatory domains in PRD-containing proteins. Shown are the *B. subtilis* antiterminator LicT (which binds to RNA) and several transcription activators (which bind to DNA), including the *B. subtilis* regulators MtlR and LevR. Antiterminators are composed of two PRDs fused to the RNA binding domain. The two PRDs usually contain four potential sites of PTS-mediated phosphorylation (73). Similarly, MtlR-like transcription activators contain two PRDs fused to the DNA binding and Mga-like domains (71). However, in MtlR-like transcription activators, the PRDs are followed by an $EIIB^{Gat}$-like domain and an $EIIA^{Mtl}$-like domain. *B. subtilis* MtlR needs to be activated both by phosphorylation at His-342 and by the interaction of its C-terminal $EIIB^{Gat}$- and $EIIA^{Mtl}$-like domains (marked with a bracket) with the $EIIB^{Mtl}$ domain of the mannitol-specific PTS permease MtlA (146) (Fig. 1). The domain order in the *B. subtilis* transcription activator LevR is different from that in MtlR: the DNA binding and NtrC-like domains are followed by PRD1, $EIIA^{Man}$- and $EIIB^{Gat}$-like domains, and finally a truncated PRD2 (which contains only one conserved His). In all of the presented proteins the known stimulating phosphorylation sites (by P∼His-HPr) are indicated by red numbers, and inhibitory phosphorylation sites (by P∼EIIA or P∼EIIB) are written in blue (pale blue in MtlR indicates slight phosphorylation). Also presented in this figure is the LevR-like transcription activator ManR from *L. monocytogenes*, which, similar to the case for MtlR from *B. subtilis*, needs to be activated by both phosphorylation by P∼His-HPr at His-585 in the $EIIA^{Man}$-like domain (A. Zébré, E. Milohanic, and J. Deutscher, unpublished results) and interaction with the EIIB component MpoB (78). It should be noted that the phosphorylation sites are not always conserved. For example, ManR from *L. innocua*, which is almost identical to *L. monocytogenes* ManR, was reported to become phosphorylated by P∼His-HPr at His-506 in the PRD1 domain (77). Finally, in some LevR-like regulators of the order *Clostridiales*, the truncated PRD2 can be replaced with an $EIIA^{Mtl}$-like domain, as is the case for the *C. beijerinckii* protein with ID number YP_001309609.1.

four regulatory domains, one of which is an EIIB of the galactitol-type PTS and one an EIIA of the mannitol/fructose class PTS (Fig. 2). According to the first members described for this family of transcription activators, they are called MtlR/LicR-type regulators (51, 56).

Depending on the phosphoryl donor, phosphorylation of the EII domains in these transcription activators either stimulates their activity (phosphorylation by P∼His-HPr) or inhibits it (phosphorylation by P∼EIIA or P∼EIIB). For example, P∼His-HPr-mediated phosphorylation of the $EIIA^{Man}$-like domain of LevR from *B. subtilis* (57) and *L. casei* (50), the first intensively studied members of the EII and NtrC domain-containing regulators, activates the expression of the *lev* operon, which encodes the proteins for the extracellular degradation of the polysaccharide levan to fructose monomers and the components for a low-efficiency PTS specific for fructose and mannose. The absence of P∼His-HPr-mediated phosphorylation during the uptake of an efficiently metabolized carbon source represents one of the carbon catabolite repression (CCR) mechanisms operative in firmicutes. Nevertheless, in many firmicutes synthesis of the PTS transport components for the major repressing carbohydrates, glucose and mannose (ManLMN), is also regulated by a LevR-like protein (ManR), which in *L. monocytogenes* needs to be activated by P∼His-HPr-mediated phosphorylation at the $EIIA^{Man}$ domain (A. Zébré, E. Milohanic, and J. Deutscher, unpublished results). An autoregulation mechanism for glucose transport therefore seems to be operative. In contrast, phosphorylation by the cognate P∼EIIB component usually inhibits the activity of transcription activators, and its absence is used for induction of the corresponding PTS operon. Such a regulatory mechanism has been reported, for example, for *B. subtilis* LicR, which controls the expression of an operon encoding the components of a cellobiose-specific PTS. The uptake of cellobiose by the PTS^{Cel} is assumed to lead to dephosphorylation of the corresponding EIIB component and therefore to prevent the $EIIB^{Cel}$-mediated phosphorylation of the C-terminal $EIIA^{Mtl}$-like domain of the transcription activator LicR, thereby allowing the expression of the *cel* (or *lic*) operon. A similar regulation mechanism is probably operative for those

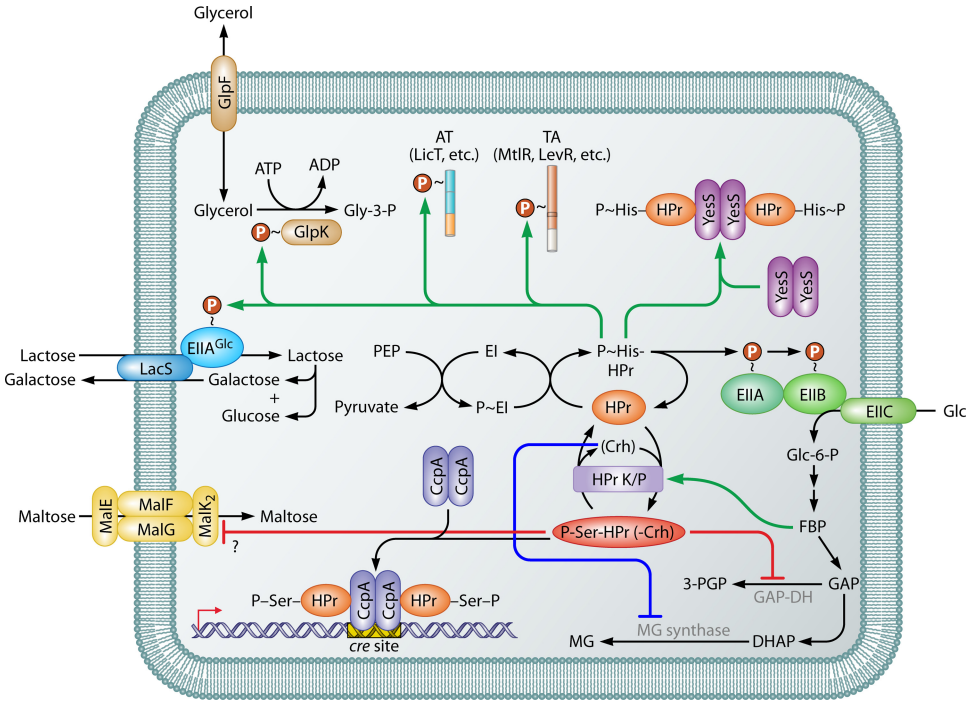

FIG 3 PTS-catalyzed glucose uptake and the HPr/Crh "regulon" in firmicutes. HPr, the central regulator of carbon metabolism in firmicutes, exists in four different forms: unphosphorylated, phosphorylated at His-15, phosphorylated at Ser-46, and doubly phosphorylated. *B. subtilis* contains in addition the HPr paralogue Crh, which lacks His-15 and therefore can be phosphorylated only at Ser-46. Histidyl-phosphorylated HPr prevails when a less favorable carbon source is utilized, whereas P-Ser-HPr and P-Ser-Crh are formed when glucose or other preferred sugars are metabolized. The utilization of glucose leads to an increase of the concentration of the glycolytic intermediate FBP, which stimulates the kinase function of HprK/P. Either the different forms of HPr (and *B. subtilis* Crh) interact with their target proteins (YesS, MG synthase, CcpA, and MalK) or P~His-HPr phosphorylates them (glycerol kinase, PRD-containing transcription activators and antiterminators, and LacS). The following regulatory functions of HPr and Crh and their various phosphorylated forms are presented. (i) Unphosphorylated Crh of *B. subtilis* interacts with methylglyoxal synthase (MG synthase) and inhibits its activity. Methylglyoxal synthase is an enzyme at the entry point of the methylglyoxal bypass of glycolysis that catalyzes the transformation of dihydroxyacetone-P into methylglyoxal. (ii) P-Ser-HPr as well as P-Ser-Crh interacts with the *B. subtilis* glycolytic enzyme glyceraldehyde-3-P DH (GAP DH) and inhibits its activity. (iii) P-Ser-HPr and in *B. subtilis* also P-Ser-Crh interact with CcpA and stimulate its repressor function for CCR by binding to the *cre* operator sites of numerous catabolic genes. (iv) P-Ser-HPr of lactobacilli and lactococci inhibits maltose uptake by an inducer exclusion mechanism by probably directly interacting with a component of the ABC transporter. (v) P~His-HPr interacts with and stimulates the *B. subtilis* transcription activator YesS, which controls the expression of the pectin/rhamnogalacturonan genes. (vi) P~His-HPr phosphorylates and activates several PRD-containing antiterminators (AT) and transcription activators (TA), and the absence of their phosphorylation during glucose metabolism is used as a CcpA-independent CCR mechanism. (vii) P~His-HPr also phosphorylates and activates glycerol kinase (GlpK). The absence of GlpK phosphorylation leads to inducer exclusion. (viii) Finally, in streptococci, P~His-HPr phosphorylates the EIIA^{Glc}-like domain of LacS and stimulates the lactose/galactose exchange reaction catalyzed by this protein.

LevR-like transcription activators that contain a C-terminal EIIA^{Mtl}-like domain (Fig. 2). In contrast, induction of the mannitol operon of *B. subtilis*, which is controlled by the transcription activator MtlR, occurs only when the P~EIIA^{Mtl}-mediated phosphorylation of MtlR at the conserved cysteyl residue in the penultimate EIIB^{Gat}-like domain is prevented by the presence of mannitol in the growth medium (58). So far, *B. subtilis* MtlR is the only example of P~EIIA-mediated phosphorylation of a transcription activator at the conserved Cys in the EIIB^{Gat}-like domain (Fig. 2).

The lactose-specific transporter LacS. LacS from *S. thermophilus* contains an EIIA^{Glc}-like domain fused to its C terminus (Table 1 and Fig. 3). This natural hybrid protein catalyzes slow H^+/lactose symport as well as fast lactose/galactose counterflow (59). Intracellular lactose is cleaved into glucose and galactose. The latter hexose cannot be metabolized by *S. thermophilus* and is therefore secreted. The export of galactose is coupled to the uptake of lactose via a counterflow mechanism. Phosphorylation of the EIIA^{Glc}-like domain of LacS was demonstrated for *S. thermophilus* (60) and *S. salivarius* (61). It increases the affinity of LacS for its substrate and the speed of the counterflow reaction (62). Efficient uptake of lactose therefore requires a functional β-galactosidase and phosphorylation of LacS by PEP, EI, and HPr (Fig. 3). By carrying out *in vitro* and *in vivo* complementation studies with truncated LacS lacking the EIIA^{Glc}-like domain, it could be established that the unphosphorylated EIIA^{Glc}-like domain does not bind to truncated LacS. In contrast, the phosphorylated EIIA^{Glc}-like domain interacted with truncated LacS and stimulated its lactose/galactose counterflow activity (63).

HPr domain-containing proteins. Several proteins were found to contain an HPr-like domain fused either to their N or C termini (Table 1). The presence of an HPr-like domain at the N terminus of an NtrC-type regulator with a C-terminal DNA binding domain (but without EII domains) in *Clostridium acetobutylicum* ATCC 824 suggested a possible cross talk between this transcription regulator (called HprR) and the PTS (52), with the HPr-like domain functioning as the receiver module (Fig. 4). His-15, the PEP-dependent phosphorylation site for P~EI, is nicely conserved in the HPr-like domain, whereas the surrounding of Ser-46

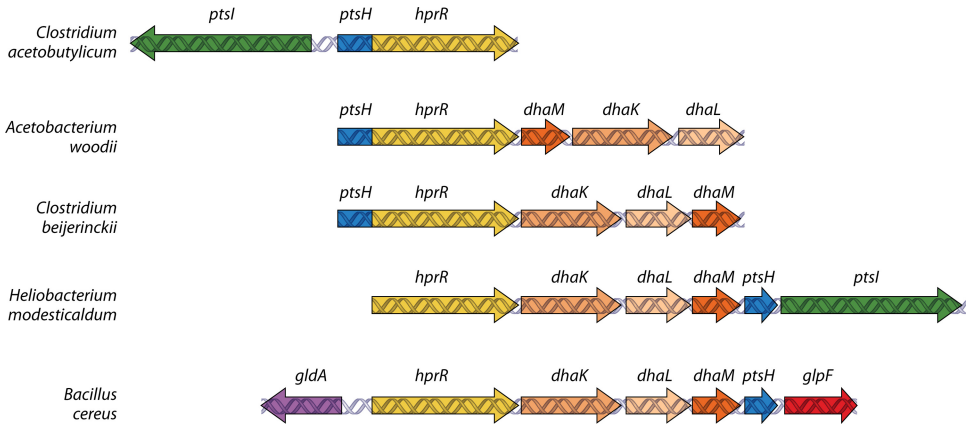

FIG 4 Gene arrangements around the *hprR* gene, encoding a σ^{54}-dependent regulator, in different organisms. The *hprR* gene is represented by the yellow arrows; when it is fused to *ptsH*, we call the gene *ptsH-hprR* and the protein accordingly PtsH-HprR. The *hprR* gene fused to *ptsH* was discovered in *C. acetobutylicum*, where a *ptsI* gene oriented in opposite direction is located upstream from *ptsH-hprR*. The *ptsI* gene is expressed from a σ^{54}-dependent promoter and therefore probably controlled by PtsH-HprR (CA_C3088). In other organisms, *ptsH-hprR* is part of the *dhaMKL* (*A. woodii*, Awo_c08990) or *dhaKLM* (*C. beijerinckii*, Cbei_2147) operon. The latter order of the *dha* genes is more common and is also found in *H. modesticaldum* and *B. cereus*. However, HprR of these two organisms (HM1_0841 and II5_05639, respectively) does not contain an HPr domain fused to its N terminus. Instead, a separate *ptsH* gene is located downstream from the *dhaKLM* genes, which is followed by a *ptsI* gene in *H. modesticaldum* and by a *glpF*-like gene in *B. cereus*. In the latter organism, a gene encoding a glycerol dehydrogenase-like protein (*gldA*) is located upstream from *hprR* and oriented in the opposite direction.

differs from that usually present in HPr of firmicutes. Indeed, a gene encoding a paralogue of EI (with 54% sequence identity to *B. subtilis* EI) is located just upstream from *hprR* and oriented in the opposite direction (Fig. 4). This is the only *ptsI* gene present in *C. acetobutylicum*, although a second *ptsH* gene is located elsewhere in the genome (unpublished observation). HPr encoded by the second *ptsH* also contains a well-conserved Ser-46. It is likely that the EI protein not only phosphorylates the second HPr but also transfers the phosphoryl group to His-15 in the HPr domain of HprR. Phosphorylation probably leads to structural changes affecting the affinity of HprR for its DNA target site. HprR does not contain the receiver module with the Asp phosphorylation site normally present in NtrC-like regulators. Activation of HprR by inter- or intramolecular transfer of the phosphoryl group from the HPr-like domain to a second phosphorylation site in the NtrC-like regulator is therefore unlikely. Similar to the case for other NtrC-type regulators, HprR probably controls the expression of RpoN (σ^{54})-dependent transcription units. Interestingly, the gene encoding the EI paralogue is preceded by a typical RpoN-dependent promoter, and its expression was therefore proposed to be controlled by HprR (52); HprR might nevertheless control the expression of other genes.

Genome sequencing revealed that HprR is present not only in *C. acetobutylicum* but also in many other bacteria of the order *Clostridiales*. In addition, an identical organization of the genes encoding HprR and the EI paralogue is found in several of them. In other *Clostridiales* the *hprR* gene is followed by the genes encoding the proteins for dihydroxyacetone phosphorylation, in the order *dhaMKL* or *dhaKLM* (Fig. 4). Interestingly, in a few species, such as *Halobacteroides halobius* and *Heliobacterium modesticaldum*, HPr is not fused to HprR, but *ptsH* and *ptsI* genes are located downstream from *hprR*, with the *dhaKLM* genes inserted between *hprR* and *ptsHI* (Fig. 4). Because in certain bacteria HprR exists without an HPr domain, we suggest that the form without the PTS protein be called HprR and that it be named PtsH-HprR when the PTS domain is fused to it. Accordingly, the gene names should be *hprR* and *ptsH-hprR* (Fig. 4). *H. halobius* and *H. modesticaldum* contain only the *ptsH* and *ptsI* genes located next to *hprR*. The gene arrangement in these organisms suggests that HprR might regulate the expression of the *dha* operon in response to dihydroxyacetone or glycerol availability. This hypothesis is further supported by our observation that in *Bacillus thuringiensis* IBL 200 and *Bacillus cereus* MSX-A1, the *hprR* gene is preceded by a gene encoding a glycerol dehydrogenase and followed by *dhaK*, *dhaL*, *dhaM*, *ptsH*, and *glpF*, with the last gene encoding a glycerol facilitator-like protein (Fig. 4). A *ptsHI* operon in these two organisms is located elsewhere on the genomes of the two bacilli. Glycerol metabolism via the enzymes encoded by the *dha* operon and the adjacent genes in the two bacilli thus resembles the glycerol utilization systems present in *L. monocytogenes* (64) and *E. faecalis* (65), which first oxidize intracellular glycerol to dihydroxyacetone, which is subsequently phosphorylated by the DhaKLM system.

HprR and PtsH-HprR probably control the expression of the glycerol/dihydroxyacetone system, depending on the phosphorylation state of the HPr-like domain or protein encoded by the associated or fused *ptsH* gene. In fact, in organisms possessing only the *ptsH* gene fused to *hprR*, such as *Clostridium beijerinckii*, the HPr-like domain of HprR probably participates in dihydroxyacetone phosphorylation by transferring the phosphoryl group received from P~EI to DhaM ($EIIA^{Dha}$), which in turn passes it on to the ADP molecule bound to DhaL. As already mentioned, the resulting ATP is used by DhaL to phosphorylate dihydroxyacetone bound to DhaK. It is therefore likely that the presence of dihydroxyacetone will lead to dephosphorylation of the HPr-like domain of HprR. Therefore, either the unphosphorylated general PTS component stimulates the transcription activator function of HprR or the phosphorylated HPr domain inhibits it. A similar activation or inhibition of HprR by HPr or P~His-HPr, respectively, probably occurs in bacteria where the phosphocarrier protein is encoded by a distinct protein. Finally, it should be noted that *C. acetobutylicum* does not possess a *dha* operon in its ge-

nome. The *ptsI* gene located upstream from *hprR* (Fig. 4) therefore remains the only presumed target for the σ^{54}-dependent regulator in this organism.

Interestingly, a protein belonging to the Fis family of transcriptional regulators from the two firmicutes *Eubacterium limosum* and *Tepidanaerobacter acetatoxydans* Re1 also contains an HPr-like domain (unpublished observation). These proteins exhibit significant sequence identity to BkdR of *B. subtilis*; however, *B. subtilis* BkdR, which controls the expression of the genes necessary for the utilization of the branched-chain amino acids valine and isoleucine (66), lacks an HPr-like domain. Unfortunately, for none of the HPr domain-containing proteins listed in Table 1 has the presumed regulatory role of the PTS domain been studied in detail.

Pyruvate kinase of firmicutes is a well-known example of proteins which do not contain an entire PTS domain fused to them but only a relatively small fragment, including the phosphorylation site with the conserved histidine or cysteine. In the case of pyruvate kinase, a peptide composed of about 50 amino acids exhibiting strong similarity to the surrounding of the EI autophosphorylation site is fused to its C terminus. The phosphorylatable His is located at position 539 in the sequence of *B. subtilis* pyruvate kinase, and the surrounding sequence (GGLTS**H**AAV) is nicely conserved compared to the phosphorylation site of *B. subtilis* EI (GGRTS**H**SAI). The role of the EI fragment is not known. Pyruvate kinase binds PEP to its active center but does not autophosphorylate at the conserved histidine in the EI fragment. The *B. subtilis* enzyme is also not phosphorylated by P∼EI or P∼His-HPr (G. Boël and J. Deutscher, unpublished results). It is therefore tempting to assume that HPr in phosphorylated or unphosphorylated form might bind to the EI-like fragment and thus affect the activity of pyruvate kinase.

Proteins fused to other PTS domains. In *Pasteurella multocida* Pm70 (6) and several other *P. multocida* strains as well as in *Actinobacillus succinogenes* 130Z, an entire $EIIB^{Glc}$-like domain is fused to another glycolytic enzyme, namely, triosephosphate isomerase. The phosphorylatable Cys is conserved in all these proteins, suggesting that their activity might be controlled by PTS-mediated phosphorylation in response to the presence or absence of glucose, which in most *Pasteuriaceae* is taken up via a PTS. The Na^+/H^+ symporter of *Borrelia burgdorferi*, the causative agent of borreliosis, and of other *Borrelia* strains carries an $EIIA^{Ntr}$-like domain fused to its C terminus (6). The phosphorylatable His residue is well conserved in the $EIIA^{Ntr}$-like domain. $EIIA^{Ntr}$ is a PTS protein assumed to be involved in the regulation of nitrogen metabolism (for a recent review, see reference 67). Finally, several *Vibrio* species possess an EAL domain characteristic of cyclic diguanylate phosphodiesterases (68), which contains fused to its C terminus an $EIIC^{Lac}$-like domain lacking about 170 amino acids at the N terminus. An $EIIB^{Lac}$-like domain usually fused to $EIIC^{Lac}$ is also absent. This protein therefore cannot be regulated by phosphorylation, because the EIIC domain does not contain a PTS phosphorylation site, but rather might respond to the binding of an extracellular substrate or interact with an $EIIB^{Lac}$ protein or domain of another PTS.

Another example of a protein containing a fragment of a PTS component is the C4-dicarboxylate transporter DcuA of *Enterobacteriaceae*, which contains at its C terminus a region of about 45 amino acids strongly resembling (45% sequence identity) the central part of $EIIA^{Glc}$, including the phosphorylation site (11). Although the surrounding of the phosphorylatable His of $EIIA^{Glc}$ is nicely conserved in DcuA (GVELFV**H**FG in $EIIA^{Glc}$ and GVALA V**C**FG in DcuA), the His itself is replaced with a Cys. However, Cys residues are also sometimes used as phosphorylation sites in PTS proteins (EIIB domain) and also in at least one PTS-controlled transcription activator (58). It is therefore tempting to assume that the DcuA transport activity might be controlled via either phosphorylation by or interaction with HPr or the $EIIB^{Glc}$ domain of the glucose-specific PTS permease. However, it should be noted that according to topology predictions, the C-terminal part of DcuA is quite hydrophobic and is thought to be embedded in the membrane.

There are numerous other proteins which contain clearly identifiable regulatory PTS domains, but their physiological role remains obscure. A list of some of these PTS domain-containing proteins of unknown function was presented by Barabote and Saier (6). Several of them contain a presumed helix-turn-helix motif fused to $EIIA^{Ntr}$; others were found to contain an $EIIA^{Fru}$-like domain at the N terminus and an FPr-like domain (fructose-specific HPr) at the C terminus. It will be interesting to unravel the role of some of these presumed regulatory PTS domains.

Proteins Containing a Specific PTS-Recognized Phosphorylation Domain, the PRD

Structures of PRD and PRD-containing proteins. In the course of evolution, bacteria must have produced a peptide composed of about 85 amino acids forming principally two slightly twisted antiparallel α-helices connected via an unstructured loop. The second α-helix contains a histidine, which is arranged in such a way that it can be phosphorylated by at least two different PTS proteins. The DNA region encoding this small peptide was probably duplicated in order to encode a protein fragment of about 170 amino acids. This polypeptide forms dimers (69, 70), thus finally providing what is known as the PTS regulation domain (PRD). Owing to duplication and dimerization, a PRD usually contains four conserved potential PTS phosphorylation sites. PRDs are frequently found to be fused to transcription regulators in firmicutes and actinobacteria but are less abundant in gammaproteobacteria (mostly in the family *Enterobacteriaceae*) and seem to be absent from alpha-, beta-, delta-, and epsilonproteobacteria. It is therefore likely that the PRDs evolved before the separation of firmicutes and actinobacteria and that some gammaproteobacteria acquired them later by horizontal gene transfer. Horizontal gene transfer probably also explains the presence of PRD-containing regulators in a few spirochaetes (treponemae and brachyspirae). Interestingly, in several pathogenic *E. coli* strains, genes encoding PRD-containing regulators are sometimes located in pathogenicity islands (55), which also supports the concept of horizontal gene transfer.

PRDs have so far been found in antiterminators and two types of transcription activators. These transcription activators were discussed in the preceding section, because they also contain EIIA and EIIB domains. Two PRDs are usually fused to the RNA binding (coantiterminator) domains of transcription antiterminators (called BglG/SacY-type antiterminators according to the first discovered members). The schematic presentation of LicT (a well-studied BglG/SacY family representative) in Fig. 2 shows only the LicT monomer. However, the crystal structure revealed that LicT forms dimers in which the two LicT subunits are aligned in paral-

lel. PRD1 and PRD2 of one subunit interact with PRD1 and PRD2, respectively, of the second subunit (70).

Two PRDs are also fused to the DNA binding domain and the Mga-like domain of MtlR/LicR type transcription activators (Fig. 2) (1). Again, only a monomer of the *B. subtilis* MtlR is presented in Fig. 2. However, the transcription activator is likely to form dimers similar to the model proposed in reference 71, with PRD1 and PRD2 of each subunit interacting with PRD1 and PRD2, respectively, of the second subunit. Finally, two PRDs are usually also present in LevR-type transcription activators, which contain a helix-turn-helix motif followed by an NtrC-like central domain and the first PRD (72) (Fig. 2). In these transcription activators, an $EIIA^{Man}$-like domain and an $EIIB^{Gat}$-like domain separate PRD1 from the usually truncated PRD2 containing only one phosphorylatable His. As already mentioned, in LevR-like proteins of several *Clostridiales* and a few other firmicutes, the second PRD is sometimes replaced with an $EIIA^{Mtl}$-like domain (Table 1) (Fig. 2).

PRD-containing antiterminators. The well-studied antiterminator LicT of *B. subtilis* controls the expression of the *bglPH* and *bglS* genes, which encode the PTS permease and catabolic enzymes for the uptake and metabolism of β-glucosides. The second PRD of LicT drastically alters its structure in response to specific mutations (His-Asp replacements) rendering the regulator constitutively active (69, 70). Similar structural changes are assumed to occur when PRD2 is phosphorylated by P-His-HPr or when other activating mutations are introduced into PRD2 (73, 74). These changes are probably transmitted to the RNA binding domain via PRD1 and finally lead to enhanced affinity of LicT for its target site, the ribonucleotidic antitermination target (RAT), on the corresponding nascent mRNA (1).

Unfortunately, LicT is so far the only PRD-containing protein for which the crystal structures of the two regulatory PRDs in active and inactive forms have been solved, but it is likely that similar phosphorylation-induced structural changes occur in PRDs of other antiterminators and probably also of the transcription activators. PRDs become phosphorylated by P∼His-HPr or P∼EIIBs. In most antiterminators, P∼His-HPr phosphorylates one or two histidine residues in PRD2 (73) and P∼EIIB phosphorylates one or two histidine residues in PRD1 (75).

PRD-containing transcription activators. The phosphorylation of PRDs in transcription activators is more variable. In some transcription activators, such as MtlR, only one PRD becomes phosphorylated by EI and HPr (58, 76), whereas in others, such as LicR, all four histidines of the two PRDs are phosphorylated by the general PTS components and mutation of either one of these histidines leads to a loss of function (56). In most LevR proteins (50, 57) and LevR-like transcription activators (77, 78), the C-terminal PRD2 is phosphorylated by EI, HPr, and the cognate EIIA and EIIB components. Surprisingly, the LevR-like ManR protein of *Listeria innocua* was reported to become phosphorylated by EI and HPr at PRD1 (77), whereas the almost identical ManR of *L. monocytogenes* was found to be phosphorylated by EI and HPr in the $EIIA^{Man}$-like domain (Fig. 2) (A. Zébré, F. Aké, E. Milohanic, and J. Deutscher, unpublished results). The general rule seems to be that phosphorylation of a PRD by P∼His-HPr stimulates the activity of the regulator, whereas phosphorylation by P∼EIIB inhibits it. There is at least one antiterminator, BglG from *E. coli*, which might not follow this rule. BglG was reported to be stimulated by the general PTS components EI and HPr in a phosphorylation-independent manner (79). However, recently BglG from *E. coli* was reported to become phosphorylated also by PEP, EI, and HPr as well as by PEP, EI, and FruB, a hybrid protein composed of the fructose-specific FPr and $EIIA^{Fru}$ (80). There is so far no explanation for these conflicting results.

In transcription activators, such as MtlR from *B. subtilis*, the stimulating signal has to be transmitted from amino acid His-342 in PRD2 across PRD1 and the Mga-like domain to the N-terminal DNA binding domain (71). The inactivating signal for LevR-type transcription activators is possibly transmitted even over a longer distance: from His-869 either to the active site for ATP hydrolysis in the NtrC-like central domain around amino acid 150 or all the way down to the N-terminal DNA binding domain (Fig. 2). As described above for the phosphorylation of EII domains in transcription activators, the absence of phosphorylation by P∼His-HPr owing to the uptake of an efficiently utilized carbon source is used as a CCR mechanism. In contrast, the absence of phosphorylation by the cognate P∼EIIB owing to the presence of the corresponding PTS substrate in the environment leads to the induction of the respective operon (1). A few antiterminators, such as *B. subtilis* GlcT, which controls the expression of the gene encoding the glucose-specific PTS permease and of *ptsHI*, do not require P∼His-HPr-mediated activation. In addition, P∼His-HPr-independent mutants affected in PRD1 or PRD2 could be obtained for antiterminators, such as LicT. These mutants allowed a conclusive confirmation that P∼His-HPr-mediated phosphorylation of these transcription regulators plays a role in CCR (74). The residual CCR observed for certain operons in firmicutes, in which the gene encoding the LacI/GalR-like catabolite control protein A (CcpA), the major player in CCR, had been deleted, disappeared when the corresponding regulator was mutated to a **P**TS-**i**ndependent **a**ntiterminator (Pia) or when the formation of P-Ser-HPr was prevented. In the latter case, the absence of P-Ser-HPr allows an efficient phosphorylation of the antiterminator by P∼His-HPr, even when glucose or other repressing sugars are present (74). It should be noted that in certain firmicutes, other pleiotropic CCR mechanisms exist, such as glucose kinase-mediated CCR in *Staphylococcus xylosus* (81). Even in a *ccpA* background, *pia* mutants might therefore not always cause a complete relief from CCR.

The Mga regulator of *S. pyogenes*. Mga, one of the regulators of *Streptococcus pyogenes* virulence genes, was also reported to be phosphorylated by EI and HPr (82). Phosphorylation was found to occur at a specific histidine in the central part of Mga, which was claimed to resemble PRDs. However, there does not seem to be significant sequence similarity between Mga and PRDs, and the alignment presented by Hondorp et al. (82) is not convincing. Significant sequence similarity between Mga and MtlR/LicR-type transcription activators was observed for only a small fragment of about 70 amino acids from the N-terminal part, which precedes PRD1 in the transcription activators (71). Hondorp et al. (82) also mention structural similarities between an *E. faecalis* Mga-like protein, for which the crystal structure has been solved, and the PRDs of LicT (69, 70). However, the tertiary structure of the dimers formed by these two proteins is completely different. The two subunits of unphosphorylated wild-type LicT are aligned in parallel, allowing the four histidines in the PRD1 and PRD2 dimers to face each other. In contrast, the two subunits of the *E. faecalis* Mga-like protein are oriented in an antiparallel fashion with mainly the C-terminal domain making contact to the other

subunit. The regions supposed to represent PRDs and containing the presumed phosphorylatable His are therefore located on opposite ends of the dimer. Again, only about 70 amino acids from the N-terminal part of the *E. faecalis* Mga-like protein exhibit structural similarity to the region preceding PRD1 in PRD-containing transcription activators (71), which might contribute to DNA binding. It is therefore likely that what the authors report in reference 82 might be more important than just another PRD. Similar to the case for GlpK, Mga and related transcription regulators, such as AtxA (83), might have developed their own PTS-specific phosphorylation sites.

Independently from the question whether Mga contains PRDs or not, Hondorp et al. unequivocally show that PEP-dependent phosphorylation of *S. pyogenes* Mga requires the general PTS components EI and HPr (82). PEP-dependent phosphorylation of Mga strongly inhibited *in vitro* expression of *emm*, one of the Mga-regulated virulence genes. However, there remain some uncertainties concerning the site of phosphorylation in Mga (82). Replacement of His-270, one of the histidines proposed to be located in the presumed PRD1, did not significantly diminish Mga phosphorylation. Surprisingly, Hondorp et al. did not change the two other histidines proposed to resemble the phosphorylatable His in PRDs based on their sequence alignment (His-175 and His-301) but instead replaced His-204 with an alanine. This mutation also had no significant effect on Mga phosphorylation compared to the wild-type protein. Accordingly, doubly mutated Mga(His-204-Ala,His-270-Ala) was also still phosphorylated. Finally, triply mutated Mga(His-204-Ala,His-270-Ala,His-324-Ala), with His-324 also not being one of the presumed conserved PRD-related histidines, was only slightly phosphorylated. However, several radioactive bands of lower molecular weight appeared, suggesting that the triply mutated protein might be unstable and be degraded. It remains to be determined whether replacement of the presumed phosphorylatable His-175 and His-301 has an effect on Mga phosphorylation. Nevertheless, the results presented in reference 82 suggest that PTS-mediated phosphorylation does not occur in the C-terminal part of Mga, because truncated Mga lacking the last 139 amino acids was as strongly phosphorylated by PEP, EI, and HPr as the full-length protein.

The utilization of glucose and other rapidly metabolizable carbon sources by *S. pyogenes* stimulates the expression of its virulence genes (84). Two regulatory mechanisms are probably responsible for this effect. First, expression of *mga* was found to be stimulated by carbon catabolite activation. The elevated amounts of glucose-6-P and FBP present in cells utilizing glucose or other efficiently metabolized carbon sources probably allow binding of CcpA to a specific operator site preceding *mga* (84). Second, in the absence of an efficiently transported PTS sugar, the PTS components are predominantly phosphorylated at their conserved His and Cys residues. The results reported previously (82) suggest that under these conditions Mga is also phosphorylated, leading to its inhibition and therefore to poor expression of virulence genes. In contrast, in the presence of glucose, firmicutes convert most of their HPr into P-Ser-HPr, and little P~His-HPr is therefore present. Mga is probably also barely phosphorylated and remains active, thus leading to strong virulence gene expression. Mutants producing Mga proteins with the above-described His-Ala replacement, but also additional mutants with presumed phosphomimetic His-Asp replacements, were tested as to whether they exhibit altered expression of Mga-regulated genes (82). The Ala replacements had only slight effects on the expression of the Mga-controlled *arp* and *sof* genes. In contrast, the presumed phosphomimetic mutants producing Mga(His-204-Asp,His-270-Asp) and Mga(His-204-Asp,His-270-Asp,His-324-Asp) exhibited very low Mga activity similar to that of an *mga* deletion mutant. However, owing to the uncertainty about the PTS phosphorylation site(s), it remains to be confirmed that there is a correlation between PTS phosphorylation and Mga activity.

REGULATION BY PROTEIN-PROTEIN INTERACTION

PTS components control the activity of their target proteins not only via phosphorylation but also via direct interaction. Interaction-mediated regulation seems to be even more frequent than regulation by phosphorylation. As described above for PTS-catalyzed phosphorylation of proteins at His or Cys residues, interactions with target proteins have been reported for HPr, EIIA, and EIIB components and in one case also for EI. Interactions occur with the unphosphorylated or phosphorylated forms of these proteins. The target proteins carry out various cellular functions, with the majority acting either as transport proteins or as transcription regulators.

Interaction with Unphosphorylated PTS Proteins

The interaction with a PTS protein and the accompanying activation or inhibition of the non-PTS target protein usually depend on the phosphorylation state of the PTS component. In fact, the phosphorylatable amino acids of PTS proteins and their surrounding always seem to be part of the interface of the protein-protein complex (85). If phosphorylation of a PTS component prevents the interaction with its target protein, the phosphorylatable amino acid is usually facing a negatively charged region. The phosphorylatable His, Cys, or Ser (in seryl-phosphorylated HPr) frequently adds to the stability of the complex by forming hydrogen bonds with amino acids of the target protein, which, of course, is prevented when the PTS protein is phosphorylated.

Inducer exclusion in *Enterobacteriaceae*. In most established PTS-mediated regulation mechanisms, PTS components interact in their unphosphorylated forms with non-PTS proteins. The first PTS components shown to interact with a non-PTS protein were the Crr proteins from *E. coli* and *S. enterica* serovar Typhimurium, which were later identified as glucose-specific EIIAs of the PTS ($EIIA^{Glc}$) (86). *E. coli* mutants defective in EI and/or HPr had lost the capacity to synthesize the proteins necessary for the uptake and metabolism of several non-PTS carbon sources, including glycerol, lactose, and maltose (87). The repressive effect of the *ptsHI* mutation could be overcome by a second mutation located in a gene close to the *ptsHI* operon. This gene was called *crr* for **c**atabolite **r**epression **r**esistant, because *crr* mutations also prevented the repressive effect of efficiently metabolizable carbohydrates on the synthesis of the enzymes necessary for the transport and metabolism of less favorable carbon sources.

Inactivation of *ptsHI* prevents the phosphorylation of $EIIA^{Glc}$, and it was therefore thought that under conditions where $EIIA^{Glc}$ would be present mainly in unphosphorylated form, it would inhibit the synthesis of the catabolic enzymes required for the metabolism of non-PTS carbon sources. Unphosphorylated $EIIA^{Glc}$ indeed prevails when an efficiently metabolized carbon source, such as glucose, is taken up (28). This concept was also in agreement with the observation that inactivation of the *crr* gene could overcome the repressive effect of the *ptsHI* mutation. However,

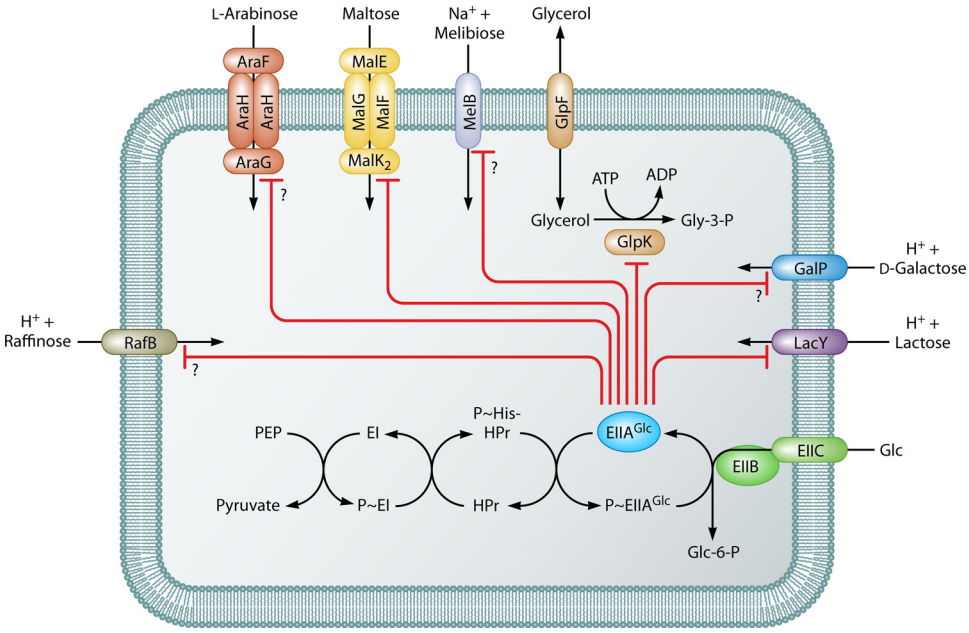

FIG 5 PTS-catalyzed glucose uptake and the EIIA^{Glc} inducer exclusion "regulon" of *E. coli*. Unphosphorylated EIIA^{Glc} but not phosphorylated EIIA^{Glc} interacts with several transport proteins or catabolic enzymes and inhibits their activity. Direct interactions have been shown for the catabolic enzyme glycerol kinase GlpK (88), the ATP binding subunit MalK of the maltose/maltodextrin-specific ABC transport system (89, 90), and the lactose permease LacY (91, 92). The crystal structures of the complexes formed between EIIA^{Glc} and the ATP-hydrolyzing MalK protein (106) as well as EIIA^{Glc} and GlpK, which catalyzes the ATP-dependent phosphorylation of glycerol to glycerol-3-P, have been solved (85). The uptake of the other four carbohydrates, L-arabinose, D-galactose, melibiose, and raffinose, is also subject to inducer exclusion, which is prevented when the EIIA^{Glc}-encoding *crr* gene is deleted. For the transporters labeled with a question mark, a direct interaction with EIIA^{Glc} is suggested by genetic data but has so far not been established by using biochemical or immunological methods.

mutants in which the repression caused by the *ptsHI* mutation was relieved only for one specific carbon source and not the others could be isolated. These mutations mapped not within *crr* but within the genes encoding the corresponding transporter, which therefore suggested that EIIA^{Glc} might interact with and inhibit proteins necessary for the uptake of the various carbon sources affected by the *ptsHI* mutation. Indeed, it was subsequently demonstrated that in *E. coli* and *S. enterica* serovar Typhimurium EIIA^{Glc} interacts with glycerol kinase (GlpK) (88) and the ATP binding protein of the maltose/maltodextrin ABC transport system (MalK) (89, 90) and that in *E. coli* EIIA^{Glc} interacts also with the lactose permease (LacY) (91, 92). In all cases, the interaction with unphosphorylated EIIA^{Glc} leads to inhibition of the non-PTS proteins (93, 94), which are usually transporters or transporter-associated components. Their inhibition prevents the uptake of the corresponding carbon source and consequently the formation of the necessary inducer; this regulation mechanism was therefore called inducer exclusion. In subsequent studies it was found that in *Enterobacteriaceae*, melibiose (95, 96), raffinose (97), galactose (98), and arabinose (99) also belong to the set of non-PTS sugars subject to inducer exclusion. The different carbohydrate utilization systems controlled by EIIA^{Glc} in *E. coli* are summarized in Fig. 5. EIIA^{Glc}-mediated inducer exclusion seems to be restricted to the family *Enterobacteriaceae*, indicating that this regulatory mechanism evolved relatively recently. It nevertheless represents the major CCR mechanism operative in *Enterobacteriaceae* (100). The term inducer exclusion is also used for EIIA^{Glc}-mediated GlpK inhibition, although GlpK is not a transport protein. Nevertheless, GlpK catalyzes the ATP-dependent phosphorylation of glycerol to glycerol-3-P (Fig. 5), the inducer of the glycerol operon in proteobacteria and most other bacteria. Because glycerol enters bacteria by facilitated diffusion, GlpK activity is important for efficient glycerol uptake. The transport-related activity of GlpK also explains why GlpK inhibition in firmicutes, which is not mediated via interaction with EIIA^{Glc} but occurs when the conserved phosphorylatable histidine in GlpK is not efficiently phosphorylated by P~His-HPr (see "Proteins Containing a Specific PTS-Recognized Phosphorylation Domain, the PRD," above), is also considered inducer exclusion (40). It is interesting to note that regarding GlpK regulation, two completely different inducer exclusion mechanisms evolved in firmicutes and *Enterobacteriaceae*, both of which nevertheless are mediated by PTS proteins: activation by P~His-HPr-mediated phosphorylation in firmicutes and inhibition by interaction with unphosphorylated EIIA^{Glc} in *Enterobacteriaceae*.

Other EIIA^{Glc} interaction partners. A regulatory role of EIIA^{Glc} completely different from that in inducer exclusion was reported for this PTS component in *Vibrio vulnificus*. EIIA^{Glc} as well as P~EIIA^{Glc} was found to interact with a bacterial 100-kDa protein which exhibits strong similarity to mammalian insulysins (101). Insulysins are peptidases that degrade insulin, and the *V. vulnificus* protein was therefore called vIDE for *Vibrio* insulin-degrading enzyme. Although both unphosphorylated and phosphorylated EIIA^{Glc} interact with vIDE, only the unphosphorylated PTS protein stimulated its insulin-degrading activity. Deletion of the vIDE-encoding *ideV* gene significantly lowered the survival and virulence of *V. vulnificus* in mice. It was therefore concluded that the vIDE/EIIA^{Glc} complex plays an important role in the survival of the bacterium in the host cell by sensing glucose (101).

In a more general study, it was attempted to detect interaction

partners of wild-type and unphosphorylatable (with replacement of the phosphorylatable His-90 with Ala) EIIA^{Glc} (11) of the pathogen *Vibrio cholerae* in planktonic and biofilm cells by carrying out a tandem affinity analysis. Several EIIA^{Glc} interaction partners were identified, some of which had already previously been shown to bind to the PTS protein in *Enterobacteriaceae*, such as glycerol kinase (see above) and adenylate cyclase (see "Interaction with phosphorylated EIIA components" below). However, several other proteins were also found to interact with EIIA^{Glc}. Interaction partners in planktonic cells include the glycolytic enzyme 6-phosphofructokinase, the purine repressor PurR, and a protein containing the GGDEF motif characteristic of diguanylate cyclases (VC0900) (102). In biofilm cells, wild-type and His-91-Ala mutant EIIA^{Glc} interacted with the gluconeogenic enzyme PEP carboxykinase. The two forms of EIIA^{Glc} also interacted with MshH, a homologue of the carbon storage regulator CsrD (103), a Mg^{2+} transporter, and a protein of unknown function (VC1291). Because for these three proteins the number of peptides found to interact with wild-type EIIA^{Glc} was significantly higher than the number of peptides binding to His-91-Ala mutant EIIA^{Glc}, it is likely that they interact primarily with P∼EIIA^{Glc}.

Among the proteins so far identified as EIIA^{Glc} interaction partners, the *E. coli* fermentation/respiration switch protein FrsA seems to have the highest affinity for unphosphorylated EIIA^{Glc}. A dissociation constant as low as 2×10^{-7} M was measured for the FrsA (previously called YafA) complex with EIIA^{Glc} (104). This protein is present in most facultative anaerobic gammaproteobacteria and is also found in a few alpha- and betaproteobacteria, such as *Thauera selenatis* and *Hirschia maritima*. Its deletion in *E. coli* leads to increased cellular respiration, whereas its overexpression causes elevated fermentation. FrsA was therefore proposed to regulate the partitioning of the flux through either the respiration or fermentation pathways in response to the phosphorylation state of EIIA^{Glc} (104).

The EIIA^{Glc} interface in protein-protein interactions. The surface of EIIA^{Glc} interacting with the various target proteins is almost always the same. For the complex formed between *E. coli* EIIA^{Glc} and GlpK, the crystal structure has been solved (85). The phosphorylatable His-90 of EIIA^{Glc} (11) is part of the interface and is exposed to a negatively charged surface of GlpK, including Glu-478. This explains why phosphorylation of EIIA^{Glc} strongly lowers its affinity for GlpK. In contrast, the affinity of EIIA^{Glc} for GlpK was strongly enhanced by the presence of a Zn^{2+} ion in the complex, which was found to be liganded by amino acid residues from both proteins: His-90 of EIIA^{Glc} and Glu-478 of GlpK (105). Recently, the structure of the maltose-specific ABC transporter with bound EIIA^{Glc} has also been determined (106). Each of the two EIIA^{Glc} molecules bound to the ABC transporter interacts with both MalK subunits. The surface of EIIA^{Glc} containing the phosphorylatable His-90 (11) interacts with the nucleotide binding domain of MalK, and a second region centered around Lys-130 interacts with the dimerization domain of the other subunit. Binding of EIIA^{Glc} prevents MalK closure by stabilizing a resting state of the ABC transporter. MalK closure is required for the interaction of the maltose-loaded periplasmic maltose binding protein with the transmembrane proteins MalF and MalG and for the binding of ATP to MalK (Fig. 5). These two events induce the outward-facing conformation of the membrane proteins, which is essential for transferring maltose from the maltose binding protein to the binding site in the transmembrane proteins (106). Interestingly, out of the 17 residues of EIIA^{Glc} making contacts with the nucleotide binding domain of MalK, 11 are also involved in the interaction with GlpK (85), HPr (107), and EIIB^{Glc} (108), thus confirming that EIIA^{Glc} uses nearly identical surfaces for the interaction with its partner proteins in order to exert its catalytic and regulatory functions.

PTS^{Ntr}. The other EIIA component that has been shown to interact with numerous non-PTS proteins in an unphosphorylated form is EIIA^{Ntr}, an EIIA of the fructose/mannitol PTS family, which exhibits neither sequence nor structure similarity to EIIA^{Glc} (109). In addition, the phosphorylation sites of the two proteins are quite different (11, 19, 110). In alpha-, beta-, and gammaproteobacteria, the *ptsN* gene, which encodes EIIA^{Ntr}, is frequently located downstream from *rpoN* (111). The RpoN protein, also known as σ^{54}, is required for the expression of several genes encoding enzymes for the utilization of nitrogen sources, but RpoN also regulates the expression of many other genes and operons. Inactivation of the *ptsN* gene from *Klebsiella pneumoniae* lowered the expression of several RpoN-controlled transcription units (112), suggesting that EIIA^{Ntr} might directly or indirectly affect RpoN activity. Also sometimes associated with the *rpoN* region is the *npr* gene (also called *ptsO*), which encodes NPr, a paralogue of HPr containing a phosphorylatable His-15 and, with the exception of *Enterobacteriaceae*, also a phosphorylatable Ser-46. In *Enterobacteriaceae*, a paralogue of EI is encoded by the *ptsP* gene (usually located distant from *rpoN*, *ptsO*, and *ptsN*). Together the three proteins EI^{Ntr} (PtsP), NPr (PtsO), and EIIA^{Ntr} (PtsN) form what is referred to as the nitrogen PTS (PTS^{Ntr}) (113). In *Enterobacteriaceae*, phosphorylation of NPr at His-15 is catalyzed mainly by EI^{Ntr}. Nevertheless, in bacteria also possessing EI, HPr, and sugar-specific EIIB and EIIC components, such as most *Enterobacteriaceae*, a cross talk between the two types of PTS exists (114, 115). The activity of EI^{Ntr} was recently shown to be antagonistically regulated by the metabolic intermediates situated at the intersection between carbon and nitrogen metabolism, namely, α-ketoglutarate and glutamine (116). Components of the PTS^{Ntr} are found not only in *Enterobacteriaceae* but also in many proteobacteria that are devoid of any known EIIB and EIIC components. In these organisms, the proteins of the PTS^{Ntr} are sometimes called EI, HPr, and EIIA^{Fru}. An HPr kinase able to phosphorylate NPr at Ser-46 and an EIIA of the mannose class PTS are usually also found in organisms containing the incomplete PTS^{Ntr} (111), except in *Enterobacteriaceae*, which are devoid of HprK/P. The genes encoding these five proteins are usually organized in two operons, as was shown for *Brucella melitensis* (19) and *Ralstonia eutropha* (110). In most alphaproteobacteria, such as *B. melitensis* and *Agrobacterium tumefaciens*, the *hprK* and *ptsO* genes are located downstream from the genes encoding a two-component system called *chvI*/*chvG* in *A. tumefaciens* (for chromosomal virulence) (111). In most betaproteobacteria, such as *R. eutropha* and *Neisseria meningitidis*, the EIIA^{Man}-encoding gene as well as *ptsO* and *ptsP* seem to form an operon, whereas *ptsN* and *hprK* are located in a second operon with *hprK* being followed by the *rapZ* (former *yhbJ*) gene (110). In *E. coli*, RapZ acts as adaptor protein allowing RNase E to degrade the small RNA GlmZ, which is required for the translation of the glucosamine-6-P synthase-encoding RNA (117).

Surprisingly, only EIIA^{Ntr} and not EIIA^{Man} of *R. eutropha* could be phosphorylated with PEP and purified EI^{Ntr} and NPr (110), although the phosphorylatable His residue is conserved in

$EIIA^{Man}$. Owing to the absence of an entire PTS able to transport carbohydrates, it is not clear what exactly controls the phosphorylation states of the usually four soluble PTS components in these organisms. It is likely that the PEP/pyruvate ratio (28) and also the α-ketoglutarate/glutamine ratio (116) affect the phosphorylation states of these PTS proteins. In *E. coli*, the two metabolites α-ketoglutarate and glutamine bind to EI^{Ntr} and exert antagonistic effects on its activity. In contrast, in *Sinorhizobium meliloti* only glutamine was found to bind to the N-terminal GAF domain of EI^{Ntr} and to stimulate its autophosphorylation activity (118). In addition, it seems that the ATP-dependent phosphorylation of NPr affects the PTS phosphorylation cascade, because deletion of the *hprK* gene in *B. melitensis* led to significantly increased phosphorylation of $EIIA^{Ntr}$ compared to that in the wild-type strain (19). In *R. eutropha*, an *hprK* mutant could be isolated only in an *npr* (*ptsO*) background, suggesting that elevated phosphorylation of HPr at His-15 owing to the absence of phosphorylation at Ser-46 negatively affects growth of this organism (110). Indeed, the *npr* mutant could easily be complemented with wild-type *npr* and the *npr*(His15Ala) allele, whereas no transformants could be obtained with the *npr*(Ser46Ala) allele.

Regulation of K^+ transport. The components of the PTS^{Ntr} are involved not only in the regulation of nitrogen-related genes and proteins (113). $EIIA^{Ntr}$ also seems to play an important role in the control of low- and high-affinity potassium (K^+) transport systems. Indeed, the PTS component was shown to interact with TrkA (119), a soluble protein required for K^+ uptake via the transporter TrkH (120). TrkA forms a tetrameric ring which needs to bind ATP in order to exert its positive effect on the single-channel activity of TrkH (121). It is therefore tempting to assume that the interaction of $EIIA^{Ntr}$ with TrkA prevents its stimulating effect on TrkH and therefore on the uptake of K^+. Indeed, a *ptsN* mutant accumulates high concentrations of K^+ inside the cells. K^+ differentially affects the binding of σ^{70} and σ^S to apo RNA polymerase and thus influences σ factor selectivity (122). In contrast to $EIIA^{Ntr}$, phosphorylated $EIIA^{Ntr}$ does not interact with TrkA.

In addition to the low-affinity Trk system, *E. coli* also possesses a high-affinity transporter for K^+ composed of the KdpFABC components, which form a P-type ATPase (123). The expression of the genes encoding these proteins is regulated by the two-component system KdpD/KdpE, with KdpD being a kinase sensing extracellular K^+ probably via aspartyl residues located in its periplasmic loops (124) and KdpE being the response regulator. The two regulator genes *kdpD* and *kdpE* are located downstream from *kdpFABC*. When the K^+ concentration in the medium is low, KdpD autophosphorylates and subsequently passes the phosphoryl group on to KdpE. The phosphorylated response regulator promotes transcription initiation of the *kdpFABCDE* genes (125). Unphosphorylated $EIIA^{Ntr}$ was shown to bind to KdpD and to stimulate its kinase activity, thereby increasing the amount of phosphorylated KdpE and hence the expression of the *kdpFABCDE* genes (126). In agreement with this result, inactivation of the *ptsN* gene caused strongly reduced expression of the *kdpFABCDE* genes. As observed for other $EIIA^{Ntr}$ interaction partners, phosphorylation of the PTS component prevents its interaction with KdpD. Owing to the already-mentioned cross talk in *E. coli* between the components of the regular PTS and the PTS^{Ntr}, growth on glucose also leads to dephosphorylation of $EIIA^{Ntr}$ and consequently to elevated expression of the *kdpFABCDE* genes (126). The opposing regulation of the two K^+ transport systems by $EIIA^{Ntr}$ allows *E. coli* to shut down the low-affinity transporter TrkA when the bacterium is exposed to low K^+ concentrations and to shut down KdpFABC when facing high K^+ concentrations.

Interestingly, mutants of *Rhizobium leguminosarum* lacking both $EIIA^{Ntr}$ proteins present in this organism were unable to grow at low K^+ concentrations (127). Unphosphorylated $EIIA^{Ntr}$ was assumed to interact with KdpD/E and to stimulate the expression of the genes encoding the high-affinity K^+ transporter. Bacterial two-hybrid experiments indeed revealed an interaction of $EIIA^{Ntr}$ with the transcription regulator proteins KdpD/E. In agreement with this concept, a *ptsP* mutant containing only unphosphorylated $EIIA^{Ntr}$ due to the absence of EI^{Ntr} grew normally at low K^+ concentrations. Interestingly, $EIIA^{Ntr}$ was found to regulate several other ABC transporters in *R. leguminosarum* (127).

Other $EIIA^{Ntr}$ interaction partners. Another *E. coli* sensor kinase that was reported to be regulated by $EIIA^{Ntr}$ is PhoR (128). Together with the response regulator PhoB, PhoR controls the expression of the *pho* regulon, which includes more than 30 genes organized in nine transcription units. When *E. coli* cells are exposed to low phosphate concentrations, the kinase activity of PhoR increases, which results in an elevated amount of P∼PhoB. The phosphorylated response regulator binds to specific sites on the DNA, the so-called Pho boxes, and stimulates the expression of the genes of the *pho* regulon. Mutants devoid of $EIIA^{Ntr}$ exhibit reduced expression of the *pho* regulon. Deletion of *ptsN* lowers the expression of the *pho* regulon because in order to be fully active, the sensor kinase PhoR needs to interact with $EIIA^{Ntr}$. This activation seems to occur with unphosphorylated $EIIA^{Ntr}$, because mutants unable to phosphorylate the EIIA component also exhibited increased expression of the *pho* regulon (128).

Unphosphorylated $EIIA^{Ntr}$ was also found to interact with the E1 subunit of *Pseudomonas putida* pyruvate dehydrogenase and to inhibit its activity (129). This enzyme produces acetyl coenzyme A (acetyl-CoA) from pyruvate, and the presence of $EIIA^{Ntr}$ therefore inhibits the metabolism through the tricarboxylic acid cycle. Interestingly, through yeast two-hybrid experiments the $EIIA^{Man}$ component of the incomplete *B. melitensis* PTS was demonstrated to interact with the E1 subunit (SucA) of 2-oxoglutarate dehydrogenase, which has a subunit composition similar to that of pyruvate dehydrogenase (19). In addition, a fusion protein composed of SucA and DivIVA sequestered $EIIA^{Man}$ to the two cell poles, thus confirming the interaction of $EIIA^{Man}$ with SucA. 2-Oxoglutarate dehydrogenase is part of the tricarboxylic acid cycle and catalyzes the transformation of 2-oxoglutarate into succinyl-CoA. The effect of phosphorylation of $EIIA^{Man}$ on this protein-protein interaction and its physiological role have not been studied.

The virulence of the pathogen *S. enterica* serovar Typhimurium is also controlled by PTS proteins. First, it has been reported that inactivation of the general PTS components EI and HPr prevents the replication of this bacterium in macrophages (130). Second, deletion of $EIIA^{Ntr}$ was found to indirectly affect the virulence of this pathogen. $EIIA^{Ntr}$ was reported to interact with the response regulator SsrB, which is required for the expression of numerous genes located within pathogenicity island 2 of this bacterium (131). The interaction with $EIIA^{Ntr}$ prevents SsrB from stimulating the expression of the genes in pathogenicity island 2.

Unphosphorylated $EIIA^{Ntr}$ was also reported to be necessary for the derepression of the *E. coli ilvBN* genes, which encode acetohydroxy acid synthase I, the first common enzyme for the synthesis of branched-chain amino acids (132). Mutants with *ptsN*

deletions were extremely sensitive to leucine-containing peptides, whereas *ptsP* and *npr* (*ptsO*) mutants were more resistant than the wild-type strain. However, the interaction partner of $EIIA^{Ntr}$ for the regulation of the *ilvBN* genes has not yet been identified.

In the betaproteobacterium *R. eutropha*, $EIIA^{Ntr}$ was found to interact with the bifunctional ppGpp synthase/hydrolase (SpoT1), an enzyme involved in the stringent response. Bacterial two-hybrid assays indicated that this interaction occurs only with the unphosphorylated form of $EIIA^{Ntr}$ (133).

Interaction with EIIB components. There are also several examples where EIIB components (as distinct proteins or as domains) interact with transcription regulators. When the EIIB component is fused to the cognate transmembrane permease, the target proteins are sequestered to the membrane. The first such example was the *E. coli* repressor Mlc, which was reported at nearly the same time by three different groups (134–136) to interact with the $EIIB^{Glc}$ domain of PtsG, the glucose-specific PTS permease. Mlc is a repressor of the ROK (**r**epressors, **o**pen reading frames, and **k**inases) family, and it was discovered because transformation of an *E. coli* wild-type strain with a plasmid into which the *mlc* gene had been inserted led to the formation of colonies with increased size when the transformants were grown on glucose-containing solid medium (137). The name *mlc* (**m**aking **l**arge **c**olonies) was deduced from this phenotype. The *mlc* gene turned out to be allelic with the previously described *dgsA* (2-**d**eoxy-D-**g**lucose **s**ensitive) locus (138).

E. coli Mlc is a regulator which represses the expression of several genes, including *mlc*, *ptsG* (*ptsG* encodes the $EIIB^{Glc}$ and $EIIC^{Glc}$ domains of the glucose-specific PTS permease), *ptsHI-crr*, *manXY*, and *malT* (1, 2, 4). The interaction of Mlc with the unphosphorylated $EIIB^{Glc}$ domain inhibits its repressor function (for reviews, see references 1, 2, and 4). Deletion of the *ptsG* gene therefore prevented the expression of all Mlc-controlled genes (139, 140).

An interesting aspect of this regulation mechanism is that Mlc needs to be sequestered to the membrane by the $EIIB^{Glc}$ domain in order to become inactive. When $EIIB^{Glc}$ was produced as a distinct cytoplasmic protein instead of being fused to the membrane-integral $EIIC^{Glc}$, it was still able to bind Mlc but no longer inhibited its activity (141). Possibly, cytoplasmic $EIIB^{Glc}$ has a lower affinity for Mlc and might therefore have lost its inhibiting effect. However, fusing the $EIIB^{Glc}$ domain to another membrane protein prevented Mlc activity, suggesting that the interaction with the membrane environment is important for Mlc regulation (141, 142). The crystal structure of the complex formed by the $EIIB^{Glc}$ domain and Mlc revealed that Mlc forms tetramers with each subunit binding an $EIIB^{Glc}$ domain. In fact, $EIIB^{Glc}$ binds to Mlc in the central part of the protein between the N-terminal DNA binding motif and the C-terminal dimerization domain. This interaction lowers the affinity of the repressor for its target sites (142). However, interaction with the $EIIB^{Glc}$ domain alone does not seem to be sufficient for Mlc inactivation. Interaction with the hydrophobic membrane environment also seems to be required (143). $EIIB^{Glc}$-mediated membrane sequestration was proposed to cause structural restrictions leading to reduced flexibility and therefore to lower affinity for its DNA targets (142). Arg-424 located close to the phosphorylatable Cys-421 in PtsG makes polar contacts with the carboxylate of the C-terminal glycine of Mlc (136, 144). Unphosphorylated $EIIB^{Glc}$ prevails when the cells take up glucose. As a consequence, the uptake of glucose leads to $EIIB^{Glc}$-mediated sequestration of Mlc to the membrane and therefore to increased expression of Mlc-repressed genes (140). It should be noted that Mlc activity is also inactivated by the interaction with the cytoplasmic protein MtfA (Mlc titration factor A) when this protein is overproduced (145).

Other proteins regulated by interaction with unphosphorylated EIIB domains or distinct EIIB proteins include PRD-containing transcription activators. The first example was MtlR from *B. subtilis*, the DeoR-type transcription activator of the mannitol operon, which is also regulated via phosphorylation by P∼His-HPr and $P{\sim}EIIA^{Mtl}$ (see "Proteins Containing a PTS Component Fused to the N or C Terminus" and "Proteins Containing a Specific PTS-Recognized Phosphorylation Domain, the PRD" above). In addition, the $EIIB^{Mtl}$ domain of the mannitol-specific PTS permease interacts with the two C-terminal regulatory domains of MtlR and thereby stimulates its transcription activation function (146). $EIIB^{Mtl}$ fused to the tyrosine kinase modulator YwqC (147), a transmembrane protein not related to mannitol transport, also interacted with MtlR and stimulated its activity. However, similar to what was reported for Mlc, interaction with the $EIIB^{Mtl}$ domain is not sufficient for MtlR activation, because overproduction of $EIIB^{Mtl}$ as a distinct cytoplasmic protein in the *B. subtilis* wild-type strain 168 prevented induction of the *mtl* operon. In this strain, soluble and membrane-associated $EIIB^{Mtl}$ probably compete for binding MtlR, and owing to the excess of soluble $EIIB^{Mtl}$, little MtlR is sequestered to the membrane and activated. The interaction with the membrane environment is therefore also required for MtlR stimulation. This interaction seems to occur only with unphosphorylated $EIIB^{Mtl}$, because first, the Cys-Asp replacement in $EIIB^{Mtl}$ prevented the interaction with MtlR in yeast-two hybrid experiments (146) and second, replacement of the phosphorylatable Cys with a Ser in soluble $EIIB^{Mtl}$ prevented the inhibitory effect on P*mtlA* expression observed with wild-type $EIIB^{Mtl}$ (71). In contrast to cysteyl-phosphorylated $EIIB^{Mtl}$, seryl-phosphorylated $EIIB^{Mtl}$ cannot transfer its phosphoryl group to mannitol (148) and, owing to the low-energy phosphate bond, probably cannot pass it back to $EIIB^{Mtl}$. As a consequence, P-Ser-$EIIB^{Mtl}$ accumulates in the cytoplasm, and the phosphorylated mutant $EIIB^{Mtl}$ apparently no longer competes with the $EIIB^{Mtl}$ domain of MtlA for binding MtlR and rendering it inactive (71).

Several results contradictory to the above model have been published by the group of Altenbuchner. For example, it was reported that the simultaneous deletion of *mtlA* and *mtlF* causes strong constitutive expression from the P*mtlA* promoter (149), whereas according to the results reported in reference 58, deletion of both *mtlA* and *mtlF* led to MtlR inactivation and only the single deletion of *mtlF* caused constitutive MtlR activity. In a more recent study from the Altenbuchner laboratory, deletion of the entire *mtl* operon, including *mtlD*, was found to inhibit expression from P*mtlA* (76). Although MtlR regulation in other bacterial species, such as *Geobacillus stearothermophilus* and *L. casei* (P. Joyet and J. Deutscher, unpublished results), significantly differs from that observed in *B. subtilis*, it is unlikely that the use of different *B. subtilis* strains (3NA and 168) might account for the observed differences in MtlR regulation. A more likely explanation might be the use of a plasmid-borne reporter gene fusion with an optimized P*mtlA* promoter in one study and a chromosome-integrated reporter gene fusion with wild-type P*mtlA* in the other. Heravi and Altenbuchner also recently reported that a *B. subtilis*

mutant MtlR in which His-342 was replaced with an Asp and Cys-419 with an Ala was active even when produced in a mutant lacking MtlA (EIICB^{Mtl}) (76). These results were also thought to be contradictory to the above model of EIIB^{Mtl}-mediated MtlR activation. A possible explanation for this EIIB^{Mtl}-independent MtlR activity could be that the *mtlR*(His-342-Ala,Cys-419-Ala) double mutation not only prevents the inactivating phosphorylation at Cys-419 but also induces structural changes in the transcription activator resembling those caused by EIIB^{Mtl}-mediated membrane sequestration.

The second PRD-containing transcription activator regulated by the interaction with an EIIB domain is the LevR-like ManR from *L. monocytogenes*. This protein controls the expression of the *manLMNO* operon, which encodes the major glucose transporter of this human pathogen (78, 150). However, ManR activity is regulated not by the components of the mannose-type PTS ManLMN but by the proteins of another mannose-type PTS, MpoABCD, a low-affinity glucose/mannose-specific PTS that functions mainly as glucose sensor (78). An interaction between EIIB^{Mpo} (MpoB) and ManR was suggested by results from genetic experiments. While deletion of EIIA^{Mpo} led to constitutive expression of the *man* operon, deletion of EIIB^{Mpo} or of both EIIA^{Mpo} and EIIB^{Mpo} completely inhibited it, suggesting that EIIB^{Mpo} is required for ManR activity (78). An interaction of EIIB^{Mpo} with ManR could indeed be established by carrying out yeast two-hybrid experiments. Similar to the interaction of EIIB^{Mtl} with MtlR, EIIB^{Mpo} also interacts with two of the four regulatory domains of ManR (A. C. Zébré, M. Ventroux, M.-F. Noirot-Gros, J. Deutscher and E. Milohanic, unpublished results). However, in contrast to EIIB^{Mtl}, EIIB^{Mpo} is not fused to one of the two membrane components MpoC or MpoD but is a distinct cytoplasmic protein. As a consequence, it is unlikely that membrane sequestration plays a role in listerial ManR regulation.

PTS components also play a role in biofilm formation, as has been shown for EI and HPr of *V. cholerae* (151). In a recent study, the utilization of mannitol by this organism was reported to activate biofilm formation and the expression of the *vps* genes, which encode the proteins required for the synthesis of the exopolysaccharides forming the biofilm matrix (152). Even when grown on other carbon sources, the mannitol-specific PTS permease MtlA, an EIICBA protein, was found to be necessary for biofilm formation. In fact, it turned out that the EIIB^{Mtl} domain was sufficient for the stimulating effect. Synthesis of this domain from an IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible allele in cells growing in the absence of mannitol also caused elevated biofilm formation. Phosphorylation of the EIIB^{Mtl} domain is not required for this effect, because a mutant overproducing EIIB^{Mtl}, in which the phosphorylatable His of the EIIA domain was replaced with an alanine, also exhibited elevated biofilm formation (152).

Finally, an interesting presumably phosphorylation-independent interaction occurs between the peptide SgrT and the EIIC-EIIB domains of the glucose/*N*-acetylglucosamine family of PTS proteins. SgrT is encoded by the 5′ end of the small RNA SgrS, whereas its 3′ part is complementary to the 5′ end of the *ptsG* mRNA and thus allows the formation of an RNA-RNA hybrid, which inhibits translation of *ptsG* and is a target for RNase E (153). The small peptide SgrT inhibits the transport function of the PTS transporter probably by binding to the linker region connecting the EIIC^{Glc} and EIIB^{Glc} domains. Indeed, replacement of a proline (Pro-384) present in a conserved region (-L-K-T-**P**-G-R-E-D-) of the linker in *E. coli* PtsG with an arginine prevented the repressive effect of SgrT on glucose utilization (154). Surprisingly, the conserved linker motif is also found in the PTS permeases PtsG, GamP, and NagP of *B. subtilis*, although there is no evidence for the presence of an SgrT homologue in firmicutes. The conserved linker motif might therefore carry out additional functions.

Interaction with HPr and its paralogues Crh and NPr. A highly interesting interaction of HPr from enterococci with the response regulator of the CroRS two-component system was recently detected by carrying out a protein fragment complementation assay (155). CroR is required for intrinsic cephalosporin resistance in *E. faecalis*. It was therefore not surprising that deletion of HPr causes a cephalosporin hyperresistance phenotype, confirming that the interaction of HPr with CroR diminishes the intrinsic cephalosporin resistance of *E. faecalis*. Hyperresistance toward cephalosporins was also observed when either one of the two phosphorylatable amino acids of HPr, His-15 or Ser-46, was replaced with alanine. This result suggests that phosphorylation of HPr by either PEP and EI or ATP and HprK/P prevents the interaction with CroR. In agreement with these results, cephalosporin resistance of *E. faecalis* was also influenced by the utilization of carbon sources (155). While growth on the non-PTS carbon sources pyruvate and citrate, which leads to high levels of P∼His-HPr, caused enhanced cephalosporin resistance, growth on the PTS substrates glucose and *N*-acetylglucosamine, which leads to low levels of P∼His-HPr, had no such effect. The nutrient effect disappeared in a *croR* mutant, suggesting that it depends on the interaction between HPr and CroR.

Unphosphorylated HPr from *E. coli* and the two unphosphorylated HPr paralogues Crh from *B. subtilis* and NPr from *E. coli* were also found to interact with non-PTS target proteins. HPr from *E. coli* interacts with a regulatory protein and an enzyme involved in carbon storage. In the latter case, unphosphorylated as well as phosphorylated *E. coli* HPr was reported to bind with high affinity to glycogen phosphorylase (156). However, only the unphosphorylated PTS protein has a significant stimulating effect on glycogen phosphorylase activity (157). Neither NPr nor FPr (an *E. coli* HPr homologue specific for the transport of fructose) binds to glycogen phosphorylase. Interaction with HPr increases the V_{max} and lowers the K_m of glycogen phosphorylase for its substrate. It was proposed that under conditions where the PEP level drops and the PTS proteins are barely phosphorylated, the interaction with HPr will favor the breakdown of glycogen. Recently, HPr from *E. coli* was also reported to interact with the anti-σ^{70} factor Rsd (31). Only unphosphorylated HPr formed a tight complex with Rsd and thus prevented the inhibitory effect of Rsd on σ^{70}-dependent transcription, as was shown in *in vivo* and *in vitro* experiments.

B. subtilis Crh contains a well-conserved Ser-46 phosphorylation site, but lacks His-15, which is replaced with a glutamine. Crh is therefore not phosphorylated by PEP and EI. However, similar to the case for *B. subtilis* HPr, this protein is phosphorylated by ATP and HprK/P. Unphosphorylated Crh was reported to interact with the methylglyoxal synthase MgsA, an enzyme of the methylglyoxal bypass of glycolysis, and to inhibit its activity (158). Phosphorylation of Crh at Ser-46 by ATP and HprK/P prevents this interaction. As a consequence, flux through the methylglyoxal bypass is probably low when no efficiently metabolized carbohydrate is taken up via the PTS. Under these conditions, *B. subtilis* cells contain high levels of unphosphorylated Crh (35), which inhibits

MgsA. Interestingly, regulation of MgsA is a Crh-specific phenomenon because no interaction occurs between MgsA and HPr (158). It is therefore likely that Gln-15 of Crh, which distinguishes it from the His-15-containing HPr, plays an important role in this interaction.

Unphosphorylated NPr from *E. coli* was reported to regulate the production of lipid A, a component of the lipopolysaccharide layer. For that purpose, NPr interacts with the enzyme LpxD (159), an acetyltransferase essential for the synthesis of lipid A (160). This interaction seems to inhibit the activity of LpxD, because an *npr* mutant showed increased lipopolysaccharide synthesis. Phosphorylated NPr as well as unphosphorylated HPr did not interact with the acetyltransferase. The physiological role of PTS-mediated regulation of the production of the bacterial endotoxin lipid A is not yet understood. By carrying out yeast two-hybrid experiments, it was found that NPr from *B. melitensis* interacts with a pyrophosphate phosphatase (Ppa) (19). No further studies were carried out to demonstrate the potential physiological relevance of this protein-protein interaction.

Interaction with EI and EI^{Ntr}. The PTS is also involved in carbohydrate-mediated chemotaxis, a regulatory phenomenon in which the frequency of the change of the direction of flagellar rotation (change between tumbling and swimming) determines the speed at which a bacterium approaches an attracting carbon source or moves away from a repellent (161). A central protein in the chemotactic signaling pathway is the autophosphorylating sensor kinase CheA, which passes its phosphoryl group on to the CheY protein. P∼CheY interacts with the lower part of the switch complex, which is composed of the FliG, FliM, and FliN proteins and associated with the flagella rotor, thus inducing clockwise rotation and therefore tumbling (162). *In vitro* studies with purified enzymes showed that *E. coli* EI, but not P∼EI, inhibits the autophosphorylation activity of CheA (163). Similar experiments with the *B. subtilis* proteins revealed an opposite mechanism, i.e., inhibition of CheA activity by P∼EI and not EI (164). These contradictory results are not surprising since in *B. subtilis* CheY exerts an effect on flagellar rotation antagonistic to that in *E. coli*. However, for neither the *E. coli* nor the *B. subtilis* system has a direct interaction between CheA and EI or P∼EI, respectively, been established by using biochemical or immunological methods, although the inhibiting effect of EI on CheA activity in *in vitro* experiments suggests a direct interaction of the two proteins (163).

EI^{Ntr} and aspartokinase from *Bradyrhizobium japonicum* were also reported to interact with each other (165). This interaction, which was confirmed by *in vitro* pulldown experiments, inhibits the autophosphorylation activity of EI^{Ntr}. Aspartokinase is encoded by the *lysC* gene, and its deletion prevented the uptake of δ-aminolevulinic acid and the utilization of prolyl-glycyl-glycine as a proline source. This phenotype could be compensated for by expressing *ptsP* in *trans* from a multicopy plasmid. The result suggests that aspartokinase exerts an effect on EI^{Ntr} that can be mimicked by the overproduction of the PTS protein.

Interaction with Phosphorylated PTS Proteins

Interactions with phosphorylated PTS components seem to be less frequent than interactions with the unphosphorylated proteins. One reason might be that they are more difficult to detect. Nevertheless, several interactions with phosphorylated HPr and HPr paralogues as well as phosphorylated EIIA and EIIB components have been reported. HPr of firmicutes as well as its paralogue Crh (catabolite repression HPr) present in bacilli is also phosphorylated at Ser-46 by ATP and HprK/P. This modification also occurs with the HPr paralogue NPr of HprK/P-containing proteobacteria (19, 110). The seryl-phosphorylated PTS components also carry out important regulatory functions by interacting with several specific target proteins. The phosphorylation sites of the PTS components are usually part of the interface, and the phosphoryl group faces positively charged amino acids in the target proteins, mostly arginine, which increases the stability of the protein-protein complex (166).

Interaction with phosphorylated EIIA components. Genetic data had suggested that in *Enterobacteriaceae* phosphorylated EIIA^{Glc} stimulates the activity of adenylate cyclase (167). This enzyme converts ATP into cyclic AMP (cAMP), which is an important second messenger in prokaryotes and eukaryotes. Low intracellular levels of cAMP are observed in *E. coli* and *S. enterica* serovar Typhimurium cells grown on an efficiently metabolized carbon source or in mutants preventing the formation of P∼EIIA^{Glc}. In *Enterobacteriaceae*, cAMP binds to the cAMP receptor protein (Crp), and the resulting complex functions as transcription activator for numerous catabolic genes and operons (for a review, see reference 1). These results therefore strongly suggested that P∼EIIA^{Glc} interacts with adenylate cyclase and stimulates its catalytic activity. More specifically, cAMP formation seems to affect the lag phase characteristic for diauxie, which is observed when cells grow on a mixture of a preferred and a less favorable carbon source. Under these conditions, bacteria utilize the less favorable carbon source only when the preferred carbon source is exhausted (168), usually leading to two growth phases (hence the name diauxie) separated by a lag phase, during which the enzymes required for the transport and metabolism of the less-favorable carbon source are synthesized. Addition of extracellular cAMP either shortens or entirely eliminates the lag phase (169). However, addition of extracellular cAMP cannot prevent CCR, i.e., the preferred use of, for example, glucose over a less favorable carbon source, such as lactose (170). The interaction of EIIA^{Glc} with adenylate cyclase could indeed be confirmed by fusing adenylate cyclase to the *E. coli* integral membrane protein Tsr. Unphosphorylated EIIA^{Glc} as well as P∼EIIA^{Glc} was found to bind to the C-terminal regulatory domain of membrane-anchored adenylate cyclase. However, when adenylate cyclase activity assays were carried out in the presence of *E. coli* crude extracts, only P∼EIIA^{Glc} was able to stimulate the enzyme activity (171). An interaction of unphosphorylated EIIA^{Glc} with adenylate cyclase could also be demonstrated by *in vivo* experiments with the His-91-Ala EIIA^{Glc} mutant from *V. cholerae* by using the tandem affinity purification method (102).

Interaction with phosphorylated EIIB components. While some PRD-containing transcription activators are regulated via interaction with unphosphorylated EIIB domains or proteins, antiterminators seem to be regulated rather by the interaction with phosphorylated EIIB. A well-established example of the interaction of an antiterminator with a phosphorylated EIIB component is the *E. coli* antiterminator BglG. Similar to the case for other PRD-containing antiterminators, BglG activity is inhibited by phosphorylation catalyzed by the P∼EIIB^{Bgl} domain of BglF (see "Proteins Containing a Specific PTS-Recognized Phosphorylation Domain, the PRD" above). In addition, BglG binds to the P∼EIIB^{Bgl} domain of the β-glucoside-specific PTS permease BglF

(172). In the absence of a BglF-recognized substrate (salicin or arbutin), the $EIIB^{Bgl}$ domain of BglF is mainly phosphorylated and interacts with the antiterminator BglG. While $EIIB^{Mtl}$-mediated membrane sequestration leads to activation of *B. subtilis* MtlR, $EIIB^{Bgl}$-mediated membrane sequestration inhibits the antiterminator activity of BglG. As soon as a substrate for the PTS^{Bgl} (salicin or arbutin) becomes available, the $EIIB^{Bgl}$ domain of BglF will be dephosphorylated, which weakens its affinity for BglG. The antiterminator is therefore released into the cytoplasm, thereby regaining its activity and allowing efficient expression of the *bgl* operon.

In a recent study, the *B. subtilis* antiterminator LicT was also found to change its cellular localization in response to substrate availability. In the absence of a substrate, LicT is phosphorylated in its PRD1 by the $P\sim EIIB^{Bgl}$ domain, and the antiterminator is equally distributed in the cell. In the presence of a substrate, LicT is no longer phosphorylated by the $P\sim EIIB^{Bgl}$ domain and is located in subpolar regions (173). Although there is no evidence that the presence or absence of $EIIB^{Bgl}$ or $P\sim EIIB^{Bgl}$ affects the activity of LicT by direct interaction, phosphorylation of the PRD1 of LicT by $P\sim EIIB^{Bgl}$ seems to play an important role in the cellular localization of the antiterminator.

Interaction with histidyl-phosphorylated HPr. Presently, there is only one reported example where P∼His-HPr interacts with and controls a target protein. This is the *B. subtilis* transcription activator YesS, which belongs to the AraC/XylS family of regulators (174). YesS contains a C-terminal DNA binding domain, and it controls the expression of the pectin/rhamnogalacturonan utilization genes of *B. subtilis* (175). A *ptsH* deletion mutant exhibited significantly reduced YesS activity. Yeast two-hybrid and "three-hybrid" experiments revealed that YesS interacts with HPr and P∼His-HPr but not with P-Ser-HPr. In three-hybrid experiments, the additional synthesis of HprK/P prevented the interaction of HPr with YesS, whereas the synthesis of EI had no effect (175). The interaction with HPr and P∼His-HPr occurs within a central regulatory domain of YesS (amino acids 406 to 510), whose sequence seems to be unique to YesS. Although HPr and P∼His-HPr bind to YesS, only phosphorylated HPr stimulates its activity (Fig. 3). This was inferred from experiments with a *ptsI* mutant and a *ptsH*(H15A) mutant, neither of which allowed formation of P∼His-HPr and which exhibited significantly reduced YesS activity compared to that of the wild-type strain. In contrast, inactivation of the HprK/P-encoding *hprK* gene or of *crh*, which encodes the HPr paralogue Crh, did not lower YesS activity (175).

Interactions with P-Ser-HPr and P-Ser-Crh. As mentioned above, HPr of firmicutes becomes phosphorylated not only at His-15 by PEP and EI but also at Ser-46 by ATP and HprK/P (21). This is also true for the HPr paralogue Crh present in bacilli, in which His-15 is replaced with a Gln, whereas Ser-46 and its surrounding region are well conserved compared to those in HPr of firmicutes (41). Homologues of Crh are found in bacilli, geobacilli, and oceanobacilli (1). HprK/P is a bifunctional enzyme also capable of dephosphorylating P-Ser-HPr and P-Ser-Crh by catalyzing a phosphorolysis reaction producing unphosphorylated protein and pyrophosphate (22). Numerous proteobacteria contain the HPr paralogue NPr (PtsO), in which Ser-46 and its surrounding region are well conserved. They also contain an HprK/P homologue, and *in vitro* phosphorylation of NPr has been established in the two alphaproteobacteria *B. melitensis* (19) and *Gluconobacter oxydans* (176) and the betaproteobacterium *R. eutropha* (110). A physiological function of P-Ser-NPr has so far not been established, but this posttranslational modification seems to be essential for growth of *R. eutropha* (110). In contrast, the role of P-Ser-HPr as a corepressor in CCR in firmicutes is well established and has been extensively reviewed (1, 177, 178). We therefore provide here only a brief description of this regulation mechanism.

The uptake of an efficiently metabolized carbon source by firmicutes leads to an increase of the FBP and a decrease of the phosphate concentration. These conditions favor the kinase activity of the bifunctional HprK/P (Fig. 1) and therefore cause an increase of the amount of P-Ser-HPr in the cells. P-Ser-HPr interacts with the catabolite control protein A (CcpA) (179) and allows this LacI-type repressor to bind to the catabolite response elements (*cre*), its target sites on the DNA (Fig. 3) (180). The *cre* site either can overlap the promoter region, in which case CcpA/P-Ser-HPr prevents binding of the RNA polymerase holoenzyme, or can be located downstream from the transcription initiation site with CcpA/P-Ser-HPr functioning as a roadblock. Mutants in which *ccpA* or *hprK* is deleted or Ser-46 of HPr is replaced with an alanine (181) are relieved from CcpA-mediated carbon catabolite repression. As already mentioned, carbon catabolite repression in firmicutes can be mediated by other mechanisms based on PRD-containing regulators, on inducer exclusion (1), or, in some bacteria, on glucose kinase (81). For certain catabolic genes or operons, the CcpA/P-Ser-HPr complex binds upstream from the promoter, which leads to catabolite activation instead of catabolite repression (182). In bacilli and a few other firmicutes, P-Ser-Crh can also bind to CcpA and function as a corepressor or coactivator for certain catabolic genes and operons during the utilization of an efficient carbon source (Fig. 3) (183, 184).

P-Ser-HPr of *B. subtilis* as well as its paralogue P-Ser-Crh has also been reported to interact with the glycolytic enzyme glyceraldehyde-3-P dehydrogenase (GapA) (185). In fact, both proteins interact with GapA in their unphosphorylated and seryl-phosphorylated forms, but only the seryl-phosphorylated forms of the two proteins were able to inhibit GapA (Fig. 3). P-Ser-HPr probably also plays an indirect role in *S. pyogenes* (84, 186) and *Bacillus anthracis* (187) virulence regulation, because in these pathogens CcpA controls the expression of several virulence genes, one being the streptolysin S-encoding gene of *S. pyogenes* (186).

There is strong evidence that in certain lactic acid bacteria P-Ser-HPr is also involved in inducer exclusion. Similar to what has been reported for *Enterobacteriaceae*, the uptake of the non-PTS sugar maltose by *L. casei* or *Lactococcus lactis* is immediately arrested when glucose or another efficiently metabolized carbon source is added (188–190). A similar observation was also made for the transport of ribose by *L. lactis* (190). These non-PTS sugars are taken up by ABC transport systems (191). Mutations in the *hprK* or *ptsH* gene preventing the formation of P-Ser-HPr also prevented the inhibitory effect of glucose on the uptake of the non-PTS sugars (188, 190). In contrast, mutations which caused an increase of the intracellular amount of P-Ser-HPr permanently inhibited the uptake of the non-PTS sugar (192). These results strongly suggest that similar to inducer exclusion in *Enterobacteriaceae*, where $EIIA^{Glc}$ interacts with several non-PTS permeases, inducer exclusion in firmicutes is mediated by the interaction of P-Ser-HPr with a component of the ABC transporters (Fig. 3); unfortunately, this protein has not yet been identified.

Phosphorylation of HPr was discovered in connection with another regulatory phenomenon called inducer expulsion, which occurs in several firmicutes, including streptococci, lactococci, and lactobacilli (21, 193). Most of these bacteria transport the nonmetabolizable carbohydrate derivatives 2-deoxy-D-glucose (2-DG) and thiomethyl-β-D-galactopyranoside (TMG) via a PTS and therefore accumulate them as P derivatives in the cell. Addition of glucose or another efficiently metabolizable carbon source to cells preloaded with P-2DG or P-TMG led to rapid expulsion of the nonmetabolizable carbon source (194, 195). In fact, inducer expulsion was found to be a two-step process (193). In the first step, accumulated P-2DG or P-TMG is dephosphorylated by a P-sugar phosphatase, and in the second step, 2-DG or TMG is expelled. The conditions triggering inducer expulsion (metabolism of an efficiently utilized carbon source) also lead to the formation of P-Ser-HPr, and it was therefore suspected that P-Ser-HPr might be involved in inducer expulsion. Indeed, several P-sugar phosphatases of streptococci, lactococci, and enterococci were reported to be regulated by P-Ser-HPr (196–198). However, *L. casei* and *L. lactis* mutants unable to form P-Ser-HPr (because of *ptsH1* mutation or *hprK* deletion) still exhibited inducer expulsion, establishing that P-Ser-HPr is not essential for this regulatory process. Interestingly, a second type of inducer expulsion was reported for some heterofermentative lactobacilli, such as *Lactobacillus brevis*. P-Ser-HPr was reported to be involved in this second type of inducer expulsion, which affects only non-PTS carbohydrates (199). The galactose/H^+ symporter of *L. brevis* catalyzes not only galactose transport but also the uptake of the nonmetabolizable TMG, which in this organism is accumulated in the unphosphorylated form. The presence of glucose prevents the accumulation of TMG and causes the efflux (expulsion) of already-accumulated thio-sugar. Binding of P-Ser-HPr to the non-PTS permease was assumed to transform the symporter into a uniporter (facilitator) by uncoupling sugar transport from proton symport, thereby allowing the efflux of accumulated substrates. This concept was supported by the reconstitution of the HPr regulatory system (HPr, EI, and HprK/P) of *L. brevis* in a *B. subtilis* mutant devoid of HprK/P but still containing HPr. The strain also produced the *L. brevis* galactose permease GalP (200). When this strain was transformed with a plasmid encoding wild-type *L. brevis* HPr, it was not able to accumulate TMG when glucose was present in the medium. In contrast, glucose could not prevent TMG accumulation by a strain producing Ser-46-Ala mutant HPr. A strain transformed with a plasmid encoding *L. brevis* Ser-46-Asp mutant HPr was not able to accumulate TMG even when glucose was absent.

CONCLUSIONS AND PERSPECTIVES

The PTS was discovered 50 years ago in the laboratory of Saul Roseman at the University of Michigan, Ann Arbor, with the first article describing a PTS component, the HPr from *E. coli*, and its role in hexose phosphorylation being published in 1964 (20). Three years later, evidence for the presence of a PTS in a firmicute was obtained in the laboratory of Melvine Laurance Morse at the University of Colorado, Denver. They identified a PTS catalyzing the transport and phosphorylation of lactose in *Staphylococcus aureus* (201). In the following years, the proteins forming the glucose- and mannose-specific PTSs in *E. coli* (202, 203) and the lactose-specific PTS in *S. aureus* (204) were identified, and their role in transport and phosphorylation of the two hexoses and the disaccharide was established. Since then, a huge number of PTSs transporting a large variety of substrates, including hexoses, 6-deoxy-hexoses (14), amino sugars, *N*-acetyl-amino sugars, gluconic acids (205), pentitols (206, 207), ascorbate (208), and disaccharides, have been identified. We recently obtained evidence that the *E. faecalis* maltose-specific $EIICBA^{Mal}$ (MalT) (15) also transports and phosphorylates the trisaccharide maltotriose and the tetrasaccharide maltotetraose (J. Deutscher, A. Hartke, J. Thompson, C. Magni, C. Henry, V. Blancato, G. Repizo, N. Sauvageot, A. Pikis, T. Kentache, and A. Mokhtari, unpublished results). A previous report had already suggested that the homologous maltose transporter MalT from *Streptococcus mutans* might take up maltotriose and maltotetraose (209).

PTS proteins are also involved in the phosphorylation of intracellular dihydroxyacetone (7), which enters the cells by facilitated diffusion or is formed from glycerol in a reaction catalyzed by glycerol dehydrogenase (64). Soon after its discovery, it was realized that the PTS not only transports and phosphorylates carbohydrates but also carries out regulatory functions related to carbon metabolism and sugar transport, such as catabolite repression and inducer exclusion. As described in the preceding sections, in the last 25 years PTS proteins were found also to regulate numerous cellular functions not or only indirectly related to carbon metabolism and transport, and this number increases steadily. PTS components control nitrogen and phosphate metabolism as well as potassium transport, antibiotic resistance, biofilm formation, and endotoxin production and also regulate the virulence of several pathogens. Given the high physiological impact of its regulatory functions, the PTS can no longer be considered merely a sugar transport and phosphorylation system that also plays some regulatory roles. The two functions rather seem to be of equal importance. In addition, in view of the tight connection between carbohydrate transport and metabolism and the regulatory functions of the PTS, it seems inappropriate to ask which of the two activities of the PTS is more important. Nevertheless, from an evolutionary point of view it would be interesting to understand how the multicomponent or multidomain PTS developed its different activities. Was the PTS originally a transport system that acquired regulatory functions, or was it an early regulatory system responding to environmental signals that later developed carbohydrate transport and phosphorylation activities, or, finally, did the transport and regulation systems evolve separately and became connected at a later stage in evolution in order to allow a correlation of transport and phosphorylation of carbohydrates with the different regulatory functions? The occurrence of an "incomplete PTS" composed of EI, HPr, and two EIIAs in numerous proteobacteria seems to favor the last hypothesis. In addition, many regulatory functions of the PTS seem to have developed late in evolution.

Concerning the PEP-requiring dihydroxyacetone phosphorylation system, we might be witnessing the creation of a novel PTS. At the present stage, transport and phosphorylation of dihydroxyacetone are two distinct steps, with the second being catalyzed by EI, HPr, EIIA, and DhaL and the first by facilitated diffusion. To make the system resemble a classical PTS and thus provide it with high transport efficiency, the dihydroxyacetone facilitator might integrate the dihydroxyacetone binding domain of DhaK. DhaL then needs to transform its ADP binding site into an EIIA-recognized Cys or His phosphorylation site in order to make it an EIIB-like protein. Given the slow pace of evolution, we will certainly not

witness whether one day indeed a classical dihydroxyacetone-specific PTS with the proposed characteristics will be formed.

Whatever the course of evolution of the PTS was, regulatory functions probably played an important role in early stages of the PTS development. The fact that all phosphoryl group transfer steps, with the exception of the last one, i.e., the phosphorylation of the carbohydrate substrate, are reversible makes the PTS an efficient sensor and a fast signal transduction system. In addition, the PTS responds to the most significant signal for bacterial cell growth and proliferation, i.e., the availability of carbon sources in the environment and their efficient metabolism. Together these characteristics explain why the PTS controls so many cellular functions and developed so many different regulatory mechanisms, each probably optimally adapted to the needs of different bacterial species. By developing an additional signal entry via the metabolite-controlled HprK/P in firmicutes and numerous proteobacteria, the complexity of PTS-mediated regulation grew even larger. HprK/P probably evolved from an ancestral kinase and retained the capacity to utilize pyrophosphate as a phosphoryl donor for its kinase activity. Oligophosphates were probably already abundant when life developed on our planet. By reversing the phosphorylation reaction, HprK/P uses P_i for the dephosphorylation of P-Ser-HPr, thereby producing pyrophosphate (22) (Fig. 1). HprK/P probably gained the ability also to utilize ATP when this nucleoside triphosphate became available.

Despite extensive efforts in numerous laboratories, we understand only a few PTS-mediated regulation systems. Little is known about the role of HprK/P in proteobacteria, and the different functions of the PTS^{Ntr} in *Enterobacteriaceae* are barely understood (113). In addition, what is the role of the N-terminal domain of HprK/P, which is absent in HprK/Ps of alphaproteobacteria and which can be deleted from HprK/Ps from firmicutes without affecting their known activities (210)? In the crystal structure, this domain binds P_i to conserved arginine residues, but P_i does not seem to be a physiological effector. PTS domains are found in numerous proteins, and the list of Table 1 is far from being complete; there are numerous other proteins containing a domain of a PTS protein or a small fragment of it composed of about 50 amino acids. Only in a few cases has the regulatory function of these PTS domains been determined.

Phosphoproteome analyses revealed that numerous PTS components become phosphorylated at Ser or Thr residues, including the general PTS proteins EI and HPr as well as many sugar-specific PTS components (211–217). In firmicutes, HPr was found to be phosphorylated not only at Ser-46 but also at position 12, which can be Ser or Thr (212, 213). Other HPr phosphorylation sites were also found (213). Other frequent targets of phosphorylation are the EII components of the mannose PTS (211, 213, 215–217). With the exception of P-Ser46-HPr and P-Ser46-Crh, nothing is known about the enzymes catalyzing these protein phosphorylations and the physiological role of Ser/Thr-phosphorylated PTS components.

Highly interesting is the role of the PTS in certain pathogens, such as *S. enterica* serovar Typhimurium, *V. cholerae*, *V. vulnificus*, *S. pyogenes*, or *L. monocytogenes*. In *L. monocytogenes*, the utilization of an efficient carbon source, such as cellobiose, glucose, fructose, mannose, etc., inhibits the expression of the virulence genes, which are controlled by PrfA, a Crp-like transcription activator (218, 219). The detailed mechanism is not yet understood, but it is likely that a PTS component is involved (220, 221). Many other PTS-related regulatory systems exist for which the mechanism and/or function are not understood, and new systems are constantly being discovered, clearly showing that 50 years after the discovery of the PTS, its regulatory functions are far from being fully understood.

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REFERENCES

1. **Deutscher J, Francke C, Postma PW.** 2006. How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. Microbiol. Mol. Biol. Rev. **70:**939–1031. http://dx.doi.org/10.1128/MMBR.00024-06.
2. **Erni B.** 2013. The bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS): an interface between energy and signal transduction. J. Iran. Chem. Soc. **10:**593–630. http://dx.doi.org/10.1007/s13738-012-0185-1.
3. **Pickl A, Johnsen U, Schönheit P.** 2012. Fructose degradation in the haloarchaeon *Haloferax volcanii* involves a bacterial type phosphoenolpyruvate-dependent phosphotransferase system, fructose-1-phosphate kinase, and class II fructose-1,6-bisphosphate aldolase. J. Bacteriol. **194:**3088–3097. http://dx.doi.org/10.1128/JB.00200-12.
4. **Plumbridge J.** 2002. Regulation of gene expression in the PTS in *Escherichia coli*: the role and interactions of Mlc. Curr. Opin. Microbiol. **5:**187–193. http://dx.doi.org/10.1016/S1369-5274(02)00296-5.
5. **Saier MH, Jr, Reizer J.** 1990. Domain shuffling during evolution of the proteins of the bacterial phosphotransferase system. Res. Microbiol. **141:**1033–1038. http://dx.doi.org/10.1016/0923-2508(90)90077-4.
6. **Barabote RD, Saier MH, Jr.** 2005. Comparative genomic analyses of the bacterial phosphotransferase system. Microbiol. Mol. Biol. Rev. **69:**608–634. http://dx.doi.org/10.1128/MMBR.69.4.608-634.2005.
7. **Gutknecht R, Beutler R, Garcia-Alles LF, Baumann U, Erni B.** 2001. The dihydroxyacetone kinase of *Escherichia coli* utilizes a phosphoprotein instead of ATP as phosphoryl donor. EMBO J. **20:**2480–2486. http://dx.doi.org/10.1093/emboj/20.10.2480.
8. **Alpert C-A, Frank R, Stüber K, Deutscher J, Hengstenberg W.** 1985. Phosphoenolpyruvate-dependent protein kinase enzyme I of *Streptococcus faecalis*. Purification and properties of the enzyme and characterization of its active center. Biochemistry **24:**959–964.
9. **Gassner M, Stehlik D, Schrecker O, Hengstenberg W, Maurer W, Rüterjans H.** 1977. The phosphoenolpyruvate-dependent phosphotransferase system of *Staphylococcus aureus*. 2. ^{1}H and ^{31}P nuclear-magnetic-resonance studies on the phosphocarrier protein HPr, phosphohistidines and phosphorylated HPr. Eur. J. Biochem. **75:**287–296. http://dx.doi.org/10.1111/j.1432-1033.1977.tb11528.x.
10. **Deutscher J, Beyreuther K, Sobek MH, Stüber K, Hengstenberg W.** 1982. Phosphoenolpyruvate-dependent phosphotransferase system of *Staphylococcus aureus*: factor III^{Lac}, a trimeric phospho-carrier protein that also acts as a phase transfer catalyst. Biochemistry **21:**4867–4873. http://dx.doi.org/10.1021/bi00263a006.
11. **Dörschug M, Frank R, Kalbitzer HR, Hengstenberg W, Deutscher J.** 1984. Phosphoenolpyruvate-dependent phosphorylation site in enzyme III^{glc} of the *Escherichia coli* phosphotransferase system. Eur. J. Biochem. **144:**113–119. http://dx.doi.org/10.1111/j.1432-1033.1984.tb08438.x.
12. **Pas HH, Robillard GT.** 1988. *S*-Phosphocysteine and phosphohistidine are intermediates in the phosphoenolpyruvate-dependent mannitol transport catalyzed by *Escherichia coli* EII^{Mtl}. Biochemistry **27:**5835–5839. http://dx.doi.org/10.1021/bi00416a002.
13. **Charrier V, Deutscher J, Galinier A, Martin-Verstraete I.** 1997. Protein phosphorylation chain of a *Bacillus subtilis* fructose-specific phosphotransferase system and its participation in regulation of the expression of the *lev* operon. Biochemistry **36:**1163–1172. http://dx.doi.org/10.1021/bi961813w.
14. **Rodríguez-Díaz J, Rubio-del-Campo A, Yebra MJ.** 2012. *Lactobacillus casei* ferments the N-Acetylglucosamine moiety of fucosyl-α-1,3-N-acetylglucosamine and excretes L-fucose. Appl. Environ. Microbiol. **78:**4613–4619. http://dx.doi.org/10.1128/AEM.00474-12.
15. **Mokhtari A, Blancato VS, Repizo GD, Henry C, Pikis A, Bourand A, Álvarez Md Immel FS, Mechakra-Maza A, Hartke A, Thompson J,**

Magni C, Deutscher J. 2013. *Enterococcus faecalis* utilizes maltose by connecting two incompatible metabolic routes via a novel maltose 6′-phosphate phosphatase (MapP). Mol. Microbiol. **88:**234–253. http://dx.doi.org/10.1111/mmi.12183.

16. **Cao Y, Jin X, Levin EJ, Huang H, Zong Y, Quick M, Weng J, Pan Y, Love J, Punta M, Rost B, Hendrickson WA, Javitch JA, Rajashankar KR, Zhou M.** 2011. Crystal structure of a phosphorylation-coupled saccharide transporter. Nature **473:**50–54. http://dx.doi.org/10.1038/nature09939.
17. **Cai L, Cai S, Zhao D, Wu J, Wang L, Liu X, Li M, Hou J, Zhou J, Liu J, Han J, Xiang H.** 2014. Analysis of the transcriptional regulator GlpR, promoter elements, and posttranscriptional processing involved in fructose-induced activation of the phosphoenolpyruvate-dependent sugar phosphotransferase system in *Haloferax mediterranei*. Appl. Environ. Microbiol. **80:**1430–1440. http://dx.doi.org/10.1128/AEM.03372-13.
18. **Bächler C, Flükiger-Brühwiler K, Schneider P, Bähler P, Erni B.** 2005. From ATP as substrate to ADP as coenzyme: functional evolution of the nucleotide binding subunit of dihydroxyacetone kinase. J. Biol. Chem. **280:**18321–18325. http://dx.doi.org/10.1074/jbc.M500279200.
19. **Dozot M, Poncet S, Nicolas C, Copin R, Bouraoui H, Mazé A, Deutscher J, De Bolle X, Letesson JJ.** 2010. Functional characterization of the incomplete phosphotransferase system (PTS) of the intracellular pathogen *Brucella melitensis*. PLoS One **5:**e12679. http://dx.doi.org/10.1371/journal.pone.0012679.
20. **Kundig W, Ghosh S, Roseman S.** 1964. Phosphate bound to histidine in a protein as an intermediate in a novel phospho-transferase system. Proc. Natl. Acad. Sci. U. S. A. **52:**1067–1074. http://dx.doi.org/10.1073/pnas.52.4.1067.
21. **Deutscher J, Saier MH, Jr.** 1983. ATP-dependent protein kinase-catalyzed phosphorylation of a seryl residue in HPr, a phosphate carrier protein of the phosphotransferase system in *Streptococcus pyogenes*. Proc. Natl. Acad. Sci. U. S. A. **80:**6790–6794. http://dx.doi.org/10.1073/pnas.80.22.6790.
22. **Mijakovic I, Poncet S, Galinier A, Monedero V, Fieulaine S, Janin J, Nessler S, Marquez JA, Scheffzek K, Hasenbein S, Hengstenberg W, Deutscher J.** 2002. Pyrophosphate-producing protein dephosphorylation by HPr kinase/phosphorylase: a relic of early life? Proc. Natl. Acad. Sci. U. S. A. **99:**13442–13447. http://dx.doi.org/10.1073/pnas.212410399.
23. **Fox DK, Meadow ND, Roseman S.** 1986. Phosphate transfer between acetate kinase and enzyme I of the bacterial phosphotransferase system. J. Biol. Chem. **261:**13498–13503.
24. **Kirkland PA, Gil MA, Karadzic IM, Maupin-Furlow JA.** 2008. Genetic and proteomic analyses of a proteasome-activating nucleotidase A mutant of the haloarchaeon *Haloferax volcanii*. J. Bacteriol. **190:**193–205. http://dx.doi.org/10.1128/JB.01196-07.
25. **Simoni RD, Hays JB, Nakazawa T, Roseman S.** 1973. Sugar transport. VI. Phosphoryl transfer in the lactose phosphotransferase system of *Staphylococcus aureus*. J. Biol. Chem. **248:**957–965.
26. **Hengstenberg W, Schrecker O, Stein R, Weil R.** 1976. Lactose transport and metabolism in *Staphylococcus aureus*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Sect. I **5:**203–219.
27. **Lolkema JS, ten Hoeve-Duurkens RH, Robillard GT.** 1993. Steady state kinetics of mannitol phosphorylation catalyzed by enzyme II^{mtl} of the *Escherichia coli* phosphoenolpyruvate-dependent phosphotransferase system. J. Biol. Chem. **268:**17844–17849.
28. **Hogema BM, Arents JC, Bader R, Eijkemans K, Yoshida H, Takahashi H, Aiba H, Postma PW.** 1998. Inducer exclusion in *Escherichia coli* by non-PTS substrates: the role of the PEP to pyruvate ratio in determining the phosphorylation state of enzyme IIA^{Glc}. Mol. Microbiol. **30:**487–498. http://dx.doi.org/10.1046/j.1365-2958.1998.01053.x.
29. **Bettenbrock K, Fischer S, Kremling A, Jahreis K, Sauter T, Gilles ED.** 2006. A quantitative approach to catabolite repression in *Escherichia coli*. J. Biol. Chem. **281:**2578–2584. http://dx.doi.org/10.1074/jbc.M508090200.
30. **Vadeboncoeur C, Brochu D, Reizer J.** 1991. Quantitative determination of the intracellular concentration of the various forms of HPr, a phosphocarrier protein of the phosphoenolpyruvate: sugar phosphotransferase system in growing cells of oral streptococci. Anal. Biochem. **196:**24–30. http://dx.doi.org/10.1016/0003-2697(91)90112-7.
31. **Park YH, Lee CR, Choe M, Seok YJ.** 2013. HPr antagonizes the anti-σ^{70} activity of Rsd in *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A. **110:**21142–21147. http://dx.doi.org/10.1073/pnas.1316629111.
32. **Doucette CD, Schwab DJ, Wingreen NS, Rabinowitz JD.** 2011. α-Ketoglutarate coordinates carbon and nitrogen utilization via enzyme I inhibition. Nat. Chem. Biol. **7:**894–901. http://dx.doi.org/10.1038/nchembio.685.
33. **Deutscher J, Pevec B, Beyreuther K, Kiltz H-H, Hengstenberg W.** 1986. Streptococcal phosphoenolpyruvate-sugar phosphotransferase system: amino acid sequence and site of ATP-dependent phosphorylation of HPr. Biochemistry **25:**6543–6551. http://dx.doi.org/10.1021/bi00369a031.
34. **Deutscher J, Kessler U, Alpert CA, Hengstenberg W.** 1984. Bacterial phosphoenolpyruvate-dependent phosphotransferase system: P-Ser-HPr and its possible regulatory function. Biochemistry **23:**4455–4460. http://dx.doi.org/10.1021/bi00314a033.
35. **Monedero V, Poncet S, Mijakovic I, Fieulaine S, Dossonnet V, Martin-Verstraete I, Nessler S, Deutscher J.** 2001. Mutations lowering the phosphatase activity of HPr kinase/phosphatase switch off carbon metabolism. EMBO J. **20:**3928–3937. http://dx.doi.org/10.1093/emboj/20.15.3928.
36. **Deutscher J, Engelmann R.** 1984. Purification and characterization of an ATP-dependent protein kinase from *Streptococcus faecalis*. FEMS Microbiol. Lett. **23:**157–162. http://dx.doi.org/10.1111/j.1574-6968.1984.tb01053.x.
37. **Reizer J, Novotny MJ, Hengstenberg W, Saier MH, Jr.** 1984. Properties of ATP-dependent protein kinase from *Streptococcus pyogenes* that phosphorylates a seryl residue in HPr, a phosphocarrier protein of the phosphotransferase system. J. Bacteriol. **160:**333–340.
38. **Deutscher J, Sauerwald H.** 1986. Stimulation of dihydroxyacetone and glycerol kinase activity in *Streptococcus faecalis* by phosphoenolpyruvate-dependent phosphorylation catalyzed by enzyme I and HPr of the phosphotransferase system. J. Bacteriol. **166:**829–836.
39. **Yeh JI, Charrier V, Paulo J, Hou L, Darbon E, Claiborne A, Hol WGJ, Deutscher J.** 2004. Structures of enterococcal glycerol kinase in the absence and presence of glycerol: correlation of conformation to substrate binding and a mechanism of activation by phosphorylation. Biochemistry **43:**362–373. http://dx.doi.org/10.1021/bi034258o.
40. **Darbon E, Servant P, Poncet S, Deutscher J.** 2002. Antitermination by GlpP, catabolite repression via CcpA, and inducer exclusion elicited by P∼GlpK dephosphorylation control *Bacillus subtilis glpFK* expression. Mol. Microbiol. **43:**1039–1052. http://dx.doi.org/10.1046/j.1365-2958.2002.02800.x.
41. **Charrier V, Buckley E, Parsonage D, Galinier A, Darbon E, Jaquinod M, Forest E, Deutscher J, Claiborne A.** 1997. Cloning and sequencing of two enterococcal *glpK* genes and regulation of the encoded glycerol kinases by phosphoenolpyruvate-dependent, phosphotransferase system-catalyzed phosphorylation of a single histidyl residue. J. Biol. Chem. **272:**14166–14174. http://dx.doi.org/10.1074/jbc.272.22.14166.
42. **Yu P, Pettigrew DW.** 2003. Linkage between fructose 1,6-bisphosphate binding and the dimer-tetramer equilibrium of *Escherichia coli* glycerol kinase: critical behavior arising from change of ligand stoichiometry. Biochemistry **42:**4243–4252. http://dx.doi.org/10.1021/bi027142l.
43. **Deutscher J, Aké FMD, Zébré AC, Cao TN, Kentache T, Monniot C, Ma Pham QM, Mokhtari A, Joyet J, Milohanic E.** 2014. Carbohydrate utilization by *Listeria monocytogenes* and its influence on virulence gene expression, p 49–76. *In* Hambrick EC (ed), *Listeria monocytogenes*: food sources, prevalence and management strategies. Nova Science Publishers, Hauppauge, NY.
44. **Deutscher J, Bauer B, Sauerwald H.** 1993. Regulation of glycerol metabolism in *Enterococcus faecalis* by phosphoenolpyruvate-dependent phosphorylation of glycerol kinase catalyzed by enzyme I and HPr of the phosphotransferase system. J. Bacteriol. **175:**3730–3733.
45. **Reizer J, Novotny MJ, Stuiver I, Saier MH, Jr.** 1984. Regulation of glycerol uptake by the phosphoenolpyruvate-sugar phosphotransferase system in *Bacillus subtilis*. J. Bacteriol. **159:**243–250.
46. **Wehtje C, Beijer L, Nilsson R-P, Rutberg B.** 1995. Mutations in the glycerol kinase gene restore the ability of a *ptsGHI* mutant of *Bacillus subtilis* to grow on glycerol. Microbiology **141:**1193–1198. http://dx.doi.org/10.1099/13500872-141-5-1193.
47. **Yeh JI, Kettering R, Saxl R, Bourand A, Darbon E, Joly N, Briozzo P, Deutscher J.** 2009. Structural characterizations of glycerol kinase: unraveling phosphorylation-induced long-range activation. Biochemistry **48:**346–356. http://dx.doi.org/10.1021/bi8009407.
48. **Poolman B, Royer TJ, Mainzer SE, Schmidt BF.** 1989. Lactose transport system of *Streptococcus thermophilus*: a hybrid protein with homology to the melibiose carrier and Enzyme III of phosphoenolpyruvate-dependent phosphotransferase systems. J. Bacteriol. **171:**244–253.

49. **Deutscher J, Galinier A, Martin-Verstraete I.** 2002. Carbohydrate uptake and metabolism, p 129–150. *In* Sonenshein AL, Hoch JA, Losick R (ed), *Bacillus subtilis* and its closest relatives: from genes to cells. ASM Press, Washington, DC.
50. **Mazé A, Boël G, Poncet S, Mijakovic I, Le Breton Y, Benachour A, Monedero V, Deutscher J, Hartke A.** 2004. The *Lactobacillus casei ptsHI*47T mutation causes overexpression of a LevR-regulated but RpoN-independent operon encoding a mannose class phosphotransferase system. J. Bacteriol. **186:**4543–4555. http://dx.doi.org/10.1128/JB.186.14.4543-4555.2004.
51. **Henstra SA, Tuinhof M, Duurkens RH, Robillard GT.** 1999. The *Bacillus stearothermophilus* mannitol regulator, MtlR, of the phosphotransferase system. A DNA-binding protein, regulated by HPr and IICB^{mtl}-dependent phosphorylation. J. Biol. Chem. **274:**4754–4763.
52. **Reizer J, Schneider B, Reizer A, Saier MH, Jr.** 1999. A hybrid response regulator possessing a PEP-dependent phosphorylation domain. Microbiology **145:**987–989. http://dx.doi.org/10.1099/13500872-145-5-987.
53. **Poolman B, Modderman R, Reizer J.** 1992. Lactose transport system in *Streptococcus thermophilus*. The role of histidine residues. J. Biol. Chem. **267:**9150–9157.
54. **Cochu A, Roy D, Vaillancourt K, Lemay JD, Casabon I, Frenette M, Moineau S, Vadeboncoeur C.** 2005. The doubly phosphorylated form of HPr, HPr(Ser-P)(His~P), is abundant in exponentially growing cells of *Streptococcus thermophilus* and phosphorylates the lactose transporter LacS as efficiently as HPr(His~P). Appl. Environ. Microbiol. **71:**1364–1372. http://dx.doi.org/10.1128/AEM.71.3.1364-1372.2005.
55. **Rouquet G, Porcheron G, Barra C, Répérant M, Chanteloup NK, Schouler C, Gilot P.** 2009. A metabolic operon in extraintestinal pathogenic *Escherichia coli* promotes fitness under stressful conditions and invasion of eukaryotic cells. J. Bacteriol. **191:**4427–4440. http://dx.doi.org/10.1128/JB.00103-09.
56. **Tobisch S, Stülke J, Hecker M.** 1999. Regulation of the *lic* operon of *Bacillus subtilis* and characterization of potential phosphorylation sites of the LicR regulator protein by site-directed mutagenesis. J. Bacteriol. **181:**4995–5003.
57. **Martin-Verstraete I, Charrier V, Stülke J, Galinier A, Erni B, Rapoport G, Deutscher J.** 1998. Antagonistic effects of dual PTS-catalysed phosphorylation on the *Bacillus subtilis* transcriptional activator LevR. Mol. Microbiol. **28:**293–303. http://dx.doi.org/10.1046/j.1365-2958.1998.00781.x.
58. **Joyet P, Derkaoui M, Poncet S, Deutscher J.** 2010. Control of *Bacillus subtilis mtl* operon expression by complex phosphorylation-dependent regulation of the transcriptional activator MtlR. Mol. Microbiol. **76:**1279–1294. http://dx.doi.org/10.1111/j.1365-2958.2010.07175.x.
59. **Veenhoff LM, Poolman B.** 1999. Substrate recognition at the cytoplasmic and extracellular binding site of the lactose transport protein of *Streptococcus thermophilus*. J. Biol. Chem. **274:**33244–33250. http://dx.doi.org/10.1074/jbc.274.47.33244.
60. **Gunnewijk MGW, Postma PW, Poolman B.** 1999. Phosphorylation and functional properties of the IIA domain of the lactose transport protein of *Streptococcus thermophilus*. J. Bacteriol. **181:**632–641.
61. **Lessard C, Cochu A, Lemay JD, Roy D, Vaillancourt K, Frenette M, Moineau S, Vadeboncoeur C.** 2003. Phosphorylation of *Streptococcus salivarius* lactose permease (LacS) by HPr(His~P) and HPr(Ser-P)(His~P) and effects on growth. J. Bacteriol. **185:**6764–6772. http://dx.doi.org/10.1128/JB.185.23.6764-6772.2003.
62. **Gunnewijk MGW, Poolman B.** 2000. HPr(His~P)-mediated phosphorylation differently affects counterflow and proton motive force-driven uptake via the lactose transport protein of *Streptococcus thermophilus*. J. Biol. Chem. **275:**34080–34085. http://dx.doi.org/10.1074/jbc.M003513200.
63. **Geertsma ER, Duurkens RH, Poolman B.** 2005. The activity of the lactose transporter from *Streptococcus thermophilus* is increased by phosphorylated IIA and the action of beta-galactosidase. Biochemistry **44:**15889–15897. http://dx.doi.org/10.1021/bi051638w.
64. **Monniot C, Zébré AC, Aké FMD, Deutscher J, Milohanic E.** 2012. Novel listerial glycerol dehydrogenase- and phosphoenolpyruvate-dependent dihydroxyacetone kinase system connected to the pentose phosphate pathway. J. Bacteriol. **194:**4972–4982. http://dx.doi.org/10.1128/JB.00801-12.
65. **Sauvageot N, Ladjouzi R, Benachour A, Rincé A, Deutscher J, Hartke A.** 2012. Aerobic glycerol dissimilation via the *Enterococcus faecalis* DhaK pathway depends on NADH oxidase and a phosphotransfer reaction from PEP to DhaK via EIIA^{Dha}. Microbiology **158:**2661–2666. http://dx.doi.org/10.1099/mic.0.061663-0.
66. **Débarbouillé M, Gardan R, Arnaud M, Rapoport G.** 1999. Role of BkdR, a transcriptional activator of the SigL-dependent isoleucine and valine degradation pathway in *Bacillus subtilis*. J. Bacteriol. **181:**2059–2066.
67. **van Heeswijk WC, Westerhoff HV, Boogerd FC.** 2013. Nitrogen assimilation in *Escherichia coli*: putting molecular data into a systems perspective. Microbiol. Mol. Biol. Rev. **77:**628–695. http://dx.doi.org/10.1128/MMBR.00025-13.
68. **Bellini D, Caly DL, McCarthy Y, Bumann M, An S-Q, Dow JM, Walsh RPRMA.** 2014. Crystal structure of an HD-GYP domain cyclic-di-GMP phosphodiesterase reveals an enzyme with a novel trinuclear catalytic iron centre. Mol. Microbiol. **91:**26–38. http://dx.doi.org/10.1111/mmi.12447.
69. **Graille M, Zhou CZ, Receveur-Brechot V, Collinet B, Declerck N, van Tilbeurgh H.** 2005. Activation of the LicT transcriptional antiterminator involves a domain swing/lock mechanism provoking massive structural changes. J. Biol. Chem. **280:**14780–14789. http://dx.doi.org/10.1074/jbc.M414642200.
70. **van Tilbeurgh H, Le Coq D, Declerck N.** 2001. Crystal structure of an activated form of the PTS regulation domain from the LicT transcriptional antiterminator. EMBO J. **20:**3789–3799. http://dx.doi.org/10.1093/emboj/20.14.3789.
71. **Joyet P, Bouraoui H, Aké FM, Derkaoui M, Zébré AC, Cao TN, Ventroux M, Nessler S, Noirot-Gros MF, Deutscher J, Milohanic E.** 2013. Transcription regulators controlled by interaction with enzyme IIB components of the phosphoenolpyruvate:sugar phosphotransferase system. Biochim. Biophys. Acta **1834:**1415–1424. http://dx.doi.org/10.1016/j.bbapap.2013.01.004.
72. **Débarbouillé M, Martin-Verstraete I, Klier A, Rapoport G.** 1991. The transcriptional regulator LevR of *Bacillus subtilis* has domains homologous to both σ^{54}- and phosphotransferase system-dependent regulators. Proc. Natl. Acad. Sci. U. S. A. **88:**2212–2216. http://dx.doi.org/10.1073/pnas.88.6.2212.
73. **Lindner C, Galinier A, Hecker M, Deutscher J.** 1999. Regulation of the activity of the *Bacillus subtilis* antiterminator LicT by multiple PEP-dependent, enzyme I- and HPr-catalysed phosphorylation. Mol. Microbiol. **31:**995–1006. http://dx.doi.org/10.1046/j.1365-2958.1999.01262.x.
74. **Lindner C, Hecker M, Le Coq D, Deutscher J.** 2002. *Bacillus subtilis* mutant LicT antiterminators exhibiting enzyme I- and HPr-independent antitermination affect catabolite repression of the *bglPH* operon. J. Bacteriol. **184:**4819–4828. http://dx.doi.org/10.1128/JB.184.17.4819-4828.2002.
75. **Tortosa P, Declerck N, Dutartre H, Lindner C, Deutscher J, Le Coq D.** 2001. Sites of positive and negative regulation in the *Bacillus subtilis* antiterminators LicT and SacY. Mol. Microbiol. **41:**1381–1393. http://dx.doi.org/10.1046/j.1365-2958.2001.02608.x.
76. **Heravi KM, Altenbuchner J.** 2014. Regulation of the *Bacillus subtilis* mannitol utilization genes: promoter structure and transcriptional activation by the wild-type regulator (MtlR) and its mutants. Microbiology **160:**91–101. http://dx.doi.org/10.1099/mic.0.071233-0.
77. **Xue J, Miller KW.** 2007. Regulation of the *mpt* operon in *Listeria innocua* by the ManR protein. Appl. Environ. Microbiol. **73:**5648–5652. http://dx.doi.org/10.1128/AEM.00052-07.
78. **Aké FM, Joyet P, Deutscher J, Milohanic E.** 2011. Mutational analysis of glucose transport regulation and glucose-mediated virulence gene repression in *Listeria monocytogenes*. Mol. Microbiol. **81:**274–293. http://dx.doi.org/10.1111/j.1365-2958.2011.07692.x.
79. **Raveh H, Lopian L, Nussbaum-Shochat A, Wright A, Amster-Choder O.** 2009. Modulation of transcription antitermination in the *bgl* operon of *Escherichia coli* by the PTS. Proc. Natl. Acad. Sci. U. S. A. **106:**13523–13528. http://dx.doi.org/10.1073/pnas.0902559106.
80. **Rothe FM, Bahr T, Stülke J, Rak B, Görke B.** 2012. Activation of *Escherichia coli* antiterminator BglG requires its phosphorylation. Proc. Natl. Acad. Sci. U. S. A. **109:**15906–15911. http://dx.doi.org/10.1073/pnas.1210443109.
81. **Wagner E, Marcandier S, Egeter O, Deutscher J, Götz F, Brückner R.** 1995. Glucose kinase-dependent catabolite repression in *Staphylococcus xylosus*. J. Bacteriol. **177:**6144–6152.
82. **Hondorp ER, Hou SC, Hause LL, Gera K, Lee CE, McIver KS.** 2013. PTS phosphorylation of Mga modulates regulon expression and viru-

lence in the group A streptococcus. Mol. Microbiol. **88:**1176–1193. http://dx.doi.org/10.1111/mmi.12250.
83. **Tsvetanova B, Wilson AC, Bongiorni C, Chiang C, Hoch JA, Perego M.** 2007. Opposing effects of histidine phosphorylation regulate the AtxA virulence transcription factor in *Bacillus anthracis*. Mol. Microbiol. **63:**644–655. http://dx.doi.org/10.1111/j.1365-2958.2006.05543.x.
84. **Almengor AC, Kinkel TL, Day SJ, McIver KS.** 2007. The catabolite control protein CcpA binds to P*mga* and influences expression of the virulence regulator Mga in the group A streptococcus. J. Bacteriol. **189:**8405–8416. http://dx.doi.org/10.1128/JB.01038-07.
85. **Hurley JH, Faber HR, Worthylake D, Meadow ND, Roseman S, Pettigrew DW, Remington SJ.** 1993. Structure of the regulatory complex of *Escherichia coli* III^{Glc} with glycerol kinase. Science **259:**673–677. http://dx.doi.org/10.1126/science.8430315.
86. **Saier MH, Jr, Roseman S.** 1976. Sugar transport. The *crr* mutation: its effect on repression of enzyme synthesis. J. Biol. Chem. **251:**6598–6605.
87. **Saier MH, Jr, Roseman S.** 1976. Sugar transport. Inducer exclusion and regulation of the melibiose, maltose, glycerol, and lactose transport systems by the phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **251:**6606–6615.
88. **Postma PW, Epstein W, Schuitema ARJ, Nelson SO.** 1984. Interaction between III^{Glc} of the phosphoenolpyruvate:sugar phosphotransferase system and glycerol kinase of *Salmonella typhimurium*. J. Bacteriol. **158:**351–353.
89. **Dean DA, Reizer J, Nikaido H, Saier MH, Jr.** 1990. Regulation of the maltose transport system of *Escherichia coli* by the glucose-specific enzyme III of the phosphoenolpyruvate-sugar phosphotransferase system. Characterization of inducer exclusion-resistant mutants and reconstitution of inducer exclusion in proteoliposomes. J. Biol. Chem. **265:**21005–21010.
90. **Kühnau S, Reyes M, Sievertsen A, Shuman HA, Boos W.** 1991. The activities of the *Escherichia coli* MalK protein in maltose transport, regulation, and inducer exclusion can be separated by mutations. J. Bacteriol. **173:**2180–2186.
91. **Osumi T, Saier MH, Jr.** 1982. Regulation of lactose permease activity by the phosphoenolpyruvate:sugar phosphotransferase system: evidence for direct binding of the glucose-specific enzyme III to the lactose permease. Proc. Natl. Acad. Sci. U. S. A. **79:**1457–1461. http://dx.doi.org/10.1073/pnas.79.5.1457.
92. **Sondej M, Weinglass AB, Peterkofsky A, Kaback HR.** 2002. Binding of enzyme IIA^{Glc}, a component of the phosphoenolpyruvate:sugar phosphotransferase system, to the *Escherichia coli* lactose permease. Biochemistry **41:**5556–5565. http://dx.doi.org/10.1021/bi011990j.
93. **Saier MH, Jr, Novotny MJ, Comeau-Fuhrman D, Osumi T, Desai JD.** 1983. Cooperative binding of the sugar substrates and allosteric regulatory protein (enzyme III^{Glc} of the phosphotransferase system) to the lactose and melibiose permeases in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **155:**1351–1357.
94. **de Boer M, Broekhuizen CP, Postma PW.** 1986. Regulation of glycerol kinase by enzyme III^{Glc} of the phosphoenolpyruvate:carbohydrate phosphotransferase system. J. Bacteriol. **167:**393–395.
95. **Sutrina SL, Inniss PI, Lazarus LA, Inglis L, Maximilien J.** 2007. Replacing the general energy-coupling proteins of the phospho-enolpyruvate:sugar phosphotransferase system of *Salmonella typhimurium* with fructose-inducible counterparts results in the inability to utilize nonphosphotransferase system sugars. Can. J. Microbiol. **53:**586–598. http://dx.doi.org/10.1139/W07-020.
96. **Kuroda M, De Waard S, Mizushima K, Tsuda M, Postma P, Tsuchiya T.** 1992. Resistance of the melibiose carrier to inhibition by the phosphotransferase system due to substitutions of amino acid residues in the carrier of *Salmonella typhimurium*. J. Biol. Chem. **267:**18336–18341.
97. **Titgemeyer F, Mason RE, Saier MH, Jr.** 1994. Regulation of the raffinose permease of *Escherichia coli* by the glucose-specific enzyme IIA of the phosphoenolpyruvate:sugar phosphotransferase system. J. Bacteriol. **176:**543–546.
98. **Misko TP, Mitchell WJ, Meadow ND, Roseman S.** 1987. Sugar transport by the bacterial phosphotransferase system. Reconstitution of inducer exclusion in *Salmonella typhimurium* membrane vesicles. J. Biol. Chem. **262:**16261–16266.
99. **Hoischen C, Levin J, Pitaknarongphorn S, Reizer J, Saier MH, Jr.** 1996. Involvement of the central loop of the lactose permease of *Escherichia coli* in its allosteric regulation by the glucose-specific enzyme IIA of the phosphoenolpyruvate-dependent phosphotransferase system. J. Bacteriol. **178:**6082–6086.
100. **Inada T, Kimata K, Aiba H.** 1996. Mechanism responsible for glucose-lactose diauxie in *Escherichia coli*: challenge to the cAMP model. Genes Cells **1:**293–301. http://dx.doi.org/10.1046/j.1365-2443.1996.24025.x.
101. **Kim YJ, Ryu Y, Koo BM, Lee NY, Chun SJ, Park SJ, Lee KH, Seok YJ.** 2010. A mammalian insulysin homolog is regulated by enzyme IIA^{Glc} of the glucose transport system in *Vibrio vulnificus*. FEBS Lett. **584:**4537–4544. http://dx.doi.org/10.1016/j.febslet.2010.10.035.
102. **Pickering BS, Smith DR, Watnick PI.** 2012. Glucose-specific enzyme IIA has unique binding partners in the *Vibrio cholerae* biofilm. mBio **3**(6):e00228–12. http://dx.doi.org/10.1128/mBio.00228-12.
103. **Suzuki K, Babitzke P, Kushner SR, Romeo T.** 2006. Identification of a novel regulatory protein (CsrD) that targets the global regulatory RNAs CsrB and CsrC for degradation by RNase E. Genes Dev. **20:**2605–2617. http://dx.doi.org/10.1101/gad.1461606.
104. **Koo BM, Yoon MJ, Lee CR, Nam TW, Choe YJ, Jaffe H, Peterkofsky A, Seok YJ.** 2004. A novel fermentation/respiration switch protein regulated by enzyme IIA^{Glc} in *Escherichia coli*. J. Biol. Chem. **279:**31613–31621. http://dx.doi.org/10.1074/jbc.M405048200.
105. **Pettigrew DW, Meadow ND, Roseman S, Remington SJ.** 1998. Cation promoted association of *Escherichia coli* phosphocarrier protein IIA^{Glc} with regulatory target protein glycerol kinase: substitutions of a Zn(II) ligand and implications for inducer exclusion. Biochemistry **37:**4875–4883. http://dx.doi.org/10.1021/bi971634u.
106. **Chen S, Oldham ML, Davidson AL, Chen J.** 2013. Carbon catabolite repression of the maltose transporter revealed by X-ray crystallography. Nature **499:**364–368. http://dx.doi.org/10.1038/nature12232.
107. **Wang GS, Louis JM, Sondej M, Seok Y-J, Peterkofsky A, Clore GM.** 2000. Solution structure of the phosphoryl transfer complex between the signal transducing proteins HPr and IIA^{Glucose} of the *Escherichia coli* phosphoenolpyruvate:sugar phosphotransferase system. EMBO J. **19:**5635–5649. http://dx.doi.org/10.1093/emboj/19.21.5635.
108. **Cai M, Williams DC, Jr, Wang G, Lee BR, Peterkofsky A, Clore GM.** 2003. Solution structure of the phosphoryl transfer complex between the signal transducing protein IIA^{Glucose} and the cytoplasmic domain of the glucose transporter IICB^{Glucose} of the *Escherichia coli* glucose phosphotransferase system. J. Biol. Chem. **278:**25191–25206. http://dx.doi.org/10.1074/jbc.M302677200.
109. **Ren J, Sainsbury S, Berrow NS, Alderton D, Nettleship JE, Stammers DK, Saunders NJ, Owens RJ.** 2005. Crystal structure of nitrogen regulatory protein IIA^{Ntr} from *Neisseria meningitidis*. BMC Struct. Biol. **5:**13. http://dx.doi.org/10.1186/1472-6807-5-13.
110. **Krausse D, Hunold K, Kusian B, Lenz O, Stülke J, Bowien B, Deutscher J.** 2009. Essential role of the *hprK* gene in *Ralstonia eutropha* H16. J. Mol. Microbiol. Biotechnol. **17:**146–152. http://dx.doi.org/10.1159/000233505.
111. **Boël G, Mijakovic I, Mazé A, Poncet S, Taha M-K, Larribe M, Darbon E, Khemiri A, Galinier A, Deutscher J.** 2003. Transcription regulators potentially controlled by HPr kinase/phosphorylase in Gram-negative bacteria. J. Mol. Microbiol. Biotechnol. **5:**206–215. http://dx.doi.org/10.1159/000071072.
112. **Merrick MJ, Taylor M, Saier MH, Jr, Reizer J.** 1995. The role of genes downstream of the σ^N structural gene *rpoN* in *Klebsiella pneumoniae*, p 189–194. *In* Tikhonovitch IA, Provorov NA, Romanov VI, Newton WE (ed), Nitrogen fixation: fundamentals and applications. Kluwer Academic Publishers, Dordrecht, The Netherlands.
113. **Pflüger-Grau K, Görke B.** 2010. Regulatory roles of the bacterial nitrogen-related phosphotransferase system. Trends Microbiol. **18:**205–214. http://dx.doi.org/10.1016/j.tim.2010.02.003.
114. **Pflüger K, de Lorenzo V.** 2008. Evidence of *in vivo* cross talk between the nitrogen-related and fructose-related branches of the carbohydrate phosphotransferase system of *Pseudomonas putida*. J. Bacteriol. **190:**3374–3380. http://dx.doi.org/10.1128/JB.02002-07.
115. **Zimmer B, Hillmann A, Görke B.** 2008. Requirements for the phosphorylation of the *Escherichia coli* EIIA^{Ntr} protein *in vivo*. FEMS Microbiol. Lett. **286:**96–102. http://dx.doi.org/10.1111/j.1574-6968.2008.01262.x.
116. **Lee CR, Park YH, Kim M, Kim YR, Park S, Peterkofsky A, Seok YJ.** 2013. Reciprocal regulation of the autophosphorylation of enzyme I^{Ntr} by glutamine and α-ketoglutarate in *Escherichia coli*. Mol. Microbiol. **88:**473–485. http://dx.doi.org/10.1111/mmi.12196.
117. **Göpel Y, Papenfort K, Reichenbach B, Vogel J, Görke B.** 2013. Tar-

geted decay of a regulatory small RNA by an adaptor protein for RNase E and counteraction by an anti-adaptor RNA. Genes Dev. **27:**552–564. http://dx.doi.org/10.1101/gad.210112.112.

118. **Goodwin RA, Gage DJ.** 14 March 2014. Biochemical characterization of a nitrogen-type phosphotransferase system reveals enzyme EI^{Ntr} integrates carbon and nitrogen signaling in *Sinorhizobium meliloti*. J. Bacteriol. http://dx.doi.org/10.1128/JB.01489-14.
119. **Lee CR, Cho SH, Yoon MJ, Peterkofsky A, Seok YJ.** 2007. *Escherichia coli* enzyme IIA^{Ntr} regulates the K^+ transporter TrkA. Proc. Natl. Acad. Sci. U. S. A. **104:**4124–4129. http://dx.doi.org/10.1073/pnas.0609897104.
120. **Cao Y, Jin X, Huang H, Derebe MG, Levin EJ, Kabaleeswaran V, Pan Y, Punta M, Love J, Weng J, Quick M, Ye S, Kloss B, Bruni R, Martinez-Hackert E, Hendrickson WA, Rost B, Javitch JA, Rajashankar KR, Jiang Y, Zhou M.** 2011. Crystal structure of a potassium ion transporter, TrkH. Nature **47:**336–340. http://dx.doi.org/10.1038/nature09731.
121. **Cao Y, Pan Y, Huang H, Jin X, Levin EJ, Kloss B, Zhou M.** 2013. Gating of the TrkH ion channel by its associated RCK protein TrkA. Nature **496:**317–322. http://dx.doi.org/10.1038/nature12056.
122. **Lee CR, Cho SH, Kim HJ, Kim M, Peterkofsky A, Seok YJ.** 2010. Potassium mediates *Escherichia coli* enzyme IIA^{Ntr}-dependent regulation of sigma factor selectivity. Mol. Microbiol. **78:**1468–1483. http://dx.doi.org/10.1111/j.1365-2958.2010.07419.x.
123. **Haupt M, Bramkamp M, Coles M, Kessler H, Altendorf K.** 2005. Prokaryotic Kdp-ATPase: recent insights into the structure and function of KdpB. J. Mol. Microbiol. Biotechnol. **10:**120–131. http://dx.doi.org/10.1159/000091559.
124. **Laermann V, Cudic E, Kipschull K, Zimmann P, Altendorf K.** 2013. The sensor kinase KdpD of *Escherichia coli* senses external K^+. Mol. Microbiol. **88:**1194–1204. http://dx.doi.org/10.1111/mmi.12251.
125. **Heermann R, Altendorf K, Jung K.** 2003. The N-terminal input domain of the sensor kinase KdpD of *Escherichia coli* stabilizes the interaction between the cognate response regulator KdpE and the corresponding DNA-binding site. J. Biol. Chem. **278:**51277–51284. http://dx.doi.org/10.1074/jbc.M303801200.
126. **Lüttmann D, Heermann R, Zimmer B, Hillmann A, Rampp IS, Jung K, Görke B.** 2009. Stimulation of the potassium sensor KdpD kinase activity by interaction with the phosphotransferase protein IIA^{Ntr} in *Escherichia coli*. Mol. Microbiol. **72:**978–994. http://dx.doi.org/10.1111/j.1365-2958.2009.06704.x.
127. **Prell J, Mulley G, Haufe F, White JP, Williams A, Karunakaran R, Downie JA, Poole PS.** 2012. The PTS^{Ntr} system globally regulates ATP-dependent transporters in *Rhizobium leguminosarum*. Mol. Microbiol. **84:**117–129. http://dx.doi.org/10.1111/j.1365-2958.2012.08014.x.
128. **Lüttmann D, Göpel Y, Görke B.** 2012. The phosphotransferase protein $EIIA^{Ntr}$ modulates the phosphate starvation response through interaction with histidine kinase PhoR in *Escherichia coli*. Mol. Microbiol. **86:**96–110. http://dx.doi.org/10.1111/j.1365-2958.2012.08176.x.
129. **Pflüger-Grau K, Chavarría M, de Lorenzo V.** 2011. The interplay of the $EIIA^{Ntr}$ component of the nitrogen-related phosphotransferase system (PTS^{Ntr}) of *Pseudomonas putida* with pyruvate dehydrogenase. Biochim. Biophys. Acta **1810:**995–1005. http://dx.doi.org/10.1016/j.bbagen.2011.01.002.
130. **Bowden SD, Rowley G, Hinton JC, Thompson A.** 2009. Glucose and glycolysis are required for the successful infection of macrophages and mice by *Salmonella enterica* serovar Typhimurium. Infect. Immun. **77:**3117–3126. http://dx.doi.org/10.1128/IAI.00093-09.
131. **Choi J, Shin D, Yoon H, Kim J, Lee CR, Kim M, Seok YJ, Ryu S.** 2010. *Salmonella* pathogenicity island 2 expression negatively controlled by $EIIA^{Ntr}$-SsrB interaction is required for *Salmonella* virulence. Proc. Natl. Acad. Sci. U. S. A. **107:**20506–20511. http://dx.doi.org/10.1073/pnas.1000759107.
132. **Lee CR, Koo BM, Cho SH, Kim YJ, Yoon MJ, Peterkofsky A, Seok YJ.** 2005. Requirement of the dephospho-form of enzyme IIA^{Ntr} for derepression of *Escherichia coli* K-12 *ilvBN* expression. Mol. Microbiol. **58:**334–344. http://dx.doi.org/10.1111/j.1365-2958.2005.04834.x.
133. **Karstens K, Zschiedrich CP, Bowien B, Stülke J, Görke B.** 2014. The phosphotransferase protein $EIIA^{Ntr}$ interacts with SpoT, a key enzyme of the stringent response, in *Ralstonia eutropha* H16. Microbiology **160:**711–722. http://dx.doi.org/10.1099/mic.0.075226-0.
134. **Tanaka Y, Kimata K, Aiba H.** 2000. A novel regulatory role of glucose transporter of *Escherichia coli*: membrane sequestration of a global repressor Mlc. EMBO J. **19:**5344–5352. http://dx.doi.org/10.1093/emboj/19.20.5344.
135. **Lee SJ, Boos W, Bouche JP, Plumbridge J.** 2000. Signal transduction between a membrane-bound transporter, PtsG, and a soluble transcription factor, Mlc, of *Escherichia coli*. EMBO J. **19:**5353–5361. http://dx.doi.org/10.1093/emboj/19.20.5353.
136. **Nam TW, Cho SH, Shin D, Kim JH, Jeong JY, Lee JH, Roe JH, Peterkofsky A, Kang SO, Ryu S, Seok Y-J.** 2001. The *Escherichia coli* glucose transporter enzyme $IICB^{Glc}$ recruits the global repressor Mlc. EMBO J. **20:**491–498. http://dx.doi.org/10.1093/emboj/20.3.491.
137. **Hosono K, Kakuda H, Ichihara S.** 1995. Decreasing accumulation of acetate in a rich medium by *Escherichia coli* on introduction of genes on a multicopy plasmid. Biosci. Biotechnol. Biochem. **59:**256–261. http://dx.doi.org/10.1271/bbb.59.256.
138. **Plumbridge J.** 1998. Control of the expression of the *manXYZ* operon in *Escherichia coli*: Mlc is a negative regulator of the mannose PTS. Mol. Microbiol. **27:**369–380. http://dx.doi.org/10.1046/j.1365-2958.1998.00685.x.
139. **Zeppenfeld T, Larisch C, Lengeler JW, Jahreis K.** 2000. Glucose transporter mutants of *Escherichia coli* K-12 with changes in substrate recognition of $IICB^{Glc}$ and induction behavior of the *ptsG* gene. J. Bacteriol. **182:**4443–4452. http://dx.doi.org/10.1128/JB.182.16.4443-4452.2000.
140. **Plumbridge J.** 1999. Expression of the phosphotransferase system both mediates and is mediated by Mlc regulation in *Escherichia coli*. Mol. Microbiol. **33:**260–273. http://dx.doi.org/10.1046/j.1365-2958.1999.01462.x.
141. **Seitz S, Lee SJ, Pennetier C, Boos W, Plumbridge J.** 2003. Analysis of the interaction between the global regulator Mlc and $EIIB^{Glc}$ of the glucose-specific phosphotransferase system in *Escherichia coli*. J. Biol. Chem. **278:**10744–10751. http://dx.doi.org/10.1074/jbc.M212066200.
142. **Nam TW, Jung HI, An YJ, Park YH, Lee SH, Seok YJ, Cha SS.** 2008. Analyses of Mlc-IIB^{Glc} interaction and a plausible molecular mechanism of Mlc inactivation by membrane sequestration. Proc. Natl. Acad. Sci. U. S. A. **105:**3751–3756. http://dx.doi.org/10.1073/pnas.0709295105.
143. **Tanaka Y, Itoh F, Kimata K, Aiba H.** 2004. Membrane localization itself but not binding to IICB is directly responsible for the inactivation of the global repressor Mlc in *Escherichia coli*. Mol. Microbiol. **53:**941–951. http://dx.doi.org/10.1111/j.1365-2958.2004.04179.x.
144. **Pennetier C, Domínguez-Ramírez L, Plumbridge J.** 2008. Different regions of Mlc and NagC, homologous transcriptional repressors controlling expression of the glucose and N-acetylglucosamine phosphotransferase systems in *Escherichia coli*, are required for inducer signal recognition. Mol. Microbiol. **67:**364–377. http://dx.doi.org/10.1111/j.1365-2958.2007.06041.x.
145. **Göhler AK, Staab A, Gabor E, Homann K, Klang E, Kosfeld A, Muus JE, Wulftange JS, Jahreis K.** 2012. Characterization of MtfA, a novel regulatory output signal protein of the glucose-phosphotransferase system in *Escherichia coli* K-12. J. Bacteriol. **194:**1024–1035. http://dx.doi.org/10.1128/JB.06387-11.
146. **Bouraoui H, Ventroux M, Noirot-Gros M-F, Deutscher J, Joyet P.** 2013. Membrane sequestration by the EIIB domain of the mannitol permease MtlA activates the *Bacillus subtilis mtl* operon regulator MtlR. Mol. Microbiol. **87:**789–801. http://dx.doi.org/10.1111/mmi.12131.
147. **Mijakovic I, Poncet S, Boël G, Mazé A, Gillet S, Jamet E, Decottignies P, Grangeasse C, Doublet P, Le Maréchal P, Deutscher J.** 2003. Transmembrane modulator-dependent bacterial tyrosine kinase activates UDP-glucose dehydrogenases. EMBO J. **22:**4709–4718. http://dx.doi.org/10.1093/emboj/cdg458.
148. **Opacić M, Vos EP, Hesp BH, Broos J.** 2010. Localization of the substrate-binding site in the homodimeric mannitol transporter, EII^{mtl}, of *Escherichia coli*. J. Biol. Chem. **285:**25324–25331. http://dx.doi.org/10.1074/jbc.M110.122523.
149. **Heravi KM, Wenzel M, Altenbuchner J.** 2011. Regulation of *mtl* operon promoter of *Bacillus subtilis*: requirements of its use in expression vectors. Microb. Cell Fact. **10:**83. http://dx.doi.org/10.1186/1475-2859-10-83.
150. **Stoll R, Goebel W.** 2010. The major PEP-phosphotransferase systems (PTSs) for glucose, mannose and cellobiose of *Listeria monocytogenes*, and their significance for extra- and intracellular growth. Microbiology **156:**1069–1083. http://dx.doi.org/10.1099/mic.0.034934-0.
151. **Houot L, Chang S, Pickering BS, Absalon C, Watnick PI.** 2010. The phosphoenolpyruvate phosphotransferase system regulates *Vibrio chol-*

erae biofilm formation through multiple independent pathways. J. Bacteriol. **192:**3055–3067. http://dx.doi.org/10.1128/JB.00213-10.
152. **Ymele-Leki P, Houot L, Watnick PI.** 2013. Mannitol and the mannitol-specific enzyme IIB subunit activate *Vibrio cholerae* biofilm formation. Appl. Environ. Microbiol. **79:**4675–4683. http://dx.doi.org/10.1128/AEM.01184-13.
153. **Morita T, Kawamoto H, Mizota T, Inada T, Aiba H.** 2004. Enolase in the RNA degradosome plays a crucial role in the rapid decay of glucose transporter mRNA in the response to phosphosugar stress in *Escherichia coli*. Mol. Microbiol. **54:**1063–1075. http://dx.doi.org/10.1111/j.1365-2958.2004.04329.x.
154. **Gabor E, Göhler AK, Kosfeld A, Staab A, Kremling A, Jahreis K.** 2011. The phosphoenolpyruvate-dependent glucose-phosphotransferase system from *Escherichia coli* K-12 as the center of a network regulating carbohydrate flux in the cell. Eur. J. Cell Biol. **90:**711–720. http://dx.doi.org/10.1016/j.ejcb.2011.04.002.
155. **Snyder H, Kellogg SL, Skarda LM, Little JL, Kristich CJ.** 25 November 2013. Nutritional control of antibiotic resistance via an interface between the phosphotransferase system and a two-component signaling system. Antimicrob. Agents Chemother. http://dx.doi.org/10.1128/AAC.01919-13.
156. **Seok Y-J, Sondej M, Badawi P, Lewis MS, Briggs MC, Jaffe H, Peterkofsky A.** 1997. High affinity binding and allosteric regulation of *Escherichia coli* glycogen phosphorylase by the histidine phosphocarrier protein, HPr. J. Biol. Chem. **272:**26511–26521. http://dx.doi.org/10.1074/jbc.272.42.26511.
157. **Koo BM, Seok Y-J.** 2001. Regulation of glycogen concentration by the histidine-containing phosphocarrier protein HPr in *Escherichia coli*. J. Microbiol. **39:**24–30.
158. **Landmann JJ, Busse RA, Latz JH, Singh KD, Stülke J, Görke B.** 2011. Crh, the paralogue of the phosphocarrier protein HPr, controls the methylglyoxal bypass of glycolysis in *Bacillus subtilis*. Mol. Microbiol. **82:**770–787. http://dx.doi.org/10.1111/j.1365-2958.2011.07857.x.
159. **Kim HJ, Lee CR, Kim M, Peterkofsky A, Seok YJ.** 2011. Dephosphorylated NPr of the nitrogen PTS regulates lipid A biosynthesis by direct interaction with LpxD. Biochem. Biophys. Res. Commun. **409:**556–561. http://dx.doi.org/10.1016/j.bbrc.2011.05.044.
160. **Bartling CM, Raetz CR.** 2009. Crystal structure and acyl chain selectivity of *Escherichia coli* LpxD, the N-acyltransferase of lipid A biosynthesis. Biochemistry **48:**8672–8683. http://dx.doi.org/10.1021/bi901025v.
161. **Hazelbauer GL.** 2012. Bacterial chemotaxis: the early years of molecular studies. Annu. Rev. Microbiol. **66:**285–303. http://dx.doi.org/10.1146/annurev-micro-092611-150120.
162. **Paul K, Brunstetter D, Titen S, Blair DF.** 2011. A molecular mechanism of direction switching in the flagellar motor of *Escherichia coli* Proc. Natl. Acad. Sci. U. S. A. **108:**17171–17176. http://dx.doi.org/10.1073/pnas.1110111108.
163. **Lux R, Jahreis K, Bettenbrock K, Parkinson JS, Lengeler JW.** 1995. Coupling of the phosphotransferase system and the methyl-accepting chemotaxis protein-dependent chemotaxis signaling pathways of *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. A. **92:**11583–11587. http://dx.doi.org/10.1073/pnas.92.25.11583.
164. **Garrity LF, Schiel SL, Merrill R, Reizer J, Saier MH, Jr, Ordal GW.** 1998. Unique regulation of carbohydrate chemotaxis in *Bacillus subtilis* by the phosphoenolpyruvate-dependent phosphotransferase system and the methyl-accepting chemotaxis protein McpC. J. Bacteriol. **180:**4475–4480.
165. **King ND, O'Brian MR.** 2001. Evidence for direct interaction between enzyme I^{Ntr} and aspartokinase to regulate bacterial oligopeptide transport. J. Biol. Chem. **276:**21311–21316. http://dx.doi.org/10.1074/jbc.M101982200.
166. **Schumacher MA, Allen GS, Diel M, Seidel G, Hillen W, Brennan RG.** 2004. Structural basis for allosteric control of the transcription regulator CcpA by the phosphoprotein HPr-Ser46-P. Cell **118:**731–741. http://dx.doi.org/10.1016/j.cell.2004.08.027.
167. **Krin E, Sismeiro O, Danchin A, Bertin PN.** 2002. The regulation of enzyme IIA^{Glc} expression controls adenylate cyclase activity in *Escherichia coli*. Microbiology **148:**1553–1559.
168. **Mostovenko E, Deelder AM, Palmblad M.** 2011. Protein expression dynamics during Escherichia coli glucose-lactose diauxie. BMC Microbiol. **11:**126. http://dx.doi.org/10.1186/1471-2180-11-126.
169. **Ishizuka H, Hanamura A, Kunimura T, Aiba H.** 1993. A lowered concentration of cAMP receptor protein caused by glucose is an important determinant for catabolite repression in *Escherichia coli*. Mol. Microbiol. **10:**341–350. http://dx.doi.org/10.1111/j.1365-2958.1993.tb01960.x.
170. **Fraser ADE, Yamazaki H.** 1982. Significance of β-galactosidase repression in glucose inhibition of lactose utilization in *Escherichia coli*. Curr. Microbiol. **7:**241–244. http://dx.doi.org/10.1007/BF01568806.
171. **Park Y-H, Lee BR, Seok Y-J, Peterkofsky A.** 2006. *In vitro* reconstitution of catabolite repression in *Escherichia coli*. J. Biol. Chem. **281:**6448–6454. http://dx.doi.org/10.1074/jbc.M512672200.
172. **Lopian L, Nussbaum-Shochat A, O'Day-Kerstein K, Wright A, Amster-Choder O.** 2003. The BglF sensor recruits the BglG transcription regulator to the membrane and releases it on stimulation. Proc. Natl. Acad. Sci. U. S. A. **100:**7099–7104. http://dx.doi.org/10.1073/pnas.1037608100.
173. **Rothe FM, Wrede C, Lehnik-Habrink M, Görke B, Stülke J.** 2013. Dynamic localization of a transcription factor in *Bacillus subtilis*: the LicT antiterminator relocalizes in response to inducer availability. J. Bacteriol. **195:**2146–2154. http://dx.doi.org/10.1128/JB.00117-13.
174. **Egan SM.** 2002. Growing repertoire of AraC/XylS activators. J. Bacteriol. **184:**5529–5532. http://dx.doi.org/10.1128/JB.184.20.5529-5532.2002.
175. **Poncet S, Soret M, Mervelet P, Deutscher J, Noirot P.** 2009. Transcriptional activator YesS is stimulated by histidine-phosphorylated HPr of the *Bacillus subtilis* phosphotransferase system. J. Biol. Chem. **284:**28188–28197. http://dx.doi.org/10.1074/jbc.M109.046334.
176. **Zhang P, Ma Y, Wang F, Wei D.** 11 February 2014. Phosphorylation of HPr by HPr kinase in *Gluconobacter oxydans* 621H. Protein Pept. Lett. http://dx.doi.org/10.2174/0929866521666140212111059.
177. **Görke B, Stülke J.** 2008. Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. Nat. Rev. Microbiol. **6:**613–624. http://dx.doi.org/10.1038/nrmicro1932.
178. **Deutscher J.** 2008. The mechanisms of carbon catabolite repression in bacteria. Curr. Opin. Microbiol. **11:**87–93. http://dx.doi.org/10.1016/j.mib.2008.02.007.
179. **Deutscher J, Küster E, Bergstedt U, Charrier V, Hillen W.** 1995. Protein kinase-dependent HPr/CcpA interaction links glycolytic activity to carbon catabolite repression in Gram-positive bacteria. Mol. Microbiol. **15:**1049–1053. http://dx.doi.org/10.1111/j.1365-2958.1995.tb02280.x.
180. **Fujita Y, Miwa Y, Galinier A, Deutscher J.** 1995. Specific recognition of the *Bacillus subtilis gnt cis*-acting catabolite-responsive element by a protein complex formed between CcpA and seryl-phosphorylated HPr. Mol. Microbiol. **17:**953–960. http://dx.doi.org/10.1111/j.1365-2958.1995.mmi_17050953.x.
181. **Eisermann R, Deutscher J, Gonzy-Tréboul G, Hengstenberg W.** 1988. Site-directed mutagenesis with the *ptsH* gene of *Bacillus subtilis*. Isolation and characterization of heat-stable proteins altered at the ATP-dependent regulatory phosphorylation site. J. Biol. Chem. **263:**17050–17054.
182. **Presecan-Siedel E, Galinier A, Longin R, Deutscher J, Danchin A, Glaser P, Martin-Verstraete I.** 1999. Catabolite regulation of the *pta* gene as part of carbon flow pathways in *Bacillus subtilis*. J. Bacteriol. **181:**6889–6897.
183. **Galinier A, Haiech J, Kilhoffer M-C, Jaquinod M, Stülke J, Deutscher J, Martin-Verstraete I.** 1997. The *Bacillus subtilis crh* gene encodes a HPr-like protein involved in carbon catabolite repression. Proc. Natl. Acad. Sci. U. S. A. **94:**8439–8444. http://dx.doi.org/10.1073/pnas.94.16.8439.
184. **Martin-Verstraete I, Deutscher J, Galinier A.** 1999. Phosphorylation of HPr and Crh by HprK, early steps in the catabolite repression signalling pathway for the *Bacillus subtilis* levanase operon. J. Bacteriol. **181:**2966–2969.
185. **Pompeo F, Luciano J, Galinier A.** 2007. Interaction of GapA with HPr and its homologue, Crh: novel levels of regulation of a key step of glycolysis in *Bacillus subtilis*? J. Bacteriol. **189:**1154–1157. http://dx.doi.org/10.1128/JB.01575-06.
186. **Shelburne SA, III, Keith D, Horstmann N, Sumby P, Davenport MT, Graviss EA, Brennan RG, Musser JM.** 2008. A direct link between carbohydrate utilization and virulence in the major human pathogen group A Streptococcus. Proc. Natl. Acad. Sci. U. S. A. **105:**1698–1703. http://dx.doi.org/10.1073/pnas.0711767105.
187. **Chiang C, Bongiorni C, Perego M.** 2011. Glucose-dependent activation of *Bacillus anthracis* toxin gene expression and virulence requires the carbon catabolite protein CcpA. J. Bacteriol. **193:**52–62. http://dx.doi.org/10.1128/JB.01656-09.
188. **Viana R, Monedero V, Dossonnet V, Vadeboncoeur C, Pérez-**

Martínez G, Deutscher J. 2000. Enzyme I and HPr from *Lactobacillus casei*: their role in sugar transport, carbon catabolite repression and inducer exclusion. Mol. Microbiol. **36:**570–584. http://dx.doi.org/10.1046/j.1365-2958.2000.01862.x.

189. **Dossonnet V, Monedero V, Zagorec M, Galinier A, Pérez-Martínez G, Deutscher J.** 2000. Phosphorylation of HPr by the bifunctional HPr kinase/P-Ser-HPr phosphatase from *Lactobacillus casei* controls catabolite repression and inducer exclusion but not inducer expulsion. J. Bacteriol. **182:**2582–2590. http://dx.doi.org/10.1128/JB.182.9.2582-2590.2000.
190. **Monedero V, Kuipers OP, Jamet E, Deutscher J.** 2001. Regulatory functions of serine-46-phosphorylated HPr in *Lactococcus lactis*. J. Bacteriol. **183:**3391–3398. http://dx.doi.org/10.1128/JB.183.11.3391-3398.2001.
191. **Mazé A, Boël G, Zúñiga M, Bourand A, Loux V, Yebra MJ, Monedero V, Correia K, Jacques N, Beaufils S, Poncet S, Joyet P, Milohanic E, Casarégola S, Auffray Y, Pérez-Martínez G, Gibrat JF, Zagorec M, Francke C, Hartke A, Deutscher J.** 2010. Complete genome sequence of the probiotic *Lactobacillus casei* strain BL23. J. Bacteriol. **192:**2647–2648. http://dx.doi.org/10.1128/JB.00076-10.
192. **Monedero V, Yebra MJ, Poncet S, Deutscher J.** 2008. Maltose transport in *Lactobacillus casei* and its regulation by inducer exclusion. Res. Microbiol. **159:**94–102. http://dx.doi.org/10.1016/j.resmic.2007.10.002.
193. **Reizer J, Novotny MJ, Panos C, Saier MH, Jr.** 1983. Mechanism of inducer expulsion in *Streptococcus pyogenes*: a two-step process activated by ATP. J. Bacteriol. **156:**354–361.
194. **Reizer J, Panos C.** 1980. Regulation of β-galactoside phosphate accumulation in *Streptococcus pyogenes* by an expulsion mechanism. Proc. Natl. Acad. Sci. U. S. A. **77:**5497–5501. http://dx.doi.org/10.1073/pnas.77.9.5497.
195. **Thompson J, Chassy BM.** 1982. Novel phosphoenolpyruvate-dependent futile cycle in *Streptococcus lactis*: 2-deoxyglucose uncouples energy production from growth. J. Bacteriol. **151:**1454–1465.
196. **Ye J-J, Minarcik J, Saier MH, Jr.** 1996. Inducer expulsion and the occurrence of an HPr(Ser-P)-activated sugar phosphate phosphatase in *Enterococcus faecalis* and *Streptococcus pyogenes*. Microbiology **142:**585–592. http://dx.doi.org/10.1099/13500872-142-3-585.
197. **Ye J-J, Saier MH, Jr.** 1995. Purification and characterization of a small membrane-associated sugar phosphate phosphatase that is allosterically activated by HPr(Ser(P)) of the phosphotransferase system in *Lactococcus lactis*. J. Biol. Chem. **270:**16740–16744. http://dx.doi.org/10.1074/jbc.270.28.16740.
198. **Cook GM, Ye J-J, Russell JB, Saier MH, Jr.** 1995. Properties of two sugarphosphate phosphatases from *Streptococcus bovis* and their potential involvement in inducer expulsion. J. Bacteriol. **177:**7007–7009.
199. **Ye J-J, Reizer J, Cui X, Saier MH, Jr.** 1994. ATP-dependent phosphorylation of serine-46 in the phosphocarrier protein HPr regulates lactose/H^+ symport in *Lactobacillus brevis*. Proc. Natl. Acad. Sci. U. S. A. **91:**3102–3106. http://dx.doi.org/10.1073/pnas.91.8.3102.
200. **Djordjevic GM, Tchieu JH, Saier MH, Jr.** 2001. Genes involved in control of galactose uptake in *Lactobacillus brevis* and reconstitution of the regulatory system in *Bacillus subtilis*. J. Bacteriol. **183:**3224–3236. http://dx.doi.org/10.1128/JB.183.10.3224-3236.2001.
201. **Hengstenberg W, Egan JB, Morse ML.** 1967. Carbohydrate transport in *Staphylococcus aureus*. V. The accumulation of phosphorylated carbohydrate derivatives, and evidence for a new enzyme-splitting lactose phosphate. Proc. Natl. Acad. Sci. U. S. A. **58:**274–279.
202. **Kundig W, Roseman S.** 1971. Sugar transport. I. Isolation of a phosphotransferase system from *Escherichia coli*. J. Biol. Chem. **246:**1393–1406.
203. **Kundig W, Roseman S.** 1971. Sugar transport. II. Characterization of constitutive membrane-bound enzymes II of the *Escherichia coli* phosphotransferase system. J. Biol. Chem. **246:**1407–1418.
204. **Hengstenberg W, Penberthy WK, Hill KL, Morse ML.** 1969. Phosphotransferase system of *Staphylococcus aureus*: its requirement for the accumulation and metabolism of galactosides. J. Bacteriol. **99:**383–388.
205. **Brockmeier A, Skopnik M, Koch B, Herrmann C, Hengstenberg W, Welti S, Scheffzek K.** 2009. Activity of the *Enterococcus faecalis* $EIIA^{gnt}$ PTS component and its strong interaction with $EIIB^{gnt}$. Biochem. Biophys. Res. Commun. **388:**630–636. http://dx.doi.org/10.1016/j.bbrc.2009.08.100.
206. **London J, Chace NM.** 1979. Pentitol metabolism in *Lactobacillus casei*. J. Bacteriol. **140:**949–954.
207. **Bourand A, Yebra MJ, Boël G, Mazé A, Deutscher J.** 2013. Utilization of D-ribitol by *Lactobacillus casei* BL23 requires a mannose-type phosphotransferase system and three catabolic enzymes. J. Bacteriol. **195:**2652–2661. http://dx.doi.org/10.1128/JB.02276-12.
208. **Zhang ZG, Aboulwafa M, Smith MH, Saier MH, Jr.** 2003. The ascorbate transporter of *Escherichia coli*. J. Bacteriol. **185:**2243–2250. http://dx.doi.org/10.1128/JB.185.7.2243-2250.2003.
209. **Webb AJ, Homer KA, Hosie AH.** 2007. A phosphoenolpyruvate-dependent phosphotransferase system is the principal maltose transporter in *Streptococcus mutans*. J. Bacteriol. **189:**3322–3327. http://dx.doi.org/10.1128/JB.01633-06.
210. **Fieulaine S, Morera S, Poncet S, Monedero V, Gueguen-Chaignon V, Galinier A, Janin J, Deutscher J, Nessler S.** 2001. X-ray structure of HPr kinase: a bacterial protein kinase with a P-loop nucleotide binding domain. EMBO J. **20:**3917–3927. http://dx.doi.org/10.1093/emboj/20.15.3917.
211. **Soufi B, Gnad F, Jensen PR, Petranovi D, Mann M, Mijakovic I, Macek B.** 2008. The Ser/Thr/Tyr phosphoproteome of *Lactococcus lactis* IL1403 reveals multiply phosphorylated proteins. Proteomics **8:**3486–3493. http://dx.doi.org/10.1002/pmic.200800069.
212. **Macek B, Mijakovic I, Olse JV, Gnad F, Kumar C, Jensen PR, Mann M.** 2007. The serine/threonine/tyrosine phosphoproteome of the model bacterium *Bacillus subtilis*. Mol. Cell. Proteomics **6:**697–707. http://dx.doi.org/10.1074/mcp.M600464-MCP200.
213. **Misra SK, Milohanic E, Francine A, Mijakovic I, Deutscher J, Monnet V, Henry C.** 2011. Analysis of the serine/threonine/tyrosine phosphoproteome of the pathogenic bacterium *Listeria monocytogenes* reveals phosphorylated proteins related to virulence. Proteomics **11:**4155–4165. http://dx.doi.org/10.1002/pmic.201100259.
214. **Schmidl SR, Gronau K, Pietack N, Hecker M, Becher D, Stülke J.** 2010. The phosphoproteome of the minimal bacterium *Mycoplasma pneumoniae*: analysis of the complete known Ser/Thr kinome suggests the existence of novel kinases. Mol. Cell. Proteomics **9:**1228–1242. http://dx.doi.org/10.1074/mcp.M900267-MCP200.
215. **Macek B, Gnad F, Soufi B, Kumar C, Olsen JV, Mijakovic I, Mann M.** 2008. Phosphoproteome analysis of *E. coli* reveals evolutionary conservation of bacterial Ser/Thr/Tyr phosphorylation. Mol. Cell. Proteomics **7:**299–307. http://dx.doi.org/10.1074/mcp.M700311-MCP200.
216. **Sun X, Ge F, Xiao CL, Yin XF, Ge R, Zhang LH, He QY.** 2010. Phosphoproteomic analysis reveals the multiple roles of phosphorylation in pathogenic bacterium *Streptococcus pneumoniae*. J. Proteome Res. **9:**275–282. http://dx.doi.org/10.1021/pr900612v.
217. **Lin MH, Hsu TL, Lin SY, Pan YJ, Jan JT, Wang JT, Khoo KH, Wu SH.** 2009. Phosphoproteomics of *Klebsiella pneumoniae* NTUH-K2044 reveals a tight link between tyrosine phosphorylation and virulence. Mol. Cell. Proteomics **8:**2613–2623. http://dx.doi.org/10.1074/mcp.M900276-MCP200.
218. **Park SF, Kroll RG.** 1993. Expression of listeriolysin and phosphatidylinositol-specific phospholipase C is repressed by the plant-derived molecule cellobiose in *Listeria monocytogenes*. Mol. Microbiol. **8:**653–661. http://dx.doi.org/10.1111/j.1365-2958.1993.tb01609.x.
219. **Milenbachs AA, Brown DP, Moors M, Youngman P.** 1997. Carbon-source regulation of virulence gene expression in *Listeria monocytogenes*. Mol. Microbiol. **23:**1075–1085. http://dx.doi.org/10.1046/j.1365-2958.1997.2711634.x.
220. **Herro R, Poncet S, Cossart P, Buchrieser C, Gouin E, Glaser P, Deutscher J.** 2005. How seryl-phosphorylated HPr inhibits PrfA, a transcription activator of *Listeria monocytogenes* virulence genes. J. Mol. Microbiol. Biotechnol. **9:**224–234. http://dx.doi.org/10.1159/000089650.
221. **Mertins S, Joseph B, Goetz M, Ecke R, Seidel G, Sprehe M, Hillen W, Goebel W, Müller-Altrock S.** 2007. Interference of components of the phosphoenolpyruvate phosphotransferase system with the central virulence gene regulator PrfA of *Listeria monocytogenes*. J. Bacteriol. **189:**473–490. http://dx.doi.org/10.1128/JB.00972-06.