Journal of Cellular Biochemistry 51:47–54 (1993)

Gene Regulation by Phosphate in Enteric Bacteria

B.L. Wanner

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

Abstract The *Escherichia coli* phosphate (PHO) regulon includes 31 (or more) genes arranged in eight separate operons. All are coregulated by environmental (extra-cellular) phosphate and are probably involved in phosphorus assimilation. Pi control of these genes requires the sensor PhoR, the response regulator PhoB, the binding protein-dependent Pi-specific transporter Pst, and the accessory protein PhoU. During Pi limitation, PhoR turns on genes of the PHO regulon by phosphorylating PhoB that in turn activates transcription by binding to promoters that share an 18-base consensus PHO Box. When Pi is in excess, PhoR, Pst, and PhoU together turn off the PHO regulon, presumably by dephosphorylating PhoB. In addition, two Pi-independent controls that may be forms of cross regulation turn on the PHO regulon in the absence of PhoR. The sensor CreC, formerly called PhoM, phosphorylates PhoB in response to some (unknown) catabolite, while acetyl phosphate may directly phosphorylate PhoB. Cross regulation of the PHO regulon by CreC and acetyl phosphate may be examples of underlying control mechanisms important for the general (global) control of cell growth and metabolism.

Key words: acetyl phosphate, cross regulation, phosphate control, phosphorus metabolism, protein phosphorylation, two-component regulatory systems

Escherichia coli uses three kinds of phosphorus compounds for growth: inorganic phosphate (Pi), organophosphates, and phosphonates (Pn). When Pi, the preferred phosphorus (P) source, is in excess, Pi is taken up by the low affinity Pi transporter, Pit. Under these conditions, the genes for the high affinity Pi-specific transporter, Pst, and ones for use of alternative P sources are repressed. The latter genes are coregulated as members of the phosphate (PHO) regulon and are induced more than 100-fold during Pi limitation. Altogether 31 genes belonging to the *E. coli* PHO regulon have now been cloned and sequenced. Most of the corresponding gene products have also been characterized. They are transcribed as eight separate genes and operons. All probably have a role in the assimilation of different P sources from the environment (Table I).

Cellular P metabolism is complex. Hence, it is likely that multiple controls may act on the PHO regulon. Basically, the assimilation of any P compound involves two early steps. First, Pi or an alternative P compound must be taken up. And, second, the Pi, or the P in the alternative compound must be incorporated into ATP, the primary phosphoryl donor in metabolism. Eventually, P is incorporated into essential components in membrane lipids, complex carbohydrates such as lipopolysaccharides, and nucleic acids. P also forms high energy bonds and is incorporated into many proteins posttranslationally.

The PHO regulon is controlled by PhoR and PhoB that are similar to the respective partner proteins in the large family of two-component regulatory systems of sensors that act as histidine protein kinases and of response regulators [Stock et al., 1989]. PhoR is the sensor and PhoB is the response regulator, a DNA binding protein that acts as a transcriptional activator. Transcription of PHO regulon genes requires the phosphorylated form of PhoB [Makino et al., 1989]; and controls that regulate the PHO regulon affect the amount of phosphorylated PhoB. Three controls act on the PHO regulon. One control is Pi-dependent and requires the sensor PhoR. Two others are Pi-independent and activate transcription in the absence of PhoR. One Pi-independent control requires the sensor CreC, formerly called PhoM, and the other requires acetyl phosphate [Wanner et al., 1988; Wanner and Wilmes-Riesenberg, 1992].

Pi control of the PHO regulon is a form of transmembrane signal transduction. It is coupled to the first step in P metabolism, Pi uptake. The Pi-independent controls are examples of cross regulation. They appear to be coupled to

Received August 25, 1992; accepted August 25, 1992.

TABLE I. Phosphate-Starvation-Inducible (*psi*) Genes of the *E. coli* PHO Regulon*

Name	Map	Function
phnCDEFGHIJKLMNOP (psiD)	93.3′	Pn uptake and breakdown
phoA (psiA)-psiF	8.7′	Bap, unknown
phoBR	9.0′	Regulator, sensor
phoE	5.8′	Polyanion porin
phoH (psiH)	23.6′	Unknown
psiE	91.5′	Unknown
pstSCAB-phoU	84.0′	Pi uptake, Pi repression
ugpBAECQ (psiB, psiC)	75.8′	G-3-P uptake, phospho-diesterase

*Only sequenced *psi* genes are listed.

subsequent steps in P metabolism. Both may be connected to central metabolic pathways for the incorporation of intracellular Pi into ATP. The PHO regulon and its control are summarized below. See Wanner [1987a], Wanner [1990], and Wanner [1992] for earlier reviews.

THE PHO REGULON AND P ASSIMILATION

Depending upon the external Pi concentration, Pi is taken up by the Pit or Pst transporter [Rosenberg, 1987]. The Pit transporter is a single component, proton motive force-driven transporter similar to LacY [Kaback, 1990]. Pit has a 10 μM K_d, is made constitutively, and is not part of the PHO regulon. Also, Pit has no role in Pi control since *pit* mutations are without effect on gene regulation. In contrast, the Pst transporter is a multicomponent system that is similar to ones for histidine, maltose, ribose, and other bacterial periplasmic transport systems [Landick et al., 1989]. Pst has a 0.8 μM K_d, is made during Pi limitation, and is a member of the PHO regulon. PstS is the periplasmic Pi binding-protein [Luecke and Quiocho, 1990], PstC and PstA are integral membrane proteins, and PstB is the permease. Like permeases for similar binding protein-dependent transporters, PstB shares sequence similarities at the protein level to the products of the mammalian multidrug resistance and cystic fibrosis genes [Hyde et al., 1990]. The Pst system is encoded by an operon that also encodes a protein called PhoU, which has no role in transport. Even a complete deletion of the *phoU* gene is without effect on Pi uptake by the Pst system (PM Steed and BLW, unpublished data). Both Pst and PhoU are required for repression, but not for activation, of the PHO regulon.

Under conditions of Pi limitation, both transportable and nontransportable organophosphates can serve as P sources. *sn*-glycerol-3-phosphate (G-3-P) is taken up by the binding protein-dependent Ugp transporter of the PHO regulon. UgpB is the periplasmic G-3-P binding protein, UgpE and UgpA are integral membrane proteins, and UgpC is the permease. G-3-P transported by the Ugp system may directly enter the biosynthetic pool for membrane phospholipid biosynthesis, without the release of free Pi inside the cell. Glycerophosphoryl diesters (deacylated phospholipids) are also transported by the Ugp system, but they are immediately hydrolyzed by the UgpQ phosphodiesterase, which is closely associated with the Ugp transporter [Brzoska and Boos, 1988].

Nontransportable organophosphates are hydrolyzed in the periplasm by the non-specific phosphomonoesterase bacterial alkaline phosphatase [Bap; Reid and Wilson, 1971; Coleman, 1992], the *phoA* gene product of the PHO regulon, and the Pi released is taken up by the Pit or Pst transporter. Complex P anions probably diffuse into the periplasm through the PhoE (or another) porin, where they are broken down by degradative enzymes such as Bap [Rao and Torriani, 1988]. Also, other periplasmic phosphatases, e.g., an acid phosphatase, AppA, and a glucose-1-phosphate phosphatase, Agp [Dassa et al., 1990], can hydrolyze these and other esters. In addition, some organophosphates, e.g., G-3-P and hexose phosphates, are taken up by other transport systems. However, these other phosphatases and transport systems probably have no role in P assimilation because they are not under PHO regulon control [Wanner, 1990].

Pn are structural analogs of organophosphates that have a direct carbon-phosphorus (C-P) bond in place of the carbon-oxygen-phosphorus ester linkage. Many bacteria can use natural or synthetic Pn as a sole P source under conditions of Pi limitation. The use of Pn as a P source requires breakage of the C-P bond, for which two pathways exist, a phosphonatase pathway and a C-P lyase pathway. These pathways are distinguishable by their strikingly different substrate specificities, which results from differences in the biochemical mechanism for breakage of the C-P bond. The phosphonatase pathway has a narrow substrate specificity, while the C-P lyase pathway has a very broad substrate

specificity. Some bacteria such as *E. coli* and *Salmonella typhimurium* have only one pathway, while other bacteria such as *Enterobacter aerogenes* have both pathways. When cloned into *E. coli,* genes for both pathways are under PHO regulon control [Metcalf and Wanner, 1991; Lee et al., 1992; K-S Lee, WW Metcalf, and BLW, unpublished data].

In *E. coli,* all genes for Pn uptake and degradation are in the fourteen gene *phnC*-to-*phnP* operon, which encodes a binding protein-dependent Pn transporter and a C-P lyase [Wackett et al., 1987; Wanner and Boline, 1990; Chen et al., 1990; Metcalf et al., 1990; Wanner and Metcalf, 1992; WWM and BLW, unpublished data]. The transport system is composed of three proteins: PhnC, PhnD, and PhnE. PhnD is the periplasmic binding protein, PhnE is an integral membrane protein, and PhnC is the permease. The C-P lyase is an apparent membrane-associated enzyme complex composed of nine polypeptides: PhnG, PhnH, PhnI, PhnJ, PhnK, PhnL, PhnM, PhnN, and PhnP. Two other gene products, PhnF and PhnO, have no (apparent) role in uptake or degradation. On the basis of sequence similarities, they may have roles in gene regulation. Actually, Pn utilization is cryptic in the common laboratory strain *E. coli* K-12, though it is functional in most natural strains of *E. coli,* and in many other members of the family *Enterobacteriaceae* [Wanner and Boline, 1990]. The cryptic Pn phenotype for *E. coli* K-12 is due to a frameshift mutation in the *phnE* (EcoK) gene [Makino et al., 1991].

The *phoBR* operon [Wanner and Chang, 1987] is autogenously regulated [Guan et al., 1983]. Thus, the synthesis of the regulatory proteins PhoB and PhoR is under PHO regulon control. On the basis of studies using genetic fusions, the amounts of PhoB and PhoR increase more than 100-fold during Pi limitation. PhoB synthesis is also subject to translational control by a process that may involve down regulation of the *phoBR* operon by an antisense RNA [Wanner, 1990; B-D Chang and BLW, unpublished data]. Increased PhoB synthesis during induction of the PHO regulon is probably responsible, at least in part, for an unusual phenomenon associated with certain regulatory mutants. Null *phoR* mutations [Wanner, 1987b] as well as particular mutations of the *pstSCAB-phoU* operon [Wanner, 1986] lead to a "clonal variation" phenotype in which induction leads to an "induced state." In such mutants, conditions that turn on the PHO regulon lead to the formation of cells in which induction is maintained in the absence of the inducing signal.

Three additional sequenced genes (*psiE, psiF,* and *phoH*) whose functions are unknown are also coregulated as members of the PHO regulon. These genes were originally identified in a set of 55 mutants made with the transposon Mu d1 (*lacZ*) that showed a phosphate-starvation-inducible (*psi*) Lac$^+$ phenotype. The *psiE* gene corresponds to an open reading frame between the *malG* and *xylE* genes near 91.5′. Two mutants had insertions in the *psiE* gene. The *psiF* gene corresponds to an open reading frame immediately downstream of the *phoA* gene, which showed that the *phoA* gene itself was in an operon. One mutant had an insertion in the *psiF* gene. The *phoH* gene corresponds to a new gene, formerly called *psiH* [Metcalf et al., 1990; PMS, WWM, and BLW, unpublished data]. Three mutants had insertions in the *phoH* gene. Also, seven *psiA* mutants had insertions in the *phoA* gene, one *psiB* and one *psiC* mutant had insertions in the *ugpB* gene, and three *psiD* mutants had insertions in the *phnD* gene. Early studies on these and other *psi* genes also showed that several were induced by other stresses, in addition to Pi limitation. For example, the *ugpB* (*psiB* and *psiC*) fusions were induced by both Pi and carbon limitations [Wanner and McSharry, 1982]. In agreement, two laboratories recently showed that the ugpBAECQ operon has two promoters. One is induced by Pi limitation and the other, by carbon limitation [Kasahara et al., 1991; Su et al., 1991].

Pi-REGULATED PROMOTERS

All PHO regulon promoters except one have an 18-base consensus "PHO Box." No PHO Box lies immediately upstream of the *psiE* gene; it may be a distal gene in an operon, however. The in vitro transcription of the *phnC, phoA, phoB, phoH, pstS,* and *ugpB* promoters requires phosphorylated PhoB, which on the basis of DNAse I protection and methylation interference studies binds to the corresponding PHO Boxes (Fig. 1). In vitro studies on the *phoE* and *psiE* genes have not been done. Also, the in vivo mRNA start sites were determined for the *phoA, phoB,* and *pstS* promoters [Makino et al., 1986], for the *phoE* promoter [Tommassen et al., 1987], and for the *phoH* promoter (H. Shinagawa, personal communication). That the PHO Box is required for transcriptional activation in vivo

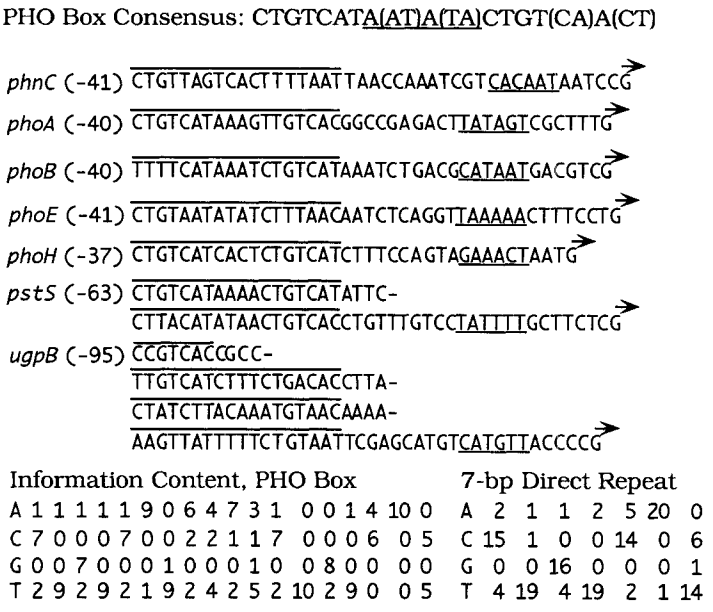

Fig. 1. Sequences of PhoB-activatable promoters. The mRNA start sites were determined for the *phnC* promoter [Makino et al., 1991], the *phoA, phoB,* and *pstS* promoters [Makino et al., 1986], the *phoE* promoter [Tommassen et al., 1987], and the *ugpB* promoter [Kasahara et al., 1991]. Phosphorylated PhoB protects bases −55 to −12 of the *phnC* promoter [Makino et al., 1991], bases −44 to −11 of the *phoB* promoter [Makino et al., 1988], bases −65 to −16 of the *pstS* promoter [Makino et al., 1988], and bases −100 to −14 of the *ugpB* promoter [Kasahara et al., 1991]. The complete DNA sequence of the *phoH* gene, its mRNA start site and protection of bases −41 to −14 of the *phoH* promoter by phosphorylated PhoB were determined by Kim, Makino, Amemura, Shinagawa, and Nakata (H Shinagawa, personal communication). The PHO Box regions are marked with lines above them. The −10 regions are underlined. The information content is described by Schneider et al. [1986]. The −35 region in the PHO Box consensus is underlined.

was shown by studying the effects of 5′ deletions on the expression of the *phnC* promoter (D Agrawal and BLW, unpublished data), the *phoE* promoter [Tommassen et al., 1987], and the *pstS* promoter [Kimura et al., 1989].

The PHO Box in the *phnC, phoA, phoB, phoE,* and *phoH* promoters is composed of two 7-bp direct repeats separated by 4-bp that is part of the −35 region (Fig. 1). The *pstS* promoter has an additional PHO Box that was also protected by PhoB; the *pstS* upstream region contains three additional half sites that were not protected (not shown). The *ugpB* promoter has one additional PHO Box and one additional half site that were protected. Interestingly, the *phoE* promoter has an additional PHO Box in the opposite orientation more than 100 bases upstream of the mRNA start site (not shown). This additional PHO Box may act as a transcriptional enhancer [Tommassen et al., 1987].

Pi CONTROL BY TRANSMEMBRANE SIGNAL TRANSDUCTION

Pi control of the PHO regulon involves two processes: repression by the Pst system, PhoU, and PhoR when Pi is in excess and activation by PhoR when Pi is limiting. That Pi control involves transmembrane signal transduction is evident by the requirement for the Pst transporter. The vast majority of *pst* mutations simultaneously abolish both transport and repression. Missense changes in PstS and PstB, including ones in the nucleotide binding domain of the PstB permease [Cox et al., 1989], abolish both processes. Yet, repression is independent of transport per se because a missense change in PstA abolishes transport without affecting repression [Cox et al., 1988]. Further, Pi repression involves detection of environmental Pi. An extracellular Pi concentration of 4 μM represses PHO regulon gene expression. This corresponds to an amount that would fully saturate the Pi binding-protein PstS (0.8 μM K_d). Derepression by Pi limitation is also not accompanied by a lowering of the internal Pi concentration. Further, PhoU is required for repression, although PhoU has no role in transport. Since PhoU is a product of the *pstSCAB-phoU* operon and is loosely associated with the inner membrane, it is reasonable to suppose that PhoU interacts with the Pst system. An interaction of PhoU

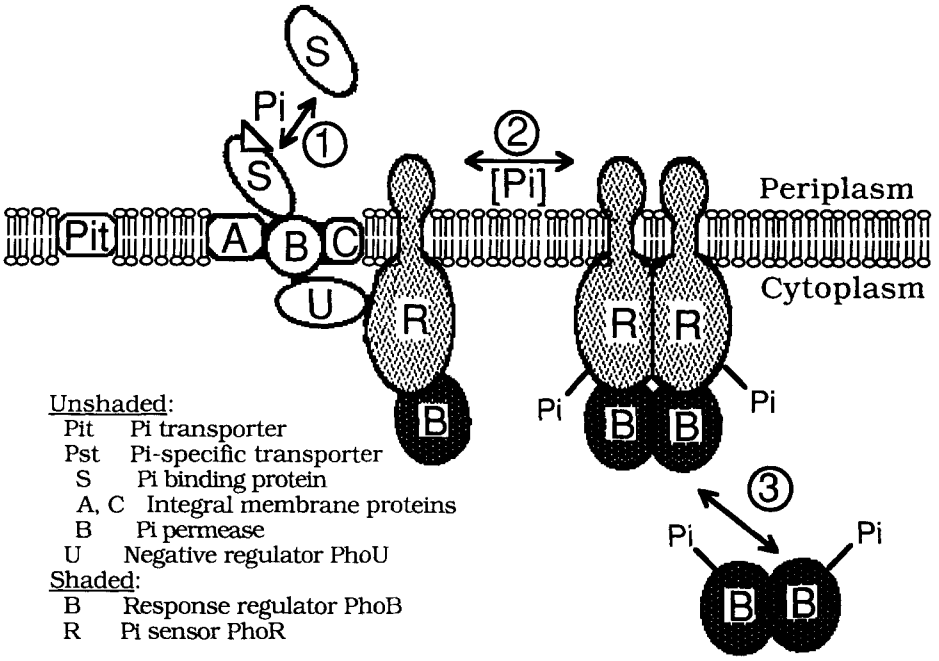

Fig. 2. Pi control of PHO regulon by transmembrane signal transduction. See text.

with both Pst and PhoR may be important in Pi control [Wanner, 1990].

PhoR has a dual role in Pi control. Therefore PhoR probably exists in two forms, a repressor, $PhoR^R$, and an activator, $PhoR^A$, form [Wanner, 1987a]. These two forms may correspond to monomer and dimer forms, though no evidence indicates which is the repressor or activator form. In any case, $PhoR^A$ probably activates PhoB by phosphorylation during Pi limitation and $PhoR^R$ probably inactivates PhoB by dephosphorylation when Pi is in excess. Accordingly, Pi control would involve the interconversion of PhoR between its $PhoR^R$ and $PhoR^A$ forms by a mechanism involving environmental Pi, the Pst system, and PhoU.

A model for Pi transmembrane signal transduction is shown in Figure 2, in which $PhoR^R$ and $PhoR^A$ are arbitrarily indicated as monomer and dimer forms, respectively. This model depicts three events important in Pi repression: saturation of PstS by environmental Pi, binding of Pi bound PstS with PstABC complexes in the membrane, and the formation of a "repressor complex" containing Pi bound PstS, PstABC, PhoU, and PhoR. Such a complex would maintain PhoR in its repressor form that would dephosphorylate PhoB. Accordingly, Pi limitation, or mutations in the Pst system or PhoU, would cause PhoR to be released from the complex. This would lead to its conversion to $PhoR^A$ that would autophosphorylate and would phosphorylate PhoB.

That Pi control probably involves protein-protein interactions with PhoR is supported by its (apparent) domain structures. PhoR is 431 amino acids in length and probably consists of three domains (Fig. 3). It has near its N-terminus a highly hydrophobic region of about 50 amino acids. This segment may form a do-

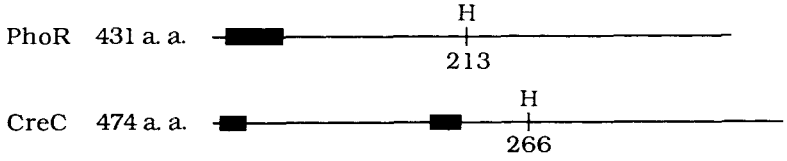

Fig. 3. Simple diagrams for the sensors PhoR and CreC. Filled boxes are hydrophobic segments. The sensor CreC is like many sensors in that it has two transmembrane domains and a short stretch of about 50 amino acids after its second transmembrane domain and before its kinase domain. The histidine residues that are probably phosphorylated are shown. See text.

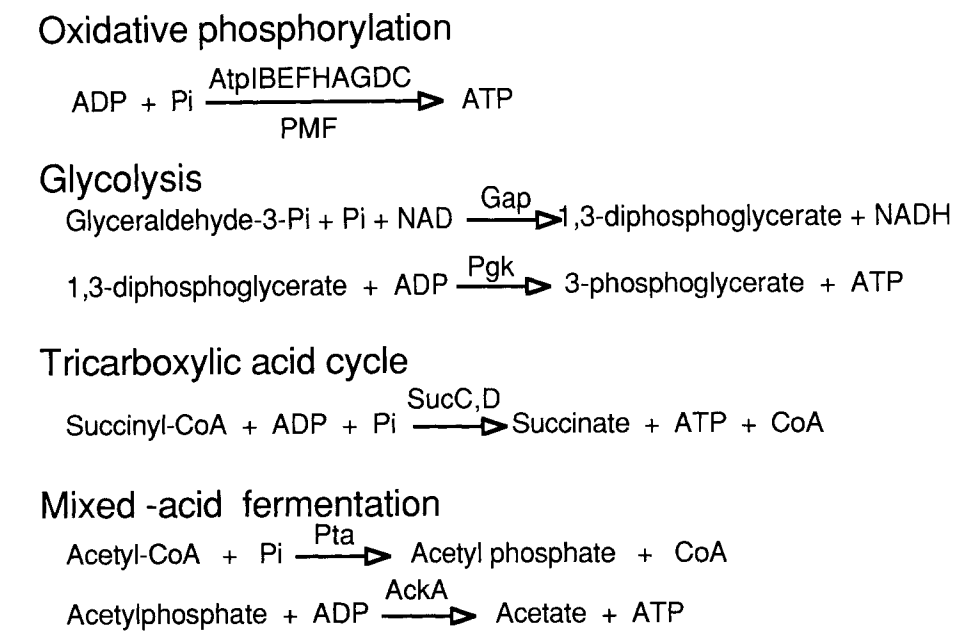

Fig. 4. Pathways for incorporation of Pi into ATP. Gene symbols are used as enzyme names. AckA, acetate kinase; AtpIBEFHAGDC, ATP synthase; Gap, glyceraldehyde 3-phosphate dehydrogenase; Pgk, phosphoglycerate kinase; Pta, phosphotransacetylase; SucC, D, succinyl-CoA synthetase.

main with two α-helices spanning the membrane and a short six or seven amino acid region exposed to the periplasm, or, it may form a domain that is entirely in the membrane. Regardless, the bulk of PhoR is in the cytoplasm and is likely to consist of two additional domains. Many sensors have N-terminal domains followed by regions of about 50 amino acids preceding their kinase domains, e.g., see the structure for CreC in Figure 3. In contrast, PhoR has an unusually large region of about 150 amino acids immediately following its membrane domain. Further, mutational studies imply that this "linker domain" in PhoR is both necessary for repression and important for protein-protein interaction(s). The changes L146P, L147P, and R148C in this region eliminate repression and are partially dominant to the wild-type protein. The dominance of such mutant proteins implies that this region has a role in protein-protein interactions, which may involve interactions with PhoU or PhoR dimerization. The carboxyl terminal domain of PhoR is its kinase domain, in which a conserved histidine, H213, is probably the site of autophosphorylation. The changes T217A, P218L, P218S, T220N, and Y225C in this region also abolish repression (but not activation) and are recessive to wild-type. They may affect the PhoR kinase or phosphatase function (B-DC and BLW, unpublished data).

Pi-INDEPENDENT CONTROLS AND CROSS REGULATION

Pi control is abolished and two Pi-independent controls are apparent in *phoR* mutants. These Pi-independent controls are the basis for cross regulation. That they are likely important in wild-type cells is evident from physiology and mutational studies. Both are regulated by the carbon and energy source, but in different ways. One is induced by glucose and involves the sensor CreC; the other is induced by pyruvate and involves acetyl phosphate synthesis. Both appear to be coupled to steps in central metabolism that are also steps in intracellular Pi metabolism. CreC may phosphorylate PhoB in response to a signal for the incorporation of Pi into ATP by oxidative phosphorylation or substrate-level phosphorylation, in glycolysis or the TCA cycle. Acetyl phosphate may directly phosphorylate PhoB when large amounts are made by substrate-level phosphorylation in mixed-acid fermentation. In this regard, acetyl phosphate is an intermediate in a pathway for the entry of Pi into ATP. Therefore acetyl phosphate synthesis

is formally a pathway for both carbon and Pi metabolism [Fig. 4; Wanner, 1992].

Mutational studies showed that these Pi-independent controls are separate and that they are coupled to central metabolism. CreC is a product of the *creABCD* operon, in which mutations are without effect on control by acetyl phosphate. Conversely, mutations in the *pta* or *ackA* gene, for acetyl phosphate metabolism, are without effect on CreC-dependent control [Wanner et al., 1988; Wanner and Wilmes-Riesenberg, 1992]. Also, mutations in other genes that are likely to affect central metabolism alter Pi-independent control of the PHO regulon involving CreC or acetyl phosphate synthesis. These other genes include ones for aerobic respiratory control (*arcA*), adenylate cyclase (*cya*), cAMP receptor protein (*crp*), isocitrate dehydrogenase (*icd*), malate dehydrogenase (*mdh*), outer membrane regulator (*ompR*), exopolysaccharide production (*ops*), phosphotransferase enzyme I, and the phosphohistidinoprotein HPr (*ptsHI*), and others. Further, there may be a regulatory coupling between genes for acetyl phosphate metabolism and Pi uptake, at least under some growth conditions. Even though *ackA* and *pta* mutations are without effect on the use of Pi as a P source, a mutation that abolishes *ackA* gene expression simultaneously abolishes the use of Pi as a P source [Wanner et al., 1988; Wanner, 1992; MR Wilmes-Riesenberg and BLW, unpublished data]. Accordingly, there may exist a common regulator for genes for acetyl phosphate metabolism and Pi utilization.

PROSPECTS

Many PHO regulon genes have been analyzed in great detail. Yet, the roles of some (*phoH, psiE,* and *psiF* and perhaps others) are not understood. The biochemical mechanism for transcriptional activation of these genes is also fairly well established. In response to Pi limitation, the sensor PhoR autophosphorylates and phosphorylates PhoB that in turn activates transcription by binding to the respective promoter sites. How PhoR detects environmental Pi levels is particularly unclear, although both the Pst transporter and PhoU are known to be required. Somehow the Pst system and PhoU must communicate a signal for Pi repression to PhoR. This process probably involves protein-protein interactions. Future studies on signal transduction in the PHO regulon should include determination of which proteins (PstA, PstB, PstC, PstS, PhoU, and PhoR) interact and how they interact in regards to Pi control.

In addition, the PHO regulon is controlled by two forms of cross regulation that are apparent in *phoR* mutants. Both are connected to carbon and energy metabolism in ways that imply a role for cross regulation in the overall control of the PHO regulon. CreC autophosphorylates and phosphorylates PhoB in response to some (unknown) catabolite, while high levels of acetyl phosphate may directly phosphorylate PhoB. These controls by CreC and acetyl phosphate synthesis may be coupled to different pathways for intracellular Pi metabolism. Regulatory interactions of this sort may be especially important in the coordinate control of cell metabolism and growth in regard to Pi availability. Cross regulation may also be important as a general (global) control in other gene systems regulated by protein phosphorylation, as it seems to be in the PHO regulon. Future studies on cross regulation of the PHO regulon should include determination of the signal for the sensor CreC as well as the role for CreC and acetyl phosphate in wild-type cells.

ACKNOWLEDGMENTS

This laboratory is supported by Public Health Service grant GM35392 from the National Institutes of Health. I especially thank H. Shinagawa for personal communications and W.W. Metcalf for helpful comments on the manuscript.

REFERENCES

Brzoska P, Boos W (1988): Characteristics of a *ugp*-encoded and *phoB*-dependent glycerophosphoryl diester phosphodiesterase which is physically dependent on the Ugp transport system of *Escherichia coli*. J Bacteriol 170:4125–4135.

Chen C-M, Ye Q, Zhu Z, Wanner BL, Walsh CT (1990): Molecular biology of carbon-phosphorus bond cleavage: Cloning and sequencing of the *phn* (*psiD*) genes involved in alkylphosphonate uptake and C-P lyase activity in *Escherichia coli* B. J Biol Chem 265:4461–4471.

Coleman JE (1992): Structure and mechanism of alkaline phosphatase. Annu Rev Biophys Biophys Chem 21:441–483.

Cox GB, Webb D, Godovac-Zimmermann J, Rosenberg H (1988): Arg-220 of the PstA protein is required for phosphate transport through the phosphate-specific transport system in *Escherichia coli* but not for alkaline phosphatase repression. J Bacteriol 170:2283–2286.

Cox GB, Webb D, Rosenberg H (1989): Specific amino acid residues in both the PstB and PstC proteins are required for phosphate transport by the *Escherichia coli* Pst system. J Bacteriol 171:1531–1534.

Dassa J, Marck C, Boquet PL (1990): The complete nucleotide sequence of the *Escherichia coli* gene *appA* reveals

significant homology between pH 2.5 acid phosphatase and glucose-1-phosphatase. J Bacteriol 172:5497–5500.

Guan C-D, Wanner B, Inouye H (1983): Analysis of regulation of *phoB* expression using a *phoB-cat* fusion. J Bacteriol 156:710–717.

Hyde SC, Emsley P, Hartshorn MJ, Mimmack MM, Gileadi U, Pearce SR, Gallagher MP, Gill DR, Hubbard RE, Higgins CF (1990): Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. Nature 346:362–365.

Kaback HR (1990): The *lac* permease of *Escherichia coli:* A prototypic energy-transducing membrane protein. Biochim Biophys Acta Bio-Energetics 1018:160–162.

Kasahara M, Makino K, Amemura M, Nakata A, Shinagawa H (1991): Dual regulation of the *ugp* operon by phosphate and carbon starvation at two interspaced promoters. J Bacteriol 173:549–558.

Kimura S, Makino K, Shinagawa H, Amemura M, Nakata A (1989): Regulation of the phosphate regulon of *Escherichia coli:* Characterization of the promoter of the pstS gene. Mol Gen Genet 215:374–380.

Landick R, Oxender DL, Ames GF-L (1989): Bacterial amino acid transport systems. In Martonosi AN (ed): "The Enzymes of Biological Membranes Second Edition," Volume 3. New York: Plenum Press, pp 577–615.

Lee K-S, Metcalf WW, Wanner BL (1992): Evidence for two phosphonate degradative pathways in *Enterobacter aerogenes.* J Bacteriol 174:2501–2510.

Luecke H, Quiocho FA (1990): High specificity of a phosphate transport protein determined by hydrogen bonds. Nature 347:402–406.

Makino K, Shinagawa H, Amemura M, Nakata A (1986): Nucleotide sequence of the phoB gene, the positive regulatory gene for the phosphate regulon of *Escherichia coli* K-12. J Mol Biol 190:37–44.

Makino K, Shinagawa H, Amemura M, Kimura S, Nakata A, Ishihama A (1988): Regulation of the phosphate regulon of *Escherichia coli:* Activation of *pstS* transcription by PhoB protein in vitro. J Mol Biol 203:85–95.

Makino K, Shinagawa H, Amemura M, Kawamoto T, Yamada M, Nakata A (1989): Signal transduction in the phosphate regulon of *Escherichia coli* involves phosphotransfer between PhoR and PhoB proteins. J Mol Biol 210:551–559.

Makino K, Kim S-K, Shinagawa H, Amemura M, Nakata A (1991): Molecular analysis of the cryptic and functional *phn* operons for phosphonate use in *Escherichia coli* K-12. J Bacteriol 173:2665–2672.

Metcalf WW, Steed PM, Wanner BL (1990): Identification of phosphate-starvation-inducible genes in *Escherichia coli* K-12 by DNA sequence analysis of *psi::lacZ*(Mu d1) transcriptional fusions. J Bacteriol 172:3191–3200.

Metcalf WW, Wanner BL (1991): Involvement of the *Escherichia coli phn* (*psiD*) gene cluster in assimilation of phosphorus in the form of phosphonates, phosphite, Pi esters, and Pi. J Bacteriol 173:587–600.

Rao NN, Torriani A (1988): Utilization by *Escherichia coli* of a high-molecular-weight, linear polyphosphate: Roles of phosphatases and pore proteins. J Bacteriol 170:5216–5223.

Reid TW, Wilson IB (1971): *E. coli* alkaline phosphatase. In Boyer PD (ed): "The Enzymes IV." New York: Academic Press, pp 373–415.

Rosenberg H (1987): Phosphate transport in prokaryotes. In Rosen BP, Silver S (eds): "Ion Transport in Prokaryotes." San Diego, California: Academic Press, Inc., pp 205–248.

Schneider TD, Stormo GD, Gold L (1986): Information content of binding sites on nucleotide sequences. J Mol Biol 188:415–431.

Stock JB, Ninfa AJ, Stock AM (1989): Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol Rev 53:450–490.

Su T-Z, Schweizer HP, Oxender DL (1991): Carbon-starvation induction of the *ugp* operon, encoding the binding protein-dependent *sn*-glycerol-3-phosphate transport system in *Escherichia coli.* Mol Gen Genet 230:28–32.

Tommassen J, Koster M, Overduin P (1987): Molecular analysis of the promoter region of the *Escherichia coli* K-12 *phoE* gene: Identification of an element, upstream from the promoter, required for efficient expression of PhoE protein. J Mol Biol 198:633–641.

Wackett LP, Wanner BL, Venditti CP, Walsh CT (1987): Involvement of the phosphate regulon and the *psiD* locus in the carbon-phosphorus lyase activity of *Escherichia coli* K-12. J Bacteriol 169:1753–1756.

Wanner BL, McSharry R (1982): Phosphate-controlled gene expression in *Escherichia coli* using Mud*1*-directed *lacZ* Fusions. J Mol Biol 158:347–363.

Wanner BL (1986): Novel regulatory mutants of the phosphate regulon in *Escherichia coli* K-12. J Mol Biol 191:39–58.

Wanner BL (1987a): 82. Phosphate regulation of gene expression in *Escherichia coli.* In Neidhardt FC, Ingraham J, Low KB, Magasanik B, Schaechter M, Umbarger HE (eds): "*Escherichia coli* and *Salmonella typhimurium* Cellular and Molecular Biology," Volume 2. Washington, D.C.: Am. Soc. Microbiol., pp 1326–1333.

Wanner BL (1987b): Control of *phoR*-dependent bacterial alkaline phosphatase clonal variation by the *phoM* region. J Bacteriol 169:900–903.

Wanner BL, Chang B-D (1987): The *phoBR* operon in *Escherichia coli* K-12. J Bacteriol 169:5569–5574.

Wanner BL, Wilmes MR, Young DC (1988): Control of bacterial alkaline phosphatase synthesis and variation in an *Escherichia coli* K-12 *phoR* mutant by adenyl cyclase, the cyclic AMP receptor protein, and the *phoM* operon. J Bacteriol 170:1092–1102.

Wanner BL (1990): Phosphorus assimilation and its control of gene expression in *Escherichia coli.* In Hauska G, Thauer R (eds): "The Molecular Basis of Bacterial Metabolism." Heidelberg: Springer-Verlag, pp 152–163.

Wanner BL, Boline JA (1990): Mapping and molecular cloning of the *phn* (*psiD*) locus for phosphonate utilization in *Escherichia coli.* J Bacteriol 172:1186–1196.

Wanner BL (1992): Minireview: Is cross regulation by phosphorylation of two-component response regulator proteins important in bacteria? J Bacteriol 174:2053–2058.

Wanner BL, Metcalf WW (1992): Molecular genetic studies of a 10.9-kbp operon in *Escherichia coli* for phosphonate uptake and biodegradation. FEMS Microbiol Lett 100: in press.

Wanner BL, Wilmes-Riesenberg MR (1992): Involvement of phosphotransacetylase, acetate kinase, and acetyl phosphate synthesis in the control of the phosphate regulon in *Escherichia coli.* J Bacteriol 174:2124–2130.