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# Signal transduction in the control of phosphate-regulated genes of *Escherichia coli*

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Bacteria such as *Escherichia coli* regulate the synthesis of a large number of proteins in response to extracellular inorganic phosphate ($P_i$) levels by activating transcription of co-regulated genes. More than 80 proteins are synthesized in increased amounts in response to $P_i$ limitation in the gram-negative bacterium *E. coli* [1–3]. Many of these proteins are the products of a set of co-regulated genes known as the phosphate (Pho) regulon. Altogether 38 Pho regulon genes have now been characterized (by cloning, sequencing, mutational analysis, and/or examination of their gene products) in *E. coli*, or the closely related bacteria *Enterobacter aerogenes* and *Salmonella typhimurium* [4]. These genes are arranged in eight (or more) transcriptional units, and their gene products probably all have a role in the assimilation of alternative phosphorus (P) sources from the environment. Their transcription requires an upstream activation site (called a Pho Box) and a DNA-binding protein (called PhoB) that acts as a transcriptional activator only when it is phosphorylated (Fig. 1). The level of expression of Pho regulon genes is regulated by the amount of phospho-PhoB.

### $P_i$ control of the Pho regulon involves a "two-component regulatory system"

The activation of Pho regulon genes such as *phoA* (encoding bacterial alkaline phosphatase, Bap) under conditions of $P_i$ limitation requires two proteins: PhoR and PhoB. These proteins are members of the large family of two-component regulatory systems in bacteria. These systems are important in a variety of adaptive responses and signal transduction pathways [5]. All of them are likely to share a common biochemical signaling mechanism in which a sensor kinase (usually membrane-associated, like PhoR) is autophosphorylated on a conserved histidine residue in response to an environmental stimulus (the extracellular $P_i$ level in the case of the Pho regulon) and then transfers the phosphoryl group to a conserved aspartate residue on its partner response regulator (commonly a transcriptional activator, like PhoB).

As many as 50 pairs of these sensor and regulator proteins can exist in a single bacterium. Twenty-five potential signaling kinases and 37 response regulator (receiver domain) proteins have been identified on the basis of the *E. coli* genome sequence, which is now more than 50% known. A few examples of two-component regulatory systems shown to act by a common phosphorylation mechanism are listed in Table 1. Altogether, more than 110 unique proteins with signatures of a sensor kinase (transmitter) domain and 170 with signatures of a receiver domain have been identified on the basis of sequence similarities at the protein level (as determined from searching DNA databases for homologous proteins). Analogous signaling systems exist in eucaryotic cells including *Saccharomyces cerevisiae* [6–8], *Arabidopsis thaliana* [9], and *Neurospora crassa* [10]. In yeast, a two-component system (SLN1 and SSK1) can provide signaling input to a MAP kinase cascade that is responsive to small molecules [7, 11]. MAP kinase cascades are extremely important in signaling; they are key components of convergent signal transduction pathways in mammalian cells [12, 13]. Although histidine phosphorylation has also been recently found in human cells [14], the phosphorylation chemistry of that system is distinctively different from two-component regulatory systems.

### $P_i$ control is a paradigm of a signal transduction pathway in which occupancy of a cell surface receptor(s) regulates gene expression in the cytoplasm

$P_i$ control of the Pho regulon involves two processes: inhibition when environmental $P_i$ is in excess (greater than a few $\mu$M) and activation under conditions of $P_i$ limitation (Fig. 2). It is controlled by the extracellular $P_i$ level. The phosphate-specific transporter (the Pst system) plays a crucial role in the process of inhibition; yet, transport *per se* is not involved. The expression of genes of the Pho regulon is inhibited when environmental $P_i$ (the preferred P source) is in excess (greater than ca. 4 $\mu$M). This inhibition requires an inhibitor form of PhoR (called $PhoR^R$), a protein called PhoU, and all components of the Pst system. By analogy to other two-component regulatory systems, PhoR is thought to act as the $P_i$ sensor. Whether PhoR detects $P_i$ directly (perhaps via a regulatory site on PhoR) or indirectly via an interaction with PhoU (or the Pst transporter) is unknown. The expression of Pho regulon genes is activated 100-fold or more during growth after exhaustion of environmental $P_i$. This activation requires the activator form of PhoR (called $PhoR^A$) and PhoB.

Therefore, $P_i$ control involves the interconversion of $PhoR^R$ and $PhoR^A$. This interconversion is likely to involve protein-protein interactions between PhoR, PhoU, and Pst components and conformational changes brought about in response to the extracellular $P_i$ level. Accordingly, $PhoR^R$ predominates when $P_i$ is in excess and $PhoR^A$ predominates under conditions of $P_i$ limitation. Formation of $PhoR^R$ can result due to association of PhoR with PhoU and the Pst system upon full $P_i$ occupancy of the

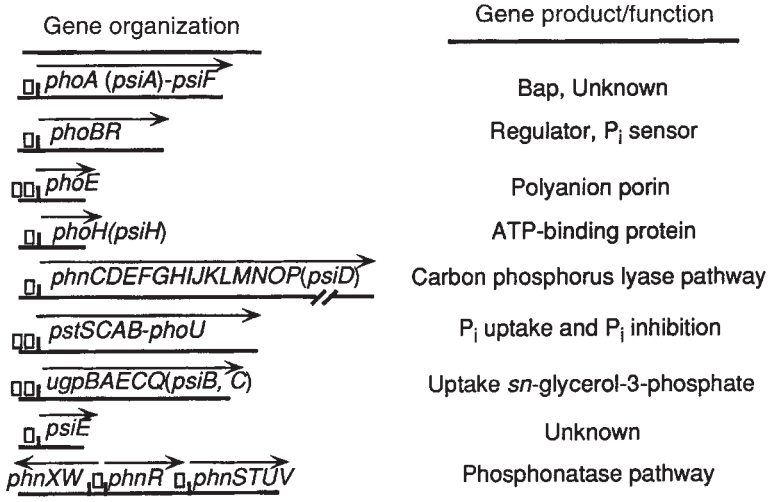


**Fig. 1.** *Organization of sequenced genes of the Pho regulon.* (Adapted from Figure 5 in [3]. Used with permission from the American Society for Microbiology.)

**Table 1.** Examples of two-component regulatory systems

| Regulatory system | Sensor kinase | Response regulator(s) |
|---|---|---|
| Chemotaxis | CheA | CheY, CheB |
| Nitrogen control | NtrB ($NR_{II}$) | NtrC ($NR_I$) |
| Osmoregulation | EnvZ | OmpR |
| $P_i$ control | PhoR | PhoB |
| Catabolite control | CreC (PhoM) | CreB |
| Aerobic respiratory control | ArcB | ArcA |

Pst system, or binding of $P_i$ to a hypothetical regulatory site on PhoR. In the latter case, $P_i$ saturation of the Pst system can facilitate $P_i$ binding to the regulatory site. A conformational change leading to formation of $PhoR^A$ can result due to release of PhoR from the inhibition complex, low $P_i$ occupancy of the Pst system, or low $P_i$ occupancy of a PhoR regulatory site.

### PhoR is a cytoplasmic membrane protein that acts as a sensor kinase

PhoR is a cytoplasmic integral membrane protein of 431 amino acids in length. It appears to be composed of three domains: an N-terminal "membrane-binding domain" and two cytoplasmic domains. Its N-terminal domain of about 50 amino acids has two predicted $\alpha$-helical transmembrane segments capable of forming a helical hairpin with a few amino acids exposed to the periplasm. In agreement, its N-terminal segment is capable of translocating a reporter protein to the cell surface; in-frame fusions of Tn*phoA* [15, 16] to residues 29, 37, and 40, but not to 54 or beyond, produce PhoR'-'PhoA fusion proteins with Bap activity, indicating localization of these hybrid proteins to the periplasm. Full-length PhoR is localized to the cytoplasmic membrane, while a truncated protein (denoted 'PhoR) lacking this domain is cytoplasmic. These 'PhoRs lead to low level activation ("constitutive signaling") of PhoB *in vivo* [17; A. Haldimann and B.L. Wanner, unpublished data]. PhoR is unusual among membrane-associated sensor kinases, for it has no large extracellular (periplasmic) domain. This has been verified by its proteinase K inaccessibility in spheroplasts [16]. Most similar sensor proteins appear to have periplasmic domains longer than ca. 125 residues, or to have multiple membrane-spanning segments.

The cytoplasmic domains of PhoR include an unusually large "linker domain" of about 150 residues following its membrane domain and a C-terminal kinase domain beginning near residue 159 that is highly conserved among family members. Further, in-frame deletions of its linker domain (Δ160 to 167, Δ123 to 167, Δ110 to 157, and Δ160 to 174) lead to low level activation, suggesting that it has a regulatory role [16]. The linker domain can interact with PhoU. A truncated form of PhoR lacking its N-terminal 83 amino acids is radiolabeled with $\gamma$-[$^{32}$P]-ATP *in vitro* on His-213 [18] and phospho-'PhoR rapidly transfers the phosphoryl group to PhoB. It is reasonable to suppose that PhoR facilitates the dephosphorylation of phospho-PhoB, yet no such activity has been demonstrated. Numerous *phoR* missense changes have been characterized, including partially dominant ones (suggestive of oligomerization) and ones causing constitutive signaling. All of these alter residues of its cytoplasmic domain(s) [3].

### The Pho regulon is subject to additional controls that can be forms of "cross regulation"

PhoR is required for inhibition when $P_i$ is in excess. In its absence, two other signaling pathways–involving CreC (formerly called PhoM) or acetyl phosphate–lead to high level activation of PhoB in response to carbon sources [19–21]. Therefore, the Pho regulon appears to be subject to multiple controls, each of which affect the phosphorylation of PhoB or dephosphorylation of phospho-PhoB. Its control by $P_i$ involves detection of the extracellular $P_i$ level and is coupled to the first step in $P_i$ metabolism, $P_i$ uptake. Its additional controls are $P_i$ independent and can be forms of cross regulation. This is because Pho regulon control by CreC and acetyl phosphate (which are independent of each other) involves regulatory couplings between $P_i$, carbon, and energy metabolism. Both are coupled to subsequent steps in $P_i$ metabolism, the entry of $P_i$ into ATP, the primary phosphoryl donor in cellular metabolism. CreC is the catabolite regulatory sensor, which (like PhoR) can phosphorylate PhoB. Acetyl phosphate is an intermediate in the phosphotransacetylase-acetate kinase (Pta-AckA) pathway for $P_i$ incorporation into ATP. Acetyl phosphate can activate Pho regulon gene expression either directly by acting as a chemical phosphorylating agent or indirectly via an unknown signaling histidine kinase.

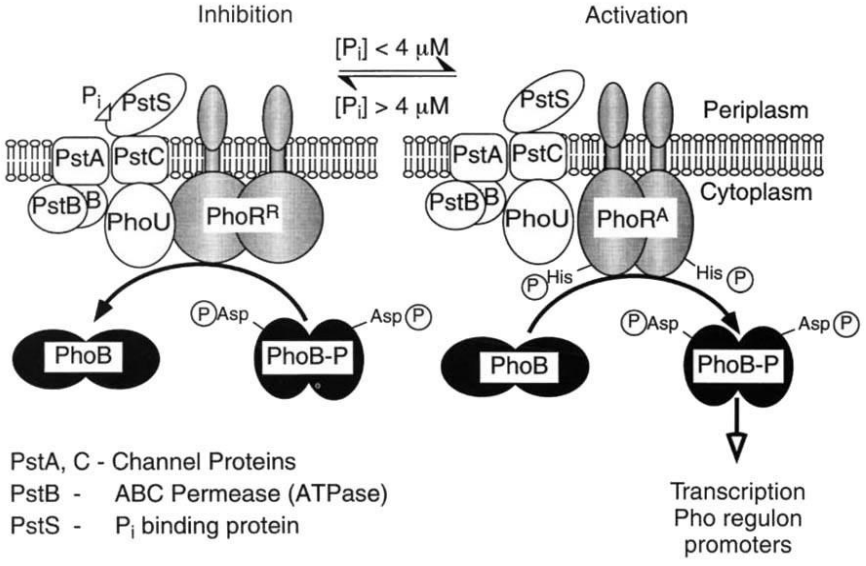


**Fig. 2.** *Transmembrane signal transduction in $P_i$ control of the Pho regulon.* PhoR is thought to exist in two forms; $PhoR^R$ refers to the inhibitor (formerly repressor) form of PhoR and $PhoR^A$ refers to the activator form of PhoR. These were named $PhoR^R$ and $PhoR^A$ long before it was understood how they can act. (Adapted from Figure 4 in [3]. Used with permission of the American Society for Microbiology.)

## Many unanswered questions remain in regard to our understanding of Pho regulon control in *E. coli*

In spite of the wealth of information on the molecular genetics, molecular biology, and biochemistry of the Pho regulon and other signal transduction pathways in bacteria, many questions remain unanswered. Several Pho regulon genes are yet unidentified. Are any of them involved in intracellular $P_i$ metabolism? Both PhoR and the $P_i$ binding protein PstS (see the article by F. Quiocho, this issue) are required for $P_i$ sensing. Does PhoR detect $P_i$ directly? Or, does PhoR "sense" full $P_i$ occupancy of the Pst system only indirectly? An inhibition complex composed of PhoR, PhoU, and the Pst system has been proposed to control the Pho regulon. Do all of these proteins interact? If so, which ones interact directly? How do they interact? Do the cytosolic domains interact? Or, do membrane spanning domains interact within the membrane? In any case, extracellular $P_i$ is detected. How is the signal transmitted across the membrane in order to regulate gene expression in the cytoplasm? Cross regulation can be a form of global control resulting from phosphorylation of PhoB by a nonpartner sensor kinase(s) or acetyl phosphate. Is this kind of regulation of fundamental importance, or does it merely reflect nonspecific biochemistry reactivities between highly similar proteins?

New studies are aimed towards elucidating the answers to these and many other questions. For the sake of brevity, citations are given only for research reported within the past five years. Six recent reviews [2, 3, 21–24] cover these and other aspects of $P_i$ regulated genes of the Pho regulon in more detail. They also include additional citations of the primary research.

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