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Global regulation by the seven-component P_i signaling system

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This review concerns how *Escherichia coli* detects environmental inorganic orthophosphate (P_i) to regulate genes of the phosphate (Pho) regulon by the PhoR/PhoB two-component system (TCS). P_i control by the PhoR/PhoB TCS is a paradigm of a bacterial signal transduction pathway in which occupancy of a cell surface receptor(s) controls gene expression in the cytoplasm. The P_i signaling pathway requires seven proteins, all of which probably interact in a membrane-associated signaling complex. Our latest studies show that P_i signaling involves three distinct processes, which appear to correspond to different states of the sensory histidine kinase PhoR: an inhibition state, an activation state, and a deactivation state. We describe a revised model for P_i signal transduction of the *E. coli* Pho regulon.

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Introduction

How cells respond to environmental (extracellular) signals is of fundamental importance in biology. The control of the *Escherichia coli* phosphate (Pho) regulon by extracellular inorganic orthophosphate (P_i) is of special interest for it serves as a paradigm for a two-component system (TCS) in which signaling is mediated by an ABC (ATP-binding cassette) transporter, the Pst (phosphate-specific transport) system, in the absence of transport.

The *E. coli* Pho regulon is comprised of a large number of genes that are coregulated by environmental P_i, the preferred P source, and that are required for the assimilation of a variety of phosphorus (P) sources for growth. Signal transduction by environmental P_i requires seven proteins, which are thought to interact in a membrane-associated signaling complex. These P_i signaling proteins include: firstly, two that are members of the large family of TCSs, namely the sensory histidine kinase (HK) PhoR (an integral membrane protein) and its partner DNA-binding response regulator (RR) PhoB (a transcription factor); secondly, four components of the ABC transporter Pst; and thirdly, the chaperone-like PhoR/PhoB inhibitory protein called PhoU.

The PhoR HK is required for the activation (phosphorylation) of the PhoB RR under conditions of P_i limitation. Other (nonpartner) HKs, for example, the CreC HK of the CreC/CreB TCS, can also activate (phosphorylate) PhoB, both *in vivo* and *in vitro*. The finding of such interactions has lead to the suggestion that 'crossregulation' can occur between different TCSs, which may play a role in the integration of multiple signals. For example, crossregulation of the PhoR/PhoB TCS may be important for connecting different steps of P_i metabolism [1]. Similar interactions have been seen among nonpartner proteins of other TCSs (e.g. the NarX/NarL and NarQ/NarP TCSs [2•]). DNA microarray studies have provided further evidence for crossregulation among the BaeS/BaeR, PhoR/PhoB, and CreC/CreB TCSs [3]. Other data suggest that crossregulation of the PhoR/PhoB TCS is likely to be even more extensive [4]. Thus, further studies of the Pho regulon can serve as a model for crossregulation among different TCSs.

This review covers the period from when inorganic orthophosphate when P_i control of the *E. coli* Pho regulon was last reviewed in 1996 [1] through 2009. It includes new information on genes controlled by the PhoR/PhoB TCS, crossregulation and stochasticity in the control of P_i-regulated genes, and our current understanding of how environmental P_i regulates the *E. coli* Pho regulon.

The PhoR/PhoB TCS controls genes for phosphorus assimilation

Estimates for the number of P_i-regulated genes vary widely. Proteome profiles of cells grown under P_i excess and limited conditions revealed nearly 400 proteins (almost 10% of the *E. coli* proteome) whose amounts varied in response to the environmental P source [5]. Results from DNA microarray experiments have also shown the number of PhoR/PhoB-regulated genes to be large (Y Jiang *et al.*, unpublished data). These data are consistent with computational predictions of a large number of PhoB-binding sites on the genome [6]. However, in the absence of direct evidence, it is difficult to provide a complete catalog of Pho regulon genes. To date, only 31 genes (9 transcriptional units: *eda*, *phnCDEFGHIJKLMNOP*, *phoA*, *phoBR*, *phoE*, *phoH*, *psiE*, *pstSCAB-phoU*, and *ugpBAECQ*) have been

Table 1

Genes of the *E. coli* K-12 phosphate regulon

Gene	ECK number[a]	Product description[b]	References
amn	ECK1977	AMP nucleosidase	[7]
eda	ECK1851	Aldolase	[8]
phnC	ECK4099	Phosphonate transporter subunit, predicted ATP-binding component	[1]
phnD	ECK4098	Phosphonate transporter subunit, periplasmic-binding component	[1]
phnE	ECK4096	Phosphonate transporter subunit, membrane component	[1]
phnF	ECK4095	Predicted transcription regulator, GntR/HutC family	[1,9]
phnG	ECK4094	Carbon-phosphorus lyase complex subunit	[1]
phnH	ECK4093	Carbon-phosphorus lyase complex subunit	[1,10]
phnI	ECK4092	Carbon-phosphorus lyase complex subunit	[1]
phnJ	ECK4091	Carbon-phosphorus lyase complex subunit	[1]
phnK	ECK4090	Carbon-phosphorus lyase complex subunit, predicted ATP-binding component	[1]
phnL	ECK4089	Carbon-phosphorus lyase complex subunit, predicted ATP-binding component	[1]
phnM	ECK4088	Carbon-phosphorus lyase complex subunit, membrane component	[1]
phnN	ECK4087	Carbon-phosphorus lyase complex subunit, predicted ATP-binding component, ribose 1,5-bisphosphokinase activity protein	[1,11]
phnO	ECK4086	Carbon-phosphorus lyase complex subunit, predicted acyltransferase with acyl-CoA *N*-acyltransferase domain	[1]
phnP	ECK4085	Carbon-phosphorous lyase complex accessory protein, phosphodiesterase activity protein	[1,12]
phoA	ECK0378	Bacterial alkaline phosphatase	[1]
phoB	ECK0393	DNA-binding response regulator	[1]
phoE	ECK0242	Outer membrane phosphoporin protein E	[1]
phoH	ECK1010	Conserved protein with nucleoside triphosphate hydrolase domain	[1]
phoR	ECK0394	Sensory histidine kinase	[1]
phoU	ECK3717	Chaperone-like PhoR/PhoB inhibitory protein	[1,13,14]
psiE	ECK4022	Predicted phosphate starvation-inducible protein E	[1]
psiF	ECK0379	Predicted phosphate starvation-inducible protein F	[1]
pstA	ECK3719	Phosphate transporter subunit, membrane component	[1]
pstB	ECK3718	Phosphate transporter subunit, ATP-binding component	[1]
pstC	ECK3720	Phosphate transporter subunit, membrane component	[1]
pstS	ECK3721	Phosphate transporter subunit, periplasmic-binding component	[1]
ugpA	ECK3436	Glycerol-3-phosphate transporter subunit	[1]
ugpB	ECK3437	Glycerol-3-phosphate transporter subunit, periplasmic-binding component	[1]
ugpC	ECK3434	Glycerol-3-phosphate transporter subunit, ATP-binding component	[1]
ugpE	ECK3435	Glycerol-3-phosphate transporter subunit, membrane component	[1]
ugpQ	ECK3433	Glycerol-3-phosphate transporter subunit, membrane component	[1]
yibD	ECK3605	Predicted glycosyl transferase	[7]
ytfK	ECK4213	Conserved protein	[7]

[a] ECK numbers are in accordance with Riley *et al.* [15].
[b] Product descriptions are in accordance with Riley *et al.* [15], the latest GenBank record for *E. coli* K-12 MG1655 (U00096 dated July 2009), and the EcoGene (www.ecogene.org) and PEC (Profiling of *E. coli* Chromosome [16]; http://www.shigen.nig.ac.jp/ecoli/pec/) databases (December 2009 version).

shown to be directly controlled by the PhoR/PhoB TCS (Table 1). Although strong evidence exists for several others (such as *amn*, *psiF*, *yidD*, and *yibD*), direct evidence for their control by PhoB is lacking. In this regard, expression of the acid-inducible *asr*, which had been previously reported to be transcriptionally controlled by the PhoR/PhoB TCS [17], is now known to be instead regulated by the stationary phase sigma factor RpoS [18]. Earlier interpretations from the same investigators were based on indirect effects of the PhoR/PhoB TCS under conditions of P_i limitation.

The Pst system is the predominant system for P_i uptake

Nearly all genes directly controlled by the PhoR/PhoB TCS have a role in assimilation of P_i or an alternative P source for growth (Table 1). The most strongly activated promoter *pstSp* (for the *pstSCAB-phoU* operon) governs expression of the ABC transporter Pst and PhoU [1]. It had until recently been thought that the Pst system has a role in P_i uptake only under conditions of P_i limitation. A variety of data now show that the Pst system, not the low affinity 'phosphate inorganic transporter' PitA, serves as the primary P_i transporter when P_i is in excess. PitA is unlikely to act primarily as a P_i transporter, but rather as a transporter of divalent metal cations (Zn^{2+}) that are transported in complex with P_i [19]. A primary role for PitA as a Zn^{2+}, and not a P_i, transporter is supported by the finding that *pitA* expression is activated by Zn^{2+}, and not by P_i limitation [20,21]. Likewise, *pitB* [22,23] probably has no role in P_i uptake in normal cells, as it is not expressed under normal growth conditions.

The PhoB RR acts as a transcription factor for Pho regulon promoters

PhoB belongs to the OmpR/PhoB subfamily, the largest group of RRs. PhoB is comprised of an N-terminal receiver domain and a C-terminal DNA-binding domain. Its activity as transcription factor depends upon its state of phosphorylation (D53) of the PhoB receiver domain. Several structures of PhoB have been determined of both its receiver and DNA-binding domains (without and with Mg^{2+} and DNA; www.prfect.org/EcoliProteins), including those of two 'constitutively active' mutants [24–27]. NMR studies have also examined the activation mechanism for receiver domain ([28]; see also [29] in this issue) and the mechanism of DNA binding [30].

The PhoR HK lacks a P_i sensory domain

PhoR acts as the P_i sensory HK and is essential for three distinct processes that control PhoB activity as a transcription factor: inhibition (prevention of PhoB phosphorylation), activation (phosphorylation of PhoB), and deactivation (dephosphorylation of phospho-PhoB). As shown in Figure 1, PhoR is comprised of five domains (or regions). Its N-terminal transmembrane (TM) domain is required solely for the association of PhoR to the membrane. Presumably, membrane localization of PhoR is necessary for interaction with the Pst transporter. PhoR acts as a sensory protein via an interaction between a cytosolic domain of PhoR (possibly its PAS domain; YH and BLW, manuscript in preparation) and the Pst transporter (possibly the ABC component PstB; Figure 2) and/or PhoU.

Crossregulation of Pho regulon by nonpartner HKs

PhoB can also be activated in the absence of PhoR. Activation of PhoB in the absence of PhoR is due to crossregulation (PhoB phosphorylation [1]) by nonpartner HKs such as CreC [31] or small molecule phosphoryl donor(s) such as acetyl phosphate [32]. When PhoR is absent, the nonpartner HKs ArcB, CreC, KdpD, and QseC can lead to moderate activation of PhoB in response to different growth conditions, while the nonpartner HKs BaeS and EnvZ can lead to low level activation [4,33]. It should be noted that these studies were carried out by examining gene expression in cultures, in which gene expression levels reflect only population averages and not the dynamics of gene expression in single cells.

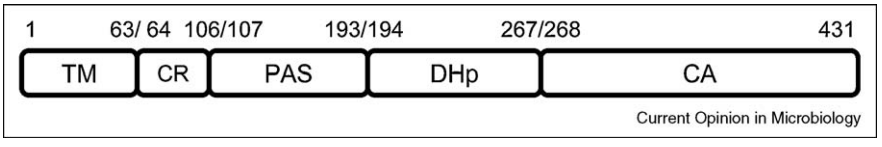
Stochastic expression of the Pho regulon

Single-cell profiling by using flow cytometry to monitor gene expression in single cells has revealed an unforeseen stochastic, 'all-or-none,' character for the activation of PhoB by nonpartner HKs [4]. Modeling has shown that stochastic behavior can result not only from TCSs that have a positive feedback loop (i.e. phospho-PhoB leads to autoamplification of PhoB synthesis) but also from systems in which the rate of HK translation initiation is limited (as appears to be the case for PhoR [34••]). Accordingly, the low amounts of PhoR resulting from low rates of PhoR translation are expected to lead to the formation of occasional cells in a population having no PhoR protein. Activation of PhoB by nonpartner HKs in these cells would lead to stochastic activation of PhoB and to the emergence of multiple stable phenotypes within a population of genetically identical cells. Such behavior at the cellular level is likely to be of fundamental importance not only in the recovery of cells from periods of stress but also in persistence, host–phage interactions and pathogenesis [35–38]. While other TCSs have not been similarly tested for stochasticity, it is reasonable to propose that several are likely to exhibit similar bimodal expression patterns. Two characteristics that appear to be important for stochastic behavior are: firstly, the presence of an autoregulatory loop controlling expression of the TCS and secondly, low translation rates for the HK mRNA [34••].

The Pst transporter is required for P_i signal transduction

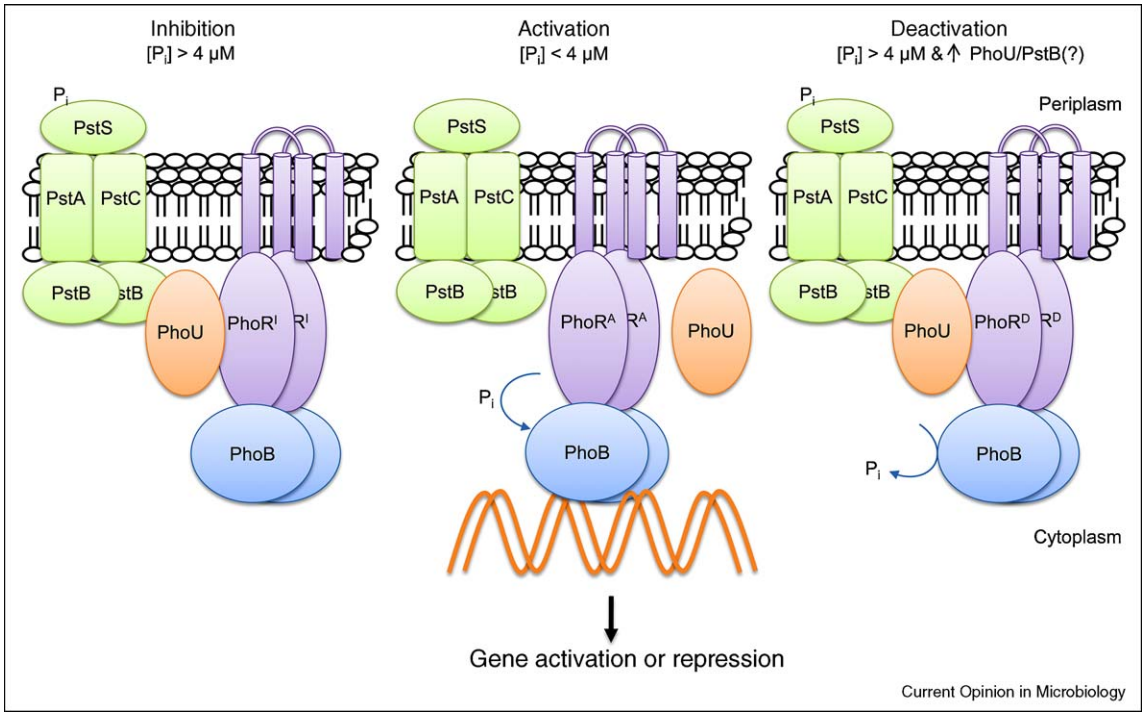
Early studies showed that the Pst transporter is essential for detecting environmental P_i. Also, recent data show that PhoR detects P_i only indirectly (YH and BLW, manuscript in preparation). Further, the Pst system but not P_i uptake *per se* is essential for P_i signaling by the Pst system [1]. By analogy to the ABC (MalEFGK) transporter for maltose [39], we propose that the Pst transporter exists in two distinct states: in one state, the Pst transporter is both transport and signaling active; and in the other, the Pst transporter is both transport and signaling inactive. These states would correspond to closed (transport active) conformation when P_i is bound and an open (resting state) conformation in the absence of bound P_i. Thus, mutations of the Pst system that abolish P_i uptake without affecting P_i signaling block uptake but yet allow formation of the closed and open conformations [1].

Figure 1



Domain organization of PhoR. TM, transmembrane-anchoring domain; CR, positively charged linker region; PAS, Per-Arnt-Sim domain; DHp, dimerization and histidine phosphoacceptor domain; CA, a catalytic domain.

Figure 2



Model for transmembrane signal transduction by environmental P_i. The signaling processes of inhibition, activation, and deactivation are proposed to correspond to different states of PhoR: an inhibition state ($PhoR^I$), an activation state ($PhoR^A$), and a deactivation state ($PhoR^D$). The P_i binding protein PstS is fully saturated when P_i is in excess. Under these conditions, a signal is propagated to PhoR leading to formation of $PhoR^I$, which interferes with phosphorylation of PhoB. No such signal exists under conditions of P_i limitation (or absence of a Pst component), leading to formation of the default state $PhoR^A$ which acts as a phospho-donor for autophosphorylation of PhoB. Following a period of P_i limitation, $PhoR^D$ promotes dephosphorylation of phospho-PhoB. Formation of $PhoR^D$ requires an increased amount of PhoU or PstB in addition to excess P_i.

A model for P_i signaling

Mechanistically, P_i signaling is a negative process. Excess P_i is required for turning the system off. Activation is the default state and results under conditions of P_i limitation. The Pst transporter is essential for inhibition, as well as deactivation [1]. Deactivation resets the PhoR/PhoB system to its inhibition state (Figure 2). That activation (phosphorylation) of PhoB leads to a conformational change in PhoB has been shown by the examination of the structural changes brought about by phosphoryl group analog BeF_3^- [28] and the structure of constitutively active PhoB proteins [27].

Like the Pst transporter, PhoU also has an obligatory role in both inhibition and deactivation of PhoB. The finding that PhoU-like proteins from *Aquifex aeolicus* and *Thermotoga maritima* share structural similarity with proteins belonging to the eukaryotic chaperone Hsp70 family [13,14] supports a chaperone-like role for PhoU. The action of PhoU as an accessory protein is fully compatible with PhoU being a chaperone. Accordingly, PhoU probably acts together with PhoR to promote autodephosphorylation of PhoB-P [40•].

A caveat of P_i signaling by the proposed PhoR/PhoB/PstSCAB/PhoU complex is that individual complexes can exist in different states within a cell. Accordingly, when P_i is in excess, all complexes probably exist in the transport and signaling active state, in which PhoR would be in the inhibition state. Under conditions of P_i limitation, these complexes probably exist in different states within the same cell. That is, under these conditions, some complexes would be in the transport and signaling inactive (PhoR activation) state. Other complexes would be in the transport and signaling active (PhoR inhibition) state. The existence of complexes in both states within the same cell would be necessary to permit simultaneous activation of PhoR/PhoB-regulated genes and growth on limiting amounts of P_i.

Conclusions

Much new information has been learned about the molecular control of the Pho regulon over the past decade, especially with respect to signaling by environmental P_i. Three areas are likely to contribute substantial new information about the Pho regulon and its control in the future (Box 1).

Box 1 Key problems for future studies of the PhoR/PhoB TCS

- The advent of genome-wide mRNA analysis by deep sequencing (RNA-seq) coupled with chromatin immunoprecipitation (ChIP-seq) can provide unprecedented sensitivity and specificity for protein–DNA interactions on a genome-wide scale [41]. Application of such technology to P_i signal transduction should provide comprehensive identification of genes controlled by the PhoR/PhoB TCS.
- Studying single-cell gene expression by the PhoR/PhoB TCS under diverse environmental conditions is likely to provide definitive results regarding the role of crossregulation among different TCSs.
- Studying the different states of the proposed seven-component P_i signaling complex is likely to require development of new technologies that enable examination of single protein complexes inside living cells that are similar to ones now being used to study activities of other machines at the single molecule level [42].

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