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MINIREVIEW

Is Cross Regulation by Phosphorylation of Two-Component Response Regulator Proteins Important in Bacteria?†

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INTRODUCTION

A large family of structurally and functionally similar two-component regulatory systems exists in bacteria. In general, these systems consist of pairs of partner proteins called sensors and response regulators. Sensors are important in signal transduction and share sequence similarities at the protein level. They probably all act as histidine protein kinases that can phosphorylate themselves and as phosphotransferases that can interact with and phosphorylate partner regulators, which also share sequence similarities among themselves. Sensors detect extracellular (environmental) or intracellular stimuli and transfer signals to response regulators by phosphorylation, a process that in turn controls the activity of regulators of chemotaxis and gene expression. In addition, response regulators may receive input signals from different regulatory systems, which in at least one case involves a sensor kinase that is a member of another two-component regulatory system.

"Cross regulation" may be a form of control of response regulators by a signal that does not involve phosphorylation by its partner sensor. Such regulatory interactions may be especially important as a way of directly linking different systems in a network to coordinate cell growth and metabolism. In this minireview I will describe evidence for what may be examples of cross regulation in bacterial two-component regulatory systems. I will primarily describe examples of systems that control gene expression in *Escherichia coli* or *Salmonella typhimurium*. Two-component regulatory systems, signal transduction, and protein phosphorylation in bacteria have been reviewed elsewhere (2, 5, 6, 13, 26, 32, 37, 38).

WHAT IS CROSS REGULATION?

I will use the term cross regulation to refer to the control of a response regulator of one two-component regulatory system by a different regulatory system. By definition, cross regulation must act by controlling the activity of the response regulator; the other regulatory system may or may not also be a two-component regulatory system. By the term cross regulation, I do not intend to imply any particular control mechanism, except that it must involve a control other than phosphorylation by its partner sensor. Cross regulation probably usually does involve phosphorylation, however. This is likely because phosphorylation is the only means that is known to control a response regulator. It may involve the phosphorylation of a response regulator by a nonpartner sensor, it may involve a different covalent modification, or it may involve the binding of an effector molecule.

TWO-COMPONENT REGULATORY SYSTEMS

Primarily on the basis of sequence comparisons at the protein level, 16 (or more) two-component regulatory systems have been inferred to exist in *E. coli* or *S. typhimurium* (Fig. 1). For 14 systems, genes for both partner proteins have been sequenced. One sensor (CheA) is paired with two regulators (CheB and CheY); two sensors (ArcB and CpxA) share a common regulator (ArcA, also called SfrA). No gene for a sensor has been found for Orf2 or TctD. With the exception of CheA-CheB and CheA-CheY, all two-component regulatory systems probably control gene expression. Although no target gene has been identified for CreB (formerly called PhoM-Orf2) or Orf2, both contain structural motifs common to a number of DNA-binding proteins. It is common that the genes for partner sensors and response regulators are linked; in some cases, the genes are part of an operon.

Ten regulators with known target genes are probably transcriptional activators, including NtrC, OmpR, PhoB, ArcA (SfrA), NarL, RcsB, UhpA, PgtA, PhoP, and TctD. Some regulators also act as repressors. NtrC specifically activates the $glnAp_2$ promoter and represses the $glnAp_1$ promoter; ArcA (SfrA) activates the *traY* promoter of the F plasmid (34) and represses genes of aerobic pathways during anaerobic growth; NarL activates the nitrate reductase (*narGHJI*) and formate dehydrogenase-N (*fdnGHI*) operons and represses the fumarate reductase (*frdABCD*) operon (9).

Six regulators (CheB, CheY, NtrC, OmpR, PhoB, and CreB) are phosphorylated in vitro in ATP-dependent reactions catalyzed by their partner sensors. In vitro transcription by NtrC, OmpR, and PhoB requires the phosphorylated regulator. Phosphorylation activates regulators; dephosphorylation inactivates them. Controls may affect either process.

Phosphorylated regulators have very different half-lives, depending on how the regulator is dephosphorylated. Dephosphorylation may or may not involve the partner sensor. In some cases, but not in others, an accessory protein is required. In chemotaxis, CheY but not CheB dephosphorylation is stimulated by a protein called CheZ, while their sensor (CheA) is without effect. In N control, NtrC dephosphorylation is stimulated by its sensor (NtrB), ATP, and a protein called PII (GlnB). In osmoregulation, OmpR dephosphorylation is stimulated by its sensor (EnvZ) plus ATP. In P_i control, PhoB dephosphorylation may require its sensor

† This paper is dedicated to Professor H. E. Umbarger in celebration of his 28 years of studying bacterial physiology at Purdue.

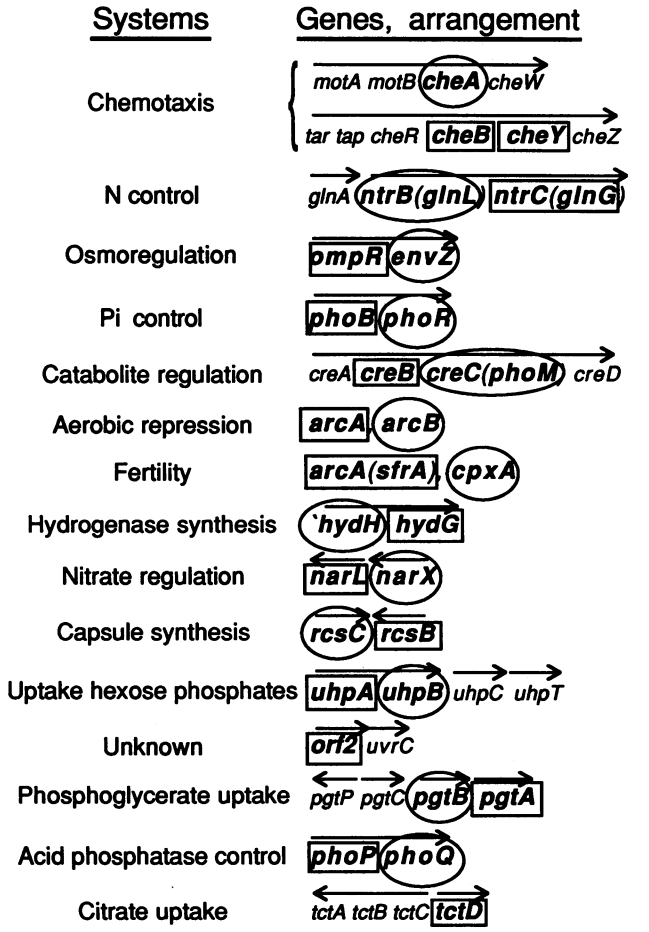

FIG. 1. Two-component regulatory systems in *E. coli* and *S. typhimurium*. Sensors are circled; regulators are boxed. The *cheA*, *cheB*, *cheY*, *ntrB*, and *ntrC* genes were studied in both *E. coli* and *S. typhimurium*; the *ompR*, *envZ*, *phoB*, *phoR*, *creB*, *creC*, *arcA*, *arcB*, *cpxA*, *hydH*, *hydG*, *narL*, *narX*, *rcsC*, *rcsB*, *uhpA*, *uhpB*, and *orf2-uvrC* genes were studied primarily in *E. coli*; the *pgtB*, *phoP*, *phoQ*, and *tctD* genes were studied in *S. typhimurium*. The following systems have been studied: ArcA-ArcB (19, 20), ArcA (SfrA)-CpxA (34), CheA-CheB-CheY (16, 36), CreB-CreC (4, 43), HydH-HydG (39), NarL-NarX (15, 29, 35), NtrB-NtrC (21, 27), OmpR-EnvZ (1, 10, 17), RcsC-RcsB (40), Orf2-UvrC (25, 41), PgtB (48), PhoB-PhoR (22), PhoP-PhoQ (12, 23), TctD (47), and UhpA-UhpB (46). The *arcA* gene and *creABCD* operon are adjacent (3, 8). Only the C terminus of the *'hydH* gene was sequenced (39).

(PhoR) and a protein called PhoU, as indicated by mutant phenotypes (43).

P_i CONTROL AND CROSS REGULATION OF THE PHO REGULON

The best example of cross regulation is in the phosphate (PHO) regulon of *E. coli* (42, 43). The PHO regulon includes the gene, *phoA*, for bacterial alkaline phosphatase (Bap), as well as several other genes for the acquisition (degradation and uptake) of environmental P sources. Extracellular P_i [$P_{i(ext)}$] is the preferred P source for growth; $P_{i(ext)}$ is taken up via the Pit or PstSCAB system. Intracellular P_i [$P_{i(int)}$] is then incorporated into ATP, which is the primary phosphoryl donor in metabolism. This can occur via one of several

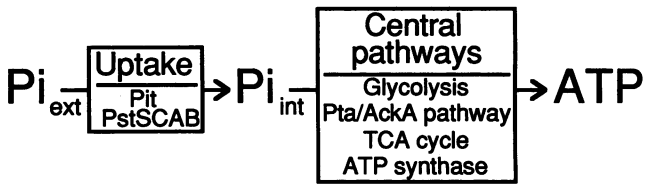

FIG. 2. P_i uptake and assimilation into ATP. P_i is incorporated into ATP via glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase in glycolysis, via the phosphotransacetylase and acetate kinase (Pta-AckA) pathway (except during growth on acetate), via succinyl-coenzyme A synthetase in the tricarboxylic acid (TCA) cycle during aerobic growth, or via the F_1F_0 ATP synthase.

distinct routes in central metabolism (Fig. 2). Which pathway is used depends upon the carbon source, other conditions of growth, and the growth phase. For example, P_i may enter ATP via glycolysis, the tricarboxylic acid cycle, or the ATP synthase during aerobic growth on glucose or via the Pta-AckA pathway during growth on pyruvate.

Three controls act on the PHO regulon (Fig. 3). Two of these involve cross regulation; the way in which they may interact is diagramed in Fig. 4. P_i control involves the PstSCAB transporter and the sensor, PhoR. P_i limitation leads to induction in wild-type cells. Repression is abolished in *pstSCAB* and *phoU* mutants; both induction and repression are abolished in *phoR* mutants, in which two P_i-independent controls become apparent. Each of the latter involves a central pathway in metabolism and is regulated by the carbon source, but in different ways. One control involves the sensor CreC (formerly called PhoM) and is induced during growth on glucose (44). The other is CreC independent and is induced during growth on pyruvate but not during growth on glucose. The latter probably detects acetyl phosphate, an intermediate in the Pta-AckA pathway (Fig. 5). This control may involve an unknown sensor, X, that detects acetyl phosphate and activates PhoB by phosphorylation, or acetyl phosphate may directly activate PhoB via a different mechanism (45).

PHO regulon control by the Pta-AckA pathway is associated with acetyl phosphate synthesis. Conditions expected to cause an accumulation of acetyl phosphate lead to induction; conditions expected to decrease acetyl phosphate synthesis do not (Table 1). Acetyl phosphate is made via Pta and degraded via AckA during growth on glucose or pyruvate; the converse is true during growth on acetate. Much more acetyl phosphate is made during growth on pyruvate, which is (primarily) metabolized via the Pta-AckA pathway, than

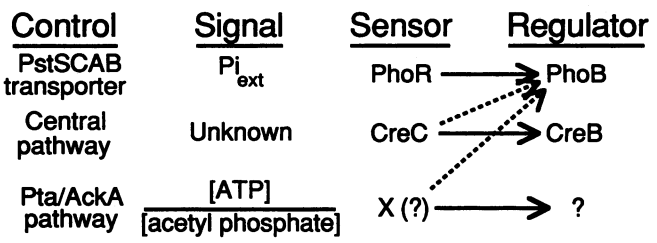

FIG. 3. Three controls on the PHO regulon. Solid arrows show interactions between partner proteins. Dashed arrows show cross regulation between the nonpartner protein CreC and PhoB or between the Pta-AckA pathway and PhoB. The latter which may or may not involve an unknown sensor kinase, X (?).

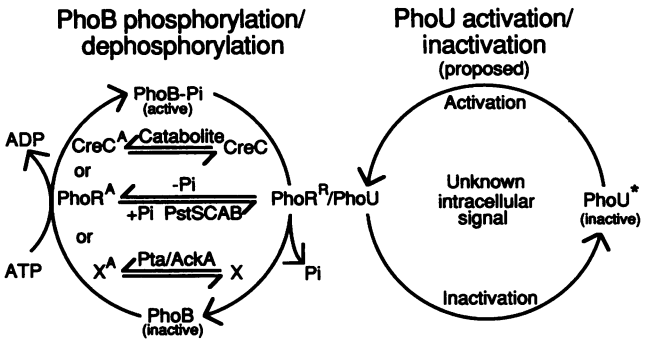

FIG. 4. Controls on the regulator PhoB. Two or more sensors may phosphorylate PhoB when converted into their respective activator forms ($CreC^A$, $PhoR^A$, and a putative one, X^A) in response to different stimuli. PhoB dephosphorylation may involve the repressor form of only one sensor, $PhoR^R$, together with PhoU. An activation-inactivation of PhoU would allow for independent control over PhoB dephosphorylation in the presence of $PhoR^R$. Such a control may be necessary to allow cross regulation by phosphorylation of PhoB in a wild-type cell. PhoU* and X are hypothetical. The mechanism of PhoB activation by the Pta-AckA pathway is not understood (45).

during growth on glucose. Therefore, pyruvate leads to induction via the Pta-AckA pathway, while glucose and acetate do not. By preventing breakdown, an *ackA* mutation leads to induction by glucose but not by acetate. By preventing synthesis, a *pta* mutation abolishes induction by pyruvate; by preventing breakdown, this mutation leads to induction by acetate. All effects due to this control are abolished in a Δ(*pta ackA*) mutant (45).

Even though cross regulation of the PHO regulon involving the sensor CreC or the Pta-AckA pathway is apparent only in *phoR* mutants, cross regulation is likely to play a role in wild-type cells. No effect may have been seen in $phoR^+$ cells because the appropriate gene or growth condition had not been tested. Earlier studies on cross regulation in the PHO regulon concerned primarily *phoA* gene expression during aerobic growth on glucose (42).

IS THERE A TELEOLOGICAL BASIS FOR CROSS REGULATION?

The most compelling evidence that cross regulation is important in wild-type cells is the finding that each control of the PHO regulon appears to involve P_i metabolism. In particular, PHO regulon control by the Pta-AckA pathway is coupled to the incorporation of P_i into ATP via this pathway. This leads to the hypothesis that there is a regulatory link between the uptake of $P_{i(ext)}$ across the cell membrane (which involves PstSCAB, PhoU, PhoR, and PhoB) and the incorporation of P_i into ATP (which involves acetyl phosphate synthesis and PhoB). Cross regulation involving the Pta-AckA pathway may detect the ratio of ATP to acetyl phosphate, with a lowered ratio causing induction. This is expected for a control that involves the synthesis of the end product of a pathway. Accordingly, cross regulation with

FIG. 5. P_i entry into ATP via the Pta-AckA pathway.

TABLE 1. PhoR- and CreC-independent control of the PHO regulon

Additional mutation[a]	Carbon source	Bap phenotype[b]
None	Glucose or acetate	Negative
None	Pyruvate	Induced
ackA	Glucose or pyruvate	Induced
ackA	Acetate	Negative
pta	Glucose or pyruvate	Negative
pta	Acetate	Induced
Δ(*ackA pta*)	Glucose, pyruvate, or acetate	Negative

[a] All cells were *phoR* and Δ*creABCD*.
[b] Induction always led to a 100-fold or greater effect. The fold induction depends on the mutation and the carbon source (45).

CreC, which appears to be linked to a different central pathway, may detect a signal for incorporation of P_i into ATP via glycolysis, the tricarboxylic acid cycle, or the ATP synthase (Fig. 2).

ADDITIONAL EVIDENCE FOR CROSS REGULATION

Since phosphorylation dictates whether a response regulator is functional, the mutational loss of its cognate sensor should impose a null phenotype. Therefore, depending upon whether a regulator is an activator or repressor, sensor mutants are expected to display an uninducible or constitutive phenotype, respectively. Like sensor mutants in the PHO regulon, sensor mutants in N control, osmoregulation, and nitrate regulation do not show null phenotypes (9, 30, 31). Since the control(s) that is seen in such sensor mutants is quite substantial, N control, osmoregulation, and nitrate regulation may also involve examples of cross regulation. Accordingly, nonpartner sensors may also phosphorylate the regulators NtrC, OmpR, and NarL.

Cross regulation involving the regulators NtrC, OmpR, and NarL may also have a physiological basis. Importantly, cross regulation is seen in cells that contain a normal amount of the regulator. In general, experiments were carried out with mutants in which each gene was in single copy. This is an arrangement in which cells are expected to make normal amounts of the regulator, as well as of the (presumed) nonpartner sensor. Cross regulation in these experiments is also controlled in ways that imply an in vivo role. NtrB-independent control of an NtrC-regulated promoter is N regulated (30), EnvZ-independent control of an OmpR-regulated promoter is osmotically regulated (31), and NarX-independent control of NarL-regulated promoters is nitrate regulated (9). Cross regulation is also seen in an *envZ* null mutant when OmpR is overexpressed. In this case OmpR is phosphorylated in vivo and the phosphorylation state is osmotically regulated (11). Thus, there may exist a sensor for osmoregulation which is functionally similar to EnvZ. Unfortunately, it is unclear what specific signals are detected by EnvZ and NarX or what are the (presumed) sensors of cross regulation in these systems.

N control and cross regulation of the regulator NtrC share some common features and also display differences. NH_3 is the preferred N source and is incorporated into glutamine and glutamate (the primary N donors in metabolism) via reduction of 2-ketoglutarate, an intermediate in the tricarboxylic acid cycle. The intracellular ratio of glutamine to 2-ketoglutarate is a signal for N limitation involving the sensor NtrB (and PII); a lowered ratio leads to induction. Although cross regulation is N regulated, induction is much

slower in the absence of NtrB than in its presence (30). Curiously, GltF of the glutamate synthetase (*gltBDF*) operon has been implicated in cross regulation of NtrC (7), but no mechanism has been proposed. GltF is not a sensor as judged by its sequence. It has also been shown that the response of an N-regulated, NtrC-dependent promoter to different environmental stimuli depends on the promoter structure. To account for this, it was proposed that two (or more) sensors may phosphorylate NtrC and that the degree of phosphorylation may determine when a particular promoter is activated (33). Nevertheless, the mechanistic basis of cross regulation of NtrC, like that of OmpR or NarL, is poorly understood. No sensor for cross regulation of NtrC, OmpR, or NarL has been identified.

Is cross regulation of response regulators general? No evidence of cross regulation exists for the regulators ArcA (SfrA), UhpA, PgtA, and PhoP. However, it is possible that cross regulation in these systems may occur under different growth conditions. Unexpectedly, a cell mutated in the sensor for capsule synthesis (RcsC) displays a constitutive phenotype. To account for this, it was suggested that the particular mutation, the *rcsC137* allele, is an allele-specific mutation (40). Alternatively, capsule synthesis in this mutant may be an additional example of cross regulation.

Is cross regulation due to nonspecific interactions? The term "cross talk" has been used to describe interactions between response regulators and nonpartner sensors which may or may not be indicative of cross regulation. The term was first used in 1887 to describe the unwanted transfer of signals from one circuit to another on the telephone. Some cross talk may be due to "noise" which can result from cross-specificities that occur in biochemical reactions in which sensors of similar sequence phosphorylate nonpartner regulators. Such interactions may be insignificant in vivo. In contrast, cross regulation may have evolved as a form of cross talk which confers selective advantages. Regulatory interactions of this sort may be of fundamental importance in global control and would explain why particular sequence similarities have been conserved.

A number of studies have provided evidence that nonspecific as well as specific interactions may occur between nonpartner proteins in vitro. Sensors are able to phosphorylate not only their partner regulators but their nonpartner regulators as well. The phosphorylation of nonpartner regulators by CheA, EnvZ, and NtrB is probably due to cross-specificities. This is because much greater amounts of the nonpartner protein are needed. Also, the rates of phosphotransfer between nonpartner proteins are much slower than those of phosphotransfer between partner proteins in these reactions (18, 28). Interaction between NtrB and CheY because of overexpression of NtrB is possibly also due to cross-specificity (28).

Interactions suggestive of cross regulation are observed in vitro between the sensor CreC and the regulator PhoB. CreC can phosphorylate both its partner CreB and the nonpartner PhoB. Also, even though the rate of phosphotransfer from CreC to CreB is more efficient than that from CreC to PhoB, both CreB and PhoB stimulate dephosphorylation of the sensor CreC (4). In contrast, no dephosphorylation of the sensor was observed in the studies cited above on the phosphorylation of nonpartner regulators by CheA, EnvZ, and NtrB (18, 28). On these grounds, interactions between CreC and PhoB are more likely to reflect cross regulation.

CROSS REGULATION AND CELL METABOLISM

Cross regulation may be especially important in the control of central pathways of energy and carbon metabolism. In this regard, it should be noted that most, and perhaps all, two-component regulatory systems are related to central metabolism (Fig. 1). CheA-CheB and CheA-CheY are connected via the phosphotransferase system (14). NtrB-NtrC and PhoR-PhoB are connected via pathways by which N and P are assimilated. ArcB-ArcA, HydH-HydG, and NarX-NarL are connected via pathways in energy (H and O) metabolism. UhpB-UhpA, PgtB-PgtA, and TctD are connected via transport of substrates (hexose phosphates, phosphoglycerate, and citrate) in central metabolism. RcsC-RcsB is connected via synthesis of polysaccharide. EnvZ-OmpR, CreC-CreB, and PhoQ-PhoP may be related in other ways.

It is also noteworthy that protein phosphorylation involving either two-component regulatory systems or the phosphotransferase system controls pathways for assimilation of C, H, N, O, and P but not for assimilation of S. A repressor that is not modulated by phosphorylation controls genes in S metabolism (24). This is fully consistent with the concept of cross regulation because S is not assimilated via a central metabolic pathway, unlike C, H, N, O, and P.

PERSPECTIVES

In summary, cross regulation may be an important form of global control that links response regulators of two-component regulatory systems to each other or to other general regulatory systems. Its functional basis in the PHO regulon may be to provide a regulatory coupling(s) between P_i uptake and the incorporation of P_i into ATP via the Pta-AckA pathway and other central pathways. There may be a similar basis for cross regulation in other two-component regulatory systems as well.

I have described one viewpoint on cross regulation of response regulators. In this regard, I should point out that the importance of cross regulation is not at all clear. Indeed, some have argued that cross regulation (a form of cross talk) may be unimportant. This is because it has been seen only in null-type sensor mutants that probably lack both the kinase and phosphatase of that sensor. Surely it is conceivable that one reason a sensor may have a phosphatase is to prevent the phosphorylation of its partner regulator by a nonpartner sensor, because of cross-specificities. Alternatively, the phosphatase on a sensor may provide an additional site of regulation. At least two simple conditions may lead to activation of a response regulator by cross regulation in wild-type cells. Cross regulation may occur upon inhibition of the phosphatase, which may be indirect as illustrated in Fig. 4, or upon destruction of the sensor by proteolysis. Many sensors, such as PhoR, are made in very low amounts under conditions of repression (42, 43). They may therefore may be subject to rapid turnover under certain (unknown) growth conditions.

Even though cross regulation in the PHO regulon was first reported 12 years ago, many unanswered questions remain. What is the signal for the CreC-CreB system? What genes are regulated by the CreC-CreB system? What is the mechanism by which acetyl phosphate activates PhoB? Does acetyl phosphate have another regulatory role in the cell? The answers to these and other questions may also show how important cross regulation is in normal bacteria in their natural environment(s).

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