Specificity in Two-Component Signal Transduction Pathways

Michael T. Laub[1] and Mark Goulian[2]

[1]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; email: laub@mit.edu

[2]Department of Biology and Department of Physics, University of Pennsylvania, Philadelphia, Pennsylvania 19104

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Abstract

Two-component signal transduction systems enable bacteria to sense, respond, and adapt to a wide range of environments, stressors, and growth conditions. In the prototypical two-component system, a sensor histidine kinase catalyzes its autophosphorylation and then subseqeuntly transfers the phosphoryl group to a response regulator, which can then effect changes in cellular physiology, often by regulating gene expression. The utility of these signaling systems is underscored by their prevalence throughout the bacterial kingdom and by the fact that many bacteria contain dozens, or sometimes hundreds, of these signaling proteins. The presence of so many highly related signaling proteins in individual cells creates both an opportunity and a challenge. Do cells take advantage of the similarity between signaling proteins to integrate signals or diversify responses, and thereby enhance their ability to process information? Conversely, how do cells prevent unwanted cross-talk and maintain the insulation of distinct pathways? Here we address both questions by reviewing the cellular and molecular mechanisms that dictate the specificity of two-component signaling pathways.

OVERVIEW

Two-component signal transduction systems are one of the most prevalent means by which bacteria sense, respond, and adapt to changes in their environment or in their intracellular state. These signaling systems, comprised of sensor histidine kinases (HK) and their cognate response regulator (RR) substrates, are found in nearly every sequenced bacterial genome, with some encoding as many as 200. The prevalence of these systems underscores their tremendous versatility and their utility to bacteria. These pathways have been implicated in mediating the response of bacteria to a wide range of signals and stimuli, including nutrients, cellular redox state, changes in osmolarity, quorum signals, antibiotics, and more. Studies of specific two-component pathways have revealed the fundamental molecular mechanisms through which these signaling proteins can sense and transduce signals, but how an organism simultaneously coordinates the activity of so many highly-related signaling systems is just beginning to be explored. As histidine kinases and response regulators each comprise paralogous gene families that share significant sequence and structural similarity, there is the potential for considerable cross-phosphorylation, or cross-talk. Yet cells somehow ensure the high-fidelity transmission of signals so that specific stimuli produce the desired, beneficial response. How then do cells avoid unwanted cross-talk? Or, under certain circumstances, do cells exploit cross-talk and the similarity of two-component signaling systems to integrate different signals or to diversify the response to a single signal? Here we review the evidence for and against cross-talk in two-component signaling pathways and describe recent efforts to understand, at the cellular and molecular levels, how specificity in two-component signaling systems is maintained to enable cells to sense, respond, and adapt to changes in their environments.

Receiver domain: the domain of a response regulator that receives a phosphoryl group from a histidine kinase (HK)

Bifunctional histidine kinase: a histidine kinase that also stimulates the dephosphorylation of its cognate response regulator

Hybrid histidine kinase: a histidine kinase containing a phospho-accepting receiver domain, typically at the C-terminal end of the protein. Following autophosphorylation, these kinases usually pass their phosphoryl group intramolecularly to the receiver domain

HPT: histidine phosphotransferase

Phosphorelay: a common architecture for two-component signaling proteins in which a phosphoryl group is sequentially transferred from a histidine kinase to a receiver domain, to a histidine phosphotransferase, and finally to the receiver domain of a soluble response regulator. The histidine kinase and first receiver domain are often fused as a hybrid histidine kinase

INTRODUCTION TO TWO-COMPONENT SIGNAL TRANSDUCTION

In the prototypical two-component signaling pathway, activation of a sensor histidine kinase leads to autophosphorylation on a conserved histidine residue followed by transfer of the phosphoryl group to a cognate response regulator (**Figure 1**). Phosphorylation of the response regulator on a conserved aspartate residue within its receiver domain typically activates an attached output domain. Many different types of output domains exist, but they are frequently DNA-binding domains so that phosphorylation of the response regulator is coupled to changes in transcription. In many cases, histidine kinases are bifunctional and can catalyze both the phosphorylation and dephosphorylation of their cognate response regulator. For bifunctional histidine kinases, input stimuli can regulate either the kinase or phosphatase activity. A common variant of the typical two-component pathway is the so-called phosphorelay (**Figure 1**). These pathways are usually initiated by a hybrid histidine kinase that autophosphorylates and then transfers its phosphoryl group intramolecularly to a response regulator-like receiver domain. The phosphoryl group is then shuttled to a histidine phosphotransferase (HPT) and subsequently to a terminal response regulator, which can evoke the desired response. The general structure, functions, and chemistry of two-component signaling pathways have been previously reviewed (7, 12, 39, 73, 89, 106).

CROSS-TALK, CROSS-REGULATION, AND BRANCHED PATHWAYS

Histidine kinases and response regulators each comprise paralogous gene families and the members of each family share significant homology at both the primary sequence level and the structural level. The similarity

of these signaling proteins raises the possibility of cross-talk between different two-component pathways. Although the existence of such cross-talk has been widely suggested, it is unclear to what extent it occurs in vivo. In many cases, confusion arises from what is meant by the term cross-talk. Here, we define cross-talk as the communication between two pathways that, if eliminated, would leave intact two distinct, functioning pathways (**Figure 2**). In general, cross-talk between distinct pathways must be kept to a minimum, otherwise an organism would not be able to evoke the necessary response to a specific input stimulus. However, under some conditions, it may be advantageous to an organism to permit or use cross-talk as a means of either integrating multiple signals or of diversifying the response to a single input; we refer to such cases, where cross-talk benefits the organism, as "cross-regulation" to distinguish them from detrimental, unwanted cross-talk (100).

We also distinguish cross-talk and cross-regulation from pathways that are inherently, or necessarily, branched, i.e., cases where the topology of a signaling pathway includes one-to-many or many-to-one relationships that are required for an organism to mount a proper response to a given stimulus (**Figure 2**). For instance, chemotaxis in *Escherichia coli* involves a histidine kinase CheA that phosphorylates two response regulators, CheY and CheB. Phosphorylation of each regulator by CheA is necessary for a proper chemotactic response. We would not consider this cross-talk, but rather a one-to-many relationship between kinase and substrates that is physiologically relevant and beneficial to the wild-type organism.

Below we review the evidence for and against cross-talk in a variety of two-component pathways and discuss both the cellular- and molecular-level mechanisms that dictate the specificity of interaction in two-component signaling systems. We focus predominantly on cross-talk and specificity at the

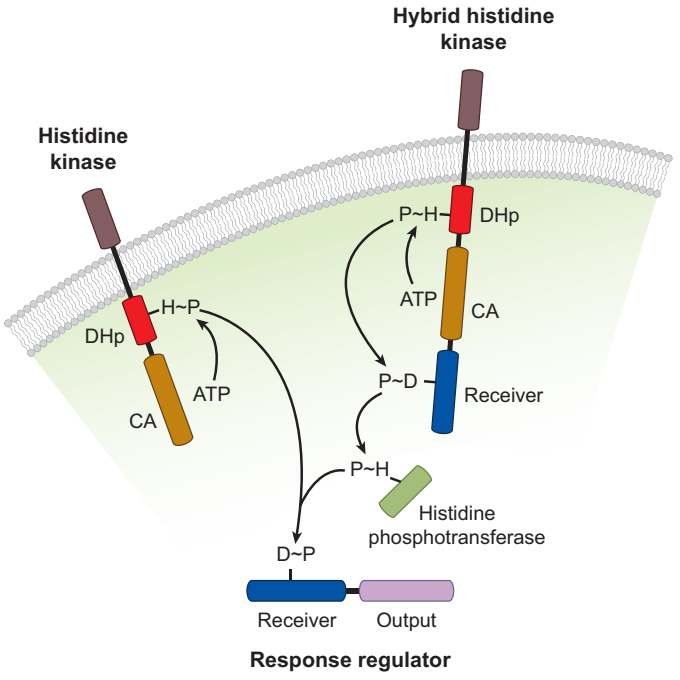

Figure 1

Schematic overview of the two-component signal transduction paradigm and the domain structure of each component. In the prototypical two-component pathway (*left*), a histidine kinase autophosphorylates on a conserved histidine residue with subsequent transfer of the phosphoryl group to a cognate response regulator. Input domains on histidine kinases vary widely and typically do not share substantial homology to one another. The catalytic and ATPase (CA) domain of the histidine kinase is responsible for binding ATP and catalyzing autophosphorylation of a conserved histidine found within the dimerization and histidine phosphotransferase (DHp) domain. The DHp domain mediates homodimerization and serves as the phosphodonor for a cognate response regulator. Many histidine kinases are bifunctional and also dephosphorylate their cognate response regulator. Input signals can stimulate the kinase or phosphatase activity of a histidine kinase. Response regulators typically contain two domains, a receiver domain and an output domain. Receiver domains contain the phosphoacceptor aspartate and several other highly conserved amino acids that catalyze phosphotransfer from a histidine kinase. Output domains, which are activated by phosphorylation of the receiver domain, are varied, but are often involved in binding DNA. Two-component pathways thus often enable cells to sense and respond to stimuli by inducing changes in transcription. Phosphorelays (*right*) are a common variant of the two-component signaling paradigm. Receipt of a stimulus activates autophosphorylation of a hybrid histidine kinase. The phosphoryl group is then passed intramolecularly to a C-terminal receiver domain, similar to that found in response regulators. A histidine phosphotransferase (HPT) then shuttles the phosphoryl group from the hybrid kinase to a soluble response regulator containing an output domain.

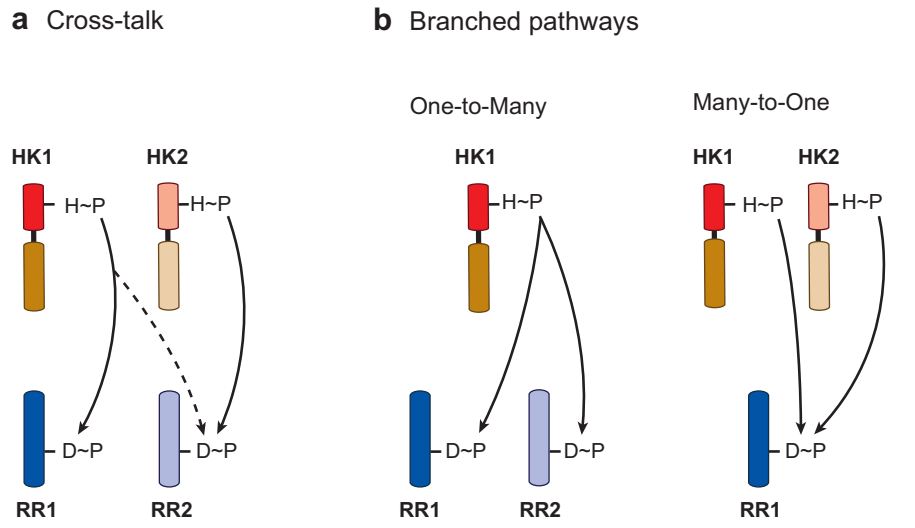

Figure 2

Cross-talk versus branched pathways. The schematics show different possible connectivities of two-component signaling proteins. (*a*) As described in the text, we define cross-talk as the communication between pathways that, if eliminated, would leave intact two distinct pathways. In most cases, cross-talk is probably detrimental to the cell. However, in a few cases cross-talk is beneficial—these situations are referred to as cross-regulation. (*b*) We distinguish both cross-talk and cross-regulation from those pathways that are inherently, or obligately, branched. There are numerous examples of cases where one kinase has multiple bona fide response regulator substrates (one-to-many) or where multiple kinases phosphorylate the same response regulator (many-to-one).

level of phosphotransfer, but also describe interactions between two-component pathways at other levels.

Cross-talk: the communication between pathways that, if eliminated, leaves intact two distinct, functioning pathways

CA: catalytic and ATP-binding

DHp: dimerization and histidine phosphotransfer

Autophosphorylation: the process by which a histidine kinase transfers the gamma phosphoryl group from ATP to a conserved histidine residue

PREVENTING CROSS-TALK IN VIVO – ROLE OF PHOSPHATASES AND SUBSTRATE COMPETITION

In many cases, cross-talk has been seen only after introducing various genetic perturbations and is thus unlikely to be present in, or physiologically relevant to, the wild-type organism. However, these studies help to unveil the cellular mechanisms that ensure the specificity and insulation of two-component signaling pathways. Studies of the PhoR-PhoB and the VanS-VanR two-component signaling systems have been particularly useful in highlighting the mechanisms used by cells to prevent cross-talk (**Figure 3**) (27, 28, 35, 36, 83). The PhoR-PhoB two-component system is endogenous to *E. coli* and allows the organism to sense and respond to changes in phosphate availability. The VanS-VanR two-component system regulates the expression of genes conferring vancomycin resistance in enterococci and other Gram-positive bacteria, but can be expressed and studied in *E. coli*. Cross-talk from the kinase VanS to the response regulator PhoB can occur in *E. coli*, but only in the absence of PhoR (27, 83). Similarly, the kinase PhoR can cross-talk to the response regulator VanR, but only in the absence of VanS (35, 83). In each case, the cross-talk is likely a consequence of eliminating the phosphatase activity normally provided by the other histidine kinase, which is bifunctional (**Figure 3**). In other words, any inadvertent cross-phosphorylation of PhoB by VanS is eliminated by PhoR-dependent dephosphorylation of PhoB, and vice versa for cross-phosphorylation of VanR by PhoR.

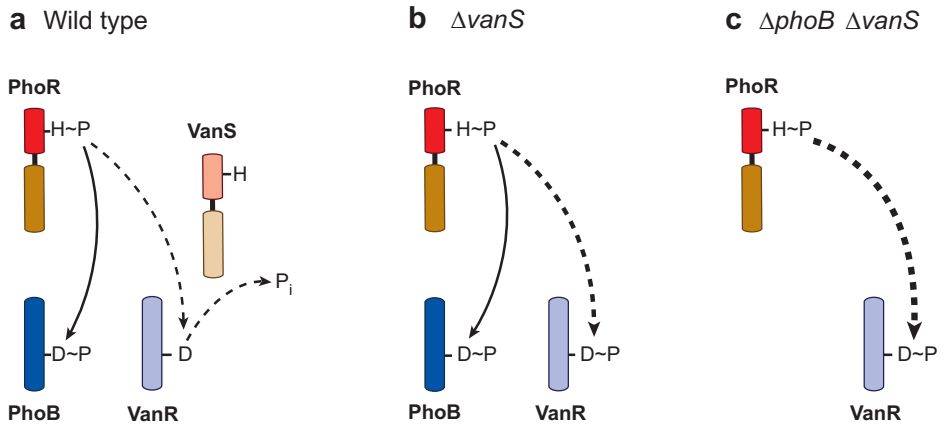

Figure 3

Cellular mechanisms for preventing cross-talk. Studies of the PhoR-PhoB and VanS-VanR two-component pathways in *E. coli* demonstrate two major mechanisms by which cells prevent unwanted cross-talk between different pathways. (*a*) In wild-type cells any inadvertent cross-phosphorylation of VanR~P is eliminated by the phosphatase activity of VanS, thereby preventing the accumulation of phosphorylated VanR. (*b*) Eliminating VanS can thus lead to increased cross-talk, represented by thicker arrows, from PhoR~P (or acetyl-phosphate). (*c*) Competition between response regulators for the phosphodonor PhoR~P also helps prevent cross-talk. PhoR~P has a higher affinity for PhoB relative to VanR, and so preferentially phosphorylates PhoB. Eliminating PhoB therefore leads to even more cross-phosphorylation of VanR.

This mechanism of preventing cross-talk—a bifunctional histidine kinase acting as a phosphatase for its cognate regulator—has been seen in a number of systems and appears to be a general phenomenon. For example, in *E. coli* the histidine kinase CreC (formerly PhoM) can also phosphorylate the response regulator PhoB, but as with VanS, it does so only in the absence of PhoR (5, 101, 103). In a systematic search for cross-talk between four two-component systems in *E. coli*, UhpB-UhpA, NtrB-NtrC, PhoR-PhoB, and ArcB-ArcA, no evidence for cross-talk in wild-type strains was observed (96). However, in a strain lacking the bifunctional histidine kinase NtrB, there was evidence of cross-talk from UhpB and PhoR to the response regulator NtrC. The role of phosphatases in preventing cross-talk was also explored in a mathematical analysis of two-component signaling that compared the behavior of monofunctional (kinase only) and bifunctional histidine kinases (4). At least for the class of models considered in that analysis, bifunctional systems were indeed much more effective at suppressing cross-talk. It was further suggested that monofunctional histidine kinases are therefore more likely to appear in two-component systems where the response regulator integrates inputs from multiple kinases. However, even for systems with monofunctional histidine kinases, there may be additional phosphatases (e.g., CheZ in the case of *E. coli* chemotaxis) that protect against unwanted cross-talk (89).

In addition to the phosphatase activity of bifunctional histidine kinases, there is evidence that response regulator competition also helps limit cross-talk between some signaling pathways. For example, the cross-talk from VanS to PhoB described above is enhanced not just by the absence of PhoR (the cognate kinase/phosphatase for PhoB) but also by the absence of VanR, the cognate response regulator for VanS. The fact that cross-talk was not observed when both response regulators were present suggests that the cognate regulator normally out-competes the

Cross-regulation: communication between distinct pathways that, under certain circumstances, provides a physiological benefit to the organism

Phosphotransfer: the reaction in which a phosphorylated histidine kinase transfers its phosphoryl group to a response regulator

Acetyl-phosphate: a high-energy, low-molecular-weight compound that can serve as a relatively nonspecific phosphodonor to many response regulators

noncognate regulator, aiding in the prevention of cross-talk (**Figure 3**). Similarly, recent studies of the CpxA-CpxR and EnvZ-OmpR systems in *E. coli* have shown that cross-talk from the kinase CpxA to the regulator OmpR requires the absence of both EnvZ and CpxR, whereas, conversely, cross-talk from EnvZ to CpxR requires the absence of CpxA and OmpR (A. Siryaporn & M.G., manuscript in preparation). The effect of competition on cross-talk is enhanced by the low abundance in vivo of histidine kinases relative to response regulators. In *E. coli*, EnvZ and OmpR are present at roughly 100 and 3500 molecules per cell, respectively (20), and similar ratios are observed in other systems (M.T.L, unpublished data). This relatively low kinase concentration minimizes cross-talk to noncognate substrates without affecting the extent of phosphorylation of the cognate response regulator (8; A. Siryaporn & M.G., manuscript in preparation). At least in the case of EnvZ and OmpR, the stoichiometric ratio is probably due, in part, to the overlap between the start codon of *envZ* and the stop codon of *ompR*, along with a weaker ribosome binding site for *envZ*. Many other two-component signaling genes have a similar operon structure, suggesting that histidine kinases may generally be of lower abundance than response regulators, helping to prevent cross-talk.

Consistent with the notion that low concentrations of histidine kinases help to suppress cross-talk to noncognate substrates, many groups have reported cross-talk after a histidine kinase is overproduced. For instance, overproducing a constitutively active variant of the histidine kinase NtrB in a *cheA*$^-$ strain of *E. coli* suppressed smooth swimming behavior, indicating cross-phosphorylation of CheY (69). This result was consistent with in vitro experiments demonstrating that phosphotransfer from NtrB~P to CheY can occur, but at significantly lower efficiency than phosphotransfer from CheA~P to CheY (69). Because overexpression of *ntrB* was required to see activation of CheY in vivo, there is likely minimal cross-talk between these systems in wild-type cells. However, these results and other similar experiments (43, 69) provided early support for the notion that all two-component regulators share a common mechanism of phosphotransfer. Moreover, the ability of some histidine kinases, when overproduced, to cross-phosphorylate noncognate regulators was used as a tool to identify new histidine kinases prior to the era of whole-genome sequences. For example, a screen in *E. coli* for multicopy suppressors of an *envZ* deletion or *phoR creC* double deletion resulted in the identification of numerous histidine kinases, including BarA, BaeS, BasS, and EvgS (1, 66, 67, 68, 95).

CROSS-TALK FROM ACETYL-PHOSPHATE

There is substantial evidence that many response regulators can be cross-phosphorylated by the small molecule phosphodonor acetyl-phosphate [26, 55, 61, 102; see (109) for a comprehensive review]. Acetyl-phosphate concentrations are sensitive to the metabolic state of the cell (109), and it is an attractive idea that this small molecule could function as a global signal that feeds into various two-component systems (61, 100, 109). One recent genetic analysis has provided strong support for a model in which acetyl phosphate regulates flagellar and capsule biosynthesis as a phosphodonor for the Rcs phosphorelay in *E. coli* (29). However, in most cases, the effects of acetyl phosphate on response regulator phosphorylation have only been observed in the absence of the cognate histidine kinase. As with the PhoR-PhoB and VanS-VanR systems described earlier, the phosphatase activity of the cognate bifunctional histidine kinase presumably offsets any inappropriate phosphorylation by acetyl-phosphate. Hence, for wild-type cells grown in conditions that produce high acetyl-phosphate in vivo and that do not activate a given histidine kinase, the kinase's cognate response regulator will not be stably phosphorylated. However, under the same

growth conditions, but in a strain lacking the histidine kinase, acetyl-phosphate can act as an efficient phosphodonor, leading to a phosphorylated response regulator (21, 26, 35, 42). This still leaves open the possibility that acetyl-phosphate plays a role in wild-type cells under conditions in which response regulator dephosphorylation, either from the cognate histidine kinase or from a dedicated phosphatase, is slow, but no such cases have been reported.

EXAMPLES OF CROSS-REGULATION IN VIVO

The cases described above demonstrate that a variety of cellular mechanisms exist to limit cross-talk and to insulate pathways from one another. In each case, cross-talk was observed only after introducing a genetic perturbation, indicating that two-component signaling pathways are usually insulated against cross-phosphorylation. There are, however, a few cases, described below, where cross-phosphorylation between otherwise distinct pathways likely does occur in wild-type cells.

Under anaerobic conditions *E. coli* can respire using nitrate or nitrite as electron acceptors. Regulation of genes necessary for this process are controlled by the NarX and NarQ histidine kinases and the response regulators NarL and NarP (87). NarX is preferentially stimulated by nitrate and phosphorylates NarL. NarQ, on the other hand, is stimulated by both nitrite and nitrate and phosphorylates both NarL and NarP (88). Hence, NarX is required to discriminate between nitrate and nitrite stimuli in the differential regulation of Nar-regulated genes. NarP and NarL share the same DNA binding site consensus sequences, although they bind specific sites with different affinities. NarL can also bind additional sites that are not recognized by NarP (22). This suggests multiple interactions between these two systems at the level of both cross-phosphorylation and differential regulation of promoters in the Nar regulon. The cross-regulation presumably enables a finely tuned response to conditions of high or low nitrate (87).

A recent study has uncovered evidence of cross-regulation between the phosphate-responsive PhoR-PhoP two-component system and the essential YycG-YycF system in *Bacillus subtilis* (40). Transcription of the gene *yocH* is stimulated under conditions of phosphate limitation. This stimulation requires the phosphate sensor kinase PhoR, but does not depend on PhoR's cognate response regulator PhoP. Transcription of *yocH*, however, is sensitive to levels of the response regulator YycF, and PhoR can phosphorylate YycF in vitro. These, and additional results, suggest a model for cross-regulation of YycF by PhoR in response to low phosphate (41).

In *E. coli*, there is evidence for cross-regulation between the ArcB-ArcA and EnvZ-OmpR systems (60). The ArcB-ArcA phosphorelay is a central regulator of genes associated with the switch from aerobic to anaerobic growth. The EnvZ-OmpR system regulates expression of a number of genes, including the porin genes *ompF* and *ompC*. Several lines of evidence support a model of cross-phosphorylation of OmpR by ArcB. Cells grown in anaerobic conditions showed increased expression of the porin OmpC and decreased expression of the porin OmpF, consistent with increased OmpR phosphorylation. These changes in OmpC and OmpF were seen even in the absence of the usual OmpR cognate kinase EnvZ, suggesting that OmpR receives phosphate from an alternative source. Overproducing the histidine phosphotransferase (HPT) domain of ArcB, a tripartite protein containing histidine kinase, receiver, and HPT domains (see **Figure 1**) during aerobic growth also leads to high OmpC and low OmpF, in a manner independent of ArcB's usual cognate response regulator ArcA. Finally, purified ArcB phosphorylates both ArcA and OmpR in vitro, but does not phosphorylate other response regulators such as UvrY or KdpE. Taken together, these results are strongly suggestive of cross-phosphorylation of OmpR

by ArcB. However, the possibility that, in wild-type cells, ArcB affects OmpR activity and porin expression *via* its cognate substrate ArcA has not yet been ruled out. ArcB has also been implicated in cross-phosphorylation of RssB (62), but in vivo evidence to support this conclusion was obtained in cells expressing RssB at approximately threefold higher concentrations than in wild type (50).

Cross-regulation has also been suggested to occur in the regulation of nitrogen metabolism in *Rhodobacter capsulatus* (25). In this organism, the two-component system NtrB-NtrC regulates genes involved in nitrogen assimilation and fixation. Cells lacking the response regulator NtrC are unable to grow with N_2 or urea as a nitrogen source, whereas cells lacking NtrB are able to grow on these compounds. However, cells lacking both NtrB and another histidine kinase, NtrY, are unable to grow on N_2 or urea, indicating that both NtrY and NtrB can phosphorylate NtrC in vivo. NtrY is encoded in an operon with the response regulator NtrX, which suggests that the effect on NtrC, the usual cognate substrate of NtrB, represents cross-regulation. However, the conditions under which NtrY is active and the extent to which it cross-phosphorylates NtrC in wild-type cells is not yet known (25).

INTRINSICALLY BRANCHED PATHWAYS

In addition to these few cases of cross-regulation between distinct pathways, there are numerous examples of inherently, or obligately, branched pathways with "many-to-one" and "one-to-many" relationships. That is, pathways in which multiple histidine kinases phosphorylate a single response regulator or one histidine kinase phosphorylates multiple response regulators, respectively (**Figure 2**). One of the first two-component systems to be discovered, the chemotaxis system in *E. coli*, is an example of one-to-many signaling. In this case, the histidine kinase CheA phosphorylates two response regulators, CheY and CheB [see (6) for a general review]. Phosphorylation of CheY enables it to bind the flagellar motor and control the switching bias between tumbling and straight swimming. Phosphorylation of CheB, on the other hand, enhances its activity as a methylesterase for the chemoreceptors, which is important for precise adaptation. Phosphorylation of both response regulators by CheA is necessary for chemotaxis and thus this signaling pathway is inherently branched. This basic structure of chemotaxis signaling is found quite broadly among bacteria, although in many cases there appear to be even more kinases and response regulators involved, and hence possibly even more branching (90).

There are several well-studied examples of the many-to-one structure in two-component systems. In *B. subtilis*, at least four histidine kinases, KinA–KinD, are able to phosphorylate the response regulator Spo0F, an intermediary in the sporulation phosphorelay [see (46) and references therein]. The phosphoryl group on Spo0F is transferred to the phosphotransferase Spo0B and then to the response regulator Spo0A, which functions as a transcriptional regulator that controls entry into stationary phase and sporulation. Presumably, the large number of kinases enables multiple input signals to control the level of Spo0A∼P. Indeed, it appears that KinC and KinD produce sufficient amounts of Spo0A∼P to initiate entry into stationary phase, but not sporulation, whereas the higher amounts of Spo0A∼P achieved by activation of KinA and KinB activity trigger sporulation (46). A more complete picture awaits determination of the specific stimuli that regulate this phosphorelay, and the roles of the various kinases in responding to each stimulus.

Another well-studied system with multiple histidine kinases converging on the same phospho-transfer pathway is the quorum sensing network in *Vibrio harveyi*. In this case, three distinct hybrid histidine kinases, LuxN,

LuxQ, and CqsS, phosphorylate a single histidine phosphotransferase, LuxU, which in turn phosphorylates the response regulator LuxO (38). The three kinases respond to three distinct autoinducers. For both sporulation of *B. subtilis* and quorum sensing networks in *V. harveyi*, there is evidence that the different sensor kinases activate different programs of gene expression despite converging on a single response regulator. In both systems, it appears that this differential regulation is due to differences in the concentration of phosphorylated response regulator that can be achieved by the different histidine kinases (30, 31, 46, 104). For quorum sensing in *V. harveyi*, it has been proposed that the many-to-one architecture also enables coincidence detection; when each kinase is active, phosphorylated LuxO can accumulate, but when any one kinase is not active, it can function as a phosphatase to prevent accumulation of phosphorylated LuxO (38, 63).

A somewhat different example of a many-to-one branched pathway can be found in the regulation of development in *Caulobacter crescentus*. In this case, the histidine kinases DivJ and PleC each regulate the phosphorylation state of the response regulator DivK, which controls cell cycle progression and cell differentiation (37, 108). DivJ acts mainly as a kinase for DivK, whereas PleC acts predominantly as a phosphatase. In predivisional cells, DivJ and PleC are localized to opposite poles of the cell, the stalked and swarmer poles, respectively (108). Cell division thus produces a stalked cell that phosphorylates DivK and a swarmer cell that dephosphorylates DivK. The many-to-one relationship of these proteins coupled with the differential inheritence of DivJ and PleC is crucial to the establishment of asymmetric *Caulobacter* daughter cells.

An elaborate example of branched regulation of two response regulators by a single histidine kinase has recently been described in *Rhodopseudomonas palustris* (80, 81). The CbbRRS system consists of a histidine kinase CbbSR and two response regulators, CbbRR1 and CbbRR2. These proteins are involved in Rubisco biosynthesis, although neither response regulator has an identifiable DNA binding domain. CbbSR has an autophosphorylation domain and a receiver domain, CbbRR1 has an HPt domain and a receiver domain, and CbbRR2 has two receiver domains. Analysis of various histidine kinase and response regulator mutants in vitro suggests that CbbSR can mediate several different phosphotransfer reactions to the two response regulators. It appears that this enables the system to switch between multiple signaling states. However, the precise outputs controlled by the response regulators, as well as the signal inputs for the histidine kinase, have not yet been determined.

Many more examples of branched regulation will likely emerge as two-component signaling continues to be explored in diverse bacteria. A recent analysis of genes predicted to encode two-component signaling proteins in 207 genomes revealed many instances in which there is a large disparity between the number of response regulators and the number of histidine kinases (3). Assuming these "orphans" do in fact participate in phosphotransfer-mediated signal transduction, a large number of highly branched signaling circuits will likely be found in these bacteria.

KINETIC PREFERENCE IN PHOSPHOTRANSFER PATHWAYS

Although a number of cellular mechanisms, such as phosphatases and substrate competition, help to prevent unwanted cross-talk in vivo, histidine kinases also have an intrinsic ability to discriminate, at a molecular level, cognate substrates from noncognate substrates. The inherent preference of a kinase for its cognate substrate prevents unwanted cross-talk between different pathways. Structural analyses, mutagenesis, and computational approaches have begun to reveal the basis for this discrimination at the level of individual amino acids.

Phosphotransfer profiling: a technique in which a histidine kinase is tested simultaneously and in parallel for the ability to phosphorylate a comprehensive panel of response regulators (RR). This technique allows the rapid mapping of cognate histidine kinase-response regulator pairs

Histidine kinases will phosphorylate a wide range of response regulators in vitro, consistent with the fact that histidine kinases and response regulators comprise paralogous gene families. However, many analyses have demonstrated that histidine kinases exhibit a remarkable kinetic preference in vitro for their exclusive, in vivo cognate substrates. For example, early studies on cross-talk between the NtrB-NtrC and CheA-CheY pathways found that both NtrB and CheA kinases can phosphorylate NtrC, but that NtrB does so more efficiently in vitro (69). Similar results were also observed in comparisons of OmpR phosphorylation by EnvZ and CheA, and of NtrC phosphorylation by NtrB and EnvZ (43). Detailed analysis of the two-component signaling proteins controlling sporulation in *B. subtilis* demonstrated that the histidine kinase KinA can phosphorylate either Spo0F or Spo0A, but with a more than 50,000-fold preference for Spo0F (19, 33). In fact, identifying this kinetic preference was a crucial step in establishing the order of phosphoryl group flow in this pathway, ultimately leading to the delineation of the first multi-component phosphorelay, a common pathway architecture for two-component signaling molecules (see **Figure 1**). Similarly, studies of the vancomycin resistance signaling pathway in enterococci demonstrated that the histidine kinase VanS has a 10,000-fold preference for phosphorylation of its in vivo cognate substrate VanR relative to the noncognate substrate PhoB from *E. coli* (27, 28). Detailed kinetic studies of the phosphorelay controlling the osmolarity response in *Saccharomyces cerevisiae* extended these observations to histidine phosphotransferases, showing that YPD1 preferentially transfers a phosphoryl group to SSK1 relative to SKN7, consistent with observations that SSK1 is the preferred in vivo target of YPD1 (44).

More recently, these observations of kinetic preference in two-component pathways have been extended to a global, system-wide level using a technique called phosphotransfer profiling (15, 84). In this assay, a purified histidine kinase is autophosphorylated and then tested, simultaneously and in parallel, for phosphotransfer to each response regulator encoded in a genome of interest (**Figure 4*a***). As many organisms encode 30 or more response regulators, it is not feasible to precisely measure the kinetic constants for each possible HK-RR pair. However, by examining phosphotransfer from an autophosphorylated histidine kinase to each response regulator at even two or three time points, the kinetically preferred substrates are usually easily identified (**Figure 4*b***). This system was tested using several well-characterized HK-RR pairs that are encoded in operons, including EnvZ-OmpR, CpxA-CpxR, and CheA-CheY from *E. coli*. In each case, the histidine kinase exhibited a strong kinetic preference in vitro for its in vivo cognate substrate over all other response regulator substrates. This assay was similarly applied to two-component pathways in *C. crescentus*. For branched pathways, where a histidine kinase has two bona fide substrates in vivo, the phosphotransfer profiling demonstrated an equivalent preference for each substrate in vitro relative to all other possible substrates. An earlier system-wide analysis of phosphotransfer relationships was conducted in *E. coli*, but in that study phosphotransfer was only examined at a single time point, precluding an assessment of kinetic preference (111). The correspondence between in vitro and in vivo cognate pairings in two-component pathways in different organisms indicates that kinetic preference is likely to be a universal property of two-component signaling in all organisms. This further suggests that phosphotransfer profiling can be used to systematically map the cognate substrates of orphan kinases, those that are not encoded in an operon along with their cognate substrate. In contrast to *E. coli*, many organisms such as *C. crescentus* encode large numbers of orphan kinases; phosphotransfer profiling has proved valuable in mapping their cognate regulators (14, 15, 84).

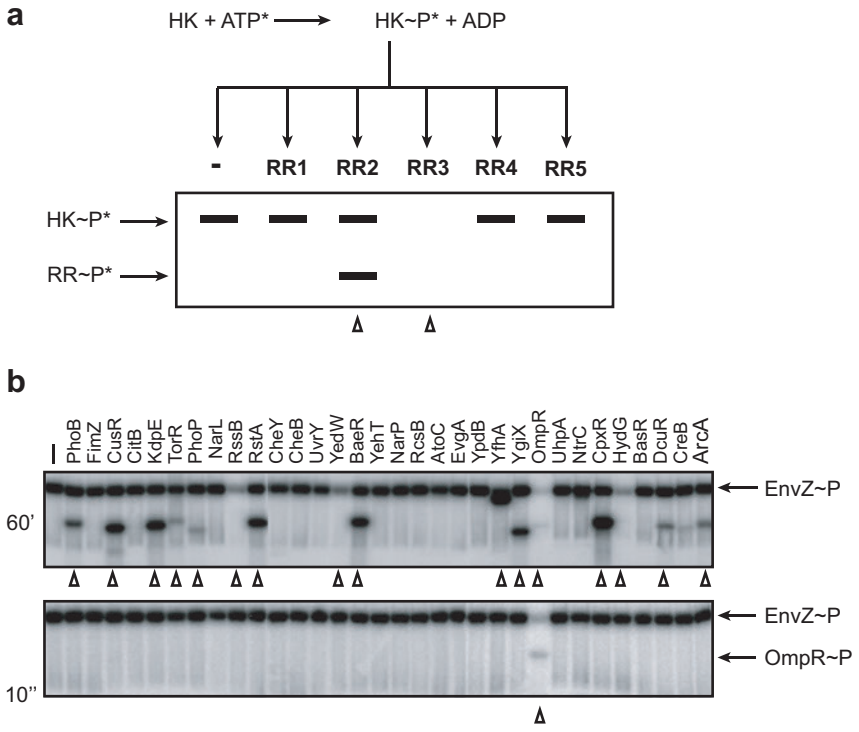

Figure 4

System-wide kinetic preference of phosphotransfer in vitro. (*a*) Schematic of phosphotransfer profiling, a technique for assessing the global substrate preference of a given histidine kinase (84). The cytoplasmic portion of a histidine kinase is purified and incubated in vitro with radiolabeled ATP, leading to autophosphorylation. Phosphorylated histidine kinase is then incubated alone or individually with one of the response regulators from a genome of interest. Each reaction is examined for phosphotransfer by electrophoresis and autoradiography. In the absence of a response regulator, a single band is seen corresponding to the autophosphorylated histidine kinase. Phosphotransfer to a response regulator is manifested by the appearance of a band corresponding to the response regulator or by disappearance of the autophosphorylated histidine kinase band. (*b*) Example of using phosphotransfer profiling to identify the cognate response regulator for the histidine kinase EnvZ from *E. coli*. With 60 minute phosphotransfer incubations, EnvZ phosphorylates at least 16 response regulators (marked with *open arrowheads*), but with 10 second incubations, EnvZ manifests a strong preference for OmpR, its in vivo cognate partner.

MOLECULAR RECOGNITION IN TWO-COMPONENT SIGNALING PATHWAYS

The exquisite biochemical selectivity of histidine kinases in vitro means that the specificity of two-component signaling pathways is dictated primarily at the level of molecular recognition. While other mechanisms such as scaffolds may exist in vivo, the primary determinants of specificity are intrinsic to the molecules involved and independent of additional factors. To date, there has been no atomic-level structure solved for a histidine kinase in complex with a cognate response regulator. However, through structural analysis of the individual components and a combination of mutagenesis and computational approaches, a picture is beginning to emerge of how kinases accurately discriminate between cognate and noncognate response regulators.

It should be noted that, although a histidine kinase catalyzes its autophosphorylation, phosphotransfer is catalyzed primarily by amino acids in the response regulator. The evidence for this comes from numerous studies showing that response regulators can be phosphorylated by small molecules, such as acetyl-phosphate and carbamoyl-phosphate, which have no catalytic capacity. The specificity of phosphotransfer, however, requires proper pairing of amino acids on both histidine kinases and response regulators. Below, we summarize the attempts to identify these amino acids in both molecules and the recent progress made in mapping the molecular basis of specificity in two-component signaling pathways.

One of the first insights into specificity at a molecular level came from the study of a functional chimeric receptor in which the periplasmic domain of the aspartate chemoreceptor Tar from *E. coli* was fused to the cytoplasmic domain of the kinase EnvZ (94). This chimera, dubbed Taz, induced transcription of the OmpR-regulated gene *ompC* when aspartate was added to the growth media. This, along with studies of similarly constructed chimeras (11, 75), demonstrated that the cytoplasmic portions of histidine kinases dictate their specificity, with no contribution from the periplasmic and transmembrane domains. These studies also suggest that the mechanism of transducing a signal across the membrane is conserved between chemoreceptors and histidine kinases. Finally, these studies with chimeras demonstrate that sensor histidine kinases are inherently modular, opening the door to rational design of kinases with novel sensing capabilities.

The cytoplasmic portion of all histidine kinases contains two highly conserved domains: (*i*) an N-terminal dimerization and histidine phosphotransfer (DHp) domain that contains the histidine residue subject to autophosphorylation in a conserved region called the H-box and (*ii*) a C-terminal catalytic and ATP-binding (CA) domain that catalyzes transfer of the gamma phosphoryl group from ATP to the H-box. Structures of several CA domains have been solved, revealing a common mixed α/β-sandwich fold (13, 56, 85). Structural studies of the DHp domain demonstrated that it dimerizes to form a four-helix bundle (91, 92). The phosphorylatable histidine lies in a solvent exposed position within the first alpha helix of the DHp domain. Recently, the first structure of an entire cytoplasmic portion of a histidine kinase was solved (57).

Of these two conserved domains, a variety of studies indicate that the DHp domain dictates the specificity of phosphotransfer to response regulators. The histidine kinase EnvZ still functions as an auto-kinase in vitro when the DHp and CA domains are purified and incubated as separate polypeptides, and the phosphorylated DHp domain can still transfer a phosphoryl group to OmpR (72). These findings suggest that the specificity of phosphotransfer, with respect to the histidine kinase, is determined primarily by the DHp domain. However, purified EnvZ DHp domain was tested only for phosphotransfer to the cognate substrate OmpR, and not to noncognate substrates. Additional evidence for the DHp domain as the main arbiter of specificity for the histidine kinase has come from several yeast two-hybrid screens in which response regulators used as bait have isolated interacting clones that contain only the DHp domain of a cognate histidine kinase (58, 59, 70, 82). Further, NMR titration experiments highlighted a region at the base of the EnvZ DHp domain's four-helix bundle that likely interacts with its cognate response regulator OmpR (92).

Response regulators are typically comprised of two domains, a receiver domain and an output domain. As noted earlier, the output domains do not form a single, paralogous family. The receiver domains, by contrast, do comprise a large, paralogous family with highly similar structures [reviewed in (89)]. Several amino acids are completely conserved among all response regulators, including the phosphorylation site aspartate. The receiver

domain dictates the specificity of interaction with histidine kinases as chimeric response regulators behave according to the identity of their receiver domain (2, 17, 40, 79, 99).

Despite the elucidation of structures for many individual proteins and domains involved in phosphotransfer from histidine kinases to response regulators, there is not yet an atomic-level structure of the two domains in complex. There are, however, two structures of receiver domains in complex with cognate histidine phosphotransferases, the YPD1:SLN1 complex from *S. cerevisiae* (110) and the Spo0F:Spo0B complex from *B. subtilis* (112). As histidine phosphotransferases form four-helix bundles similar to those found in the DHp domains of histidine kinases (105, 110, 112), the structures of them in complex with response regulators provide excellent insight into the specificity of phosphotransfer reactions at a detailed, atomic level. The structure of the Spo0F:Spo0B complex from *B. subtilis* has been particularly helpful because the phosphotransferase Spo0B dimerizes in a similar manner to histidine kinases and is generally considered a suitable proxy for the DHp domain of histidine kinases (**Figure 5**). In support of this notion, the four-helix bundle structure of Spo0B looks remarkably similar to the DHp domain from *Thermotoga maritima* HK853, suggesting that the structure of the Spo0F:Spo0B complex will be representative of histidine kinase-response regulator interactions in general (57). In the Spo0F:Spo0B complex, the response regulator Spo0F docks primarily to the base of the four-helix bundle of Spo0B, placing the active site aspartate of Spo0F within 5 Å of the phosphorylation site histidine on Spo0B (**Figure 5**). Spo0F makes additional contacts with the C-terminal α/β domain of Spo0B. However, as described earlier, the DHp domain of a histidine kinase is likely sufficient to dictate substrate specificity, suggesting that the interactions made between Spo0F and the four-helix bundle of Spo0B will be of most relevance to understanding HK:RR specificity. This interface involves a mixture of hydrophobic and hydrogen-bond interactions. For the response regulator, most of the residues in direct contact lie within alpha helix 1 and in the five β-α loops that surround the aspartate phosphorylation site. For the histidine phosphotransferase, the majority of interacting residues reside within helix 1, although significant contributions appear to be made by some residues of helix 2 as well (**Figure 5**).

The residues in direct contact between Spo0F and Spo0B correlate to some extent with alanine-scanning mutagenesis studies aimed at identifying amino acids important for phosphotransfer from Spo0F to Spo0B (93). Alanine- and cysteine-scanning mutagenesis of other phosphotransfer pairs have also identified residues that correspond to the interfacial residues of Spo0F and Spo0B (45, 76–78). These mutagenesis studies do not, however, directly pinpoint specificity determinants. They identify amino acids required for binding or for catalyzing phosphotransfer but do not directly address how a response regulator distinguishes between cognate and noncognate kinases, or, vice versa, how a kinase distinguishes between possible response regulator partners. For instance, there may be amino acids on a response regulator that influence specificity by preventing interaction with a noncognate substrate without significantly influencing interaction with the cognate substrate; such sites would be missed by alanine-scanning mutagenesis. In a more directed search for amino acids that dictate specificity of phosphotransfer, Wanner and colleagues screened for mutations in the response regulator PhoB in *E. coli* that would allow it to accept a phosphoryl group from the noncognate kinase VanS instead of the usual, cognate kinase PhoR (36). Several of the amino acids identified correspond to amino acids in Spo0F that directly contact Spo0B, consistent with their playing a major role in specificity and the choice of histidine kinase phosphodonor by PhoB. Additional genetic screening and/or rational mutagenesis will ultimately be crucial for producing a detailed

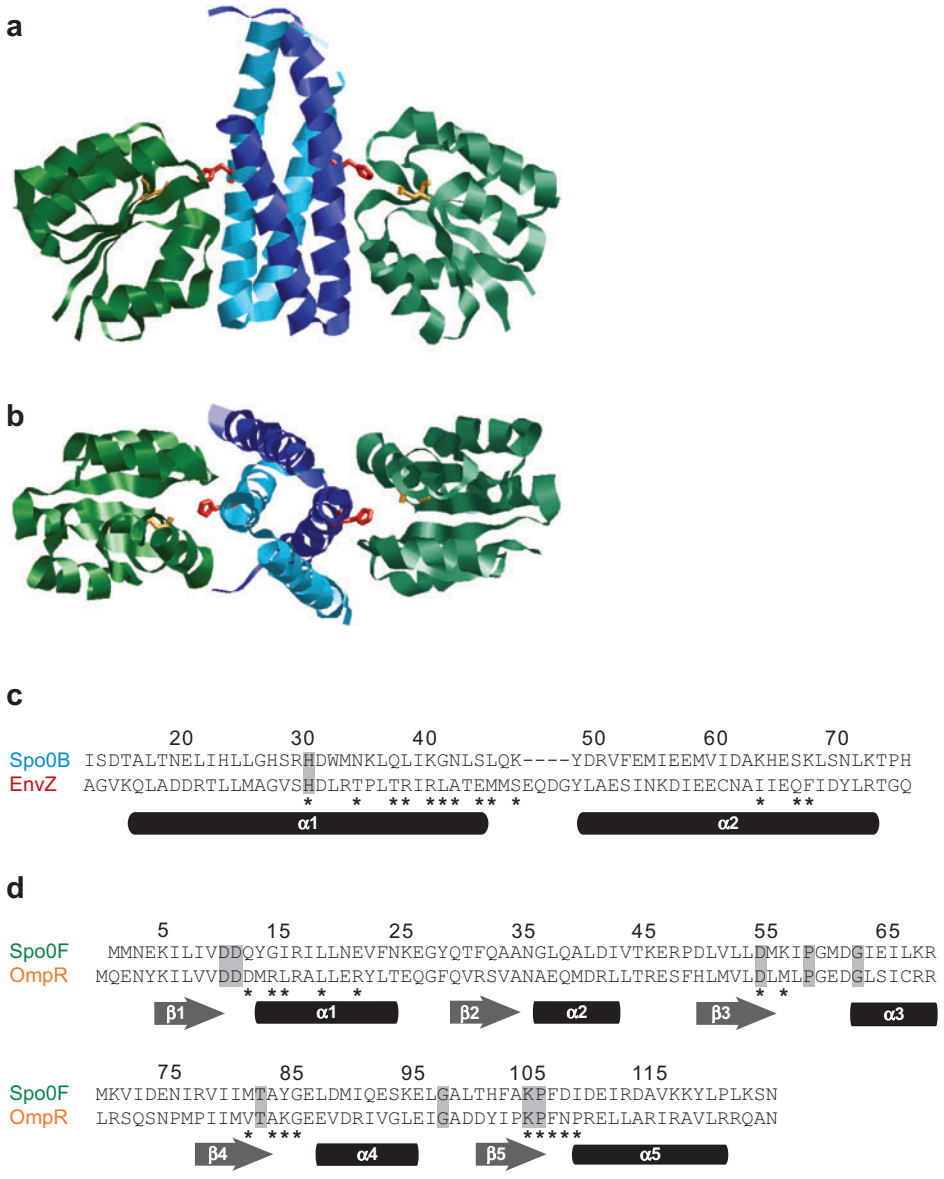

Figure 5

Structure of the histidine phosphotransferase Spo0B in complex with the response regulator Spo0F. (*a*) Shown are two Spo0B molecules (*blue*, *light blue*) in complex with two Spo0F molecules (*green*, *light green*), PDB: 1F51. Two Spo0B molecules dimerize by forming a four-helix bundle. The second domain of Spo0B, which is present in the co-crystal, was removed to highlight the interface between Spo0F and helix 1 and 2 of Spo0B. The phosphorylation site histidine (*red*) of Spo0B is shown protruding from helix 1 of each Spo0B protomer and in close proximity to the phospho-accepting aspartate (*orange*) of Spo0F. (*b*) Orthogonal view of structure in panel (*a*). (*c*) Sequence alignment of Spo0B and the prototypical histidine kinase EnvZ. (*d*) Sequence alignment of Spo0F and the prototypical response regulator OmpR. Residues shaded in grey are highly conserved in all histidine kinases or response regulators. Asterisks indicate residues in direct contact in the Spo0B:Spo0F co-crystal structure (112). The secondary structure of EnvZ and OmpR are shown below their respective alignments.

molecular-level understanding of specificity in phosphotransfer relationships.

COMPUTATIONAL APPROACHES TO SPECIFICITY

Computational analyses have also shed light on the molecular basis of specificity in two-component signaling systems. Early sequence analyses by Grebe & Stock identified 11 subfamilies of histidine kinases and 8 subfamilies of response regulators (32), and with the recent explosion in the number of whole-genome sequences available, histidine kinase classification has been expanded to include 13 distinct subfamilies. As certain subfamilies of histidine kinases tend to interact with certain subfamilies of response regulators, the sequences that define or distinguish subfamilies may well include amino acids involved in mediating specificity (52). A number of investigators have also combined structural studies with sequence alignment analyses to identify possible specificity-determining residues. Those residues that are variable between response regulators (or between histidine kinases) and that are solvent-exposed in solved structures, or in homology-modeled structures, have been predicted to mediate specificity (39, 51, 65).

In a more systematic effort to identify putative specificity-determining residues, an algorithm was developed to search for amino acids that are conserved within sets of orthologous histidine kinases but variable between different sets of paralogous kinases (53). An identical analysis was done for response regulators. These analyses identified 10 amino acids in histidine kinases and 6 in response regulators that may be specificity determinants. Many of these residues map to the molecular surfaces surrounding the active site that catalyzes phosphotransfer and are likely to be in contact with one another during phosphotransfer. These observations strongly implicate these residues in contributing to specificity and they make clear predictions of mutations that could be introduced to alter or to design the specificity of interaction. This computational approach could, however, be missing certain key residues as the method assumes that specificity-determining residues only vary between paralogous HK-RR pairs and not between orthologous HK-RR pairs. Specificity residues could vary between orthologous pairs, but presumably any change in the histidine kinase will be accompanied by a corrresponding, or compensatory, change in the cognate response regulator. An analysis of coevolving amino acids in histidine kinases and response regulators may therefore help further pinpoint specificity residues.

In sum, the combination of mutagenesis, structural analysis, and bioinformatic approaches is beginning to reveal the atomic-level basis of specificity in phosphotransfer between cognate histidine kinases and response regulators. The ultimate test of whether we understand specificity in two-component pathways will be whether histidine kinases and response regulators can be rationally designed to have altered specificity, as has been done with transcription factors and other families of proteins [for examples, see (24, 54, 107)].

OTHER MECHANISMS FOR DICTATING SPECIFICITY AND PATHWAY INSULATION

In addition to the inherent discrimination of kinases for their cognate substrates, there may be additional means of ensuring the specificity of two-component signaling pathways. In eukaryotic cell signaling, there are numerous examples in which specificity is controlled through spatial localization of regulatory proteins. Such localization, which is often mediated by adaptor or scaffold proteins, can target proteins to specific regions of the cell, thereby preventing cross-talk to proteins localized to other regions. Spatial localization could play a similar role in controlling specificity in two-component signaling in prokaryotes. There are many reports of histidine kinases

and response regulators that are subcellularly localized in bacteria, but in most cases the significance with respect to signaling specificity is not well understood (9, 18, 49).

In bacterial chemotaxis, signaling proteins associate in clusters. Current models suggest this clustering is important for signal amplification [reviewed in (86)]. In at least one system it has also been suggested that clustering may play a role in limiting cross-talk. *Rhodobacter sphaeroides* has two separate chemotaxis pathways (98). One of the pathways localizes to the cell pole, which is similar to the chemotaxis systems in other bacteria. The second pathway also assembles in a cluster; however, it is localized to the cytoplasm. Thus, targeting the two pathways to distinct cellular locations in *R. sphaeroides* may be a mechanism for avoiding inappropriate cross-talk between these pathways (98).

For branched pathways, localization can be used to select for signaling through one or the other branch. This appears to be the case for PleC and DivJ interactions with DivK in *C. crescentus*, discussed above. The localization of the histidine kinases PleC and DivJ to the stalked and flagellar poles, respectively, effectively enforces position-dependent specificity for their action on DivK. DivK is only phosphorylated by DivJ on one end of the cell and only dephosphorylated by PleC on the other end. It is perhaps not surprising that this form of regulation appears in the context of cellular differentiation and an asymmetric cell division, and it will be interesting to see if this emerges as a common theme among bacteria that have complex developmental programs.

In addition to spatial localization, specificity could be enforced through temporal control. To prevent unwanted cross-talk between two different two-component pathways, cells could, in principle, ensure that only one of the two systems is expressed at a given time. Such temporal control could similarly be used for branch selection in branched pathways. At present, however, we are not aware of any reports of this form of regulation providing insulation from cross-talk.

CROSS-TALK AND CROSS-REGULATION AT OTHER LEVELS

Thus far we have focused on cross-talk and specificity with regard to phosphotransfer from histidine kinases to response regulators. Although two-component signaling pathways do not show extensive cross-talk at this level, there is growing evidence for other mechanisms of cross-regulation and signal integration. In many cases, two-component signaling pathways converge at the transcriptional level so that response regulators from different pathways regulate overlapping sets of genes (10, 16, 23, 34, 47, 64, 97). For example, in *Salmonella* the response regulators PmrA and RcsB can each activate the genes *ugd* and *wzz* to effect changes in the composition of outer-membrane lipopolysaccharide (23, 64). These response regulators are phosphorylated by distinct, insulated pathways (the PmrB histidine kinase and the RcsC-YojN phosphorelay, respectively), but directly regulate some of the same genes by binding to different *cis*-regulatory sites in their promoters. Transcriptional cross-regulation by different two-component pathways has also been described recently for the EnvZ-OmpR and CpxA-CpxR systems in *E. coli*. Although there is minimal cross-talk at the level of phosphotransfer in these systems, certain genes, such as *ompF* and *csgD*, are directly regulated by both OmpR and CpxR (10, 47). Systematic microarray analysis of all two-component signaling mutants in *E. coli* suggests there may be overlap of target genes for a number of two-component pathways (71). Cross-regulation at the level of transcription allows distinct pathways to elicit some of the same responses. However, because cross-talk is minimal at the level of phosphotransfer, bacteria can still ensure that a given stimulus results in a specific

output that is appropriate to, or tailored to, the input stimulus.

Two-component pathways can also converge and influence one another at levels other than transcription. For instance, in *B. subtilis*, activation of the competence response regulator ComA by its cognate histidine kinase ComP triggers synthesis of the RapA phosphatase, which in turn down-regulates the response regulator Spo0F, a critical intermediate in the phosphorelay that activates sporulation (74). This inhibition through cross-regulation ensures that *B. subtilis* cells committed to competence do not also commit to sporulation. In *Salmonella*, activation of the PhoQ-PhoP two-component pathway leads to synthesis of the protein PmrD, which binds another response regulator, PmrA, to protect it from dephosphorylation (48). The phosphorylation of PmrA is controlled by a separate, cognate kinase called PmrB, but the persistence of this pathway's output is dictated by PmrD, the transcriptional target of a different two-component pathway. In *C. crescentus* a complex network of two-component signaling pathways coordinates cell cycle progression and development. Again, there appears to be minimal cross-talk at the level of phosphotransfer, but distinct pathways nevertheless cross-regulate one another at other levels. For instance, the response regulator DivK, as described above, is phosphorylated by a cognate kinase DivJ, but can regulate the major cell cycle phosphorelays by modulating the activity of the histidine kinase CckA and the response regulator CtrA (14). Collectively, these recent studies suggest that two-component pathways are often arranged into complex circuits with extensive cross-regulation at a variety of levels, thereby endowing cells with the ability to perform sophisticated information processing tasks.

FINAL PERSPECTIVES

Genome sequencing projects have demonstrated that two-component signal transduction systems represent the single largest paralogous family of signaling proteins in the bacterial kingdom. Given the highly similar sequences and structures of these proteins, there is seemingly a great potential for cross-talk, but cells have evolved a variety of mechanisms to minimize unwanted, detrimental communication between distinct pathways. This review focused mainly on the mechanisms that ensure specificity with respect to phosphotransfer. However, there are other aspects of two-component signaling that demand precise specificity as well. For instance, histidine kinases and most response regulators function as homodimers—what ensures the specificity of dimerization and prevents unproductive heterodimers from forming? As with phosphotransfer, it will be important to probe the structural, atomic-level basis for the specificity of these protein-protein interactions. As emphasized already, the ultimate test of whether we understand the specificity of protein-protein interactions throughout two-component pathways will be whether we can redesign or rationally engineer these systems. Can pathways, in effect, be rewired to execute novel functions? A more complete understanding of specificity may also enable the prediction of kinase-regulator pairs in any genome. As noted, many bacterial genomes encode numerous orphan two-component signaling genes. Methods now exist to systematically map the cognate pairings among these orphans, but the ability to predict cognate pairs from sequence alone would greatly facilitate the mapping of regulatory networks in these bacteria.

Understanding how two-component signaling pathways evolve also promises to be a major focus of future studies. One recent study probed the contribution of gene duplication and lateral gene transfer to the expansion of two-component signaling pathways throughout the bacterial kingdom (3). In terms of signaling specificity, the expansion of two-component pathways during

evolution raises interesting questions. How do recently duplicated pathways become insulated from one another? How many mutations are required? What constraints are imposed by existing pathways?

Finally, although there is minimal cross-talk between distinct two-component pathways, not all of these pathways are linear with simple one-to-one relationships between kinases and regulators. There are a growing number of examples of more complicated signaling topologies, and genome gazing suggests that many more remain to be discovered. In addition, there are numerous examples now of two-component systems that cross-regulate one another at levels other than cross-phosphorylation. Understanding all of the connections between two-component pathways will be crucial to developing a more complete understanding of how bacteria sense, adapt, and respond to changes in their environment.

SUMMARY POINTS

1. Cross-talk between different two-component signaling pathways at the level of phosphorylation is rare and often seen only in mutants lacking mechanisms that ensure pathway insulation.
2. Some two-component pathways are inherently, or necessarily, branched at the level of phosphorylation and involve many-to-one or one-to-many relationships between histidine kinases and response regulators.
3. Many histidine kinases are bifunctional and can stimulate the dephosphorylation of their cognate response regulators. Hence, when not active for autophosphorylation, bifunctional histidine kinases can effectively suppress any inadvertent cross-phosphorylation of their cognate regulators.
4. Response regulators are typically more abundant than histidine kinases and so compete for phosphorylation by histidine kinases. Removing the cognate regulator for a kinase or overproducing a histidine kinase can result in cross-phosphorylation of noncognate regulators.
5. Despite the relative rarity of cross-phosphorylation, there are numerous cases of cross-regulation at other levels, particularly the transcriptional level.
6. Complex circuits of two-component signaling proteins help cells to respond and adapt to a wide range of environments, stressors, and growth conditions.
7. Histidine kinases exhibit a large, system-wide kinetic preference in vitro for phosphotransfer to their in vivo cognate substrate, implying that specificity of two-component pathways is dictated primarily at the level of molecular recognition.
8. Structural analysis of two-component signaling proteins, particularly co-crystal structures of histidine phosphotransferases and their cognate response regulators, help pinpoint amino acids that dictate specificity.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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