Cell, Vol. 86, 845–848, September 20, 1996, Copyright ©1996 by Cell Press

Signal Transduction via the Multi-Step Phosphorelay: Not Necessarily a Road Less Traveled

Minireview

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Signal Transduction by Two-Component Regulatory Systems

All living cells must sense changes in their environment and respond appropriately. To meet this need, prokaryotic organisms commonly employ a sophisticated signal transduction strategy known as the two-component regulatory system (reviewed in Parkinson, 1993; Hoch and Silhavy, 1995). This signaling mechanism is ubiquitous in bacteria, and homologous pathways have recently been identified in several eukaryotic organisms as well, including Saccharomyces cerevisiae, Arabidopsis thaliana, Neurospora crassa, and Dictyostelium discoideum (see Alex et al., 1996, and references therein). This minireview focuses on an emerging class of complex signaling pathways built from two-component circuit elements, the multi-step His-Asp phosphorelay.

The prototypical two-component pathway is comprised of two proteins: a histidine protein kinase, also called a sensor kinase, and a response regulator (Figure 1). The N-terminal portion of the histidine protein kinase functions as an input domain, detecting environmental stimuli directly or interacting with an upstream receptor. At the C-terminal end of the protein is the transmitter module. This domain is generally about 240 amino acids long and contains several blocks of residues that are conserved among histidine protein kinases. One of these, termed the H box, includes a histidine residue at which the protein autophosphorylates using the γ-phosphoryl group of ATP. The G1 and G2 boxes are glycine-rich sequences that bear resemblance to the nucleotide-binding motifs of other proteins. The specific functions of the conserved F and N boxes are not readily apparent by inspection of their sequences, but are presumably involved in the catalytic activity of the protein.

The response regulator has a receiver domain, generally at its N-terminus, which is approximately 120 amino acids in length and contains the conserved sequences that define membership in the response regulator family. The receiver domain catalyzes transfer of the phosphoryl group from the histidine of the sensor kinase to a conserved aspartate residue, and can also utilize a variety of small molecules (but not ATP) as alternate phosphodonors (Lukat et al., 1992). The phosphorylation state of the receiver modulates the activity of a unique C-terminal output domain, commonly a transcriptional regulator, to elicit an adaptive response to the stimulus.

The two-component signaling strategy governs responses to a wide array of environmental stimuli, including changes in osmolarity, nutrient availability, and host proximity. Although the general sequence of events is similar for all of these systems, each pathway exhibits variations on this basic scheme that tailor it to a particular sensory task. The modular design of the two-component paradigm has facilitated the evolutionary construction of a variety of signal transduction circuits, all of which utilize phosphoryl group transfer as the mechanism of communication between modules.

The His-Asp-His-Asp Phosphorelay

One circuitry motif that exemplifies this versatility is the four-step His-Asp-His-Asp phosphorelay. Burbulys et al. (1991) reported the first known phosphorelay built from sensor kinase and response regulator family members. This pathway, which governs initiation of sporulation in Bacillus subtilis, involves a chain of four proteins through which a phosphoryl group is transferred before ultimately activating the Spo0A transcriptional regulator (Figure 2A). The relay begins with the activation and concomitant autophosphorylation of one of three sensor kinases, KinA, KinB, or KinC. The phosphoryl group is then transferred to an aspartate residue in Spo0F, a response regulator that is comprised entirely of a conserved receiver domain. Spo0F serves as a phosphodonor for the next link in the chain, Spo0B, which shares no sequence similarity with sensor kinases, but nonetheless appears to be phosphorylated on a histidine residue. Finally, the phosphoryl group completes its course by transfer to an aspartate in Spo0A. The sporulation initiation pathway of B. subtilis remained the only characterized example of a His-Asp-His-Asp phosphorelay for several years. There have now been several recent reports of similar or related circuits in a variety of two-component pathways (Tsuzuki et al., 1995; Uhl and Miller, 1996; Posas et al., 1996, this issue).

The BvgS-BvgA two-component system modulates the transcriptional regulation of virulence factors in Bordetella pertussis. In this pathway, the first three steps of the four-step phosphorelay occur within a single protein, BvgS (Uhl and Miller, 1996). BvgS resides in the cytoplasmic membrane and contains both a transmitter module and a receiver module, as well as a C-terminal domain that is characteristic of a family of signaling proteins with similar architecture and, perhaps, phosphorelay patterns. One feature of the C-terminal domain that is shared with all known members of the BvgS family (which includes the bacterial proteins ArcB, BarA, EvgS,

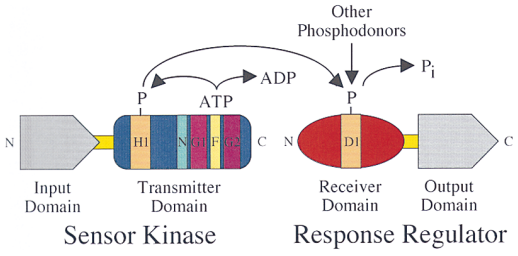

Figure 1. Organization of a Prototypical Two-Component Regulatory System

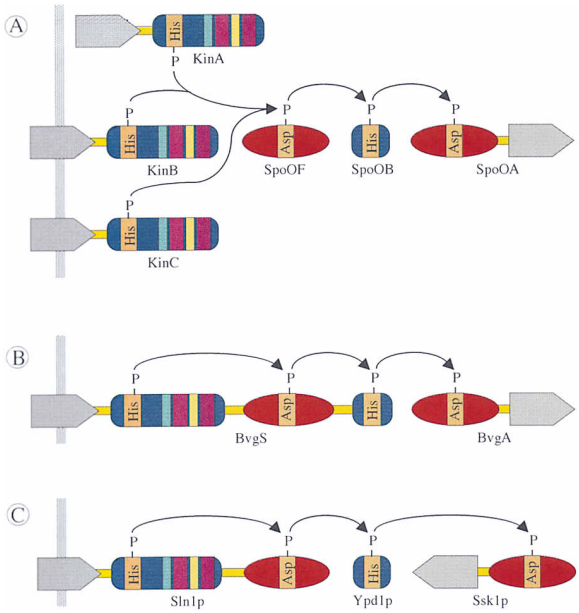

Figure 2. Signaling in the Kin-Spo0 (A), BvgS-BvgA (B), and Sln1p-Ypd1p-Ssk1p (C) Phosphorelays

LemA, RpfC, RteA, and TorS) is an invariant histidine residue flanked by conserved sequences. In the BvgS-BvgA phosphorelay (Figure 2B), BvgS autophosphorylates a histidine residue in its transmitter module in accordance with environmental signal input. The phosphoryl group is then sequentially transferred to an aspartate in the BvgS receiver domain, from this aspartate to a histidine in the C-terminal domain, and finally to an aspartate in the receiver domain of a physically independent response regulator, BvgA. Therefore, despite the apparent economy of this system relative to the B. subtilis Kin-Spo0 pathway in that the entire relay is comprised of only two proteins, four phosphorylation events occur in sequence, and the same alternating pattern of histidine and aspartate phosphorylation sites is observed for both.

A Phosphorelay in Yeast Osmoregulation

In this issue of Cell, Posas et al. (1996) report a His-Asp-His-Asp phosphorelay which governs osmoregulation in the yeast Saccharomyces cerevisiae. The Sln1p-Ypd1p-Ssk1p pathway, which is currently the best characterized eukaryotic two-component system, represents yet another organizational design of the phosphorelay (Figure 2C). In this case, the first two phosphorylation sites are located in Sln1p, a transmembrane protein that contains a cytoplasmic transmitter domain as well as an attached receiver domain. The Sln1p protein autophosphorylates at a histidine within its transmitter domain and the phosphoryl group is then transferred to an aspartate in the Sln1p receiver domain. From the Sln1p receiver domain, the phosphoryl group is then passed to a histidine residue in a novel protein called Ypd1p. Ypd1p is a small cytoplasmic protein that has a short sequence of weak, but functionally significant, similarity to the histidine phosphorylation sites of some sensor kinases. From Ypd1p, the phosphoryl group is transferred to the final member of the phosphorelay, Ssk1p, in which the receiver domain is on the C-terminal side of the putative output domain, modulates the activity of a downstream MAP kinase cascade (Maeda et al., 1995).

Phosphorelay Architecture

The discovery that the yeast Sln1 pathway employs a phosphorelay mechanism with the same histidine (H1) →aspartate (D1)→histidine (H2)→aspartate (D2) configuration reported for the Kin-Spo0 and BvgS-BvgA systems suggests that this signaling strategy may be widely utilized by eukaryotic as well as prokaryotic organisms. Two basic elements are common to the known phosphorelays: they employ four sequential phosphorylation events, and these phosphotransfers alternate between histidine and aspartate residues. These features invite speculation about the evolutionary origins of the relays. It may be that these pathways arose in a single event by combining pairs of two-component systems to permit phosphate flow from one H-D pair to a second H-D pair. Alternatively, the relays may have arisen through incremental addition of communication modules to a two-component pathway, for example, through gene fusions that created proteins with multiple modules, such as BvgS and Sln1p. These historical possibilities are certainly not mutually exclusive. In addition to evolutionary implications, the alternation of histidine and aspartate phosphorylation sites may reflect some mechanistic constraints. The H1 and H2 components may be simple substrate sites, with no catalytic functions, whereas the D1 and D2 receiver domains are probably responsible for catalyzing all phosphotransfers subsequent to the initial autophosphorylation event. The ability of the receiver modules to utilize either phosphoramidates or acyl phosphates as phosphodonors (Lukat et al., 1992) raises the possibility of phosphoryl group transfer between aspartate residues, as well as between aspartate and histidine. However, the H and D modules of the two-component system are presumably designed to recognize and dock with one another during phosphotranfer; the complementary surface shapes of the H and D modules would most likely preclude Asp→Asp and His→His phosphotransfers.

An intriguing architectural feature of these three reported phosphorelays is that each utilizes a different pattern of covalent linkage between modules. The four phosphorylation sites of the Kin-Spo0 pathway are found on independent proteins, whereas the Sln1p-Ypd1p-Ssk1p pathway fuses the first two members of the relay into one protein, and the BvgS-BvgA pathway conjoins the first three. This observation may provide an avenue by which other potential phosphorelays are recognized. Presumably, any of eight permutations of connections between phosphotransfer domains may be used to form a linear His-Asp-His-Asp phosphorelay (Figure 3). Comparison of these possible domain combinations with two-component proteins of unique configuration may implicate some of these as participants in phosphorelays. One example is the RcaE-RcaC pathway which governs chromatic adaptation in cyanobacteria. The RcaC protein was previously reported to contain two intact receiver domains, one at each terminus (Chiang et al., 1992). This may be consistent with a phosphorelay pattern like that depicted in Figure 3G. In

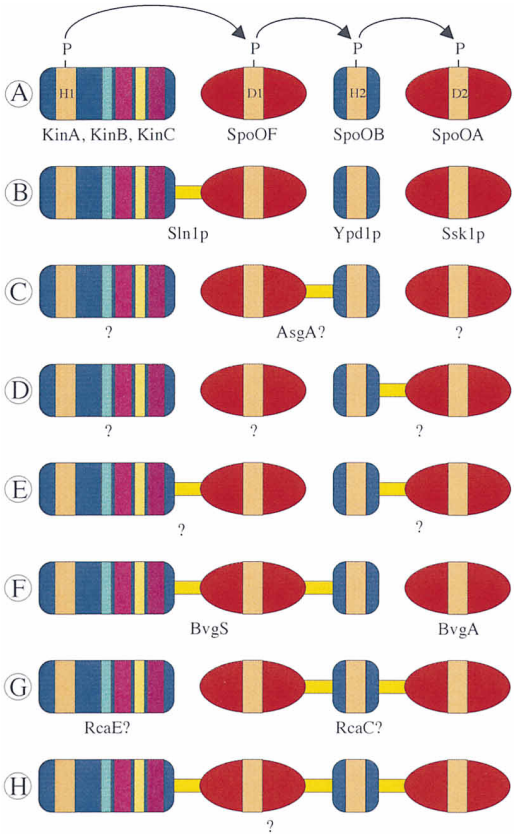

Figure 3. Possible Distributions of the His-Asp-His-Asp Phosphorelay Elements among Different Proteins

See text for details. Note that input and output domains are not depicted.

recognition of RcaC as a potential phosphorelay candidate, we examined the midsection of this protein more closely and discovered a sequence including His316 that conforms in 9/11 positions to the weak consensus H2 phosphorylation site proposed by Ishige et al. (1994). Furthermore, Kehoe and Grossman (1996) have recently reported the identification of RcaE, a sensor kinase that could function upstream of RcaC in this proposed phosphorelay.

Another protein which might reasonably be expected to participate in a phosphorelay is AsgA, which is involved in regulating the production of cell density signals necessary for fruiting body development in Myxococcus xanthus. AsgA is currently the only published example of a protein that is comprised of an N-terminal receiver domain and a C-terminal transmitter domain (Plamann et al., 1995). This unique configuration is consistent with AsgA providing the D1 and H2 sites in a relay like that depicted in Figure 3C. Its candidacy as the middle link in a phosphorelay is supported by the absence of input or output domains. Figure 3 predicts, then, that the as yet unidentified members of this putative phosphorelay are a typical sensor kinase and response regulator. It should be mentioned, however, that AsgA contains a fully functional transmitter domain (Li and Plamann, 1996), in contrast to the context of known H2 sites. Thus, it may be that phosphorylation of the AsgA receiver instead regulates the autokinase activity of the transmitter, which then serves as a phosphodonor for a putative downstream response regulator.

Hybrid kinases, i.e., sensor kinases that contain attached receiver modules, account for roughly 30% of the currently reported sensor kinases in bacteria. Moreover, many of the eukaryotic sensor kinases that have been identified so far are of this class. Although it is provocative to speculate about the potential involvement of these kinases in His-Asp-His-Asp phosphorelays, not all hybrid kinases are relay participants, and the phosphorelay is certainly not the only use of this architectural design. For example, the attached receiver module of VirA, a transmembrane hybrid kinase of Agrobacterium tumefaciens, functions as an autoinhibitory domain (Chang et al., 1996). In its unphosphorylated state, this receiver domain interacts with the transmitter module and prevents the transmitter from autophosphorylating and serving as a phosphodonor to its cognate response regulator VirG.

It should also be noted that there is no mechanistic necessity for a linear rather than branched phosphorelay. For example, the ArcB-ArcA phosphorelay is architecturally similar to the BvgS-BvgA pathway, but exhibits both H1→D2 and H2→D2 phosphotransfer (Tsuzuki et al., 1995).

Phosphorelay Attributes

It is worth noting that the reported phosphorelays, as well as the putative phosphorelay candidates mentioned above, govern major developmental commitments, decisions that should not be lightly made. For instance, activation of the Kin-Spo0 pathway of B. subtilis or the AsgA pathway of M. xanthus ultimately results in sporulation. BvgS, as well as several other BvgS family members, is involved in regulation of a wide variety of virulence factors. Likewise, the RcaE-RcaC chromatic adaptation system modulates dramatic changes in the ratio of the two major chromoproteins of the light harvesting system, which accounts for up to 50% of the total cellular protein. Inappropriate activation of any of these pathways would undoubtedly have deleterious effects on the cell, at the very least a grave misuse of cellular resources.

The realization that phosphorelays may be more common than previously appreciated, coupled with the identification of multiple architectural configurations, suggests that these circuits have special signaling properties. In contrast to, for example, a MAP kinase phosphorylation cascade, where activation of downstream kinases by phosphorylation increases the flow of phosphoryl groups through the pathway, the relay approach offers no signal amplification beyond the initial autophosphorylation of H1. Perhaps instead the relay establishes a threshold that requires a signal of minimum proportion or duration to be overcome before a response is seen. The most important advantage of the multi-step relay, however, may be that it provides the potential for multiple regulatory checkpoints (Grossman, 1995; Hoch, 1995). In systems with unlinked components, transcriptional regulation of relay components provides one mechanism of control, as has been demonstrated in the Kin-Spo0 pathway. In cases such as BvgS or Sln1p, the presence of several relay steps in one

protein may increase signaling efficiency and reduce non-specific crosstalk from other pathways. Phosphatases that act on specific relay sites provide another versatile mechanism for regulating signal flow in phosphorelays. Finally, the multiple phosphorylation sites of the phosphorelay could provide junction points for communicating with other signaling pathways, perhaps endowing the cell with sophisticated information-processing capabilities far beyond the simplistic linear sequence of events depicted in Figure 3.

The elegant characterization of the yeast Sln1 pathway by Posas et al. (1996) is doubly noteworthy. It provides the first demonstration that proteins of the eukaryotic two-component family undergo phosphorylation reactions characteristic of their bacterial counterparts, and at the same time broadens our view of signal transduction mechanisms in bacteria to emphasize the importance of the multi-step phosphorelay as a versatile and sophisticated signaling strategy exploited by prokaryotes and eukaryotes alike.

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