

Update on Signal Transduction

The Two-Component System¹

Regulation of Diverse Signaling Pathways in Prokaryotes and Eukaryotes

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SENSORY-RESPONSE CIRCUITS: REALITY CHECKS AT THE CELLULAR LEVEL

Unicellular microorganisms experience “life on the edge,” as they have little ability to change their environment and face fierce competition for limited resources. They must therefore respond to a barrage of environmental cues in a rapid and accurate manner. Among multicellular organisms, plants in particular cannot escape their environment and so must be masters at adapting and coordinating cellular events to accommodate prevailing conditions. The penalty for losing touch with reality is often death. The study of sensory-response systems has defined the basics of how many organisms detect and respond with exquisite sensitivity to changes in their chemical or physical environments. Such studies have recently focused on events that occur at the cellular and molecular levels, elucidating the mechanisms of detecting extracellular signals and transducing such signals into the appropriate intracellular events. In a large number of cases, these signaling pathways involve phosphorylation of key effector proteins by protein kinases. In bacteria numerous sensory-response circuits operate by making use of a phosphorylation control mechanism referred to as the “two-component system” (Nixon et al., 1986; Parkinson and Kofoid, 1992).

The basic biochemical events of two-component signal transduction were first established by Ninfa and Magasanik (1986) for the NR system, a regulatory system that controls gene expression in response to nitrogen-source availability in *Escherichia coli*. At about the same time, Ausubel and co-workers (Nixon et al., 1986) recognized amino acid sequence similarities between the components of the NR system and components of numerous other bacterial sensory systems that had not been characterized at a biochemical level. Such similarities raised the exciting possibility that these other systems operated via a signaling mechanism analogous to that utilized by the NR system. Subsequent work has borne out this idea, and the list of

two-component systems has expanded to include hundreds of distinct systems. Fueled in part by the explosion of sequence information provided by various genome projects, the number of two-component systems continues to grow at a rapid pace, and numerous review articles on the topic have been published (e.g. Stock et al., 1990; Bourret et al., 1991; Parkinson and Kofoid, 1992; Hoch and Silhavy, 1995).

Whereas the two-component system has been firmly established as a prevalent signaling mechanism in bacteria, the existence of two-component regulators in eukaryotic systems has been uncovered only very recently and, to date, only in a limited number of organisms (fungi, slime molds, and plants) (Loomis et al., 1997; Wurgler-Murphy et al., 1997). In the first part of this review, we outline the basics of how two-component systems operate in well-characterized bacterial systems. In the second part, we review the emerging picture of two-component signaling in the context of eukaryotic cells, particularly in higher plants.

BASIC PLAYERS: IT TAKES TWO TO SIGNAL

The basic two-component system involves a sensor kinase, or HPK, as well as an RR. As depicted in Figure 1, the role of the HPK is to direct phosphorylation of its cognate RR in response to a specific environmental signal; this phosphorylation regulates the activity of the RR. Some bacteria make extensive use of such systems. For example, inspection of the complete genome of *E. coli* indicates that over 30 distinct HPK-RR circuits operate in this single bacterium. Basic Local Alignment Search Tool (BLAST) searches of the *Mycoplasma genitalium* genome database, however, revealed no likely HPK homologs, suggesting that not all prokaryotes utilize two-component systems as extensively as *E. coli*. Similar surveys of other sequence databases indicate that while some eukaryotes (e.g. *Arabidopsis thaliana*) may have a number of two-component systems, others (e.g. *Saccharomyces cerevisiae*) appear to have only a single two-component system. Here we outline

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Abbreviations: HPK, His protein kinase; MAP, mitogen-activated protein; MAPK, MAP kinase; NR, nitrogen regulatory protein; RR, response-regulator protein.

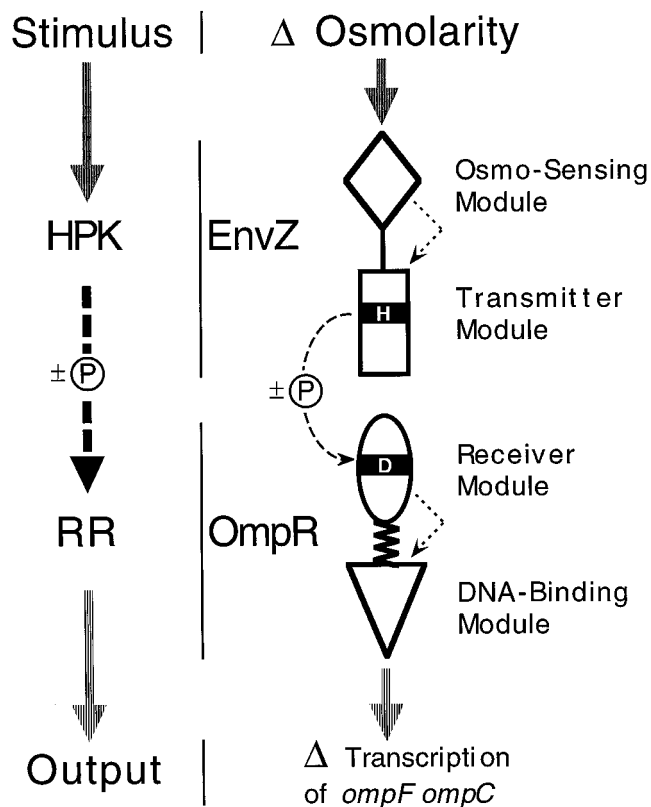


Figure 1. Example of a basic two-component system. The *E. coli* osmolarity-response system consists of an HPK osmosensor (EnvZ) and an RR transcription factor (OmpR) (Pratt and Silhavy, 1995). EnvZ autophosphorylates using ATP as the phosphate donor. The phosphate from the transmitter module of EnvZ is then transferred to an Asp residue in the receiver module of OmpR, thereby affecting the promoter interactions of the OmpR DNA-binding module, which regulates the transcription of two porin genes, *ompF* and *ompC*. Changes in osmolarity are perceived by the amino-terminal module of EnvZ. In response to such changes, EnvZ changes the level of phosphorylated OmpR. The dotted lines depict intra-protein regulatory interactions. The dashed line depicts phosphorylation/dephosphorylation events. P, Phosphoryl group; H, His; D, Asp.

some of the basic characteristics common to the large families of two-component HPKs and RRs.

Sensor HPKs

In several respects, HPKs are similar to the well-defined family of receptor Tyr kinases (Stock et al., 1991): HPKs operate as dimers and autophosphorylate; they are associated with the cytoplasmic membrane, usually via one or two membrane-spanning sequences; and they typically contain extracellular sensory input modules fused to the protein kinase catalytic module (Bourret et al., 1991). This arrangement makes it easy to envision environmental stimuli impinging on the HPK in a manner that regulates its kinase activity. However, there are relatively few cases in which we have much understanding of the actual ligands that interact directly with the HPKs, and in several cases a distinct protein serves as the primary receptor for the stim-

ulus (Fig. 2, A and B). Because of this, it has been difficult to determine the exact relationship between signal perception and catalytic activity of the HPK (e.g. whether the signal stimulates HPK activity). Despite the general mechanistic similarities shared by HPKs and other types of protein kinases, sequence analysis indicates that HPKs are only distantly related to Tyr kinases and Ser/Thr kinases (Stock et al., 1995).

There are also operational features that distinguish HPKs from other protein kinases. First, HPKs do not catalyze direct transfer of a phosphate from ATP to their "substrate" RR; rather, each HPK must first autophosphorylate, and then the phosphoryl group from HPK-P is passed to the RR. A second difference is that the site of HPK autophosphorylation is a His residue, and the site of RR phosphorylation is an Asp residue (Bourret et al., 1991). The energetics and chemical stabilities of phospho-His and phospho-Asp differ significantly from those of "more traditional" phospho-amino acids (phospho-Tyr, phospho-Ser, and phospho-Thr) (Stock et al., 1990, 1995).

Several hundred HPKs (some well characterized, some surmised based on sequence analysis) have been found in bacteria, and amino acid sequence comparisons have identified a common 250-amino acid "transmitter module" in each of these. This module is thought to encompass the autokinase active site and, in most cases, the His-phosphorylation site. Excluding sequences of closely related homologs, the transmitter modules from any two HPKs typically share 20 to 50% sequence identity (average sequence identity, 25%). Five blocks of 5 to 10 amino acids with higher conservation have been identified in most transmitter modules (Parkinson and Kofoid, 1992; Stock et al., 1995). Some HPKs also have phosphatase activities, i.e. they can catalyze dephosphorylation of their cognate RRs (Igo et al., 1989; Makino et al., 1989). This dephosphorylation appears to involve a mechanism that is distinct from simple reversal of the HPK-RR phosphotransfer reaction (Hsing and Silhavy, 1997).

RRs

The sensor HPK regulates the activity of a cytoplasmic RR by directing its phosphorylation as depicted in Figure 1. GenBank now contains over 400 different examples of RRs. Analysis of the amino acid sequences of known and suspected RRs has established two general themes: (a) RRs have an approximately 110-amino acid domain referred to as a "receiver module" that contains the Asp-phosphorylation site; and (b) most RRs are two-domain proteins in which the receiver module is fused to a second domain having some kind of output or effector activity (Parkinson and Kofoid, 1992). In many cases, the output domain is a DNA-binding module whereby the RR functions as a transcription factor, and Asp phosphorylation serves to control its ability to either bind its target DNA sequence or interact with other components of the transcription machinery (Hakenbeck and Stock, 1996).

There are also RRs that have nothing to do with transcription. For example, *E. coli* CheB demethylates the chemotaxis-receptor proteins, and phosphorylation of the

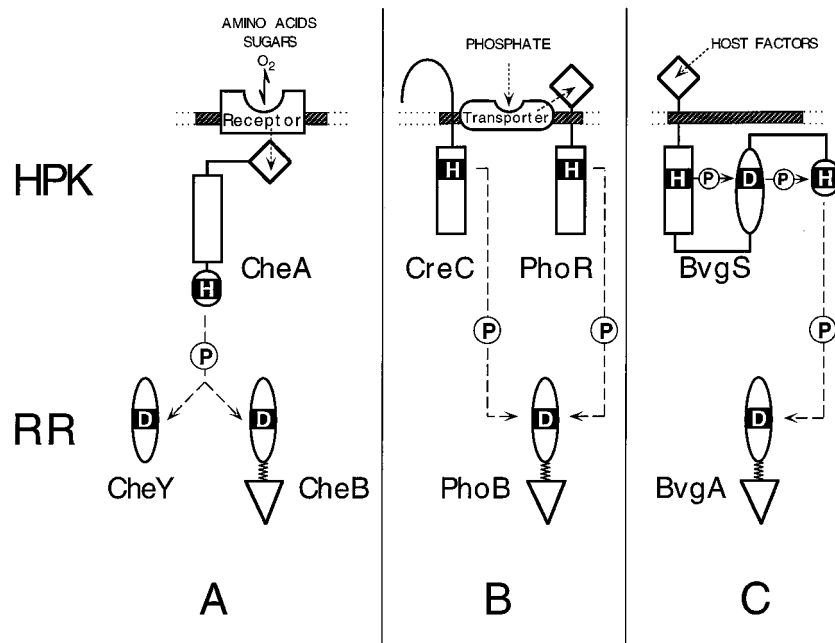


Figure 2. Examples of diversity in the two-component system. In A and B, a cell-surface receptor or transport protein is responsible for direct binding/detection of a stimulus, and this information is then conveyed to the appropriate HPK via protein-protein interactions. A, Part of the *E. coli* chemotaxis signaling pathway, a system in which the CheA HPK phosphorylates either of two different RRs; the CheY RR promotes changes in swimming direction, whereas the CheB RR promotes sensory adaptation (Bourret et al., 1991; Stock et al., 1991). The location of the phosphorylation site in CheA is atypical in that it is located outside of the transmitter module (Parkinson and Kofoid, 1992). B, Basic elements of the *E. coli* Pho(phosphate) system, which is responsible for controlling gene expression in response to phosphate availability. In this system, two distinct HPKs, PhoR and CreC, can phosphorylate the RR transcription factor PhoB. CreC responds to the intracellular concentration of some unknown metabolite, whereas PhoR is regulated by a phosphate-specific transport system (Wanner, 1994). C, BvgS, a hybrid HPK that regulates expression of virulence determinants of the human pathogen *Bordetella pertussis* in response to as-yet-unknown host factors. BvgS undergoes His to Asp to His phosphotransfer prior to phosphorylation of the RR transcription factor BvgA (Uhl and Miller, 1996). H, His; P, phosphoryl group; D, Asp.

CheB receiver module serves to enhance this activity (Fig. 2A) (Lupas and Stock, 1989). In the case of *E. coli* SprE, the output module regulates the activity of a protease (Pratt and Silhavy, 1996). Thus, the basic conformational changes associated with receiver phosphorylation are able to control a variety of activities (Lowry et al., 1994). If one excludes sequences of closely related homologs (e.g. NR₁ from two closely related bacterial species), receiver modules from any two RRs share sequence identity at only 20 to 30% of the positions, but all receiver modules are thought to have a similar three-dimensional structure (Stock et al., 1990; Volz, 1993). X-ray crystal structures and/or NMR-derived three-dimensional structures have been obtained for CheY (Stock et al., 1989; Volz and Matsumura, 1991), Spo0F (Feher et al., 1997), and NarL (Baikalov et al., 1996) proteins. These structures indicate a common $\alpha\beta$ protein structure for the receiver modules in RRs, with the phosphorylation site located in the loop connecting two of the central strands of β sheet that comprise the core of the receiver module structure. The three-dimensional structures of receiver modules are strikingly similar to that of the small GTP-binding protein Ras (Stock et al., 1991). This similarity is especially interesting in view of the ability of Ras to control MAPK pathways in several eukaryotic systems as we discuss later (Avruch et al., 1994).

A "Simple" Example

The EnvZ-OmpR system of *E. coli* provides a relatively straightforward example of the basics of two-component signaling (Fig. 1). This system regulates the expression of the *ompF* and *ompC* porin genes in response to changes in extracellular osmolarity (Pratt and Silhavy, 1995). EnvZ is an autophosphorylating HPK that serves as an osmosensor. The osmo-sensing module of EnvZ is located in the periplasmic space of the bacterial cell, and the EnvZ transmitter domain is situated in the cytoplasm. The actual signal perceived by EnvZ has not been determined, but under conditions of high osmolarity, autophosphorylated EnvZ readily transfers its high-energy His-phosphoryl group to the conserved Asp in the receiver module of the RR OmpR. P-OmpR binds to sequences upstream of the *ompF* and *ompC* genes, regulating their expression.

There are numerous complexities to this regulation that are beyond the scope of this review, but, overall, this two-component system controls the relative levels of OmpF and OmpC proteins. OmpF and OmpC form homotrimeric pores in the outer bacterial membrane, and by regulating the relative levels of larger (OmpF) and smaller (OmpC) pores, the EnvZ-OmpR systems restricts the rate of diffusion of materials through the outer membrane un-

der conditions of high osmolarity. In addition to serving as an HPK, EnvZ also catalyzes dephosphorylation of P-OmpR. The response of this system to osmotic conditions results from control of the two opposing enzymatic activities of EnvZ: kinase versus phosphatase (Pratt and Silhavy, 1995).

LESSONS LEARNED FROM BACTERIAL TWO-COMPONENT SYSTEMS

Modularity

Because RRs and HPKs are modular, it has been possible to determine the activities of isolated domains of these proteins. For example, in vitro studies on HPK activity have often been carried out on modified versions of HPKs that lack membrane-associated regions. In many cases (including that of EnvZ), deletion of such regions removes the sensory-input modules, resulting in a partially or completely active form of the kinase (Parkinson and Kofoid, 1992). This indicates that the input domain may often serve to inhibit kinase activity, and suggests that a common mechanism of kinase regulation could be the removal of this inhibition. Without knowing the ligands or "direct stimuli" for most HPKs, however, it is difficult to test these ideas. An added complication in such analyses is that the input domain may simultaneously regulate phosphatase activity of the HPK.

In a similar strategy, removal of the receiver modules from RRs has been useful in defining whether the receiver has a positive or negative influence on the activity of the output module. As a result of such studies, there are examples in which the receiver module operates as an inhibitor of RR output (Lupas and Stock, 1989; Kahn and Ditta, 1991; Baikolov et al., 1996), and those in which it operates in a positive manner to stimulate RR output (Drummond et al., 1990; Tsuzuki et al., 1994).

More-than-Two-Component Systems

Although some two-component systems appear to be as simple as indicated in Figure 1, many systems are more complex and involve either additional two-component modules or a variety of accessory proteins (e.g. Fig. 2). Some two-component systems, for instance, require an additional phosphatase to control the phosphorylation level of the RR. There are also numerous examples of systems that utilize more than one HPK or RR. These examples include: (a) multiple HPKs directing a single RR, (b) multiple RRs directed by a single HPK, (c) multistep phosphotransfer relays, and (d) hybrid sensor HPKs (Parkinson and Kofoid, 1992). Many of the sensor kinases identified in eukaryotic systems are hybrid HPKs, in which a receiver module is fused to the HPK such that a single protein encompasses both of the two-component elements. Most hybrid kinases appear to phosphorylate a receiver module on a second distinct protein; this opens up the possibility of having different pathways by which a phosphate can be transferred from a His-phosphorylation site to a receiver module (Appleby et al., 1996). FrzE in *Myxococcus xanthus*

provides an interesting exception to this generality in that this hybrid kinase appears to be capable of carrying out effector functions without the help of a distinct RR (Ward and Zusman, 1997).

Control Points

Different two-component systems appear to control RR phosphorylation levels via somewhat distinct mechanisms. For example, in response to a stimulus some systems alter RR-phosphorylation levels by controlling the rate of HPK autophosphorylation (Borkovich and Simon, 1990), whereas in other systems it is the phosphatase activity of the HPK or an additional component that is regulated in response to a stimulus (Atkinson et al., 1994; Perego and Hoch, 1996). This diversity underscores the impressive flexibility of two-component circuitry; it can be modified to operate in a variety of different contexts using different aspects of the basic protein structures of receiver and transmitter modules and the basic biochemistry of the phosphorylation/dephosphorylation chemistry.

Science by Analogy: Proceed with Caution

Several HPK-RR pairs have been subjected to extensive random and site-directed mutagenesis. The resulting mutants have helped to define functionally important positions within the respective transmitter and receiver modules of each HPK-RR pair. It seems reasonable to expect that such positions identified in one system would also play important roles in other two-component systems, and that mutations at such sites could be useful starting points for analyzing newly discovered two-component systems. In practice, however, such an approach has not been very successful. For mutation sites that are outside of the immediate vicinity of the active sites of HPKs and RRs, there are numerous examples of mutations that have a strong phenotype in one system but not in another (Parkinson and Kofoid, 1992; Stock et al., 1995). Results obtained with the HPK homolog SpoIIAB in *Bacillus subtilis* further underscore the need for caution when using sequence homology information to make predictions about function or mechanism: SpoIIAB actually operates as a Ser protein kinase, phosphorylating another protein (SpoIIAA) without any involvement of the His-Asp phosphorelay that is the hallmark of traditional two-component systems (Min et al., 1993).

EUKARYOTIC TWO-COMPONENT SYSTEMS

Members of the two-component family are now starting to be found with increasing frequency in eukaryotes, suggesting that the basic His-to-Asp phosphotransfer mechanism is employed by a variety of eukaryotic sensory-response pathways (Loomis et al., 1997; Wurgler-Murphy and Saito, 1997). A number of genes encoding HPKs, hybrid HPKs, and RRs have been reported in yeasts (*S. cerevisiae*, *Schizosaccharomyces pombe*, and *Candida albicans*), in the slime mold *Dictyostelium discoideum*, in *Neurospora crassa*, and in higher plants (e.g. Brown et al., 1993; Chang

et al., 1993; Ota and Varshavsky, 1993; Wilkinson et al., 1995; Alex et al., 1996; Kakimoto, 1996; Posas et al., 1996; Schuster et al., 1996; Shaulsky et al., 1996; Wang et al., 1996; Sakakibara et al., 1998).

In all but a few cases, the designation of a component as an HPK or an RR has been based exclusively on sequence similarities to known bacterial HPKs and RRs. One of the tacit assumptions in making such assignments is that "if it looks like a duck, it will quack like a duck." However, we really know very little concerning the input stimuli, output activities, biochemical properties, and signaling activities of most eukaryotic HPKs and RRs. Such information will provide much-needed tests of whether each of these HPK and RR "look-alikes" operate biochemically in the same manner as the well-characterized bacterial proteins. In this regard, we point out that in addition to SpoIIAB (the *B. subtilis* HPK "look-alike" mentioned above), there is a family of mitochondrial protein kinases that has sequence similarity to HPKs, but that actually functions as Ser/Thr kinases (Popov et al., 1993).

Mindful that the "logic of the duck" can lead to a certain amount of egg-laying, we note here that there do seem to be some general trends emerging in eukaryotic two-component systems. First, amino acid sequence similarities shared by any two eukaryotic two-component regulators are about the same as those shared by any two bacterial regulators or any eukaryotic-bacterial comparison. Second, arrangements of the protein modules in eukaryotic two-component systems are similar to those encountered in prokaryotes. Third, prokaryotic and eukaryotic two-component systems may respond to the same or similar signals. For example, the two-component system is known to play an important role in controlling responses to osmotic stress in bacteria (Pratt and Silhavy, 1995), in the budding yeast *S. cerevisiae* (Posas et al., 1996), in the fission yeast *S. pombe* (Shieh et al., 1997), and in the slime mold *D. discoideum* (Schuster et al., 1996). In addition, an HPK in *D. discoideum* regulates gene expression in prestalk cells and controls terminal differentiation of prespore cells (Wang et

al., 1996) in a manner that generally resembles the two-component pathway controlling sporulation in the bacterium *B. subtilis* (Hoch, 1995). Fourth, several eukaryotic two-component systems appear to regulate extended downstream effector cascades; that is, the two-component system may comprise only the upstream portion of a more extensive signaling pathway. This situation represents a clear difference from most prokaryotic two-component systems, in which the HPK-RR circuit comprises most or all of the sensory-response pathway, with the RR components serving as the end-of-the-line effectors. Several eukaryotic two-component pathways, including their output activities, are outlined in Table I.

TWO-COMPONENT REGULATORS IN HIGHER PLANTS

Receptors for Ethylene

In the ethylene signal transduction pathway of *Arabidopsis* there are at least four related HPK genes, *ETR1*, *ERS*, *ETR2*, and *EIN4*, which are thought to encode ethylene receptors (Chang et al., 1993; Hua et al., 1995, 1997; Schaller and Bleecker, 1995). Their predicted protein sequences are most similar to one another in the amino-terminal "sensory input" module (67–82% amino acid similarity). For *ETR1*, this region was shown to bind ethylene in a reversible and saturable manner, providing compelling evidence that *ETR1* is an ethylene receptor (Schaller and Bleecker, 1995). It seems likely that *ERS*, *ETR2*, and *EIN4* will also be found to bind ethylene, based on their sequence similarities with *ETR1* as well as their similar ethylene-insensitive mutant phenotypes. The carboxyl-terminal portion of each of these ethylene receptors contains a putative HPK transmitter module. *ETR1*, *ETR2*, and *EIN4* also have a carboxyl-terminal receiver module fused to the transmitter. Thus, *ETR1*, *ETR2*, and *EIN4* have adopted the hybrid HPK arrangement, whereas *ERS* has the appearance of a typical HPK. The *ETR2* sequence is the most diverged from the bacterial HPK consensus se-

Table I. Some of the known eukaryotic two-component systems and their output activities

Organism	Probable Signal	Pathway			Output Signaling	References
		Hybrid HPK (His→Asp)		RR (→Asp)		
<i>S. cerevisiae</i>	Osmolarity	SLN1	YPD1 (→His)	SSK1	MAPK cascade	Maeda et al. (1994); Posas et al. (1996)
<i>S. pombe</i>	Various stresses	?		MCS4	MAPK cascade	Shieh et al. (1997)
<i>A. thaliana</i>	Ethylene	ETR1		?	MAPK cascade (?)	Chang et al. (1993); Hua et al. (1995); Hua et al. (1997)
		ERS ^a		?	MAPK cascade (?)	
		ETR2		?	MAPK cascade (?)	
		EIN4		?	MAPK cascade (?)	
<i>D. discoideum</i>	Osmolarity Secreted peptide	DOKA		?	Cytoskeletal alteration (?)	Schuster et al. (1996)
		DHKA		REGA	cAMP-dependent protein kinase activity	Wang et al. (1996); Shaulsky et al. (1996); Shaulsky et al. (1998)

^a A typical (not a hybrid) HPK.

quences, lacking, for example, the conserved His autophosphorylation site (Hua et al., 1997). Although RRs would be the predicted effectors for the ethylene receptors, there is currently no evidence that RRs function in ethylene signal transduction.

Notably, all of the mutants that have been isolated for each of the Arabidopsis ethylene-receptor genes are dominant to the wild type and display ethylene insensitivity. Moreover, all of the mutations that have been identified are located within one of three hydrophobic regions in the amino-terminal ethylene-binding region. It is possible that recessive loss-of-function mutations, including those that would fall within the transmitter or receiver modules, have not been isolated due to the redundancy of the ethylene receptors. Indeed, there is evidence (from intragenic suppressor mutations of the dominant mutant *etr1* gene) that null *etr1* mutants have a wild-type phenotype (Hua et al., 1997). It is unclear why plants have multiple receptors for ethylene. Conceivably, the different receptors have tissue- or stage-specific functions (partially redundant) or act together as a hetero-multimeric receptor complex. A similar family of two-component ethylene receptors exists in tomato (Wilkinson et al., 1995; Yen et al., 1995; Zhou et al., 1996), and one of these homologs was identified as the gene for *Never-Ripe* (Wilkinson et al., 1995). *Never-Ripe* mutants have a dominant ethylene-insensitive phenotype, which includes a severe delay in fruit ripening (Yen et al., 1995).

Cytokinin Signaling

Recently, another two-component gene, *CK11*, was identified in Arabidopsis. The *CK11* gene was isolated from an enhancer-tagged line on the basis of cytokinin-independent hypocotyl growth (Kakimoto, 1996). This particular phenotype suggests that a two-component system might be involved in cytokinin signal transduction. *CK11* encodes a hybrid HPK comprised of a unique amino-terminal domain followed by a transmitter domain and a receiver domain. The amino-terminal portion of *CK11* (the presumed sensory-input module) has no sequence similarity to that of the ethylene-receptor family, and one attractive hypothesis is that *CK11* serves as a receptor for cytokinin. In maize there is an RR gene that might be involved in cytokinin-mediated nitrogen signaling from root to shoot; expression of this RR gene was induced by nanomolar concentrations of t-zeatin in detached maize leaves (Sakakibara et al., 1998).

Clues to Plant Phytochrome Action

The mechanism of plant phytochrome signaling has long remained elusive. One suggested mechanism is Ser protein kinase activity, but this has not been firmly established (Quail, 1997). In 1991, Schneider-Poetsch noted limited but discernable sequence similarity (roughly 25% identity) between bacterial HPKs and the carboxyl-terminal portion of plant phytochromes, leading to the proposal that phytochrome action might involve a two-component mechanism (Schneider-Poetsch et al., 1991). Currently, there is no evidence that plant phytochromes possess such activity; how-

ever, recent work in cyanobacteria strongly suggests that higher plant phytochromes are at least derived from an ancestral HPK-RR system. The strongest evidence comes from studies on the cyanobacterial *Synechocystis cph1* gene, which codes for a spectrally functional phytochrome (the only such protein currently known in prokaryotes) (Hughes et al., 1997; Yeh et al., 1997).

The *cph1* gene product is a two-component sensor that possesses light-responsive His autokinase activity (Yeh et al., 1997). The amino-terminal domain of Cph1 has sequence similarity to plant phytochromes and is capable of binding chromophores and of undergoing red/far-red light-induced reversible absorbance changes (Hughes et al., 1997; Yeh et al., 1997). Moreover, the phosphate on Cph1 is transferred from the His to an Asp residue in the separate RR Rcp1 (Yeh et al., 1997). Cph1 and Rcp1 thus form a light-regulated two-component system, which has implications for the activity of phytochromes in higher plants. His autokinase activity is exhibited by the Pr form of Cph1 rather than by the Pfr form, even though Pfr is normally thought of as the light-activated form; this suggests that the dark (Pr) form is the active form of phytochrome and that red light reduces or shuts off its activity.

Another light-responsive two-component system is the *rcaE* gene product from the cyanobacterium *Fremyella diplosiphon*. RcaE appears to be a sensor for chromatic adaptation. The amino-terminal portion of RcaE has small regions of sequence similarity to plant phytochromes and even to plant ethylene receptors (Kehoe and Grossman, 1996). RcaC is a response regulator that might act downstream of RcaE in regulating light responses in *F. diplosiphon* (Kehoe and Grossman, 1996).

DIVERSITY IN SIGNALING OUTPUT

As we have discussed, in most prokaryotic two-component systems, a membrane-associated HPK directs the activity of an RR that functions as a transcription factor. Thus, the typical output of the prokaryotic HPK-RR circuit is direct control of gene expression. What about the immediate output activity of two-component systems in eukaryotes? So far, only *S. cerevisiae* RR Skn7 appears to fit the "classic" prokaryotic model, operating as a transcription factor (Brown et al., 1994). However, even with Skn7 there are indications of intriguing complexities such as regulation by multiple sensory inputs (Brown et al., 1994; Page et al., 1996) and involvement in a diversity of processes ranging from cell wall biosynthesis (Brown et al., 1993) to oxidative stress responses (Krems et al., 1996; Morgan et al., 1997) and even G1 cyclin expression (Morgan et al., 1995). None of the other known eukaryotic RRs resembles a transcription factor, and none of the known eukaryotic HPK proteins appears to contain an output module. Based on the few available examples (described below), the trend in eukaryotes is that the immediate/direct output activities of two-component systems lie farther upstream of the ultimate regulators of gene expression (Table I).

MAPK Cascades

In three different pathways, the identification of downstream signaling elements has revealed coupling of the two-component system with the distinctly eukaryotic MAPK cascade. This is a new twist on the two-component system, as bacteria are not known to contain MAPK cascades. This also represents a new type of regulation of these cascades, which are more typically known to be regulated by upstream Tyr kinase receptors or seven-transmembrane (G-protein-coupled) receptors (Blumer and Johnson, 1994). The most established example of two-component regulation of a MAPK cascade is the *S. cerevisiae* osmolarity-response pathway, which controls adaptive responses to high osmolarity. The pathway involves a multistep phosphorelay from His to Asp to His to Asp residues (Posas et al., 1996). This multistep phosphorelay system can be found with slight variations in several bacterial two-component pathways such as that shown in Figure 2C (Appleby et al., 1996).

In the yeast osmolarity-response pathway, the phosphorelay begins with a hybrid HPK, SLN1, which is considered to be a transmembrane osmosensor. SLN1 is thought to undergo His autophosphorylation in low-osmolarity conditions (Maeda et al., 1994). Similar to the *Synechocystis* Cph1 phytochrome, SLN1 has His autokinase activity in the absence of the apparent signal (high osmolarity), suggesting that the SLN1 HPK is inactivated by the signal. In the next step of the phosphorelay, the phosphate is transferred from the His to an Asp in the SLN1 receiver module. The phosphate is then transferred to a His residue on a small intermediary protein called YPD1, and finally the phosphate is transferred to an Asp residue on a separate RR called SSK1 (Posas et al., 1996). Such an elaboration on the basic two-component system may allow for additional regulation, including the integration of different signals.

The output activity of SSK1 is the regulation of a MAPK cascade (Maeda et al., 1994, 1995). Under low-osmolarity conditions, the phosphorylation described above renders SSK1 inactive; under high-osmolarity conditions, SSK1 is unphosphorylated and activates two redundant MAPKKs, SSK2 and SSK22. SSK1 is known to physically interact with the regulatory domains of both of these MAPKKs, although the mechanism of stimulation is unclear. Next, SSK2 and SSK22 activate the MAPKK PBS2, which in turn activates the MAPK HOG1. The action of this MAPK pathway results in the expression of *GPD1*, which encodes a key enzyme in glycerol biosynthesis, leading to adaptive responses to high osmolarity (Wurgler-Murphy and Saito, 1997).

In *S. pombe*, a similar story is unfolding for a MAPK pathway that is activated by a range of stresses, including osmotic stress, oxidative stress, UV light, heat shock, and the protein-synthesis inhibitor anisomycin. This MAPK pathway, comprised of Wak1 (MAPKKK), Wis1 (MAPKK), and *StyI* (MAPK), was found to be regulated by an RR, Mcs4 (Shieh et al., 1997). Msc4 and Wak1 are structurally and functionally homologous to the SSK1 RR and the SSK2/SSK22 MAPKKs, respectively. These parallels with the *S.*

cerevisiae osmolarity-response pathway suggest that there may be one or more two-component sensors controlling the *S. pombe* pathway. In addition to this stress-activated pathway, Mcs4 controls the timing of mitotic initiation via an *StyI*-independent pathway that has yet to be defined (Shieh et al., 1997).

Another example of possible two-component regulation of the MAPK pathway is the Arabidopsis ethylene-response pathway. Based on genetic-epistasis analysis, the ethylene receptors act upstream of CTR1. CTR1 is a negative regulator of ethylene responses and encodes a Ser/Thr protein kinase most similar to the Raf family of MAPKKs (Kieber et al., 1993). Thus, it is likely that the ethylene-response pathway contains a MAPK cascade controlled by the two-component ethylene receptors. So far, a MAPKK and MAPK for this pathway have not been conclusively identified. There is evidence that the putative regulatory domain of CTR1 can physically associate with the transmitter domains of ETR1 and ERS, as well as with the receiver domain of ETR1, raising the possibility that the regulation of CTR1 activity involves direct interaction of CTR1 with the receptors (Clark et al., 1998). It remains to be seen whether the receptors provide direct "output" to CTR1 or whether additional two-component proteins such as RRs are involved. However, in view of the remarkable adaptability of the basic two-component elements, it would not be surprising if ethylene signal transduction reveals yet another variation on two-component signaling pathways.

Other Pathways

Given the above examples of MAPK regulation by eukaryotic two-component systems, it is important to point out that this is not always the case and may not even be a common situation. Other familiar eukaryotic signaling cascades may also be regulated by two-component systems. For example, the DhkA-RegA two-component system in the slime mold *D. discoideum* regulates cAMP phosphodiesterase activity of RR RegA (Shauly et al., 1998). Adjustments of cAMP levels via DhkA-RegA are responsible for controlling the activity of a cAMP-dependent protein kinase, which plays a key role in controlling the complex morphogenetic events that result in sporulation (Shauly et al., 1996, 1998). Another two-component system that points to diversity in signaling output is the osmotic-response system of *D. discoideum*. In this system, the hybrid HPK DokA may regulate events that are quite different from those described above for the yeast osmosensor SLN1. This slime mold does not appear to cope with conditions of high osmolarity by accumulating compatible osmolytes such as glycerol, but by events involving the cytoskeleton (Schuster et al., 1996). The pathway linking DokA to such events remains to be determined, but may provide yet another example of the diversity of signaling output regulated by two-component systems in eukaryotes.

SUMMARY

The basic two-component system involves two large families of signaling modules that build upon a His-to-Asp

phosphotransfer theme. Bacteria display numerous variations on this theme, illustrating the flexibility of the system. There is growing evidence, including a number of unpublished reports, that two-component regulators and distant relatives play important sensory-response roles in eukaryotes. These eukaryotic systems reveal further diversification of the two-component-based circuitry, most notably in the regulation of MAPK modules. Although quite a lot has been learned about how two-component systems operate, there remain numerous fundamental questions in both eukaryotic and prokaryotic systems; for example: How is HPK activity regulated by sensory input? What is the nature of the structural change resulting from receiver module phosphorylation, and how does this change result in the activation/deactivation of output activity? Are there "one-component" systems in which an "orphan" receiver module or transmitter module operates without a partner? In cells containing multiple two-component systems that respond to different stimuli, how is specificity maintained so as to minimize inappropriate "cross-talk"? Are there examples of two-component systems in which the transmitter and receiver modules direct protein-protein interactions but do not involve protein phosphorylation? As more and more two-component systems are discovered, and as the number of researchers in this field grows, we look forward to the resolution of these issues, as well as to further surprises from these versatile signaling modules.

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