Plant Two-Component Signaling Systems and the Role of Response Regulators[1]

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THE "PROKARYOTIC" LIFESTYLE OF PLANTS

At first glance, a 100-year-old tree and a bacterium do not have much in common. However, both plants and bacteria face a similar problem: They are confined to their environment and have to endure and adapt to a broad array of environmental conditions. To manage this complex task, prokaryotic organisms rely on the two-component signaling system, which is involved in such diverse processes as chemotaxis, osmotic sensing, and light perception (Stock et al., 2000). As shown in recent years, all canonical elements of two-component signaling systems are also found in plants (Schaller et al., 2001). This *Update* focuses on recent advances in our understanding of plant two-component systems, with particular emphasis on the function of plant response regulators.

PROTEIN ELEMENTS AND BIOCHEMISTRY OF TWO-COMPONENT SIGNALING SYSTEMS

In its simplest form, the two-component signaling system is composed of two elements: a His kinase and a response regulator (Fig. 1A; for review, see Stock et al., 2000). Although the majority of prokaryotic two-component signaling systems are composed of just these two proteins, there also exist more complex signaling systems that include additional elements, which are referred to as multistep, two-component systems (Fig. 1B). The biochemistry of two-component signal transduction is a phosphorelay: After a signal-induced activation by the input-domain, the transmitter of a His kinase autophosphorylates at a conserved His residue (Fig. 1A). The phophoryl group is then transferred to an invariant Asp within a conserved domain of the receiver (Fig. 1A). This His-to-Asp phosphorelay controls the activity of the output domain of the response regulator, which results, for example, in interaction with other proteins or binding to DNA (Stock et al., 2000).

The outlined one-step, two-component signaling system can be extended into a multistep system by including additional protein modules containing phosphorylatable Asp or His residues. The second

Asp is again included in a receiver module. In plants, the additional receiver module is often carboxyterminally fused to the His kinase, thereby creating a hybrid type of sensor kinase (Fig. 1B). The second His is embedded in a domain known as the HPt domain. The HPt domain enables the transfer of a phosphoryl group between two receiver modules (Stock et al., 2000). Many of the prokaryotic HPt domains are physically linked to other two-component signaling elements, but the Arabidopsis HPt domains are all individual proteins (Schaller et al., 2001). Why are some two-component systems of the simple and others of the complex type? The reason for extending the complexity of two-component systems does not apparently lie in signal amplification. Within a two-component system, the signal is relayed as a phosphoryl group in a strict one-to-one manner. Thus, the signaling output of the simple and the multistep, two-component system is the same. If it is not the gain in signal amplification, what could be the reason behind the apparent preference of multistep systems in plants? The answer to this question will become clear in subsequent sections.

TWO-COMPONENT SIGNALING ELEMENTS OF ARABIDOPSIS

In the early 1990s, the cloning of three genes led to the breakdown of a dogma. Until then, it was believed that two-component signaling systems were restricted to prokaryotes. However, with the realization that the Arabidopsis ethylene receptor ETR1 (Chang et al., 1993), the phytochrome photoreceptors (Schneider-Poetsch, 1992), and the yeast (*Saccharomyces cerevisiae*) osmonsensor SLN1 (Ota and Varshavsky, 1993) were all related to His kinases, it became clear that some genera of eukaryotes depended on two-component systems as well. With sequencing of the Arabidopsis genome now completed, 17 His and hybrid kinase-like proteins (AHK), five HPt proteins (AHP), and 23 response regulators (ARR) have been identified in Arabidopsis (for a complete list and phylogenetic trees, see Schaller et al., 2001). Although analysis has just begun, it is evident that, with the exception of phytochromes (Yeh and Lagarias, 1998), most AHKs, AHPs, and ARRs in principle function as phosphotransfer proteins (Schaller et al., 2001).
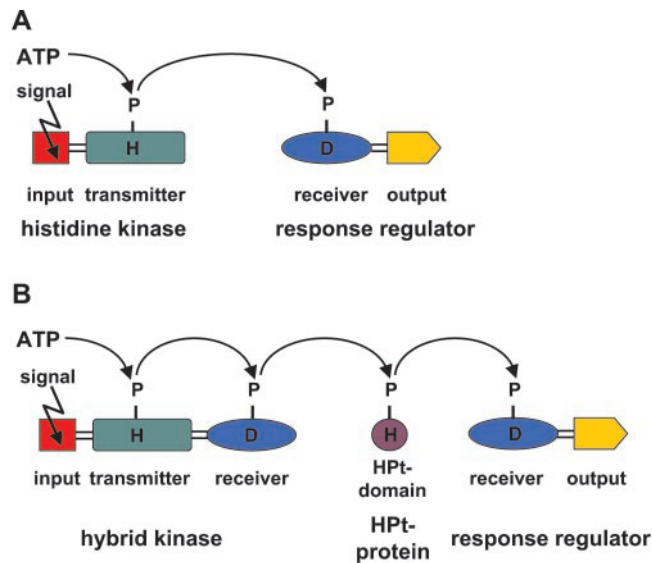
Figure 1. Schematic representation and basic features of the simple (A) and the multistep (B) two-component signaling systems. Signal perception by the input domain of the His or hybrid kinase induces autophosphorylation of the transmitter at a conserved His residue (H). The phosphate (P) is relayed to a conserved Asp residue (D) that is localized either in the receiver of the cognate response regulator (A) or, in the case of hybrid kinases, in an attached receiver domain (B). In the multistep, two-component signaling system, His-containing phosphotransfer (HPt) domain proteins serve as phospho-His intermediates between the hybrid kinase and the response regulator.

## ETHYLENE PERCEPTION AND SIGNALING

The ethylene perception and transduction system is one of the best understood plant signaling pathways (for review, see Chang and Stadler, 2001). The initial identification of ETR1 (Fig. 2A) was followed by the description of four additional ethylene receptors either belonging to the hybrid kinase type (ETR2 and EIN4) or the His kinase type (ERS1 and ERS2). By generating ethylene-binding sites in a heterologous yeast system (Schaller and Bleecker, 1995) and in genetic studies, it was elegantly shown that ETR1 is a membrane-associated ethylene receptor (Fig. 2A; Chang and Stadler, 2001). ETR1 exhibits His autophosphorylation activity in vitro (Gamble et al., 1998) and interacts with several AHP proteins in the yeast two-hybrid system (Urao et al., 2000). However, it has not yet been demonstrated that ETR1 and the other members of the ethylene receptor family act as ethylene-triggered hybrid kinases in plants.

If ethylene is signaling through a two-component system, one would expect to identify mutants affected in genes coding for HPt domain proteins and response regulators. Such mutants have not been reported, potentially because of functional redundancy within these signaling elements. The element shown to act downstream of the ethylene receptors and found capable of physically interacting with ETR1 and ERS1 is CTR1 (constitutive triple response

1), which is thought to function as a RAS-like mitogen-activated protein kinase kinase kinase (Chang and Stadler, 2001). Thus, it appears that the hybrid kinase-like ethylene receptors can function in a unique way linking the perception of the hormone to the activity of a mitogen-activated protein kinase cascade (for additional details on ethylene signaling downstream of CTR1, see Chang and Stadler, 2001). However, although CTR1 is a central component in ethylene signal transduction, there are several indications for the existence of additional signaling elements: (a) *ctr1*-null mutants are still slightly responsive to ethylene (Kieber et al., 1993; Larsen and Chang, 2001), and (b) the phenotype of quadruple *etr1-etr2-ein4-ers2* receptor mutants is more pronounced in comparison with that of *ctr1* (Hua and Meyerowitz, 1998). With AHP and ARR knockout lines now in hand (for review, see http://plantsp.sdsc.edu), it should soon be possible to determine whether two-component proteins represent these additional ethylene signaling components.

CYTOKININ PERCEPTION

The recent advances in our understanding of cytokinin signaling offer important insights into plant two-component systems (for recent reviews, see Schmülling, 2001; Haberer and Kieber, 2002). In 1996, a putative hybrid kinase (CKI1) was described by Kakimoto (1996), which, when overexpressed, resulted in cytokinin-independent growth of calli. However, most of the important advances came just recently. By screening for the loss of cytokinin-dependent greening and shoot formation of hypocotyl explants from Arabidopsis, the *cre1* (cytokinin response 1) mutant was identified. The *CRE1* gene codes for a hybrid kinase with an aminoterminal cytokinin-binding domain (Yamada et al., 2001) flanked by two transmembrane stretches (Fig. 2A). In contrast to ETR1, CRE1 contains a second receiver-like domain downstream of the transmitter (Fig. 2A). When expressed in yeast or fission yeast (*Schizosaccharomyces pombe*) strains, in which the endogenous hybrid kinases were knocked out, the *CRE1* gene product rescued the altered growth phenotypes of these mutants in the presence of cytokinin (Inoue et al., 2001; Suzuki et al., 2001). These results indicate that (a) CRE1 can bind cytokinin, (b) cytokinin binding induces the His kinase activity of CRE 1, and (c) CRE1 can transfer the phosphoryl group to the intrinsic HPt domain proteins of yeast and fission yeast initiating multistep, two-component signaling cascades. The function of CRE1 as a sensor His kinase in planta is also suggested by recent studies performed in Arabidopsis protoplasts (Hwang and Sheen, 2001). However, although CRE1 is a cytokinin receptor with His kinase activity, it is probably not responsible for mediating all of the plant cytokinin responses (Schmülling, 2001; Haberer and Kieber, 2002). Two
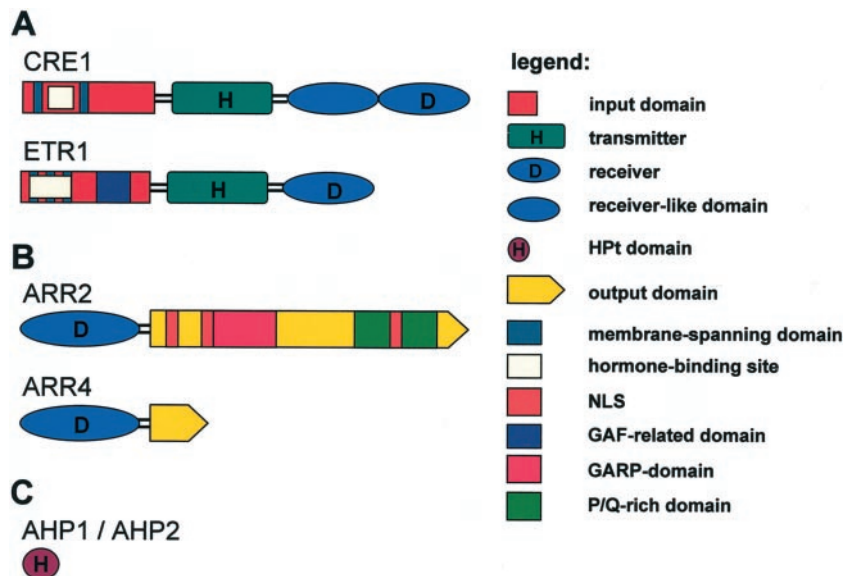
Figure 2. Structure of representative members of two-component signaling elements from Arabidopsis. A, Hybrid kinases: CRE1 and ETR1. The function of the GAF-related domain of ETR1 is unknown. B, ARR: ARR2, type-B response regulator; ARR4, type-A response regulator. The GARP motif of type-B response regulators is responsible for DNA binding and the nuclear localization sequences (NLS) for nuclear targeting. The Pro- and Gln-rich (P/Q-rich) domain serves as transactivation domain, which may interact with the basal transcription machinery. C, AHP: AHP1 and AHP2. The conserved amino acid residues (H, His; D, Asp) of the different two-component elements are indicated.

additional CRE1-like His kinases in Arabidopsis, AHK2 and AHK3, presumably also function as cytokinin receptors (Hwang and Sheen, 2001; Suzuki et al., 2001; Ueguchi et al., 2001).

## ARABIDOPSIS RESPONSE REGULATORS AND HPT DOMAIN PROTEINS

Although His kinase-like proteins were described in the early 1990s, it required almost a decade for other two-component signaling elements to be identified in higher plants, namely response regulators and HPt domain proteins. According to sequence homology, domain composition, and transcriptional regulation, most Arabidopsis ARRs can be described as either type A or B (Schaller et al., 2001). The type-A response regulators are relatively small and contain a receiver domain along with short C-terminal extensions (Fig. 2B). The mRNA of type-A ARRs is rapidly accumulating to varying extent after cytokinin treatment. The type-B ARRs have receiver domains along with an extended C-terminal output domain and are not induced by cytokinin (Fig. 2B). Recent microarray studies, however, indicate that the expression of the type-B response regulator ARR2 is down-regulated by the phytochrome-A photoreceptor in seedlings (Tepperman et al., 2001). We will now highlight recent insights into the function of type-A and type-B ARRs.

TYPE-B ARRS: RESPONSE REGULATORS WITH TRANSCRIPTION FACTOR PROPERTIES

The comparison of the amino acid sequences of the output domains of type-B ARRs revealed a highly conserved stretch of about 60 amino acids. This stretch was given the name GARP motif according to the founding members Golden2, ARR, and Psr1 (Fig. 2B; Riechmann et al., 2000). The GARP motif is pre-

dicted to fold into an α-helical structure and is related to the DNA-binding motifs of the basic helix-loop-helix and the MYB classes of transcription factors (Lohrmann et al., 1999, 2001; Sakai et al., 2000). Thus, it was proposed that the GARP motif represented a novel DNA-binding domain, and that type-B ARRs might function as transcription factors. By studying the subcellular distribution and transactivation capability in vivo, it became evident that type-B ARRs localized to the nucleus and were transcriptionally active (Lohrmann et al., 1999, 2001; Sakai et al., 2000). The first hint pointing to potential target promoters came with the histochemical analysis of the expression pattern of an *ARR2* promoter::*uidA* construct in transgenic Arabidopsis plants. Although the *ARR2* gene was expressed in almost all Arabidopsis organs, particularly high β-glucuronidase activity was observed in pollen grains (Lohrmann et al., 2001). A similar expression pattern is described for the nuclear genes for components of the mitochondrial respiratory complex I (*nCI* genes). The pollen-specific activity of the *nCI* genes is mediated by a conserved promoter element called the pollen box (Zabaleta et al., 1998). Lohrmann and colleagues (2001) demonstrated sequence-specific binding of ARR2 to the pollen box element and narrowed down the binding sequence to a 5'-W/GAT/W-3' core, where W is either A or T. This sequence matches the consensus binding site identified independently by Sakai et al. (2000) using the GARP domains of ARR1 and ARR2 in an oligo-selection approach. With the identification of the *nCI* genes as targets and with the demonstration of transactivation capability, it is evident that ARR1, ARR2, and presumably the other type-B ARRs function as transcription factors inside the nucleus (Fig. 3).

Recent evidence suggests that the type-B response regulators ARR1 and ARR2 are involved in cytokinin
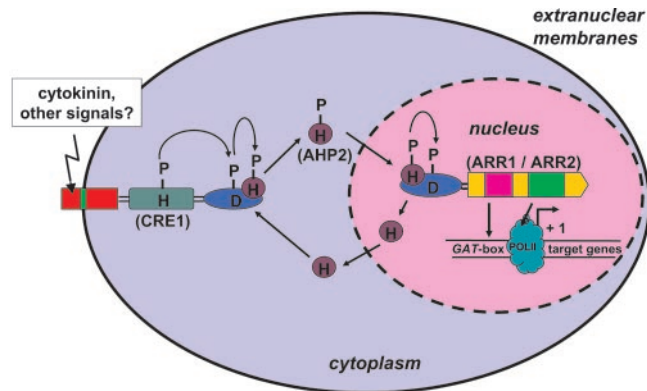
Figure 3. Model of an Arabidopsis multistep phosphorelay, demonstrating signal transduction from the extranuclear membranes to the nucleus. Signal perception by the input domain of a membrane-bound sensor His kinase (e.g. CRE1) induces autophosphorylation of the transmitter. The phosphate (P) is relayed to the cognate AHP (e.g. AHP2) interacting with the cytosolic receiver module of the kinase. The phosphorylated AHP shuttles to the nucleus and transfers the phosphate to the receiver domain of its cognate type-B response regulator (e.g. ARR1 and ARR2). The de-phosphorylated AHP shuttles back to the cytosol where it can be rephosphorylated. Phosphorylation of the receiver domain of the response regulator results in a conformational change in its output domain. This conformational change may either induce binding of type-B ARRs to their target promoter sequence (e.g. the GAT box in the case of ARR1 and ARR2) and/or activation of their transcriptional capacity. The promoter-associated, active response regulators triggers the transcription of its target genes (e.g. *nCI* genes, type-A *ARR* genes). The conserved amino acid residues (H, His; D, Asp) of the different two-component moduls are indicated. POLII, RNA polymerase II-dependent basal transcription machinery.

signaling. An *arr1*-null mutant was found to be partially resistant to cytokinin in shoot regeneration and root elongation, whereas ARR1-overexpressing lines displayed the opposite, hypersensitive phenotype (Sakai et al., 2001). Similar alterations in sensitivity to cytokinin were observed when the expression of several type-A *ARR* genes including *ARR4* were analyzed in the *arr1* mutant and ARR1-overexpressing lines (Sakai et al., 2001). A comparable cytokinin-inducible effect of ARR1 and ARR2 on the expression of an *ARR6* (type-A ARR)::reporter construct was observed in Arabidopsis mesophyl protoplasts (Hwang and Sheen, 2001). These results suggest that ARR1 and ARR2 mediate the cytokinin-induced expression of type-A *ARR*s (Fig. 4). It is of interest to note that ARR2 very strongly induced the *ARR6*::reporter gene expression in Arabidopsis protoplasts even in the absence of cytokinin (Hwang and Sheen, 2001). This observation is indicative for the presence of a further stimulus that enables the activation of ARR2 and, in turn, ARR2-regulated target genes in protoplasts.

From the study in Arabidopsis protoplasts, it is not clear whether the activities of ARR1 and ARR2 are regulated by Asp phosphorylation (Hwang and Sheen, 2001). This raises the question of whether

cytokinin signals through a canonical phosphorelay to regulate the transcription factor properties of type-B response regulators. However, the conservation of the canonical phosphorylation sites in the type-B ARRs (and also the type-A ARRs) are strongly indicative of the functional relevance of these sites. Moreover, Sakai and colleagues (2001) provided in planta evidence that the phospho-accepting receiver domain suppresses the function of ARR1 in the absence of cytokinin and that the cytokinin signal releases this suppression. This release could potentially be induced by phosphorylation of the highly conserved Asp in the receiver domain of ARR1.

The finding that type-B ARRs are localized to the nucleus raises the question as to how the signal information perceived by the extranuclear and membrane-bound sensor His kinases is transduced to the nuclear compartment. For instance, one of the cognate sensor His kinases of ARR1 and ARR2 is very likely the cytokinin receptor CRE1, which is predicted to be present on an extranuclear membrane (Yamada et al., 2001). Moreover, protein-protein interaction studies revealed that the sensor His kinases CKI1, CRE1, and ETR1 cannot directly interact with ARR1 and ARR2 (Lohrmann et al., 2001; C. Grefen and K. Harter, unpublished data). As outlined in Figure 1B, the Arabidopsis HPt domain proteins could perform the information transfer as part of a
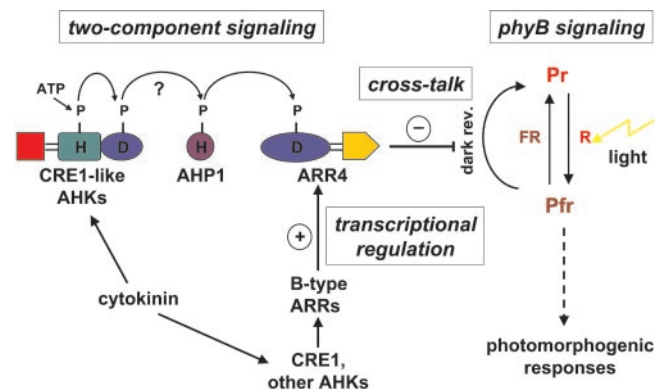
Figure 4. Model for the action of an ARR4-comprising two-component signaling system on phytochrome B signal transduction. Red light (R) induces the photoconversion of the inactive Pr form of phytochrome B (phyB) in the physiologically active Pfr form. Phosphorylated ARR4 then stabilizes the active Pfr form by inhibition of phyB dark reversion (dark rev.). Pfr stabilization enhances the sensitivity of plants to red light and induces more intense photomorphogenic responses (phyB signaling). The phosphorylation state and the activity of ARR4 is potentially regulated by a multistep phosphorelay comprising of the HPt protein AHP1 and presumably CRE1-like hybrid kinases (two-component signaling). Therefore, ARR4 enables cross talk and thus integration of signal information provided by hormone (cytokinin) and the red-light signaling cascades (cross talk). The expression of ARR4 and the protein composition of the two-component system are regulated by cytokinin (transcriptional regulation). The conserved amino acid residues (H, His; D, Asp) of the different two-component moduls are indicated. FR, Far-red light; P, phosphate residue.

multistep, two-component phosphorelay. His kinases (ETR1, CKI1, and CRE1) as well as response regulators (ARR2) can interact with AHPs, indicating that HPt domain proteins may serve as molecular adaptors (Urao et al., 2000; Lohrmann et al., 2001; Suzuki et al., 2001). AHPs are localized to the cytosol as well as the nucleus of the plant cell, and thus have the ability to perform functions in both compartments (Hwang and Sheen, 2001; Lohrmann, 2001; Sweere, 2001). Although the AHP proteins have a size of around 12 kD (Schaller et al., 2001) and may enter the nucleus by diffusion, at least some AHPs transiently accumulate in the nucleus of Arabidopsis protoplasts in response to cytokinin application (Hwang and Sheen, 2001). These observations support the following model of AHP action (Fig. 3). After activation by the specific signal the sensor His-kinase autophosphorylates and then transfers the phosphoryl group to the cognate AHP. The AHP then shuttles into the nucleus and relays the phosphate to nuclear type-B ARRs. Phosphorylation of the receiver module triggers the transcription factor properties of type-B ARRs, which results in an altered activity of their target genes. The dephosphorylated AHP then moves back to the cytoplasmic compartment to be rephosphorylated. This model would indicate that higher plants have adapted the ancestral two-component systems to a novel type of eukaryotic intracellular signaling mechanism. Because of the necessity for a shuttling HPt domain protein, this adaptation could only be achieved by use of multistep, two-component systems.

TYPE-A ARRS: RESPONSE REGULATORS MODULATING OTHER SIGNAL TRANSDUCTION PATHWAYS

As mentioned above, the transcription of type-A response regulator genes like *ARR4* is rapidly induced by cytokinin. This and some additional criteria established the hypothesis that type-A ARRs are primary response genes of cytokinin. Recent studies suggest that the cytokinin-regulated transcriptional control of type-A *ARR* genes is mediated by type-B response regulators (Fig. 4; Hwang and Sheen, 2001; Sakai et al., 2001). An effector protein recently was identified for a type-A response regulator, thereby lending insight into the signaling mechanisms that operate downstream of these response regulators. Sweere and colleagues (2001) demonstrated that ARR4 specifically interacts with the plant photoreceptor phytochrome B. It is interesting that ARR4 did not interact with the COOH terminus of phytochrome B, which is related to transmitter domains of His kinases (Schneider-Poetsch, 1992), but with the extreme NH$_2$-terminus.

Phytochrome B is a red/far-red reversible chromoprotein, which regulates red light-specific photomorphogenetic responses in plants (Neff et al., 2000).

It is synthesized in the inactive Pr form and photoconverted by red light into the physiologically active Pfr form. Far-red light is able to induce the photoconversion of the active Pfr form back to the inactive Pr form. In addition, active Pfr converts back to inactive Pr in a light-independent manner known as dark reversion, which is thought to be an additional switch-off mechanism for phytochromes (Eichenberg et al., 2000). Independent approaches in yeast and in Arabidopsis indicated that the interaction of ARR4 with phytochrome B resulted in the stabilization of the physiologically active Pfr form by inhibition of Pfr-to-Pr dark reversion (Fig. 4; Sweere et al., 2001).
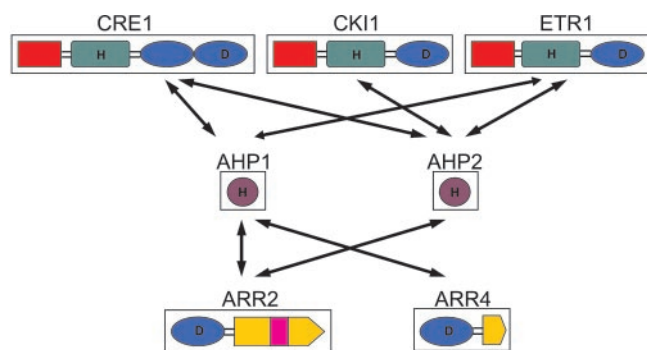
The functional relevance of the phytochrome B:ARR4 interaction and Pfr stabilization was examined in transgenic plants overexpressing ARR4. These plants displayed a hypersensitivity to red light in respect to all photomorphogenic responses tested (e.g. hypocotyl elongation, root growth, and flowering time). Moreover, transgenic Arabidopsis plants expressing a mutant form of ARR4, which was not phosphorylated at the conserved Asp residue in a plant cell-free phosphorelay system, displayed hyposensitive phenotypes to red light in hypocotyl elongation and flowering time (Sweere, 2001; V. Mira-Rodado and K. Harter, unpublished data). These data suggests that the mutant protein exerts a dominant-negative effect on the function of endogenous ARR4 and that Asp phosphorylation of ARR4 plays an important role in modulating phytochrome B activity (Sweere, 2001). It is interesting that the above-mentioned wild-type and mutant ARR4-overexpressing lines also displayed weak hyposensitive and hypersensitive, respectively, phenotypes in response to cytokinin treatment (Sweere, 2001). Recent studies in protoplasts have shown that the overexpression of type-A ARRs represses their own expression, suggesting that type-A ARRs may also provide a negative feedback regulation in cytokinin signaling (Hwang and Sheen, 2001; Haberer and Kieber, 2002).

Because no His kinase activity of phytochrome B has been reported and because of the primary cytokinin response properties of ARR4, the photoreceptor may be a target of a cytokinin-modulated two-component signaling system (Fig. 4). CRE1-like cytokinin receptors might regulate the activity of ARR4 by a His-Asp phosphorelay and thereby modulate phytochrome-B signaling. Such a phosphorelay may involve the HPt domain protein AHP1, which has the ability to function in a phosphorelay with CRE1 (Suzuki et al., 2001) and also acts upstream of ARR4 (Sweere, 2001). AHP1 and ARR4 are found in both the cytosol and nucleus (Hwang and Sheen, 2001; Sweere et al., 2001). Thus, the putative AHK-AHP1-ARR4 phosphorelay, as well as the interaction of ARR4 with phytochrome B, may occur in the cytosol to modulate cytosolic, phytochrome B-dependent processes. On the other hand, AHP1 may also be able

to transfer the phosphoryl group from the cytosol to the nucleus, where ARR4 may then modulate nuclear, phytochrome B-dependent responses. Regardless of which sensor His kinase regulates ARR4 activity, the studies by Sweere and colleagues suggest a novel type of cross talk between an ancestral two-component system and the phytochrome B-dependent light-signaling pathway (Fig. 4). This cross talk may enable the integration of signal information provided by hormone (cytokinin) and red light (phytochrome B) signal transduction cascades. It is now of major interest to identify the effector proteins of the remaining type-A response regulators and to clarify the underlying cross talk mechanisms.

A COMPLEX PLANT TWO-COMPONENT SIGNALING NETWORK EMERGES: CONCLUSIONS AND PERSPECTIVES

As described above, there is now the first experimental evidence that hybrid kinases like CRE1, AHK2, and AHK3 signal through multistep phosphorelays containing AHPs and ARRs. In addition, a wealth of yeast two-hybrid and in vitro protein-protein interaction studies indicate the possibility of a complex two-component signaling network in cells of higher plants (Urao et al., 2000; Lohrmann et al., 2001). A detail of such a potential network is shown in Figure 5. Specificity to some of these interactions can be recognized. For example, ETR1 and CRE1 interact with both AHP1 and AHP2, whereas CKI1 interacts with only AHP2 (Fig. 5). Specificity is also apparent for the interaction of ARRs with AHPs. ARR2 interacts with AHP1 and AHP2, whereas the interaction of ARR4 is restricted to AHP1 (Fig. 5). The physiological relevance of these interactions is not yet clear, because two-component elements tend to be notoriously promiscuous. For instance, as indicated in previous sections, two-component signaling

elements from Arabidopsis can participate in phosphorelays from biologically irrelevant fungal systems. However, even given the preliminary nature of this emerging picture, cross talk between different two-component signaling pathways would have major functional implications. Two-component systems may form a complex signaling network capable of sensing, computing, and integrating a broad range of signals. The sessile lifestyle of plants makes it necessary that every cell monitors and integrates a multitude of inputs to reach a finely balanced decision on how to react at the cellular and the whole-plant level. The increased potential for cross talk potentially represents an additional reason as to why multistep two-component systems rather than simple two-component systems were utilized in plants. We have already received our first glance at this complexity from studies revealing cross talk between the ARR4-comprising two-component system with phytochrome B-dependent signal transduction. Overlapping sets of synergistic and antagonistic physiological responses are also described for many other stimuli such as ethylene and cytokinin (Gilroy and Trewavas, 2001). Complexly interconnected two-component systems could participate in the generation of these overlapping physiological responses.

In the near future, the combinatorial use of genetic, molecular, cell biological, and biochemical approaches should clarify the in planta signaling pathways, in which two-component signaling elements are involved in. Furthermore, these approaches will reveal how the two-component network is weaved and linked to other eukaryotic signal transduction cascades.

Figure 5. Detail from the emerging network of two-component signaling systems in Arabidopsis. Lines indicate direct protein-protein interactions detected in the yeast two-hybrid system and in vitro. Proteins that are not connected do not display physical interaction. ETR1, CRE1, and CKI hybrid kinase suggested to be involved cytokinin signaling. AHP1 and AHP2, HPt domain proteins. ARR2 and ARR4, Response regulators. The conserved amino acid residues (H, His; D, Asp) of the different two-component moduls are indicated.

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