J Plant Growth Regul (2002) 21:32–39
DOI: 10.1007/s003440010045

J P G R
Journal of Plant Growth Regulation

Molecular Mechanisms of Cytokinin Signaling

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Abstract

Cytokinins regulate a myriad of plant growth and developmental processes. Recent molecular genetic studies in *Arabidopsis* have begun to unravel the molecular mechanisms underlying cytokinin perception and signal transduction. A family of cytokinin receptors has been identified, and these are homologous to bacterial two-component sensor kinases. Events immediately downstream of cytokinin binding are similar to the classic phosphorelay paradigm. The cytokinin signal appears to be transduced from the membrane-localized histidine kinase-like receptors into the nucleus via a transient translocation of the AHP proteins, which are *Arabidopsis* homologs of histidine phosphotransfer proteins. Once in the nucleus, the AHPs activate the type-B class of *Arabidopsis* response regulators (ARRs), which in turn activate the transcription of a second class of *Arabidopsis* response regulators, the type-A ARRs. A model of cytokinin signaling from perception at the plasma membrane to activation of gene expression in the nucleus is beginning to emerge.

Key words: *Arabidopsis*; Hormones; Cytokinin signal transduction; Two-component; Histidine kinases

Introduction

Cytokinins were first identified by the pioneering work of Skoog and Miller in their search for factors that could promote the proliferation of cultured tobacco pith cells (see Armstrong, this issue). They have since been implicated in the regulation of many plant growth and developmental processes, including the control of leaf senescence, cell proliferation, bud opening, apical dominance, chloroplast development, and sink/source relationships (Mok and Mok 2001; Mok and Mok 1994). Rapid progress has recently been made in the characterization of a cytokinin signaling pathway that is similar to two-component systems. This review will discuss our current understanding of cytokinin signaling, drawing mainly from molecular genetic studies in *Arabidopsis*.

The cytokinin receptor and downstream elements are similar to two-component signaling systems. Two-component systems are prevalent prokaryotic signaling pathways that mediate adaptive cellular responses to environmental stimuli (reviewed by Stock and others 2000; West and Stock 2001). Typically, signaling involves two partners, the sensor kinase and the response regulator, and proceeds through an alternating His–Asp phosphorylation (Figure 1). The sensor kinase consists of an input and a transmitter domain. Detection of the signal by the input domain controls the catalytic activity of the transmitter domain, which is a histidine kinase. Sensor kinases, which associate into dimers, transphosphorylate onto a conserved His residue located

Received: 11 December 2001; Accepted: 12 December 2001
Online publication: 30 April 2002
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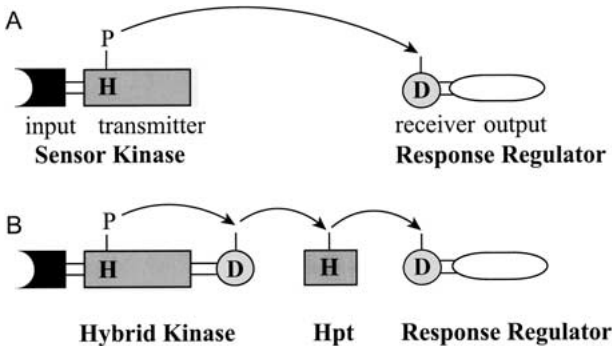

Figure 1. Carton representations of two-component phosphotransfer schemes. **(A)** A basic prokaryotic two-component system with a sensor histidine kinase and a response regulator. H and D represent the conserved phospho-accepting histidine and aspartate residues involved in the phosphorelay. The input, transmitter, receiver, and output domains are indicated. **(B)** A multistep phosphorelay system involving a hybrid sensor kinase, with input, transmitter and receiver domains, a histidine-containing phosphotransfer protein (Hpt), and a response regulator. Arrows depict phosphotransfer reactions.

in the transmitter domain. The phosphoryl group is then transferred onto the Asp residue within the receiver domain of a cognate response regulator. The phosphorylation state of the receiver domain regulates the activity of the output domain, which often directly regulates gene transcription.

A more complex version of the two-component system is the multistep phosphorelay in which the His–Asp unit has been duplicated. Although all phosphorelays involve four sequential phosphorylation events that alternate between His and Asp, the architecture of the multistep system components is variable. That is, a phosphorelay can comprise two, three, or four distinct proteins. *Arabidopsis* phosphorelay components are similar to those of the budding yeast osmosensing pathway (reviewed in Lohrmann and Harter 2001; Schaller and others 2001). They consist of a sensor histidine kinase with a fused receiver domain, an arrangement known as a hybrid kinase, a histidine phosphotransfer protein (AHP) and two classes of response regulators, called type-A and type-B ARRs. All these components have been shown to participate in cytokinin signaling as discussed in the following sections.

The Cytokinin Receptor is a Histidine Kinase

The *cytokinin response 1* (*cre1*) mutant was isolated in a screen for mutants impaired in the cell division, greening, and shoot formation responses of callus tissue to cytokinin (Inoue and others 2001). Root growth in *cre1* mutants is also partially resistant to inhibition by cytokinin. Genetic and molecular analysis showed that CRE1 corresponds to the histidine kinase AHK4 (Inoue and others 2001). The predicted secondary structure of CRE1 consists of two trans-membrane domains in the N-terminal region separated by an extracellular loop (Inoue and others 2001; Mähönen and others 2000). The histidine kinase domain is followed by two receiver-like domains that are predicted to be located on the cytoplasmic size of the plasma membrane.

The CRE1 extracellular domain belongs to a ligand-binding domain family termed CHASE for Cyclase/Histidine kinases Associated Sensing Extracellular (Anantharaman and Aravind 2001; Mougel and Zhulin 2001). CHASE domains have been identified in a wide range of prokaryotic and eukaryotic organisms and are predicted to bind diverse low-molecular-weight ligands. Membranes prepared from fission yeast expressing CRE1 specifically bind isopentyl adenine with a high affinity (Yamada and others 2001), confirming that CRE1 is capable of binding cytokinin. A single amino acid substitution in the CHASE domain eliminates *in vitro* cytokinin binding, *in vivo* function, as well as the ability of the protein to complement an *E. coli* mutant disrupted in the RcsC histidine kinase (Mähönen and others 2000; Yamada and others 2001). These data suggest that the CHASE domain of CRE1 is the site of cytokinin binding and that cytokinin binding is required for CRE1 function.

Elegant experiments involving the ability of CRE1 to complement histidine kinase-deficient yeast and *E. coli* mutants in a cytokinin-dependent manner provided compelling evidence that CRE1 is a cytokinin receptor (reviewed by Haberer and Kieber 2002; Schumülling 2001). Mutation of the CRE1 phosphorylation sites, either His459 or Asp973, abolished complementation in these systems, indicating that CRE1 function requires His–Asp phosphorylation (Inoue and others 2001; Suzuki and others 2001b). Furthermore, disruption of the relevant host HPt or response regulator abolished the ability of CRE1 to restore growth in these heterologous systems, which suggests that CRE1 is capable of transferring a phosphoryl group to the host HPt molecule.

Hwang and Sheen (2001) have developed a transient expression assay using *Arabidopsis* leaf mesophyll protoplast that allows the monitoring of transcriptional activation of a cytokinin primary response gene, *ARR6*. In this system, overexpression of CRE1 results in increased induction of *ARR6*

expression by cytokinin, confirming that CRE1 is a cytokinin receptor in *Arabidopsis* cells. As in yeast and *E. coli,* kinase activity and His–Asp phosphoryl transfer appears to be necessary for CRE1 activity in plant cells, as mutation of the targets of phosphorylation eliminates activity. In fact, overexpression of these mutant forms of CRE1 results in a negative dominant effect, suggesting that CRE1 may use a bimolecular phosphoryl transfer process as described for prokaryotic histidine kinases. In these prokaryotic systems, sensor kinases are dimers that transphosphorylate on histidine. The *Arabidopsis* ethylene receptor ETR1 is a histidine kinase that forms disulfide-linked dimers (Schaller and others 1995). Association of a mutant and wild-type CRE1 receptor may result in nonfunctional dimers, which would explain the dominant negative effect observed when mutant forms of CRE1 are overexpressed.

The *cre1/wol* mutants display a root phenotype, but aerial parts of the plant are unaffected, which suggests that CRE1 is not the only cytokinin receptor in *Arabidopsis* (Hwang and Sheen 2001; Inoue and others 2001). Two other closely related kinases, AHK2 and AHK3, both of which contain a CHASE domain, have also been implicated in cytokinin signaling. Both of these histidine kinases are able to complement yeast and *E. coli* mutants as CRE1 does, and both bind to cytokinin when expressed in heterologous systems (Suzuki and others 2001b; Ueguchi and others 2001; Yamada and others 2001; T. Mizuno, personal communication). However, overexpression of AHK2 and AHK3 in protoplasts has little effect on the induction of *ARR6* by cytokinin (Hwang and Sheen 2001). Loss-of-function mutations of these two AHK genes should reveal the function of these histidine kinases and their role in cytokinin responsiveness.

CKI1 was the first histidine kinase homolog implicated in cytokinin signaling. It was isolated in an activation tagging screen for mutants that proliferated shoots from cultured cells in the absence of exogenously supplied cytokinin (Kakimoto 1996). When CKI1 is overexpressed in *Arabidopsis* protoplasts, *ARR6* transcription is elevated independently exogenous cytokinin, suggesting that CKI1 is constitutively active or is saturated by endogenous cytokinin levels (Hwang and Sheen 2001). This constitutive, cytokinin-insensitive activity of CKI1 has also been reported in *E. coli* complementation assays (Yamada and others 2001). Thus, the role of CKI1 as a cytokinin receptor remains unclear. Heterologous complementation studies indicate that when histidine kinases are overexpressed, they can transfer phosphate groups to non-cognate substrates in the host cell; therefore, the effect of CKI1 overexpression *in planta* should be interpreted with caution. Recently, a loss-of-function allele of CKI1 has been identified and shown to result in a female gametophytic lethal phenotype (T. Kakimoto, personal communication). Further analysis of this mutant should clarify the role of CKI1 in cytokinin signaling.

Arabidopsis Histidine Phosphotransfer Proteins Implicated As Downstream Targets of CRE1

In bacterial and yeast multistep phosphorelays, histidine phosphotransfer proteins (HPt) act downstream of hybrid kinases. HPts mediate transfer of the phosphoryl group from the receiver domain of the hybrid kinase to the receiver domain of a response regulator (Figure 1). The *Arabidopsis* genome contains five genes encoding HPt-like proteins; they are called AHPs (*Arabidopsis* HPts). The predicted amino acid sequences indicate that these five AHPs consist of a single phosphotransmitter domain containing a conserved His residue that is the putative phosphorylation site (Suzuki and others 2001a).

Several recent reports have provided evidence that AHPs are likely involved in cytokinin signaling. Overexpression of AHP2 in *Arabidopsis* results in a slight increase in the sensitivity of the transgenic seedling to cytokinins (Suzuki and others 2001a). More importantly, when expressed in *Arabidopsis* protoplasts, AHP1–GFP and AHP2–GFP fusion proteins transiently traffic from the cytoplasm to the nucleus following exogenous application of cytokinin (Hwang and Sheen 2001). These results suggest that these two AHPs act as shuttles between the hybrid kinases located at the plasma membrane and the response regulators located in the nucleus (Hwang and Sheen 2001; Lohrmann and others 1999; Sakai and others 1998).

A large number of interactions have been detected among various AHPs and several histidine kinases and among AHPs and type-A and type-B ARRs using the yeast two-hybrid assay. These data are consistent with AHPs acting as intermediates in the signaling from histidin kinases such as CRE1 to the *Arabidopsis* response regulators (ARRs), as is the case with their bacterial counterparts. Although there appears to be some specificity in these interactions (that is, individual AHPs interact with only subsets of the histidine kinases), such specificity may be artifactual because a negative result in a yeast two-hybrid may not accurately reflect a lack of *in vivo* interaction.

The ability of AHPs to function as phosphotransmitters between hybrid kinases and response regulators has also been investigated. Several of the AHPs are able to complement a deletion of YPD1, the histidine transfer protein of the budding yeast osmosensing phosphorelay pathway (Miyata and others 1998; Suzuki and others 1998). Coexpression of various AHPs with CRE1 in *E. coli* compromises the ability of CRE1 to complement a mutation in the endogenous *E. coli* RcsC histidine kinase; mutation of the histidine phosphorylation site of AHP2 abolishes this effect (Suzuki and others 2001b). These results suggest that AHPs can compete with the endogenous *E. coli* HPt protein for phosphorylation by CRE1 on their conserved histidine residue. Phosphorylation of AHP at the conserved phosphoaccepting histidine residue has been demonstrated *in vitro* by incubation of AHP1 and AHP2 with crude *E. coli* membrane preparations (Imamura and others 1999; Suzuki and others 1998). Phosphotransfer from AHP1 and AHP2 (both phosphorylated by *E. coli* microsomes) to type-A and type-B ARRs *in vitro* also been demonstrated (Imamura and others 1999; Suzuki and others 1998, 2001b). Taken together these results suggest that AHPs can function as phosphotransmitters.

The emerging model for the early steps of the cytokinin-signaling pathway is as follows (Figure 2): CRE1 is activated by cytokinin binding to the CHASE domain and *trans*-autophosphorylates. Activated CRE1 phosphorylates a subset of the AHPs, which then move from the cytoplasm to the nucleus where they transfer the phosphate group to the ARRs. Molecular mechanisms by which AHPs are mobilized in the cell by cytokinin have not yet been studied, and there is as yet no direct data to show that phosphorylation of the AHPs is important in their relocalization.

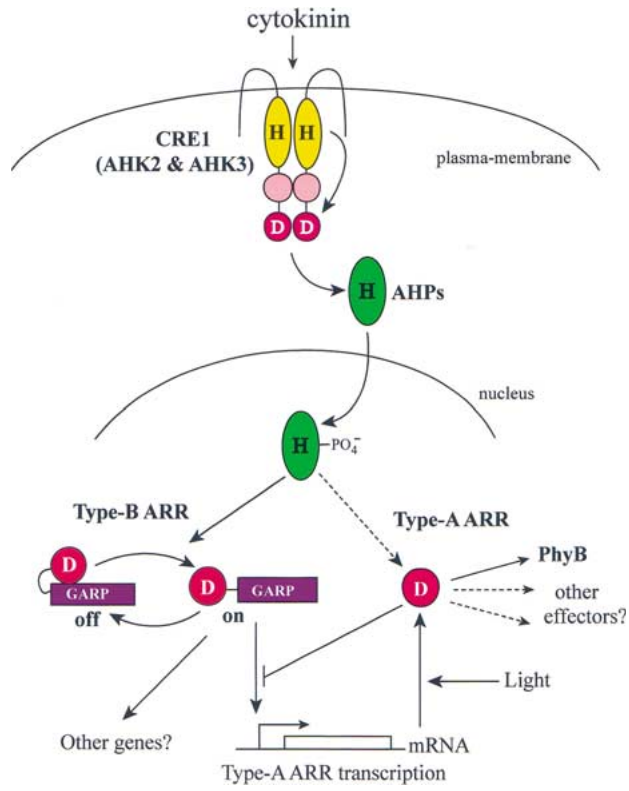

Figure 2. Proposed model for two-component pathway in cytokinin signaling. Cytokinin binds to CREl and possibly to other related histidine kinases such as AHK2 and AHK3 in the CHASE domain. CRE1, by analogy to CKI1 (Hwang and Sheen 2001), is likely to be located in the plasma membrane where it forms dimers. Binding of cytokinin activates the kinase activity within the CRE1 transmitter domain. The kinase catalyzes the autophosphorylation *in trans* of a His (indicated by an H) within the transmitter domain (yellow). The phosphate is then transferred to an Asp residue (indicated by a D) within the fused receiver domain (red). A second degenerated receiver domain (lighter red) is also present in CRE1 and its homologs. The phosphate is then likely to be transferred to an AHP protein (green), which transiently translocates to the nucleus where it activates type-B ARRs, possibly via phosphorylation of their receiver domains (red). The receiver domain of type-B ARRs inhibits the activity of the output domain in the absence of signal. The activated type-B ARRs increased the transcription of type-A ARRs, whose feedback inhibits their own transcription. Light appears to regulate the level of ARR4 protein, as indicated by the arrow. The functional interaction between AHPs and type-A ARRs is unclear. The output of the signaling pathway is mostly unknown, although one likely target is PhyB. See text for additional details.

Role of *Arabidopsis* Response Regulator

In typical two-component pathways, the last effector is the response regulator that triggers the response to the signal initiated by the histidine kinase. The *Arabidopsis* genome encodes a large response regulator gene family comprising 22 members (Schaller and others 2001). These genes have been divided into two groups, type-A and type-B, based on a phylogenic analysis of their amino acid sequences and their domain structures (D'Agostino and Kieber 1999; D'Agostino and others 2000; Imamura and others 1999; Schaller and others 2001). The type-A ARRs consist of a receiver domain with short N- and C-terminal extensions, whereas the type-B ARRs contain a receiver domain and a C-terminal output domain displaying similarity to transcription factors (D' Augastino and

Kieban 1999). Furthermore, expression of most type-A genes is inducible by cytokinin, whereas type-B expression is unaffected by cytokinin.

Sequence analysis of the type-B output domain reveals the presence of potential nuclear localization signals (Lohrmann and others 1999; Sakai and others 1998), and indeed several type-B ARRs have been shown to localize to the nucleus (Hwang and Sheen 2001; Lohrmann and others 1999, 2001; Sakai and others 2000). The type-B ARR output domains contain a conserved ''GARP domain'', so called because it is found in GOLDEN2 in maize, the ARRs, and the Psr1 protein from *Chlamydomonas* (Reichmann and others 2001). The GARP domain of ARR1 and ARR2 binds DNA in a sequence-specific manner, preferentially binding to the core sequence AGATT in gel-shift assays (Sakai and others 2000). The presence of multiple AGATT motifs within the promoter *ARR6* and other type-A ARR genes indicates that type-B ARRs may regulate directly transcription of type-A ARR genes which is consistent with the observation that the induction of expression of type-A ARR genes by cytokinin is not inhibited by cycloheximide (Brandstatter and Kieber 1998). The C-terminal part of ARR1, ARR2, and ARR11 output domain can activate transcription from a GAL4-driven promoter when fused to the GAL4 DNA-binding domain in both yeast and tobacco cells (Lohrmann and others 1999, Saki and others 2000), indicating that it contains transcription activator activity.

A loss-of-function mutation in *ARR1* was found to result in reduced sensitivity to cytokinin in shoot regeneration assays and showed reduced transcript levels of *ARR6* (a cytokinin-inducible type-A gene). Conversely, overexpression of *ARR1* in transgenic plants increased sensitivity to cytokinin and resulted in an elevated level of *ARR6* transcript, both in the presence and absence of exogenous cytokinin (Sakai and others 2001). Overexpression of a truncated version of ARR1, in which the receiver domain has been removed, resulted in a much stronger phenotype compared with overexpression of the full-length gene, suggesting that the receiver domain may negatively regulate the transcriptional activity of the output domain (Sakai and others 2000). Similarly, overexpression of *ARR2* in *Arabidopsis* protoplasts also elevated both the basal and cytokinin-induced level of a cytokinin-responsive *ARR6* reporter (Hwang and Sheen 2001). Overexpression of other type-B genes in protoplasts (*ARR1* and *ARR10*) in this protoplast system also increased *ARR6* expression, although to a lesser extent than did overexpression of *ARR2* (Hwang and Sheen 2001). Taken together, these results indicate that type-B ARRs are involved in cytokinin signaling leading to the induction of expression of type-A ARRs.

In prokaryotes, response regulators are in equilibrium between inactive and active states (reviewed by Foussard and others 2001; West and Stock 2001). Nonphosphorylated receiver domains generally repress the activity of the output domain, whereas phosphorylation leads to activation of the output domain. Surprisingly, overexpression of a mutant of *ARR2,* in which the conserved phosphorylation site in the receiver domain is altered, was just as efficient as wild-type *ARR2* in stimulating the induction of *ARR6* expression by cytokinin in isolated *Arabidopsis* protoplasts (Hwang and Sheen 2001). This result suggests that phosphorylation of ARR2 is not required for its activation. However, this conclusion should be viewed with caution as it is based on overexpression in an artificial system.

Although phosphorylation results in an activation of bacterial response regulators, the molecular mechanisms by which activation is achieved are quite diverse (reviewed by Foussard and others 2001; West and Stock 2001). In some cases, activation consists of the formation of response regulator oligomers. For example, upon phosphorylation, the *E. coli* response regulator ArcA multimerizes into an active complex that is composed of both phosphorylated and nonphosphorylated ArcA proteins in a ratio of 1:1, suggesting that phosphorylated ArcA can recruit nonphosphorylated ArcA for assembly (Jeon and others 2001). In such a model, overexpression of a nonphosphorylatable *ARR2* mutant in a wild-type cell might still be able to stimulate transcription in a phosphorelay-dependent manner by association of the mutant *ARR2* with endogenous, wild-type phosphorylatable *ARR2*. Conservation of the phosphorylation site among type-B ARRs and demonstration that kinase and phosphotransfer activity are required for CRE1 function suggests that the phosphorelay mechanism is functional in at least a subset of the *Arabidopsis* two-component elements. Further study should reveal whether phosphorylation is sufficient or whether other factors are required to control the transcriptional activity of type-B ARRs.

Cytokinin signaling through a two-component pathway leads to the induction of the type-A ARR gene expression. Ten genes encoding type-A ARRs are present in the *Arabidopsis* genome and these fall into five pairs with highly similar amino acid sequences (D'Agostino and others 2000). The amino acid sequences of the type-A receiver domains are very similar to each other, though there is a variable region of 11–21 amino acids in the central portion.

The sequences of the C-terminal domain are also more divergent and are not highly similar to any known proteins in GenBank (D'Agostino and others 2000; Imamura and others 1999). These C-terminal domains vary in size, but all are less than 100 amino acids and do not appear to act as classical output domains.

Overexpression of several type-A ARRs in *Arabidopsis* protoplasts suppressed cytokinin induction of *ARR6* expression, indicating that type-A ARRs negatively regulate their own expression (Hwang and Sheen 2001). In bacteria, dephosphorylation of the response regulator leads to its deactivation (reviewed by Foussard and others 2001; West and Stock 2001). Most of the systems described require a specific phosphatase for this dephosphorylation reaction, but in a few cases a reverse phosphorelay process has been implicated (Ansaldi and others 2001; Georgellis and others 1997; Sourjik and Schmitt 1998). In *E. coli*, chemotactic stimuli leads to the autophosphorylation of the histidine kinase CheA, which phosphorylates the response regulator CheY (reviewed by Bourret and others 1991). CheY is dephosphorylated by the phosphatase CheZ. In contrast, *Rhizobium meliloti* does not have a CheZ ortholog but rather a second response regulator called CheY1 (Sourjik and Schmitt 1998). Dephosphorylation of CheY2, the ortholog of the *E. coli* CheY, involves a reverse phosphorelay in which the phosphoryl group is transferred from CheY2 to CheY1 via an HPt domain intermediate. Similarly, type-A ARRs could act as a sink for phosphate, promoting the dephosphorylation, and thus inactivation of the type-B ARRs, which would result in a suppression of their own (that is type-A ARR) transcription. Because both type-A and type-B ARRs are located in the nucleus, the reverse phosphorelay may employ the histidine of the AHPs; the phosphoryl group would be transferred back from type-B ARR to an AHP and then to the type-A ARRs. Alternatively, the type-A ARRs may compete with the type-B ARRs for phosphotransfer from the AHPs. Consistent with both of these models, interactions between AHPs and type-A ARRs have been reported and phosphotransfer from AHPs to type-A ARRs occurs *in vitro* (Imamura and others 1999; Suzuki and others 1998; Urao and others 2000).

Downstream Pathway

Though the early events of cytokinin signal transduction have been elucidated, the outputs of this pathway remain obscure. ARR2 has been implicated in the control of the expression of genes encoding several components of the *Arabidopsis* mitochondrial respiratory chain complex; however, it is unknown whether the expression of these genes is related to cytokinin signaling (Lohrmann and others 2001). Apart from the type-A ARR genes, no other genes whose expression is controlled by type-B ARRs and cytokinin have been identified. Type-B ARR loss-of-function mutants and the use of *Arabidopsis* gene microarrays should provide powerful tools for the identification of such genes.

The other possible output of the phosphorelay pathway may be via the type-A ARRs. The presence of a histidine kinase-like domain in the red light receptor PhyB led Sweere and coworkers (2001) to investigate the potential interaction of PhyB and the ARRs. Direct interaction of ARR4 and PhyB was shown to occur in both yeast cells and *Arabidopsis.* Surprisingly, ARR4 does not interact with the histidine kinase domain but rather with the extreme N-terminus of PhyB. Photoconversion experiments revealed that the interaction with ARR4 stabilizes the active Pfr form of PhyB. Consistent with this, transgenic *Arabidopsis* overexpressing ARR4 displayed hypersensitivity to red light. Many examples of interactions between light and cytokinin on plant development and gene expression have been reported (reviewed by Thomas 1997), and ARR4 may play a role in the integration of red light and cytokinin signals.

ARR4 transcript is present at equal levels in etiolated and light-grown seedlings (D'Agostino and others 2000). In contrast, ARR4 protein is detectable only in light-grown seedlings (Sweere and others 2001). Thus, ARR4 protein accumulation requires light. Regulation of *ARR4* gene expression therefore appears to be complex, with transcription controlled by cytokinin and protein accumulation controlled by light. Although overexpression of ARR4 in *Arabidopsis* protoplasts reduced the induction of expression of type-A ARR gene by cytokinin. (Hwang and Sheen 2001), it remains unclear whether *in vivo* ARR4 is involved in both feedback on cytokinin signaling and light signaling. More generally, the results obtained with ARR4 suggest that other type-A ARRs might be acting to propagate the cytokinin signal to distinct effector pathways.

Conclusions

Two-component signaling pathways are widespread in prokaryotes. The evolution of the original two-step His–Asp phosphotransfer into more sophisticated multistep phosphorelays occurred by the multiplication of the receiver and HPt modules.

Recent advances in cytokinin signaling have revealed how the modular organization of the multistep phosphorelay has been adapted to the eukaryotic cell and its compartmentation. Sensor kinases and ARRs are physically separated by the plasma and nuclear membranes. Interaction between these elements is mediated by the shuttling of AHP modules between the cytoplasm and nucleus. Further studies should reveal if the architectural constraints of the eukaryotic cell have led to the development of new regulatory mechanisms distinct from those of the typical phosphorelay.

Arabidopsis phosphorelay elements constitute a complex network with each component belonging to a multigene family. It is not clear yet what the extent of the functional redundancy is within each of these families. Several lines of evidence suggest that a least some of the components are involved in specific functions in some cell types: (1) Despite the fact that CRE1 appears to be expressed in most tissues, *wol* mutant displays a root-specific phenotype, suggesting that other cytokinin receptors provide overlapping functions (Mähönen and others 2000). (2) Interaction of PhyB with ARR4 may be specific to that response regulator since no interaction was observed between PhyB and ARR5 (Sweere and others 2001). (3) In contrast to AHP1 and AHP2, AHP5 does not move to the nucleus in response to cytokinin (Hwang and Sheen 2001). The question of the specificity of the function of the various gene family members remains open.

The hybrid sensor kinase family contains additional receptors, including members of the ethylene receptor subfamily. The ethylene receptor ETR1 displays histidine kinase activity and could potentially interact with downstream phosphorelay components (Gamble and others 1998). Signals other than cytokinin may be transduced by the two-component system. Dissection of these pathways, whether each signal involves specific components or whether components are shared allowing cross-talk between different inputs, and how specificity of interaction among the members of the different transduction chains is achieved will be two of the challenges of future research.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (grants MCB-9996354 and IBN-0114965 to J. Kieber).

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