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 phosphorylation 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...
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 phosphorelay systems 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 (HPT) and two classes of response regulators, called type-A and type-B ARRPs. 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/Hisidine 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*