Cytokinin receptor: **Just another histidine kinase**

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The cytokinin family of plant hormones is involved in diverse aspects of plant growth and development *in vivo* and in culture. Two groups have recently shown that a two-component histidine kinase functions as a cytokinin receptor specifically required for vascular development.

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Investigators interested in plant development tend to fall into two broad groups. One group is interested in physiological aspects of growth and development and uses genetic and molecular approaches to elucidate the mechanisms of light and hormone action. In contrast, the other group seeks to establish the genetic framework for development, typically focussing on a particular structure such as the flower or the root. Although these two approaches have been quite distinct, with separate sessions at major meetings for example, there is a growing awareness that plant development depends on the integration of hormonal and light signals with the genetic program.

A recent study of root development [1] beautifully illustrates this new approach. In *Arabidopsis*, root development involves a highly predictable series of cell divisions that suggests strict genetic control [2]. The recent work of Sabatini *et al.* [1], however, demonstrates that the plant hormone auxin has a key role in determining the behavior of cells in the root meristem. And two studies [3,4] have now revealed that vascular development in the *Arabidopsis* root also requires a protein that appears to function as a receptor for the plant hormone cytokinin.

The story starts with the isolation of an *Arabidopsis* mutant called *wooden leg* (*wol*). Recessive *wol* mutants display defects in the root vascular system, including a reduction in the total number of cells and the absence of phloem and procambium — the primary meristem which gives rise to vascular tissue. Genetic studies [2] suggest that *WOL* is not directly involved in cell fate determination, but is required for specific cell divisions in the developing vascular system. According to this model, the absence of phloem and procambium in the vascular system is an indirect effect of having fewer cells.

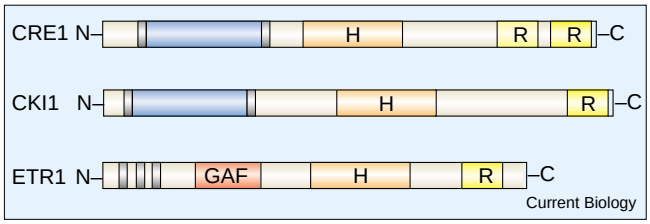
In their recent paper, Mahonen *et al.* [4] show that *WOL* is required for a series of asymmetric divisions in the root meristem that are necessary for development of the phloem and procambium. The authors have characterized *WOL* by a positional cloning approach and show that it encodes a member of the two-component family of signaling proteins found in plants, fungi and bacteria. In plants, the best-characterized members of the family are the ethylene receptors [4].

WOL has a novel extracellular domain flanked by two transmembrane regions, a histidine kinase domain, and two response regulator domains (Figure 1). The gene is expressed in the vascular cylinder and in the pericyle — a tissue between the endodermis and phloem which gives rise to lateral roots — consistent with its function in these tissues (Figure 2). From the domain content and organization of WOL, Mahonen *et al.* [4] propose that the protein is a receptor required for vascular morphogenesis. Presumably the extracellular region interacts with a ligand, but what ligand?

The answer to this question is found in another recent study [3] in which a genetic approach was taken to identify genes required for response to the cytokinin group of plant hormones. Cytokinins are aminopurine derivatives — discovered in 1955 — that can stimulate division of tobacco cells in culture [6]. Physiological studies have implicated the cytokinins in various growth processes, but until recently we knew remarkably little about how these compounds function. This began to change in 1996 with the isolation of a gene called *CKI1* [7].

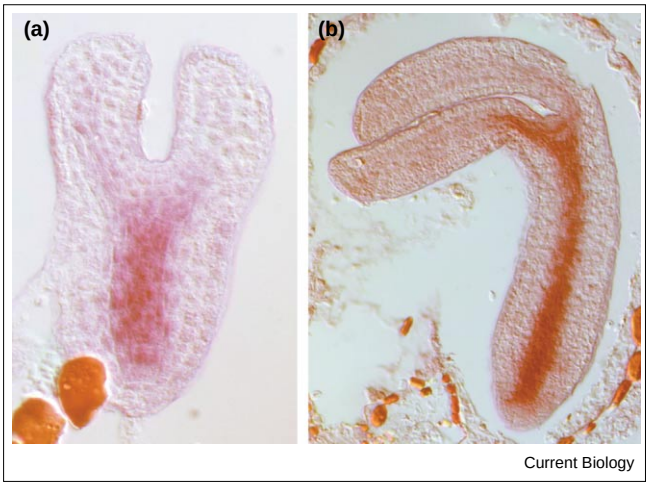
Over-expression of *CKI1* in *Arabidoipsis* confers cytokinin-independent cell division upon transgenic tissues. CKI1 is

Figure 1



Three two-component histidine kinase proteins in *Arabidopsis*. CKI1 and CRE1 are similar in overall organization but quite diverged in sequence. The ETR1 protein is a representative of the ethylene receptor family. These proteins do not have an extracellular ligand binding domain since ethylene binding occurs within the hydrophobic regions [3]. The grey bars represent hydrophobic regions and the blue section is presumed to be extracellular. The GAF domain is a conserved motif that may function in cyclic-GMP binding. H and R represent the histidine kinase and response regulator domains respectively.

Figure 2



Localization of *WOL/CRE1* mRNA during embryogenesis by *in situ* hybridization. (a) Torpedo stage, (b) bent-cotelydon stage. mRNA accumulation is evident in the procambium of cotyledon shoulders, prospective hypocotyl, and embryonic root. Photos courtesy of Yka Helariutta and Marjukka Riikonen.

a two-component histidine kinase with an overall organization similar to WOL, except that CKI1 has a single response regulator. The sequence of the protein and the effects of over-expression suggest that it may function as a cytokinin receptor, but this has not been demonstrated. In the latest chapter of this developing story, however, Kakimoto and collaborators [3] present convincing evidence that a different two-component protein involved in root development, CRE1, is a cytokinin receptor.

The *cytokinin response 1* (*cre1*) mutant was isolated in a screen that involved an *Arabidopsis* version of replica plating [3]. Approximately 19,000 mutagenized seedlings were cut into two parts. The upper part of the seedling — containing the shoot meristem and cotyledons — was placed on medium without cytokinin. This section grew and eventually produced seed. The other explant, a section of the seedling stem or hypocotyl, was placed on medium containing cytokinin. Wild-type hypocotyl explants grown on this medium rapidly proliferate into a dark green mass of callus tissue. In contrast *cre1* explants produce lighter green tissue with numerous roots, behavior that is normally observed on medium without cytokinin. Subsequent analysis using intact seedlings showed that *cre1* plants are less sensitive to the inhibitory effects of cytokinin on root growth, but display normal responses to other plant hormones.

Taken together, these results indicate that the *cre1* mutant is deficient in cytokinin response. *CRE1* was mapped to the top of chromosome 2 in a region that also contains the *WOL* gene. Since Kakimoto's earlier studies had implicated two-component proteins in the cytokinin response, it was logical to check to see if *CRE1* and *WOL* were identical. DNA sequencing revealed that the *cre1-1* allele has a glycine to aspartate (G467D) substitution in the histidine kinase domain of WOL. In addition, a genomic fragment containing the wild-type kinase gene restored normal cytokinin response when introduced into *cre1-1* plants, confirming that CRE1 is WOL. Surprisingly CRE1 and CKI1 are quite diverged, with approximately 25% amino acid identity in the amino-terminal half of the proteins — the region containing the histidine kinase and regulatory domains. Most striking, the putative extracellular domains of the two proteins are completely different, a fact that argues against a common ligand.

In the case of CRE1, the ligand does appear to be cytokinin. To demonstrate this Inoue *et al.* [3] used a yeast strain deficient in SLN1 — a two component histidine kinase that functions in osmosensing. At normal osmolarity, SLN1 initiates a phosphorylation cascade that results in inhibition of a mitogen activated protein (MAP) kinase pathway. The *sln1Δ* mutant is lethal because the MAP kinase pathway is overactive. Remarkably, expression of *CRE1* in this mutant restores viability in a cytokinin-dependent manner. Mutant forms of CRE1 in which conserved residues are replaced in both the kinase domain and the response regulator domain lack the ability to rescue the *sln1Δ* strain. Similarly the *cre1-1* mutant allele will not rescue the yeast mutant. These results provide convincing evidence that CRE1 is a cytokinin receptor. Rescue by CRE1 is also dependent upon a protein called YPD1, a phosphotransfer protein that facilitates phosphate transfer from SLN1 to the response regulator SSK1. This result suggests that CRE1 can transfer a phosphate to YPD1 in yeast and therefore may interact with a similar protein in *Arabidopsis*.

Both of the two recent studies of *WOL* [3,4] note that there are two other genes in the *Arabidopsis* genome that are closely related to *WOL/CRE1*. As the function of *WOL/CRE1* appears to be largely restricted to the vascular system, it is likely that the two related genes function as cytokinin receptors in other tissues in the plant. This still leaves the question of CKI1 function unanswered. It is possible that CKI1 does interact with cytokinin even though the putative ligand-binding region is quite different from WOL/CRE1. It should be possible to test this by asking if CKI1 will function in place of WOL/CRE1 in either *Arabidopsis* or yeast. Alternatively, a recombinant protein in which the amino-terminal region of WOL/CRE1 is replaced by the corresponding CKI1 sequence can be tested for function.

What about the rest of the cytokinin signaling pathway? The function of WOL/CRE1 in the *slnΔ* mutant suggests that WOL/CRE1 interacts with a YPD-like protein. In

addition, there are a number of response regulator genes in *Arabidopsis* and transcription of some of these is rapidly induced by cytokinin [8]. The possibility that some of these proteins act downstream of WOL/CRE1 must also be addressed. Finally, to return to my earlier theme, we can now begin the difficult task of understanding how cytokinin interacts with the genetic program to regulate processes such a vascular development.

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