

Receptor kinases in plant development

Philip W. Becraft

Receptor protein kinases (RPKs) are components of signal transduction pathways that elicit cellular responses to extracellular information. These proteins play an essential role in animal development, but the traditional thinking has been that the cell wall is a barrier to this type of signaling in plants and that most intercellular communication must therefore occur via the plasmodesmata. However, recent evidence indicates that RPKs are also essential for a variety of plant processes, including development. A combination of molecular and genetic analysis has revealed the existence of several plant proteins that possess the characteristics of RPKs, although their function as a receptor has yet to be demonstrated.

Receptor protein kinases (RPKs) are a diverse group of proteins that span the plasma membrane and allow cells to recognize and respond to their extracellular environment¹. They contain a cytoplasmic protein kinase domain that is activated by ligand binding to the extracellular receptor domain (Fig. 1). The first plant receptor-like kinase (RLK) to be identified was ZmPK1 of maize². This was isolated using degenerate PCR primers to kinases, and subsequent analysis revealed that it contained similarity to the S-locus glycoprotein (SLG) in its predicted extracellular domain. Since then, a great many more RLKs have been identified by both molecular and genetic means. Two main features are noteworthy³. First, with the exception of PRK1, which has dual specificity for serine/threonine and tyrosine⁴, all other plant RLKs characterized to date belong to the serine-threonine kinase family. This contrasts with the situation in animals, where most RPKs of developmental importance are tyrosine kinases¹ – a notable exception being the TGF- β receptor. Second, plant RLKs can be classified according to features of the predicted extracellular domain (Fig. 2). The majority contain leucine rich repeat (LRR) sequences, which are implicated

in protein-protein interactions⁵. Another group contain an S-domain with similarity to SLG, and several others contain unique features such as an epidermal growth factor (EGF)-like motif⁶, a tumor necrosis factor receptor (TNFR)-like motif⁷ or a lectin-binding domain⁸.

Here we examine the likely role that RLKs play in important aspects of signal recognition and plant development.

Receptor-like kinases and plant development

Pollen development in petunia

PRK1 was isolated from *Petunia inflata* in a differential screen to identify genes specifically expressed in pollen tubes⁴. The sequence of one clone suggested it encoded an RLK, designated PRK1 ('pollen receptor-like kinase 1'). *PRK1* is expressed in mature pollen and growing pollen tubes. Expression of an antisense cDNA to *PRK1* resulted in 50% pollen abortion⁹. Microspore meiosis proceeded normally but half of the microspores were arrested at the uninucleate stage. Neither the transgene nor the pollen abortion phenotype was transmitted through the pollen, indicating that pollen expressing the transgene aborted. Surprisingly, the transgene showed reduced transmission through the female and subsequent analysis revealed that embryo sac development was also arrested postmeiotically, usually during the maturation stages¹⁰. These results indicate that *PRK1* is required for postmeiotic gametophyte development and raise the intriguing possibility that it might coordinate gametophytic development with that of the surrounding sporophytic tissue¹⁰.

The predicted *PRK1* protein consists of 720 amino acids⁴. The extracellular domain contains five LRRs but no other similarity with known proteins has been reported. The sequence of the kinase domain predicted a serine/threonine kinase, but phosphoamino acid analysis indicated that tyrosine residues were also phosphorylated.

ERECTA1 and the regulation of organ shape

The *erecta1* mutation is present in the common Landsberg *erecta* ecotype. The mutant phenotype consists of a compact inflorescence, and shortened siliques and leaf petioles¹¹. In other respects the flowers appear normal, suggesting that the mutant decreases post-fertilization carpel elongation. Thus *ERECTA1* is required for proper specification of organ shape. *ERECTA1* has been cloned by T-DNA tagging and found to encode a predicted RLK of 976 amino acids. The extracellular domain contains 20 LRRs and the cytoplasmic domain contains a serine/threonine kinase catalytic domain¹¹.

CRINKLY4 and the differentiation of aleurone and shoot epidermis

The phenotype of maize *crinkly4* (*cr4*) mutants includes defects in the aleurone and shoot epidermis⁷. In mutant kernels, sporadic patches of the aleurone fail to differentiate and *cr4* plants have crinkled leaves that often adhere to one another (Fig. 3). Analysis of the original mutant allele suggested that the plant phenotype was primarily a consequence of epidermal defects; epidermal cells were

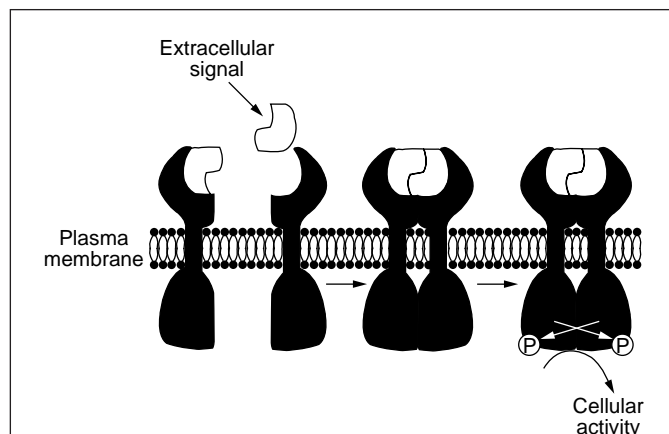


Fig. 1. A general model for how receptor protein kinases (RPKs) function. RPKs form a large and multivariuous class of proteins that function in diverse and often complex signaling systems¹. They are plasma membrane-spanning proteins with an extracellular receptor domain and an intracellular protein kinase domain. The general mechanism of RPK function is that binding of an extracellular signal ligand induces receptor dimerization. This brings the intracellular kinase domains into close proximity, facilitating trans-phosphorylation, which activates the kinase domains and allows them to regulate a cellular response. Different RPKs form either homodimers or heterodimers. Some dimerize with several partners, activating different responses accordingly and some participate in more than one signal transduction pathway depending on cell type.

It is enticing to speculate that SERK is required to make cells competent to undergo embryogenesis and that this competence reflects the ability to perceive an inductive signal. At this juncture, the only evidence for this is a correlation between expression and embryogenic competence; however, if SERK is shown to be required

for competence, it will be interesting to determine the nature and source of the signal that triggers embryogenesis. It is difficult to imagine that a molecule present in callus cultures and ovules could be a specific inducer of embryogenesis, suggesting that SERK might confer an embryogenic response to a non-specific signal.

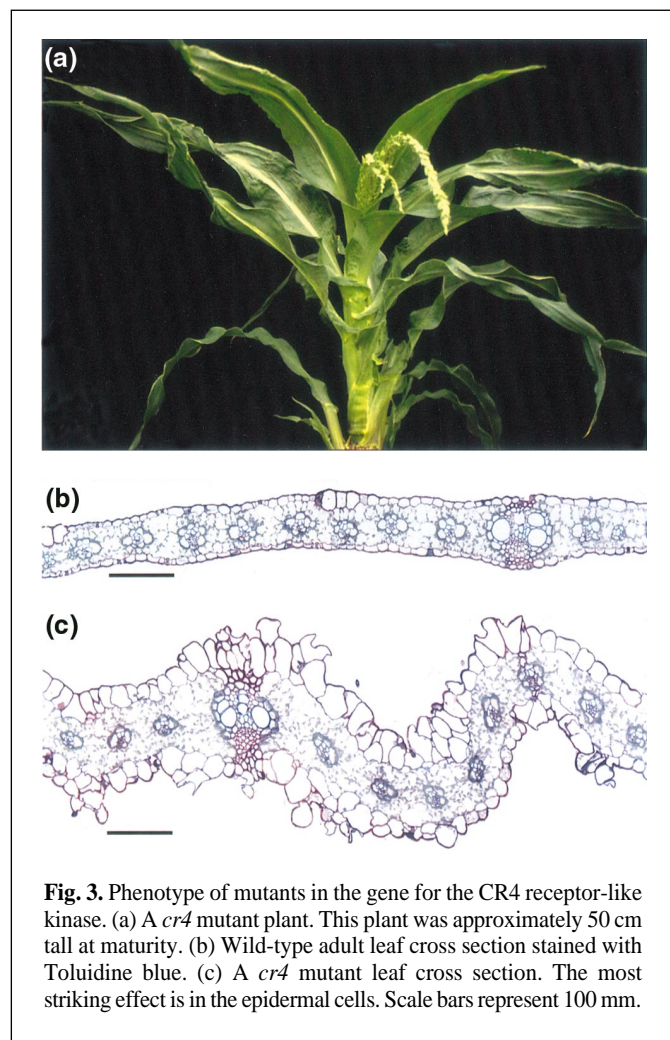


Fig. 3. Phenotype of mutants in the gene for the CR4 receptor-like kinase. (a) A *cr4* mutant plant. This plant was approximately 50 cm tall at maturity. (b) Wild-type adult leaf cross section stained with Toluidine blue. (c) A *cr4* mutant leaf cross section. The most striking effect is in the epidermal cells. Scale bars represent 100 mm.

Wall associated kinases connect the cytoplasm and extracellular matrix

A protein designated PRO25 was isolated fortuitously in a screen for *Arabidopsis* proteins that interact with the light-harvesting complex in chloroplasts⁶. Subsequently it was found to be located in the plasma membrane and to be tightly associated with the cell wall; hence it was renamed WAK1 (wall associated kinase 1)²¹. WAK1 belongs to a family of RLKs of which one, WAK2, appears to be required for normal development²² (B. Kohorn, pers. commun.). An embryo lethal phenotype is linked to a T-DNA insertion in the WAK2 gene indicating it is essential for embryo development.

WAKs possess extracellular domains that include two EGF-like repeats^{21,22} (B. Kohorn, pers. commun.). Different WAKs also possess other motifs that are commonly associated with the extracellular matrix. In addition to the EGF repeats, WAK2 also contains collagen, extensin and neurexin motifs. Features that are common among WAKs are their tight association with the extracellular matrix and their identical cytoplasmic domains.

Signal ligands

No ligand has yet been identified for a plant RLK but clues exist as to their nature. Sequence motifs in the extracellular domains of the developmental RLKs suggest that they may all recognize peptide signal molecules. The LRRs are a common motif involved in protein-protein interactions⁵, but their sequences do not help to identify any specific proteins as potential ligands. The similarity between CR4 and the TNFR ligand-binding domain implies that the CR4 ligand might be related to TNF, but TNF belongs to a family of peptide signals that share little primary sequence conservation²³. Similarly, one can speculate that the WAK ligands might be related to EGF, but this also defines a family with only scattered regions of sequence conservation. Thus, although RLK sequences provide clues, they do not encourage the use of sequence-based strategies to isolate the ligands; instead, identification of the ligands will have to rely on biochemical or genetic approaches.

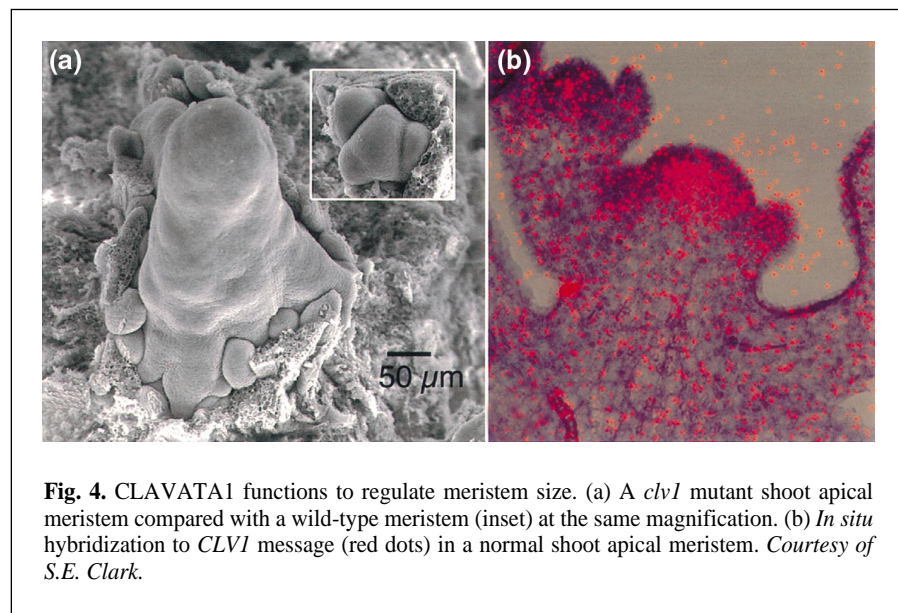


Fig. 4. CLAVATA1 functions to regulate meristem size. (a) A *clv1* mutant shoot apical meristem compared with a wild-type meristem (inset) at the same magnification. (b) *In situ* hybridization to *CLV1* message (red dots) in a normal shoot apical meristem. Courtesy of S.E. Clark.

Genetic evidence identifies CLV3 as a likely candidate for the CLV1 ligand¹³. Both have nearly identical mutant phenotypes and double mutants are no worse than strong single mutants, indicating that both gene products function in the same process of meristem regulation. Furthermore, semidominant alleles of both genes suggest that both gene products function as multimers, while non-complementation between particular *clv1* and *clv3* mutant alleles suggests a physical interaction between the respective gene products. Thus it is likely that CLV1 and CLV3 either form a ligand/receptor pair or function as heterodimeric RLKs. Similar genetic evidence has identified *dek1* as a candidate gene encoding the CR4 ligand; loss of function mutants have similar phenotypes, the recessive mutants show dominant interactions (P. Beraft, unpublished) and *Dek1*⁺ might act non-cell-autonomously²⁴, consistent with it acting as a diffusible signal.

Several examples of peptide signals are known in plants. Systemin is a short peptide signal molecule involved in the wound response²⁵. A number of other peptides of similar size are likely to have developmental roles. ENOD40 is a ten amino acid peptide that is induced during root nodulation but which is also present in non-nodulating species²⁶. Other biologically active plant peptides include PNP (plant natriuretic peptide) from ivy²⁷ and leginsulin from soybean²⁸. Leginsulin can bind and activate a seed kinase, basic 7S globulin (Bg). A 7 kDa peptide from *Brassica* pollen coat binds SLG (Ref. 29), but the physiological significance of this is unknown and binding studies to the S-domain RLKs (with extracellular similarity to SLG) have not been reported.

Although small peptides clearly function as plant signals, the EGF- or TNF-like molecules suggested by WAK and CR4 are larger. Mature TNF is a trimer consisting of 17 kDa monomers³⁰. A common perception of the cell wall is that of a static barrier surrounding the cell that would pose an impediment to signaling by such molecules. However, our current understanding of the cell wall is that of a complex, dynamic structure more akin to an extracellular matrix, and some proteins are clearly able to move through it^{22,31,32}. Fluorescently labeled molecules of approximately 17 kDa diffuse freely through the cell wall of many cells, and molecules up to 41 kDa were able to move at reduced rates^{33,34}. Cell wall porosity is controlled by pectins³⁴. These are in a semi-fluid gelatinous state in meristematic regions but greater crosslinking occurs in older tissues where most of the diffusion assays were conducted³¹. Thus, in growing regions, diffusion should occur more freely, allowing larger molecules to act as signaling ligands.

By contrast, contact-dependent signaling appears less likely. The primary cell wall in most cells is of the order of 100 nm thick³¹. An extracellular domain of 450 amino acids in a continuously linear α -helix would span a distance of approximately 68 nm (Ref. 35). In a more realistic structural configuration there would be little opportunity for proteins within the plasma membranes of adjacent cells to interact directly.

Downstream signal transduction events

The cellular processes regulated by RLKs, and the mechanisms by which regulation occurs are largely unknown in plants. The RLK sequences do not contain any obvious motifs, such as the SH2 domains found in animal signal transduction molecules. Some of the developmental RLKs regulate cell shape or morphology, implying that cytoskeletal functions must lie downstream of the receptors. The relatively few signal transduction molecules that are known to function downstream of plant RLKs have all been identified by protein interaction strategies using the RLK cytoplasmic domains.

Recent evidence suggests that a protein phosphatase functions in the CLV1 signal transduction system. A type 2C protein phosphatase called KAPP (kinase associated protein phosphatase) was identified by screening an expression cDNA library with the radiolabeled cytoplasmic domain of RLK5, an *Arabidopsis* RLK of unknown function³⁶. RLK5 is very similar in sequence to CLV1 and several lines of evidence suggest that KAPP functions in the CLV1 signaling pathway *in vivo*. KAPP binds to CLV1 *in vitro*, in a manner that is dependent on the phosphorylation status of CLV1 (Refs 17, 18). Both are also expressed simultaneously in shoot apical meristems, and the KAPP overexpression phenotype in transgenic plants mimics CLV1 loss of function¹⁸. Furthermore, CLV1 and KAPP co-immunoprecipitate from plant extracts and reduction of KAPP transcript levels by sense suppression alleviates the *clv1* mutant phenotype¹⁷. Size fractionation studies indicate that CLV1 is present in two populations: one is approximately 210 kDa in size, and may correspond to a dimer; and a 425 kDa complex that appears to include KAPP (S. Clark, pers. commun.). Because increased

KAPP expression gives rise to a phenotype similar to a reduction in CLV1 function and reducing KAPP expression suppresses the *clv1* loss of function phenotype it appears that KAPP functions to negatively regulate the CLV1 signaling pathway^{17,18}.

Four factors have been identified that bind to the cytoplasmic domain of SRK (involved in pollen self-incompatibility). Three of the proteins were identified by yeast two-hybrid screens and include, THL-1 and THL-2, which are similar to thioredoxin-H (Ref. 37). A third protein, ARC1, contains arm repeats³⁸. Thioredoxins modulate protein activity by reducing disulfide bonds, which allows them to transduce signals³⁹. Arm repeat proteins act as adapter molecules in cadherin-mediated cell adhesion and other signal transduction pathways⁴⁰. Binding of THL-1 and THL-2 to SRK was not phosphorylation dependent³⁷, but ARC1 only bound to phosphorylated SRK (Ref. 38). Both THL-1 and ARC1 are phosphorylated by SRK (Refs 37, 38). The fourth protein to bind SRK is KAPP (Ref. 41).

KAPP binds to several RLKs including members of LRR and S-domain classes but it appeared unable to bind to CR4 or ZmPK1, an S-domain RLK of unknown function⁴¹. The RLKs from *Arabidopsis* and maize were included in this study and the *Arabidopsis* and maize KAPP proteins both bound the same subset of RLKs indicating that the interactions are evolutionarily conserved.

Interestingly, KAPP was shown to bind to the kinase domains of the LRR-type RLK5 and the S-type RLK4 and SRK-A14 proteins⁴¹. THL-1 and THL-2, on the other hand, bound SRK-A14 and SRK-910 but not RLK4 or RLK5 (Ref. 37). Although these interactions have not yet been demonstrated *in vivo*, several important conclusions can be drawn from these studies.

- In addition to the different classes of extracellular domains, there are different classes of cytoplasmic domains and a particular cytoplasmic class does not coincide with a specific extracellular class.
- There is the potential for more than one signal transduction pathway to be regulated by a single receptor.
- The signal transduction systems may overlap, and thereby complicate genetic interpretations of RLK functions if partial compensation occurs by another signaling pathway.
- There may be factors that function in multiple pathways.

This last point has important implications for genetic strategies to dissect these pathways. The mutant phenotype of a factor that functions in multiple pathways might not resemble the phenotype of mutations in the receptor, and perturbations targeted towards one pathway might also affect others. Consistent with this is the observation that KAPP underexpressing lines show several phenotypes in addition to suppression of the *clv1* mutant phenotype¹⁷.

Future perspectives

Receptor protein kinases are key molecules in animal cell interactions and it is now clear they also have critical roles in plant cell interactions. For example, the CLV1 receptor-like kinase is required to regulate the relative rates of proliferation and differentiation of meristem cells. Because these activities occur in spatially separated regions of the meristem, cell interactions are necessary to maintain the proper balance of activities and CLV1 may mediate the intercellular signaling necessary for this process. Likewise, cell interactions are required to establish organ shape and cellular pattern. Receptor-like kinases such as ER and CR4 are critical for these processes and therefore are probably involved in the required cell interactions. New plant RLKs of unknown function are being reported at a rapid rate and it is likely that many will turn out to have additional developmental roles. The molecular mechanisms that underly plant RLK functions are still poorly understood and the extent to which these processes are conserved in plant and animal systems remains an open question. Some extracellular motifs of plant RLKs resemble animal motifs whereas others have only

been identified in plants. The cytoplasmic domains of plant RLKs do not appear to have motifs in common with animal RPKs, but the recently identified arm repeat motif in a plant RLK-interacting protein³⁸ suggests that the signal transduction pathways may have some similarity with animals. The degree of similarity or difference between plants and animals will become apparent as we learn more, but it is evident that complexity is one feature they do have in common.

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Philip Becraft is at the Zoology and Genetics, and Agronomy Depts, 2116 Molecular Biology Building, Iowa State University, Ames, IA 50011, USA (tel +1 515 294 2903; fax +1 515 294 6755; e-mail becraft@iastate.edu).