

RECEPTOR KINASE SIGNALING IN PLANT DEVELOPMENT

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■ **Abstract** The *Arabidopsis* genome sequence has revealed that plants contain a much larger complement of receptor kinase genes than other organisms. Early analysis of these genes revealed involvement in a diverse array of developmental and defense functions that included gametophyte development, pollen-pistil interactions, shoot apical meristem equilibrium, hormone perception, and cell morphogenesis. Amino acid sequence motifs and binding studies indicate that the ectodomains are capable of binding, either directly or indirectly, various classes of molecules including proteins, carbohydrates, and steroids. Genetic and biochemical approaches have begun to identify other components of several signal transduction pathways. Some receptor-like kinases (RLKs) appear to function with coreceptors lacking kinase domains, and genome analysis suggests this might be true for many RLKs. The KAPP protein phosphatase functions as a negative regulator of at least two RLK systems, and *in vitro* studies suggest it could be a common component of more. Whether plant signaling systems display a modularity similar to animal systems remains to be determined. Future efforts will reveal unknown functions of other RLKs and elucidate the relationships among their signaling networks.

CONTENTS

INTRODUCTION	164
THE EVOLUTION OF PLANT RLKS	165
RLKs in Plants Versus Animals	165
Families of Plant RLKS	165
DEVELOPMENTAL FUNCTIONS OF PLANT RLKS	167
Meristem Development	167
Pollen/Pistil Interactions	168
Hormone Signaling	169
Gametophyte Development	170
Cell Morphogenesis and Differentiation	171
Organ Shape	172
Organ Abscission	172
Somatic Embryogenesis	173
RLK SIGNALING PATHWAYS	174

Signal Ligands	174
Other Components of RLK Signal Transduction Systems	177
SUMMARY AND FUTURE PROSPECTS	183

INTRODUCTION

Receptor protein kinases (RPKs) are important mediators of paracrine signaling in metazoans. The advent of the *Arabidopsis* genome sequence revealed a surprisingly extensive array of receptor-like kinase (RLK) genes. Although no functional information is available for the majority of these RLKs, different members of this family are known to function in various aspects of development and plant defense. This review provides an overview of receptor kinases and their signal transduction systems that function in plant development.

Receptor kinases contain a protein kinase that is modulated in response to a stimulus. Historically, the first receptor kinases identified were integral plasma membrane proteins containing a single transmembrane domain. Such proteins contain an extracellular receptor domain (ectodomain) and a cytoplasmic protein kinase domain (Figure 1). Thus many proteins that perform receptor functions and have protein kinase activity are not considered in the receptor kinase class. This review considers only the single-pass transmembrane type of RPKs. The two major classes of RPKs show different substrate specificity in the kinase domain. Receptor tyrosine kinases (RTKs) phosphorylate tyrosine residues, whereas serine/threonine kinase receptors (STKRs) phosphorylate serine and threonine residues. Plant genomes encode a large number of proteins with the predicted topologies of receptor kinases but for which receptor function has not been demonstrated; such proteins are referred to as RLKs.

The dogma for RPK function is that they exist in the membrane as inactive monomers (Becraft 1998). Ligand binding induces dimerization, which brings the intracellular kinase domains into proximity and allows them to transphosphorylate and activate one another. There are exceptions to this general model. For example, several plant RLKs are multimers in their inactive form (Giranton et al. 2000, Trotochaud et al. 1999) (discussed below). One appears to function through an intramolecular kinase activity (Schulze-Muth et al. 1996). Additionally, some RLK proteins have aberrant kinase domains that might be inactive (Barre et al. 2002, Valon et al. 1993; X. Cao & P.W. Becraft, unpublished). Thus the actual mode of action for each RLK cannot be assumed but must be demonstrated on an individual basis.

The first plant RLK was identified in maize using degenerate polymerase chain reaction primers to the protein kinase domain (Walker & Zhang 1990). Subsequently, several RLKs were isolated from *Arabidopsis* and *Brassica* (Chang et al. 1992, Goring & Rothstein 1992, Kohorn et al. 1992, Stein et al. 1991, Walker 1993). The *Brassica* S-locus receptor-like kinase (SRK) was the only one for which a function could be hypothesized by virtue of its association with the

self-incompatibility locus. Nonetheless, their discovery was significant because it was not previously clear whether such signaling mechanisms would exist in plants. Many had assumed that because of the plant cell wall, most plant cell communication would occur via plasmodesmata or small molecules such as hormones.

THE EVOLUTION OF PLANT RLKS

RLKs in Plants Versus Animals

Genomic sequence analysis revealed that *Arabidopsis* contains 417 genes encoding RLKs (Shiu & Bleecker 2001b). This compares with 43 (40 RTKs and 3 STKRs) in *Caenorhabditis elegans* (Plowman et al. 1999), 25 (20 RTKs and 5 STKRs) annotated in *Drosophila melanogaster* (FlyBase 1999), and 70 (58 RTKs and 12 STKRs) in humans (Massagué 1998, Robinson et al. 2000, Smith et al. 1997). In addition to the large number of RLK genes in plants, many of the RLK transcripts are alternatively spliced (Bassett et al. 2000, Giranton et al. 1995, Kumar & Trick 1994, Stein et al. 1991, Tobias & Nasrallah 1996), adding even more complexity. In animals, receptor kinases function predominantly as growth factor receptors, regulating developmental processes and homeostasis. Plant RLKs are known to function in development, hormone perception, and pathogen response, although this list is likely to grow as more are studied.

A major distinction between receptor kinases in animals and plants is the prevalence of STKRs in plants versus RTKs in animals. In mammals, there are 20 classes of RTKs based on ectodomain sequences (Plowman et al. 1999), whereas the TGF β receptor family represents the only class of STKRs. In contrast, all known plant RLKs contain serine/threonine kinase consensus sequences. However, at least two appear to be dual specificity kinases. PRK1 (pollen receptor-like kinase1) autophosphorylates on serine and tyrosine residues (Mu et al. 1994) and SERK (somatic embryogenesis receptor-like kinase) phosphorylates serines, threonines, and tyrosines (Shah et al. 2001b,c). Yeast similarly contains kinases with only serine/threonine consensus sequences, yet 30 of 119 were capable of phosphorylating tyrosine residues (Zhu et al. 2000). Thus it is possible that more plant RLKs might also have tyrosine kinase activity, again highlighting the necessity to characterize the biochemical activity of each RLK.

Families of Plant RLKs

A recent analysis of kinases revealed that all known plant RLKs are of monophyletic origin in a clade that contains Pelle cytoplasmic kinases of animals (Shiu & Bleecker 2001b). The clade also contains a large number of cytoplasmic plant kinases [receptor-like cytoplasmic kinases (RLCKs)]. Interestingly, animal RTKs form a distinct lineage from plant RLKs, suggesting that different kinases were independently recruited to function as receptor kinases in animals and plants. Most plant RLKs are members of distinct families, some quite extensive. There was a

strong tendency for proteins that grouped together by kinase relatedness to also have similar ectodomains, indicating that most existing families underwent expansion after the initial event that fused the kinase and ectodomains (Shiu & Bleecker 2001a,b).

Sequences for RLK genes have been reported as ESTs (expressed sequence tags) for over two dozen plant species. To date there have been no reports from any plant of a class of RLK that does not have a closely related member in *Arabidopsis*. There are over 21 different classes of *Arabidopsis* RLK ectodomains. Several select examples are shown in Figure 1. The most common extracellular motif is the leucine-rich repeat (LRR), present in over half the *Arabidopsis* RLKs (Shiu & Bleecker 2001a,b). LRRs are a common signal transduction motif thought to be involved in protein-protein interactions (Kobe & Deisenhofer 1994); they are not found on any animal receptor kinases. There are at least 8 classes of LRR ectodomains with different numbers and arrangements of LRRs, some interspersed with other sequences (Shiu & Bleecker 2001a,b).

The second largest class of ectodomains contains lectin motifs (Barre et al. 2002; Hervé et al. 1996, 1999). This class includes 42 members of the lectin-receptor kinases (LecRLKs), with an ectodomain similar to legume lectins (Barre et al. 2002, Shiu & Bleecker 2001a). Amino acid substitutions in the putative monosaccharide-binding site suggest that LecRLKs might not bind simple sugars as do true legume lectins (Barre et al. 2002, Hervé et al. 1999). A B-lectin, or agglutinin motif, is present in the ectodomain of the 40 RLKs containing the cysteine-rich S-domain (Shiu & Bleecker 2001a). The S-domain is found on the *Brassica* SRK involved in pollen self-incompatibility and is similar to the S-locus glycoprotein (SLG) (Stein et al. 1991). SRKs also have a PAN motif (Shiu & Bleecker 2001a). PAN domains function in protein-protein interactions or protein-carbohydrate interactions and are found in the ectodomain of several animal receptors, but not RPKs (Tordai et al. 1999). Other motifs suggestive of carbohydrate binding include a chitinase domain in CHRK1 (chitinase-related receptor-like kinase) (Y.S. Kim et al. 2000, Shiu & Bleecker 2001a), a thaumatin domain (Osmond et al. 2001, Shiu & Bleecker 2001a, Wang et al. 1996), and a lysin domain (Bateman & Bycroft 2000, Shiu & Bleecker 2001a). Extensin motifs also suggest interactions with cell walls (Shiu & Bleecker 2001a,b).

Several other extracellular motifs are predicted to be involved in protein interactions. Wall-associated kinases (WAKs) all contain epidermal growth factor (EGF) repeats (He et al. 1999, Kohorn et al. 1992) in addition to various other motifs such as collagen, neurexin, and tenascin, similar to extracellular matrix protein motifs of metazoans (He et al. 1999). The CRINKLY4 (CR4) family contains cysteine-rich repeats found in tumor necrosis factor receptor (TNFR) (Becraft et al. 1996) and a putative RCC1 propeller domain (McCarty & Chory 2000).

The DUF26 domain, also called CRR (cysteine-rich repeat), contains four conserved cysteines with a C-X8-C-X2-C signature (Chen 2001; Shiu & Bleecker 2001a,b). This motif is found in at least 42 *Arabidopsis* RLKs (Chen 2001; Czernic et al. 1999; Du & Chen 2000; Ohtake et al. 2000; Shiu & Bleecker 2001a,b;

Takahashi et al. 1998), appears to be specific to plants, and has an unknown function (Apweiler et al. 2001). Other plant-specific domains with unknown functions include proline-rich motifs, as well as CrRLK1-like (Schulze-Muth et al. 1996; Shiu & Bleecker 2001a,b) and LRK10-like (Feuillet et al. 1998; Shiu & Bleecker 2001a,b) ectodomains.

Interestingly, of all the extracellular motifs found in plant RLKs, only the EGF motif is also found in RPKs of animals. This, in combination with the divergent lineages of plant and animal RPK kinase domains, supports the notion that receptor kinases arose independently in plants and animals.

DEVELOPMENTAL FUNCTIONS OF PLANT RLKS

From the handful of RLKs with known functions, it is clear they are involved in diverse processes. Several others show differential expression patterns that suggest additional functions (summarized in Table 1). RLKs with genetically defined functions are considered in detail below.

Meristem Development

Shoot apical meristems contain a population of stem cells that divide to replace cells incorporated into differentiated structures. Proper meristem function requires a balance between stem cell proliferation and cell differentiation. The *CLAVATA1*

TABLE 1 RLKs showing tissue- or cell-specific expression

RLK	Tissue/inducer	RLK type	Species	Reference
LePRK1, 2	Pollen	LRR	<i>Lycopersicon esculentum</i>	(Muschiatti et al. 1998)
LTK1, 2, 3	Endosperm	LRR	<i>Z. mays</i>	(Li & Wurtzel 1998)
RKF1	Flowers	LRR	<i>A. thaliana</i>	(Takahashi et al. 1998)
SbRLK1	Mesophyll	LRR	<i>Sorghum bicolor</i>	(Annen & Stockhaus 1999)
RLK4	SAM ^a , lateral root	S-domain	<i>A. thaliana</i>	(Coello et al. 1999)
RPK1	ABA ^a	LRR	<i>A. thaliana</i>	(Hong et al. 1997)
OsTMK	GA ^a	LRR	<i>O. sativa</i>	(van der Knaap et al. 1999)
NTS16	Pollination	atypical ^b	<i>N. tabacum</i>	(Li & Gray 1997)
LRRPK	Light (repressor)	LRR	<i>A. thaliana</i>	(Deeken & Kaldenhoff 1997)
INRPK1	Photoperiod (short day)	LRR	<i>Ipomoea nil</i>	(Bassett et al. 2000)
SARK	Senescence	LRR	<i>Phaseolus vulgaris</i>	(Hajouj et al. 2000)

^aSAM, shoot apical meristem; ABA, abscisic acid; GA, gibberellic acid.

^bThe N terminus appears to contain either a very small receptor domain or just a membrane anchor.

(*CLV1*) gene in *Arabidopsis* functions in maintaining shoot apical meristem equilibrium by inhibiting proliferation; *clv1* mutants have abnormally large meristems because stem cell proliferation outpaces differentiation (Clark et al. 1993). The increase in meristem size produces secondary effects including altered phyllotaxy, increased floral organ numbers, and the club-shaped siliques from which the name *CLAVATA* derives. *CLV1* encodes a RLK and is expressed in the corpus region of the meristem (Figures 1, 2) (Clark et al. 1997). The ectodomain contains 23 LRRs, and the cytoplasmic kinase domain autophosphorylates on serine residues (Stone et al. 1998, Williams et al. 1997). In vitro phosphorylation of an inactive mutant *CLV1* kinase by wild-type indicates that autophosphorylation occurs intermolecularly (Williams et al. 1997). *CLV1* exists in plant extracts as a 185-kDa disulfide-linked multimer (Trotochaud et al. 1999). *CLV1* protein fails to accumulate in *clv2* mutants, suggesting that *CLV1* is intrinsically unstable and requires the presence of the receptor-like *CLV2* protein for stability (Jeong et al. 1999) (see below). Thus it is likely that *CLV2* protein is a subunit of a *CLV1* heteromeric receptor.

OsLRK1 was isolated from rice and shows 55% amino acid identity with *Arabidopsis* *CLV1* (C. Kim et al. 2000). This gene is expressed in growing regions of the shoot, and antisense suppression caused the production of extra floral organs, a phenotype reminiscent of *clv1*. Thus *CLV1* function may be conserved between monocots and dicots.

Pollen/Pistil Interactions

The *Brassica* *SRK* functions in stigmatic cells for pollen recognition in the sporophytic self-incompatibility system (Goring et al. 1993, Nasrallah et al. 1994). Self-incompatibility is a mechanism that promotes outcrossing and involves an active rejection of self-pollen. This phenotype is controlled by the haplotype of a complex locus called the *S-locus* (Nasrallah 1997). The stigma rejects pollen produced by any plant carrying the same *S* haplotype. For example, the stigma of a *S₆S₉* plant rejects pollen produced by any plant carrying the *S₆* or *S₉* haplotype but accepts pollen from plants carrying different haplotypes.

Two related genes, *SRK* and *SLG* (*S-locus glycoprotein*), are encoded at the *S-locus* and expressed in the stigma (Nasrallah et al. 1987, Stein et al. 1991). The ectodomain of *SRK* is similar to *SLG*, a secreted glycoprotein, and both are highly polymorphic (Stein et al. 1991, Watanabe et al. 1994, Yamakawa et al. 1995). Extracellular motifs include an agglutinin (B-lectin)-like domain, the cysteine-rich S-domain, and a PAN domain (Figure 1).

SRK is the major female determinant of the self-incompatibility phenotype. Mutations in the *SRK* gene are associated with loss of the self-incompatibility response (Goring et al. 1993, Nasrallah et al. 1994), and suppression of *SRK* with a transgene suppresses self-incompatibility (Stahl et al. 1998). Transformation of the *SRK₂₈* gene into *S₆₀* haplotype *Brassica rapa* conferred a rejection response to *S₂₈* pollen (Takasaki et al. 2000). Plants remained receptive to *S₂₈* pollen when transformed with *SLG₂₈*. Similar results were obtained with *B. napus*

(Cui et al. 2000, Silva et al. 2001). Furthermore, transgenic *SRK* was shown to contribute to the stigmatic function in the complex dominance relationship between various *S* haplotypes (Hatakeyama et al. 2001).

SRK encodes a serine/threonine kinase that is targeted to the plasma membrane (Cabrilac et al. 2001, Delorme et al. 1995, Giranton et al. 2000, Goring & Rothstein 1992, Stein et al. 1996, Stein & Nasrallah 1993). The *SRK* transcript is differentially spliced (Giranton et al. 1995, Stein et al. 1991) and produces both a full-length membrane localized RLK (Delorme et al. 1995, Stein et al. 1996) and a secreted product corresponding to the ectodomain (Giranton et al. 1995). Whether these variant forms are of functional significance is not yet known. In insect cell microsomes, *SRK* has constitutive kinase activity and autophosphorylates intermolecularly, whereas *SRK* spontaneously forms oligimers in unpollinated stigmas. Thus *SRK* protein is intrinsically active (Giranton et al. 2000).

Interestingly, a gene related to *SRK* is linked to the self-incompatibility locus in *Arabidopsis lyrata* (Schierup et al. 2001). The RLK encoded by this locus also shows allelic polymorphisms with even higher sequence diversity than in *Brassica*. This locus does not appear to contain a haplotype structure or encode a SLG ortholog. Yet another RLK, *IRK*, is genetically linked to the self-incompatibility locus in *Ipomoea trifida* and expressed in stigmas, consistent with its function in self-incompatibility (Kowyama et al. 1996).

Biochemical evidence suggests that RLKs might also function in compatible pollinations. The tomato *LePRK1* and *LePRK2* transcripts are specifically expressed in mature pollen and germinating pollen tubes (Musciatti et al. 1998). Both encode LRR RLKs that localize to the plasma membrane of pollen tubes. Labeled *LePRK2* was immunoprecipitated from pollen microsomal membranes following incubation with γ -³²P-ATP, showing that *LePRK2* was actively phosphorylated in these membrane fractions. Addition of stylar extracts inhibited *LePRK2* phosphorylation, and sequential additions demonstrated that they could promote the dephosphorylation of *LePRK2*. This activity was impervious to phosphatase inhibitors and boiling and was not present in leaf extracts. These results suggest that *LePRK2* may be involved in pollen-pistil interactions. Expression of another RLK gene, *NTS16*, is induced in tobacco styles upon pollination (Li & Gray 1997), suggesting that RLKs might function on both sides of compatible pollen-pistil interactions.

Hormone Signaling

The *Arabidopsis BRI1* gene was identified in genetic screens for mutants insensitive to exogenous brassinolide (Clouse et al. 1996, Li & Chory 1997). Brassinolide is a type of brassinosteroid, plant steroid hormones that are involved in growth regulation. Brassinosteroids promote cell elongation; deficient plants grow as dwarfs in the light and have a light-grown phenotype in the dark (Li et al. 1996, Szekeres et al. 1996). *bri1* mutants are phenotypically similar to plants that have a brassinolide deficiency (Clouse et al. 1996, Li & Chory 1997).

The *BRI1* gene was cloned and found to encode a LRR RLK (Li & Chory 1997). The ectodomain contains 25 LRRs with a unique island of 70 amino acids between repeats 21 and 22 (Figure 1). At least 3 mutant alleles contain lesions in this island, which indicates its functional significance (Friedrichsen et al. 2000, Li & Chory 1997). A *BRI1*-GFP fusion protein, controlled by the *BRI1* 5' promoter, showed ubiquitous expression and was targeted to the plasma membrane (Friedrichsen et al. 2000).

Phosphoamino acid analysis of autophosphorylated recombinant *BRI1* kinase domain showed phosphorylation primarily on serine residues, with a minor amount on threonine (Oh et al. 2000). MALDI-MS analysis of tryptic fragments mapped at least 12 autophosphorylated residues to small peptides. Three sites occurred in the activation segment of the kinase domain. One of the phosphorylation sites occurred within the N-terminal calmodulin-binding protein tag of the recombinant protein. Synthetic peptides from this sequence were used to derive a consensus recognition site that resembled targets of SNF1-related kinases.

A rice dwarf mutant, *d61*, is caused by lesions in a *BRI1* homologous gene (Yamamuro et al. 2000). The rice protein contains 22 LRRs, with a 70-amino-acid island between repeats 18 and 19. Similar to *Arabidopsis bri1* mutants, *d61* shows reduced sensitivity to exogenous brassinolide and dwarfism owing to decreased internode elongation and decreased mesocotyl elongation in the dark.

Gametophyte Development

PRK1 was isolated from a petunia pollen tube cDNA library, and RNA gel blot analysis detected *PRK1* transcripts in both ungerminated and germinated pollen (Mu et al. 1994). A transgene containing the ectodomain-coding region in antisense orientation was unable to transmit through the pollen (Lee et al. 1996). The pollen mother cells underwent normal meiosis and produced microspores; however, subsequent development was abnormal, and half the microspores (presumably those carrying the transgene) failed to undergo mitosis, remaining uninucleate and finally aborting.

Surprisingly, the *PRK1* antisense transgene also showed greatly reduced transmission through the female gametophyte (Lee et al. 1996). This was unexpected because *PRK1* expression was thought to be pollen specific and because the *Lat52* promoter used to drive transgene expression was also thought to be pollen specific. Closer examination revealed that *PRK1* transcripts were present in normal ovaries (Lee et al. 1997). Embryo sacs of antisense lines usually undergo normal mitoses, although some arrest after the first or second division. However, nuclear migration, embryo sac maturation, and differentiation are disrupted. Thus *PRK1* is required for postmeiotic development of both male and female gametophytes.

PRK1 contains 720 amino acids and is detected as a 69-kDa band on immunoblots of petunia microsomal proteins (Mu et al. 1994). The 328-amino-acid ectodomain contains five interspersed LRRs (Figure 1). The sequence of the cytoplasmic kinase domain predicts a serine/threonine kinase; however, it is autophosphorylated on serine and tyrosine residues.

Cell Morphogenesis and Differentiation

The maize *CRINKLY4* (*CR4*) gene is important for a complex array of processes in plant and endosperm development. *CR4* is required in the endosperm for aleurone cell fate specification because in *cr4* mutants, the fate of the peripheral cell layer switches from aleurone to starchy endosperm (Becraft & Asuncion-Crabb 2000, Becraft et al. 1996). Mutant plants are short, with crinkled leaves that often form graft-like fusions (Becraft et al. 1996). An allelic series showed that *CR4* functions preferentially in the epidermis but is required for diverse processes of cellular development throughout the shoot (Jin et al. 2000). Functions include the regulation of cell proliferation, fate, patterning, morphogenesis, and differentiation, all of which suggest that *CR4* functions in a growth-factor-like response. Genetic mosaic analysis demonstrated that *CR4* acts cell autonomously in leaves, indicating that it does not regulate a secondary intercellular signal (Becraft et al. 2001).

CR4 was cloned by transposon tagging and encodes a RLK (Becraft et al. 1996). *CR4* is expressed in the growing regions of the shoot, particularly in the shoot apical meristem and lateral organ primordia (Becraft et al. 2001, Jin et al. 2000). The encoded RLK of 901 amino acids contains a functional serine/threonine kinase in the cytoplasmic domain (Jin et al. 2000). The cysteine-rich ectodomain contains a motif similar to the ligand-binding domain of mammalian tumor necrosis factor receptor (Figure 1), suggesting the ligand for *CR4* may be a peptide related to tumor necrosis factor (Becraft et al. 1996). A second motif containing repeats of approximately 37 amino acids may form a RCC1-like propeller structure, another protein interaction motif (McCarty & Chory 2000). *Arabidopsis* contains an orthologous gene, *ACR4*, that is expressed in protodermal cells of the embryo and shoot (Tanaka et al. 2002).

The *WAK1* (*WALL-ASSOCIATED KINASE1*) cDNA was isolated fortuitously and initially called *pro25* (Kohorn et al. 1992). Subsequent analysis showed that the *WAK1* RLK localized to the plasma membrane and associated tightly with the cell wall (He et al. 1996). *WAK1* belongs to a cluster of five related genes (He et al. 1999), and antibodies generated against the *WAK1* kinase domain recognize other members of this cluster (Anderson et al. 2001). Thus the wall association represents a collective property of the *WAKs*. Another key feature of *WAKs* is an EGF motif in the ectodomain (Figure 1) (He et al. 1999, Kohorn et al. 1992). Other ectodomain sequence motifs specific to different *WAKs* are related to the metazoan extracellular matrix proteins tenascin, collagen, and neurexin (He et al. 1999).

RNA gel blots detected transcripts of *WAK1-3* and *WAK5* primarily in leaves and stems, with trace amounts in other tissues (He et al. 1999). *WAK4* expression was highest in siliques (He et al. 1999), but RT-PCR also revealed low levels in leaves (Lally et al. 2001). In situ hybridization and promoter::GUS fusions showed *WAK1* and *WAK2* expression in the shoot apical meristem, expanding leaves, and lateral root meristems (Wagner & Kohorn 2001). The expression patterns were overlapping but distinct. For example, in leaves *WAK1* is primarily expressed in the vasculature, whereas *WAK2* expression is strongest at the margins, and *WAK3* is expressed sporadically.

It has been hypothesized that WAKs may function to link the cell wall to the cytoplasm (He et al. 1996). This link could be purely structural, independent of an additional signaling function, or the link could be an integral part of a putative signaling function, perhaps acting as a tension sensor (Anderson et al. 2001). At least some WAKs are required for normal cell expansion (Lally et al. 2001, Wagner & Kohorn 2001). Antisense suppression of both *WAK2* (Wagner & Kohorn 2001) and *WAK4* (Lally et al. 2001) caused a dwarf phenotype, the result of a decrease in cell expansion. Leaf, stem, and root cells all showed the effect, with leaf cells being most sensitive to reductions in WAK levels (Lally et al. 2001). In both cases, the antisense transgene included conserved gene sequences and thus would be expected to suppress multiple WAK family members. Therefore, it is not clear which, or how many, WAKs are involved in controlling cell expansion. Inducible antisense expression was used because transgenic plants could not be obtained with the CaMV 35S promoter, most likely indicating that WAKs are essential for viability (Wagner & Kohorn 2001). Induced antisense expression also resulted in sterility, although the basis of this phenotype was not reported (Lally et al. 2001).

WAKs also appear to function in plant defense. *WAK1* transcript is pathogen inducible, and *WAK1*, 2, and 3 are inducible by salicylic acid (He et al. 1998, 1999), a pathogen-induced signaling molecule. *WAK1* expression was found to be required for plants to survive on medium containing salicylic acid. Thus *WAK1* may function to protect plant cells from their own defense response to pathogens. A *WAK2* promoter::GUS reporter is also wound inducible, suggesting other possible functions in plant defense (Wagner & Kohorn 2001).

Organ Shape

The Landsberg *erecta* ecotype is a popular laboratory strain because of its convenient growth habit. The *erecta* (*er*) phenotype includes short, compact inflorescences, thickened stem, short petioles, rounded leaves, and short, thick siliques (Lease et al. 2001a, Torii et al. 1996). Phyllotaxy is unchanged, indicating that the major function of *ER* is to regulate the ultimate shape of various shoot organs. The cellular basis of this phenotype has not been reported, but presumably some aspect of cell division or cell expansion is affected.

The *ER* gene was cloned by T-DNA insertional mutagenesis and encodes a LRR RLK (Torii et al. 1996). *ER* is expressed in the shoot apical meristem and organ primordia (Yokoyama et al. 1998). The *ER* ectodomain contains 20 LRRs. In the genomic organization of the *ER* locus, each LRR represents a separate exon. The cytoplasmic kinase domain autophosphorylates on serine and threonine residues *in vitro* (Lease et al. 2001a).

Organ Abscission

RLK5 was isolated from *Arabidopsis* by hybridization to the maize *ZmPK1* kinase domain (Walker 1993). Subsequent antisense suppression of the gene revealed a function in floral organ abscission; abscission of sepals, petals, and stamens was

prevented or delayed (Jinn et al. 2000). Therefore, RLK5 was renamed HAESA, from a Latin word meaning “to adhere to.” It was not reported whether the morphological differentiation of an abscission zone or just the actual cell separation was blocked.

HAESA is expressed in the abscission zones of floral organs in a developmentally regulated manner (Jinn et al. 2000). Expression coincides with the stages when flowers gain competence to self-pollinate and around the time abscission zones differentiate. *HAESA* expression is also observed at the sites of leaf attachment to the stem. *Arabidopsis* leaves do not normally abscise, and the significance of this expression is not known. *HAESA* protein has 999 amino acids and contains 21 LRRs in the ectodomain (Walker 1993). The protein is localized to the plasma membrane, and the cytoplasmic kinase domain autophosphorylates on serine and threonine residues *in vitro* (Jinn et al. 2000).

Somatic Embryogenesis

SERK (*SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE*) was isolated from embryogenic cells of carrot suspension cultures (Schmidt et al. 1997). Embryogenic cells were sorted by morphology and used to construct a cDNA library. Differential screening identified one clone that was expressed in embryogenic but not in non-embryogenic cultures. *In situ* hybridization showed that *SERK* was expressed in cells predicted to be embryogenic by morphological criteria, and *SERK* expression was induced in tissue explants placed in embryogenic culture conditions. Similar results were observed in *Arabidopsis* (Hecht et al. 2001) and in the monocot *Dactylis glomerata* L. (Somleva et al. 2000). Cell tracking experiments using a *SERK* promoter::luciferase reporter construct verified that somatic embryos were indeed derived from *SERK*-expressing cells (Schmidt et al. 1997). Overexpression increased the embryogenic potential of *Arabidopsis* cultures, indicating that *SERK* functions to promote embryogenic competence.

In normal *Arabidopsis* plants, *SERK* is expressed in developing ovules (Hecht et al. 2001). In mature ovules, expression is restricted to the embryo sac, where it is expressed in all cells. Following fertilization, *SERK* is expressed in the endosperm and the zygote, and in embryos through the heart stage, at which time expression ceases. A *SERK* promoter::GUS reporter was expressed postembryonically in the vasculature. No developmental abnormalities were reported for *35S*::*SERK* transgenic plants, indicating that *SERK* does not interfere with postembryonic development.

The *Arabidopsis* *SERK1* (*AtSERK1*) protein contains 625 amino acids. The characteristic ectodomain configuration consists of a leucine zipper motif near the amino terminus just after the signal peptide, five LRRs, and a proline-rich region between the LRRs and transmembrane domain (Figure 1). The proline-rich region contains two copies of a SPP sequence motif found in extensins (Baudino et al. 2001, Hecht et al. 2001, Schmidt et al. 1997). The cytoplasmic domain contains a dual specificity protein kinase that autophosphorylates serines and threonines, with

trace tyrosine phosphorylation (Shah et al. 2001b,c), but strongly phosphorylates tyrosines in myelin basic protein (Shah et al. 2001c). In vitro phosphorylation of an inactive mutant kinase domain by a normal kinase domain indicated that autophosphorylation occurs intermolecularly.

YFP (yellow fluorescent protein)-tagged AtSERK1 localized to the plasma membranes of insect cells and plant protoplasts (Shah et al. 2001a). Yeast two-hybrid analysis showed that the ectodomain of AtSERK1 could homodimerize; FRET (fluorescence resonance energy transfer) analysis of coexpressed AtSERK1-YFP and AtSERK1-CFP (cyan fluorescent protein) indicated a close association of different AtSERK1 molecules in cowpea protoplasts. Deletion of the leucine zipper did not inhibit membrane localization but abolished FRET. These results suggest that AtSERK1 homomultimerizes in plant cells and that multimerization requires the leucine zipper motif. However, the majority of AtSERK1 molecules appeared to be in monomeric form, suggesting that a signal ligand might induce dimerization.

RLK SIGNALING PATHWAYS

The identity of a protein as a RLK implies that it belongs to a signal transduction system. To fully understand the function of the RLK, it is necessary to understand other components of the system, how they interact, the activating signal, and which downstream events it regulates. Genetic and biochemical approaches are beginning to yield information on several RLK signal transduction systems.

Signal Ligands

One of the most important and interesting components of any signal transduction system is the signaling molecule that initiates the response. In the case of RLKs, ligand binding induces RLK activation and downstream signal transduction. Knowing the ligand opens the way to understanding the cells or tissues that are involved in a particular signaling system, as well as the potential for manipulating it for experimental or biotechnological purposes.

CLV3 IS THE LIGAND FOR CLV1 Mutants in the *Arabidopsis CLAVATA3 (CLV3)* gene showed the same enlarged meristem phenotype as *CLV1*, which places them in the same pathway (Clark et al. 1995). This was further supported with biochemical evidence showing that a 450-kDa active CLV1 complex did not form in *clv3* mutants (Trotochaud et al. 1999). *CLV3* was cloned by transposon tagging and encodes a 96-amino-acid protein (Fletcher et al. 1999). *CLV3* is predicted to be secreted and cleaved to produce a 78-amino-acid protein, although it has been speculated that the signal peptide is not actually removed from the protein (Cock & McCormick 2001). The apparent molecular mass of the *CLV3* monomer is 6 kDa, but it is present in plant tissues as a 25-kDa multimer (Trotochaud et al. 2000). It is not yet known whether this is a homomeric or heteromeric complex. *CLV3* bound to yeast cells expressing *CLV1* and *CLV2*, cofractionated with the active

CLV1 complex in gel filtration chromatography, and coimmunoprecipitated with CLV1 from plant extracts (Trotochaud et al. 2000). That CLV3 binds to CLV1 and is required for the formation of an active complex strongly suggests that CLV3 is the ligand for CLV1. Interestingly, CLV3 did not bind to *clv1-10* mutant protein in plants or yeast cells. This allele contains an amino acid substitution in the kinase domain that abolishes kinase activity. These results suggest that activity of the cytoplasmic kinase domain is required for efficient ligand binding by the ectodomain (Trotochaud et al. 2000).

CLV3 is expressed in a small group of central zone cells believed to represent the stem cell population (Figure 2). This expression domain overlaps slightly with the *CLV1* expression domain in the corpus layer (Fletcher et al. 1999). Thus stem cell proliferation appears to be regulated by signaling between layers.

CLV3 belongs to a family of related molecules designated CLE (*CLAVATA3/ESR*) that contain a conserved stretch of 15 amino acids near the carboxy terminus; all are predicted to be secreted (Cock & McCormick 2001). *Arabidopsis* contains 28 genes of this family. Three maize *ESR* (embryo surrounding region) proteins also belong to this family. The *ESR* proteins of unknown function are expressed in the developing maize endosperm surrounding the young embryo (Bonello et al. 2000).

SP11/SCR IS THE LIGAND FOR SRK From genetic evidence, it was long known that the pollen determinant of the *Brassica* self-incompatibility system was encoded by the complex *S-locus* (Nasrallah 1997 for review). The *SCR* (*S-locus cysteine-rich*) gene, also called *SP11* (*S-locus protein 11*), was discovered when sequence analyses of the region containing *SRK* and *SLG* revealed a cysteine-rich open reading frame (Schopfer et al. 1999, Suzuki et al. 1999). The encoded protein is similar (or identical) to a previously identified pollen coat protein (PCP), PCP-A1, which inhibits cross pollination when applied to stigmas (Stephenson et al. 1997). *SP11/SCR* is expressed in anthers and shows a high degree of allelic sequence variation (Schopfer et al. 1999, Takayama et al. 2000). When the *Brassica oleracea* *SCR*₆ allele was transformed into *S*₂ homozygotes, the transgenic plants could no longer pollinate *S*₆ females but were fully fertile as females in the reciprocal cross (Schopfer et al. 1999). Similar results were obtained with *B. rapa* *S*₈ and *S*₉ allele *SCR* (Shiba et al. 2001). Application of recombinant *SCR* protein elicited the self-incompatibility response when applied to stigmas of the same haplotype, thereby preventing germination of compatible cross pollen (Kachroo et al. 2001, Takayama et al. 2000). Thus *SP11/SCR* is the pollen determinant for self-incompatibility.

SP11/SCR is a small protein of 74–83 amino acids, depending on the allele (Schopfer et al. 1999, Takayama et al. 2000). Following cleavage of the signal peptide, the mature *SCR/SP11*₈ protein has a relative molecular mass of 5.7 kDa and contains four disulfide bonds among the eight cysteine residues (Takayama et al. 2001). The sequence and arrangements of cysteine disulfide bonds are similar to those of defensins (Bruix et al. 1993, Takayama et al. 2001), small proteins with antimicrobial activity (Broekaert et al. 1995). *Arabidopsis* contains two large families, 114 genes, that encode proteins related to *SCR/SP11* (Vanoosthuysse et al.

2001). The *SCRL* family is most closely related and contains 28 genes. Eighty-six genes show greater similarity to other PCPs, and it is hypothesized that these might represent ligands for other members of the S-domain RLK family.

Because SRK is the female determinant of self-incompatibility, it was suspected that the male determinant would be the ligand for this RLK. Support for this came from the demonstration that PCPs can activate SRK phosphorylation in an in vitro microsomal assay (Cabrillac et al. 2001). Activation occurred in a haplotype-specific manner; PCPs from compatible cross haplotypes did not induce SRK activation, whereas PCPs from self-haplotype pollen did. Direct interaction between SCR and SRK was demonstrated with pull-down experiments and ELISA (Kachroo et al. 2001). An epitope-tagged SRK₆ ectodomain, expressed and purified from tobacco leaves, was able to interact with bacterially expressed SCR₆. Interaction with the self SCR₆ was approximately 10-fold stronger than with the cross haplotype SCR₁₃. SRK₉ ectodomain expressed in silkworm larvae was not able to bind a biologically active SCR/SP11₉, suggesting that plant-specific modifications might be critical for SRK receptor function (Takayama et al. 2001). Finally, chemically synthesized SCR/SP11₈ induced autophosphorylation of SRK₈ in stigmatic plasma membrane fractions; SCR/SP11₉ did not (Takayama et al. 2001). ¹²⁵I-labeled SCR/SP11₈ specifically bound stigmatic microsomal membranes from S₈ homozygotes. Scatchard analysis indicated high- and low-affinity-binding sites with dissociation constants of 1.2 and 32 nM, respectively (Takayama et al. 2001). After chemical cross-linking, SRK immunoprecipitation pulled down the labeled SCR/SP11, as well as SLG. Thus SCR/SP11 binds SRK and activates autophosphorylation.

BRI1 BINDS BRASSINOLIDE The fact that *bri1* mutants are brassinolide insensitive indicates that the BRI1 receptor kinase functions at or downstream of the site of perception (Li & Chory 1997). Brassinolide application triggered pathogen defense responses in rice cells containing a chimeric RLK with the *Arabidopsis* BRI1 ectodomain fused to the cytoplasmic domain of XA21, a rice pathogen resistance RLK (He et al. 2000). This indicated that BRI1 was directly involved in brassinolide perception and suggested that it might function as a steroid receptor. *Arabidopsis* membrane fractions bound tritiated brassinolide with a dissociation constant near 10 nM, and the number of binding sites increased in transgenic plants overexpressing BRI1 (Wang et al. 2001). Binding was shown to be specific by competition with unlabeled brassinolide, whereas other steroids competed poorly. Membrane fractions from *bri1* mutants with disrupted ectodomains, including substitutions in the 70-amino-acid island, did not bind brassinolide. Anti-GFP antibodies coimmunoprecipitated tritiated brassinolide from extracts of plants expressing a BRI1-GFP fusion protein, demonstrating that BRI1 binds brassinolide directly or as part of a complex. Finally, treatment of *Arabidopsis* seedlings with brassinolide caused a mobility shift of BRI1 on SDS-PAGE immunoblots, which was reversible with alkaline phosphatase treatment and was not observed in mutants with inactive kinase domains. This suggests that brassinolide induces autophosphorylation of BRI1.

In sum, these results show that BRI1 functions as the site of brassinolide perception. That steroid perception occurs at the plasma membrane is interesting because steroids are able to freely cross membranes. In animals, the best-studied steroid receptors are cytosolic or nuclear (see commentary by Becraft 2001). Steroid responses initiated by membrane receptors are poorly understood, and it will be interesting to see whether they show any similarity to plant steroid perception.

GRP AND PECTIN BIND THE WAK ECTODOMAIN Two molecules have been identified that bind the ectodomain of WAKs. The first is pectin, a carbohydrate that forms part of the cell wall matrix (Wagner & Kohorn 2001). Pectinase treatment released WAKs from the cell wall fraction, and the anti-pectin antibodies, JIM5 and JIM7, recognized bands of the same mobility as WAK on immunoblots of cell wall-released proteins. The majority of pectin-bound WAK appears to be phosphorylated, as determined by immunoblotting of pectinase-treated cell wall fractions with anti-phosphothreonine antibodies; a band of similar mobility to WAK's was detected (Anderson et al. 2001). Whether pectin binding causes phosphorylation has not been determined so the functional significance of the interaction between WAK and pectin remains obscure. It is also possible that WAK could modify pectin (Wagner & Kohorn 2001).

The second molecule, an extracellular glycine-rich protein (GRP), was identified in yeast two-hybrid screens with the *WAK1* ectodomain (Park et al. 2001, Wagner & Kohorn 2001). AtGRP-3 interacted with WAK1, 3, and 5; WAK1 interacted only with AtGRP-3 and not -2, -4, -6, -7, or -8 (Park et al. 2001). WAK1 coimmunoprecipitated from plant extracts with GRP-3 antibodies, which suggests that the two proteins indeed interact *in vivo*. In seedling protein extracts, WAK1 is found in two gel filtration chromatography fractions of approximately 200 and 500 kDa, but in protoplast extracts it is present only in the 200-kDa fraction. Following application of AtGRP3 to the protoplasts, WAK1 is again found in both fractions, suggesting that GRP3 induces the formation of a high-molecular-weight WAK1 complex (Park et al. 2001). The functional significance of this complex formation is not yet known but likely represents receptor activation.

Other Components of RLK Signal Transduction Systems

Once activated, RLKs initiate downstream signal transduction pathways leading to cellular responses. This typically involves recruiting proteins to the activated receptor complex, triggering regulatory cascades of protein phosphorylation or other biochemical reactions, finally leading to alterations in gene expression and other cellular functions. Understanding the factors involved, the regulatory interactions among them, and their targets of regulation is essential to understanding RLK function.

CLV1 When plant extracts were size-fractionated by gel filtration, CLV1 was found in two fractions of approximately 185 and 450 kDa (Trotochaud et al.

1999). The 185-kDa complex is present in *clv3* mutants and in some alleles of *clv1*, depending on the lesion (Trotochaud et al. 1999). Therefore, this complex represents the inactive, disulfide-linked multimer mentioned above and is likely made up of CLV1 and CLV2 subunits (Jeong et al. 1999).

CLV2 is a receptor-like protein (RLP) with a LRR-containing predicted ectodomain and a transmembrane domain, but no cytoplasmic kinase domain (Jeong et al. 1999). Although a direct interaction between CLV1 and CLV2 has not yet been reported, genetic and biochemical evidence are consistent with this scenario. Strong *clv1* and *clv3* alleles are epistatic to *clv2* mutants in the shoot apical meristem (Kayes & Clark 1998). Weak *clv1* alleles showed allele-specific interactions with *clv2*; *clv1-7*, *clv2-2* double mutants showed greatly enlarged meristems comparable to strong *clv1* mutants. Although the expression of *CLV1* transcripts is expanded in *clv2* mutants, concomitant with the increase in meristem size (Kayes & Clark 1998), CLV1 protein levels are drastically diminished (Jeong et al. 1999). These data could be explained if CLV2 were a subunit of a heteromeric CLV1 receptor and required for CLV1 protein stability. Whereas *CLV1* functions exclusively in the shoot apical meristem, *CLV2* has additional functions in organ development (Clark et al. 1993, 1997; Jeong et al. 1999; Kayes & Clark 1998). It is hypothesized that CLV2 heterodimerizes with other RLKs during organ development. A similar function was recently discovered in maize. The *fasciated ear2* (*fea2*) mutant has enlarged female inflorescence meristems, indicating that *FAE2* functions to restrict meristem size (Taguchi-Shiobara et al. 2001). The gene was cloned by transposon tagging and encodes a RLP similar to CLV2. The *Arabidopsis* genome encodes 30 LRR RLPs, suggesting that heterodimers may be a common feature of LRR RLKs (Taguchi-Shiobara et al. 2001).

The 450-kDa fraction appears to represent an active receptor complex because this complex is absent in loss-of-function *clv1* and *clv3* mutants (Trotochaud et al. 1999). In addition to the 185-kDa disulfide-linked CLV1 multimer, several other components of this complex have been identified. As discussed above, CLV3 is part of the 450-kDa active complex (Trotochaud et al. 2000). ROP, a Rho-like GTPase, is also part of the active CLV1 complex. ROP antiserum detected a 25-kDa band that coimmunoprecipitated with CLV1 from the 450-kDa fraction but not from the 185-kDa fraction. The function of ROP in CLV1 signaling is not known (Trotochaud et al. 1999).

A noteworthy component of the active CLV1 complex is KAPP (kinase-associated protein phosphatase). KAPP is a type 2C protein phosphatase, first isolated by screening an *Arabidopsis* cDNA expression library for interactions with the cytoplasmic domain of HAESA, then known as RLK5 (Stone et al. 1994). KAPP bound phosphorylated but not dephosphorylated HAESA. The interaction occurred via a region of KAPP called the kinase interaction (KI) domain. Deletion analysis defined a 119-amino-acid region necessary for interaction with RLK kinase domains (Li et al. 1999). The center of this region contains a 52-amino-acid forkhead-associated (FHA) domain. FHA domains mediate interactions with

phosphoproteins in a wide variety of prokaryotes and eukaryotes (Li et al. 2000), and site-directed mutagenesis of conserved FHA residues abolished KAPP interactions with RLKs (Li et al. 1999). Both the *Arabidopsis* and maize KAPP KI domains interacted in vitro with the same subset of five out of seven diverse RLKs tested (Braun et al. 1997). Interactions have subsequently been reported between KAPP and a number of additional RLKs, including WAK1 (Park et al. 2001); OsTMK, a gibberellin-induced RLK in rice (van der Knaap et al. 1999); and CLV1 (Stone et al. 1998, Trotochaud et al. 1999, Williams et al. 1997).

The KAPP KI domain interacted with phosphorylated CLV1 in filter-binding assays (Stone et al. 1998, Williams et al. 1997) and coimmunoprecipitated with CLV1 antibodies from plant extracts (Stone et al. 1998, Trotochaud et al. 1999). *KAPP* RNA is expressed throughout shoot apical meristems and in provascular tissue, a distribution that includes, but is not limited to, the *CLV1* expression domain (Williams et al. 1997). *KAPP* was present in a broader range of high-molecular-weight fractions than *CLV1*, and was not eliminated from the high-molecular-weight fractions in *clv1* mutants, which suggests that *KAPP* interacts with multiple proteins (Trotochaud et al. 1999). Transgenic studies indicate that *KAPP* functions as a negative regulator of *CLV1* signaling. Overexpression of *KAPP* produced a slight increase in the number of carpels per flower, a phenotype similar to weak *clv1* mutants (Williams et al. 1997), whereas cosuppression of the endogenous *KAPP* transcript led to suppression of the *clv1-1* mutant phenotype (Stone et al. 1998).

Two targets of the *CLV* signal transduction system are the *WUSCHEL* (*WUS*) (Schoof et al. 2000) and *POLTERGEIST* (*POL*) genes (Yu et al. 2000). *pol* mutants partially suppress *clv1*, 2, and 3 mutants but have no phenotypic defects alone. It is hypothesized that they function redundantly with *WUS*. *WUS* promotes stem cell proliferation in the shoot apical meristem; *wus* mutant meristems become depleted of cells (Laux et al. 1996). *WUS* encodes a homeodomain protein and is expressed in a small group of cells in the interior region of the central zone, just beneath the *CLV3* expression domain (Mayer et al. 1998). In *clv* mutants, the *WUS* expression domain is expanded, whereas *CLV3* overexpression inhibits *WUS* expression (Brand et al. 2000). Thus *CLV* signaling inhibits cell proliferation through negative regulation of the *WUS* gene. A feedback loop from *WUS* conversely acts as a positive regulator of *CLV3* (Brand et al. 2000, Schoof et al. 2000). Overexpression of *WUS* expands the *CLV3* expression domain, and *CLV3* is not expressed in *wus* mutants.

The reciprocal signaling between the peripheral layers and internal cells is analogous to the regulation of cell proliferation in vertebrate limb buds, where FGF8 signals from the apical ectodermal ridge control proliferation of the mesodermal progress zone (Crossley et al. 1996). FGF10 signals back from the mesoderm to the apical ectodermal ridge to stimulate FGF8 expression, forming a feedback loop that maintains the proliferative activity of the limb bud (Ohuchi et al. 1997). However, whereas the ectodermal signals in limb buds act to promote proliferation, *CLV3* signaling limits cell proliferation.

SRK Another gene encoded by the *S-locus* is *SLG*. Although *SLG* appears to contribute to the self-incompatibility response, its function is not clear (Cui et al. 2000; Shiba et al. 2000; Takasaki et al. 1999, 2000). Self-compatible mutants that express low levels of *SLG* showed normal levels of *SRK* transcript but did not accumulate *SRK* protein (Dixit et al. 2000). Expression of *SRK* alone in tobacco resulted in the production of aberrant disulfide-linked *SRK* aggregates that were prevented by co-expressing *SLG* and *SRK*, which suggests that *SLG* expression might facilitate the processing or accumulation of *SRK*. These results have yet to be reconciled with the report that because some self-incompatible haplotypes lack *SLG*, it is dispensable (Suzuki et al. 2000). Furthermore, other studies indicate that genetic transformation with *SRK* alone was sufficient to confer a self-incompatibility phenotype (Takasaki et al. 2000). One possible explanation is that certain allelic variants of *SRK* are inherently more stable than others and thus show varying requirements for *SLG* (Dixit et al. 2000). As mentioned above, *SLG* coimmunoprecipitated as part of a chemically cross-linked *SRK* complex, suggesting that *SLG* may function as a coreceptor with *SRK* (Takayama et al. 2001). Thus coreceptors might not be limited to *LRR RLK* complexes.

Several other components of the *SRK* signal transduction pathway have been identified. *KAPP* interacts with *SRK* in filter-binding assays (Braun et al. 1997) but whether this has functional relevance is not yet known. *ARC1* (*ARM REPEAT CONTAINING1*) was identified by screening a yeast two-hybrid library with the *SRK* cytoplasmic domain (Gu et al. 1998). The interaction required an active *SRK* kinase domain, suggesting that it was phosphorylation dependent, and the *SRK* kinase phosphorylated *ARC1* in vitro (Gu et al. 1998). The interaction occurred in the carboxy-terminal region of *ARC*, which contains five *ARM* repeats, a protein-protein interaction motif (Gu et al. 1998, Mazzurco et al. 2001). *ARC1* interacted in yeast two-hybrid assays with all five *SRK* allelic variants tested and with related *RLKs*, *SFR1*, and *SFR2*; however, whether these latter interactions have biological relevance is unknown (Mazzurco et al. 2001). *ARC1* transcript is expressed specifically in stigmatic tissues (Gu et al. 1998). Although the biochemical function of *ARC1* is unknown, antisense suppression of *ARC1* partially disrupts the self-incompatibility response (Stone et al. 1999). Antisense has no effect on compatible pollinations and overexpression does not affect either incompatible or compatible pollinations. Thus *ARC1* is a specific and essential component of the *SRK* signal transduction pathway and acts as a positive effector of the self-incompatibility response.

Two *THIOREDOXIN-H* clones, *THL1* and *THL2*, were also isolated in yeast two-hybrid screens with the *SRK* cytoplasmic domain (Bower et al. 1996). They interact specifically with *SRK* and not with other *RLKs* tested (Bower et al. 1996, Mazzurco et al. 2001). *SRK* interacts with two of the five *THIOREDOXIN-H* proteins of *Arabidopsis* (Mazzurco et al. 2001). A kinase-inactive mutant *SRK* failed to interact with both clones in yeast but did interact with *THL1* in vitro, suggesting the interaction may not be phosphorylation dependent. *THL1* was weakly phosphorylated by *SRK* and shown to have thioredoxin activity in vitro (Bower et al.

1996). The interaction required an active catalytic site on *THL1* and a specific cysteine residue on *SRK*, indicating that the interaction involved redox activity (Mazzurco et al. 2001). Although *THL2* showed preferential expression in floral tissues, neither was specific to the pistil, suggesting they have additional functions outside *SRK* signaling (Bower et al. 1996).

Recent evidence indicates that thioredoxin inhibits *SRK* (Cabrillac et al. 2001). Full-length *SRK* constitutively autophosphorylates in stigma or insect cell microsomes, but only in response to pollination in intact stigma cells. Addition of soluble stigma extract inhibited autophosphorylation, which suggests that an inhibitor present in stigma cells prevents phosphorylation in the absence of ligand. Depletion of thioredoxin from the extract with affinity resin removed the inhibitor; addition of recombinant *THL1* inhibited the *in vitro* autophosphorylation of *SRK*. When pollen coat proteins containing *SCR/SP11*, the *SRK* ligand, were added to the *in vitro* phosphorylation reactions in the presence of inhibitory stigma extracts, autophosphorylation was detected, but only when PCPs were isolated from a self-incompatible haplotype relative to the *SRK* allelic variant. Thus ligand binding overcomes the inhibitory effects of thioredoxin.

The *SRK*, *SLG*, and *ARC1* proteins are not sufficient to confer self-incompatibility to female *Arabidopsis* tissues. Transgenic *Arabidopsis* plants expressing these genes did not block hydration and germination of *Brassica* pollen of an incompatible haplotype (Bi et al. 2000). Therefore, additional components to this system, present in *Brassica*, are missing in *Arabidopsis*.

It was previously hypothesized that the *SRK* signaling system might regulate an aquaporin protein (Ikeda et al. 1997). An aquaporin gene was tightly linked to, and not transcribed in, a *mod* mutant that eliminated the self-incompatibility response. One of the early events in pollen germination is hydration, so it was logical to hypothesize that an aquaporin could be important for this process and would be a likely point to block pollen germination in the self-incompatibility response. However, antisense suppression of the aquaporin gene did not inhibit self-incompatibility, and a new γ -ray-induced *mod* mutation had an intact aquaporin gene expressed at normal levels (Fukai et al. 2001). Therefore, *MOD* does not correspond to aquaporin, and the product of this gene remains unknown.

BRI1 No proteins that directly interact with *BRI1* have been reported, yet several factors that appear to function in *BRI1* signal transduction have been identified. An activation-tagging screen for genetic suppressors of the weak *bri1-5* mutant yielded *brs1-1D* (*bri1 suppressor1-1Dominant*), which dominantly suppresses multiple aspects of the *bri1* phenotype (J. Li et al. 2001). Overexpression of *BRS1* suppresses the *bri1-5* phenotype; a *brs1* knockout produced no obvious phenotypic effects, which suggests that this gene may be redundant. Neither the activation-tagged nor overexpressed *BRS1* had any effect on the phenotype of wild-type plants. *brs1-1D* did not suppress the *bri1-5* phenotype in a brassinolide-deficient mutant background and only suppressed *bri1* alleles with extracellular lesions but not with a deficient kinase domain. Thus *brs1-D* appears to affect an early step specific to

brassinolide signaling. *BRS1* is predicted to encode a secreted carboxy peptidase, and site-directed mutagenesis demonstrated that carboxy peptidase activity is required for suppression of *bri1-5*. Whether this activity is required for processing of BRI1, another component of BRI1 signal transduction, or another function is not yet known.

A second mutant gene that causes brassinolide insensitivity was recently reported. *bin2* (*brassinolide insensitive2*) is a semidominant gain-of-function mutant. The gene was cloned and found to encode a previously described kinase, ASK η , related to GSK3/SHAGGY (Li & Nam 2002). Overexpression of a wild-type allele recapitulated the *bin2* phenotype and enhanced the weak phenotype of *bri1-301* mutants, whereas reduced expression of *BIN2* suppressed *bri1-301*. Thus *BIN2* acts as a negative regulator of BRI1 signaling. Efforts to demonstrate direct interactions between BRI1 and *BIN2* were unsuccessful.

The *TRIP-1* gene was isolated by subtractive hybridization as an mRNA induced by brassinolide treatment in bean cell suspension cultures (Jiang & Clouse 2001). *Arabidopsis* contains two highly related *TRIP-1* genes with 90% amino acid identity to one another. *TRIP-1* transcript was induced by brassinolide in *Arabidopsis* cell cultures in the presence of cycloheximide, indicating that protein synthesis was not required. Antisense suppression produced dwarf plants with phenotypes similar to brassinolide deficiency, suggesting that *TRIP-1* has a key function in the brassinolide response. The encoded protein of 326 amino acids contains five WD repeats and is similar to TGF- β receptor interacting protein-1 (*TRIP-1*). *TRIP-1* is identical to a translation factor, eIF3-i, which is a conserved component of eIF3 in plants, mammals, and yeast (Burks et al. 2001). This suggests that translational regulation might be a key aspect of brassinolide signaling. *TRIP-1* is also a substrate for the TGF- β receptor serine/threonine kinase (Chen et al. 1995), suggesting that phosphorylation might also regulate translational activity.

Biochemical analysis of *BRI1* antisense rice plants showed alterations in calcium-dependent protein kinase (CDPK) and MAP kinase activity (Sharma et al. 2001). The activity of a 60-kDa membrane-associated MAP kinase was induced by brassinolide treatment, but activity and brassinolide responsiveness were decreased in *BRI1* antisense plants. Conversely, the activity of a 50-kDa cytosolic CDPK decreased with brassinolide treatment and increased in *BRI1* antisense plants. On two-dimensional gels, the Ca²⁺-dependent phosphorylation of several proteins increased in *BRI1* antisense plants. Thus *BRI1* appears to positively regulate MAP kinase activity and negatively regulate CDPK activity.

Expression of constitutively active and dominant-negative forms of *ROP2* causes alterations in photomorphogenic responses and sensitivity to brassinolide, which suggests that *ROP* may function in the *BRI1* signal transduction pathway (H. Li et al. 2001). The possibility that *ROP* may be a direct signal transducer is intriguing given the demonstrated interaction between *ROP* and *CLV1* (Trotochaud et al. 1999).

PRK1 Yeast two-hybrid screens have identified two factors that interact with *PRK1*. The first, *NeIF2B β* , shows homology to *eIF2B β* from mammals and yeast

(Park et al. 2000). eIF2B β is a subunit of eIF2B, a guanine exchange factor that is an essential component of the translation initiation machinery. Mammalian eIF2B can be regulated by phosphorylation, albeit on the α -subunit (Kleijn et al. 1998). Nonetheless, it suggests the possibility that PRK1 regulates translation. The second factor is KIP1 (kinase-interacting protein1) (Skirpan et al. 2001). Both *KIP1* and *PRK1* transcripts are specifically expressed in pollen grains. The interaction is much weaker with a kinase-impaired PRK1 bait than with wild-type, suggesting that the interaction is phosphorylation dependent. PRK1 phosphorylates KIP1 in vitro. The function of KIP1 is unknown, but recognizable motifs include an EF hand calcium-binding motif, 9 coiled coils (a protein-protein interaction motif), and 7 tandem repeats of an 11-amino-acid sequence similar to a feature on Tau, a protein involved in microtubule assembly. Given the requirement for PRK1 in gametophytic mitosis, it is intriguing to speculate that PRK1 might signal through KIP1 to regulate the microtubule cytoskeleton in postmeiotic divisions.

ERECTA *AGB1* was identified as a possible component of the ER signal transduction pathway in a genetic screen for mutants with phenotypes similar to the *er* phenotype (Lease et al. 2001b). *elk* (*erecta-like*) mutants fell into five complementation groups. *elk1* was allelic to the *tir3* mutant with reduced sensitivity to the auxin transport inhibitor, *N*-1-naphthylphthalamic acid (NPA) (Ruegger et al. 1997). The *elk4* phenotype was the most similar to *er*, and double mutants suggest that the two genes function in the same pathway controlling silique development (Lease et al. 2001b). Other organs showed more severe double-mutant phenotypes than either single mutant, which suggests that the genes may function in parallel pathways in leaves and stems. The *ELK4* locus was cloned (Lease et al. 2001b) and encodes a previously described heterotrimeric G protein β -subunit *AGB1* (Weiss et al. 1994). It remains to be determined how *AGB1* fits into the ER pathway, but it does not appear to be a downstream target gene because *AGB1* transcript was at nearly normal levels in *er* mutants and vice versa (Lease et al. 2001b).

SUMMARY AND FUTURE PROSPECTS

Plant genomes encode a much larger complement of receptor kinase-like proteins than other organisms. Post-transcriptional processing further increases the complexity of the “RLKome.” It is evident from the relatively few studied plant RLKs that this class of proteins has many diverse functions that will be revealed only through functional analyses. As such, it is likely that an equally diverse array of signaling pathways will emerge. In mammals, signaling systems are often modular, and many divergent pathways share multiple components. Early indications are that plants similarly have components that function in multiple pathways. KAPP interacts with several RLKs in vitro, and functional analyses indicate it is a negative regulator of at least two pathways. How widespread KAPP function is remains to be determined. Several RLKs, including *CLV1* and *SRK*, may function with coreceptors that lack kinase domains, and genome analysis suggests this

might be a common feature of RLK signaling. Genetic, molecular, and biochemical approaches are identifying components of RLK signaling pathways. The functions of several are known, but how most of these factors fit into the respective signaling systems remains to be determined. Rapidly developing tools of genomics and proteomics promise rapid advancement of this field.

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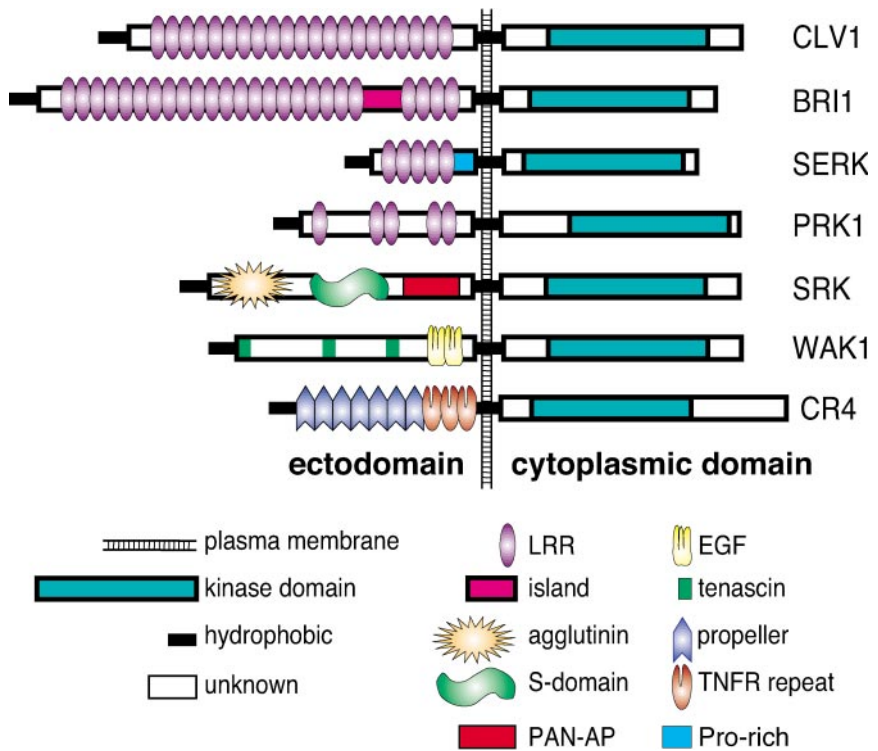


Figure 1 Protein domain configurations of several select receptor-like kinases.

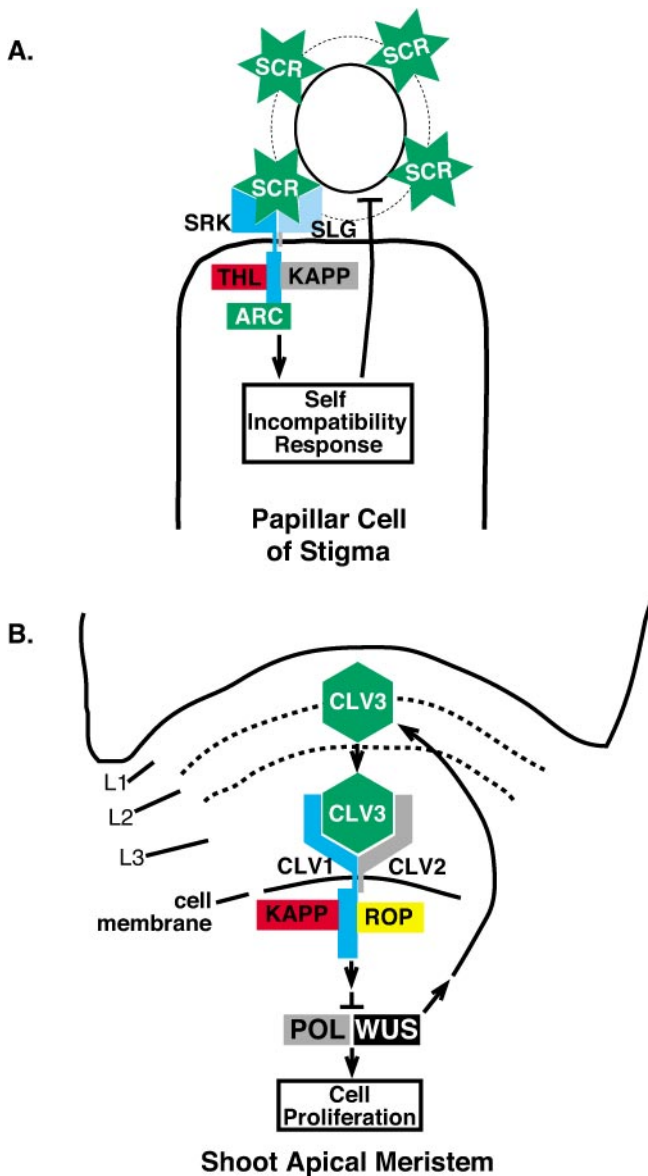


Figure 2 Diagram of proposed signaling pathways for SRK (A) and CLV1 (B). RLKs are shown in dark blue, co-receptors in light blue, positive effectors are green, negative effectors are red, and factors with unknown functions are yellow. Downstream targets of the signaling pathway are in black. Components in gray remain to be verified. Arrows and blocking symbols do not necessarily denote a specific number of steps because in most cases the number of steps between events is unknown. Dotted lines represent hypothetical relationships.